CARDIOVASCULAR EFFECTS OF EXERCISE IN HAMSTERS WITH EXPERIMENTAL THIAMINE DEFICIENCY

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The hemodynamic effects of daily treadmill exercise were evaluated in hamsters with experimental thiamine deficiency to test the hypothesis that increased energy consumption might be a contributory factor in the pathogenesis of beriberi heart disease.

Daily exercise enhanced thiamine deficiency and was manifested by earlier development of symptoms of neuropathy compared to non-exercised animals. Hemodynamics of exercised thiamine deficient animals were characterized by significantly lower O₂ consumption, lower cardiac output, and lower left ventricular minute work, compared to exercised, pair-fed control animals. Left ventricular end-diastolic pressure was slightly but not significantly higher in thiamine deficient animals. Left ventricular function, therefore, was depressed in this group. There was no evidence of hyperkinetic circulation, cardiomegaly or congestive heart failure.

Neuropathy and depressed ventricular function, characteristic of pure thiamine deficiency, were observed in the absence of high cardiac output or high output failure, the pathogenesis of which may require other unknown factors.

NUMEROUS attempts to produce beriberi heart disease by inducing thiamine deficiency in experimental animals have failed.¹⁻¹⁴ Whereas the pathophysiology of this disease has been well studied in human investigations,¹⁵⁻¹⁸

the etiology has not yet been clearly identified. Co-factors other than thiamine appear necessary for the development of beriberi heart disease. Consideration of the basic biochemical roles of thiamine, as well as clinical observations,¹⁹⁻²¹ have led to the hypothesis that increased energy consumption might be such a co-factor in beriberi heart disease. The present study was designed to test this hypothesis.

Key Words:

Beriberi heart disease,
High output heart failure
Left ventricular function

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MATERIALS AND METHODS

The thiamine deficient test diet, otherwise nutritionally well balanced, and the control diet, sufficient in thiamine but otherwise identical to the test diet, were obtained from Nutritional Biochemicals, Inc., Cleveland, Ohio. The thiamine content measured by the formalin-azo reaction method²² was 1 microgram/gm in the test diet and 15 microgram/gm in the control diet.

Sixty male LHC/LAK creamy hamsters (Lakeview Hamster Colony, Newfield, New Jersey), 10 weeks of age, were divided into five groups, and six or seven animals were caged together. The first group of animals (group 1) received the thiamine deficient test diet, the second group (group 2) was pair-fed the control diet in sufficient quantities to maintain them at the same body weight as group 1 animals, and the third group (group 3) was fed the control diet *ad libitum*. These three groups were exercised on a treadmill (Warren E. Collins, Inc., Braintree, Mass.) from the first day on the test diet. Each animal ran 300 meters per day at a rate of 10 meters per minute without electrical stimulation. The exercise was continued every day until group 1 animals showed specific signs of thiamine deficiency. The fourth group of animals (group 4) was given the control diet *ad libitum* and was not exercised. Body weights were measured twice a week. When group 1 animals became symptomatic, cardiovascular investigations were performed. The other 3 groups of animals were tested as soon as they had been subject to their respective diets as long as group 1. The fifth group of animals (group 5) was given the thiamine deficient test diet *ad libitum* and observed without exercise. In 3 symptomatic animals of this group, 2 mg of thiamine HCl were injected intraperitoneally.

The methods for electrocardiographic and hemodynamic studies have been described previously.^{14,23,24} Animals were anesthetized with sodium pentobarbital, 50 mg/kg intraperitoneally. The electrocardiogram was obtained as soon as the animals became anesthetized. Standard limb leads, right parasternal (V_1), and left mid-chest (V_4) leads were recorded by means of a specially constructed preamplifier connected to a polyrecorder (Hewlett Packard, Model 868) at a paper speed of 100 mm/sec. The trachea was intubated via tracheotomy, and the animals were ventilated mechanically with ambient air, using a rodent respirator (Model 680, Harvard Apparatus Co. Inc., Dover, Mass.). The left parasternal skin

was incised, and 26-gauge needles, connected to a fiberoptic pressure transducer via short (10 cm) polyethylene tubings (PE 90: i.d. 0.086 cm, o.d. 0.127 cm) were inserted into the right (RV) and left (LV) ventricular cavities under oscilloscopic pressure monitoring. The frequency response of the entire system was shown to be linear up to 100 Hz ("pop" test).

RV and LV pressures were recorded with high and low gain at a paper speed of 100 mm/sec. An electrocardiographic monitor lead was simultaneously recorded. The left ventricular pressure curve was differentiated continuously by a R-C differentiator, and the peak of the first derivative (dp/dt) was computed in terms of mmHg/sec.

0.2 ml of left ventricular blood was drawn for analyses of pO_2 , pCO_2 , and pH by a gas analyzer (Model 113, Instrumentation Laboratory, Boston, Mass.), and the ventilator was adjusted according to the results of these analyses. Expired air was collected in a rubber bag for 5 minutes, and during this period, 50 microliters of RV and then LV blood were withdrawn for the determination of O_2 content by micro Van Slyke Method. Expired air O_2 and CO_2 contents were analyzed by Scholander's method. Cardiac output (ml/min) was calculated by the direct Fick method. Stroke index (ml/gm) were expressed per gram of body weight. LV minute work was calculated by the formula: LV minute work (gm·m/min) = $CO \times (LVSM - LVEDP) \text{ (mmHg)} \times 13.6/1000$, where CO = cardiac output, LVSM = LV mean systolic pressure and LVEDP = LV end-diastolic pressure. After the hemodynamic study, the thoracic cage was opened, and whole blood was drawn from the right ventricle into a heparinized syringe for determination of red blood cell transketolase by the method of Brin et al.²⁵ Heart, lung, and liver were excised, blotted on dry gauze, and weighed on a Mettler balance (H54). The hearts were fixed with 10% buffered formalin, and an area free of puncture wounds was stained with hematoxylin and eosin, and Masson trichrome for study under light microscopy. Data were expressed as mean $\pm$ standard error of the mean ($Mn \pm SEM$), and Student's *t* test was used to compare groups.

RESULTS

All animals used in the study adapted quite well to exercise, as evidenced by the fact that none had to be electrically stimulated. Group 1 animals continued to exercise and appeared healthy until they developed neurological signs

Fig. 1. The effects of diet and exercise upon body weight. Group 1 (thiamine deficient) and group 2 (pair fed) animals lost weight ($p < 0.025$ and $p < 0.001$, respectively) and group 3 (ad lib fed) animals gained weight ($p < 0.01$) during 25 days of exercise. Group 4 (ad lib fed, non-exercised) animals also gained weight during this period ($p < 0.005$). A comparison of the growth curves of groups 3 and 4 indicates that exercise itself induces no deleterious effect upon growth.

Fig. 2. The effect of exercise upon body weight in thiamine deficiency. Horizontal axis expresses the days after the initiation of the deficient diet. Group 1 (thiamine deficient, exercised) animals maintained body weight initially, and developed symptoms by the 25th day of diet. Group 5 (thiamine deficient, non-exercised) animals developed symptoms from the 32nd to the 45th day of the diet. By day 45, all but 3 of the 8 animals in this group had died. The neurological symptoms of these 3 animals disappeared within 4 hours after thiamine HCl administration, and the animals regained weight rapidly.

Fig. 3. Red blood cell hemolysate transketolase (TK) activity and thiamine pyrophosphate (TPP) adding effect in thiamine deficiency. The significantly lower TK level in group 1 ($p < 0.025$ compared to all other groups) and higher TPP adding effect represent chemical evidence of thiamine deficiency.

on the 25th day. Peripheral neuropathy was manifested by limping, and some animals showed ataxia. Upon appearance of signs of thiamine

deficiency, the hemodynamic study was carried out.

The effects of diet and activity upon body

TABLE I EFFECTS OF EXERCISE ON ELECTROCARDIOGRAPHIC FINDINGS IN HAMSTERS WITH EXPERIMENTAL THIAMINE DEFICIENCY. (Mn $\pm$ SEM)

Group	No.	Heart Rate (beats/min)	P-R Interval (sec)	QRS Duration (sec)
1	10	418 $\pm$ 11	0.054 $\pm$ 0.001	0.028 $\pm$ 0.001
	<i>p</i>	NS	NS	NS
2	8	432 $\pm$ 10	0.055 $\pm$ 0.001	0.031 $\pm$ 0.001
3	8	502 $\pm$ 13	0.046 $\pm$ 0.001	0.023 $\pm$ 0.001
	<i>p</i>	< 0.01	< 0.005	< 0.005
4	10	438 $\pm$ 16	0.051 $\pm$ 0.001	0.029 $\pm$ 0.001

Group 1: exercised, thiamine deficient animals, Group 2: exercised, pair-fed control animals, Group 3: exercised, ad lib fed control animals, Group 4: non-exercised, ad lib fed control animals. Statistical analysis was made of the comparisons between groups 1 and 2, and group 3 and 4, employing Student's *t* test. NS: not significant.

TABLE II HEMODYNAMIC AND AUTOPSY FINDINGS IN EXERCISED HAMSTERS WITH EXPERIMENTAL THIAMINE DEFICIENCY. (Mn $\pm$ SEM)

Group	1		2		3		4	
No.	10	<i>p</i>	8		8	<i>p</i>	10	
RVSP (mmHg)	23 $\pm$ 3	NS	28 $\pm$ 2		34 $\pm$ 2	NS	32 $\pm$ 2	
EDP (mmHg)	1.5 $\pm$ 0.6	NS	3.4 $\pm$ 1.1		1.6 $\pm$ 0.6	NS	1.7 $\pm$ 0.5	
LVSP (mmHg)	111 $\pm$ 9	NS	108 $\pm$ 13		82 $\pm$ 5	NS	101 $\pm$ 9	
EDP (mmHg)	5.3 $\pm$ 1.0	NS	3.5 $\pm$ 0.7		3.2 $\pm$ 0.7	NS	4.4 $\pm$ 1.2	
dp/dt _{max} (mmHg/sec)	3552 $\pm$ 407	NS	3975 $\pm$ 793		2793 $\pm$ 357	NS	3797 $\pm$ 477	
O ₂ Consumption (ml/min)	0.91 $\pm$ 0.14	< 0.005	1.98 $\pm$ 0.26		1.98 $\pm$ 0.54	NS	1.49 $\pm$ 0.15	
A-V O ₂ Difference (vol%)	6.59 $\pm$ 0.84	NS	6.55 $\pm$ 1.13		6.92 $\pm$ 1.23	NS	8.36 $\pm$ 0.80	
Cardiac Output (ml/min)	17.14 $\pm$ 4.16	< 0.025	37.81 $\pm$ 7.63		32.10 $\pm$ 6.86	NS	19.14 $\pm$ 2.55	
Stroke Index $\times 10^{-4}$ (ml/gm)	6.57 $\pm$ 1.48	NS	14.79 $\pm$ 3.60		9.19 $\pm$ 2.27	NS	7.31 $\pm$ 1.28	
LV minute work (gm $\cdot$ m/min)	17.08 $\pm$ 5.00	< 0.05	37.41 $\pm$ 8.18		22.05 $\pm$ 3.89	NS	17.66 $\pm$ 3.20	
Heart Weight (mg)	315.6 $\pm$ 10.8	NS	269.9 $\pm$ 8.6		375.3 $\pm$ 4.1	< 0.001	314.8 $\pm$ 4.5	
Heart Weight/Body Weight $\times 10^{-4}$	39.7 $\pm$ 1.2	NS	38.9 $\pm$ 0.9		36.6 $\pm$ 0.7	NS	38.1 $\pm$ 0.9	

RVSP=right ventricular systolic pressure, EDP=end-diastolic pressure, LVSP=left ventricular systolic pressure, dp/dt_{max}=maximal rate of the left ventricular pressure rise, O₂=oxygen, A-V=arteriovenous, Group identifications: see foot note of Table I.

weight are shown in Figure 1 as a function of the duration of the diet. A paired *t* test analysis of each group, comparing body weight on day 1 of the diet with that on day 25, revealed that every group had undergone significant changes in weight. Both groups 1 and 2 had lost considerable weight, but their respective weights by day 25 did not differ significantly. Exercise alone was not seen to have a deleterious effect upon the animals, as judged from the weight curves of groups 3 and 4. As is shown in Figure 2, non-exercised thiamine deficient animals (group 5) developed signs from 1 to 3 weeks later than

exercised animals. Neurological manifestations progressed rapidly, and without treatment, death occurred in 3 to 5 days. In all 3 animals treated with thiamine HCl, neurological manifestations disappeared in 4 hours, and these animals regained weight rapidly.

Red blood cell transketolase activity and the effect of adding thiamine pyrophosphate are shown in Figure 3. The transketolase activity of group 1 was observed to be lower than both groups 2 and 3, and also lower than that of group 4. The thiamine pyrophosphate adding effect was observed to be greater than 15% for 6 out of

Fig.4. Evidence of depressed left ventricular function in exercised, thiamine deficient hamsters. Left ventricular (LV) minute work, as an index of LV performance, in group 1 (thiamine deficient, exercised) animals was significantly lower than that of group 2 (pair fed, exercised) animals ($p < 0.025$) at a slightly but non-significantly ($p < 0.2$) higher LV end-diastolic pressure, as an index of LV preload. The figure indicates that the left ventricles of thiamine deficient animals operate on the different function curve compared to the pair fed controls.

9 animals in group 1.

The resting EKG findings for all groups, determined prior to tracheotomy, are presented in Table I. The P-R intervals of groups 1 and 2 were not significantly different from each other, but they were significantly longer than those of groups 3 and 4. This delay in A-V conduction seems not to be specific to thiamine deficiency so much as to starvation in general. In looking at the effects of exercise alone, the exercised control group 3 had a higher resting heart rate ($p < 0.01$) and a shorter QRS duration ($p < 0.005$) than the non-exercised control group 4.

The ventricular pressures of all groups are given in Table II. There was a 78% success rate in the execution of this hemodynamic study. The two major technical problems included malpositioning of the needles in the cavities, necessitating repeated punctures of the myocardium, and inability to draw RV blood. Groups 1 and 2 showed no significant differences in RV and LV pressures and LV dp/dt. Similarly, groups 3 and 4 showed no differences in these parameters, and thus exercise was not observed to induce any specific pressure changes at rest.

The cardiac performance of each group is

indicated in Table II and Figure 4. The oxygen consumption of group 1 was 54% lower than that of group 2. The CO of group 1 was 56% lower than that of group 2, and its left ventricular minute work was 54% below that of group 2 animals. Figure 4 displays these results graphically. Group 1 appears to be operating on a different left ventricular function curve from the paired exercised control animals (group 2). In comparing the cardiac performance of groups 3 and 4, animals which had exercised (group 3) showed a trend toward an increase in O_2 consumption and cardiac output and a decrease in arteriovenous O_2 difference, compared to the unexercised control animals (group 4), but these differences are not significant statistically.

The autopsies summarized in Table II yielded no evidence for either right or left heart failure in the thiamine deficient animals. None had hepatosplenomegaly, ascites, pulmonary congestion or effusion, and the heart weight/body weight ratio did not differ significantly from the starvation group. The heart of all animals were within normal limits by gross and histological examination.

DISCUSSION

The clinical manifestations of beriberi heart disease range from severe peripheral edema and anasarca due to right ventricular failure to fulminant, often fatal, biventricular failure (Shoshin). The pathophysiology of this condition is characterized by peripheral vasodilatation and high output failure.¹⁵⁻¹⁸ Beriberi heart disease is more prominent in patients with mild neuropathy and quite rare in patients who have disabling neurological symptoms or who are bed-ridden. Shoshin especially is more frequent in otherwise healthy men and pregnant or lactating women.²¹ There has been good agreement that basal metabolic rate is normal or high in wet (cardiac type) and low in dry beriberi (neuropathic type).²¹

As thiamine is an essential co-enzyme of pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and transketolase for the formation of acetyl-CoA and succinyl-CoA, and use in the hexose monophosphate shunt, respectively, the deficiency of this vitamin results in disturbed glucose metabolism and blocked Krebs cycle. A decrease in the supply of NADH, and therefore ATP production from oxidative phosphorylation, enhances the Embden-Meyerhof pathway and causes further increases in pyruvate concentration. Hence, an increase in energy demand may

be expected to enhance thiamine deficiency. Therefore, it has been postulated that disabling neurological manifestations protect heart failure by reducing O_2 demand and cardiac load.^{19-21,26}

Numerous attempts have been made to produce this disease in experimental animal models. Electrocardiographic abnormalities,^{1,3} cardiac hypertrophy,^{3,5,7,10,12} myocardial necrosis and degeneration^{3,10,12} and electron microscopic alterations^{11,12} have been reported. Disturbances of myocardial metabolism in thiamine deficiency include diminished thiamine dependent enzyme activity,^{7,27} increase in tissue pyruvate concentration,^{1,2,12} and decrease in ATP concentration.^{4,9,11} Impairment of mechanical properties of myocardial muscle was observed in rats with severe thiamine deficiency in this laboratory,⁸ as well as by others.⁶ Recently, Phornphutkul et al¹³ reported normal myocardial contractility and increase in myocardial oxygen consumption and coronary blood flow in isolated perfused hearts of thiamine deficient rats. The evidence from these studies suggests that thiamine deficiency has a deleterious effect upon the myocardium. However, none of these experimental models appear comparable to human beriberi heart disease, and congestive heart failure has not been observed.

Little is known about the overall hemodynamic effect of thiamine deficiency in experimental animal models. Hackel et al² reported unchanged cardiac output and decreased arterial pressure in dogs with thiamine deficiency compared to ad lib fed controls. However, 3 starvation control animals manifested an elevated cardiac output, and their arterial pressures were between those of thiamine deficient and ad lib fed controls. Therefore, that model was not comparable to human beriberi heart disease. We investigated hemodynamic changes in Syrian golden hamsters after 21 days on a deficient diet.¹⁴ In that observation, none had clinical signs of thiamine deficiency, and cardiac performance was identical to that of the control group.

The current study was undertaken to investigate the hemodynamics of symptomatic thiamine deficient animals. Exercise was performed with the expectation of findings that the disease comparable to that of humans. No deleterious effects of exercise stress upon either the cardiovascular system or the animals' general condition were observed. Nor did exercise stress have any deleterious effects upon the thiamine deficient animals prior to the symptomatic stage. Com-

pared to non-exercised, deficient animals, they maintained good food intake and body weight. However, the exercised animals developed symptoms on or about day 25 of the diet, compared to 35 to 45 days on the diet for the same strain of animals without exercise.

The cardiac performance in thiamine deficient animals was characterized by low O_2 consumption and low cardiac output compared to paired controls. Whereas there were no differences in pressures and/or the contractility index (dp/dt), left ventricular function was slightly impaired in this group compared to starvation animals. This was manifested by significantly lowered LV work at slightly higher LV filling pressure (Figure 4). Autopsy showed no evidences suggestive of congestive heart failure, such as hepatomegaly, cardiomegaly, or pulmonary congestion.

Once again clinical beriberi heart disease in experimental animals could not be produced with thiamine deficiency, although there was evidence for mild left ventricular dysfunction. Persistence of low cardiac output biventricular heart failure after successful treatment of high output failure with thiamine in alcoholic patients with beriberi heart disease has been reported in the literature,^{2,8} and there could be arguments that these animals have passed through the stage of high output and they were too sick when hemodynamic study was performed. The deficient animals had maintained good dietary intake, general conditions, and exercise performance until one day prior to the study, however, and we are sure that we did not miss the stage of high output failure in this observation. Low cardiac output could be attributed primarily to low O_2 consumption. Low O_2 consumption found in this study is in agreement with observations in patients with neuropathy without heart failure.²¹ It may be attributed to interference with NADH production, resulting in lack of substrate and impaired electron transport.

The inability of previous investigators to produce heart failure in experimental animals by means of thiamine deficient diets could have been attributed to marked decrease in bodily activity secondary to peripheral neuropathy and lethargy. This explanation cannot be applied to the present investigation. Thiamine deficient animals maintained daily activity until they became symptomatic from neurological disturbances. Our observations coincide with two old experiments reported in the literature. Fowls fed

polished rice developed symptoms earlier when they were exercised,²⁹ and in exercised dogs with thiamine deficiency, neuropathy was observed earlier than control animals without cardiomegaly.³⁰

There was no evidence to suggest peripheral vasodilatation in this study. The peripheral vasodilatation observed in human beriberi is reversible with administration of thiamine,^{16-18,31} and therefore we must accept that this phenomenon is thiamine mediated.

Although beriberi heart disease was produced in human experiments with "vitamin B deficiency" in the 1920's and 30's,³²⁻³⁴ it has never been produced in experimental animals with pure "thiamine deficiency". Peripheral vasodilatation and high cardiac output may be species specific effects of thiamine deficiency. However, it would be reasonable to consider the possibility of as yet unknown cofactor(s) in the pathogenesis of peripheral vasodilatation.

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