Serum lipids in choline-deficient male and female rats

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SUMMARY Serum lipids from normal and choline-deficient male and female rats were fractionated on silicic acid. Total cholesterol, phosphorus, and fatty acid compositions were determined on serum cholesterol esters, triglycerides, phospholipids, and a small mixed fraction. Choline deficiency reduced the concentration of all classes of lipids in both sexes. Decreases in the phospholipid fractions of both sexes, and in cholesterol ester fractions of female rats, were largely due to preferential decreases in their arachidonic acid components, but the fatty acids in triglycerides decreased uniformly in both sexes.

CHOLINE DEFICIENCY produces a striking decrease in blood lipids of the rat (1, 2) and dog (3) and causes fatty livers in most of the species examined. This deficiency particularly impairs triglyceride mobilization from the liver, although fatty livers may also result, in general, from increased net transport into the liver, increased hepatic synthesis of lipid, or reduced hepatic oxidation of lipid (4). Deficiencies of essential fatty acids (5) or essential amino acids (6), for example, may produce fatty livers, suggesting that in these three deficiencies, lipoprotein does not enter the circulation until it is complete in its phospholipid (which contains choline and essential fatty acid) and protein moieties. Sex hormones have been observed to influence liver lipid accumulation in choline-deficient rats (7).

It has previously been observed that in rats, the sex of the animal markedly influences the saturated fatty acids of phospholipids in liver (8, 9), plasma (9–12), and red blood corpuscles (12). It was found that the ratio of stearic to palmitic acid in phospholipids was invariably higher in females than in male. In male rats, the ratio of stearic to palmitic was often about 1.0, and in females the ratio was as high as 2.3. Experiments on normal animals and castrates treated with estrogen or androgen indicated that estrogen increased the amounts of stearic and arachidonic acids in rat plasma phospholipids (11).

Rat liver lecithins display metabolic heterogeneity. Collins (13) showed, for example, that the amount of incorporation of P^{32} into rat liver lecithin in vivo is dependent upon the fatty acid composition of the lecithin, and isozaki et al. demonstrated (14) that lecithins containing higher proportions of stearic and arachidonic acids incorporated P^{32}-activity at a different rate from lecithins composed largely of palmitic and linoleic acids; the specific activity derived from methyl-C^{14} methionine was greater in the first group of lecithins than in the second. These observations suggested that a sex difference in formation of lecithins, due to a sex difference in choline metabolism, might be responsible for the observed influence of sex on saturated fatty acids of rat phospholipids. It has been observed that male rats accumulate more liver lipid in choline deficiency than females do, and that this sex difference may be removed by estrogen (7). The serum lipids in choline-deficient male and female rats were therefore examined and interpreted in terms of two different pathways known to exist (15–17) in the formation of lecithin.

METHODS

Male and female weanling Long-Evans rats, averaging 48 and 47 g, respectively, were housed individually in wire-bottomed galvanized cages. All animals were given stock diet (Diablo Labration[1]) for the 1st week,

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[1] Diablo Laboratories, Inc., Berkeley, California.

TABLE 1 COMPOSITION OF DIET

Major Components	*% by Weight*
C-1 Assay protein (Archer-Daniels-Midland Co.)	18.0
Safflower oil (Pacific Vegetable Oil Co.)	10.0
Sucrose	66.0 ∓ 0.61
Minerals, USP XIV	4.0
Minor Components	*μg/g diet*
Choline bitartrate	±2500 as choline
Thiamine HCl	4.0
Riboflavin	4.0
Folic acid	2.0
Pyridoxine	2.0
Calcium D-pantothenate	10.0
Niacin	10.0
p-Aminobenzoic acid	10.0
Biotin	1.5
i-Inositol	500
Ascorbic acid	100
Vitamin A acetate, 500,000 IU/g (0.50 IU/μg)	34
Vitamin D, 400,000 IU/g (0.40 IU/μg)	26
α-Tocopherol	71
Menadione	50

and experimental diets[2] (Table 1) for the following 2 weeks. The experimental diets were low in methionine, contained no added vitamin B_{12}, and differed only in choline content. The control diet contained choline bitartrate (0.25% of the diet as choline), added at the expense of sucrose to the choline-deficient diet. Half of each sex group received the choline-deficient diet, and the others were given the control diet. After the rats had been given the experimental diets for 2 weeks, they were fasted for 18 hr before they were killed by heart puncture exsanguination while under Nembutal (sodium pentobarbital, Abbott) anesthesia.

Serum was separated and pairs of samples from matching rats were pooled when necessary to attain at least 1.5 ml serum/sample. Serum was measured dropwise into 10 volumes of 95% ethanol and allowed to stand 4 hr in the dark. Hydroquinone was added to the ethanol as an antioxidant. One-tenth milligram was used per serum extract regardless of serum volume, which ranged from 1.5 to 3.1 ml. The alcohol extract was decanted through Whatman No. 1 filter paper into a collecting flask, and the proteinaceous residue was reextracted with 10 volumes of 95% ethanol–diethyl ether 3:1. This mixture was allowed to stand 4 hr or overnight in the dark, after which the solvent was decanted through the filter into the collecting flask. After one more 4 hr extraction with alcohol–ether, the whole mixture was decanted into the filter and rinsed with solvent. Between decantations, filters were covered with watch glasses. The combined filtrate was evaporated under vacuum (water pump) at temperatures below 55° until about 1 ml liquid remained.[3] Nitrogen was released into the flask to break the vacuum, and petroleum ether was immediately added to the warm flask. The aqueous material in the flask was extracted 3 times with warmed petroleum ether, and 3 times with petroleum ether at room temperature. Petroleum ether extracts were combined, evaporated to a convenient volume, and stored below 0°. The petroleum ether extract was then ready for fractionation on silicic acid.[4]

Each individual serum lipid extract was fractionated on silicic acid into cholesterol esters, triglycerides, phospholipids, and a mixed fraction containing mono- and diglycerides, unesterified cholesterol, and free fatty acids (20). The fatty acids of each fraction were analyzed by gas-liquid chromatography[5] (10), and cholesterol (21) and phosphorus (22) were measured when appropriate. The amount of each fatty acid in the triglyceride fraction was calculated from its ratio to a known amount of an added internal standard (23). The amounts

[2] This experimental design was adapted from a report by Olson, Jablonski, and Taylor (18). These workers used male weanling rats, and fed them stock diet one week and experimental diets for the following two weeks. Their experimental diets were similar to those used here, except that they fed about 40% corn oil as compared to the 10% safflower oil used by us. They added vitamin B_{12}, which we did not. Thus their diet contained more linoleic acid, and more total fat, than the present diet. In their work, the amount of fat in the diet and its fatty acid composition had much smaller effects than the choline deficiency. Our use of safflower oil had little if any specific effect on serum lipid compositions, because our observed fatty acid patterns were much like those found in serum lipids from rats fed stock diets (19).

[3] It has been observed that a higher proportion of arachidonic acid is obtained in the cholesterol ester fraction if a little liquid is retained when the extraction solvent is removed. Replicate extracts were either evaporated completely to dryness or allowed to retain some liquid. Those remaining wet showed, after fractionation and GLC analysis, much larger proportions of arachidonic acid as cholesterol ester. The cholesterol content of this fraction revealed no loss, although the "loss" of arachidonic acid sometimes amounted to 1/3 of the total fraction, an accurately measurable difference. There was no increase in cholesterol or arachidonic acid in the chromatographic fraction containing free fatty acids and free cholesterol. These results exclude hydrolysis of the cholesterol esters as a cause of this phenomenon. The mechanism of the effect remains unknown to us. It is possible to obtain precise and reproducible results using either evaporation procedure, but we feel that the procedure producing the higher yield of arachidonic acid in this fraction is the more accurate.

[4] We have found that this procedure, involving alcohol and alcohol–ether as primary extractants, does not produce quantitative yields of plasma phospholipid soluble in petroleum ether. Cholesterol is completely transferred from the aqueous material in the extraction flask to petroleum ether solution. Investigations of the problem in this laboratory show that the phospholipids that are not extracted into petroleum ether are intact and are very similar in composition and distribution to those that do dissolve. The phospholipid data reported here should therefore be considered minimum values, with fatty acid compositions representative of the total amount.

[5] Aerograph A-90-P, Wilkens Instrument Co., Walnut Creek, California. The liquid phase was poly(diethylene glycol succinate) supported on siliconized Chromosorb W, operated at 180–200°. The apparatus was calibrated daily by analysis of fatty acid esters in a mixture of accurately known composition.

TABLE 2 CHOLESTEROL ESTERS, UNESTERIFIED CHOLESTEROL, PHOSPHOLIPID, AND TRIGLYCERIDE IN SERUM OF CHOLINE-DEFICIENT RATS

	Sex and Diet	Cholesterol as Ester	Unesterified Cholesterol	Phospholipid	Triglyceride
			mg/100 ml serum		
M	Control	62.7 ± 3.3*	19.3 ± 1.7	117 ± 5.4	52 ± 7.4†
M	Deficient	40.3 ± 4.0	11.4 ± 2.8	85 ± 8.1	14 ± 2.1†
F	Control	56.0 ± 4.0	16.0 ± 2.4	89 ± 12.0	24 ± 2.9†
F	Deficient	36.8 ± 1.5	11.3 ± 1.2	75 ± 5.8	16 ± 5.4

* Values ± standard errors. P values in text determined by "t" test (39).

† Preparation and analysis were performed on sera from individual animals when the quantity was sufficient. When the volume of a single serum was too low for accurate analysis, equal volumes from 2 animals were pooled. The values marked† represent the mean of 4 separate preparations. All other values were obtained from 5 separate preparations.

of each fatty acid in the cholesterol ester and phospholipid fractions were calculated from their percentage compositions and from their cholesterol and phosphorus contents.

RESULTS

Choline deficiency produced hypolipidemia in both male and female rats (Table 2). Lipids of all classes decreased significantly (p <0.05 or less) in deficient male rats, but only esterified cholesterol decreased significantly (p <0.01) in deficient females. The only sex difference that was statistically significant was in the triglycerides of the control animals (p <0.02). Olson, Jablonski, and Taylor (18) found sharp decreases in serum cholesterol and phospholipid in male rats on a similar regimen.

Cholesterol ester fatty acid patterns (Table 3) revealed effects of choline deficiency in female rats. In these animals, the proportion of arachidonic acid decreased (p <0.01) and the proportion of linoleic acid increased (p <0.01). Control animals showed a significant sex difference. Male rats had a higher proportion of linoleic acid (p <0.01) and a lower proportion of arachidonic acid (p <0.05) than females. This difference was not observed in choline-deficient rats.

Both diet and sex influenced the patterns of phospholipid fatty acids (Table 3). Choline deficiency increased the proportions of palmitic acid both in males (p <0.05) and in females (p <0.01), and decreased the proportion of stearic acid in females (p <0.01). The proportion of arachidonic acid dropped in deficient animals of both sexes (p <0.01), and in deficient females the proportion of linoleic acid increased (p <0.01). Sex differences were most marked in saturated fatty acids of control animals. Male animals had lower proportions of stearic acid than females (p <0.01) and perhaps slightly higher proportions of palmitic acid (p <0.1). In other words, the ratio of stearic to palmitic acids was higher in phospholipids

TABLE 3 FATTY ACID PATTERNS OF SERUM LIPID FRACTIONS IN CHOLINE-DEFICIENT RATS

Fraction	Sex and Diet	Fatty Acids*,† 14:0	16:0	16:1	18:0	18:1	18:2	20:4
Cholesterol Ester	M Control	0.46 ± 0.05	6.24 ± 0.16	2.08 ± 0.21	1.08 ± 0.27	4.68 ± 0.60	17.2 ± 0.42	67.2 ± 1.4
	M Deficient	0.42 ± 0.05	7.30 ± 0.31	1.92 ± 0.14	1.62 ± 0.26	4.80 ± 0.28	18.2 ± 1.1	65.1 ± 1.2
	F Control	0.40 ± 0.03	5.24 ± 0.42	1.64 ± 0.31	1.56 ± 0.31	3.44 ± 0.36	13.9 ± 0.69	72.8 ± 1.8
	F Deficient	0.58 ± 0.07	6.16 ± 0.39	2.26 ± 0.16	1.48 ± 0.16	4.30 ± 0.41	18.2 ± 0.74	66.1 ± 0.66
Phospholipid	M Control	—	20.5 ± 1.2	0.86 ± 0.10	21.2 ± 1.1	4.32 ± 0.18	15.4 ± 0.18	36.2 ± 0.68
	M Deficient	0.64 ± 0.07	23.8 ± 0.42	1.16 ± 0.17	20.7 ± 0.89	4.38 ± 0.27	16.4 ± 0.67	31.9 ± 0.72
	F Control‡	0.32 ± 0.03	17.2 ± 0.66	0.50 ± 0.04	27.2 ± 0.73	3.55 ± 0.19	13.5 ± 0.16	36.9 ± 0.92
	F Deficient	0.56 ± 0.05	22.7 ± 0.60	1.12 ± 0.13	22.8 ± 0.57	4.22 ± 0.45	17.2 ± 0.53	29.9 ± 1.0
Triglyceride	M Control‡	1.22 ± 0.17	18.7 ± 1.0	2.38 ± 0.03	3.35 ± 0.23	15.5 ± 0.56	43.7 ± 0.68	13.0 ± 1.6
	M Deficient‡	2.00 ± 0.79	20.4 ± 0.94	2.50 ± 0.33	4.25 ± 0.41	13.6 ± 0.28	40.6 ± 1.7	15.0 ± 1.8
	F Control‡	1.10 ± 0.18	19.4 ± 0.10	2.08 ± 0.26	3.30 ± 0.12	15.5 ± 1.0	41.8 ± 0.33	15.2 ± 1.7
	F Deficient	—	19.8 ± 0.61	2.30 ± 0.18	3.46 ± 0.25	15.0 ± 0.81	41.1 ± 1.1	14.3 ± 1.5

* Values ± standard errors. P values in text determined by "t" test (39).

† Percentages by weight of total fatty acids in fraction. Some minor components are not shown here.

‡ Preparation and analysis were performed on sera from individual animals when the quantity was sufficient. When the volume of a single serum was too low for accurate analysis, equal volumes from 2 animals were pooled. The values marked‡ represent the mean of 4 separate preparations. All other values were obtained from 5 separate preparations.

from female rats, as has been observed previously (8–12). This sex effect, like that found in cholesterol ester fatty acid patterns, almost disappeared in deficient rats.

Triglyceride fatty acid patterns seemed to depend very little either on diet or sex, except for a possible decrease in oleic and linoleic acids in male deficient animals as compared to male controls.

In general, choline deficiency appeared to reduce the average chain length of fatty acids in cholesterol esters and phospholipids. Decreases in percentages of arachidonic acid in these fractions were roughly balanced by increases in the percentages of linoleic acid. In the phospholipid fraction, choline deficiency decreased the proportion of stearic acid while increasing the proportion of palmitic acid. The deficiency reduced sex differences, so that male and female deficient rats approached a common pattern.

Concentrations of fatty acids in each fraction were computed for individual preparations and are summarized in Table 4. Fatty acid patterns (Table 3) were more strictly regulated than concentrations (Table 4), as is apparent from the sizes of the standard errors. The triglyceride fraction illustrates this contrast most strikingly. Concentrations (mg/100 ml serum) fluctuated even within groups (standard errors often larger than 10%) while percentage values were relatively constant.

Cholesterol ester fatty acid levels (Table 4) were reduced in choline deficiency. Total decreases in fatty acids were about 35% of control values in both sexes. Arachidonic acid, the largest component, accounted for most of the decrease in this fraction. Sex differences were small in both control and deficient rats.

Phospholipid fatty acid levels decreased in deficient rats of both sexes (Table 4). The absolute changes (mg/100 ml) were probably greater[4] than in the fatty acids of cholesterol esters. Choline deficiency produced an uneven decrease in fatty acids of the phospholipid fraction. Stearic and arachidonic acids accounted for most of the decrease, especially in females (Table 5). Control male rats (Table 4) had more palmitic ($p < 0.05$) and more linoleic ($p < 0.02$) acids than female controls. These sex differences disappeared in deficient animals.

The decreases in linoleic and arachidonic acids produced by choline deficiency were parallel in phospholipids and cholesterol esters of corresponding groups of rats.

Triglyceride total fatty acids decreased in choline deficiency, particularly sharply in males. Thus the sex difference evident in control animals was removed, and deficient animals of both sexes approached the same level of circulating triglyceride. No fatty acid in this fraction appeared to be specifically affected by the deficiency; that is, each fatty acid decreased in the same proportion as the total.

TABLE 4 LEVELS OF SERUM FATTY ACIDS IN CHOLINE-DEFICIENT RATS*

Fraction	Sex and Diet	Fatty Acids†							
		14:0	16:0	16:1	18:0	18:1	18:2	20:4	Total
		mg/100 ml serum							
Cholesterol Ester	M Control	—	3.1 ± 0.17	1.0 ± 0.08	0.52 ± 0.04	2.2 ± 0.16	8.3 ± 0.41	34 ± 2.1	49 ± 2.6
	M Deficient	—	2.5 ± 0.29	0.62 ± 0.06	0.58 ± 0.12	1.6 ± 0.21	6.2 ± 0.78	22 ± 1.6	32 ± 3.1
	F Control	—	2.3 ± 0.15	0.68 ± 0.09	0.64 ± 0.11	1.5 ± 0.06	6.1 ± 0.30	32 ± 3.0	44 ± 3.2
	F Deficient	—	1.8 ± 0.16	0.64 ± 0.07	0.44 ± 0.07	1.2 ± 0.14	5.2 ± 0.12	19 ± 0.91	29 ± 1.2
Phospholipid	M Control	—	17 ± 1.3	0.70 ± 0.10	18 ± 1.5	3.5 ± 0.21	13 ± 0.63	30 ± 0.88	82 ± 3.8
	M Deficient	—	14 ± 1.3	0.66 ± 0.07	12 ± 1.5	2.6 ± 0.14	9.8 ± 1.1	19 ± 2.1	60 ± 5.7
	F Control‡	—	12 ± 1.3	0.35 ± 0.05	19 ± 1.7	2.4 ± 0.26	9.3 ± 0.93	25 ± 2.8	69 ± 6.7
	F Deficient	—	12 ± 0.90	0.58 ± 0.09	12 ± 0.73	2.2 ± 0.39	8.8 ± 0.58	16 ± 1.6	52 ± 4.0
Triglyceride	M Control‡	0.60 ± 0.12	9.4 ± 1.6	1.2 ± 0.19	1.7 ± 0.33	7.8 ± 1.3	22.0 ± 3.2	6.3 ± 1.0	50 ± 7.4
	M Deficient‡	0.28 ± 0.08	2.8 ± 0.41	0.35 ± 0.09	0.58 ± 0.09	1.8 ± 0.30	5.6 ± 1.0	2.0 ± 0.29	14 ± 2.1
	F Control‡	0.25 ± 0.05	4.5 ± 0.57	0.50 ± 0.09	0.75 ± 0.10	3.6 ± 0.65	9.6 ± 1.3	3.4 ± 0.36	23 ± 2.9
	F Deficient	—	3.2 ± 1.1	0.42 ± 0.15	0.50 ± 0.13	2.6 ± 1.0	6.8 ± 2.4	2.0 ± 0.36	16 ± 5.4

* Values ± standard errors. P values in text determined by "t" test (39).

† Some minor components are not shown here.

‡ Preparation and analysis were performed on sera from individual animals when the quantity was sufficient. When the volume of a single serum was too low for accurate analysis, equal volumes from 2 animals were pooled. The values marked‡ represent the mean of 4 separate preparations. All other values were obtained from 5 separate preparations.

TABLE 5 DECREASES OF INDIVIDUAL FATTY ACID CONCENTRATIONS INDUCED BY CHOLINE DEFICIENCY. FATTY ACIDS OF SERUM PHOSPHOLIPIDS

	Fatty Acids					
	16:0	18:0	18:1	18:2	20:4	Total
Difference between male control and male deficient, mg/100 ml.....	3	6	0.9	3	11	22
Approximate* percentage of total decrease attributable to component	14	27	4	14	50	
Difference between female control and female deficient, mg/100 ml.....	0	7	0.2	0.5	9	17
Approximate* percentage of total decrease attributable to component....	0	41	1	3	53	

* The sum of the percentages is not exactly 100 because of the standard error in the values from which percentages were calculated.

Choline deficiency produced decreases in the concentration of the major classes of circulating lipids. Triglycerides, which in control animals had very similar fatty acid patterns for both sexes but significantly different concentrations, decreased in both sexes to concentrations statistically indistinguishable from each other. Cholesterol esters and phospholipids together both decreased in concentration and changed their fatty acid patterns. In this way, sex differences visible in control animals nearly disappeared.

DISCUSSION

Serum triglycerides behaved differently from the other major fractions in choline deficiency. No apparent changes in fatty acid pattern occurred, and the decrease in concentration was strongly sex-dependent. The decrease in concentration found here may be attributed to impaired mobilization from the liver, in agreement with observations of triglyceride accumulations in liver. The observed sex difference in liver lipid accumulation (male rats accumulate more than females) may be removed by estrogen (7). The present results, which show that serum triglycerides of males respond more markedly to choline deficiency than those of females, probably reflect events in the liver.

Serum phospholipid and cholesterol ester behaved similarly in several ways. These fractions contained most of the circulating arachidonic acid, and, in deficient animals of both sexes, most of the linoleic acid. In livers of choline-deficient rats, amounts of total cholesterol and phospholipid are nearly normal (24). Therefore, the decrease in circulating phospholipid is probably not attributable to impaired mobilization from the liver but to impaired phospholipid formation induced by the deficiency of precursor.

The biochemical relation between phospholipid and cholesterol esters is less direct. A clue to the connection may be found in the work of Glomset et al., who have characterized a plasma transferase found in the rat and in man (19, 25). This enzyme preferentially transfers the fatty acid from the 2-position of plasma lecithin to plasma unesterified cholesterol. The relatively high degree of unsaturation found in plasma cholesterol esters may result from this reaction, because the 2-position of plasma lecithin (as in most other lecithins) contains the more highly unsaturated fatty acids. Our observations show that in a given group, the concentration of arachidonic acid in phospholipids was approximately equal to that found in cholesterol esters (Table 4), so that the phospholipid fraction could easily have supplied substrate for the transferase reaction. The fatty acids of serum phospholipids are probably an important source of circulating cholesterol esters (19).

This fatty acid transferase reaction may explain other relations between the cholesterol ester and phospholipid fractions. The parallel behavior of linoleic acid in these fractions is also explained by its location on the 2-position in lecithins. Stearic and palmitic acids, however, are usually found on the 1-position of lecithins; these fatty acids were found only in minor quantities in cholesterol esters (Table 4).

Sex differences observed in the fatty acid compositions of phospholipids largely disappeared in choline-deficient animals. It is reasonable to assume that a decrease in serum lecithin accompanied this disappearance of sex differences. That is to say, the major sex difference in phospholipid fatty acids probably occurred in the lecithins. The question arises, at what stage in lecithin biosynthesis may sex hormones influence the fatty acid pattern? Two important pathways of lecithin biosynthesis seem pertinent to this question. Lecithin may be formed directly from diglyceride and cytidine diphosphocholine (CDP choline) (16, 17). An indirect synthesis proceeds by the stepwise methylation of phosphatidyl ethanolamine (PE), which in turn is formed from phosphatidyl serine (PS) (15, 26, 27). It has been observed that the conversion of labeled methyl groups to choline phosphatide is greater in liver from female rats than in that from males, both in vitro (28) and in vivo (29). Since methyl-labeled methionine was used, the incorporation was probably mainly through the indirect path. These results would suggest that female rats probably favor lecithin synthesis from cephalin (PS and PE) precursors, rather than direct biosynthesis

from CDP choline. Cephalins usually contain much larger proportions of stearic and arachidonic acids than lecithins or glycerides from the same tissue (30–37). The lecithins of female rats should therefore contain higher proportions of stearic and arachidonic acids, and those of male rats should have more palmitic and linoleic acids. In the serum phospholipid fraction (Table 3), control females had a larger proportion of stearic acid than males, and control males had larger proportions of both palmitic and linoleic acids than females. This type of sex difference in the fatty acids of rat phospholipids has been observed previously (8–12).

In choline deficiency, the amount of CDP choline in the rat liver is not decreased (38), so that the direct path of lecithin biosynthesis should not have been impaired. The reduced level of circulating phospholipid suggests that formation was decreased. Therefore, it is possible that in choline deficiency, it is the indirect synthesis of lecithin that is impaired, especially under our dietary conditions in which the supply of methyl groups was low. If so, the proportions of stearic and arachidonic acids would be expected to decrease in the phospholipid fraction. This was, in fact, the case (Table 3). Table 5 shows that the decrease in concentration of serum phospholipid produced by choline deficiency was accompanied mainly by decreases in the amounts of phospholipid stearic and arachidonic acids, especially in females.

The observed changes in serum lipid composition are consistent with the possibility that the indirect biosynthesis of lecithins was particularly impaired by choline deficiency. Studies of phospholipid metabolism with radioactive tracers also indicate that the formation of lecithin from cephalin precursors is impaired in choline-deficient rats (14).

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