Environmental temperature and choline requirement in rats. I: Choline deficiency in rats at various temperatures

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ABSTRACT Rats were maintained at 2°, 21°, and 33° for 3 weeks on a choline-supplemented or a choline-deficient diet. In contrast to the findings of some other workers, choline deficiency produced fatty livers at all temperatures.

The ratio of the total liver lipid to the total food intake was the same in all choline-supplemented rats. In choline-deficient rats this ratio was always higher and varied directly with temperature.

KEY WORDS choline · deficiency · fatty · liver · lipids · environmental temperature · pseudolipotropic effect · rat

In 1949 Sellers and You (1) reported that rats fed a choline-deficient diet and kept at an environmental temperature of 2.5 ± 1° did not develop the fatty infiltration of liver found at 25 ± 2°. Later, they reported that if the hypolipotropic diet contained 50% fat then excess lipid accumulated in the liver of rats at 2° but it was significantly less than at 25°; they described the cold temperature as having "pseudolipotropic" effect (2). The findings were confirmed by Treadwell, Flick, and Vahouny (3, 4). These authors (4) claimed that the degree of fatty infiltration which occurred at 25° in rats on a choline-deficient diet was lowered when the rats were transferred to a temperature of 1°. These findings suggest that the choline requirements of the rat are dependent on environmental temperature.

The present study was undertaken to see if the previous work could be extended by determining whether higher temperatures create a greater demand for choline in rats.

METHODS

Rats

The rats used in the experiments were male, white albinos originating from the Wistar strain, and inbred for more than 20 years at the Department of Physiology, University of Queensland, Brisbane. They were 4–6 weeks old and weighed between 50 and 100 g.

Diet

The hypolipotropic diet was based on the series II diet of Best, Lucas, Ridout, and Patterson (5). It consisted of casein (fat- and vitamin-free) 8%; gelatin, 12%; beef dripping, 10%; peanut oil, 2%; sucrose, 62%; salt mixture [Hubbell, Mendel, and Wakeman (6)], 4%; vitamin powder, 1%; vitamin A and D concentrate, 1%. The vitamin powder contained: thiamine hydrochloride, 50 mg; riboflavin, 25 mg; pyridoxine, 20 mg; calcium pantothenate, 90 mg; nicotinamide, 100 mg; folic acid, 5 mg; biotin, 5 mg; vitamin B_{12}, 75 μg; 2-methyl-1,4-naphthoquinone, 10 mg; *p*-aminobenzoic acid, 1 g; α-tocopherol, 250 mg; inositol, 5 g; made up to 100 g with fine sucrose.

The vitamin concentrate [Vetemul, Nicholas (Australia) Pty., Ltd.] contained 5,000 I.U. of vitamin A and 500 I.U. of vitamin D_3 per gram. When choline supplements were given, they were added at a level of 0.23 g of choline chloride per 100 g of diet, which provides 200 mg of choline per 100 g of diet.

Food and water were given ad libitum.

Treatment

Groups of 4–8 rats were maintained at 2 ± 2°, 21 ± 2°, and 33 ± 2° for 3 weeks. In Experiment 4 two additional temperatures, 10 ± 2° and 27 ± 2°, were used. Rats

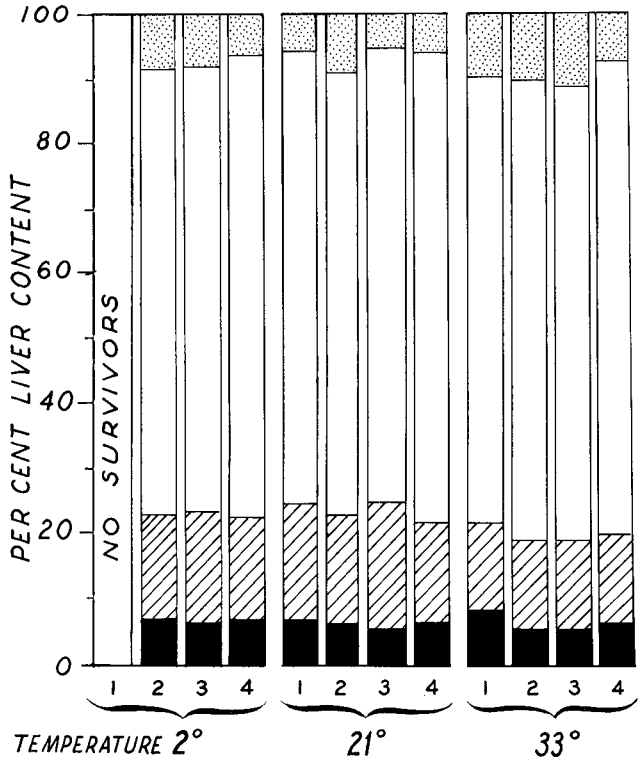

FIG. 1. Composition of liver from rats maintained at 2°, 21°, and 33° and fed a choline-supplemented diet. Dotted area, ash and carbohydrate; solid area, lipid; cross-hatching, protein; blank area, water. Figures at base of histograms refer to the experiment number. The number of rats used at each temperature range were 4 in Experiment *1*; 8 in Experiments *2* and *3*; and 6 in Experiment *4*.

were kept in individual metal cages and fed and weighed every 2nd day for 3 weeks. They were then killed without previous fasting and the livers removed for the determination of water, lipid, and nitrogen.

In the first experiment all rats kept at 2° died, so for subsequent experiments, rats used at 2° were first kept at 14° for 7–14 days before the experiment. In addition rats kept at 2° and 10° were given a block of wood on the floor of the cage to minimize heat loss by conduction. These procedures increased the survival rate of rats at 2° to 62–100%.

The colony from which the rats were obtained was normally fed a commercial poultry diet (Turkey-Growing Cubes, Barnes Milling, Brisbane, Australia) which gives good growth and breeding performance in rats.

Analyses

On removal, the livers were blotted with tissue paper, weighed, cut up, and dried over P_2O_5 in a vacuum oven at 90°. Lipid was determined gravimetrically after Soxhlet extraction with chloroform–methanol 2:1. Nitrogen was estimated by the micro-Kjeldahl method (7) in 100–150 mg portions of the dry, lipid-free residue. The protein content was then derived by multiplying the total nitrogen content of the liver by 6.25.

The remaining portion of the liver after subtracting the weight of water, lipid, and protein was regarded as ash and carbohydrate.

RESULTS

Figures 1 and 2 show the composition of rat livers in terms of wet weight when choline-supplemented and choline-deficient diets were fed to rats kept at various temperatures.

The water fraction of the liver in rats fed the choline-supplemented diet was between 68 and 71% at all temperatures. In choline-deficient groups the water content was between 52 and 67%. When there was a large accumulation of lipid, the proportion of water was lowered.

The lipid fraction of the liver in choline-supplemented groups was 5.5–7.5% at all temperatures investigated. In choline-deficient groups this invariably increased to 10–34%. Analysis of variance showed no significant temperature dependence of liver lipid concentration, but the effect of diet was highly significant ($P < 0.001$).

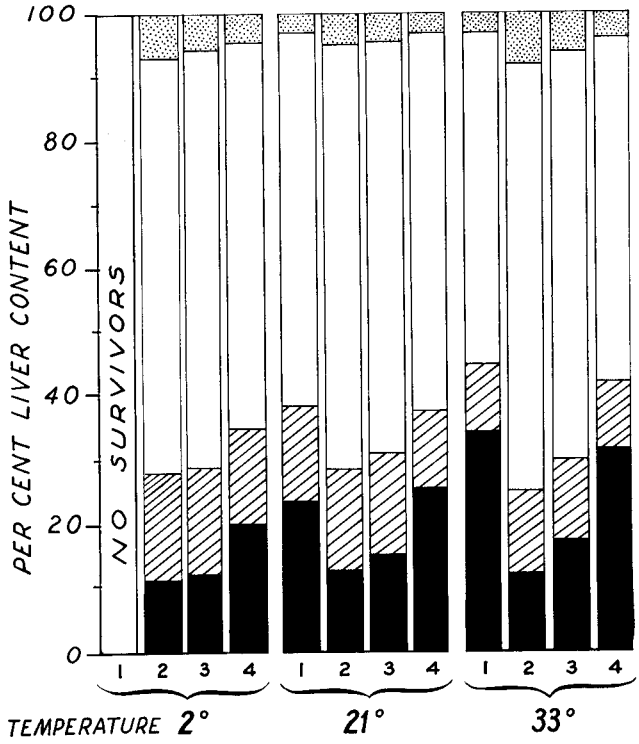

FIG. 2. Composition of liver from rats maintained at 2°, 21°, and 33° and fed a choline-deficient diet. Dotted area, ash and carbohydrate; solid area, lipid; cross-hatching, protein; blank area, water. Figures at base of histograms refer to the experiment number. The number of rats used at each temperature range were 4 in Experiment *1;* 8 in Experiments *2* and *3*; and 6 in Experiment *4*.

TABLE 1 EFFECTS ON RAT LIVER OF TEMPERATURE AND CHOLINE SUPPLEMENTATION OR DEFICIENCY IN DIET

Expt. No.	Temp.	Choline Supplement*	No. of Rats†	Increase in Body Wt.	Total Food Intake	Wet Liver Wt.	Liver Lipid	Lipid: Wet Wt. Liver	Ratio‡
	°C			g	g	g	mg	%	
1	2 ± 2	+	4 (4)	—	—	—	—	—	—
		−	4 (4)	—	—	—	—	—	—
	21 ± 2	+	4	32 ± 6	206 ± 17	4.345 ± 0.239	284 ± 32	6.53 ± 0.45	1.38 ± 0.11
		−	4	8 ∓ 11	168 ± 26	4.471 ± 0.983	1100 ± 504	23.62 ± 6.45	5.49 ± 1.40
	33 ± 2	+	4	12 ± 6	130 ± 28	3.220 ± 0.328	256 ± 19	8.04 ± 1.24	2.02 ± 0.41
		−	4	2 ± 6	108 ± 8	3.868 ± 0.890	1396 ± 727	34.06 ± 10.50	12.66 ± 6.37
2	2 ± 2	+	8 (2)	13 ± 6	282 ± 25	4.475 ± 0.107	298 ± 24	6.69 ± 0.60	1.06 ± 0.13
		−	8 (3)	11 ± 8	273 ± 19	4.695 ± 0.934	515 ± 228	10.65 ± 3.69	1.88 ± 0.83
	21 ± 2	+	8 (1)	9 ± 7	147 ± 20	2.984 ± 0.389	169 ± 36	5.76 ± 0.25	1.16 ± 0.26
		−	8	8 ± 3	136 ± 26	2.891 ± 0.892	392 ± 270	12.31 ± 5.17	2.71 ± 1.51
	33 ± 2	+	8	−6 ± 10	77 ± 13	1.974 ± 0.418	115 ± 35	5.85 ± 1.49	1.50 ± 0.42
		−	8 (1)	−4 ± 5	77 ± 9	2.286 ± 0.534	283 ± 128	11.99 ± 4.36	3.63 ± 1.67
3	2 ± 2	+	8	18 ± 10	232 ± 22	4.409 ± 0.580	274 ± 46	6.20 ± 0.28	1.18 ± 0.45
		−	8 (3)	11 ± 10	251 ± 23	4.938 ± 0.965	636 ± 456	12.01 ± 5.76	2.46 ± 0.16
	21 ± 2	+	8	10 ± 7	169 ± 26	3.863 ± 0.409	226 ± 13	5.86 ± 0.58	1.33 ± 0.68
		−	8 (1)	16 ± 10	178 ± 23	4.591 ± 0.629	707 ± 374	14.93 ± 6.18	3.47 ± 2.40
	33 ± 2	+	8 (2)	−3 ± 7	112 ± 16	3.123 ± 0.495	172 ± 28	5.53 ± 0.43	1.55 ± 0.23
		−	8 (1)	−8 ± 9	95 ± 10	3.591 ± 0.424	641 ± 412	17.16 ± 9.45	5.18 ± 3.81
4	2 ± 2	+	6 (1)	32 ± 10	264 ± 17	5.636 ± 0.644	402 ± 70	7.12 ± 0.65	1.52 ± 0.16
		−	6 (2)	30 ± 2	265 ± 5	7.463 ± 1.717	1616 ± 1154	19.92 ± 10.76	6.06 ± 4.27
	10 ± 2	+	6	32 ± 4	242 ± 24	5.036 ± 0.799	326 ± 16	6.49 ± 0.54	1.34 ± 0.13
		−	6 (1)	19 ± 11	239 ± 42	6.831 ± 3.194	1880 ± 1109	25.17 ± 8.97	7.43 ± 3.65
	21 ± 2	+	6	30 ± 13	194 ± 43	4.898 ± 1.311	310 ± 74	6.38 ± 0.58	1.60 ± 0.16
		−	6	18 ± 9	162 ± 13	5.652 ± 1.022	1431 ± 405	30.26 ± 5.39	9.02 ± 2.90
	27 ± 2	+	6	17 ± 7	146 ± 18	3.916 ± 0.664	271 ± 34	6.96 ± 0.55	1.87 ± 0.29
		−	6	6 ± 11	129 ± 32	5.856 ± 1.121	1794 ± 516	30.19 ± 1.32	13.87 ± 2.90
	33 ± 2	+	6	9 ± 9	105 ± 20	3.663 ± 0.999	230 ± 32	6.71 ± 2.32	2.25 ± 0.48
		−	6 (1)	0 ± 5	92 ± 9	5.426 ± 0.951	1740 ± 410	32.12 ± 5.87	18.99 ± 4.62

* Male 50–100 g rats at various temperatures were fed a choline-deficient diet, or the diet supplemented with 20 mg of choline per 10 g diet, for 3 weeks. Experimental results given as mean ±SD.

† The numbers in parentheses show the number of rats that died during the experiment.

‡ The ratio refers to the ratio of liver lipids in milligrams to the total food intake in grams during the 21 days of the experiment.

The liver lipid in choline-supplemented rats at 2° was 6.2–7.1%. In the choline-deficient groups at 2°, the mean liver lipid was consistently greater and all rats showed some degree of fatty liver. The concentrations of liver lipids from rats at 2° in Experiments 2, 3, and 4 have been pooled and a "*t*" test performed between the results for choline-supplemented and choline-deficient groups. The difference seen in the results is highly significant, $P < 0.001$.

In both the choline-supplemented and choline-deficient groups it was found that the percentage of protein in the liver was lower in rats kept at 33° than at 2° or 21°.

In Table 1 a comparison is made between the food intake and liver lipid. It may be seen that rats at 2° ate 1.5–2 times more food than those at 21°, and 2.5–3.5 times more than those at 33°. Weight increases were found in all rats kept at temperatures below 33° but at 33° weight gains did not always occur.

The livers were usually heavier and the amount of lipid was usually greater in the choline-deficient rats at any particular temperature. The ratio, liver lipid (milligrams) to total food intake (grams) was 1.38–2.25 in all rats fed choline in the diet. When choline was withheld from the diet this ratio was greater, 1.88–18.99, and was directly proportional to temperature (see also Fig. 3).

DISCUSSION

Since Sellers and You (1) reported their findings, considerable interest has been aroused by metabolic studies in the cold. In contradiction to earlier experiments (1–4), our work shows that fatty livers can be induced in rats kept at 2° on a choline-deficient diet. We think it unlikely that the age of the rats used was responsible for this difference in results. The temperature ranges were the same and heat conduction was minimized as suggested by Treadwell et al. (3). The strain of rats used appeared to be less able to survive in the cold than those of the previous workers, e.g., Sellers and You (1, 2).

The hypolipotropic diet used by Sellers and You (1, 2) provided greater amounts of the individual amino acids than ours, although the methionine content was comparable. However, Treadwell et al. (3) used diets which were both lower and higher in amino acid content

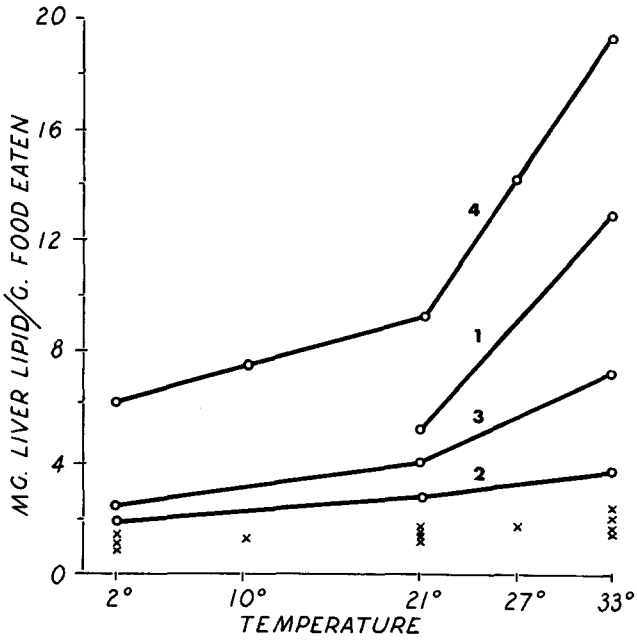

FIG. 3. The ratio, milligrams liver lipid/grams food eaten, varies with temperature when choline-deficient diets are fed to rats. On choline-supplemented diets this ratio remains constant. O—O, choline-deficient groups; *x*, choline-supplemented groups. The numbers *1–4* refer to the number of the experiment.

than those of Sellers and You (1, 2). Best et al. (5) have stated that the type of diet used in the present experiments was deficient in several essential amino acids, but the work of Cuthbertson (8) indicates that it was inadequate only in methionine and histidine. The inadequacy of these amino acids in the diet used in the present experiments should not be critical as Treadwell et al. (3) used diets containing 5 or 10% casein as against our source of 8% casein and 12% gelatin. None of the previous workers has used diets to which choline supplements were added.

Radomski and Wood (9) have recently reported that rats maintained at 2° and fed a choline-deficient diet will develop a fatty liver when the methionine intake is less than 60 mg./day. Our own work is in agreement with this observation. The findings of Sellers and You (2) also appear to be in agreement but not those of Treadwell et al. (3), for in those diets where 5 or 10% casein was the protein source, rats at 2° did not develop fatty livers despite severe restrictions of methionine intake.

It was found that the amount of dry, lipid-free residue of liver from rats at 2° was significantly greater than from those kept at 21°. Sellers and You (1) made a similar comment for their choline-deficient rats. This change may be explained in part by the lowered protein concentration of the liver with increasing environmental temperature.

The explanation offered by Treadwell et al. (3) that a lowered protein efficiency ratio (increase in body weight per gram of protein intake) was responsible for lipotropic action in the cold was based on a study with only choline-deficient rats. In the present experiments this ratio, calculated for both choline-supplemented and choline-deficient rats, varied considerably and apparently at random (see Table 2). In most cases the ratio was lower for the choline-deficient rats than for the supplemented ones. The abnormal accumulation of lipid in the liver appeared to be great even when this ratio was very low, as was seen in the choline-deficient groups at 33° in Experiments 1 and 4. We conclude that there is little correlation between this ratio and liver lipids under our experimental conditions.

The percentage of lipid content at the different temperatures was not significantly different amongst the choline-deficient groups despite the great variation of food intake at the various temperatures. Best and Ridout (10) showed that deposition of lipid in the liver was, within limits, proportional to calorie intake on choline deficient diets and on severe restriction of calorie intake the accumulation of liver lipid was reduced. In our experiments it would be expected that rats at 2° should accumulate the greatest amount of liver lipid since they consumed the greatest quantity of food. Sellers and You (1, 2) have also expressed this view, but because of the increased metabolic rate and calorie expenditure at low environmental temperature this connection between calorie intake and liver lipid accumulation may not be justified. The importance of food intake and lipid content may be assessed in another way. The present results show that the ratio, total liver lipid to total food intake, is low and does not show significant variation with environmental temperature when rats were fed choline in the diet. In rats receiving the choline-deficient diet this ratio was greater and varied directly with temperature. Data from Experiment 4, where rats were kept at five temperature ranges, emphasize this. In Fig. 3 this ratio is presented in a simplified form as the weight of liver lipid

TABLE 2 PROTEIN EFFICIENCY RATIO* FOR RATS AT 2°, 21°, AND 33° WHEN FED CHOLINE-SUPPLEMENTED AND CHOLINE-DEFICIENT DIETS

Expt. No.	Choline Supplement	2°	21°	33°
1	+	No survivors	0.78	0.50
	−		0.24	0.07
2	+	0.23	0.29	−0.38
	−	0.18	0.28	−0.29
3	+	0.37	0.28	−0.13
	−	0.22	0.46	−0.40
4	+	0.61	0.77	0.45
	−	0.56	0.56	0.02

* Increase in body wt. per gram of protein intake, as given by Treadwell et al. (3). No correlation to liver lipid concentration with choline deficiency and temperature is evident.

per gram of food eaten for both choline-supplemented and choline-deficient rats in the various experiments. Although the value of this ratio has varied in rats receiving the choline-deficient diet in the different experiments, the same temperature-dependent trend was observed in each experiment.

This ratio emphasizes the observation that rats maintained (at different environmental temperatures) on a choline-deficient diet develop fatty livers. It amplifies the finding that low environmental temperatures demonstrate some lipotropic effect and higher temperatures tend to increase the severity of fatty infiltration of the liver. The variation in lipotropic efficiency in choline-deficient rats at the different temperatures may be due to changes in the endogenous synthesis of choline or the efficiency of its utilization.

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