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THE EFFECTS OF LIPOTROPIC FACTORS ON PHOSPHOLIPIDE TURNOVER IN THE PLASMA OF NORMAL PERSONS AS INDICATED BY RADIOACTIVE PHOSPHORUS¹

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The action of choline and methionine in preventing or curing the fatty infiltration of the liver which is produced experimentally in animals by low protein diets is believed to result from an increased turnover of phospholipides in the liver (1, 2). Since this organ is the main, if not the sole, source of phospholipides in plasma (3), it would seem that changes in the formation of phospholipides in the liver would be reflected by corresponding changes in the amounts of newly formed phospholipides in the plasma (4). No methods were available for the determination of newly formed plasma phospholipides until isotopic techniques were developed and radioactive tracers became available.

It is now possible to study the formation of plasma phospholipides following the administration of radioactive phosphorus, and to obtain an indication of the turnover of these compounds in the liver. Few data of this sort are available for human subjects, however. Hence, it became necessary to estimate the turnover in normal persons, before the effects of lipotropic agents such as choline and methionine could be investigated and information could be obtained on the mode of action and possible therapeutic application of these substances.

Preliminary experiments to determine the validity of the method for determining the radioactivity and phosphorus of the plasma lipides in persons with and without liver disease were done and the results obtained were used to calculate the turnover of phospholipides (specific activity) (5).

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The study was then extended to include simultaneous determinations on the inorganic and lipide fractions of the plasma, so that the isotopic concentration in the lipides could be expressed as a function of the concentration of the inorganic phosphate—"relative specific activity" (6).

Under the conditions of our experiments, an increase in the formation of phospholipides may reasonably be assumed when higher values for the total radioactivity are found, and when the specific activity values are also higher or are the same. However, such a conclusion is valid only if the time curve of the specific activity of the inorganic fraction remains the same. Various factors, such as differences in the rate of absorption and in the equilibrium between intra- and extracellular specific activity of the inorganic phosphorus, may conceivably produce changes in the specific activity of the inorganic fraction. Comparison of the relative specific activity should provide a means of reducing these causes of error.

Preliminary study showed that the liver turnover of phospholipides as measured in the plasma varied widely in different individuals. In this study, therefore, comparisons were based on data obtained in the same individual with and without the administration of lipotropic compounds in order to interpret the results obtained in pathologic states before, during, and after treatment with these substances.

MATERIAL

Group 1: Controls not receiving choline or methionine

This group consisted of 13 persons—10 men and three women—having no clinical evidence of organic disease. The ages varied from 19 to 47 years. Preliminary data on the lipide phosphorus of seven of these individuals were used as controls for a previous study (5).

Group 2: Controls receiving choline or methionine

Six other persons, three men and three women, were studied in a similar fashion before and after the administration of a single large dose of choline chloride (or its equivalent as choline dihydrogen citrate) or methionine (dl) (10 Gm.). These persons fell in the same age group as those in Group 1, and likewise had no clinical evidence of organic disease. Data on the lipide phosphorus of three of these individuals have been reported (5). Three of the group were studied again after a two-month interval during which they received no lipotropic factors.

During the interval of study, all the controls ate their usual diet, which was assumed to be adequate in dietary essentials. They took no medication. None of them had any marked change in activity, general health, or well being.

METHODS

All individuals were given an intramuscular injection of radiophosphorus (0.5 millicuries) in the form of NaH_2PO_4 . Blood samples were taken routinely at the

time of injection,² 24 hours, and often at six, 48, 72, and 96 hour intervals. The plasma was separated from the oxalated blood by centrifugation and divided into two fractions of 5 and 8 cc. each. From one fraction the lipides were extracted with hot ethyl alcohol and alcohol-ether, and purified with chloroform (7). The radioactivity (8) and the phosphorus content (9) were determined on aliquots of the chloroform solution. The second fraction of the plasma was extracted with trichloroacetic acid, and the inorganic phosphorus was determined on an aliquot of this extract (9). Another aliquot of the trichloroacetic acid extract was treated with strychnine molybdate (10), and the precipitate dissolved in acetone. After evaporation of the solvent, the radioactivity of the inorganic phosphate was determined (8).

Urine was collected from several individuals during a three-hour period 21 to 24 hours after the injection of

² When the experiments were repeated in the same individual after a two month interval, the sample collected at the time of injection did not exhibit any radioactivity, either in the inorganic or in the lipide fraction.

TABLE I
Plasma phospholipide turnover in Group 1 individuals
(See TABLE I—Continued, next page)

Patient Name, age, sex	0 hour	24 hours							48 hours			
	Phosphorus mg./100 cc.	Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.		Specific activity‡ ×100		Relative specific activity§ ×100	Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.	
		Inorg.	Lipide	Inorg.	Lipide	Inorg.	Lipide		Lipide	Inorg.	Lipide	Inorg.
E. C. 39 M	7.88		7.4		1.116		15.1			7.29		1.560
H. P. Y. 35 M	8.25		7.68		0.476		6.2			7.11		0.838
J. H. W. 47 M			5.74		0.990		17.2					
F. R. L. 37 M	8.26		7.18		0.768		10.7					
L. M. B. 19 F	2.43		2.18		0.434		19.9					
C. W. W. 37 M	7.68		6.70		0.646		9.6					
M. S. 19 M	5.30		5.90		0.506		8.6					
B. G. S. 28 F	8.68		7.70		1.138		14.8					
N. C. T. 27 F	8.08		7.80		0.501		6.4					
R. E. K. 24 M	5.28	3.45	5.68	1.880	1.365	54.5	24.0	44.0	4.04	5.60	0.750	0.921
A. I. 47 M	6.60	1.99	7.93	0.578	1.080	29.0	13.6	46.8	2.28	7.19	0.458	1.400
J. O. D. 33 M	5.69	1.64	6.24	0.686	0.524	41.8	8.4	20.0	1.85	4.13	0.485	0.552
S. G. 34 M	3.25	1.24	2.08	0.454	0.345	36.6	16.6	45.4	2.35	3.15	0.687	0.485
Mean	6.44	2.08	6.17	0.898	0.760	40.4	13.1	39.0	2.63	5.74	0.595	0.839
G	2.0	0.95	1.97	0.65	0.35	10.9	5.4	12.7	0.96	1.7	0.14	0.41
Standard deviation %	31	46	32	72	46	27	41	33.0	37	30	24	49
Standard error of mean	0.60	0.48	0.54	0.33	0.09	5.45	1.50	6.35	0.48	0.72	0.07	0.21

* At time of injection of radioactive phosphorus.

† Expressed in relative radioactivity units (r.r.u.), the total dose injected being equal to 10^4 r.r.u. (This is 100 times the percentage of the dose of P^{32} injected which is found in the phospholipides).

TABLE I—Continued
Plasma phospholipide turnover in Group 1 individuals

Patient Name, age, sex	48 hours			96 hours						Specific activity of urine inorganic phosphorus ×100	Ratio of specific activities of urine P/plasma P	
	Specific activity† ×100		Relative specific activity‡ ×100	Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.		Specific activity† ×100				Relative specific activity‡ ×100
	Inorg.	Lipide	Lipide	Inorg.	Lipide	Inorg.	Lipide	Inorg.	Lipide			Lipide
E. C. 39 M		21.4			8.70		1.689		19.4			
H. P. Y. 35 M		11.8			7.97		0.996		12.5			
J. H. W. 47 M												
F. R. L. 37 M												
L. M. B. 19 F												
C. W. W. 37 M												
M. S. 19 M												
B. G. S. 28 F												
N. C. T. 27 F												
R. E. K. 24 M	18.5	16.4	88.6								28.8	0.53
A. I. 47 M	20.0	19.5	97.5	1.82	6.75	0.252	1.240	13.9	18.4	132.0	12.4	0.43
J. O. D. 33 M	26.2	13.4	51.1								37.3	0.89
S. G. 34 M	29.2	15.4	52.8								52.0	1.42
Mean	23.5	16.3	72.5	1.82	7.80	0.252	1.308	13.9	16.8	132.0	32.6	0.82
G	5.1	3.6	24.0									
Standard deviation %	21	22	33									
Standard error of mean	2.55	1.48	12.0									

† Specific activity is the ratio of the radioactivity (in r.r.u.) to the phosphorus (in mg.) in the extracts.

‡ Relative specific activity of plasma phospholipides is the ratio of the specific activity of the phospholipides to that of the inorganic phosphate.

radiophosphorus. One cc. of the urine samples was evaporated to dryness, and the radioactivity read directly (8). In another aliquot the phosphorus content was determined without previous mineralization (9).

In the tables the *radioactivity* has been expressed in relative radioactivity units (r.r.u.), the total dose injected being assumed to be 10^4 r.r.u. The *specific activity* is the ratio of radioactivity (in r.r.u.) to the phosphorus (in milligrams). *Relative specific activity* (6) is the ratio of the specific activity of the plasma phospholipides to that of the inorganic phosphate.

RESULTS

Turnover of plasma phospholipides in Group 1 (Table I and Figure 1)

Lipide phosphorus

The level of lipide phosphorus in the plasma of the various individuals at the time of injection of

radiophosphorus varied between 2.43 and 8.68 mg./100 cc., with a mean value of 6.44 mg./100 cc. and a standard deviation of 31 per cent. The determinations in the same individual during the 96 hour interval showed a much smaller variability. In the 13 individuals who made up Group 1, the standard deviation from one individual mean averaged 9 per cent.

The radioactivity and specific activity values determined at a given interval also showed considerable variation between individuals (43 and 41 per cent respectively at the 24th hour, and 49 and 22 per cent at the 48th hour). In Figure 1, the specific activities are plotted against time. It is apparent that by the 48th hour the curves generally tend to level into a plateau, the mean values for the specific activity at 24, 48, and 96 hours being 13.1, 16.3, and 16.8 respectively.

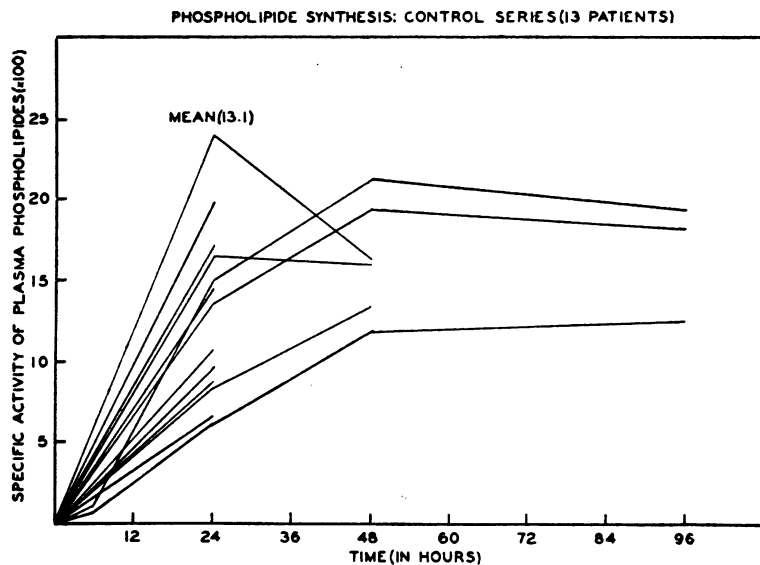


FIG. 1.

Inorganic phosphorus

The values for inorganic phosphorus varied between 1.2 and 4.0 mg./100 cc. of plasma in different individuals (average at the 24th hour 2.1 mg./100 cc., with a standard deviation of 46 per cent). In four individuals in whom repeated determinations were made, the level remained rather constant over the 96 hour period of study. The radioactivity and specific activity of the plasma inorganic phosphorus also varied considerably in the different individuals (standard deviation at the 24th hour 72 and 27 per cent respectively). In each individual subject both radioactivity and specific activity of the inorganic phosphorus showed a progressive decrease during the 96 hour period.

Relative specific activity of the lipid phosphorus

The ratio of the specific activity of the lipid phosphorus to that of the inorganic phosphorus does not adequately express the incorporation of the inorganic phosphate into the phospholipide molecule. However, it seems probable that the comparison of these ratios would minimize the influence of individual variation in the specific activity of the inorganic phosphate (11).

Urine phosphorus

Urine phosphate determinations on a three hour collection (21st to 24th hour) were made in only

four individuals. In three of these the ratio between the specific activities of the inorganic phosphorus in the urine and in the plasma varied between 0.43 and 0.89. In only one person (S. G.) was this ratio greater than 1. The possible factors influencing this ratio will be discussed in a later paper on the basis of a greater number of determinations.

Effect of a single dose of choline on the turnover of plasma phospholipides in Group 2 (Table II and Figure 2)

The administration of choline (to five individuals) or of methionine (to one individual) did not cause a significant change in the level of lipid phosphorus. In all six individuals in Group 2, the radioactivity and specific activity of the lipid phosphorus 24 and 48 hours after administration of the lipotropic agent fell within the range of the corresponding values obtained in normal individuals to whom no choline or methionine was administered (Group 1).

In the three individuals who received another injection of P^{32} two months later, no difference was noted in the values for lipid or inorganic phosphorus, radioactivity, or specific activity before or after the administration of a single large dose of choline (Figure 2).

In all six individuals the radioactivity and specific activity values of the plasma phospholipides

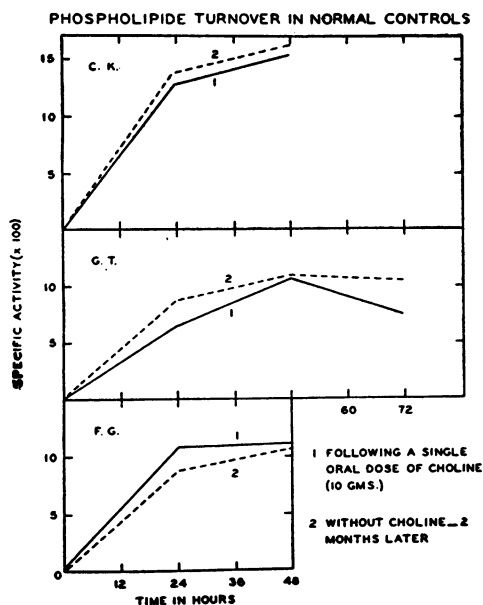


FIG. 2.

fell within the range of those obtained in the controls to whom no methionine or choline was administered (Table II). If the relative specific activities obtained at the same time interval in the

same individual are compared, the values after giving choline are either approximately the same as those obtained two months later without the administration of the lipotropic substance, or are somewhat lower.

DISCUSSION

In an effort to determine the effect of choline and methionine in stimulating phospholipide synthesis by the liver (1, 2), we have injected isotopic phosphorus in normal persons, and have determined the phosphorus level and the radioactivity both in the inorganic and in the lipide fraction of the plasma before and after the administration of lipotropic substances. Thus the isotopic concentration in the lipide phosphorus could be expressed either as specific activity or as relative specific activity. It appears that the 24 and 48 hour intervals following the injection of radio-phosphorus are the most suitable for comparison of the data. These intervals correspond to the ascending part of the specific activity-time curves, where the synthesis of phospholipides exceeds their breakdown or catabolism. Hence, the ra-

TABLE II
Phospholipide turnover in normal individuals before and after a single large dose of choline or methionine
(See TABLE II—Continued, next page)

Patient Name, age, sex	No. of injections of P ³²	Material given	0 hour	24 hours						48 hours					
			Phosphorus mg./100 cc.	Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.		Specific activity‡ ×100		Relative specific activity§ ×100		Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.	
			Lipide*	Inorg.	Lipide	Inorg.	Lipide	Inorg.	Lipide	Lipide	Inorg.	Lipide	Inorg.	Lipide	
B. J. H. 48 F	1	Choline	3.23		4.37		0.597		13.7						
O. H. H. 61 F	1	Methionine	8.85		8.40		0.493		5.9			8.73		1.090	
R. A. B. 25 M	1	Choline	4.88	3.04	5.38	0.832	0.577	27.4	10.7	39.0	3.16	6.40	0.575	0.996	
G. T. 27 M	1	Choline	6.00	3.94	6.44	0.835	0.428	21.2	6.7	31.4	3.32	6.38	0.500	0.688	
	2	Water	6.85	4.24	5.46	0.808	0.490	19.1	9.00	46.9	3.64	6.64	0.482	0.730	
F. G. 26 M	1	Choline	5.08	3.55	6.45	1.246	0.690	35.1	10.7	30.5	4.18	5.13	0.575	0.568	
	2	Water	7.85	3.90	7.86	0.855	0.690	21.9	8.8	40.0	3.38	7.68	0.485	0.840	
C. K. 25 F	1	Choline	6.63	2.80	6.64	0.800	0.860	28.6	12.9	45.1	2.50	6.02	0.476	0.924	
	2	Water	5.97	3.08	6.18	0.856	0.858	27.7	13.9	50.2	2.88	5.85	0.534	0.950	
Mean			5.78	3.33	6.28	0.928	0.608	28.1	10.1	36.5	3.29	6.53	0.530	0.853	
G			1.90	0.51	1.35	0.21	0.023	5.7	3.1	6.9	0.69	1.3	0.05	0.22	
Standard deviation %			33	15	22	23	24	20	33	19	21	20	9	26	
Standard error of mean			0.77	0.25	0.55	0.10	0.01	0.28	1.29	3.45	0.34	0.59	0.02	0.09	

*†§ See footnotes to Table I.

|| With lipotropic agent.

TABLE II—Continued

Phospholipide turnover in normal individuals before and after a single large dose of choline or methionine

Patient		No. of injections of P ³²	Material given	48 hours			72 hours						
Name, age, sex				Specific activity† ×100		Relative specific activity‡ ×100	Phosphorus mg./100 cc.		Radioactivity† r.r.u./100 cc.		Specific activity† ×100		Relative specific activity‡ ×100
				Inorg.	Lipide	Lipide	Inorg.	Lipide	Inorg.	Lipide	Inorg.	Lipide	Lipide
B. J. H.	48 F	1	Choline										
O. H. H.	61 F	1	Methionine		12.5								
R. A. B.	25 M	1	Choline	18.2	15.6	85.8							
G. T.	27 M	1	Choline	15.1	10.8	71.5	3.36	4.83	0.392	0.360	11.6	7.5	64.7
		2	Water	13.3	11.0	82.8	3.47	7.35	0.402	0.773	11.6	10.5	90.5
F. G.	26 M	1	Choline	13.8	11.1	80.5							
		2	Water	14.4	10.9	75.7							
C. K.	25 F	1	Choline	19.0	15.3	80.5							
		2	Water	18.5	16.2	87.6							
Mean				16.5	13.0	79.5	3.41	6.09	0.397	0.566	11.6	9.0	77.6
G				2.4	2.2	5.9							
Standard deviation %				15	17	7							
Standard error of mean				1.24	1.01	2.97							

dioactivity values correspond chiefly to the phospholipides synthesized from the inorganic phosphate during these intervals.

Variations in normal individuals without lipotropic substances

Our previous statement concerning the variability of the level of lipide phosphorus in different individuals and its relative constancy in the same person was confirmed and extended (5). This level was found to be comparable in the same individual even when the determinations were repeated after an interval of several months. Likewise, on the basis of the present and previous determinations, it is apparent that there is a wide variability between individuals in the turnover of phospholipides in plasma. Individual variations are still considerable, although less extensive, if the values are expressed as relative specific activity instead of specific activity.

Effect of a single large dose of lipotropic material on phospholipide synthesis

No significant difference in the plasma phospholipide phosphorus was found between the group of persons who were given a single large

dose of a lipotropic substance and those who were not. However, in view of the wide individual variability in both the level of phospholipide phosphorus and the phospholipide formation, we felt that the same individual should be used as his own control in order to detect any significant change which might occur as a result of the administration of lipotropic substances. Accordingly, the phospholipide turnover following a single large dose of choline was studied in three normal individuals and two months later the experiments were repeated in the same individuals without the administration of this substance. No significant difference in the phospholipide levels of these individuals was noted in the two experiments, and the specific activity-time curves were essentially unchanged. The values of both specific activity and relative specific activity following the administration of choline were no higher than those found in the experiment in which the individual did not receive these substances.

In experimental animals receiving a high-fat, low-choline diet, the ingestion of a single massive dose of choline has a marked stimulatory effect on the conversion of inorganic phosphate into plasma phospholipides, this effect reflecting the

increase in phospholipide synthesis in the liver (12). In animals on adequate diets containing an abundant supply of choline or choline precursors, the effect is smaller or not apparent (1). It seems that, in animals, the stimulatory action of a large dose of lipotropic substances is detectable only if the phospholipide synthesis is limited by the dietary supply of choline or choline precursors.

We have previously reported a few data on the effect of a large dose of choline or methionine on phospholipide turnover in normal persons and in patients with chronic hepatitis (5, 13). To our knowledge, no other data are available in the literature concerning the action of a single massive dose of choline or methionine on phospholipide formation in normal human subjects. In our present experiments on normal individuals the administration of a single large dose of a lipotropic substance apparently did not accelerate the turnover of phospholipides in the plasma (nor, presumably, in the liver).

If the effects of choline in human beings are analogous to those in animals, our present results obtained in persons without clinical or laboratory evidence of liver disease would indicate that the dietary supply of lipotropic substances or of their precursors was adequate.

SUMMARY

Following the administration of radioactive phosphorus to a group of normal individuals, the radioactivity and phosphorus level in the inorganic and lipide fractions of the plasma were determined. The values of the specific activity or relative specific activity have been taken as an indication of the turnover of phospholipides in the plasma, possibly reflecting the synthesis of phospholipides in the liver.

On the basis of the present study and previous experiments, it appears that the phospholipide turnover as measured in the plasma of normal persons varies widely between individuals but is fairly constant in the same individual.

In several subjects the phospholipides were studied after the ingestion of a large dose of choline or methionine. This was repeated two months later in the same individuals without the adminis-

tration of the lipotropic agent. No significant differences were noted between the results obtained in the two experiments. If these results are comparable with those obtained in experimental animals on low-choline diets, the failure of choline to produce a stimulatory effect may indicate that the dietary supply of lipotropic substances or precursors in the persons studied was probably adequate.

ACKNOWLEDGMENTS

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