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Influence of 1,3-Butanediol on Tissue Lipids of Cold-exposed Rats

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ABSTRACT The influence of feeding the polyhydric alcohol 1,3-butanediol (BD), on tissue lipids of normal and cold-exposed rats was investigated. The addition of 20% BD to a 30% fat diet lowered adipose tissue lipids with a concomitant elevation of liver lipids at either normal or cold environments. Feeding a 30% fat diet to rats for 2 weeks and then exposing them to moderate cold of 5°, or severe cold -10° without food for 72 hours produced decreases in the total lipid content of epididymal adipose tissue, liver, and muscle. Rats fed a similar diet, without cold exposure but also starved for 72 hours, showed no decrease in the total quantity of adipose tissue lipids.

The use of polyhydric alcohols as a potential new source of dietary energy was first proposed by Schlüssel (1, 2). Dymrza and Miller² showed that one of these alcohols, 1,3-butanediol, contains approximately 6 kcal/g of metabolizable energy and can be utilized by the rat when fed at levels up to 20% of the diet. Miller et al.³ have presented evidence that this compound is metabolized via carbohydrate pathways.

To observe further the utilization and metabolic effects of 1,3-butanediol when fed as a carbohydrate replacement in high energy diets, studies were conducted on rats fed this compound under the stress of a cold environment. Such studies were thought to be valuable because animals exposed to cold exhibit a marked increase in food consumption, presumably to meet their elevated caloric demands (3) due to an accelerated metabolism (4). In addition, numerous tissue lipid changes occur in rats subject to a cold environment (4, 5), and cold acclimation has a pronounced action on hepatic lipid metabolism (6-9).

The present paper demonstrates that lipid changes occur in the hepatic, muscle, and adipose tissues of rats fed 1,3-butanediol and exposed to a moderate (5°) or severe (-10°) cold environment.

EXPERIMENTAL METHODS

Male rats of the Sprague-Dawley strain were used in 2 experiments. There were

3 dietary treatments, 10% fat, 30% fat, and 30% fat plus 20% 1,3-butanediol (BD). The composition of the 3 diets is presented in table 1. The rats were housed in a thermostatically controlled room at 25 ± 2° followed by exposure in a cold room maintained at 5° ± 1° or -10° ± 1°. Meshed rubber laboratory mats were used to cover the floor bottoms of the cages in the cold rooms.

Total lipids were determined in liver, muscle and adipose tissue by the methods described by Therriault and Poe (5). In this procedure a chloroform-methanol extraction of the tissues is followed by a separation of the non-lipid components, namely, peptides, carbohydrates, purines, etc., by means of partition chromatography on Sephadex G-25⁴ columns.

Experiment 1. Effect of short-term cold and starvation. A total of 66 rats with an average body weight of 246 g were divided equally into 3 groups of 22 rats/group and placed on each of the 3 dietary treatments. These animals were fed ad libitum for 2 weeks. Then, 15 animals from each dietary treatment were divided equally into 3 subgroups of 5 animals/group and ex-

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² Dymrza, H. A., and S. A. Miller 1963 Utilization of 1,3-butanediol as a synthetic source of dietary energy. Proc. 6th International Congress of Nutrition, p. 498 (abstract).

³ Miller, S. A., A. Browning and P. Turransky 1965 The metabolism of 1,3-butanediol by the rat. Federation Proc., 24: 439 (abstract).

⁴ A. B. Pharmacia, Uppsala, Sweden.

TABLE 1
Experimental diets

	(1) 10% Fat	(2) 30% Fat + BD ²	(3) 30% Fat
	%	%	%
Casein	22.0	22.0	22.0
Glucose monohydrate	19.6	2.9	12.9
Sucrose	19.6	2.9	12.9
Dextrin ¹	19.6	3.0	13.0
Lard	7.5	22.5	22.5
Corn oil	2.5	7.5	7.5
1,3-Butanediol ²	—	20.0	—
Cellulose	—	10.0	—
Mineral mix ³	4.0	4.0	4.0
Vitamin mix ⁴	1.0	1.0	1.0
Choline chloride (50%)	0.2	0.2	0.2
Agar ⁵	4.0	4.0	4.0
Kilocalories/g	4.1	5.1	5.1

¹ Dextrin no. 7082; Corn Products Company, New York.

² 1,3-Butanediol; Celanese Corporation, New York.

³ Mineral mix contained: (in grams/kilogram of mix) CaCO₃, 292.9; KH₂PO₄, 343.1; NaCl, 250.6; MgSO₄·7H₂O, 99.8; CaHPO₄·2H₂O, 4.295; ferric citrate, 6.223; CuSO₄, 1.558; MnSO₄·H₂O, 1.209; ZnCl₂, 0.200; KI, 0.005; (NH₄)₆Mo₇O₂₄·4H₂O, 0.025; and Na₂SeO₄, 0.015.

⁴ Vitamin mix contained (in IU/kilogram) vitamin A, 5,000; vitamin D, 500; *dl*- α -tocopheryl acetate, 100; and (in milligrams/kilogram) menadione, 5; thiamine·HCl, 10; riboflavin, 20; niacin, 50; ascorbic acid, 200; pyridoxine·HCl, 10; *p*-aminobenzoic acid, 100; biotin, 0.5; Ca pantothenate, 50; folic acid, 2; inositol, 200; and vitamin B₁₂, 0.05.

⁵ USP Flakes, Penick and Company, New York; hot distilled water was added 1:1 to diet (agar-gel type diet, Miller and Allison (10)).

posed to temperatures of either 25°, 5°, or -10° without food for 72 hours. Five control rats within each dietary treatment were not starved and remained at the normal 25° environment. After this 72-hour period, all animals were killed, and total lipids determined on epididymal adipose tissue, gastrocnemius muscle, and liver.

Experiment 2. Effect of cold alone. Thirty rats with an average body weight of 110 g were divided equally into 3 groups of 10 animals/group and placed on each of the 3 dietary treatments. Animals on treatment 1 (10% fat) were fed ad libitum, whereas the rats consuming the diets of treatment 2 (30% fat + BD) and 3 (30% fat) were isocalorically and isonitrogenously pair-fed by substitution of BD and cellulose for total carbohydrates on an equivalent caloric basis (table 1). A 2-week feeding period at 25° was followed by a second 2-week feeding period at 5°. After this 4-week period, 5 animals/treatment were killed for lipid analysis of epididymal adipose tissue and liver.

Sixteen rats with an average body weight of 200 g were fed each of the 3 dietary treatments ad libitum for 2 weeks, followed by a one-week ad libitum feeding at the severe cold temperature of -10°. Five rats from each treatment were then killed for lipid analysis of epididymal adipose tissue and liver.

RESULTS AND DISCUSSION

Experiment 1. Effect of short-term cold and starvation. Tissue weights and tissue lipid analyses of rats exposed to the 3 environmental temperatures are presented in tables 2 and 3, respectively. The liver and adipose tissue weights decreased when the rats were under the dual stress of acute cold exposure and 72-hour food deprivation. The adipose tissue weights and the adipose tissue lipids of the rats consuming the diet containing BD (treatment 2) were significantly decreased, in each environmental temperature, as compared with the tissues from the rats consuming the 30% fat unsupplemented diet (treatment 3). Fasting alone for 3 days did not affect the adipose tissue lipids of the rats within each dietary treatment, but the superimposition of cold stress caused a lowering of the epididymal fat.

The muscle lipids of the rats on all dietary treatments and the liver lipids of the rats fed the 30% fat diet, with or without BD, decreased during the 3-day starvation plus cold exposure. In contrast, the liver lipids of the rats fed the 10% fat diet did not decrease when the animals were subjected either to the cold environment, or to starvation, or to both.

Fasting for 3 days within the 5° cold environment produced a mean weight loss of 72.8 g in the rats fed the 10% fat diet and a similar mean weight loss of 71.3 g in the rats fed the 30% fat + BD diet. Rats fed the 30% fat diet in this environment showed an average weight loss of only 62.8 g, which was significantly less ($P < 0.05$) than the mean weight loss of the other 2 groups.

Experiment 2. Effect of cold alone. The weight gains and the total calories consumed of the young rats fed for successive 2-week periods at 25° and 5° are pre-

TABLE 2

Liver and epididymal adipose tissue weights of rats under starvation and cold (exp. 1)

Treatment	25°, starved 72 hr		5°, starved 72 hr		-10°, starved 72 hr	
	Liver	Adipose tissue ¹	Liver	Adipose tissue	Liver	Adipose tissue
(1) 10% Fat	12.16 ± 0.54 ^a	3.70 ± 0.29 ^{wv}	7.42 ± 0.25 ^{ede}	3.13 ± 0.33 ^{uvv}	7.80 ± 0.34 ^{ed}	1.71 ± 0.50 ^y
(2) 30% Fat+ 1,3-butanediol	10.94 ± 0.33 ^{ab}	2.81 ± 0.20 ^{wvx}	8.06 ± 0.83 ^{ed}	2.73 ± 0.79 ^{wx}	6.17 ± 0.32 ^{ner}	1.42 ± 0.33 ^z
(3) 30% Fat	11.55 ± 0.35 ^a	3.99 ± 0.30 ^u	8.20 ± 0.27 ^e	3.74 ± 0.17 ^{uv}	9.93 ± 1.09 ^b	2.63 ± 0.31 ^{wxy}

¹ Dry weight.² Mean ± se. Means having common letter in superscript are not significantly different ($P > 0.05$).

TABLE 3

Tissue lipid changes of rats under starvation and cold (exp. 1)

Treatment	25°		25°, starved 72 hr		5°, starved 72 hr		-10°, starved 72 hr	
	Adipose tissue	Liver	Muscle	Adipose tissue	Liver	Adipose tissue	Muscle	Adipose tissue
(1) 10% Fat	1.17 ± 0.09 ¹	4.56 ± 0.30	3.57 ± 0.15	1.06 ± 0.09	5.96 ± 0.44	2.50 ± 0.11	0.67 ± 0.22	4.84 ± 0.28
(2) 30% Fat+ 1,3-butanediol	0.89 ± 0.05	8.06 ± 0.59	2.94 ± 0.10	0.93 ± 0.22	5.66 ± 0.25	2.50 ± 0.24	0.55 ± 0.13	5.61 ± 0.19
(3) 30% Fat	1.28 ± 0.10	6.89 ± 0.38	3.22 ± 0.26	1.23 ± 0.04	6.62 ± 0.35	2.36 ± 0.22	0.98 ± 0.13	4.41 ± 0.32

¹ Mean ± se.

sented in table 4. The rats pair-fed the 30% fat diets with and without BD (treatments 2 and 3) had statistically similar weight gains within the 2 environments. These pair-fed groups had a 28% increased food intake in the cold, whereas the ad libitum-fed rats (10% fat, treatment 1) increased their food intake by 41% in the 5° environment.

Table 5 shows that feeding the 30% fat + BD diet (treatment 2) to the young rats for 2 weeks at 5° after a preliminary 2-week feeding period at 25° resulted in a significantly lowered ($P < 0.05$) amount of adipose tissue lipids per 100 g body weight as compared with the isocalorically pair-fed rats receiving the 30% fat diet (treatment 3). The amount of epididymal adipose tissue lipids per 100 g body weight in the rats fed 30% fat + BD was similar to that of the rats fed the 10% fat diet ad libitum. However, the liver lipids of the rats fed the 30% fat + BD were significantly higher at 5°, or at -10°, as compared with the liver lipids of the rats pair-fed the 30% fat diet in these same environments.

The results of these experiments indicate that the addition of 20% 1,3-butanediol in the diet exerts an effect on lipid

metabolism in the rat. At a normal environment (25°) or at a moderately cold environment (5°), adipose tissue lipids were lowered. There was also a concomitant increase in the amount of total liver lipids in animals fed diets containing BD. Investigations in progress in our laboratory indicate that triglycerides and cholesterol are the lipid fractions that are increased in the livers of rats fed BD. It is also apparent from these studies that the physiological stress conditions of starvation and cold mediate a response of lipid metabolism in different ways. Starvation for 3 days alone does not affect the quantity of adipose tissue lipids. However, cold exposure of either starved or fed animals causes a marked decrease in the adipose tissue lipids.

The use of 1,3-butanediol and other synthetic, non-toxic, relatively energy-rich compounds as food sources may be of future importance. These data indicate that studies in lipid metabolism appear necessary in understanding the utilization of these supplements.

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TABLE 4
Average two-week weight gains and energy intake of rats at 25° and 5° (exp. 2)

Treatment	25°		5°	
	Wt gain	Energy intake	Wt gain	Energy intake
	g	total kcal	g	total kcal
(1) 10% Fat	89.4 ± 2.3 ^a	951	53.7 ± 3.9 ^c	1341
(2) 30% Fat + 1,3-butanediol	76.8 ± 4.4 ^b	964	38.0 ± 5.3 ^d	1234
(3) 30% Fat ²	84.9 ± 4.5 ^{ab}	974	32.1 ± 3.7 ^d	1250

¹ Mean ± SE. Means having common letter in superscript are not significantly different ($P > 0.05$).

² Pair-fed to treatment (2).

TABLE 5
Epididymal adipose tissue and liver lipids of rats maintained at 5° and -10° exposure (exp. 2)

Treatment	5°, 2-week exposure		-10°, 1-week exposure	
	Adipose tissue	Liver	Adipose tissue	Liver
	mg lipid/100 g body wt	%	mg lipid/100 g body wt	%
(1) 10% Fat	623.0 ± 31.8 ^a	5.07 ± 0.33 ^x	652.2 ± 68.5 ^a	5.52 ± 0.26 ^y
(2) 30% Fat + 1,3-butanediol	643.3 ± 25.3 ^a	8.04 ± 0.72 ^z	609.9 ± 84.3 ^a	6.13 ± 0.12 ^y
(3) 30% Fat	904.5 ± 53.2 ^b	6.18 ± 0.26 ^y	737.3 ± 40.4 ^a	4.99 ± 0.09 ^x

¹ Mean ± SE. Means having common letter in superscript are not significantly different ($P > 0.05$).

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