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Vitamin B_6 deficiency decreases the glucose utilization in cognitive brain structures of rats

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The effects of vitamin B_6 deficiency on metabolic activities of brain structures were studied. Male Sprague-Dawley weanling rats received one of the following diets: (1) 7 mg pyridoxine HCl/kg (control group); (2) 0 mg pyridoxine HCl/kg (vitamin B_6-deficient group); or (3) 7 mg pyridoxine HCl/kg with food intake restricted in quantity to that consumed by the deficient group (pair-fed control group). After 8 weeks of dietary treatment, rats in all three groups received an intravenous injection of 2-deoxy-[^{14}C] glucose (100 μCi/kg). Vitamin B_6 status was evaluated by plasma pyridoxal 5′-phosphate concentrations. The vitamin B_6-deficient group had significantly lower levels of plasma pyridoxal 5′-phosphate than did the control and pair-fed groups. The local cerebral glucose utilization rates in structures of the limbic system, basal ganglia, sensory motor system, and hypothalamic system were determined. The local cerebral glucose utilization rates in each of the four brain regions in the deficient animals were approximately 50% lower ($P < 0.05$) than in the control group. Results of the present study suggest that serious cognitive deficit may occur in vitamin B_6-deficient animals. (J. Nutr. Biochem. 10:525–531, 1999)

Keywords: vitamin B_6; glucose metabolism; 2-deoxyglucose; brain; rats

Introduction

Vitamin B_6 plays a crucial role in the normal functioning of the central nervous system.[1] Behavior and functional abnormalities have been observed in vitamin B_6-deficient animals. Motor impairment,[2,3] avoidance learning deficit,[4,5] attenuation of acoustic and tactile startle responses,[6] and decrement of somatosensory stimulus conduction,[7] as well as abnormal EEG[8] and convulsive seizure,[9,10] have been described. The development of these abnormalities suggests that vitamin B_6 deficiency changes the functional activities of brain structures or regions that modulate movement, cognition, and learning. Biochemical[10–12] or morphologic alterations[10] in certain brain regions were reported in progeny of rats fed a vitamin B_6-deficient diet during pregnancy and lactation. However, direct measurements or mapping of the functional activities of brain regions in vitamin B_6-deficient animals have not yet been reported.

Glucose is the exclusive substrate of energy metabolism in the brain under normal conditions. The amount of energy used or the extent of work performed by the brain cells is related to the rate of glucose consumed. A closed relationship between functional activity and energy metabolism in discrete structural and functional units of the nervous system has been demonstrated.[13–15] Based on this relationship, the functional activity of entire brain regions can be mapped by measuring local glucose consumption. The 2-deoxy-[^{14}C]-glucose (2DG) is a glucose analogue that cannot be further metabolized beyond the phosphorylation to [^{14}C]deoxyglucose-6-phosphate in the glycolytic pathway.[16] Because of this unique property of 2DG, the rates of glucose utilization in the structural and functional components of the brain can be measured quantitatively by the 2DG autoradiographic method.[16] The 2DG method has been applied in various studies of cerebral regions of glucose functional metabolism in altered physiologic and pathologic states.[17–20]

The present study applied the 2DG method to study the

This work was supported by the National Science Council of Taiwan, R.O.C., grant number NSC84-2331-B-010-115.
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Received February 23, 1999; accepted June 11, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/99/$–see front matter
PII S0955-2863(99)00040-6

metabolic activities of brain structures in the basal ganglia, the hypothalamic system, the sensory motor system, and the limbic system of vitamin B_6-deficient rats.

Materials and methods

Animal treatment

Male Sprague-Dawley rats (Experimental Animal Center of National Yang-Ming University, Taipei, Taiwan), aged 3 weeks and weighing approximately 70 grams, were randomly divided into control, pair-fed, and vitamin B_6-deficient groups. Control animals were fed the AIN-76A diet containing 7 mg PN HCl/kg (ICN Biochemicals, Cleveland, OH USA).[21] Rats in the vitamin B_6-deficient group were fed the AIN-76A diet without PN HCl (ICN Biochemicals). Both control and vitamin B_6-deficient groups had free access to food. The pair-fed rats were fed the control diet but the amount of food was restricted to the average food consumed by the vitamin B_6-deficient rats on the previous day. Animals were housed in a room maintained at constant temperature (23°C) and humidity (50%) with a 12-hour light/dark cycle. The animal care procedures followed the Guidelines for Animal Care and Use of the National Science Council (Taipei, Taiwan, ROC). Food intake was recorded daily. Body weight was measured weekly. After 8 weeks of dietary treatment, the 2DG study was performed.

Quantitative autoradiographic 2DG experiment

The 2DG experimental procedure followed the method of Sokoloff et al.[16] Rats were catheterized with polyethylene catheters through the femoral artery and vein under halothane anesthesia. At least 3 hours were allowed for rats to recover from the anesthetized state prior to the initiation of the 2DG experimental procedure. The 2DG experiment was initiated by an intravenous pulse injection of [^{14}C]-2DG (specific activity 58.0 mCi/mmol; New England Nuclear, Boston, MA USA) at a dose of 100 μCi/kg. Sixteen timed arterial blood samples were taken throughout the following 45-minute procedure. The blood samples were centrifuged immediately. For each sample, 20 μL of plasma was injected into the scintillation vial containing 4 mL of scintillation fluid (Amersham ACS II aqueous counting scintillant, Green End Aylesbury, Buckinghamshire, England) for assay of the [^{14}C]-2DG radioactivity, and 10 μL of plasma was used for subsequent glucose analysis using a Beckman glucose analyzer (Beckman Instruments Inc., Fullerton, CA USA). The blood sample (0.35 mL) taken prior to the injection of [^{14}C]-2DG was used for the analysis of pyridoxal 5′-phosphate (PLP) concentration.

Histologic and autoradiographic procedure

At the end of the 45-minute 2DG experiment, rats were euthanized by intravenous injection of 1 mL of pentobarbital sodium (65 mg/mL) and immediately perfused intracardially with 3.3% formalin (phosphate buffered to 7.4 pH) for approximately 1 minute. Immediately following the perfusion, the brains were removed and stored at −70°C. Each brain was cryosectioned coronally into 20 μm sections and every third section was saved, mounted on a coverslip, and dried on a standard slide-warming tray at 65°C. The brain sections were put into a cassette along with a set of [^{14}C]-methyl-methacrylate standards (New England Nuclear). An X-ray film (Kodak, SB-5, Eastman Kodak Co., Rochester, NY USA) was placed on the brain sections for exposure. After 10 days of exposure, the X-ray films were developed and fixed according to the manufacturer's instructions.

Local cerebral glucose utilization analysis

The values of blood glucose and plasma ^{14}C were input into a computer program of the Sokoloff equation that calculated the rate of local cerebral glucose utilization as previously described.[16] Local cerebral glucose utilization was read and analyzed using the Micro Computer Imaging Device (MCID) system with BRS2 features (Imaging Research Inc., Brock University, St. Cathrines, Ontario, Canada). A rat brain atlas[22] assisted in the identification of specific brain nuclei and regions of interest and the determination of their anatomical boundaries. The brain structures that were studied were in the limbic system, basal ganglia, sensory motor system, and hypothalamic system. Six readings were taken for a structure of interest in each brain section. The weighted average readings of the local cerebral glucose utilization for each structure were processed separately with the aid of the MCID system.

Plasma PLP analysis

Preparation of plasma samples for high performance liquid chromatography (HPLC) determination of PLP was based on the method of Furth-Walker et al.[23] The HPLC system (Waters, Milford, MA USA)[24] and the chromatographic conditions were as described previously.[24,25]

Statistical analyses

Data were analyzed by using the SPSS/PC+ statistics computer program (SPSS Inc., Chicago, IL USA). Food intake and body weight were evaluated by repeated measurement. A one-way analysis of variance was used to determine the significance of differences among group means. If the difference between groups was statistically significant, Duncan's multiple range test was used to determine the difference between means.[26] A *P*-value of less than 0.05 was considered statistically significant.

Results

The initial body weights of rats in the control, vitamin B_6-deficient, and pair-fed groups were similar. After the first week of the experiment, the body weights were significantly lower in the deficient group than in the control group. By the fourth week and thereafter, the body weights of the deficient group were significantly reduced compared with the pair-fed group (*Figure 1*). The food intake of the vitamin B_6-deficient group was significantly lower than that of the control group from the second week of the experiment. The mean food intake of the deficient group was approximately 49% of the control value (10.3 ± 0.7 g/day versus 21.1 ± 1.9 g/day, respectively). Reduction in food intake appeared to reduce body weights of the rats, whereas vitamin B_6 deficiency further suppressed the growth of the animals.

Vitamin B_6 nutritional status was evaluated by plasma PLP levels. The plasma PLP levels of vitamin B_6-deficient rats (107.5 ± 23.2 nmol/L) were 80% and 69% lower ($P < 0.05$) than those of the control (450.3 ± 167.8 nmol/L) and pair-fed animals (262.5 ± 43.5 nmol/L), respectively. This result indicated that rats in the deficient group were severely lacking in vitamin B_6. The pair-fed group also had significantly lower levels of plasma PLP than the control group.

The metabolic activities were determined in 24 structures of the four brain systems. In basal ganglia, the local cerebral glucose utilization of the caudatoputamen (CPU), globus

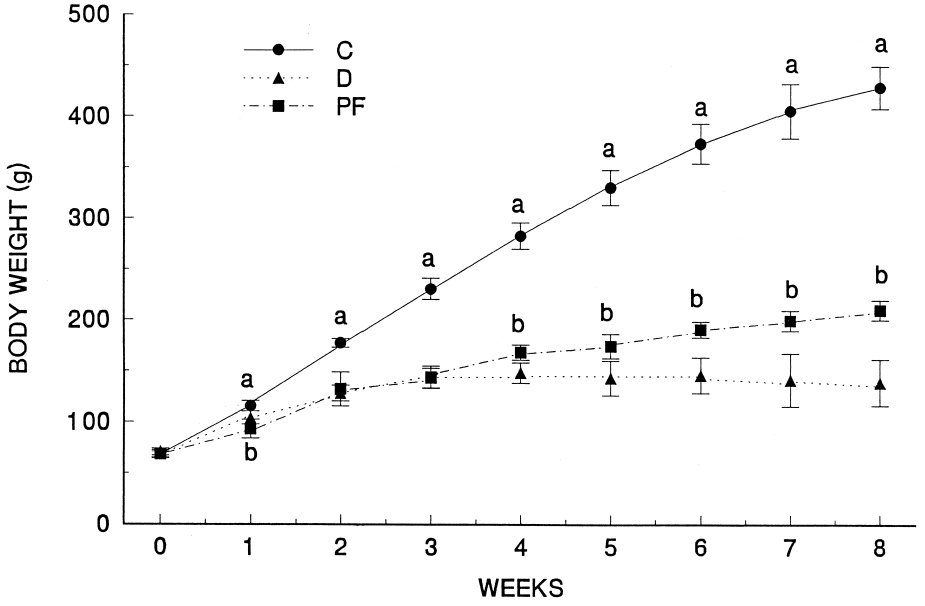

Figure 1 Mean body weight of rats. Each point represents the average body weight (mean ± SD) of six rats in the control group (C), six rats in the deficient group (D), and five rats in the pair-fed group (PF). A, Control group is different from the deficient group, $P < 0.05$. b, Pair-fed group is different from the control and deficient groups, $P < 0.05$.

pallidus, substantia nigra pas compacta, substantia nigra pas reticular, and subthalamic nucleus were measured. The local cerebral glucose utilization rates of these nuclei were significantly lower in the vitamin B_6-deficient group than in the control and pair-fed groups (*Figure 2*) but were not different between the pair-fed and control groups.

In the hypothalamic system, the local cerebral glucose utilization of the arcuate nucleus (ARC), median eminence (ME), paraventricular nucleus (PVH), and ventromedial nucleus (VMH) were analyzed. The local cerebral glucose utilization rates of the ARC, ME, PVH, and VMH of the vitamin B_6-deficient group were approximately 52%, 56%, 47%, and 50% of the control levels ($P < 0.05$), respectively (*Figure 3*). Local cerebral glucose utilization rates of the hypothalamic nuclei in the pair-fed group did not differ significantly from those of the control group. The local cerebral glucose utilization rates of the ME were comparable between the pair-fed and vitamin B_6-deficient groups.

In the sensory motor system, the local cerebral glucose utilization of the auditory cortex, motor cortex (MC), medial geniculate nucleus, paratenial nucleus thalamus, and two somatosensory cortex areas (SS1 and SS2) were examined. The vitamin B_6-deficient group had a significantly lower local cerebral glucose utilization than did the control and pair-fed groups, except that the local cerebral glucose utilization of the MC did not differ significantly between the pair-fed and vitamin B_6-deficient groups (*Figure 4*). No differences were observed in the local cerebral glucose

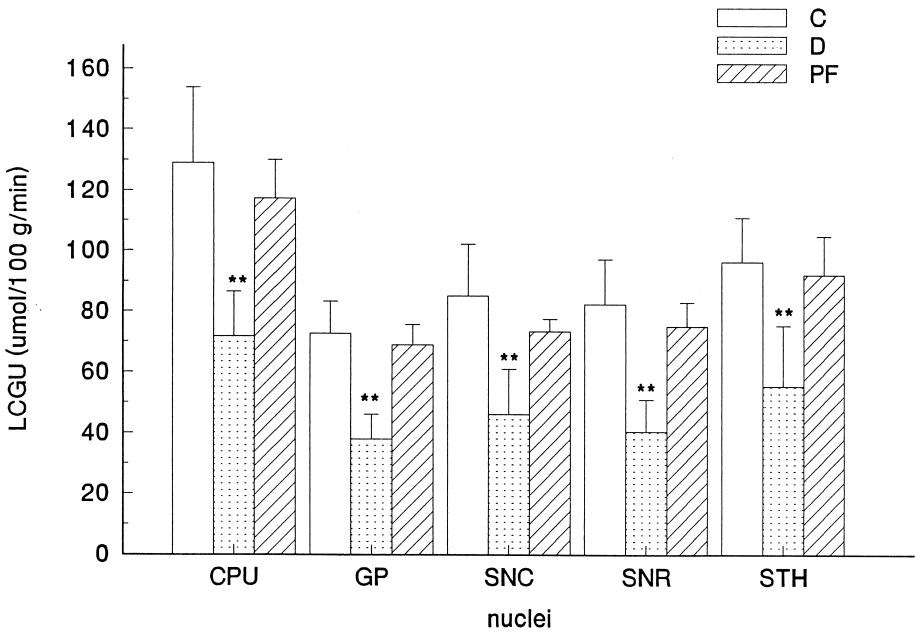

Figure 2 Local cerebral glucose utilization (LCGU) of nuclei in the basal ganglia of rats. Bars represent means ± SD of six rats in the control group (C), six rats in the deficient group (D), and four rats in the pair-fed group (PF). **Values are significantly different from those of the control and pair-fed groups ($P < 0.05$). CPU, caudatoputamen; GP, globus pallidus; SNC, substantia nigra pas compacta; SNR, substantia nigra pas reticular; STH, subthalamic nucleus.

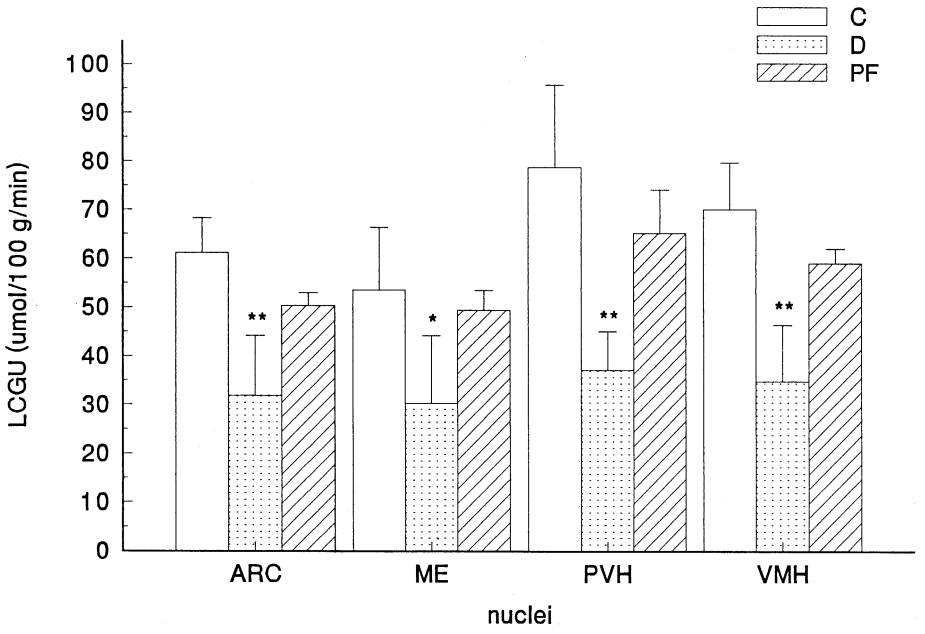

Figure 3 Local cerebral glucose utilization (LCGU) of nuclei in the hypothalamic system of rats. Bars represent means ± SD of six rats in the control group (C), six rats in the deficient group (D), and four rats in the pair-fed group (PF). *Values are significantly different from those of the control group ($P < 0.05$). **Values are significantly different from those of the control and pair-fed groups ($P < 0.05$). ARC, arcuate nucleus; ME, median eminence; PVH, paraventricular nucleus; VMH, ventromedial nucleus.

utilization of these six nuclei between the control and pair-fed groups.

In the limbic system, the frontal cortex (FC), medial prefrontal cortex (MPC), anterior cingulate, hippocampus, amygdaloid, medial preoptic nucleus (MPO), septal nucleus, supramammillary nucleus hypothalamus (SUM), and interpeduncular nucleus (IPC) were studied. Vitamin B_6-deficient animals had significantly lower local cerebral glucose utilization rates in these structures of the limbic system than did the control and pair-fed rats (*Figure 5*). The local cerebral glucose utilization rates of these nuclei in the pair-fed group were comparable to those of the control group, except that the value for MPO in the pair-fed group was significantly lower than that of the control group.

Discussion

In this study, rats fed a vitamin B_6-deficient diet experienced body size reduction and developed muscle weakness, especially in the hind legs. Deficiency in vitamin B_6 was confirmed by significantly lower levels of plasma PLP. Previous studies reported that abnormal neurologic symptoms such as high-pitched cries, hyperirritability, and con-

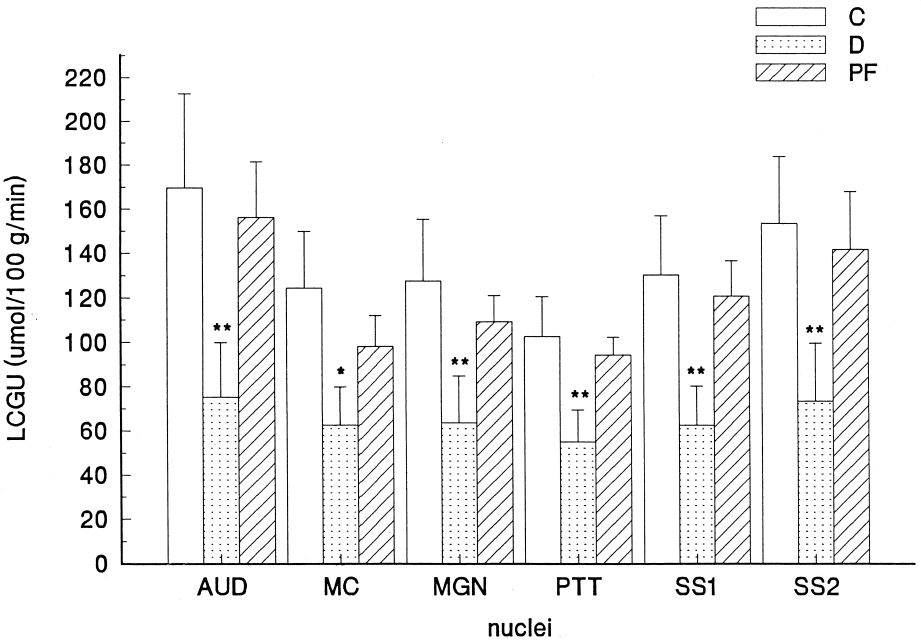

Figure 4 Local cerebral glucose utilization (LCGU) of nuclei in the sensory motor system of rats. Bars represent means ± SD of six rats in the control group (C), six rats in the deficient group (D), and four rats in the pair-fed group (PF). *Values are significantly different from those of the control group ($P < 0.05$). **Values are significantly different from those of the control and pair-fed groups ($P < 0.05$). AUD, auditory cortex; MC, motor cortex; MGN, medial geniculate nucleus; PTT, paratenial nucleus thalamus; SS1, somatosensory cortex area; SS2, somatosensory cortex area.

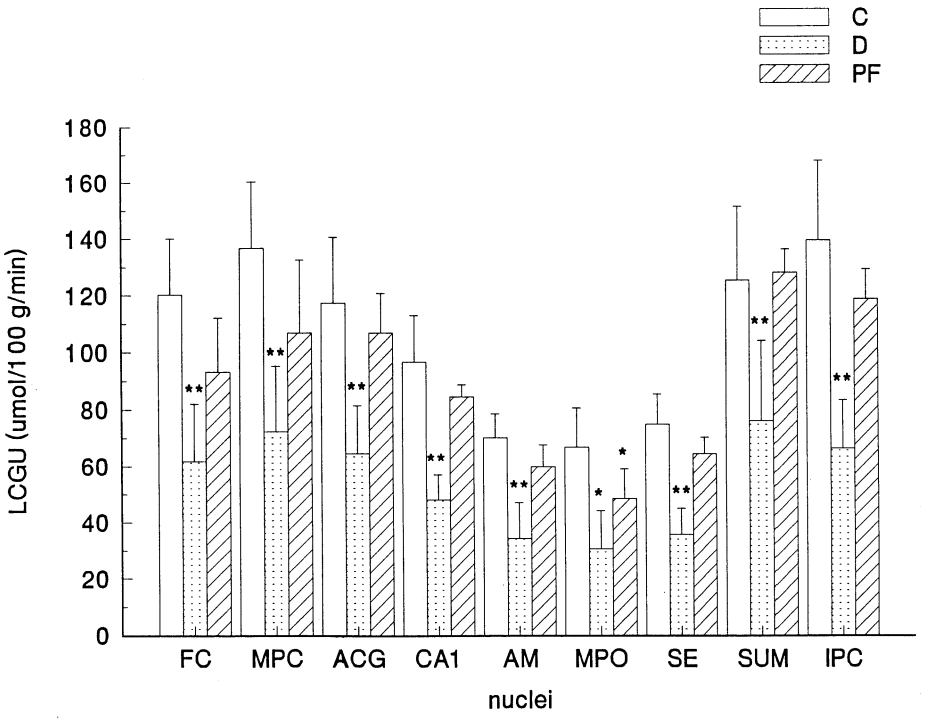

Figure 5 Local cerebral glucose utilization (LCGU) of nuclei in the limbic system of rats. Bars represent means ± SD of six rats in the control group (C), six rats in the deficient group (D), and four rats in the pair-fed group (PF). *Values are significantly different from those of the control group ($P < 0.05$). **Values are significantly different from those of the control and pair-fed groups ($P < 0.05$). FC, frontal cortex; MPC, medial prefrontal cortex; ACG, anterior cingulate; CA1, hippocampus; AM, amygdaloid; MPO, medial preoptic nucleus; SE, septal nucleus; SUM, supramammillary nucleus hypothalamus; IPC, interpeduncular nucleus.

vulsive seizure occurred in the progeny of rats fed a vitamin B_6-deficient diet during pregnancy and lactation.[9,10] In the present study, young adult rats were used and convulsive seizure was not observed in the vitamin B_6-deficient animals. This finding is in agreement with previous reports.[6,27]

The brain comprises many structural and functional components. In the present study, the effect of vitamin B_6 deficiency on the limbic system, basal ganglia, sensory motor system, and hypothalamic system was investigated. These brain regions play important roles in cognitive function. The limbic system participates in learning, memory, and emotions.[28] The basal ganglia are involved in the control of movement and also participate in learning and memory.[28–30] The hypothalamus is instrumental in maintaining body homeostasis and plays a major role in regulating emotional behavior.[28] Behavioral changes related to vitamin B_6 deficiency have been reported previously. Inferior performance in both active and passive avoidance learning were observed by Sloane and Chow[4] and Stewart et al.[5] The width of step of B_6-deficient animals has been reported to be reduced.[3] Depressed sensorimotor reactivity,[6] prolonged interwave intervals of brainstem auditory evoked potential,[31] and delayed sensory nerve responses[7] also have been demonstrated in vitamin B_6-deficient animals. These abnormalities could not have been related to developmental alterations, because adult animals were used in these studies.

The mapping of regional brain metabolic activity in the present study revealed that the glucose utilization of the four brain systems studied was significantly reduced in the vitamin B_6-deficient rats. The local cerebral glucose utilization rates of the brain structures of vitamin B_6-deficient rats were approximately one half of the values in the control animals. The pair-fed rats had values of local cerebral glucose utilization that were comparable to those of the control rats in most of the brain structures examined. In the present study, vitamin B_6 deficiency appeared to be responsible for significantly lower rates of local cerebral glucose utilization in the brain. Prior studies demonstrated that regional brain glucose consumption was associated with regional brain functional activity.[13–15] In the present study, the reduced rates of local cerebral glucose utilization observed in the basal ganglia, sensory motor system, hypothalamic system, and limbic system of vitamin B_6-deficient animals suggests that the functional activities in these brain regions were decreased.

Regional differences in brain glucose utilization were observed in the present study. In general, the sensory motor system had very high levels of glucose utilization. The CPU of the basal ganglia and FC, MPC, SUM, and IPC of the limbic system also showed relatively higher regional rates of glucose consumption. This observation is in agreement with previous reports.[16,32] Glucose consumption in the brain is required to meet the energy demands of brain cells for metabolic and physiologic processes. Differences in glucose consumption between brain structures are believed to not be due to differences in blood flow to these structures.[16] Studies have shown that the glucose utilization rate of nuclei are not positively correlated with the neuronal firing rates.[33,34] Although in the present study vitamin B_6 deficiency uniformly depressed regional glucose utilization of the brain, the expression of metabolic activities between brain structures of vitamin B_6-deficient animals after certain types of stimulation, for example, drugs or pain, may be different. Further studies of the changes in glucose utilization between brain structures of vitamin B_6-deficient ani-

mals under different treatments may help us to understand which brain regions are involved in neurologic abnormalities such as seizure.

The metabolic activity of brain structures has been shown to be independently regulated.[35] This was also evidenced in the present study. The glucose utilization rates of the brain structures of the pair-fed group were comparable to those of the control group, except that the glucose utilization rate of the MPO of the limbic system was significantly lower in the pair-fed group than in the control group. A previous study showed that food restriction did not influence whole brain PLP and pyridoxamine 5′-phosphate (PMP) levels or the activities of pyridoxal kinase and pyridoxamine (pyridoxine) 5′-phosphate oxidase.[36] It appeared that the regional glucose utilization of specific brain structures in response to food restriction might not parallel whole brain PLP and PMP concentrations. Studies have shown that the concentrations of PLP in discrete brain regions were different.[37–39] The nonuniform distribution of PLP in brain regions was suggested to meet the diversified biochemical activities of the central nervous system.[38] Further study of the relationship between changes in regional glucose utilization and vitamin B_6 vitamer concentrations in discrete brain regions or structures is needed.

In conclusion, this is the first study to report the regional brain metabolic activity in vitamin B_6 deficiency in rats. Regional glucose utilization rates in the brain structures of the basal ganglia, sensory motor system, hypothalamic system, and limbic system of vitamin B_6-deficient rats were approximately 50% lower than those of the control group. The results of the present study suggest that brain cognitive functional activities may be decreased in vitamin B_6 deficiency.

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