Influence of Sex and Dietary Phosphatides on Cholesterol Levels in Blood Plasma of Swine.* (24428)

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Substitution of vegetable oils for more saturated fats in the diet has been reported to reduce blood cholesterol in various animals and in man(1-5). A temporary reduction of serum cholesterol in human subjects was observed by Steiner and Domanski(6) following ingestion of a commercial soybean lecithin mixture. Pilgeram and Greenberg(7) observed increased phosphatidyl choline formation in rat liver slices from labeled aminoethanol and methionine, when rats were fed high cholesterol diets, in contrast to rabbits, a species more susceptible to atherosclerosis, suggesting that phosphatidyl choline is necessary for normal cholesterol metabolism. These authors(8) also suggested that inability of certain animal species to form lecithin may result in metabolic block producing atherosclerosis and that certain phosphatides may be necessary for normal cholesterol breakdown. More recently it has been reported that injection of lecithin into rabbits grown on atherogenic diets results in clearing of atherosclerotic lesions(9). We studied the effects of incorporating certain soybean phosphatide fractions in the diet on plasma cholesterol levels of a newly developed breed of small swine (10).

Materials and methods. Thirty miniature pigs were divided into 5 groups of 6 pigs each when pigs were 40 to 51 days old. Group 1 consisted of 4 boars and 2 gilts. Each other group consisted of 3 boars and 3 gilts. The boars were not castrated. It was of interest to observe sex differences, if any, in blood cholesterol values, as well as possible sex differences in response to treatment. The diets are shown in Table I. The pre-test diet was fed 33 days. Then, for the next 6 weeks, group 1 was fed the low-fat diet (5.17%) and the other 4 groups the high-fat diet (18.17%). At this time soybean phosphatides of 3 types were added at the level of 2% to diets of 3 groups fed the high-fat diet. Compositions of phosphatides were estimated to be as follows:‡ (a) Crude soybean phosphatide, estimated to approximate ⅓ phosphatidyl choline, ⅓ phosphatidyl ethanolamine, and ⅓ inositol phosphatide. Methyl esters of phosphatide fatty acids had an iodine value of 126.6, and contained 43.6% dienoic acids and 6.4% trienoic acids, measured by alkali isomerization-spectrophotometric technic(11). (b) Alcohol-insoluble fraction of soybean phosphatide, estimated to approximate ⅔ inositol phosphatide, ⅓ phosphatidyl ethanolamine, and trace of phosphatidyl choline. Methyl esters of the phosphatide fatty acids had an iodine value of 121.1, and contained 41.1% dienoic acids and 5.3% trienoic acids. (c) Alcohol-soluble fraction of soybean phosphatide, estimated to approximate ⅔ phosphatidyl choline, ⅓ phosphatidyl ethanolamine, and a trace of inositol phosphatide. The methyl esters of phosphatide fatty acids had iodine value of 104, and contained 21.1% dienoic acids and 2.7% trienoic acids. Phosphatide supplements were fed 17 weeks. All diets were fed isocalorically based on total body weight of all pigs, weighed weekly. The experiment was started with 270 calories/kg body weight/day, but because of continuous decrease in daily caloric consumption/kg body weight, it was necessary to reduce this level steadily as pigs grew older. At end of 17-week test period, the pigs were consuming 100 calories/kg body weight/day. Plasma cholesterol levels were determined(12) at 3 week intervals. Only data from end of pre-test period, the end of basal period, and end of test period are given in Table II. Data obtained at intermediate in-

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TABLE I. Composition of Rations.

Ingredient	Pre-test	Diet Low-fat	Diet High-fat
	%	%	%
Corn, ground	15	15	15
Oat groats	30	30	30
Soybean oil meal, solvent	20	20	20
Dried skim milk	10	10	10
" fish solubles	2	2	2
Trace mineralized salt	.5	.5	.5
Ca-P mineral mix (28% Ca, 6% P)	1	1	1
Steamed bone meal	1	1	1
Fortafeed 2-49c	.2	.2	.2
Vit. A and D oil (2250 A, 400 D)	.3	.3	.3
Purified beef tallow	.0	2	15
Cellulose (Alphacel)	.0	2.5	2.5
Cholesterol	.0	.5	.5
Cerelose (dextrose)	20	15	2
Calculated analysis:			
Calories/kg	3419	3099	3746
Protein, %	19.12	19.12	19.12
Fat, %	3.17	5.17	18.17
Calcium, %	.72	.72	.72
Phosphorus, %	.61	.61	.61

tervals were consistent with the data shown in the Table. Analysis of variance was made for plasma cholesterol values and body weight values for each bleeding period. Plasma lipides were extracted by cold saponification, 1 N KOH, in ethanol. Six to 8 volumes of pooled plasma were mixed with 20 volumes of alcoholic KOH and left at room temperature over night. The mixture was then brought to boiling temperaure for 15 min. and, after cooling, the fatty acids were isolated and taken to final volume of 10 ml with absolute methanol. All operations were carried out under a blanket of nitrogen. Polyunsaturated fatty acids were analyzed according to a modification of the method of Holman *et al.*(11). The final calculations were based on constants of Herb and Riemenschneider(14). Plasma phospholipide phosphorus analysis was performed by modification of the method of King(13). Phospholipides were estimated as 25 times the concentration of lipide phosphorus. In each group equal volumes of plasma, from males or females, were pooled for analyses of polyunsaturated fatty acids.

Results. Differences in body weights were nonsignificant for all periods tested, suggesting that neither differences in dietary treatment nor in sex of animals had any effect on changes in body weight.

Plasma cholesterol values of pigs in all groups increased similarly during the 6 weeks prior to incorporation of phosphatide supplements, the males averaging +50 mg% and the females +58 mg%.

Plasma cholesterol values throughout test period showed significant variation in relation to sex, with females showing higher plasma cholesterol values than males, regardless of dietary treatment. By end of third week the significance reached the 5% level, and by the

TABLE II. Blood Plasma Cholesterol.

Group		At end of 33-day pre-test period	At end of 6-wk basal period	At end of 17-wk test period	Change during 17-wk test period
		mg %	mg %	mg %	mg %
Low fat	♂ (4)	67	109	80	−29
	♀ (2)	97	144	123	−21
	Avg	77	121	94	−27
High fat	♂ (3)	78	110	119	+ 9
	♀ (3)	100	140	166	+26
	Avg	89	125	142	+17
Idem + 2% crude lecithin	♂ (3)	83	146	84	−62
	♀ (3)	91	176	186	+10
	Avg	87	161	135	−26
" + 2% alcohol-insoluble	♂ (3)	102	160	121	−39
	♀ (3)	78	148	142	− 6
	Avg	90	154	132	−22
" + 2% alcohol-soluble	♂ (3)	99	154	109	−45
	♀ (3)	93	139	174	+35
	Avg	96	147	141	− 6

TABLE III. Plasma Polyenoic Acids at End of Basal Period.*

Group		Dienes	Trienes	Tetra-enes	Penta-enes	Hexa-enes	PL†	PL/TC‡
		mg %						
Low fat	♂	23.5	.7	11.4	3.2	2.3	138.5	1.27
	♀	15.5	1.3	11.1	2.5	2.9	139.0	.97
High fat	♂	18.6	.6	8.2	2.9	2.2	173.0	1.57
	♀	24.6	.6	13.7	3.6	3.2	176.0	1.26
Idem + 2% crude lecithin	♂	14.3	.0	6.7	2.3	1.7	168.4	1.15
	♀	21.3	1.0	12.6	3.6	3.7	201.7	1.14
" + 2% alcohol-insoluble	♂	16.8	.0	11.6	3.6	3.9	191	1.19
	♀	15.6	.0	13.9	3.6	4.7	166	1.12
" + 2% alcohol-soluble	♂	28.9	.1	15.1	4.2	4.9	158	1.03
	♀	16.9	.3	12.4	3.5	3.7	152	1.09

* Pooled plasma polyenoic acid content, mg/100 ml, at end of 6-wk basal period.
† Phospholipides—mg/100 ml plasma.
‡ Phospholipide (PL)/total cholesterol (TC) ratios.

end of sixth week, the 1% level. This sexual difference in plasma cholesterol probably was due to the fact that all females were in gestation by end of experiment. In this regard it should be noted that pregnant dairy cattle have been reported to have higher cholesterol values than nonpregnant cattle(15).

Although plasma cholesterol values of all females combined were significantly higher than for males, the mean values actually were lower in 2 groups, the low-fat basal Group 1, and the high-fat basal group receiving 2% alcohol-insoluble supplement (Group 4). Statistical significance of differences in plasma cholesterol values due to dietary treatment was low ($P>.05$, $P<.10$). However, data in Table II showed definite trends, all of which were consistent except for the 3 females in Group 4. During the test period, plasma cholesterol values of both males and females decreased in the low-fat Group 1 and increased in the high-fat Group 2. That phosphatide supplements had an effect on plasma cholesterol is shown since in all 3 supplemented groups, values for males decreased appreciably. Even though, overall, females showed some increase in plasma cholesterol, those in Group 4 (supplement containing 66% inositol phosphatide) actually decreased slightly. The fact that the supplement containing large amount of inositol phosphatide led to a marked decrease in plasma cholesterol in males and inhibited increases in plasma cholesterol of females, suggests that this supplement was the most active in reducing plasma cholesterol.

During test period, plasma tetraenoic acids (arachidonic) fell in all groups, and dienoic acids (linoleic) increased in both males and females fed high fat diets (Tables III and IV). Females fed basal diets had plasma cholesterol levels equal to, or greater than, males maintained on same diets. During the test period, females maintained higher plasma tetraenoic acid levels than males. There was no apparent correlation between plasma cholesterol levels and circulating polyunsaturated fatty acids. While plasma phospholipides fell in males fed phosphatide supplements, such changes were not apparent in females. Pregnant females did not show any change in ratio of their plasma phospholipide to cholesterol. In contrast, males receiving phosphatide supplements showed a marked decrease in plasma phospholipides and in their phospholipide-cholesterol ratios. Longer term studies are in progress to determine more specifically the changes in tissue lipides of miniature pigs fed phosphatides.

Summary. 1) Body weights and blood plasma cholesterol values were observed periodically in male and female miniature swine fed high-tallow diets supplemented with dietary soybean phosphatides, for 17 weeks. Body weight changes were uniform throughout and were not related to sex or dietary treatment. The plasma cholesterol values of females as a whole after 3 weeks on the test, were significantly higher than for males ($P<.05$) and remained so after 6 weeks on test diets (P

TABLE IV. Plasma Polyenoic Acids at End of Test Period.*

Group		Dienes	Trienes	Tetra-enes	Penta-enes	Hexa-enes	PL†	PL/TC‡
		mg %						
Low fat	♂	17.7	.0	2.4	1.3	.1	121.5	1.52
	♀	19.1	.1	6.9	2.9	3.7	195.6	1.59
High fat	♂	34.6	.8	3.3	1.8	.1	170.8	1.49
	♀	27.2	.7	6.1	1.8	2.3	185.8	1.12
Idem + 2% crude lecithin	♂	32.3	.5	2.5	.1	.1	109.2	1.30
	♀	79.6	.0	15.8	3.9	6.7	192.0	1.03
" + 2% alcohol-insoluble	♂	60.2	1.1	6.6	2.8	2.4	100.5	.85
	♀	32.9	.6	9.4	2.7	3.8	150.8	1.06
" + 2% alcohol-soluble	♂	47.5	.9	8.1	2.9	2.5	140.2	.78
	♀	23.6	.0	9.4	3.3	5.3	181.8	1.05

* Pooled plasma polyenoic acid content, mg/100 ml, at end of 17-wk test period.
† Plasma phospholipides, mg/100 ml.
‡ Phospholipide (PL)/total cholesterol (TC) ratios.

$<.01$), when gilts were pregnant. 2) While differences in plasma cholesterol values of several groups, due to dietary treatment, were not statistically significant at the 5% level, definite trends were observed. Plasma cholesterol values of all pigs fed the low-fat diet were reduced during test period, while those fed the high-tallow diet increased slightly. All 3 of the dietary soybean phosphatide supplements, when added to the high-tallow diets, reduced plasma cholesterol values of males. The alcohol-insoluble fraction supplement (⅔ inositol phosphatides) also slightly reduced plasma cholesterol values of females. 3) Thus, under conditions employed, soybean phosphatides incorporated in the diet appeared to counteract the hypercholesteremic effect of the diet containing a relatively high proportion of a saturated fat. 4) The tetraenoic acid concentration of plasma tended to be higher in females than in males fed comparable diets. The feeding of phosphatide supplements, while having little effect on plasma lipides of pregnant miniature pigs, promoted a marked drop in plasma cholesterol, phospholipides, and phospholipide-cholesterol ratios in male miniature pigs. There was no apparent correlation between changes in plasma polyunsaturated acids and changes in other plasma lipides during this study.

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