CARDIAC LIPID CHANGES IN RATS AND HOW THESE CORRELATE TO MYOCARDIAL NECROSIS

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Extensive experimentation in the past decade on the safety of vegetable oils showed characteristic necrotic heart lesions in male rats related to the level and kind of oil in the diet (1-6). Certain dietary fatty acids such as linolenic (18:3n-3) (2, 7) and erucic acid (8) were identified as being involved in myocardial necrosis, while saturates (9) and linoleic acid (18:2n-6) (2) were associated with a low incidence of heart lesions. A statistical evaluation of much of the published data (23 experiments) on heart lesions in male rats fed high levels of fats and oils identified dietary 18:3n-3 and saturated fatty acids as most closely associated with differences in incidence of myocardial lesions among diets within experiments (10). In fact, 73% of the variation was explained by these fatty acids, which is almost all of the variation that can be explained by this model considering the fact that most of the residual source of variation (about 25%) is due to binomial sampling which is a function of the underlying incidence levels and sample size in each experiment (11). These results provided a model for predicting the relative cardiotoxicity of vegetable oils based on their fatty acid composition.

This experiment was designed to test the relationship of dietary fatty acids to heart lesions in male rats and determine if the cardiac lipids showed evidence of change which could be correlated to myocardial damage.

MATERIALS AND METHODS

The compositions of the dietary fats fed at 20% by weight are shown in Table 1. Soybean and LEAR (low erucic acid rapeseed) oils were selected because they contain 18:3n-3, which based on the statistical evaluation, should result in a relatively high incidence of heart lesions. The saturated fatty acid content of the two oils was increased by mixing in cocoa butter. Triolein was mixed with the oils in proportions similar to that with cocoa

butter to assure that the cardiopathogenic results were not due to dilution of other factors in the oils. In some oil mixtures the 18:3n-3 content was restored by adding an appropriate amount of linseed oil.

The semi-synthetic diets (12) were fed for 1, 2, 3, 4 and 16 weeks to male Sprague-Dawley rats. The hearts of 44 rats per diet were examined histopathologically after 16 weeks as described previously (13). The cardiac lipids of rats were analyzed at all time periods by procedures described elsewhere (14, 15). Tissue samples were also obtained from weanling rats (zero time).

Table 1. Dietary fats and their fatty acid composition.

	% by wt of the diet						
LEAR oil	20	16	16	–	–	–	–
Soybean oil	–	–	–	20	16	9.6	9.5
Cocoa butter (CB)	–	4	–	–	4	–	–
Triolein (18:1)	–	–	4	–	–	9.6	9.5
Linseed oil	–	–	–	–	–	0.8	0.8
Erucic acid (22:1)	–	–	–	–	–	–	0.2
Fatty acids (% by wt)							
Total saturates	7.2	16.6	5.4	16.2	27.6	8.7	9.1
Total monoenes	60.2	57.6	68.7	25.1	27.4	56.3	56.7
18:2n-6	22.0	17.8	17.9	51.9	40.1	28.3	27.5
18:3n-3	10.3	7.9	7.8	6.7	4.9	6.7	6.7

RESULTS AND DISCUSSION

Both soybean oil and LEAR oil fed to male Sprague-Dawley rats for 16 weeks gave a high incidence of heart lesions (Table 2). The incidence of heart lesions was significantly reduced by increasing the saturated fatty acid content of the oils by about 10%. Substitution of triolein for cocoa butter in these oils resulted in no change in the incidence of heart lesions. This indicated that changes in certain dietary fatty acids (saturates), and not dilution of factors (toxin?) in vegetable oils, influenced the incidence of heart lesions. These results are consistent with previous studies where no cardiotoxins could be removed from LEAR and soybean oils by exhaustive fractionation (16-18), and agree well with those predicted by the model (10) (Table 2).

An analysis of the heart lipids showed no significant diet differences in any of the lipid classes at any time period except in the triglyceride level (Table 3). On the other hand, the

significant time effect for all lipid classes appears to reflect only differences in water content of heart tissue, since the values were expressed as mg per g of wet heart. In any case, the cardiac lipid class changes could not be related to the observed cardiopathogenic response.

Table 2. Observed and predicted incidence of myocardial lesions in male rats fed the experimental diets for 16 weeks.

Diets	Incidence (%) (n=44) Observed	Predicted	Comparisons (d.f.)	X^2
LEAR	61	64		
LEAR + CB	36	47	All diets (6)	13.0*
LEAR + 18:1	55	62	Effect of saturates (1)	10.2**
Soybean	57	46	Effect of triolein (1)	0.1
Soybean + CB	34	27		
Soybean + 18:1	59	55		
Soybean + 18:1 + 22:1	55	55		

Table 3. Heart, lipid and cardiac lipid class weights pooled over all 7 diets.

Description	Time on diet (weeks) 0	1	2	3	4	16	P < 0.01 Diets	Time
Heart	205[a]	414	569	666	883	1107	NS	S
Lipids	35.1[b]	33.2	32.7	34.8	38.3	36.9	NS	S
CE	0.6[b]	0.2	0.2	0.2	0.2	0.3	NS	S
TG	4.6	7.7	6.5	8.2	8.6	9.9	S	S
C	3.5	3.2	2.6	2.8	3.0	2.2	NS	S
DPG	3.7	3.1	3.1	3.4	4.1	2.7	NS	S
PE	5.9	5.6	6.1	6.7	7.6	6.4	NS	S
PS + PI	0.9	0.6	1.2	1.0	0.8	0.9	NS	S
PC	12.5	9.9	10.4	10.6	11.5	9.3	NS	S
SP	1.9	1.4	1.6	1.0	1.3	0.8	NS	S

[a] mg [b] mg/g wet weight

The fatty acid composition of the cardiac lipid classes were also investigated in hopes of locating changes which would correlate to the observed cardiopathogenic response in rats. The cardiac phospholipids, generally maintain a characteristic fatty acid composition, and as membrane components, were of great interest since compositional changes might correlate to changes in heart lesion incidence. Table 4 shows that the relative concentration of cardiac arachidonic acid (20:4n-6) and the Σ saturates and ΣC22 PUFA (polyunsaturated fatty acids) remained constant throughout the experimental period. Of these, only the sum of saturates was affected by diet. It is noteworthy, that the level of saturated fatty acids in the phospholipid classes at 1 to 4 weeks and 16 weeks were significantly correlated, positively to the level of dietary saturates, and negatively to heart lesions (Table 5). This completes a rather significant correlation between dietary saturates : level of saturates in cardiac phospholipids : and heart lesions. The correlation of dietary saturates to heart lesions in this experiment was -0.81. Therefore, the results of this study suggest that the level of dietary saturates as well as the level of saturates in cardiac phospholipids could be used to predict heart lesion incidence.

Table 4. Relative concentration (%) of fatty acids and groups of fatty acids in cardiac phospholipids. Values are means of 7 diets and 5 time periods.

Description	PC	PE	DPG	PS + PI	SP	P < 0.01 Time	P < 0.01 Diet
20:4n-6	26	22	3	21	0	NS	NS
Σ Saturates	46	37	5	54	83	NS	S
Σ C22 PUFA	6	23	3	9	0	NS	NS

The C22 n-3 PUFA (i.e., 22:6n-3 and 22:5n-3) have also been considered as correlating to heart lesions, since diets which gave a high incidence of heart lesions generally contained 18:3n-3 (2, 6, 7, 10), and rats fed these diets had high levels of cardiac C22 n-3 PUFA (6, 15, 19, 20). However, as pointed out previously, the level of C22 n-3 PUFA is related to the dietary level of 18:3n-3 irrespective of the source (14). The results of this experiment support this finding as seen in Table 6. Most of the C22 PUFA (about 90-95%) in the cardiac phospholipids were derived from 18:3n-3, and only when the dietary 18:2n-6 content was greater

than 40% was the content of the C22 n-3 PUFA reduced slightly (to about 80-85%). As a comparison, a corn oil containing diet with ≤1% 18:3n-3 and 56% 18:2n-6 gave a 35 to 40% content of C22 n-3 PUFA (Table 6). It is quite evident from the results in Table 6 that the relative proportion of C22 n-3 PUFA (22:5n-3 and 22:6n-3) was not affected by the addition of dietary saturates which had resulted in a decrease in the incidence of myocardial lesions. Therefore, the results suggest that the content of the C22 n-3 PUFA in the heart phospholipids may not be a reliable indicator of heart lesions, although it appears to be a necessary component, since no dietary oils have been observed to give a high incidence of heart lesions when the content of the C22 n-3 PUFA is low. The results of this study indicate that the incidence of heart lesions in male rats can be lowered by dietary saturates even in the presence of high levels of C22 n-3 PUFA (21).

Table 5. Correlation of cardiac phospholipid saturates to dietary saturates and heart lesions.

Phospho-lipid	Time (weeks)	Dietary saturates	Heart lesions
PE	1-4	0.91	
	16	0.97	-0.80
PC	1-4	0.84	
	16	0.93	-0.77
PS + PI	1-4	0.85	
	16	0.89	-0.74

Table 6. Relative concentration (%) of C22 n-3 PUFA in the C22 PUFA in cardiac phospholipids of rats. Values are means over 5 time periods.

	Dietary		% (Σ C22 n-3/Σ C22 PUFA)		
Diet	18:3	18:2	PE	PC	PS + PI
LEAR	10.3	22.0	96	94	91
LEAR + CB	7.9	17.8	95	94	92
LEAR + 18:1	7.8	17.9	96	94	92
Soybean + 18:1	6.7	28.3	94	91	85
Soybean + 18:1 + 22:1	6.7	27.5	94	91	88
Soybean + CB	4.9	40.1	84	81	73
Corn	0.7	56.1	42	35	34

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