Effect of choline on the incorporation of ^{35}S-L-methionine into liver protein of rats with fatty livers

Recently an "antilipotropic" effect of methionine has been reported in rats fed a diet containing 9% casein[1]. In a series of experiments concerned with a study of lipotropic agents, we observed a non-lipotropic response of methionine in rats fed a diet containing 5% casein[2] (Table I). Only when methionine was added at a high level to the diet was a significant decrease in total liver lipids observed, and this effect undoubtedly resulted from a restricted food intake[3] rather than from methionine *per se*. This observation strongly suggested that in growing rats, protein metabolism involving methionine takes precedence over the lipotropic activity of methionine when both the dietary protein and choline supply are deficient.

TABLE I

EFFECT OF DIETARY DL-METHIONINE ON LIVER LIPIDS OF RATS MAINTAINED ON A 5% CASEIN–5% FAT DIET

All animals were maintained on the diet for a period of 2 weeks. The number preceded by ± represents the standard deviation.

Methionine supplement (% of diet)	*No. of animals*	*Average food consumption* (g/day)	*Change of body weight* (%)	*Liver lipids* (mg/g wet tissue)
0.0	10	10	+ 1	105 ± 32
0.3	8	15	+ 14	119 ± 22
1.3	10	13	+ 8	102 ± 24
6.0	8	5	— 44	51 ± 3*

* $P < 0.01$ when test of significance was applied to difference between means of non-supplemented and supplemented experimental values.

In order to evaluate this conclusion, the uptake of ^{35}S-labeled methionine into the sulfur-containing amino acids in liver protein was studied in rats (Sprague Dawley strain) which were maintained for a period of 5 weeks on one of the following diets: (a) containing 5% casein, 5% fat, and free from choline[2]; (b) containing 5% casein, 5% fat and supplemented with choline at a level which maintains normal liver fat[4]; (c) containing 25% casein and 5% fat[5]. At the end of the dietary regime all animals were injected with 15 μC of ^{35}S-L-methionine and, after 6 or 24 h, were killed by decapitation. A portion of the liver was rapidly excised and weighed, after which acid-soluble components were removed with cold 10% trichloroacetic acid. The lipids of the residue were then extracted for 6 h with a 2:1 (v/v) 95% ethanol: ether mixture in Soxhlet continuous extractors. Total lipids were determined gravimetrically on the extract. The lipid-free protein residue was then hydrolyzed with 20% HCl–50% HCOOH[6] for 24 h, after which the hydrolysate was decolorized with Darko KB charcoal. Aliquots of the hydrolysate were analyzed for cystine[7] and methionine[8]. Aliquots representing total ^{35}S-protein and ^{35}S-cystine, the latter isolated as the mercaptide[9], were oxidized and then precipitated and assayed as the benzidine salt[10]. ^{35}S-Methionine was calculated as the difference between these two values. Specific activity was defined

$$\text{as } \frac{(\text{counts of } ^{35}\text{S-amino acid/min}) \cdot (\text{dekagram body wt.})}{(\text{mg amino acid S}) \cdot (\text{counts/min of dose administered})}$$

As shown in Table II, choline does not alter the uptake of radioactive methionine into liver-protein methionine or cystine in rats maintained on a 5% casein diet. It is apparent that in animals which are in urgent need of methyl groups—as indicated by the significant liver-fat deposition in the animals fed a diet low in protein and unsupplemented with choline—very little methionine is available as a methyl donor for choline synthesis, since the specific activities of protein methionine in groups *a* and *b* are practically identical at either 6 or 24 h after administration of the labeled methionine.

Animals in group *c* were fed a 25% casein diet, since a reference to values obtained with an adequate protein intake seemed desirable. The specific activities of methionine and cystine in group *c* were significantly lower than the corresponding values obtained for the animals receiving the 5%-casein diet. This is in agreement with data reported by TARVER[11], who has demonstrated the incorporation of ^{35}S-methionine into liver protein to be a function of the dietary casein.

TABLE II

EFFECT OF CHOLINE ON INCORPORATION OF ^{35}S-L-METHIONINE INTO LIVER PROTEIN

Each group represents results obtained from 10 rats. Groups *c* and *b* were pair-fed with group *a*. Figures preceded by the ± sign are standard deviations

Group	*Time after injection of ^{35}S-L-methionine*	*Specific activity of protein fraction*		*Liver lipids*	*Change of body weight*
	(h)	*Methionine*	*Cystine*	*(mg/g wet tissue)*	*(%)*
a					
(5% casein–5% fat)	6	28.1 ± 1.6	14.9 ± 1.9	78 ± 28	— 10
	24	25.7 ± 1.3	17.5 ± 3.4		
b					
(5% casein–5% fat) (+ choline supplement*)	6	26.8 ± 1.0	15.6 ± 1.9	33 ± 8**	— 10
	24	23.1 ± 1.9	17.8 ± 4.2		
c					
(25% casein–5% fat)	6	15.5 ± 1.4	10.5 ± 1.7	22 ± 5**	+ 25
	24	14.6 ± 0.6	8.4 ± 0.7		

* Diet contained 0.16% choline·HCl.

** $P<0.01$ when test of significance was applied to difference between means of group *a* and groups *b* or *c*.

The evidence presented supports our hypothesis that protein metabolism takes precedence over lipotropic action when animals are subjected to a dietary stress of methyl-group dieficiency.

This work was supported in part by grants from U.S. Atomic Energy Commission, Lipotropic Research Foundation and the Department of Army, Office of Surgeon General. The authors wish to thank A. H. WALSER for his technical assistance.

Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota Medical School, Grand Forks, N.D. (U.S.A.)

FRED SNYDER*
W. E. CORNATZER

1 A. E. HARPER, D. A. BENTON, M. E. WINJE AND C. A. ELVEHJEM, *J. Biol. Chem.*, 209 (1954) 159.
2 W. E. CORNATZER AND D. G. GALLO, *Proc. Soc. Exptl. Biol. Med.*, 86 (1954) 15.
3 C. H. BEST, *Federation Proc.*, 9 (1950) 506.
4 R. J. YOUNG, C. C. LUCAS, J. M. PATTERSON, C. H. BEST, *Can. J. Biochem. and Physiol.*, 34 (1956) 713.
5 W. E. CORNATZER, G. T. HARRELL, D. CAYER AND C. ARTOM, *Proc. Soc. Exptl. Biol. Med.*, 73 (1950) 492.
6 G. L. MILLER AND V. DU VIGNEAUD, *J. Biol. Chem.*, 118 (1937) 101.
7 R. J. BLOCK AND D. BOLLING, *The Amino Acid Composition of Proteins and Foods*, C. C. Thomas, Springfield, Ill., 1951, p. 195.
8 M. J. HORN, D. B. JONES, A. E. BLUM, *J. Biol. Chem.*, 166 (1946) 313.
9 C. A. ZITTLE AND R. A. O'DELL, *J. Biol. Chem.*, 139 (1941) 753.
10 M. K. GAITONDE AND D. RICHTER, *Biochem. J.*, 59 (1955) 690.
11 H. TARVER, in J. H. LAWRENCE AND J. G. HAMILTON, *Advances in Biological and Medical Physics*, Vol. 2, Academic Press Inc., New York, 1951.

Received December 5th, 1957

* Predoctoral fellow, National Heart Institute, U.S. Public Health Service.