

The Effects of Vitamin B6 Deprivation with 4-Deoxypyridoxine in Meal-Fed Rats (42004)

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Abstract. Weanling male rats were trained to consume a control diet in one 4-hr meal a day. Rats were then fed one of three experimental diets for 7 days: (a) control diet minus B6 (-B6); (b) control diet (B6); or (c) control diet restricted to food intake of -B6 (B6R). Xanthurenic acid excretion was greater before and after a tryptophan load in -B6 than in B6R. Body weight gain, food intake, and food efficiency were not different between -B6 and B6. However, in B6R body weight gain, food intake and food efficiency were lower than that in both -B6 and B6. Serum glucose (12-16 hr after meal) and percentage body fat were similar in all three groups. No differences were found in organ size between the -B6 and both control groups indicating that short-term deprivation and deoxypyridoxine did not affect organ size. No significant differences were observed for serum growth hormone (GH), pituitary GH, serum prolactin (PRL), or pituitary PRL among the three groups. When meal fed, differences were minimized between B6 deprived and unrestricted control (B6) rats in body weight gain, food intake, food efficiency, and body composition. © 1985 Society for Experimental Biology and Medicine.

Laboratory rats fed nutrient deficient diets (1) decrease their food intake and consequently growth slows or weight loss occurs. When compared with rats fed nutritionally complete diets, symptoms of nutrient deprived rats may be due to the decrease in food intake and subsequent weight loss. When control animals are restricted to the amount of food consumed by the deprived animals, the feeding pattern in control animals changes from a "nibbling" to a meal pattern (2, 3). Many studies (3-6) have demonstrated differences in body composition and metabolism associated with these feeding patterns.

In Vitamin B6 deprived rats, food intake decreases, growth rate is depressed, and a decrease in fat accumulation occurs (7). Disturbances of carbohydrate metabolism develop including decreases in fasting blood glucose, lactate, and pyruvate, as well as liver glycogen (8). Decreases in serum insulin and growth hormone have also been reported (9-11) for B6 deprived rats. The purpose of this study was to minimize differences in the feeding pattern in B6 deficient, B6 supplemented, and B6 supplemented but restricted to the food intake of B6 deficient rats, and to determine whether parameters (body composition, carbohydrate metabolism, and

growth hormone) sensitive to feeding pattern are altered.

Methods. Weanling Wistar male rats (Hilltop, Chatsworth, Calif.) were provided with water *ad libitum* and maintained on a 12-hr light/dark cycle. Rats were trained to consume the purified control diet: 20% casein, 54.2% sucrose, 20% corn oil, 2% cellulose, 3.5% salt mix (12), B6 deficient vitamin 0.1% mix (13), and 6-mg pyridoxine/kg diet, during one 4-hr meal per day at the beginning of the dark cycle. After 3 weeks, rats (9-12/group) were then fed one of three experimental diets for 7 days: (a) control diet (B6); (b) control diet minus B6 (-B6); or (c) control diet restricted to amount consumed by -B6 group (B6R). A B6 antagonist, 4-deoxypyridoxine (12 mg/kg), was added to each diet to facilitate the onset of the B6 deficiency. The composition of the diet, specifically the 20% corn oil and deoxypyridoxine, was similar to that used by Beare *et al.* (14). Body weight and food intake were measured daily. Urine was collected for 24 hr on Day 5 before a DL-tryptophan load (1 mg/g body wt) and on Day 6 when tryptophan was added to the food. Xanthurenic acid was determined by the method of Wachstein and Gudaitis (15). Rats were killed by decapitation

and blood was collected and serum was separated. The serum was stored frozen for glucose (16), growth hormone (GH) (17), and prolactin (PRL) (18) assays. Organs were removed and weighed.

Fat cell size and number were determined in the epididymal fat pads by the osmium fixation method of Hirsch and Gallian (19) and aliquots (five) of the fixed cells were counted microscopically. Cell number was calculated from the total pad weight \times cells in the osmium sample/wet weight of the sample for osmium fixation. The percentage lipid of the adipose tissue was determined by extraction (20) of the lipid and then quantitated gravimetrically. The cell size was determined as micrograms of lipid/cell = wet weight of sample in micrograms \times the ratio of lipid to the wet weight/total number of cells in sample. Body composition was determined by drying the carcass for moisture content, grinding and mixing the dried carcass, and taking aliquots for Kjeldahl nitrogen (21), ashing (21), and lipid extraction (20). Statistical differences between treatments were determined by the *t* test (22).

Results. Body weight (Fig. 1) in the three groups was not significantly different at the start of the treatment and no significant

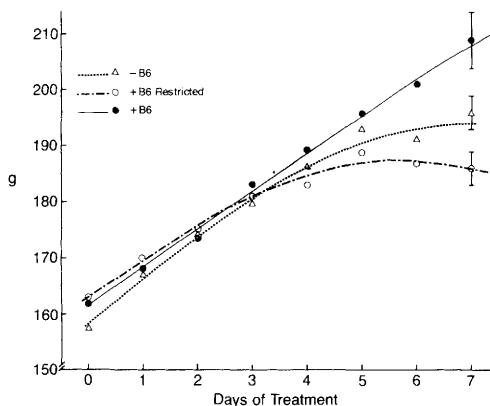


FIG. 1. Growth curve. Body weight (g) during the 7 days of treatment. Each point is the mean of 9-12 rats. The mean on Day 7 includes \pm SE for each group. The SE was similar (for each group) on previous days. Initial body weight of the three groups was not significantly different ($P > 0.05$). Final body weight of the -B6 rats was significantly less ($P < 0.025$) than that of the B6 and significantly greater ($P < 0.025$) than that of B6R.

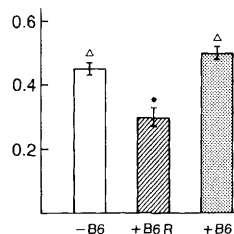


FIG. 2. Food efficiency ratio (g weight gain/g food intake). Bars are the means \pm SE of 9-12 rats. Food efficiency was significantly less ($P < 0.001$) in the B6R group (*) compared to that in the -B6 or +B6 groups (Δ).

differences were observed until Day 7 when body weight of the -B6 rats was significantly less ($P < 0.025$) than that of B6 and significantly greater ($P < 0.025$) than that of B6R. The growth rate in the B6 group did not change with the addition of the deoxyripyridoxine during the 7-day experimental period. The total weight gain for the -B6 and B6 was not significantly different ($P > 0.05$) but the total weight gain for the B6R group was significantly less ($P < 0.05$) than for the -B6 and B6 groups. Food efficiency (Fig. 2) was similar in -B6 and B6, but food efficiency was significantly ($P < 0.001$) less in B6R than in -B6 or B6. The mean daily food intake was not significantly different ($P > 0.05$) between the -B6 and B6R groups except for treatment Day 6 when the B6R group consumed 39% less food than the -B6 group. On Day 6 the tryptophan had been added to the food and apparently had a greater anorexic effect on the B6R group than on the -B6 group. The total food intake/day during the 7-day treatment period was significantly less ($P < 0.05$) in the B6R group (11.2 ± 0.3) than in the -B6 (12.3 ± 0.3) or B6 (13.3 ± 0.4) groups. The decreased food consumption by the B6R group would account for their decreased body weight gain but not decreased food efficiency. Xanthurenic acid excretion was significantly greater in the -B6 group compared to restricted controls (B6R) before ($P < 0.01$) and after ($P < 0.001$) the tryptophan load (Table I), indicating that a deficiency of vitamin B6 was present. Due to decreased food intake by the B6R group, consumption of the tryptophan dose was greater (39%) in the -B6

TABLE I. XANTHURENIC ACID EXCRETION IN B6 SUPPLEMENTED AND DEPRIVED (7 DAYS) MEAL-FED RATS

	Xanthurenic acid (μ mole/24 hr)	
	B6 deprived (-B6)	B6 supplemented (B6R)
Before tryptophan	2.33 \pm 0.60 (7) ^a	0.14 \pm 0.10 (4)
After tryptophan load (1 mg/g body wt) ^b	5.41 \pm 1.96 (6)	0.96 \pm 0.17 (11)

^a Mean \pm SE; (n) = number of rats.

^b Urine collected 24 hr after load.

group than in the B6R group, but xanthurenic acid excretion was 82% greater in the -B6 than in the B6R group.

Body composition was not different among the three groups (Fig. 3). Liver weight and epididymal fat pads (Fig. 4a) were greater in the B6 group than in the -B6 and B6R groups. Brain weight was lower in the B6 group than B6R but brain weights of -B6 and B6 were similar. When organ size was related to body weight (Fig. 4b), no differences among the three groups were observed in the fat pad weights. Liver weight as percentage body weight was lower in B6 compared to that of the B6R. Brain weight as percentage body weight was lower in the B6 than in either -B6 or B6R. Thus, in -B6, liver, fat, and brain weights expressed as absolute weight or percentage body weight were not different than those in B6R and only percentage brain weight was different in -B6 compared to B6. Epididymal fat pad cell size (-B6 = 1.01 ± 0.26 , B6R = $0.91 \pm 0.18 \mu$ g lipid/cell) and number/pad (-B6 = $2.86 \pm 0.54 \times 10^6$, B6R = $2.52 \pm 0.29 \times 10^6$) were also not different. The cell size found for the epididymal fat pad was in the range reported for rats fed high fat diets (23).

Serum glucose 12-16 hr after the last meal was not different in the three groups (-B6 = 138 ± 6 , B6R = 142 ± 5 , B6 = 145 ± 8 mg/dl). Pituitary weight (Table II) in micrograms was significantly greater ($P < 0.005$) in B6 rats than in -B6 or B6R. However,

when expressed as micrograms/100 g body wt the pituitary is proportionately larger ($P < 0.025$) in the B6R group than in the -B6 or B6 groups, indicating that food restriction of the B6R group had an effect on the rats. The concentrations of the pituitary hormones PRL and GH were not different in the serum or pituitary gland (μ g/mg) in the three groups (Table II). The total pituitary GH (μ g/pituitary) in -B6 was significantly ($P < 0.01$) lower than in B6 due to the greater pituitary weight of B6. Whether lower total GH (13%) would have an impact on function is unclear. The range of values (ng/ml in each group) for serum GH was large (-B6 = 0-320, B6R = 0-168, B6 = 1-122) and to test whether the incidence of high values was different in the treatment groups, a Fisher exact probability test (24) was used. The incidence of high values in B6 or B6R compared to those in -B6 was not significantly different ($P > 0.05$), suggesting the GH release pattern (25) was not different in the -B6 group.

Discussion. Excretion of xanthurenic acid was higher in -B6 than in B6R rats indicating a deficiency of vitamin B6 in the -B6 rats induced by dietary deprivation and addition of an antagonist, 4-deoxypyridoxine. Although differences in final body weight between the -B6, B6R, and B6 rats were significantly different, mean body weights of

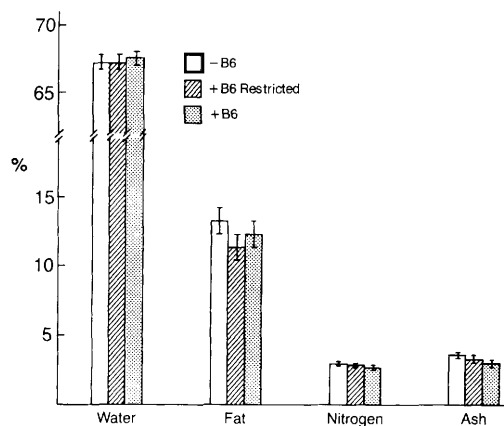


FIG. 3. Body composition (% body weight). Bars are the means \pm SE of 6-12 rats. Water, fat, nitrogen, and ash content were not significantly different ($P > 0.05$) among the three treatments.

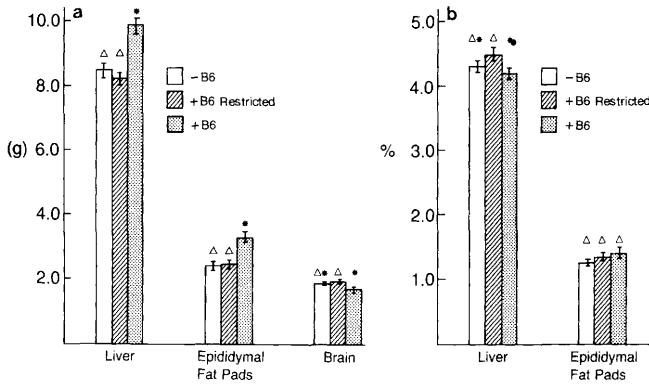


FIG. 4. (a) Organ weight (g). Bars are the means \pm SE of 9–12 rats. The weight of the liver and epididymal fat pads was significantly greater ($P < 0.005$) in the B6 rats than in the -B6 or B6R. The weight of the brain of the -B6 rats was not significantly different ($P > 0.05$) than that for B6R or B6 rats. Brain weight of B6R (Δ) rats was significantly greater ($P < 0.025$) than for B6 rats. (b) Organ weights (% body weight). Liver weight (% body weight) of -B6 rats was not significantly different than those of B6R or B6 rats. In B6R rats liver weight (% body weight) was significantly greater ($P < 0.05$) than that of B6 rats. Epididymal fat pad weight (% body weight) was not different in the three groups. Brain weight (% body weight) was significantly greater ($P < 0.01$) for -B6 and B6R rats than for B6 rats.

the three groups were within 10% of each other. Total weight gain, food intake, and food efficiency were not different between the -B6 and B6 groups, whereas, in the B6R group these parameters were lower than for both the -B6 and B6 groups. Thus, for a short-term experiment based on body weight gain, food intake, and food efficiency in meal-fed rats, the control group fed unrestricted amounts of food may be the most appropriate control group. The inability of the meal-fed restricted rats to consume all

the food provided is unclear, but has previously been observed (26). No differences in organ size between the -B6 and both the control groups were observed suggesting the short-term B6 deprivation and deoxyripyridoxine did not affect organ size. Body composition, serum glucose, pituitary PRL and GH, and serum GH and PRL values were not different for the three groups.

Previous studies (7, 14, 27) reported a decrease in body fat content in B6 deprived rats. In two studies (7, 14) body composition

TABLE II. SERUM AND PITUITARY PROLACTIN AND GROWTH HORMONE IN B6 DEPRIVED (7 DAYS) MEAL-FED RATS

	B6 deprived (-B6)	Food restricted control (B6R)	Control (B6)
Pituitary weight (mg)	6.8 \pm 0.1 (11) ^a	7.0 \pm 0.1 (12) ^a	7.8 \pm 0.3 (8) ^b
Pituitary (mg/100 g body wt)	3.5 \pm 0.1 (11) ^a	3.8 \pm 0.1 (12) ^c	3.3 \pm 0.1 (8) ^a
Serum prolactin (ng/ml)	26.0 \pm 1.7 (11) ^a	23.6 \pm 1.5 (12) ^a	28.4 \pm 3.8 (9) ^a
Pituitary prolactin (μ g/ml)	3.3 \pm 0.2 (11) ^a	3.4 \pm 0.2 (12) ^a	3.0 \pm 0.3 (8) ^a
Pituitary prolactin (μ g/pituitary)	22.6 \pm 1.7 (11) ^a	23.5 \pm 1.4 (12) ^a	23.8 \pm 2.9 (8) ^a
Serum growth hormone (ng/ml)	42.4 \pm 28.7 (11) ^a	19.4 \pm 13.6 (12) ^a	56.4 \pm 19.1 (8) ^a
Pituitary growth hormone (μ g/mg)	91.4 \pm 1.9 (11) ^a	95.0 \pm 2.5 (12) ^a	91.9 \pm 3.1 (8) ^a
Pituitary growth hormone (μ g/pituitary)	624.5 \pm 21.4 (11) ^a	61.8 \pm 23.5 (12) ^{a,d}	716.3 \pm 27.7 (8) ^d

Note. Values are the means \pm SE. Number of rats in parentheses. Different letters within a row indicate a significant difference between groups: ^b $P < 0.005$, ^c $P < 0.025$, ^d $P < 0.01$.

was determined after 7 days of deprivation, but the third study (27) was of longer duration (46–183 days). The study by Beare *et al.* (14) also added deoxypyridoxine to both control and B6 deficient diets. Although controls rats in these earlier studies were pair-fed with B6 deprived rats, the impact of pair-feeding on meal patterns was not considered. Results of the present study show that when differences in feeding pattern are minimized, body fat content is not different in rats fed an adequate B6 diet (either restricted or unrestricted fed) and rats fed a B6 deficient diet.

Makris and Gershoff (11) found no difference in serum and pituitary hormone between B6 deficient and pair-fed control rats. The results of the present study confirm these findings and suggest that the decreases in serum and pituitary growth hormone in B6 deficient rats and pair-fed controls compared with those of *ad libitum* controls found by Makris and Gershoff (11) were due to an altered feeding pattern or lower food intake by the B6 deficient rats and pair-fed controls.

In the present study fewer differences in growth, body composition, or hormone concentrations were observed in B6 deprived rats and control unrestricted-fed rats when all the rats were meal-fed. When meal fed, differences in parameters which alter carbohydrate and lipid metabolism, such as body weight gain, food intake, food efficiency, and body composition were minimized between B6 deprived and unrestricted control rats.

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