Reduction of Myocardial Necrosis in Male Albino Rats by Manipulation of Dietary Fatty Acid Levels[1]

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ABSTRACT

A comprehensive statistical analysis had shown a significant correlation between the incidence of myocardial lesions in male albino rats and the concentration of certain dietary fatty acids. To test this result under controlled conditions, male rats were fed for 16 weeks diets containing 20% by weight soybean oil or a low erucic acid rapeseed (LEAR) oil. Both dietary oils contained substantial amounts of linolenic acid, and both groups developed a high incidence of myocardial necrosis. The addition of dietary saturated fatty acids to the oil in the form of cocoa butter significantly lowered the incidence of heart lesions in both groups. The addition of cocoa butter resulted in increased absorption of saturates and increased growth. Replacement of the cocoa butter by at least an equal amount of synthetic triolein resulted in no significant changes in the cardiopathogenic response compared to the original oils, thus ensuring that the reduction in heart lesions associated with the addition of cocoa butter was not due to dilution of cardiopathogenic compounds in the original vegetable oils. These results support the hypothesis that myocardial lesions in male rats are related to the balance of dietary fatty acids and not to cardiotoxic contaminants in the oils. Changes in the dietary fatty acids did not appear to influence the proportion of the cardiac phospholipids, but their fatty acid composition was markedly influenced. Dietary linolenic acid affected the C22 polyunsaturated fatty acids (PUFA) and dietary saturates increased the level of saturates in cardiac phospholipids. The level of arachidonic acid and total C22 PUFA did not appear to be affected by diet.

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INTRODUCTION

Evidence of necrosis affecting the heart muscle (myocardial necrosis) of male rats after feeding diets rich in fat was first reported by Roine et al. (1) in 1960. These workers attributed the pathological finding to erucic (*cis*-13-docosenoic) acid (22:1) which was present at high levels in the older varieties of rapeseed oil. Subsequent studies showed that male rats fed either the new varieties of rapeseed oils which are practically devoid of 22:1 (2) or other vegetable oils (3) for at least 4 months also developed myocardial necrosis. The occurrence of myocardial necrosis has been demonstrated repeatedly in male albino rats fed corn oil (4-7), coconut oil (8), olive oil (4,9-11), peanut oil (12-14), poppyseed oil (15), safflower oil (8,10), soybean oil (4,5,8,9,16-19), sunflower oil (11,15,20), lard (21), and lard-corn oil mixtures (9,15,22).

The etiology of myocardial necrosis in male rats is still not completely understood. Of the several hypotheses advanced to explain this phenomenon, the one suggesting solely the presence of 22:1 is not supported by experimental evidence (4,7,8,11,21). The one suggesting the presence of cardiotoxic compounds in vegetable oils is highly improbable, because highly purified triglycerides from soybean oil (18) and low erucic acid rapeseed (LEAR) oil (10,18,23,24) are as cardiopathogenic as the corresponding vegetable oil. These findings led to a third hypothesis that the dietary fatty acids per se may give rise to myocardial necrosis in male rats (3,4,8,11,21). This hypothesis was strengthened by the results of a comprehensive statistical analysis of most published data on heart lesions which showed a significant correlation between the level of certain dietary fatty acids and the incidence of myocardial necrosis in male rats (25). In this statistical study, most of the variations in incidence of heart lesions among diets within experiments (59.5%) was explained by the level of saturated fatty acids (16:0 and 18:0) and linolenic acid (18:3); their effect was similar in magnitude but opposite in sign (Table 1).

The statistical analysis (25) suggests a model for the fatty acid imbalance hypothesis which was tested experimentally in this study. The present communication gives the results of this study in which dietary oils were prepared containing different levels of specific fatty acids that were fed to male rats for 16 weeks. In addition to cardiopathology, growth measurements and cardiac lipid were investigated to determine if, and to what extent they reflect changes in myocardial necrosis. Detailed nutri-

[1]Contribution no. 1004 from the Animal Research Centre and No. I-272 from the Engineering and Statistical Research Institute.

TABLE 1

A Summary of a Regression Analysis[a] of Aggregate Data Comparing Levels of Dietary Fatty Acids with Observed Incidence of Myocardial Lesions in Male Albino Rats

	Fatty acids					
	16:0 + 18:0	18:1	18:2	18:3	20:1	22:1
Overall regression coefficient	-0.013	0.002	0.001	0.016	-0.003	0.014
Partial correlation	-0.72	0.40	-0.23	0.61	0.45	0.42

[a]The regression coefficients and partial correlations were reported previously (25) except for the sum of 16:0 + 18:0 which was obtained by reevaluating the data set used in that publication. The regression coefficients are expressed as incidence of lesions/% by wt of fatty acid in test oil. Incidence is defined as number of rats affected over number of rats examined. The correlations between incidence and dietary fatty acid levels take into account experimental differences.

tional aspects have been published elsewhere (26).

MATERIALS AND METHODS

Rationale for Test Oil Mixtures

Two vegetable oils were selected which contained substanial amounts of 18:3, soybean oil and LEAR oil (Table 2). The presence of 18:3 in these oils should produce, based on the statistical evaluation (25), a relatively high incidence of myocardial necrosis in male albino rats. Based on the model, an increase in the level of saturated fatty acids in these oils should lower the incidence of myocardial necrosis. This increase in saturated fatty acids was accomplished by mixing the vegetable oils with cocoa butter (56% saturated; 26% 16:0; 29% 18:0; 1% 20:0; 0.5% 16:1; 38.5% 18:1; 5% 18:2) in a ratio of 16:4. To guarantee that the results of cardiopathology were not due to dilution of toxic substances in the 2 vegetable oils, synthetic triolein (91% 18:1; 6% 18:2; 2% 18:0; 1% 16:0) was mixed with the oils in at least an equal proportion as was the saturated fat. Oleic acid was selected because it showed a relatively low correlation to myocardial necrosis in the statistical analyses (Table 1). Soybean oil was mixed with the synthetic triolein in a ratio of 1:1 because the resultant mixture would

TABLE 2

Composition of Dietary Oils and the Fatty Acid Analysis of These Oils

	% by wt of the diet						
LEAR oil (cv. Tower)	20	16	16	–	–	–	–
Soybean oil	--	–	–	20	16	9.6	9.5
Cocoa butter	–	4	–	–	4	–	–
Triolein	–	–	4	–	–	9.6	9.5
Linseed oil	--	–	–	–	–	0.8	0.8
X:1 (20:1, 22:1 and 24:1)	–	–	–	–	–	–	0.2
Fatty acids (% by wt)							
14:0	0.1	0.1	0.1	0.1	0.1	tr	tr
16:0	4.4	8.2	3.4	12.1	17.9	5.8	5.8
18:0	1.5	7.5	1.2	3.5	9.2	2.6	2.7
20:0	0.8	0.6	0.5	0.4	0.4	0.3	0.5
22:0	0.3	0.2	0.2	0.1	tr	tr	0.1
24:0	0.1	tr	tr	tr			
Total saturates	7.2	16.6	5.4	16.2	27.6	8.7	9.1
16:1	0.4	0.2	0.2	0.2	0.3	0.1	0.1
18:1	57.5	55.8	66.9	24.6	26.9	56.1	55.3
20:1	1.9	1.2	1.2	0.3	0.2	0.1	1.0
22:1	0.6	0.4	0.4	0.1	tr	tr	0.4
24:1	0.1	tr	tr				tr
Total monounsaturates	60.5	57.6	68.7	25.1	27.4	56.3	56.7
18:2	22.0	17.8	17.9	51.9	40.1	28.3	27.5
18:3	10.3	7.9	7.8	6.7	4.9	6.7	6.7

have a fatty acid composition similar to that of the LEAR oil. The addition of a small amount (14.9 g/kg test oil) of long-chain monounsaturated fatty acids (79% 20:1; 20% 22:1 and 1% 24:1) to the latter mixture provided an even closer resemblance to LEAR oil. An oil that mimicked the fatty acid composition of a LEAR oil would be expected to give a similar incidence of heart lesions. The 18:3 content of the 2 test oils containing the mixture of soybean oil and triolein was restored to that of the original soybean oil by the addition of an appropriate amount of linseed oil (49%, 18:3).

Experimental Design

The diets were formulated to contain 20% oil and 20% casein as described previously (3, 10,26). All test oils were added to the diet at a level of 20% by weight (Table 2). The caloric content of the diets was measured by bomb calorimetry (27). Male Sprague-Dawley rats (50-60 g) were randomly allocated among 7 groups, each consisting of 50 animals, and fed ad libitum the semisynthetic diets for 16 weeks. All rats were weighed individually at the beginning and at 2-week intervals throughout the experiment. Apparent digestibilities were calculated from consumption, excretion, and composition data measured at 4, 8, and 12 weeks (28,29). Consumption data were measured from 5 groups of 10 rats per diet for the period of the whole experiment.

Analytical Procedures

Six rats from each dietary group were killed after 16 weeks by exsanguination while under CO_2 anesthesia. The hearts were removed immediately and the total lipids extracted according to a procedure intended to minimize lipolysis (30).

The cardiac lipid classes were quantitated following separation on thin silica-coated quartz rods (Chromarod S) using an Iatroscan (Technical Marketing Associates, Mississauga, Ont.) equipped with a flame ionization detector (H_2 flow rate, 175 ml/min; air flow rate, 1,850 ml/min), a scanner (scanning speed, 0.47 cm/sec), and an integrator and recorder (sensitivity, 10 mV; chart speed, 0.42 cm). The chromarods (type S) were successively developed using the following solvents: (a) hexane/diethyl ether/formic acid (85:15:0.04), (b) acetone, and (c) $CHCl_3/CH_3OH/H_2O$ (67:29:4). After each development, the chromarods were partially burned (31) to determine, in turn, neutral lipids, cholesterol and polar lipids. The phospholipids were isolated by thin layer chromatography (TLC) according to Rouser et al. (32). The fatty acid composition of cardiac lipid classes was determined by gas chromatography as described previously (7).

The hearts from the remaining 44 rats per diet group were removed and fixed in 10% neutral buffered formalin. Three histological sections were prepared from each heart as described previously (33).

Statistical Methods

The various measurements by the analytical procedures just described were analyzed using the analysis of variance. An approximate χ^2 method, described by Fienberg (34), was used to analyze the incidence data, i.e., the frequency of rats showing evidence of myocardial lesions in any of their 3 heart sections.

The data set and the statistical methods described by Trenholm et al. (25) were used to estimate a prediction equation involving levels of saturates (16:0 and 18:0) and 18:3. The original study used levels of 16:0 or 18:0 separately because they were highly correlated but, because cocoa butter contains considerable amounts of both fatty acids, it was felt that the sum of the saturate levels would be more appropriate for the analysis of this paper.

Dietary Test Oils

The proportion of fats and oils in the experimental diets and the corresponding fatty acid compositions are shown in Table 2. The addition of cocoa butter increased the percentage of saturated fatty acids of both LEAR and soybean oils by ca. 10%. The addition of triolein to soybean oil resulted in a mixture with a fatty acid composition much like that of LEAR oil. The further addition to this mixture of small amounts of long-chain monounsaturated fatty acids (20:1, 22:1 and 24:1) provided an even closer resemblance. Linseed oil was added to the mixtures containing soybean oil and triolein (1:1) to restore the level of 18:3 close to that in the original soybean oil. All diets were found to be isocaloric as determined by bomb calorimetry.

RESULTS

Cardiopathology

A high incidence of myocardial necrosis was observed in male Sprague-Dawley rats fed diets containing both LEAR and soybean oils; the difference in lesion incidence between these diets was not significant (Table 3). A 10% increase in the level of dietary saturated fatty acids of these oils, achieved by the addition of

TABLE 3

Myocardial Lesions in Male Rats Fed the Experimental Diets for 16 Weeks and Lesion Incidence Predicted

	Incidence (%)[a]		Severity[c]				
	Observed (n = 44)	Predicted[b]	1	2	3	4	>4
LEAR (cv. Tower)	61	64	10	10	3	3	1
LEAR + cocoa butter	36	47	8	3	2	2	1
LEAR + triolein	55	62	11	9	3	1	0
Soybean	57	46	10	7	7	1	0
Soybean + cocoa butter	34	27	11	1	1	1	1
Soybean + triolein	59	55	16	4	1	4	1
Soybean + triolein + X:1[d]	55	55	16	3	2	2	1

Comparisons	d.f.	χ^2[e] Incidence
All diets	6	13.0*
Diets with cocoa butter vs original oils	1	10.2**
Diets with triolein vs original oils	1	0.1

[a]The incidence of myocardial lesions is the number of rats affected over the number of rats examined per diet (44 rats).

[b]The predicted incidence of heart lesions was calculated using the following equation: $Z_i = \bar{Y} - 0.013\ (X_{1i}-\bar{X}_1) + 0.016\ (X_{2i}-\bar{X}_2)$ where Z_i is the predicted incidence of heart lesions, $\bar{Y}$ the average observed incidence of heart lesions for all diets (0.51), -0.013 and 0.016 the correlation coefficients of 16:0 + 18:0 and 18:3, respectively, X_i and X_2 the dietary concentration of 16:0 + 18:0 and 18:3 in the ith diet, and $\bar{X}$ the overall mean concentration of the specific fatty acid(s) from all diets.

[c]Severity of myocardial necrosis represents the number of rats with 1, 2, 3, 4 and >4 lesions per heart (3 sections).

[d]X:1 (20:1, 22:1, 24:1).

[e]The χ^2 analysis was according to Fienberg (34); d.f., degrees of freedom, and significance at the 5% (*), and 1% (**) level.

cocoa butter, resulted in a major decrease in the incidence of myocardial necrosis. On the other hand, using triolein in the mixture, rather than cocoa butter, gave levels of incidence in heart lesions not significantly different from those of the original oils.

The severity of myocardial necrosis, expressed as the total number of lesions per set of 3 heart sections, was relatively low in this study for rats fed soybean oil or LEAR oil compared to previous results from this laboratory (4,7, 18). Most of the affected rats had only 1 or 2 lesions per heart; those with more than 4 lesions per heart were very few. Hence, no attempt was made to analyze the severity data.

Table 3 includes the incidence of heart lesions as predicted by the regression equation (Table 1) using the fatty acid composition of the test oils (Table 2). It can be seen that the observed and predicted incidence of myocardial necrosis is in fairly close agreement.

Growth Performance and Fat Consumption

The rats fed the LEAR diet consumed less feed overall and weighed less at the end of the experiment than did rats fed the soybean diet (Table 4). The fact that only the weight data showed a significant difference here was perhaps because the consumption data, unlike the weight data, were not measured on individuals. Growth and feed consumption increased with the addition of cocoa butter to the test oils; the increase in growth was significant for the addition of saturates to LEAR oil. Substitution of triolein for the cocoa butter in the mixture with the LEAR oil appeared to have little influence on either variable. The dietary oils formulated to mimic LEAR oils seemed to reduce body weight and feed consumption, relative to soybean oil, the differences being appreciable only for the weight data.

Estimates of the consumption of 16:0 and 18:0 by the dietary groups are also given in Table 4. Rats fed soybean oil consumed much more saturated fatty acids than did those fed LEAR oil. The consumption of 16:0 and 18:0 increased markedly in rats fed diets with cocoa butter, whereas the addition of triolein reduced

TABLE 4

Body Weight, Feed Consumed and Consumption of Saturated Fatty Acids

Diets	Body weight (g)[a]	Feed consumed (g/rat)[b]	Consumption (g/rat)[c] 16:0	18:0
LEAR (cv. Tower)	416	1636	14	4
LEAR + cocoa butter	466	1684	24	17
LEAR + triolein	427	1654	12	5
Soybean	496	1709	34	10
Soybean + cocoa butter	508	1733	41	23
Soybean + triolein	461	1712	19	8
Soybean + triolein + X:1[d]	464	1681	20	8
LSD ($p<0.01$)[e]	26	70		

[a]Body weights are the mean of 50 rats per diet.

[b]Feed consumption is the total feed consumed by the rats over the entire experimental period (16 weeks) divided by the number of rats per group. Five groups of 10 rats were used for each diet.

[c]The total amount of 16:0 and 18:0 consumed was calculated by: average feed consumed (per 16 weeks) X % oil in diet X % composition of fatty acid in oil X apparent digestibility of fatty acid (average of week 4, 8 and 12).

[d]X:1, (20:1, 22:1 and 24:1).

[e]LSD, least significant difference.

TABLE 5

Weight, Lipid Content and Lipid Class Composition of the Hearts of Rats Fed the Experimental Diets for 16 Weeks

	Heart weight (g)	Lipid weight (mg/heart)	Lipid classes (mg/g wet heart)[a] CE	TG	C	DPG	PE	PS & PI	PC	SP
LEAR (cv. Tower)	1.02	31.9	0.4	8.9	2.1	2.6	6.0	0.8	9.0	0.7
LEAR + cocoa butter	1.03	34.5	0.3	11.2	2.0	2.4	6.0	0.9	8.9	0.8
LEAR + triolein	1.18	43.3	0.3	14.2	2.3	2.5	5.7	0.9	9.0	0.9
Soybean	1.09	36.1	0.3	9.3	2.2	2.7	6.6	0.8	9.8	0.7
Soybean + cocoa butter	1.25	41.2	0.4	8.9	2.2	2.7	7.1	0.9	9.3	0.8
Soybean + triolein	1.07	36.2	0.3	9.0	2.3	3.0	6.9	0.9	9.8	0.8
Soybean + triolein + X:1[b]	1.11	35.0	0.3	7.9	2.3	2.8	6.5	0.8	9.5	0.9
LSD ($p<0.01$)[c]	0.19	9.3	0.2	4.2	0.5	0.7	1.3	0.4	1.3	0.3

[a]All values are the mean of 6 rats per diet. The lipid classes are: cholesterol ester (CE), triacylglycerol (TG), cholesterol (C), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC) and sphingomyelin (SP). Trace amounts of lysophosphatidylcholine, cerebroside, diacylglycerol and free fatty acid were found but were not included in the table.

[b]X:1 (20:1, 22:1 and 24:1).

[c]LSD, least significant difference.

the absorption of these fatty acids.

Cardiac Lipid Changes

The heart and lipid weights, including the cardiac lipid class composition, are shown in Table 5. Mixing either cocoa butter or triolein with LEAR oil resulted in increased levels of cardiac lipids in the form of triacylglycerol compared to rats fed LEAR oil. The addition of cocoa butter or triolein to soybean oil appeared to have no effect on total cardiac lipids or its composition. The phospholipids were remarkably similar among all diets.

The fatty acid composition of the major cardiac phospholipids are summarized in Table 6. An analysis of variance incorporating the data from 3 phospholipids were calculated and are included in Table 6 (diphosphatidylglycerol was excluded because the composition of this polar lipid was so different). It was evident

from the statistical analysis that rats fed soybean oil showed significantly higher levels of saturated and total PUFA, and significantly lower levels of monounsaturated fatty acids in all phospholipids compared to rats fed LEAR oil. In contrast to the significant differences between the soybean and LEAR oil groups, there was a remarkable similarity in the fatty acid composition of the phospholipids of rats fed LEAR and soybean + triolein mixtures (the latter intended to mimic LEAR), there being but one significant difference in fatty acid levels. Enrichment of dietary saturated fatty acids by the addition of cocoa butter resulted in a significant increase in cardiac saturated fatty acids, apparently at the expense of monounsaturated fatty acids and linoleic acid. The most noticeable changes arising from the addition of triolein were in the increased levels of the monounsaturated fatty acids and C22 (n-3) PUFA, and in the decreased levels of saturated and (n-6) PUFA. The differences here were more pronounced in those diets with the higher proportion of triolein. The level of cardiac arachidonic acid and the total C22 PUFA did not appear to be influenced by the modifications to the dietary oils.

It should be noted that the interactions between phospholipid classes and diets were significant for most fatty acids, except saturates. However, the interactions were generally of a much smaller magnitude than the overall differences. The trends among diets for the phospholipid classes were similar; the reason for the interactions seemed to be the differences in degree of change. Transformations of the data did not remove these interactions.

Cardiac sphingomyelin consisted almost exclusively of saturated and monounsaturated fatty acids (Table 7). Rats fed soybean oil showed significantly more saturated (particularly 18:0, 22:0 and 24:0) and less monounsaturated (particularly 18:1 and 24:1) fatty acids than did rats fed LEAR oil. The addition of cocoa butter to the 2 oils had little effect on the relative proportion of saturates to monounsaturates; however, there was an increase of the C18 fatty acids at the expense of the longer chain fatty acids. The addition of triolein to LEAR oil resulted only in minor changes. On the other hand, the composition in rats fed the soybean oil triolein mixtures approached the composition of rats fed LEAR oil in the proportion of saturates and unsaturates, but there were significant differences between the kind of fatty acids within each group.

DISCUSSION

An earlier study (25) showed a relationship between levels of certain dietary fatty acids and the incidence of myocardial necrosis in male albino rats. In particular, in the presence of appreciable amounts of dietary 18:3, increases in the levels of dietary saturates were associated with a lower incidence of lesions. The nature of the study, however, precluded conclusions relating to cause and effect. The lower incidence of lesions might, e.g., be due to the type of oil, the fatty acid levels characteristic of that oil, or some other related factors. To test the "cause-effect" hypothesis properly, one must control the levels of the dietary fatty acids specifically. To this end, in this study, the level of saturated fatty acids in 2 vegetable oils, viz., soybean and LEAR oils, were increased by the addition of cocoa butter. It might still be argued that any observed change in incidence was due, not to the addition of the saturates, but to the dilution of other fatty acids or even some cardiotoxic compounds. Hence, additional mixtures were included with a synthetic triacylglycerol replacing the cocoa butter. This material was composed almost entirely of oleic acid, a fatty acid which the original study suggested was not closely related to the incidence of lesions (Table 1).

The results from this study provide convincing experimental evidence to support the hypothesis suggested by the earlier study (25). Addition of the saturates to both vegetable oils led to similar reductions (about 25%) in incidence of lesions. Furthermore, the addition of oleic acid had no discernible effect whatsoever. The fact that there are some discrepancies between the observed and predicted incidence of heart lesions (Table 3) is, indeed, not surprising. It is well established that differences in lesion incidence will occur between (35) and within (18) experiments even though all parameters were kept as nearly identical as possible. The fact is, the results of this study fit remarkably well into the continuum of points derived from the regression equations of the aggregate data of heart lesions and dietary fatty acids (25) as seen in Figure 1. Although the etiology may not be clear, it seems apparent that the manipulation of the saturate levels in the dietary oils will lead to changes in the incidence of myocardial necrosis.

The improved growth observed in rats fed the diets enriched with saturated fatty acids provided additional evidence that myocardial necrosis may be related to an improper balance of dietary fatty acids. In fact, some earlier studies had established a maximal growth in rats, provided the level of saturated fatty acids was 20-40% (36-38). It may, therefore, not be coincidental that when the level of saturates in

TABLE 6

Fatty Acid Composition (%) of the Major Cardiac Phospholipids[a]

Diets	DMA	Saturated	Monounsaturated	Polyunsaturated						
				Total	n-6	n-3	18:2	20:4	C22	C22 n-3
Phosphatidylethanolamine										
LEAR	5	33	12	50	25	25	4	19	26	24
LEAR + cocoa butter	7	34	10	48	24	25	4	19	26	24
LEAR + triolein	4	32	14	49	24	25	3	19	27	25
Soybean	7	34	7	52	31	21	8	19	24	21
Soybean + cocoa butter	9	35	7	49	30	20	6	20	23	19
Soybean + triolein	5	32	12	51	26	26	5	19	27	25
Soybean + triolein + X:1	4	33	13	51	26	26	5	19	27	26
LSD ($p<0.01$)	2	2	2	2	3	3	2	2	3	3
Phosphatidylcholine										
LEAR	0.8	40	14	45	38	7	7	30	7	7
LEAR + cocoa butter	0.9	42	12	45	38	7	6	31	8	7
LEAR + triolein	1.1	39	15	44	36	8	6	30	8	8
Soybean	1.0	42	8	49	44	5	11	32	6	5
Soybean + cocoa butter	0.8	43	7	49	44	5	8	34	6	5
Soybean + triolein	1.0	39	13	47	39	8	9	30	8	8
Soybean + triolein + X:1	1.0	39	13	47	40	8	8	31	8	8
LSD ($p<0.01$)	0.4	1	1	1	2	2	2	2	2	2
Phosphatidylserine and phosphatidylinositol										
LEAR	0.8	45	12	42	30	12	4	23	13	12
LEAR + cocoa butter	1.1	47	10	42	30	13	3	24	14	12
LEAR + triolein	1.0	45	12	42	30	12	4	23	13	12
Soybean	1.0	46	7	45	34	11	7	23	14	11
Soybean + cocoa butter	1.0	47	7	45	34	10	6	23	14	10
Soybean + triolein	1.0	44	11	44	31	13	5	23	15	13
Soybean + triolein + X:1	1.0	44	11	44	32	12	5	24	15	12
LSD ($p<0.01$)	0.3	2	1	2	2	2	1	1	2	2
Diphosphatidylglycerol										
LEAR	0.8	7	15	77	70	7	64	4	6	6
LEAR + cocoa butter	0.9	7	14	78	71	8	64	4	7	6

LEAR + triolein	0.8	7	19	74	66	8	59	4	6	7		
Soybean	0.8	6	6	88	86	3	82	2	2	2		
Soybean + cocoa butter	0.6	6	7	87	84	3	80	3	3	2		
Soybean + triolein	1.0	7	11	82	76	5	71	3	5	5		
Soybean + triolein + X:1	1.0	6	11	81	78	4	73	3	4	3		
LSD ($p<0.01$)	0.4	2	2	3	5	2	6	1	2	2		
Analysis of variance (d.f.)					Mean square							
Soybean vs LEAR (1)	5.8*	18*	247*	83*	261*	49*	111*	0.6	5.3	48*		
LEAR vs soybean-triolein mixtures (1)	0.1	3.7	6.8	27*	8.6	5.1	14	2.2	11	6.3		
Oils vs added cocoa butter (1)	7.3*	29*	20*	8.1	5.1	0.3	29*	8.1	0.1	0.1		
Oils vs added triolein (1)	3.1	32*	138*	17*	136*	57*	41*	7.9	29	60*		
Error (30)	0.4	2.1	0.9	2.1	4.1	4.4	2.1	1.9	4.6	4.5		

[a]Polyunsaturated fatty acids were grouped into those derived from the linoleic (n-6) and linolenic (n-3) acid families. The column designated DMA are the dimethyl acetals produced during acid-catalyzed transesterification of the alkenyl ethers. X:1 (20:1, 22:1 and 24:1). LSD, least significant difference at the 1% level, based on each phospholipid class separately. Analyses of variance pooled over 3 phospholipids (excluding diphosphatidylglycerol) is given at the bottom; significance is indicated at the 1% level (*).

LEAR and soybean oils was increased to the levels suggested, it was accompanied by a marked reduction in heart lesions. This appears to be consistent with the results of Hulan et al. (21) who showed that diets rich in saturates are associated with a low incidence of heart lesions in male rats. Conversely, the lack of sufficient amount of saturates may explain why previous studies (9) failed to conclude (39) any ameliorating effects when the level of saturates was raised from 6.7 or 7.7% to 11% (9).

The reasons for the limited addition of saturates to the dietary oil in a previous study (9) to test for the effect of saturates (LEAR oil to palm oil, 18 to 2) was based in part on the assumption that LEAR oils contain cardiopathogenic compounds, the effect of which would be diluted by the addition of supposedly noncardiopathogenic oil or fat. This assumption, however, is no longer tenable, as extensive studies provided no experimental evidence of the presence of cardiotoxic compounds in soybean oil (18), LEAR oils (10,18,24) or rapeseed oils high in erucic acid (24). The highly purified triglycerides from soybean oil (18) and LEAR oils (10,18) retained their cardiopathogenic properties. Furthermore, the results of this study provide additional evidence that the heart lesions were not caused by trace contaminants in the oils. Mixing either LEAR or soybean oils with cocoa butter or triolein did not result in a similar response in heart lesions as would have been expected by dilution of a toxin. From this new perspective, the data presented in Tables 3 and 4 of reference (9) can be interpreted in a different manner, i.e., LEAR oils were mixed with different proportions of saturated fat or fat-oil mixtures. If the data are thus viewed together, the results show a remarkable similarity with those presented in the present study, the correlation between levels of saturates and incidence of heart lesions being -0.76, as opposed to -0.72 in Table 1.

If dietary fatty acids are related in some way to myocardial necrosis, one might expect differences in cardiac lipid classes and/or their fatty acid composition to reflect the cardiopathological state. In general, the cardiac lipid class composition was not found to be affected by the different diets tested for heart lesions except triacylglycerol. However, the changes in the level of triacylglycerol could not be used as an indicator of cardiopathogenicity because the response to the addition of cocoa butter or triolein was unique to each oil and unlike the directional changes observed with heart lesions. Previous attempts to relate cardiac triacylglycerol levels resulting from erucic acid to heart lesions (40) were equally unsuccessful. A level of di-

TABLE 7

Fatty Acid Composition of Cardiac Sphingomyelin in Male Rats Fed the Experimental Diets for 16 Weeks[a]

Fatty acids	LEAR (cv. Tower)	LEAR + cocoa butter	LEAR + triolein	Soybean	Soybean + cocoa butter	Soybean + triolein	Soybean + triolein + (X:1)	LSD ($p<0.01$)
14:0	2.1	1.2	0.5	0.9	2.0	1.3	0.5	0.5
15:0	1.3	0.8	0.2	0.7	1.5	1.0	0.2	0.5
16:0	13.7	12.0	13.0	11.8	12.4	12.4	11.5	1.0
17:0	1.0	0.4	0.5	0.6	0.7	0.9	0.6	0.4
18:0	9.1	22.9	11.8	14.2	18.4	19.3	16.6	2.1
19:0	1.7	2.8	1.9	2.9	3.0	4.4	3.6	0.9
20:0	17.2	14.3	16.4	13.1	13.2	9.9	11.5	2.0
21:0	1.3	0.9	1.1	1.9	1.3	1.3	1.6	0.6
22:0	18.5	15.4	18.8	23.6	19.4	15.7	17.4	1.8
23:0	2.5	2.0	2.9	5.1	4.0	4.3	4.5	1.3
24:0	7.2	6.6	8.2	11.0	9.6	8.8	8.4	1.0
Total	75.6	79.3	75.3	85.8	85.5	79.3	76.4	2.7
16:1	1.9	0.9	0.9	1.3	1.3	1.3	1.1	0.5
18:1	3.5	5.7	4.6	2.9	3.9	7.1	5.8	1.1
20:1	0.3	0.3	0.4	0.2	0.2	0.3	0.5	0.2
22:1	1.2	0.7	1.0	0.7	0.1	0.7	0.9	0.3
23:1	0.3	0.2	0.4	0.2	0.2	0.2	0.2	0.2
24:1	13.4	10.8	14.5	6.3	5.2	8.0	11.3	1.5
Total	20.6	18.6	21.8	11.6	10.9	17.6	19.8	1.8
CN22.6	0.9	0.4	0.5	0.4	0.7	0.5	0.7	0.4
CN23.6	2.4	1.2	1.9	1.6	2.3	2.2	2.5	0.6

[a]All values are expressed as percent by wt and are the mean of 6 rats per diet. X:1 (20:1, 22:1 and 24:1). LSD, least significant difference. CN, carbon number.

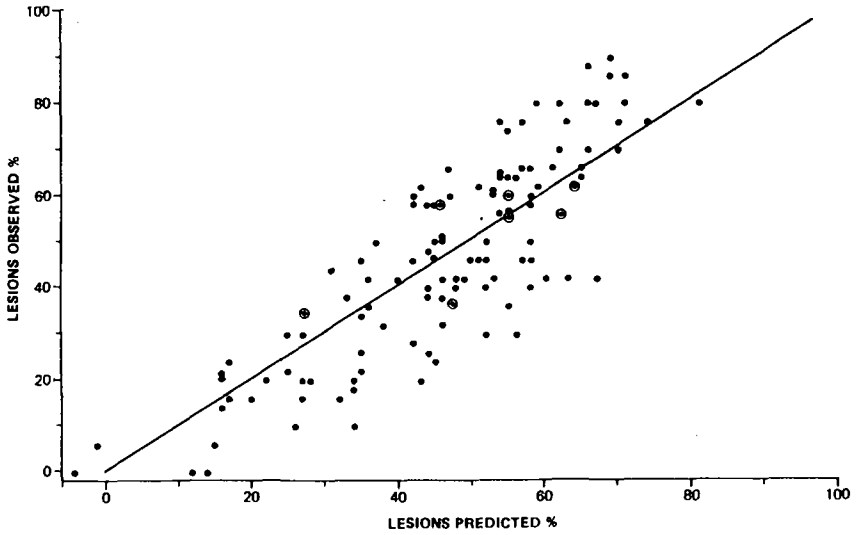

FIG. 1 Observed vs predicted incidence of myocardial lesions in male rats. The observed incidence of heart lesions was taken from published data for which regression coefficients were calculated and used to determine the predicted incidence of lesions (25). The results of each of the 7 diets of the present study (◉) are included in the previous plot (•) (25).

etary erucic acid which gave a positive cardiac triacylglycerol response (3,41) did not result in a corresponding increase in heart lesions (4,21). Furthermore, despite a similar accumulation of cardiac triacylglycerols between sexes (3) and strains (7) of rats, the lesion response was widely different.

In contrast to the cardiac lipid class composition, marked changes were observed in the fatty acid composition of the lipid classes. There was a consistent increase in the level of saturates in cardiac phospholipids with the addition of saturates to either soybean or LEAR oils. This increase parallels an increase in the consumption of saturates (Table 4) and a decrease in incidence of heart lesions (Table 3). The addition of triolein marked an increase in the level of monoenoic fatty acid as expected, as 18:1 is readily incorporated into cardiac phospholipids (42,43). The consumption of saturates was only slightly reduced by mixing triolein with LEAR oil, but a major reduction in the consumption of saturates occurred by feeding the triolein-soybean oil mixtures. The incidence in heart lesions, however, showed no significant change. Little change in heart lesion incidence was expected with the addition of triolein to LEAR oil because the level of dietary saturates was not greatly affected and 18:1 showed a low correlation to heart lesions (Table 1). On the other hand, the addition of triolein to soybean oil was expected to increase the incidence of heart lesions because the level of dietary saturates was significantly lowered. The results of this study suggest that the observed incidence of heart lesions in rats fed the soybean oil diet was relatively high—higher than expected based on previous studies (4,18). In fact, an average incidence of about 44% (4,18) would be consistent with both the consumption and fatty acid composition data. As stated previously in the discussion, the incidence of heart lesions is known to vary both within (18) and between (35) experiments, and the present value of soybean oil (57%) is within the observed variation (Fig. 1).

Dietary 18:3 resulted in a relatively high level of C22 PUFA of the linolenic acid (n-3) family with virtual exclusion of the C22 PUFA of the linoleic acid (n-6) family as seen in Table 6 and as observed previously (15,42,44). The level of (n-3) family acids appeared to be little influenced by the addition of saturates in the diet, despite the observed significant decrease in lesion incidence by the addition of saturates. This suggests that saturates are not undoing the cardiopathogenic effect of 18:3, but appear to act independently. Therefore, dietary fats or oils which contain at least 20-25% saturates, irrespective of dietary 18:3, will be associated with a low incidence of heart lesions in male rats.

It should be noted, however, that the apparent requirement of a balance of dietary fatty acids for the male albino rat is critical only when the level of fat in the diet is high, i.e., 15

or 20%. Myocardial necrosis was observed to decrease significantly in male rats when the same oil was fed at a lower level, i.e., at 5 or 10% of the diet (13,14,45,46). This would not seem unreasonable, because the de novo synthesis of saturates by the rat would be significantly reduced when a diet rich in fat was fed (47,48). Therefore, the rat might be under a nutritional stress to synthesize the required amount of saturates for its phospholipids. Proper membrane function and stability may well require a fatty acid composition within a certain range.

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