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Functional and Metabolic Consequences of Vitamin B-6 Deficiency in the Rat Heart (42442)

ARTHUR M. FELDMAN, TOMAS R. GUILARTE, KENNETH L. BAUGHMAN, AND GARY GERSTENBLITH

Department of Medicine, School of Medicine, and Department of Environmental Health Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

Abstract. The cardiac functional and metabolic consequences of pyridoxine deficiency were studied in rats maintained on a pyridoxine-deficient diet for 10 weeks. Because food intake was diminished in the pyridoxine-deficient rats, a second group of animals was fed a diet restricted to the intake of the pyridoxine-deficient animals. The inotropic response (developed pressure) to an isoproterenol or Ca^{2+} concentration response curve was measured simultaneously with high energy phosphate levels using a modified Langendorf apparatus and ^{31}P nuclear magnetic resonance spectroscopy. The inotropic response to Ca^{2+} and isoproterenol was significantly decreased relative to controls in both the food-deprived and the pyridoxine-deficient groups. Developed pressure after adrenergic stimulation was significantly less in the pyridoxine-deficient than in the food-deprived animals. Phosphocreatine and ATP levels were maintained and did not differ among the control, pyridoxine-deficient, and food-deprived groups during isoproterenol and Ca^{2+} stress, implying that the diminished inotropy was not due to an abnormality in generation of high energy phosphate levels.

Vitamin B-6 (pyridoxine) is a water soluble vitamin which is widely distributed in foods but not synthesized by mammals. Pyridoxal phosphate, the physiologically active form of vitamin B-6, is a coenzyme in a variety of metabolic reactions (1–3), including (i) amino acid transamination, the first stage in amino acid catabolism, (ii) aminolevulinic acid synthesis, a step in porphyrin synthesis, (iii) the enzymatic decarboxylation of phosphatidyl serine to form phosphatidyl ethanolamine, a precursor of the membrane component phosphatidyl choline, (iv) enzymic decarboxylation of amino acids, and (v) glycogen phosphorylation by phosphorylase, the rate-limiting reaction in glycogen catabolism. Pyridoxine deficiency has been associated clinically with abnormal central nervous system function (4), peripheral neuropathy (5), seborrheic dermatitis (6), the development of atherosclerosis (17), and systemic symptoms including nausea, vomiting, weakness, and dizziness (6).

In 1949, Agnew demonstrated cardiac hypertrophy in pyridoxine-deficient rats (8). Subsequent studies confirmed these results (9–11) and also reported histologic lesions at autopsy (12). Pyridoxine deficiency also has been associated with changes in the rate and duration of the action potential of the myocardium (13) and with cardiac hypertrophy and right ventricular dilatation in dogs (14). However, the cardiac functional and metabolic consequences of pyridoxine deficiency have not been determined.

The present study evaluated the effects of pyridoxine deficiency on isovolumic performance and high energy phosphate levels at baseline and during inotropic stress using the isolated perfused rat heart and nuclear magnetic resonance spectroscopy. Since pyridoxine deficiency is associated with diminished caloric intake (10), the effects of nutritional deprivation were also investigated.

Methods. Male Long–Evans rats (Blue Spruce Farms, Inc., Altamont, N.Y.) weighing approximately 150 g and approximately 2 months of age were randomized to individual metabolic cages. After a 1-week equilibration period, the rats were randomly divided into three groups. A control group was fed *ad libitum* a standard laboratory chow containing all nutritional constituents (AIN 76 with vitamin-free casein and complete vitamin supplementation, ICN Nutritional Biochemicals, Cleveland, Ohio). A pyridoxine-deficient group was fed *ad libitum* an identical diet that was free of pyridoxine (ICN Nutritional Biochemicals). The pyridoxine-deficient rats con-

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sumed less food than the control group. Therefore, the food consumption of each pyridoxine-deficient rat was measured daily and a corresponding amount of the control diet was fed to a paired control rat (pair-fed). All animals were weighed weekly and were allowed free access to water.

Animals were sacrificed from 10 to 11.5 weeks after starting the experimental diets and at least one animal from each group was evaluated on each day. Rats were weighed, injected intraperitoneally with 1000 units of heparin, and anesthetized with an intraperitoneal injection of pentobarbitol (1.0 mg/kg body wt). Blood (1 ml) was withdrawn from the descending aorta and placed in a heparinized collection vial. The heart was then excised. The aorta was cannulated and the heart perfused at a constant rate of 15 ml/min with 6 m*M* *N*-2-hydroxyethyl-1-piperazineethanesulfornic acid (Hepes) buffer, pH 7.4, containing 140 m*M* NaCl, 5 m*M* KCl, 1.2 m*M* $MgSO_4$, 16.7 m*M* glucose, and 0.75 m*M* CaCl. The perfusate was constantly bubbled with 100% oxygen and maintained at a temperature of 38°C. The perfusion rate provided a coronary perfusion pressure of 60–70 mm Hg. After puncturing the left ventricular apex with a 16-gauge needle, a fluid-filled latex balloon attached to the end of PE190 polyethylene tubing was inserted into the left ventricle through the mitral valve orifice and connected to a Statham P23Db transducer. Isovolumic left ventricular developed pressure was continuously recorded with a Brush direct writing recorder and the first derivative of ventricular pressure was calculated with an analog differentiator. A polyethylene tube containing a KCl-soaked wick was placed in the right ventricle and the heart paced throughout the experiment at 200 beats/min with a Grass SD-9 stimulator.

The modified Langendorf preparation was placed into the bore (25 mm) of a Bruker superconducting magnet with a field strength of 4.2 T, a phosphorous resonance of 72.89 MHz, and a pulse of approximately 45°. Nuclear magnetic resonance (NMR) spectra were obtained in the pulsed, Fourier-transformed mode on a Bruker WH-180 spectrometer interfaced with a Bruker 1080 computer. A 2-sec delay was present between pulses to provide minimally saturated spectra. Tissue levels of high energy phosphates were assessed by digitizing the areas under the phosphocreatine and ATP peaks with a Hewlett–Packard 9810A computer and results were expressed as percentage of control.

After a 30-min equilibration period, the intraventricular balloon volume was sequentially increased until a left ventricular end-diastolic pressure of 2–4 mm Hg was achieved. After obtaining baseline hemodynamics and ^{31}P NMR spectra, a Ca^{2+} concentration response curve was generated with Ca^{2+} concentrations from 0.5 to 2.0 m*M*. Each concentration was maintained for 5 min of perfusion after which the concentration was increased to the next level. Preliminary experiments demonstrated that maximal inotropic effects occurred within 5 min and all measurements were made at peak effect. At the end of the Ca^{2+} response curve the perfusate Ca^{2+} was returned to 0.75 m*M* and the heart allowed to reequilibrate. Isoproterenol was then administered in a cumulative dose fashion and each concentration was maintained for 5 min. The isoproterenol dose–response curve was performed at a Ca^{2+} concentration of 0.75 m*M* because previous studies demonstrated that a significant inotropic response to catecholamines is present at low Ca^{2+} concentrations (15). At the end of the experiment, the atria were removed, the ventricular tissue was weighed, and the heart was placed in an oven at 100°C for 24 hr for assessment of dry weight. NMR data were acquired over a 5-min period and each spectrum represented 150 acquisitions.

Plasma vitamin B6 was measured using a microbiologic assay based on the measurement of $^{14}CO_2$ generated from the metabolism of [DL-1-^{14}C]valine by *Kloeckera brevis* in the presence of varying concentrations of pyridoxine. The methodology, sensitivity, and specificity of the assay were previously described (16, 17).

Concentration responses were analyzed using analysis of variance with repeated measures (18). Individual pairwise comparisons were made between average levels at peak dose response using the mean square from the analysis of variance with repeated measures. Individual group effects were compared using one-way analysis of variance and within-group significance was determined using the Newman–Keuls test. The unpaired *t* test was used when appropriate. Analyses were performed

using program BMDP2V of the BMDP series and a Data General MV/8000 computer.

Results. Ten weeks of feeding with a pyridoxine-deficient chow resulted in a significant ($P < 0.001$) decrease in plasma vitamin B-6 levels in the deficient rats (3.0 ± 0.41 ng/ml, $n = 10$) when compared with controls (166.3 ± 8.3 ng/ml, $n = 7$). A small but significant ($P < 0.05$) decrease was also demonstrated in the pair-fed rats (135 ± 6.6 ng/ml, $n = 7$). The amount of chow consumed by the pyridoxine deficient rats was not significantly different from that consumed in the control rats until the third week of treatment (Fig. 1). However, during the subsequent weeks of the experimental period the pyridoxine-deficient rats consumed only 30% of the amount of chow eaten by the control rats. No deaths occurred in any of the animals during the experimental period and hematocrits were similar in all three groups.

Pyridoxine deficiency resulted in significant growth failure (Fig. 2). Table I presents the body weight and heart weight data from the control, pair-fed, and pyridoxine-deficient rats. The body weight of the pyridoxine deficient rats was 51.4% of the controls while the weight of the pair-fed rats was 81.8% of the controls. Although pair-fed and pyridoxine-deficient rats were fed an identical amount of chow, the pair-fed animals were able to maintain a greater body weight. Heart weights of both the pyridoxine deficient and the pair-fed rats were less than those of controls; however, when heart weight was expressed as a percentage of total body weight the values were significantly higher only in the pyridoxine-deficient hearts (Table I). No differences were seen between the wet heart weight-to-dry heart weight ratios in the three experimental groups.

Baseline left ventricular developed pressure at perfusate Ca^{2+} concentration of 0.75 m*M* was not significantly different in the control (93.0 ± 10.7, $n = 8$), pair-fed (97.6 ± 16.8, $n = 5$), and pyridoxine deficient (111.6 ± 8.6, $n = 8$) groups. Additionally, the baseline left ventricular end diastolic pressures were not significantly different in the three groups (control, 2.8 ± 0.6; pair-fed, 3.3 ± 0.6; pyridoxine deficient, 2.2 ± 0.5; $n = 8$). As the calcium concentration response curve was performed prior to the isoproterenol dose–response curve, the hearts were allowed to reequilibrate to a new baseline between stresses. The isoproterenol baselines were lower than the initial baseline; however, no significant differences were demonstrated between values in the control (51.5 ± 6.0 mm Hg, $n = 7$), pair-fed (54 ± 4.0, $n = 5$), and pyridoxine deficient (62.4 ± 2.8, $n = 8$) groups. A significant ($P < 0.01$) difference was demonstrated in the response to increasing concentrations of isoproterenol (Fig. 3) when the dose–response curves were analyzed using repeated measures analysis of variance. Both pair-fed and pyridoxine-deficient hearts generated a smaller developed pressure in response to increasing concentrations of isopro-

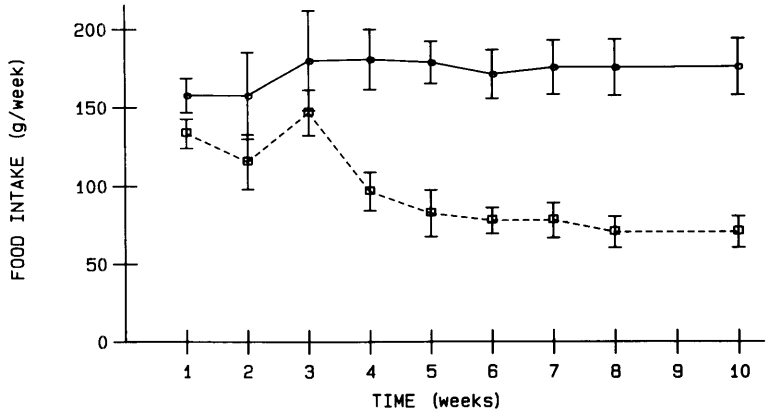

FIG. 1. Weekly food consumption of rats fed a control (○) or vitamin B-6-deficient (□) diet *ad libitum*. Values represent the means ± SEM; $n = 30$. A significant ($P < 0.001$) difference was present between the two groups.

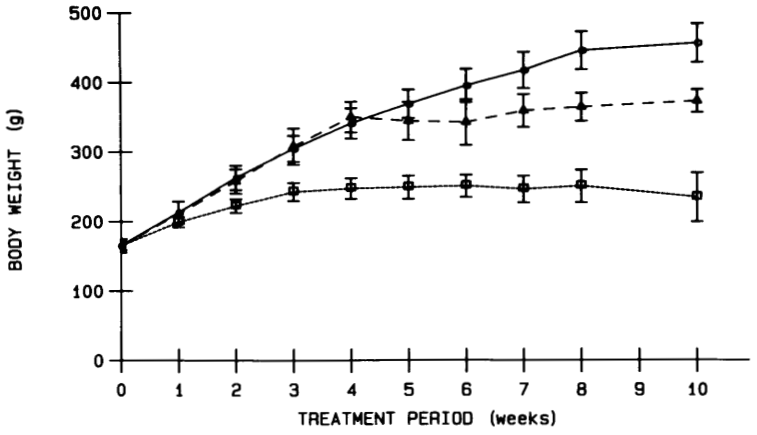

FIG. 2. Weekly growth curve (weight) for control (○), pair-fed (△), and vitamin B-6-deficient (□) rats. Values are the means ± SEM; $n = 20$.

terenol than did the control hearts. The difference was greater in the pyridoxine-deficient hearts. The mean level of developed pressure at the peak isoproterenol response (10^{-7} *M*) was 456 ± 61% of baseline value in the control group, 341 ± 27% in the pair-fed group, and 264 ± 34% in the pyridoxine-deficient group. Similar results were demonstrated when the *dP/dt* was calculated. Therefore, the diminished response to isoproterenol in the pyridoxine deficient hearts cannot be explained by nutritional deprivation alone.

The response of developed pressure to increasing Ca^{2+} concentrations was significantly lower in hearts from the pyridoxine- and food-deprived rats (Fig. 4) than the response in the control hearts. Average peak levels of developed pressure in the pyridoxine-deficient (276 ± 53, $n = 8$, % control) and pair-fed (259 ± 38, $n = 7$) hearts were significantly different from the average peak inotropic response in the control (403 ± 47, $n = 8$) hearts. The responses in the pyridoxine-deficient and pair-fed hearts were not significantly different; therefore, the diminished contractile response to increasing concentrations of Ca^{2+} in the pyridoxine-deficient rats could be explained by undernutrition.

Baseline levels of ATP and phosphocreatine were calculated based on the digitized area under the spectral peak (in.^{2}) divided by the wet weight of the ventricles (g) and the scaling factor (YF). No significant difference was demonstrated among baseline levels of phosphocreatine in the control (4.71 ± 0.7 in.^{2}), pyridoxine-deficient (5.35 ± 0.5), and pair-fed (4.1 ± 0.9) groups. Similarly, no difference was present among ATP levels in control (2.7 ± 0.3), pyridoxine-deficient (3.3 ± 0.4), and pair-fed (2.9 ± 0.5) hearts.

Contractile responses to increasing concentrations of isoproterenol or Ca^{2+} were not ac-

TABLE I. HEART AND BODY WEIGHT MEASUREMENT[a]

	BW (g)	DHW (g)	DHW/BW (mg/g)	WHW/DHW
Control	455.0 ± 9.8 (8)	0.220 ± 0.005 (9)	0.488 ± 0.104 (8)	6.11 ± 0.55 (8)
Pair-fed	372.4 ± 6.3 (7)*	0.185 ± 0.004 (8)*	0.494 ± 0.011 (7)	6.74 ± 0.65 (7)
Pyridoxine deficient	233.8 ± 12.5 (8)*	0.138 ± 0.005 (8)*	0.594 ± 0.019 (8)*	6.61 ± 0.46 (7)

[a] Results are means ± SEM (*n*). BW, body weight; DHW, dry ventricular weight; DHW/BW, dry ventricular weight to body weight ratio; WHW/DHW, wet ventricular weight to dry ventricular weight ratio. *$P = 0.05$ comparing pair-fed or pyridoxine deficient group with control.

TABLE II. BASELINE LEFT VENTRICULAR FUNCTIONAL PARAMETERS AT PERFUSATE CALCIUM CONCENTRATION OF 0.75 m*M*

	DP (mm Hg)	*dP/dt* (mm Hg sec^{-1})	LVEDP (mm Hg)
Control	93.0 ± 10.7 (8)	49.24 ± 5.2 (8)	2.8 ± 0.6 (8)
Pair-fed	97.6 ± 16.8 (5)	41.12 ± 4.6 (7)	3.3 ± 0.6 (7)
Pyridoxine deficient	111.6 ± 8.6 (8)	53.92 ± 11.3 (7)	2.2 ± 0.5 (8)
P value	NS		NS

Note. Results are means ± SEM (*n*). DP, developed pressure; dP/dt, first derivative of DP; LVEDP, left ventricular end diastolic pressure.

companied by changes in phosphocreatine or ATP levels in either the control, pair-fed, or the pyridoxine-deficient hearts (Fig. 5). Therefore, the functional abnormalities in the pyridoxine-deficient and pair-fed rats were not associated with changes in high energy phosphate levels.

Discussion. A significant decrease (98%) was demonstrated in plasma vitamin B-6 levels in rats fed a pyridoxine-deficient diet for 10 weeks. A small (18%) but significant decrease in plasma vitamin B-6 was seen in the pair-fed animals; however, it is unlikely that the small change in the pair-fed rats is of physiologic significance.

Pyridoxine deficiency was associated with significant growth failure. Although the amount of food consumed by the pyridoxine deficient and pair-fed animals was similar to that used in experimental chronic caloric restriction (21–24, 26), the diminished growth

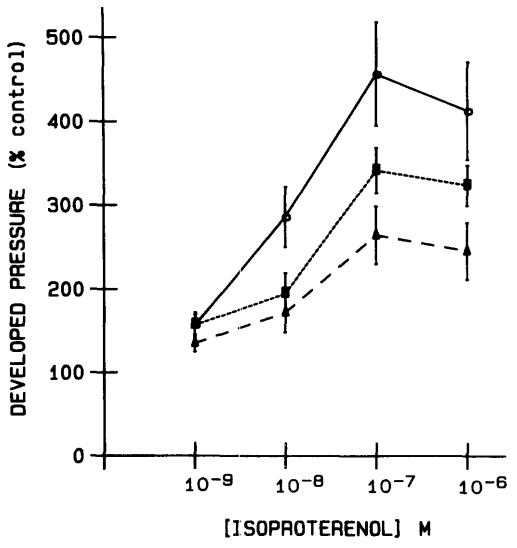

FIG. 3. Developed pressure in isolated perfused hearts from control (○) (n = 6), vitamin B-6-deficient (△) (n = 8), and pair-fed (□) (n = 5) rats during administration of isoproterenol in a cumulative dose fashion. Each concentration was maintained for 5 min and values represent the means ± SEM. A significant ($P < 0.01$) difference was present between the groups when analyzed using repeated measures analysis of variance.

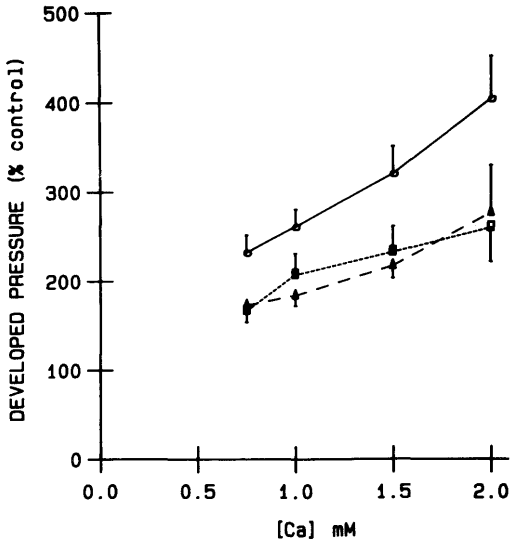

FIG. 4. Developed pressure in isolated perfused hearts from control (○) (n = 6), vitamin B-6-deficient (△) (n = 8), and pair-fed (□) (n = 5) rats during a Ca^{2+} concentration response curve. Control values were obtained at a Ca^{2+} concentration of 0.5 m*M*. Values represent the means ± SEM. Analysis of variance with repeated measures demonstrated a significant ($P < 0.01$) difference between inotropic response in hearts from control animals and those from the B-6 deficient and pair-fed groups.

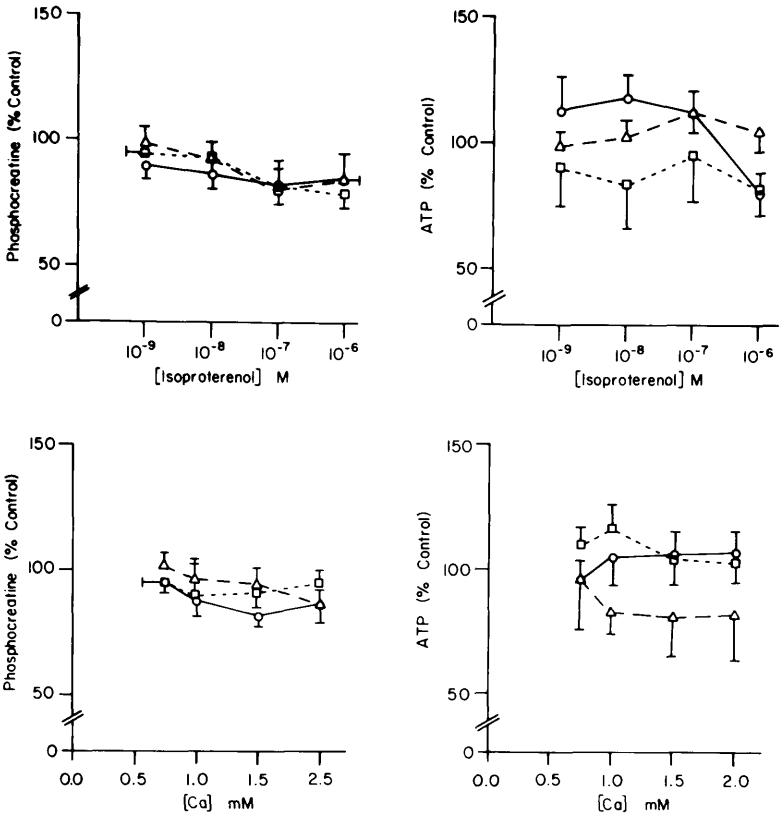

FIG. 5. Mean phosphocreatine and ATP levels (% control) during cumulative isoproterenol and Ca^{2+} concentration response curves performed on isolated perfused hearts from control (○) (n = 8), vitamin B-6-deficient (△) (n = 6), and pair-fed (□) (n = 5) rats.

in the pyridoxine-deficient rats cannot be attributed to chronic undernutrition alone because pair-fed animals demonstrated a greater ability to maintain body weight. Therefore, the increased wasting seen in the pyridoxine-deficient animals when compared with the pair-fed animals who received an identical caloric intake is probably a result of abnormalities in cellular metabolism due to the absence of the pyridoxine coenzyme.

Hearts from pair-fed and pyridoxine-deficient rats were smaller than controls. However, it is unlikely that the altered contractile response in these hearts can be explained by the small heart size. Previous studies using the isolated working heart have demonstrated that decreased heart size does not diminish myocardial contractile function (19). Hearts from pyridoxine deficient rats were heavier than those of both pair-fed and control animals when expressed as heart weight per gram of body weight. These results support the early investigations of Agnew (9) and Olsen and Martindale (10, 11) with pyridoxine-deficient rats and are similar to the relationships found between heart weights of diabetic rats and their pair-fed controls (20). That the difference is not due to selective sparing of the cardiac mass in the presence of undernutrition is supported by the similarity of heart weights in the pair-fed and control groups. Wet weight-to-dry weight ratios were similar in the three groups and therefore the difference cannot be explained by increased edema.

Adrenergic responsiveness was significantly diminished in the hearts from both the pyridoxine-deficient and the nutritionally deprived groups of animals. The decrease was greater in the pyridoxine-deficient group; therefore, caloric restriction alone cannot account for all

of the diminished response in the pyridoxine-deficient hearts. The effect of food restriction on adrenergic responsiveness is not clearly defined. Food restriction alters the sensitivity of aortic smooth muscle to isoproterenol (21, 22), and protects the myocardium from isoproterenol-induced myocardial infarction (23). However, undernutrition has a variable effect on atrial responsiveness to catecholamines (21, 22) and Crandall *et al.* (24) were unable to find a change in β-adrenergic receptor density in the ventricles of rats that were food restricted for a short period of time.

All hearts underwent isoproterenol stress subsequent to the calcium-inotropic stress. As a result of this experimental design, the baseline developed pressure prior to the determination of the isoproterenol dose–response curve was lower than its initial level. The possibility that this might have affected the isoproterenol response cannot be excluded. However, the validity of the isoproterenol data is supported by the fact that the preisoproterenol baseline developed pressures were similar in all three studied groups and that no change was seen in levels of high energy phosphates before and after the calcium stress, implying that the metabolic capacity of the hearts was unchanged.

Both pyridoxine- and food-deprived rats demonstrated a diminished sensitivity to perturbations of extracellular Ca^{2+}, suggesting that diminished caloric intake rather than vitamin B-6 deficiency is responsible for the diminished sensitivity of the heart to Ca^{2+}. Previous animal studies have evaluated the effects of protein–calorie undernutrition on cardiac function. Resting length–tension curves in isolated ventricular muscle (25) and myocardial contractility in isolated working rat hearts under baseline conditions or during increasing preload (20) were unaffected by food restriction. Intact working hearts demonstrated absolute levels of dP/dt, cardiac output, and left ventricular systolic pressures that were lower in underfed rats than in controls; however, when the indices were adjusted for body weight, ventricular function did not differ in the two groups (25). Additionally, active length–tension curves for ventricular muscle from food-deprived rats demonstrated enhanced myocardial contractility (25). Chronic food deprivation has also been associated with slowing of relaxation (26) and prolongation of contraction times (25) in isometrically contracting papillary muscles. The results of the present study are consistent with previous studies in that food deprivation did not affect baseline cardiac function. However, in contrast to prior studies, we demonstrated a diminished response to calcium associated with protein–calorie undernutrition.

The mechanism of the diminished response of pyridoxine-deficient hearts to increasing concentrations of catecholamines is unclear. Both pyridoxine deficiency and food deprivation are associated with diminished inotropic responses to both isoproterenol and Ca^{2+}. The inotropic responses to Ca^{2+} are identical in the pyridoxine-deficient and pair-fed groups, implying that the food deprivation in both groups is the cause of the diminished calcium inotropic response. However, the diminished response to adrenergic stimulation is greater in the pyridoxine-deficient than in the food-deprived animals. Therefore, Ca^{2+} insensitivity associated with food deprivation may not completely account for the diminished inotropic response to catecholamines in the pyridoxine-deficient rats. Pyridoxine is a coenzyme for a variety of metabolic pathways: amino acid transamination, amino acid decarboxylation, porphyrin synthesis, and glycogen catabolism. Therefore, a defect in a metabolic pathway would provide an attractive explanation for the diminished ventricular responses we observed in the present study. However, when hearts are stimulated with either isoproterenol or increased Ca^{2+} concentrations, there are no differences among the levels of phosphocreatine and ATP in the hearts from the control, pair-fed, or pyridoxine-deficient rats. Although energy production was not measured directly, high energy phosphate levels do not differ in the pyridoxine-deficient group despite diminished function. The NMR methodology utilized in the present study has been demonstrated to reflect changes in high energy metabolic concentrations in a direct and correlative way (27, 28). Therefore, the maintenance of high energy phosphate levels in the presence of diminished function implies an abnormality in the contractile response which is not energy dependent.

The diminished response to isoproterenol in the pyridoxine-deficient hearts might be due

to an abnormality in excitation contraction coupling proximal to Ca^{2+} generated functions, including abnormal β receptor sensitivity or decreased adenylate cyclase activity. Although β receptor responsiveness has not been evaluated directly, Ceriani *et al.* (13) speculated that pyridoxine deficiency is associated with diminished adrenergic responsiveness. They demonstrated that the adrenergic sensitive action potential configuration is altered in pyridoxine-deficient hearts in a manner similar to that seen in β-adrenergic blockade (29). Further support for abnormal receptor activity is the finding that pyridoxine deficiency is associated with a decrease in dopamine receptor binding (30) and the fact that pyridoxine is a cofactor in the synthesis of phosphatidyl inositol (3), an important membrane component.

Hearts appear to adapt to stress by altering myosin isoenzyme patterns, resulting in a change in Ca^{2+}-activated myosin ATPase activity and shortening velocity (31–33). Such a change in protein–calorie malnutrition or in pyridoxine deficiency could also account for change in response to pharmacologic stress in this study.

The present study indicates that pyridoxine deficiency and food deprivation have no effect on baseline ventricular function. However, they do diminish the cardiac inotropic response to calcium stimulation and may decrease the inotropic response to isoproterenol. Functional changes were not associated with deficiencies in high energy phosphate levels. Therefore, the diminished contractility in both pyridoxine- and food-deprived animals is probably due to an abnormality in a nonenergy-limiting step in excitation–contraction coupling. Although the precise mechanisms whereby pyridoxine deficiency and food deprivation alter cardiac function remain obscure, both provide models for altered cardiac inotropy.

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