Environmental temperature and choline requirements in rats. II: Choline and methionine requirements for lipotropic activity

J. S. CHAHL and C. C. KRATZING

Department of Physiology, University of Queensland, Brisbane, Australia

ABSTRACT Young rats were fed choline-deficient diets and maintained at different environmental temperatures. The hepatic lipid level remained normal in rats at 2° when 25 mg of choline per 100 g of food was fed; 50 mg of choline per 100 g food was required at 21° and 100 mg of choline per 100 g food at 33° to prevent excessive lipid accumulation. These values were equivalent to a mean daily intake per rat of 3 mg of choline at 2°, 5.5 mg at 21°, and 7 mg at 33° respectively.

When the growth rate was slower owing to a slight inadequacy of histidine in the basal choline-deficient diet, normal hepatic lipid was maintained by supplements of 50 mg of choline per 100 g food at 21° and 33°.

Increasing the methionine content of the diet two- or threefold from a basal value of 340 mg per 100 g food was as effective as 200 mg of choline per 100 g of food in lowering hepatic lipids at 2°, 21°, and 33°.

KEY WORDS choline · deficiency · requirement · prevention · fatty liver · environmental temperature · methionine supplementation · rat

In the previous paper (1) it was reported that in choline-deficient rats, the percentage of lipid in the liver increased with high environmental temperatures, but no direct proportionality could be found between the increase in percentage of lipid and the increase in temperature. However, when the liver lipid was expressed as a fraction of the food ingested over the experimental period, the percentage of liver lipid was more nearly directly proportional to the rise in temperature. From this it was assumed that at higher temperatures there was increased requirement for choline, at least over the range 2°–33°. Although the choline requirement at 2° was lower, it was not negligible, as the absence of choline from the diet gave rise to fatty infiltration in the liver even at 2°.

The amount of methionine provided by a choline-deficient diet is important since methionine is a precursor in the biosynthesis of choline (2, 3). Tucker and Eckstein (4) and Eckstein (5) showed that the induction of fatty liver by a choline-deficient diet depended on its methione content. The work of Radomski and Wood suggests that this is true also if the rats are maintained at low environmental temperatures (6). The adequacy of the level of other amino acids in the diet is also a factor which affects lipid deposition in the liver and has been discussed by Harper (7).

It is well established that the food intake of rats increases with decreasing environmental temperature. This was confirmed in the present study (1). It was likely that when the choline-deficient diet was fed, the availability of choline precursors, such as methionine, determined the liver lipid concentration, which increased with increasing ambient temperature when no choline was included in the diet. In the present work, the amounts of choline required to prevent lipid accumulation in the liver have been determined. In addition the effectiveness of methionine as a lipotropic agent was determined at the three temperatures.

METHODS

Rats

Male rats 4–6 weeks old weighing 60–90 g were used in groups of 6. Rats were of the same strain as in the previous work (1).

Diet

Two diets were used: diet A, whose composition is given in the preceding paper (1), and diet B, which was casein

TABLE 1 CHANGES IN LIVER LIPID WITH DIFFERENT CHOLINE INTAKES IN DIET A

Temp.	Choline Supplement	No. of Rats*	Total Food Intake	Total Choline Intake	Increase in Body Wt.	Wet Liver Wt.	Liver Lipid	Liver Lipid / Wet Liver	Liver Lipid / Food Intake†
°C	mg/100 g food		g	mg	g	g	mg	%	mg/g
21 ± 2	200	6	208 ±16‡	416	36 ±5‡	5.898 ±0.148‡	318 ±44‡	5.39 ±0.59‡	1.54 ±0.24‡
	100	6	192 ±25	192	32 ±12	5.405 ±0.553	314 ±68	5.75 ±0.74	1.63 ±0.25
	50	6	181 ±40	91	31 ±12	5.098 ±1.394	328 ±77	6.61 ±1.16	1.81 ±0.17
	0	6	164 ±25	0	22 ±10	5.588 ±0.754	1,221 ±697	21.98 ±1.47	7.54 ±1.53
33 ± 2	200	6 (1)	106 ±4	212	15 ±4	3.507 ±0.440	179 ±29	5.09 ±0.35	1.67 ±0.23
	100	6 (2)	91 ±16	91	11 ±4	3.140 ±0.875	170 ±45	5.52 ±0.89	2.02 ±0.78
	50	6	93 ±10	46	15 ±4	3.315 ±0.378	197 ±35	5.93 ±0.68	2.18 ±0.41
	0	6	88 ±12	0	6 ±4	3.352 ±0.730	706 ±330	20.74 ±6.14	0.76 ±5.84

* The numbers in parentheses show the number of rats that died during the experiment.
† Ratio of liver lipid (milligrams) to the total food intake (grams) during the 21 days of the experiment.
‡ Mean ± SD.

(fat- and vitamin-free) 6%, peanut meal (continuously extracted with hot ethanol for 48 hr) 30%, sucrose 38%, beef fat 15%, maize oil 5%, salt mixture 5%, vitamin powder 1%, and vitamin A and D concentrate 1%. Salt mixture, vitamin powder, and vitamin concentrate were the same as those used in the preceding paper.

Treatment

As shown in the tables, rats received supplements of either choline or methionine (L. Light, Colnbrook, England) in the diet. Experimental procedure and analyses were the same as those described in the accompanying paper (1).

RESULTS

In the first experiment, diet A was fed to rats maintained at either 21° or 33°. From Table 1 it may be seen that at both temperatures when the choline supplement was reduced from 200 to 50 mg per 100 g of food, there were insignificant changes in the body weight and liver lipid concentration. The food intake of all groups at 33° was approximately half that of the corresponding groups at 21°. The dependence of liver lipid on food intake can be seen in the values of the ratio of liver lipid (milligrams) to total food intake (grams), which showed only a minor increase with decreasing amounts of choline in the food. Only in groups which received no choline and whose liver lipid concentration had increased markedly did the value of the ratio increase significantly. It may be seen that the ratio at 33° was higher than at 21° for rats with no choline supplement although the increase in temperature did not produce a significant change in the concentration of lipids per gram wet weight liver.

Diet B was fed so that a state of choline deficiency might be obtained, uncomplicated by the minor deficiencies of some amino acids (notably histidine) in diet A. The second diet was calculated to provide 21% protein and was richer than the previous diet in essential amino acids.

Food intake varied inversely with temperature (see Table 2). The levels of choline in the diet produced only minor changes in food intake at the various temperatures, except at 33° where a larger variation in food intake was noted. The greatest weight increase was in rats at 21°; increases at 2° and 33° were similar to each other. The proportion and amounts of liver lipids tended to increase significantly from normal values when the total choline intake dropped below 60 mg at 2°, 120 mg at 21°, and 140 mg at 33°. The ratio of liver lipid (milligrams) to total food intake (grams), was within the range 1.24–2.31 at the three temperatures when 200 mg of choline per 100 g of food was given. It increased to 5.39 when 50 mg of choline per 100 g of food was given to rats at 33°; to 6.55 when 25 mg of choline per 100 g of food was given to those at 21°; and to 5.05 when no choline was provided in the diet of those at 2°. A proportionate increase in the ratio was noted with the further reduction of choline in the diet of those rats at 33°; and when none was provided in the diet, rats at 21° had a ratio of 14.24 and those at 33°, 20.20.

Table 3 presents the results of an experiment in which rats were fed the basal choline-deficient diet (diet A)

JOURNAL OF LIPID RESEARCH ASBMB

TABLE 2 Changes in Liver Lipid with Different Choline Intakes in Diet B

Temp.	Choline Supplement	No. of Rats*	Total Food Intake	Total Choline Intake	Increase in Body Wt.	Wet Liver Wt.	Liver Lipid	Liver Lipid / Wet Liver	Liver Lipid / Food Intake†
°C	mg/100 g food		g	mg	g	g	mg	%	mg/g
2 ± 2	200	6	263 ± 46‡	526	35 ± 20‡	4.935‡ ±1.398	332 ± 104‡	6.72 ± 0.17‡	1.24 ± 0.22‡
	100	6	271 ± 26	271	47 ± 21	5.585 ±0.297	400 ± 103	7.09 ± 0.92	1.45 ± 0.25
	50	6	260 ± 23	130	51 ± 18	6.051 ±1.263	452 ± 126	7.39 ± 1.19	1.72 ± 0.43
	25	6	273 ± 29	68	52 ± 16	6.072 ±2.764	504 ± 182	7.86 ± 1.07	1.88 ± 0.64
	0	6(1)	266 ± 32	0	52 ± 17	7.265 ±2.305	1,403 ± 945	17.41 ± 8.14	5.05 ± 3.22
21 ± 2	200	6	244 ± 34	488	71 ± 17	6.829 ±1.223	563 ± 62	8.32 ± 0.67	2.31 ± 0.16
	100	6	222 ± 24	222	59 ± 10	6.574 ±0.760	549 ± 116	8.30 ± 1.13	2.46 ± 0.39
	50	6	234 ± 34	117	65 ± 14	6.672 ±1.419	625 ± 141	9.36 ± 1.01	2.66 ± 0.41
	25	6	234 ± 21	58	62 ± 13	7.547 ±1.227	1,526 ± 445	20.06 ± 4.03	6.55 ± 1.96
	0	6	225 ± 15	0	57 ± 9	10.162 ±1.842	3,200 ± 744	31.25 ± 1.79	14.24 ± 3.08
33 ± 2	200	6(1)	121 ± 17	242	31 ± 7	3.499 ±0.383	272 ± 48	7.74 ± 0.62	1.86 ± 0.10
	100	6	142 ± 13	142	39 ± 4	3.804 ±0.261	373 ± 66	9.77 ± 0.40	2.62 ± 0.41
	50	6	163 ± 9	81	55 ± 5	5.207 ±0.611	1,051 ± 410	21.10 ± 2.65	5.39 ± 0.70
	25	6	148 ± 27	37	47 ± 13	6.033 ±1.707	1,920 ± 942	29.79 ± 9.17	12.37 ± 4.91
	0	6	128 ± 7	0	31 ± 8	7.101 ±0.877	2,560 ± 263	36.63 ± 1.99	20.20 ± 1.83

* The numbers in parentheses show the number of rats that died during the experiment.
† Ratio of liver lipid (milligrams) to the total food intake (grams) during the 21 days of the experiment.
‡ Mean ± SD.

supplemented by either choline or methionine, the latter in amounts equal to or twice the basal content of methionine in the diet. (The amount of methionine in the basal diet before supplementation was estimated to be 340 mg/100 g of food.) These supplements increased food intake in rats except for those at 2°. The rate of growth of this latter group was not affected when either choline or methionine supplements were added to the basal diet. Both supplements stimulated growth to a significant extent at 21° but at 33° only methionine had this effect, and methionine was as effective as choline in lowering the ratio (last column) as well as the amount of concentration of liver lipid at the temperatures studied. At 33° the larger supplement of methionine seemed to be more effective than the smaller in lowering liver lipids, but the difference between the two was not significant.

DISCUSSION

In these experiments it was found that over the range of temperatures studied, fatty livers developed when either of the two choline-deficient diets, unsupplemented with methionine, was fed. The hepatic lipid increased with increasing temperature. In the previous paper (1) it was suggested that the slight inadequacy of amino acids in the diet might have been the cause of this observation at 2°. When the basal diet B was used hepatic lipids still increased at 2°. This was contrary to reports by Sellers and You (8, 9) despite the close similarity of their diet to ours.

The choline requirement of rats in the present study was influenced by the nature of the diet and the environmental temperature. Administration of 50 mg of choline per 100 g of food was adequate to maintain normal liver lipid concentration in rats at 21° and 33° when diet A was fed, but not when diet B was fed. Rats fed diet B had a higher caloric intake and rate of growth than those fed diet A. Both these factors, that is increased caloric intake (10) and growth (11), have been shown to increase the choline requirement of rats.

It would be difficult to define a minimum dose of choline for the rat which would maintain a normal liver lipid concentration, since several factors seem to influence this requirement. The present study has shown that enviromental temperature also altered the choline requirement. This can be seen in Fig. 1.

Dose–response studies with choline have been reported by other workers (12, 13) but a comparison with the present investigation cannot be made since the environmental temperature of their rats is not reported. The diet used in the earlier study (12) was also deficient in the B-vitamins.

The increased choline requirements of rats with increased environmental temperature may be due to the decreased availability of choline precursors such as methionine from the diet, because food intake and therefore methionine intake were lowered with increased environmental temperature. However, no simple relationship was apparent between the increased choline requirement and the decrease in food consumption with increasing temperature. It is possible that changes in the utilization and degradation of choline might be involved.

Methionine supplements effectively replaced choline in preventing excess lipid accumulation in the liver of choline-deficient rats. It has been shown that methionine in the diet is first made available for the synthesis of protein and when this requirement has been met it is then made available for lipotropic activity (14). Our work shows that methionine supplements stimulated growth in rats maintained at higher temperatures, particularly at 33°, but not at 2°. Mills and Cottingham (15) showed that supplements of methionine produced a stimulus in growth of rats at about 33° but not at 20°. These results suggest that at 33° and possibly at 21° the methionine intake was inadequate for growth and hence the lipotropic activity of methionine was restricted. In rats maintained at 2° and therefore in a state of catabolism or decreased anabolism, their growth requirements for methionine were satisfied but a sufficient amount of it was not available for lipotropic

TABLE 3 EFFECTS OF INCREASED METHIONINE INTAKE ON LIVER LIPID

Temp.	Supplement	No. of Rats*	Total Food Intake	Increase in Body Wt.	Wet Liver Wt.	Liver Lipid	Liver Lipid / Wet Liver	Liver Lipid / Food Intake†
°C	mg/100 g food		g	g	g	mg	%	mg/g
2 ± 2	0	6 (1)	275 ± 40‡	31 ± 21‡	6.522 ±1.655‡	1,384 ± 668‡	19.80 ± 7.36‡	4.85 ± 2.68‡
	340 L-Me§	6 (1)	269 ± 16	33 ± 8	6.354 ±0.565	479 ± 57	7.55 ± 0.82	1.78 ± 0.19
	680 L-Me	6	264 ± 22	30 ± 16	6.044 ±0.847	518 ± 64	8.67 ± 1.03	1.97 ± 0.24
	200 choline	6	276 ± 41	31 ± 8	6.133 ±0.807	481 ± 93	7.83 ± 0.90	1.69 ± 0.27
21 ± 2	0	6	203 ± 19	25 ± 9	6.669 ±1.158	1,657 ± 507	24.62 ± 5.14	8.09 ± 2.16
	340 L-Me	6	221 ± 22	36 ± 7	5.700 ±0.234	463 ± 97	8.08 ± 2.75	2.10 ± 0.42
	680 L-Me	6	215 ± 14	34 ± 4	5.297 ±0.160	414 ± 62	7.80 ± 0.89	1.93 ± 0.32
	200 choline	6	235 ± 40	31 ± 10	5.201 ±0.542	380 ± 86	7.29 ± 2.05	1.63 ± 0.31
33 ± 2	0	6	117 ± 10	13 ± 5	3.980 ±0.450	967 ± 360	23.81 ± 7.24	8.43 ± 3.50
	340 L-Me	6	123 ± 11	22 ± 3	3.953 ±0.312	331 ± 79	8.33 ± 1.45	2.68 ± 0.49
	680 L-Me	6	142 ± 13	28 ± 8	4.465 ±0.501	296 ± 25	6.69 ± 0.75	2.10 ± 0.23
	200 choline	6	140 ± 22	16 ± 6	3.586 ±0.356	238 ± 44	6.62 ± 0.77	1.71 ± 0.24

* The numbers in parentheses show the number of rats that died during the experiment.
† Ratio of liver lipid (milligrams) to the total food intake (grams) during the 21 days of the experiment.
‡ Mean ± SD.
§ L-Methionine.

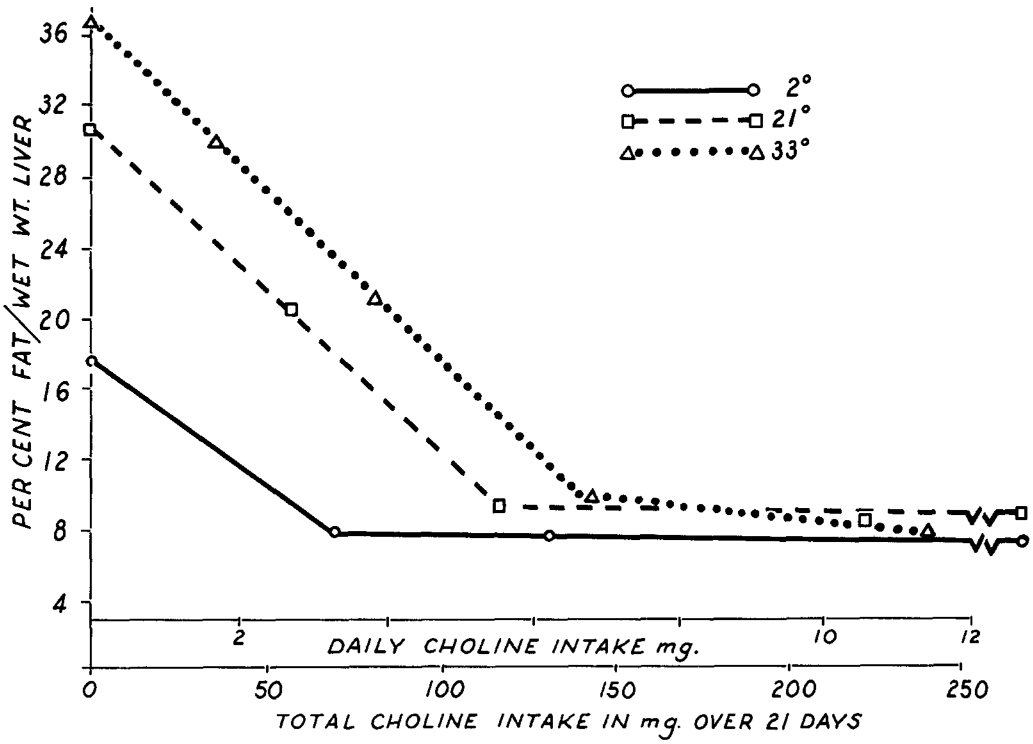

FIG. 1. Effect of environmental temperature and choline intake on the liver lipid concentration of the rat. Choline consumption of the rats was controlled by adding different concentrations to the diet. For details see Table 2. The daily choline intake has been calculated from the mean total intake over 21 days of the experiment.

activity. Supplements of methionine provided adequate amounts for both processes.

This work was supported by a University of Queensland Research Grant.

Manuscript received 4 February 1965; accepted 5 August 1965.

REFERENCES

1. Chahl, J. S., and C. C. Kratzing. *J. Lipid Res.* **7**: 17, 1966.
2. Stekol, J. A. *Am. J. Clin. Nutr.* **6**: 200, 1958.
3. Bremer, J., and D. M. Greenberg. *Biochim. Biophys. Acta* **37**: 173, 1960.
4. Tucker, H. F., and H. C. Eckstein. *J. Biol. Chem.* **126**: 117, 1938.
5. Eckstein, H. C. *J. Biol. Chem.* **195**: 167, 1952.
6. Radomski, M. W., and J. D. Wood. *Can. J. Physiol. Pharmacol.* **42**: 769, 1964.
7. Harper, A. E. *Am. J. Clin. Nutr.* **6**: 242, 1958.
8. Sellers, E. A., and R. W. You. *Science* **110**: 713, 1949.
9. Sellers, E. A., and R. W. You. *Biochem. J.* **51**: 573, 1952.
10. Best, C. H., and J. H. Ridout. *J. Physiol.* **94**: 47, 1938.
11. Griffith, W. H., and D. J. Mulford. *J. Nutr.* **21**: 633, 1941.
12. Best, C. H., C. C. Lucas, J. H. Ridout, and J. M. Patterson. *J. Biol. Chem.*, **186**: 317, 1950.
13. Best, C. H., C. C. Lucas, J. M. Patterson, and J. H. Ridout. *Can. J. Biochem. Physiol.* **3**: 613, 1958.
14. Snyder, F., and W. E. Cornatzer. *Biochim. Biophys. Acta* **27**: 650, 1958.
15. Mills, C. A., and E. Cottingham. *Arch. Biochem.* **4**: 171, 1944.