

Influence of Dietary 1,3-Butanediol on Blood Ketone Levels and on Glucose Metabolism in the Dog¹ (39892)

DALE R. ROMSOS AND GILBERT A. LEVEILLE

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

The importance of β -hydroxybutyrate and acetoacetate in cerebral metabolism of fasting humans has recently been further clarified (1-4). The brain, a major utilizer of glucose in normal postabsorptive man, utilizes increasing quantities of ketones as the fast progresses and circulating ketone levels increase. Thus, ketones replace glucose as the major oxidative fuel for the brain during a prolonged fast, which in turn contributes to body protein conservation by reducing the need for gluconeogenesis.

The dog, unlike the human, is relatively resistant to fasting ketosis (5, 6). However, it has been demonstrated that the brain of the dog can utilize ketones, provided the circulating levels are sufficiently elevated (6, 7), 1,3-Butanediol (BD) is converted to β -hydroxybutyrate and is well utilized as a source of energy in rats, chickens, and pigs (8-10). Thus, consumption of diets containing BD might elevate circulating ketone levels in dogs, thereby sparing glucose.

The purpose of this study was to examine the influence of dietary BD on β -hydroxybutyrate and acetoacetate levels in the adult dog and to investigate the effect of dietary BD on glucose utilization in this species. The dogs were fed carbohydrate-free diets to maximize the need for *de novo* glucose synthesis.

Materials and methods. Experimental animals and diet. Two-year old female beagle dogs² were housed in individual

cages with raised floors. Lights in the temperature controlled (22-23°) room were on from 7 AM to 7 PM and off from 7 PM to 7 AM. Prior to the present study, dogs were fed approximately 750 kcal of a carbohydrate-free diet daily for 60 days. The composition of this nutritionally adequate canned diet (11) has been described previously (Table 1, Diet 6 in Ref. 12). This carbohydrate-free diet contained 1.26 kcal/g and 48 and 52% of energy from protein and fat, respectively. The dogs approximately maintained their body weight during this period.

In the present study, dogs were fed 750 kcal/day; 600 kcal of the carbohydrate-free diet and 150 kcal of 1,3-butanediol³ (BD) or 150 kcal of tallow.⁴ BD was assumed to contain 6.5 kcal/g (13) and tallow was assumed to contain 9 kcal/g (11). Dogs were fed twice per day (8 AM and 5 PM) and they generally consumed their food within several minutes. Water was available *ad libitum*. Water intake was recorded for a 5-day period during the second week of the study.

Blood samples. On the 14th day of the study jugular blood samples (10 ml) were obtained just before, and 1, 3, and 5 hr after the AM meal. Blood levels of β -hydroxybutyrate (14) and acetoacetate (15) were determined by standard enzymatic procedures.

Glucose metabolism. On the 18th day of the study estimates of glucose metabolism were obtained in the dogs following a single injection of [$6\text{-}^3\text{H}$]glucose⁵ (175 μCi) and [$\text{U-}^{14}\text{C}$]glucose⁵ (15 μCi). Indwelling cathe-

¹ Supported in part by grants in aid from NIH AM 18957, by the General Foods Corporation, by the Quaker Oats Company, and by a Research Career Development Award NIH AM 00112 to D.R.R. Michigan Agricultural Experiment Station Journal Article No. 7798.

² Laboratory Research Enterprises, Inc., Kalamazoo, Michigan.

³ Generously supplied by Norman Baker, Celanese Corp., New York, New York.

⁴ Generously supplied by Oscar Mayer & Co., Madison, Wisconsin.

⁵ Amersham/Searle Corp., Arlington Heights, Illinois.

ters⁶ were inserted in the jugular vein 2 hr prior to the AM meal. One-half hour after the meal, dogs were injected with the tracer. Blood sampling procedures and methods for determining plasma glucose-specific radioactivity have been presented (16). The glucose replacement rate, transit time, total body glucose mass, and glucose-carbon recycling were calculated graphically according to Katz *et al.* (17, 18).

Statistical analysis. The data were treated statistically by Student's *t* test (19).

Results and Discussion. Dogs were fed a carbohydrate-free diet which contained 20% of metabolizable energy from added BD or from added tallow. Consumption of the 750-kcal diet containing BD resulted in a loss of 40 g of body weight per dog during the 3-week study, whereas dogs fed the diet containing added tallow gained 200 g body weight (Table I). All dogs fed BD lost weight and all dogs fed tallow gained weight. However, these small differences in body weight change should not be construed as evidence for inefficient utilization of BD in the dog. Dogs fed tallow initially weighed slightly less than dogs fed BD; consequently, energy intake of dogs fed tallow was slightly greater per unit weight than was the energy intake of dogs fed BD. Dogs fed BD also consumed nearly twice the quantity of water consumed by the other dogs (Table I). Similar results have been reported for the rat (8). The small differences in body weight observed might reflect differences in water balance of the dogs. Alternatively, the metabolizable energy value assigned to BD, which was determined in another species (13), might not be appropriate for the dog. Young rats, pigs, and chickens fed diets containing 20% of their energy in BD gain weight at a rate equal to animals fed control diets (8-10), suggesting that dietary BD is well utilized as a source of energy in these species. Although additional studies are needed, the present study similarly suggests that dietary BD is well utilized by the dog.

Dogs are relatively resistant to fasting ketosis (5, 6). Prior to the meal, circulating

TABLE I. INFLUENCE OF DIETARY 1,3-BUTANEDIOL ON BODY WEIGHT, WATER INTAKE, AND GLUCOSE METABOLISM IN THE DOG^a.

	Diet	
	Tallow	Butanediol
Initial body weight (kg)	8.66 ± 0.33	9.23 ± 0.27
Body weight change (kg)	+0.20 ± 0.03	-0.04 ± 0.01 ^b
Water intake (ml/day)	117 ± 25	200 ± 29 ^b
Plasma glucose (mg/100 ml)	92 ± 2	80 ± 2 ^b
Glucose replacement rate (mg/min/kg)		
[6- ³ H]glucose	4.93 ± 0.44	4.89 ± 0.26
[U- ¹⁴ C]glucose	3.88 ± 0.37	3.93 ± 0.23
Glucose-carbon recycling (%)	21 ± 3	20 ± 2
Glucose transit time (min)		
[6- ³ H]glucose	50 ± 4	50 ± 2
[U- ¹⁴ C]glucose	60 ± 3	59 ± 2
Glucose body mass (mg/kg)		
[6- ³ H]glucose	245 ± 23	247 ± 20
[U- ¹⁴ C]glucose	232 ± 23	233 ± 14

^a Results expressed as mean ± SEM for six dogs. Samples were obtained on the 18th day of the study.

^b Significantly different (*P* < 0.05) from values obtained when dogs were fed tallow.

ketone levels in the dogs fed the carbohydrate-free diets averaged approximately 35 μ M (Table II). This value is similar to circulating ketone levels reported in rats (8) and pigs (10) fed high-carbohydrate diets but is considerably lower than that observed in chickens (10) fed high-carbohydrate diets. Consumption of the tallow diet increased ketone levels to 63 μ M 3 hr after the meal (Table II). Acetoacetate levels increased to a greater extent than did β -hydroxybutyrate; thus, the β -hydroxybutyrate/acetoacetate ratio decreased after the meal. These results suggest that in addition to resistance to fasting ketosis the dog is relatively resistant to ketosis when fed a carbohydrate-free diet (20).

Dietary BD increased ketone levels in the dogs (Table II). Prior to the meal, circulating ketone levels were similar in tallow- and BD-fed dogs. Within 1 hr after the BD meal, β -hydroxybutyrate levels were elevated nearly 10-fold and remained elevated

⁶ Becton, Dickinson & Co., Rutherford, New Jersey.

TABLE II. INFLUENCE OF DIETARY 1,3-BUTANEDIOL ON BLOOD LEVELS OF β -HYDROXYBUTYRATE AND ACETOACETATE IN THE DOG^a.

Diet	Hours after meal			
	0	1	3	5
β -Hydroxybutyrate ($\mu\text{m/liter}$)				
Butanediol	24 \pm 2	220 \pm 12 ^b	290 \pm 12 ^b	233 \pm 6 ^b
Tallow	21 \pm 3	—	26 \pm 3 ^c	—
Acetoacetate ($\mu\text{m/liter}$)				
Butanediol	10 \pm 3	59 \pm 3 ^b	81 \pm 6 ^b	78 \pm 3 ^b
Tallow	14 \pm 3	—	37 \pm 2 ^{b,c}	—
β -Hydroxybutyrate/acetoacetate ratio				
Butanediol	2.4 \pm 0.3	3.8 \pm 0.3 ^b	3.7 \pm 0.3 ^b	3.0 \pm 0.1 ^b
Tallow	1.5 \pm 0.2 ^c	—	0.7 \pm 0.05 ^{b,c}	—

^a Results expressed as mean \pm SEM for six dogs. Samples were obtained on the 14th day of the study.

^b Significantly different ($P < 0.05$) from 0-hour value.

^c Significantly different ($P < 0.05$) from values obtained when dogs were fed butanediol.

for another 4 hr. Blood acetoacetate levels were also increased but to a lesser extent. Dogs did not excrete ketones in the urine⁷ when fed BD. The β -hydroxybutyrate/acetoacetate ratio was higher at zero hour in dogs fed BD than in dogs fed tallow. The ratio increased when the dogs consumed the BD meal and remained elevated for the 5-hr period. These results suggest that, as in the rat and chicken (8–10), BD is converted to β -hydroxybutyrate in the dog.

Plasma glucose levels were lower in dogs fed BD than in dogs fed tallow (Table I). Twenty blood samples were obtained from each dog during the 5-hr period after the meal. No differences in plasma glucose levels were observed during this time period. Rats fed approximately 20% BD also had lower plasma glucose levels than control rats (8, 9); however, differences in circulating glucose levels were not observed in chickens or pigs (10) fed BD. The reason(s) for these species-specific responses are not readily apparent.

An hypothesis tested in this study was that dietary BD would increase circulating ketone levels which in turn might alter glucose metabolism (1–4). A carbohydrate-free diet was used; thus, the glucose needs of the dog were supplied entirely through gluconeogenesis. The *in vivo* metabolism of the tracer glucose was not influenced by

consumption of BD (Table I). Rates of [$6\text{-}^3\text{H}$]glucose utilization averaged 4.9 mg/min/kg of body weight in dogs fed the two diets. Likewise, the other parameters of glucose metabolism measured, except for plasma glucose level, were not changed by the diet fed.

The percentage of glucose-carbon recycling, estimated from the difference between the rates of [$6\text{-}^3\text{H}$]- and [$\text{U-}^{14}\text{C}$]glucose, averaged 20–21% in the dogs. Glucose body mass of the dogs was not influenced by the diets fed, even though plasma glucose levels were depressed by BD (Table I). The estimates of glucose mass obtained with [$6\text{-}^3\text{H}$]- and [$\text{U-}^{14}\text{C}$]glucose were in close agreement. Other than the higher rates of glucose utilization observed in the present study with dogs in the fed state, the parameters of glucose metabolism measured were similar to those reported earlier when these dogs had been fed a carbohydrate-containing diet and then fasted for 48 hr (16).

Several possibilities exist for the failure of dietary BD to reduce glucose utilization in the adult dog. The diet contained adequate glucogenic precursors which may have precluded an observable influence of BD on glucose metabolism. Possibly BD might have influenced glucose metabolism had the dogs been fed a diet lower in protein. When ketones are infused into adult dogs circulating levels must be elevated above 1 mM before the brain is able to utilize the ketones

⁷ Labstix reagent sticks. Ames Company, Elkhart, Indiana.

as a source of energy (6, 7). The ketone levels averaged only 0.3 mM in dogs fed 20% of energy as BD. If dietary BD had elevated circulating ketone levels to a greater extent, glucose utilization may have been altered. Unfortunately, the dogs refused to consume diets containing 30–40% of energy as BD. The pig also discriminates against diets containing more than 20% of energy as BD (10). While the levels of circulating ketones observed in the present study are similar to those observed in dogs during an 8-day fast, they are much lower than the 6 mM levels observed in fasting humans (2). However, even a fast lasting 21 days did not elevate circulating ketone levels of adult dogs above 0.6 mM (21).

Summary. Adult dogs were fed carbohydrate-free diets containing 20% of metabolizable energy from 1,3-butanediol (BD) or from tallow. Each dog received a 750-kcal diet each day, of which 290 kcal were in the form of protein; consumption of these diets resulted in only slight changes in body weight during the 3-week experiment. Blood β -hydroxybutyrate and acetoacetate levels were elevated severalfold after dogs consumed a meal containing BD. Parameters of glucose metabolism were estimated after a single injection of [6-³H]- and [U-¹⁴C]glucose. The glucose replacement rate averaged 4.9 mg/min/kg and was not influenced by the diet fed. Likewise, estimates of glucose-carbon recycling and glucose body mass were not altered when BD was fed, although plasma glucose levels in the BD-fed animals were significantly lower. Consumption of a diet containing 20% of energy from BD and adequate amounts of glucogenic precursors increased circulating ketone levels but did not alter glucose metabolism in the adult dog.

The authors wish to acknowledge the technical assistance of Kathleen Muiruri and Linda Brady.

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Received September 27, 1976. P.S.E.B.M. 1977, Vol. 156.