Effect of Vitamin B_6 Deficiency on the Metabolism of Isolated Fat Cells: Response to Insulin, Epinephrine and Theophylline[1] (35307)

DENNIS J. SABO AND STANLEY N. GERSHOFF
(Introduced by Frederick J. Stare)
Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115

Relationships between vitamin B_6 nutriture and fat metabolism have been reported for 35 years. Although there may be a direct role for vitamin B_6 in fat metabolism, many investigators believe that the primary effect of vitamin B_6 deficiency on fat metabolism is through indirect mechanisms (1). In 1964, Huber *et al.* (2) reported that carcass fat content and the fatty acid composition of liver and adipose tissue from vitamin B_6 deficient rats were different from those of control animals and that injections of insulin tended to reverse these changes. Furthermore, deficient rat serum and pancreatic tissue had lower levels of insulin activity and the epididymal fat pads from deficient animals were more sensitive to insulin than those of controls. These studies suggested that lipid changes in vitamin B_6-deficient rats might be related to abnormalities in insulin availability and its relation to carbohydrate and fat metabolism. In the present study further evidence is presented to show that changes associated with lipid metabolism in vitamin B_6-deficient rats might be due to alterations in the sensitivity of adipose cells to agents which affect lipogenic and lipolytic activity.

Materials and Methods. Weanling, male Charles River CD rats were maintained on semipurified diets for 3 weeks. The basal diet contained (%): casein, 25; sucrose, 60.7; cod liver oil, 1.0; corn oil, 4.0; salts IV (3), 4.0; and choline chloride, 0.3. Vitamins were added so that each 100 g of diet contained 0.4 mg of thiamine hydrochloride, 0.8 mg of riboflavin, 4 mg of niacin, 0.02 mg of biotin, 2 mg of calcium pantothenate, 0.1 mg of menadione, 0.005 mg of vitamin B_{12}, 10 mg of DL-α-tocopherol and 0.4 mg of pyridoxine hydrochloride. The deficient groups received the basal diet without pyridoxine. When controls were pair fed they were given their last daily ration over a 24-hr period at 2-hr intervals and were sacrificed 2 hr after their last meal.

Isolated fat cells were prepared essentially as described by Rodbell (4). Epididymal fat pads were removed after decapitation, cut into small pieces and weighed. One-g portions of adipose tissue were incubated in 25-ml polyethylene vials containing 3 ml of Krebs–Ringer bicarbonate buffer with one half the usual amount of calcium, 4% dialyzed bovine albumin, and 10 mg of crude collagenase. Tissues were incubated at 37° with shaking for 1 hr and then centrifuged through gauze as described by Gliemann (5). The precipitate and supernatant fluid were removed by aspiration and the remaining free cells were suspended in the original volume of 37° Krebs–Ringer buffer containing 2% dialyzed albumin and centrifuged again. The cells were washed at least three times or until no red cells or tissue debris were visible. They were finally resuspended in an appropriate volume of buffer (Krebs–Ringer, 2% albumin) and transferred with plastic syringes to 25-ml polyethylene vials which contained all other additions except the cells. Each vial contained fat cells from approximately 35 mg of adipose tissue/ml. Vials were sealed with serum bottle stoppers and gassed with 95% O_2–5% CO_2 for 5 min at 37° and then incubated for 2 hr with shaking. All incubations were done in triplicate.

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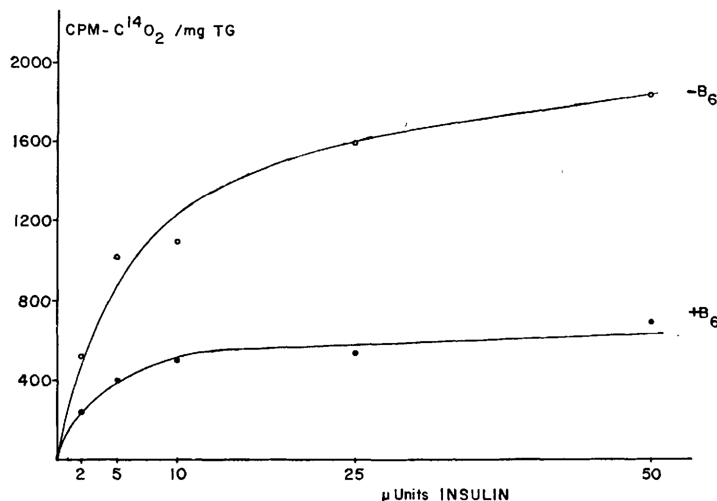

FIG. 1. The effect of insulin on $^{14}CO_2$ production per milligram of triglyeride in fat cells from vitamin B_6-deficient and control rats incubated with glucose-1-^{14}C. Values represent the mean of four determinations.

For analytical determinations, the cell suspensions were transferred to glass-stoppered centrifuge tubes and extracted with 5 ml of Dole's mixture (6). After standing for 15 min at room temperature, 3 ml of water and 3 ml of hexane were added, and the phases were allowed to separate. The lower phase was removed by aspiration and the upper phase was washed with 3 ml of water. Portions of the upper phase were used for triglyceride determinations (7), free fatty acid titrations (6), or ^{14}C lipids (4). The method of Schneider (8) was used to determine fat cell DNA and that of Layne (9) to measure protein.

Results. The results of the first experiment, shown in Fig. 1, demonstrate that in the presence of several concentrations of crystalline pork insulin, fat cells isolated from vitamin B_6-deficient rats oxidized significantly more glucose-1-^{14}C to $^{14}CO_2$ than fat cells isolated from control animals. The increase in glucose utilization in vitamin B_6-deficient fat cells existed over the entire range of insulin concentrations used and higher concentrations of the hormone were needed to reach an apparent saturation level. Similar results were obtained when the effects of increasing insulin concentrations on the conversion of glucose-1-^{14}C to lipid were determined in two experiments shown in Table I. In these studies the amount of ^{14}C from labeled glucose incorporated into triglyceride (TG) was measured. Fat cells from deficient rats were more efficient in synthesizing lipid from glucose than those from controls. This was true in the absence of insulin or in the presence of two concentrations of the hormone. The effect of vitamin B_6 deficiency was not due to inanition as can be seen when fat cells from 24-hr fasted control rats were similarly studied.

To establish the validity of using fat cell

TABLE I. The Effect of Vitamin B_6 Deficiency and Fasting on the Conversion of Glucose to Lipid by Rat Epididymal Fat Cells.

	^{14}C (cpm/mg of TG)		
Insulin (μU):	0	10	25
Expt. I			
$+B_6$	240[a]	811	1071
$+B_6$, fasted	189	442	543
$-B_6$	673	2150	2527
Expt. II			
$+B_6$	516	816	1194
$+B_6$, fasted	168	270	342
$-B_6$	972	2118	2568

[a] Values represent the mean of replicate determinations of pooled fat cell samples from six animals.

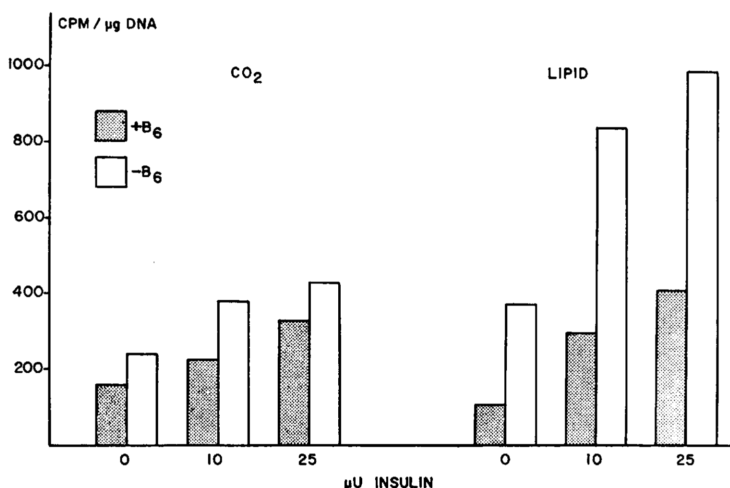

FIG. 2. The effect of insulin on $^{14}CO_2$ and ^{14}C lipid production per microgram of DNA in fat cells from vitamin B_6-deficient and control rats incubated with glucose-1-^{14}C. Values represent the mean of four determinations.

triglyceride as a means of quantifying and comparing the amount of tissue used, similar experiments were done and the results were expressed on a protein or DNA basis. These experiments provided similar data, establishing that adipocytes from vitamin B_6-deficient animals utilize more glucose than fat cells of control rats. The data of the DNA experiment are shown in Fig. 2. No significant difference in the ratios of triglycerides to DNA was observed in fat cells from deficient and control rats. The mean ratio $\pm$ SE of triglyceride (mg) to DNA (μg) in five preparations of fat cells from rats fed diets with and without vitamin B_6 were 26.2 ± 4.8 and 25.3 ± 4.9, respectively. These data indicate the validity in these studies of using fat cell triglyceride to quantify the amount of fat cells used.

In the next set of studies the effects of epinephrine and theophylline in the presence and absence of insulin on the production of free fatty acids (FFA) by epididymal fat cells from *ad libitum* and pair fed control and vitamin B_6-deficient rats were examined.

The data in Table II show that there was a tendency for fat cells from deficient rats to produce more free fatty acids than fat cells from controls in the presence of the lipolytic agents used. The increase in FFA production was not significant when epinephrine was used but was significant at the $p < .02$ level when theophylline was used. Table III shows the results of studies of the effect of insulin on the lipolytic action of theophylline and epinephrine and the effect of pair feeding the controls on the sensitivity of their fat cells to the lipolytic agents used. It appears from these data that the tendency towards increased lipolysis in fat cells from deficient rats may be a reflection of the inanition which accompanies the deficiency. The fat

TABLE II. The Effect of Epinephrine and Theophylline on the Production of FFA by Epididymal Fat Cells from Vitamin B_6-Deficient and Control Rats.

Group	FFA (μEq/mg of TG); treatment of fat cells: None	Epinephrine	Theophylline
$+B_6$	(16) 34 ± 3[a]	(11) 173 ± 16	(9) 177 ± 23
$-B_6$	(17) 44 ± 4	(12) 227 ± 22	(10) 290 ± 36

[a] Values represent means $\pm$ SE of number of studies in parentheses. When used, 0.5 μg of epinephrine or 0.1 mg of theophylline were added/ml of fat cell suspension.

TABLE III. Effects of Vitamin B_6 Deficiency and Paired Feeding on FFA Production by Rat Epididymal Fat Cells.[a]

Treatment	$+B_6$ FFA (μEq/mg of TG)	p	$-B_6$ FFA (μEq/mg of TG)	p	$+B_6$, pair fed FFA (μEq/mg of TG)	p
None	(7) 36 ± 7		(8) 49 ± 6		(3) 65 ± 8	
Epinephrine	(7) 193 ± 20	<.001	(8) 247 ± 28	<.001	(3) 308 ± 75	<.05
Epinephrine + insulin	(7) 137 ± 15	<.05	(8) 232 ± 21	NS	(3) 265 ± 55	NS
None	(4) 30 ± 4		(5) 45 ± 4		(3) 65 ± 8	
Theophylline	(4) 228 ± 30	<.001	(5) 361 ± 45	<.001	(3) 450 ± 34	<.001
Theophylline + insulin	(4) 133 ± 8	<.05	(5) 113 ± 10	<.001	(3) 178 ± 30	<.01

[a] Values represent means ± SE of number of studies in parentheses. p values obtained by a t test compare the effects of the lipolytic agents with no treatment and the effects of insulin on the activity of the lipolytic agents. When used, 0.5 μg of epinephrine, 0.1 mg of theophylline and 25 μU of crystalline pork insulin were added/ml of fat cell suspension.

cells from pair fed controls acted more like the deficient cells than the fat cells of the *ad libitum* controls. Insulin significantly inhibited the lipolytic action of epinephrine on fat cells from *ad libitum* fed controls but not on fat cells of deficient or pair fed controls. Insulin also significantly inhibited the lipolytic action of theophylline on fat cells from all three groups but appeared less effective on the fat cells of *ad libitum* controls than the other two groups.

Discussion. Free epididymal fat cells from vitamin B_6-deficient rats in these studies exhibited an increased sensitivity to insulin when compared to those from controls when glucose utilization was measured. These data agree with those of Huber *et al.* (2) who demonstrated a similar response in intact epididymal fat pads from deficient rats. In discussing vitamin deficiency experiments, the question is often asked whether the effects observed are a primary effect of the vitamin deficiency or are secondary to the decreased food intake accompanying the production of the deficiency. It seems clear that the increased lipogenic sensitivity to insulin observed with free fat cells from vitamin B_6-deficient rats are not related to inanition. In this study as in others (10, 11), it has been shown that restriction of food intake decreases the lipogenic activity of adipose tissue.

It is unlikely that the observations reported in this study are related to differences in the size of fat cells obtained from control and deficient animals. No significant difference was found in the ratio of DNA to triglyceride between control and deficient cells, and this can be interpreted as indicating that the cell sizes were similar (12).

Increased lipogenesis in vitamin B_6-deficient isolated fat cells without and in the presence of added insulin suggested the possibility that deficient fat cells might demonstrate increased lipolysis and altered sensitivity to other actions of insulin. Epinephrine promotes lipolysis by stimulating the formation of cyclic AMP by adenyl cyclase; theophylline by inhibiting cyclic-3′,5′-nucleotide phosphodiesterase which destroys cyclic AMP (13). Insulin inhibits adenyl cyclase activity (14). In the present study, it appeared that there was an increased sensitivity of fat cells from deficient rats to theophylline-stimulated lypolysis when compared to fat cells obtained from *ad libitum* fed but not pair fed controls. A tendency towards a similar pattern of sensitivity to epinephrine was also observed. It appears that all or part of the enhanced effect of lypolytic agents on vitamin B_6-deficient fat cells may be related to the inanition which accompanies the deficiency. The fat cells obtained from the food restricted, pair fed controls acted similarly to the fat cells from deficient animals. One cannot predict sensitivity of fat cells to insulin in doing lipolysis studies from the effects observed in lipogenesis studies. Insulin

was not effective in inhibiting the effect of epinephrine on fat cells from deficient or pair fed rats but was able to inhibit the epinephrine effect on fat cells from *ad libitum* controls. On the other hand, insulin appeared less effective in blocking the action of theophylline on fat cells from *ad libitum* controls than on fat cells from the other two groups of rats.

The changes observed in lipid metabolism of fat cells from vitamin B_6-deficient rats are not readily explained by current knowledge of pyridoxal dependent enzyme reactions. It appears in this study that the sensitivity of adipocytes to insulin and to a lesser extent epinephrine and theophylline was increased in vitamin B_6-deficiency and that the effects observed on lipolytic activity may be related to inanition caused by the deficiency. However, the effects of vitamin B_6-deficiency on adipose tissue metabolism cannot all be explained by such nonspecific mechanisms. This appears to be particularly true in the enhanced utilization of glucose in lipogenesis by fat cells from deficient animals both in the presence and absence of insulin.

Summary. When isolated epididymal fat cells from vitamin B_6-deficient rats were incubated with glucose, lipogenesis was greater both in the absence and presence of insulin than when fat cells from fed and fasted control animals were similarly treated. Fat cells from vitamin B_6-deficient rats also appeared more sensitive to lipolysis induced by theophylline and to a lesser extent epinephrine. This effect appeared to be related at least in part to the inanition accompanying the vitamin deficiency. Insulin was effective in partially inhibiting the epinephrine effect on fat cells from *ad libitum* contols but not on fat cells from deficient rats or pair fed controls. Conversely, insulin appeared less effective in inhibiting the theophylline effect on fat cells from *ad libitum* controls than the other groups. Ratios of triglyceride to DNA were similar in fat cells obtained from control and deficient rats indicating that in rat vitamin B_6-deficiency experiments the amount of fat cells used can be quantified by the amount of trigylceride present.

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