Gene-environment interactions in wet beriberi: effects of thiamine depletion in CD36-defect rats

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Submitted 27 February 2003; accepted in final form 22 May 2003

THIAMINE DEFICIENCY results in a broad spectrum of clinical manifestations ranging from “dry” beriberi (neuropathy and/or Wernicke-Korsakoff syndrome) to “wet” or cardiovascular beriberi. Wet beriberi, which is characterized by edema, is widespread among impoverished people in Southeast Asia, and is thus also known as oriental beriberi (1). Many patients with wet beriberi also exhibit cardiac involvement, i.e., beriberi heart disease. The high incidence of this disorder in these areas has been speculated in part to relate to the tropical climate and in part to a prevalence of lower vascular tone and lower sodium intake in Asia (4). To date, no comprehensive explanation for this selective susceptibility in Asia has been provided, and neither theoretical nor experimental data are known.

Mammals are not capable of endogenously synthesizing thiamine. Thiamine functions as an indispensable cofactor of the mitochondrial enzyme pyruvate dehydrogenase complex (PDHC), a committed step for glucose oxidation, i.e., the conversion of pyruvate into acetyl-coenzymeA (CoA), a substrate of the tricarboxylic acid (TCA) cycle, which supplies the thermodynamic driving force to generate ATP through oxidative phosphorylation. Thus a constant supply of exogenous thiamine from the diet is essential to maintain cellular function.

Fatty acids also contribute to the production of acetyl-CoA through β-oxidation. Thus in tissues that rely on glucose as the principal energy resource, such as the brain and nerve cells, thiamine depletion could result in critical consequences on their cellular function. On the other hand, the consequences of thiamine deficiency would likely be less severe in tissues that use a variety of energy substrates.

Muscles use a variety of energy substrates, with the oxidation of long-chain fatty acids (LCFAs), providing the majority of the energy requirements in some tissues, particularly in the heart (25). Therefore, in the setting of thiamine depletion, acetyl-CoA can still be produced in these cells, through β-oxidation of LCFAs. However, if the cellular availability of LCFAs was also constrained in these cells, thiamine depletion could have a severe impact on their metabolic and cellular function, which, in turn, may lead to the development of wet or cardiovascular beriberi.
LCFAs enter into several tissue cell types via both simple diffusion and protein-mediated processes (9, 19). CD36, also called fatty acid translocase (2), is one of the LCFA transporters expressed in the heart and in skeletal muscle. CD36 deficiency has been demonstrated to result in a defect of myocardial LCFA uptake in both humans and rodents (13, 18, 22, 35–37), which is counterbalanced by an increase of glucose uptake (14, 18). Humans with CD36 deficiency are found predominantly in Southeast Asia, where there is an estimated prevalence of ∼0.3% (21, 35). It is interesting to note that an overlap between the high prevalence of CD36 deficiency and the incidence of wet beriberi exists in Southeast Asia. Therefore, we hypothesized that the CD36 defect contributes to an underlying predisposition towards wet beriberi.

A defect of CD36 has previously been demonstrated in spontaneously hypertensive rats (SHR) by genetic linkage (3). This strain of rat exhibits severe uptake defects of LCFA and a rather enhanced uptake of glucose in the heart and skeletal muscle, similar to human CD36 deficiency (18). Therefore, by using CD36-defective rats (SHR/NCrj), we designed this study to test the hypothesis that the heart becomes vulnerable to thiamine deficiency when the substrate shifts from LCFA to glucose.

METHODS

Animals and rat chows. Three cognate strains of rats were purchased from two breeding suppliers [SHR/NCrj and Wistar-Kyoto (WKY)/NCrj from Charles River Japan and SHR/Izm from Japan SLC, respectively]. Normal rat chow (MM-3) and thiamine-deprived chow (AIN93G B1 deficient) were obtained from KEARI and Oriental Yeast, respectively.

The rats were housed in cages with the floor made of metal wire, at a population density of three per cage, and were allowed free access to normal or thiamine-deprived chow and water. The impact of thiamine deficiency was observed after 2 wk of treatment with the thiamine-deprived chow. Cages were placed in a temperature-controlled (26°C) environment with a 12:12-h light:dark cycle.

In this study, we addressed the issue of the impact of thiamine deficiency in two ways. In one series of experiments, we investigated the effects of thiamine-deficient chow on three cognate rat strains, i.e., SHR/NCrj, WKY/NCrj, and SHR/Izm. Three strains of rats were divided into two groups of six each. It is well established that adult rats of SHR/NCrj and SHR/Izm are hypertensive. Therefore, to eliminate the cardiovascular effects of high blood pressure, treatment with thiamine-deficient chow was started in 4-wk-old rats. In this series of experiments, we could not obtain sufficient hemodynamic data, including cardiac catheterization, due to technical limitations of performing cardiac catheterization in these smaller animals (i.e., 4–6 wk). Thus in another series of experiments, we used rats of a larger body weight, i.e., 10-wk-old SHR/NCrj rats, for hemodynamic and biochemical studies. These rats were treated in the same manner as the younger rats described above. Furthermore, the effect of thiamine resupplementation after 2 wk of thiamine deprivation was investigated by administration of thiamine hydrochloride (25 mg/kg body wt ip) dissolved in water for 3 days.

The animal experimentation protocol was approved by the institutional Animal Care and Use Committee of the Osaka Medical College Laboratory Animal Center (approval no. 262).

Analysis of genomic DNA. Genomic DNA was isolated from tail samples with the use of a QIAamp DNA Mini Kit (QIA-GEN; Hilden, Germany) by following the manufacturer’s protocol. Genomic DNA samples were analyzed on the region crossing the intron 14/exon 15 boundary with the use of the primers DelF and DelR, according to the method of Glazier et al. (15). The PCR products underwent electrophoresis on 10% acrylamide gels, stained with ethidium bromide, and were visualized with the use of UV light.

Echocardiography and pressure measurements. The animals were lightly anesthetized with pentobarbital sodium (1 mg/kg body wt ip). Two-dimensional guided M-mode echocardiography was performed with the use of an echocardiogram equipped with a 10-MHz transducer (Vivid 5, General Electronic). The heart was imaged in a two-dimensional mode in a parasternal short-axis view with a depth setting of 2 cm. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and posterior wall of the left ventricle (LV) at the level of the papillary muscles. An M-mode image was obtained at a sweep speed of 100 mm/s. LV end-diastolic dimension (Dd), LV end-systolic dimension (Ds), diastolic wall thickness of the interventricular septum (IVSd), and posterior wall thickness of the LV (PWd) were measured. To obtain the hemodynamic parameters in detail, Doppler recordings and measurements of the proximal ascending aortic diameter were performed on 12-wk-old SHR/NCrj rats. All measurements were performed from leading edge to leading edge according to the American Society of Echocardiography guidelines (28). The cross-sectional area of the aorta was calculated assuming a constant circular lumen throughout the cardiac cycle. Stroke volume was calculated on the basis of pulse wave aortic Doppler recordings, temporal integration of the Doppler velocities known as the time velocity integral (TVI) and the diameter of the proximal ascending aorta. This transthoracic echocardiographic method and pulsed Doppler at the level of the aortic annulus have been reported to accurately assess the cardiac output in rats and to show the best correlation, least bias, and best precision with thermodilution (32). Heart rate was calculated from the ECG. ECG signals were recorded with three subcutaneous brass electrodes connected to an EGG signal transducer.

After echocardiographic studies on the 12-wk-old SHR/NCrj rats, aortic and LV pressure were measured with the use of a microtip catheter transducer (model SPR 407 2F, Millar Instruments; Houston, TX), inserted in the right carotid artery and advanced to the LV. The following cardiovascular parameters were monitored in the 12-wk-old SHR/NCrj rats: aortic systolic (Ao syst), diastolic pressure (Ao diast), LV peak systolic pressure, LV end-diastolic pressure, heart rate (HR), LV contractility, i.e., peak rate of ventricular pressure rise (+dP/dt) and fall (−dP/dt), and ECG. Hemodynamic parameters, including systemic vascular resistance (SVR), were calculated as follows

\[
\text{CSA} = \frac{(\text{diameter of aorta}/2)^2 \times \pi}{80}
\]

\[
\text{SV} = \text{CSA} \times \text{TVI}
\]

\[
\text{CO} = \text{SV} \times \text{HR}
\]

Mean aortic pressure = [(Ao syst − Ao diast)/3 + Ao diast]

SVR = mean aortic pressure/CO \times 80

FS = [(Dd − Ds)/Dd] \times 100

h = (IVSd + PWd)/2
where SV is stroke volume, CO is cardiac output, CSA is the cross-sectional area of the aorta, FS is fractional shortening, and h is the mean of wall thickness.

Tissue preparation for histopathological examination. On completion of the echocardiographic studies, 6-wk-old rats were euthanized with an overdose of pentobarbital sodium. After the echocardiographic and/or hemodynamic data on 12-wk-old SHR/NCrj rats were acquired, blood samples for biochemical determination were obtained by cardiac puncture, and the animals were then euthanized with an overdose of pentobarbital sodium. The heart, lungs, and liver from 6-wk-old SHR/NCrj, WKY/NCrj, and SHR/Izm rats and 12-wk-old SHR/NCrj rats were excised and weighed. Small portions of the tissues, obtained from 12-wk-old SHR/NCrj rats, were dried overnight at 75°C and dry weight-to-wet weight ratios were assessed. Tissue weight was normalized relative to body weight (for 6- and 12-wk-old rats) and tibial length (for 12-wk-old rats) for the heart [tissue weight (mg)-to-body weight (g), tissue weight-to-body wt, and heart weight (mg)-to-tibial length (mm)] ratios. The tissues, obtained from all groups, were fixed in 10% buffered formalin overnight and embedded in paraffin, followed by staining with hematoxylin and eosin. Transverse cardiomyocyte diameter (100 cardiomyocytes/rat) was measured in sections from 6-wk-old SHR/NCrj rat hearts, and the average value of its diameter in each rat was compared between two groups (n = 6 and 6, respectively), as previously described (24).

Statistical analysis. The results for each parameter were averaged and are expressed as means ± SD. Comparisons between groups were performed with unpaired t-test; P values of <0.05 were considered significant.

RESULTS

PCR analysis of genomic DNA. PCR-amplified fragments were obtained with the use of the primers DelF and DelR, electrophoresed on a 10% polyacrylamide gel, and stained with ethidium bromide (Fig. 1). The PCR products from genomic DNA of SHR/Izm and WKY/NCrj showed the presence of both the transcribed copy (178 bp) and the untranscribed copy (170 bp) of exon 15 of CD36. On the other hand, those from SHR/NCrj rats showed only the presence of the transcribed copy (178 bp) and the untranscribed copy (170 bp). These findings from the PCR analysis of genomic DNA are consistent with the report of Glazier et al. (15). Thus genomic DNA analysis confirmed that CD36 was deficient in SHR/NCrj but not in SHR/Izm and WKY/NCrj rats.

Effects of thiamine-deficient chow on body and organ morphometrics. The effects of thiamine-deficient chow on body weight and organ morphometrics in three inbred strains of younger rats, i.e., 6-wk-old SHR/NCrj, WKY/NCrj and SHR/Izm, are presented in Table 1.

Thiamine deprivation for 2 wk did not result in reduced activity nor grossly altered appearance (ruffled fur, abnormal gait, or occasionally labored breathing) in any of the three strains of rat. Surprisingly, however, the changes in body weight with thiamine-deprived chow compared with normal chow differed.

Table 1. Morphometric effects of B1 deficiency on SHR/NCrj, WKY/NCrj, and SHR/Izm

<table>
<thead>
<tr>
<th></th>
<th>SHR/NCrj</th>
<th>B1 deficient</th>
<th>SHR/Izm</th>
<th>Control</th>
<th>B1 deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>64.7 ± 2.1</td>
<td>63.0 ± 2.8</td>
<td>77.3 ± 1.0</td>
<td>78.3 ± 3.1</td>
<td>83.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>144.2 ± 6.4</td>
<td>149.0 ± 7.2‡</td>
<td>145.0 ± 2.4</td>
<td>142.3 ± 6.7</td>
<td>171.7 ± 16.9</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>579 ± 38</td>
<td>687 ± 39*</td>
<td>499 ± 42</td>
<td>594 ± 46*</td>
<td>615 ± 34</td>
</tr>
<tr>
<td>Heart/body ratio</td>
<td>4.00 ± 0.23</td>
<td>4.69 ± 0.28*</td>
<td>3.51 ± 0.33</td>
<td>4.12 ± 0.18*</td>
<td>3.88 ± 0.26</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>11.108 ± 0.526</td>
<td>12.152 ± 0.427‡</td>
<td>985 ± 19</td>
<td>975 ± 12</td>
<td>972 ± 2</td>
</tr>
<tr>
<td>Lung, mg</td>
<td>1,028 ± 55</td>
<td>1,157 ± 106‡</td>
<td>38,54 ± 93*</td>
<td>49,00 ± 1,94</td>
<td>46,95 ± 1,89</td>
</tr>
<tr>
<td>Liver, mg</td>
<td>6,882 ± 460</td>
<td>7,179 ± 715</td>
<td>6,287 ± 52</td>
<td>5,553 ± 336*</td>
<td>7,773 ± 52</td>
</tr>
<tr>
<td>Liver/body ratio</td>
<td>47.66 ± 3.43</td>
<td>48.12 ± 3.23</td>
<td>44.18 ± 0.60</td>
<td>38.54 ± 93*</td>
<td>49.00 ± 1,94</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 rats in each group. SHR, spontaneously hypertensive; WKY, Wistar-Kyoto; diameter, transverse cardiomyocyte diameter; ratios, organ weight (mg)/body weight (g). *P < 0.001, ‡P < 0.005, §P < 0.05.

AJP-Heart Circ Physiol • VOL 285 • OCTOBER 2003 • www.ajpheart.org
quite strikingly between rats of the SHR/NCrj strain and those of the WKY/NCrj and SHR/Izm strains.

Figure 2 illustrates the gain of body weight during the 2 wk of thiamine deprivation in the three rat strains. No changes in the body weight were observed as a result of thiamine-deficient treatment in the WKY/NCrj and SHR/Izm rats, a finding that is consistent with previous studies using rodent models (11, 27). By contrast, throughout the 2-wk treatment, a thiamine-deficient chow diet resulted in an increased body weight in the SHR/NCrj rats, compared with those with normal rat chow.

Treatment with thiamine-deprived chow resulted in a significant increase in the heart weight and the ratio of heart weight to body weight in all three strains (Table 1). On the other hand, the effects of thiamine-deprived chow on the weight of lungs and liver differed between those of the SHR/NCrj rats and those of the WKY/NCrj and SHR/Izm rats.

Treatment with thiamine-deprived chow resulted in a significant increase of the lung weight in SHR/NCrj rats, but not in WKY/NCrj and SHR/Izm rats. The weight of the liver exhibited a tendency to increase with thiamine deficiency in SHR/NCrj rats, but rather to decrease in the WKY/NCrj and SHIr/Imz rats; however, this finding was not statistically significant, except for the WKY/NCrj rats (Table 1).

The findings of increased lung weight with thiamine deficiency in SHR/NCrj rats, but not in either WKY/NCrj or SHR/Izm rats, led us to examine the morphological changes of the lung.

Effects of thiamine-deficient chow on lung morphology. Microscopic findings of the hematoxylin and eosin-stained lungs from 6-wk-old SHR/NCrj, SHR/Izm, and WKY/NCrj rats are shown in Fig. 3. Microscopic examination of the lungs demonstrated that thiamine-deprived chow resulted in no discernible histological changes in WKY/NCrj and SHR/Izm rats. In sharp contrast, thiamine-deprived chow resulted in pronounced changes of lung histology in the SHR/NCrj rats: 1) medial thickening of the pulmonary arteries extending over long distances, muscularization of the arterioles, and arterialization of the veins to resemble pulmonary arteries; 2) fluid accumulation in the perivascular and alveolar spaces and edema in the alveolar walls; and 3) intravascular and intraalveolar stasis of the blood. These histological changes demonstrated substantial variation in severity but were characteristically observed to extend over the lungs. These histological changes observed in the lungs from thiamine-deficient SHR/NCrj rats were consistent with the findings of the increased fluid permeability or congestive vasculopathy of the lung, supporting the finding of increased lung weight in SHR/NCrj rats.

Echocardiographic studies demonstrated no significant findings between normal and thiamine-deficient rats in the three strains examined (data not shown). We could not perform the appropriate hemodynamic studies, including cardiac catheterization, in 6-wk-old rats. However, the increased body weight and morphological changes of the lungs observed only in SHR/NCrj rats strongly suggested the particular effects of thiamine deficiency on SHR/NCrj rats, i.e., indicative of wet or cardiac beriberi. We subsequently analyzed the effects of thiamine deprivation and thiamine resupplementation on hemodynamic and biochemical parameters in 12-wk-old SHR/NCrj rats.

Effects of thiamine deficiency and resupplementation on body and organ morphometrics and biochemical indexes in SHR/NCrj rats. The effects of thiamine deficiency and resupplementation on body and organ morphometrics of 12-wk-old SHR/NCrj rats are shown in Table 2. The intake of chow was significantly decreased in thiamine-deprived rats 3 days after the commencement of treatment, a finding that was consistent with previous studies that used rodent models (11, 27). Nevertheless, the body weight increased significantly in thiamine-deficient rats compared with normal rats. The weights of the heart, lung, and liver were also significantly increased in thiamine-deficient 12-wk-old SHR/NCrj rats, which demonstrated the reproducibility of the thiamine-deprived effects on morphometrics observed in 6-wk-old SHR/NCrj rats.

The increase in heart weight was different from those of the lungs and liver. The dry weight-to-wet weight ratio of heart did not differ between control and thiamine-deficient SHR/NCrj rats. On the other hand, the dry weight-to-wet weight ratios of the lung and liver were significantly reduced in thiamine-deficient SHR/NCrj rats compared with control rats, which in-

![Fig. 2. Chronological changes in body weight in spontaneously hypertensive (SHR)/NCrj, SHR/Izm, and Wistar-Kyoto (WKY)/NCrj rats. ○, Normal chow group; ●, thiamine deficiency group. Values are expressed as means ± SD. **P < 0.0001; *P < 0.02.](http://ajpheart.physiology.org/)
dicated the fluid retention in liver and lungs, a finding also supported by the morphological changes of lung in thiamine-deficient SHR/NCrj rats.

Blood biochemical indexes of thiamine-deficient 12-wk-old SHR/NCrj rats indicated the occurrence of lactic acidemia, which returned to control levels after thiamine resupplementation. Thiamine resupplementation resulted in significant reduction of liver weight and increase of liver dry weight-to-wet weight ratio, and a trend toward the reduction of lung weight and the increase of lung dry weight-to-wet weight ratio, which was not altered significantly in the heart. Thus we considered that the effects of thiamine deficiency on 12-wk-old SHR/NCrj rats resulted in hypertrophy of the heart, which was supported by the greater diameter of the cardiomyocytes in thiamine-deficient 6-wk-old SHR/NCrj rats (Table 1), and fluid retention in the lungs and liver.

Effects of thiamine deficiency and resupplementation on hemodynamics and biochemical indexes of SHR/NCrj rats. The effects of thiamine deficiency and resupplementation on hemodynamics of 12-wk-old SHR/NCrj rats are shown in Table 3. Thiamine deprivation for 2 wk did not affect systolic blood pressure. However, compared with control SHR/NCrj rats, the heart rate and TVI of thiamine-deficient SHR/NCrj rats increased significantly. Significant reduction of systemic vascular resistance and significant increase of cardiac output and stroke volume were evident in thiamine-deficient SHR/NCrj rats. LV end-diastolic pressure increased slightly, but significantly, in thiamine-deficient SHR/NCrj rats. Peak +dP/dt and peak −dP/dt of thiamine-deficient SHR/NCrj rats were significantly larger than those of controls, which could partly reflect the tachycardia and higher LV end-diastolic pressure in thiamine-deficient SHR/NCrj rats. Collectively,
these findings indicated that reduced systemic vascular resistance accompanied with high cardiac output and a hyperdynamic cardiac state occurred in thiamine-deficient SHR/NCrj rats. Hemodynamic parameters showed a tendency to return control levels but were not statistically significant. Taken together, these findings strongly suggested the reproduction of human wet or cardiovascular beriberi in SHR/NCrj rats.

**DISCUSSION**

Thiamine deprivation for 2 wk, which was rather shorter than the past studies, e.g., 5 wk in past studies (11, 27), brought about distinct results in CD36-defect rats (SHR/NCrj) compared with CD36-nondefect rats (WKY/NCrj and SHR/Izm). The experimental period of 2 wk was selected because a state of severe thiamine depletion emerged on a strict thiamine-deficient diet for 18 days (31). Despite the short duration of thiamine-deficient treatment, SHR/NCrj rats, but neither SHR/Izm nor WKY/NCrj rats, demonstrated increased weight of body, liver, and lungs and the histological derangement of the lungs. The biochemical and hemodynamic parameters of thiamine-deficient SHR/NCrj rats demonstrated the characteristics of human wet or cardiovascular beriberi, i.e., lactic acidemia, peripheral vasodilatation with high cardiac output and hyperdynamic state of the heart, and fluid retention of the lungs and liver. Thiamine resupplementation demonstrated the tendency to return toward, but not complete restoration of, normal biochemical and hemodynamic conditions.

Many authors (11, 27, 30) have demonstrated that it is not possible to reproduce the typical pathophysiology of human wet or cardiovascular beriberi in rats. Nev-

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**Table 2. Morphometrical and biochemical effects of B1 deficiency on SHR/NCrj**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>B1 Deficiency (n = 6)</th>
<th>P Value vs. Control</th>
<th>B1 Treatment (n = 6)</th>
<th>P Value vs. B1 deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>246.5 ± 20.3</td>
<td>244.6 ± 11.7</td>
<td>0.5826</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>280.8 ± 9.1</td>
<td>305.6 ± 6.2</td>
<td>&lt;0.0001</td>
<td>311.3 ± 4.3</td>
<td>0.4363</td>
</tr>
<tr>
<td>Intake, g/day</td>
<td>25.3 ± 0.2</td>
<td>21.3 ± 1.3</td>
<td>&lt;0.0001</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Heart, mg</td>
<td>951 ± 4</td>
<td>1,089 ± 44</td>
<td>0.0003</td>
<td>1,087 ± 26</td>
<td>0.9374</td>
</tr>
<tr>
<td>Heart/body weight, mg/g</td>
<td>3.40 ± 0.14</td>
<td>3.55 ± 0.15</td>
<td>0.0984</td>
<td>3.51 ± 0.78</td>
<td>0.6597</td>
</tr>
<tr>
<td>Heart/rib length, mm</td>
<td>28.22 ± 1.31</td>
<td>32.41 ± 1.31</td>
<td>0.0002</td>
<td>32.16 ± 0.77</td>
<td>0.9281</td>
</tr>
<tr>
<td>Dry/wet ratio</td>
<td>0.218 ± 0.004</td>
<td>0.220 ± 0.006</td>
<td>0.5995</td>
<td>0.222 ± 0.026</td>
<td>0.7342</td>
</tr>
<tr>
<td>Lung, mg</td>
<td>1.435 ± 56</td>
<td>1.555 ± 59</td>
<td>0.0047</td>
<td>1.498 ± 24</td>
<td>0.3048</td>
</tr>
<tr>
<td>Dry/wet ratio</td>
<td>0.183 ± 0.005</td>
<td>0.173 ± 0.005</td>
<td>0.0073</td>
<td>0.180 ± 0.003</td>
<td>0.0734</td>
</tr>
<tr>
<td>Liver, mg</td>
<td>10,792 ± 396</td>
<td>11,683 ± 447</td>
<td>0.0044</td>
<td>11,133 ± 384</td>
<td>0.0456</td>
</tr>
<tr>
<td>Dry/wet ratio</td>
<td>0.337 ± 0.008</td>
<td>0.320 ± 0.006</td>
<td>0.0027</td>
<td>0.330 ± 0.006</td>
<td>0.0209</td>
</tr>
<tr>
<td>pH</td>
<td>7.443 ± 0.016</td>
<td>7.322 ± 0.026</td>
<td>&lt;0.0001</td>
<td>7.412 ± 0.018</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>1.20 ± 0.11</td>
<td>2.42 ± 0.28</td>
<td>&lt;0.0001</td>
<td>1.25 ± 0.19</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. ND, not determined.

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**Table 3. Echocardiographic and hemodynamic effects of B1 deficiency on SHR/NCrj**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>B1 Deficiency (n = 6)</th>
<th>P Value vs. Control</th>
<th>B1 Treatment (n = 6)</th>
<th>P Value vs. B1 deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak LV pressure, mmHg</td>
<td>138 ± 12</td>
<td>139 ± 14</td>
<td>0.8977</td>
<td>146 ± 18</td>
<td>0.4712</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>325.1 ± 17.3</td>
<td>372.2 ± 16.7</td>
<td>&lt;0.0001</td>
<td>366.9 ± 8.7</td>
<td>0.4517</td>
</tr>
<tr>
<td>Dp, mm</td>
<td>6.46 ± 0.48</td>
<td>6.40 ± 0.84</td>
<td>0.8794</td>
<td>6.91 ± 0.19</td>
<td>0.1410</td>
</tr>
<tr>
<td>Ds, mm</td>
<td>3.08 ± 0.46</td>
<td>2.88 ± 0.56</td>
<td>0.4707</td>
<td>3.60 ± 0.21</td>
<td>0.0055</td>
</tr>
<tr>
<td>FS, %</td>
<td>52.5 ± 3.6</td>
<td>55.2 ± 4.4</td>
<td>0.2314</td>
<td>47.9 ± 2.6</td>
<td>0.0015</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>1.285 ± 0.13</td>
<td>1.485 ± 0.109</td>
<td>0.0037</td>
<td>1.459 ± 0.116</td>
<td>0.4233</td>
</tr>
<tr>
<td>LV Pwst, mm</td>
<td>1.338 ± 0.043</td>
<td>1.453 ± 0.195</td>
<td>0.1833</td>
<td>1.424 ± 0.086</td>
<td>0.7155</td>
</tr>
<tr>
<td>l, mm</td>
<td>1.312 ± 0.074</td>
<td>1.474 ± 0.095</td>
<td>0.0029</td>
<td>1.437 ± 0.079</td>
<td>0.4039</td>
</tr>
<tr>
<td>Ao, mm</td>
<td>3.85 ± 0.055</td>
<td>3.90 ± 0.082</td>
<td>0.2071</td>
<td>3.90 ± 0.10</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>TVI, cm</td>
<td>3.478 ± 0.481</td>
<td>4.614 ± 0.536</td>
<td>0.0008</td>
<td>3.968 ± 0.428</td>
<td>0.0183</td>
</tr>
<tr>
<td>Output, ml/min</td>
<td>135.4 ± 27.2</td>
<td>186.0 ± 24.7</td>
<td>0.0019</td>
<td>171.1 ± 13.4</td>
<td>0.4039</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>0.416 ± 0.073</td>
<td>0.500 ± 0.069</td>
<td>0.0357</td>
<td>0.485 ± 0.042</td>
<td>0.5714</td>
</tr>
<tr>
<td>Aovent, mmHg</td>
<td>135.3 ± 15.8</td>
<td>139.0 ± 14.2</td>
<td>0.8516</td>
<td>146.0 ± 18.0</td>
<td>0.4712</td>
</tr>
<tr>
<td>Aovent/mass, mmHg</td>
<td>83.0 ± 4.5</td>
<td>86.0 ± 9.7</td>
<td>0.5084</td>
<td>91.0 ± 10.3</td>
<td>0.4063</td>
</tr>
<tr>
<td>Mean Ao pressure, mmHg</td>
<td>100.4 ± 7.5</td>
<td>103.7 ± 10.7</td>
<td>0.5603</td>
<td>109.3 ± 12.5</td>
<td>0.4195</td>
</tr>
<tr>
<td>SVR, dyn s/cm⁻⁵ (×10⁴)</td>
<td>6.55 ± 1.36</td>
<td>6.41 ± 0.42</td>
<td>0.0076</td>
<td>5.12 ± 2.52</td>
<td>0.0856</td>
</tr>
<tr>
<td>LV end systolic, mmHg</td>
<td>5.33 ± 1.506</td>
<td>9.000 ± 1.673</td>
<td>0.0158</td>
<td>6.667 ± 1.633</td>
<td>0.0346</td>
</tr>
<tr>
<td>Peak ± dP/dt, mmHg/s</td>
<td>5,646 ± 709.7</td>
<td>10,343 ± 587.5</td>
<td>&lt;0.0001</td>
<td>9,200 ± 609.8</td>
<td>0.1320</td>
</tr>
<tr>
<td>Peak ± dP/dt, mmHg/s</td>
<td>2,698 ± 394.9</td>
<td>5,790 ± 484.6</td>
<td>&lt;0.0001</td>
<td>4,873 ± 500.3</td>
<td>0.0091</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. LV, left ventricle; Dp, diastolic dimension of LV cavity; Ds, systolic dimension of LV cavity; FS, fractional shortening; IVSd, interventricular septum dimension; Pwst, posterior wall dimension; l, mean of IVSd; and Pwst; Ao, proximal ascending aortic diameter; TVI, the time velocity integral; Aovent, systolic pressure of aorta; Aovent/mass, diastolic pressure of aorta; mean Ao pressure, mean aortic pressure; SVR, systemic vascular resistance; LV end systolic pressure; dP/dt, rate of change of LV pressure.
ertheless, because of the vulnerability of SHR/NCrj rats to thiamine deficiency as discussed below, we believe that thiamine-deficient SHR/NCrj rats recapitulate the pathophysiology of human wet or cardiovascular beriberi.

In this study, the effects of thiamine deprivation were investigated in three cognate rat strains, i.e., SHR/NCrj, WKY/NCrj, and SHR/Izm rats. SHR were derived from their normotensive progenitor strain of WKY. SHR/NCrj and SHR/Izm were both derived from their hypertensive progenitor strain of SHR. Thus their genetic backgrounds are very close. However, SHR/NCr strain rats have been shown to possess a defective CD36 gene (3). In contrast, no CD36 mutation has been demonstrated in the original SHR (16). The loss of the transcribed copy of exon 15 of CD36 in SHR/NCrj rats and the presence of both the transcribed copy and the untranscribed copy of CD36 exon 15 in both SHR/Izm and WKY/NCrj rats were confirmed by genomic analysis (Fig. 1).

CD36 is known to exhibit diverse cellular functions (17), one of which is to facilitate transport of LCFAs in the heart and in skeletal muscle (9). CD36 defect results in the shift of energy substrates from LCFAs to glucose in the heart (13, 14, 18). The myocytes of SHR/NCrj rats, but not of SHR/Izm and WKY/NCrj rats, thus rely primarily on glucose as their energy substrate. PDHC is a key step in the generation of acetyl-CoA from glucose, and thiamine is its obligatory cofactor. It is therefore not surprising that the blockade of PDHC in SHR/NCrj rats leads to severe depletion of acetyl-CoA without alternative exogenous sources of acetyl-CoA and, moreover, to the concomitant accumulation of its upstream metabolites. Thus the alteration of intracellular metabolism could affect cellular function and may lead to the development of wet or cardiovascular beriberi. Indeed, adenosine accumulation due to the decreased conversion to ATP has been speculated to result in the leakage of adenosine from the peripheral tissues, leading to systemic vasodilatation (5).

In this study, deterioration of the cardiac function, i.e., cardiovascular collapse and death, known as “shoshin” beriberi, was not observed. Beriberi heart disease is described to be due to severe thiamine deficiency persisting for at least 3 mo (8). Thus, less involvement of the heart might be, at least partially, explained by the short experimental duration under cool, dry, and sedentary conditions. Alternatively, the defect in myocardial LCPA uptake in SHR/NCrj rats is less pronounced than that in humans with CD36 deficiency [70% defect in humans (14) vs. 25% defect in the SHR (18)].

Interestingly, but unexpectedly, thiamine deficiency brought about cardiac hypertrophy in all three strains of SHR/NCrj, IShI/Izm, and WKY/NCrj rats. Although the mechanism(s) of cardiac hypertrophy in altered myocardial glucose metabolism remain(s) unknown, a mismatch of glucose metabolism, i.e., low coupling of glucose oxidation to glycolysis, may be responsible for this finding. In the setting of thiamine deficiency, i.e., blockade of PDHC by thiamine deficiency, theoretically a significant amount of glucose may shunt away from glucose oxidation to the hexosamine pathway, which may, in turn, generate abundant levels of its end product, UDP-β-N-acetylglucosamine and UDP-N-acetylglucosamine. UDP-β-N-acetylglucosamine is known to modulate some regulator proteins, including transcriptional factors, through glycosylation modification (23, 40). This series of reactions may result in the initiation of cardiac hypertrophy. A mismatch of glucose metabolism, frequently observed in hypertrophied hearts, has been reported not to result from a reduction in PDHC activity or its subunit expression; thus as yet unknown mechanism(s) may be responsible (26). Thus the cardiac hypertrophy observed in this study may suggest that a mismatch of glucose metabolism is not a consequence of cardiac hypertrophy but rather an initiator of cardiac hypertrophy. Further studies are needed to elucidate the mechanism(s) relevant to cardiac hypertrophy in thiamine deficiency.

Clinical implications. The shift of a major myocardial energy substrate from LCFAs to glucose has been demonstrated in the hypertrophied and failing heart (6, 10, 12, 20). Thus not only a genetic defect of CD36 but also the pathological heart is vulnerable to thiamine deficiency. In the present day, thiamine deficiency is considered to be a disease of the past, but any status of suboptimal thiamine levels, even well above those causing deficiency syndromes, is easily encountered. First, thiamine, a water-soluble vitamin, is easily lost into the urine as a result of diuretics (29, 34, 39, 41). Indeed, thiamine deficiency is demonstrated in 21% of patients with congestive heart failure who take loop diuretics (7). Second, thiamine is synthesized by intestinal bacteria. Therefore, antibiotic therapies are particularly at risk of attenuating thiamine levels. Third, parenteral nutrition without adequate supplementation of thiamine results in thiamine deficiency (38). Thus it is likely that deficient or suboptimal levels of thiamine are not as rare as it may seem (33). In the context of shifting the substrate metabolism from lipid to carbohydrate, we believe thiamine deficiency is not only historically important but that it remains a concern of the present.

In summary, the genetic defect of LCPA uptake in CD36 deficiency is a potential underlying pathophysiology for the pathogenesis of wet or cardiovascular beriberi. In the present study, we provide the first direct in vivo evidence of a close gene-environment interaction involved in the genesis of cardiovascular beriberi.

REFERENCES

3. Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majali KM, Trembling PM, Mann CJ,


