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Phospholipid Analysis of Cellular Fractions of the Liver Following Administration of Single Dose of Choline.* (26632)

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Liver mitochondria, nuclei and microsomes are composed of 16-32% of phospholipids on a dry weight basis(1,2). These intracellular units contain enzymes which can oxidize fatty acids and other metabolites(3). Choline-containing phospholipids (lecithins) are probably an integral part of an enzyme system necessary for oxidization of fats and fatty acids since this is only phospholipid, except phosphatidylinositol which is lipotropic. The lipotropic effect of lecithins appears to act on the metabolism of fatty acids in the liver, rather than enhancing their mobilization in the form of plasma phospholipids(4). There is a progressive decrease in concentration in the whole liver of the lecithins and a diminished ability of the liver to synthesize these phospholipids in the dog(5) or rat(6) maintained on a choline-deficient diet. Diets low in protein reduce the level of total liver phospholipids(7,8). Administration of a single dose of choline stimulated lipid phosphorylation as shown by the increase of radioactive uptake of P^{32}(9,10,11) which occurs primarily in the lecithin fraction(12), involving mitochondria and nuclei(13). In view of the above findings, experiments were undertaken to determine the phospholipid analysis of various cellular fractions of liver following administration of a single dose of choline in an attempt to ascertain the role of these lipids in mitochondria and nuclei.

Methods. Male albino rats of the Wistar strain weighing 100-110 g were maintained on 5% casein-5% fat diet(10) supplemented with 1% guanidoacetic acid (a methyl group acceptor) for 2.5 weeks and divided into 2 groups. To one group various single doses of choline in 1 ml of water (0, 40, 75 and 150 mg) was administered by stomach tube and the animal sacrificed 6 hours later. A single

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dose of 150 mg of choline in 1 ml was administered to the other group and the animals sacrificed at 0, 3, 6, 10 and 18 hours later.

Three additional groups of rats were maintained on a dietary regime of 5% casein-5% fat(10), 25% casein-5% fat(14) and stock diet, Dakota Maid Fox Feed‡ (30% protein-5% fat) and sacrificed at the end of 4 weeks.

The livers were quickly removed, weighed on a Sartorius Balance. In most cases it was necessary to pool 2 livers for analyses. The liver was minced in an iced beaker and diluted 10-fold with cold 0.88 M sucrose. The mince was homogenized in a Potter-Elvehjem type tissue grinder with Teflon pestle for approximately 4 minutes in an ice bath, forced through a No. 20 gauge hypodermic needle to remove stroma, and centrifuged in a Servall Angle Centrifuge (Type RR-1), according to a modification of the procedures of Hogeboom *et al.*(15) and Griffiths and Pace(16) for separation of the cellular fractions by differential centrifugation. Mitochondria fraction: An aliquot of the homogenate was centrifuged at 1,000 × G for 15 minutes to remove nuclei. The supernatant was then centrifuged at 10,000 × G for 30 minutes and the mitochondria precipitate was washed twice with cold 0.15 M NaCl and finally recentrifuged at 10,000 × G for 20 minutes. The mitochondria fraction was then treated with 10% trichloroacetic acid (TCA) containing 0.4 M $MgCl_2$(17) to remove the acid-soluble phosphorus and was then recentrifuged at 10,000 × G for 10 minutes. The precipitate was washed with 5% TCA containing 0.4 M $MgCl_2$ and recentrifuged at 10,000 × G for 15 minutes. The mitochondria precipitate was then covered with ethanol for dehydration for 6 hours and then washed quantitatively into Soxhlet extracting thimbles with ethanol. Nuclear fraction: An aliquot of the homogenate was recentrifuged at 10,000 × G for 30 minutes. The precipitate was washed with cold 0.15 M NaCl and 10% and 5% TCA solutions and recentrifuged. The precipitate represents nuclei and mitochondria. The value of the nuclei component was obtained by difference. Homogenate fraction: The homogenate fraction was precipitated by addition of 10% TCA, centrifuged at 1,000 × G for 15 minutes, washed with 5% TCA and recentrifuged. The lipids from the cellular fractions were extracted with 95% ethanol for 6 hours in Soxhlet continuous extractors. The lipids were purified with chloroform(18). Lipid P(19), lecithin and cephalin(20) were determined on aliquots of the chloroform solution. Histological examination showed that the mitochondria and nuclear fractions obtained corresponded in appearance and staining properties with those described by Claude (21) and Hogeboom(15).

‡ North Dakota State Mill and Elevator Co., Grand Forks.

Results. Table I shows the effect of dietary protein level on total lipid P, lecithin P and cephalin of mitochondria, nuclei and homogenates. To evaluate the significance of results, the *t* test of significance(22) was applied to the difference between mean of controls and experimental values. The data show that a reduction of protein concentration in the diet produces a statistically significant decrease in total lipid P, lecithin P and cephalin P in liver mitochondria. There is a statistically significant decrease in lecithin P of nuclei and homogenates of liver of the animals fed the 5% casein diet as compared to the 30% protein. These data indicate that the lipid P concentration in mitochondria is directly related to dietary protein intake. This observation is in agreement with the studies carried out on feeding various proteins and analyzing total lipid phosphorus; the quantity and quality of dietary protein determines phospholipid content of the liver cell(7,8,23).

The effect of a single dose of choline at various time intervals on the phospholipids of mitochondria, nuclei and homogenates in choline deficient animals is shown in Table II. It is apparent that administration of a single dose of choline by stomach tube increases total lipid P and lecithin P concentration in the liver in 3 hours. There is a statistically significant increase in total lipid P and lecithin P at 3, 6, 10 and 18 hours in the mitochondria following administration of a single dose of choline when the animals

TABLE I. Effect of Dietary Protein Level on Phospholipids of Mitochondria, Nuclei and Homogenates.

		mg of P/g of wet tissue								
		Mitochondria			Nuclei			Homogenates		
Dietary protein, %	No. of rats	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P
30	20 (8)	.29 ± .06	.12 ± .03	.16 ± .03	.27 ± .10	.14 ± .04	.12 ± .06	1.07 ± .09	.54 ± .07	.49 ± .09
25	25 (9)	.17 ± .03*	.07 ± .02*	.09 ± .02*	.43 ± .05*	.21 ± .03*	.20 ± .03*	1.03 ± .02	.54 ± .06	.42 ± .03
5	31 (11)	.16 ± .07*	.05 ± .03*	.10 ± .03*	.22 ± .06	.09 ± .03†	.11 ± .03	.65 ± .05*	.27 ± .04*	.34 ± .04

Values in parentheses indicate No. of experiments. Figures preceded by ± sign indicate stand. dev. Test of significance was applied to difference between mean of control (stock diet) and experimental values. Probability for chance occurrence of this difference was: * <.01; † <.02.

TABLE II. Effect of Single Dose of Choline (150 mg) on Phospholipids of Mitochondria, Nuclei and Homogenates at Various Time Intervals.

		mg of P/g of wet tissue								
		Mitochondria			Nuclei			Homogenates		
Time, hr	No. of rats	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P
0	34 (10)	.17 ± .03	.04 ± .02	.13 ± .03	.13 ± .03	.05 ± .02	.06 ± .02	.55 ± .07	.14 ± .06	.37 ± .09
3	8 (8)	.22 ± .03†	.09 ± .01*	.12 ± .03	.14 ± .04	.06 ± .02	.06 ± .02	.71 ± .10*	.33 ± .04*	.34 ± .07
6	26 (13)	.26 ± .04*	.11 ± .02*	.14 ± .03	.11 ± .04	.05 ± .02	.05 ± .02	.75 ± .13*	.30 ± .05*	.38 ± .08
10	16 (12)	.22 ± .03*	.12 ± .02*	.10 ± .03‡	.19 ± .07‡	.08 ± .03†	.09 ± .04	.81 ± .17*	.37 ± .08*	.38 ± .09
18	8 (6)	.26 ± .02*	.09 ± .01*	.15 ± .03	.12 ± .02	.04 ± .01	.07 ± .01	.83 ± .03*	.32 ± .04†	.40 ± .05

Values in parentheses indicate No. of experiments. Figures preceded by ± sign indicate stand. dev. Test of significance was applied to difference between mean of controls and experimental values. Probability for chance occurrence of this difference was: * <.01; † <.02; ‡ <.05.

TABLE III. Effect of Single Dose of Choline on Phospholipids of Mitochondria, Nuclei and Homogenates in 6 Hours.

		mg of P/g of wet tissue								
		Mitochondria			Nuclei			Homogenates		
Choline admin., mg	No. of rats	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P	Total lipid P	Lecithin P	Cephalin P
0	34 (10)	.17 ± .03	.04 ± .02	.13 ± .03	.13 ± .03	.05 ± .02	.06 ± .02	.55 ± .07	.14 ± .06	.37 ± .09
40	16 (10)	.21 ± .04†	.10 ± .02*	.11 ± .02	.15 ± .04	.06 ± .02	.08 ± .03	.73 ± .28	.27 ± .10*	.35 ± .15
75	8 (6)	.24 ± .02*	.12 ± .0 *	.11 ± .02	.13 ± .03	.06 ± .01	.06 ± .01	.53 ± .08	.21 ± .02‡	.22 ± .06†
150	26 (13)	.26 ± .04*	.11 ± .02*	.14 ± .03	.11 ± .04	.05 ± .02	.05 ± .02	.75 ± .13*	.30 ± .05*	.38 ± .08

Values in parentheses indicate No. of experiments. Figures preceded by ± sign indicate stand. dev. Test of significance was applied to difference between mean of controls and experimental values. Probability for chance occurrence of this difference was: * <.01; † <.02; ‡ <.05.

have been made choline deficient by administration of a methyl group acceptor, 1% guanidoacetic acid in the diet.

The effect of smaller doses of choline on total lipid P, lecithin P and cephalin P in mitochondria, nuclei and homogenates is shown in Table III. It is apparent that administration of single dose of 40 mg of choline in 6 hours produces a statistically significant increase in concentration of total lipid P and lecithin P in liver mitochondria. This increase of lecithin P concentration in the mitochondria following administration of single dose of choline gives further proof that the composition of these cellular units is affected by dietary intake. Recently Hartroft (24) has shown that liver mitochondria have an oblong or oval profile in electronmicrographs but their shapes become rounded in profile in early choline deficiency. In later stages of choline deficiency a striking increase in size of these mitochondria was observed.

Summary. 1. The effect of a single dose of choline on total lipid phosphorus, lecithin P, and cephalin P of mitochondria, nuclei and homogenates of the liver was studied in choline deficient rats. 2. Administration of single dose of choline 40, 75 and 150 mg significantly increased in 6 hours the total lipid P, and lecithin P concentration of liver mitochondria. 3. A statistically significant increase in total lipid P and lecithin P of liver mitochondria and homogenates occurred in 3, 6, 10 and 18 hours following administration of a single dose of choline. 4. Diets low in protein reduce the level of total lipid P, lecithin P and cephalin P in liver mitochondria.

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