Increased glycolytic metabolism in cardiac hypertrophy and congestive failure

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Bishop, Sanford P., and Ruth A. Altschuld. Increased glycolytic metabolism in cardiac hypertrophy and congestive failure. Am. J. Physiol. 218(1): 153-159. 1970.—Lactate dehydrogenase isozymes and potential for glycolytic metabolism in vitro were studied in right and left ventricles of 11 dogs with experimental right ventricular hypertrophy (RVH) and congestive heart failure (CHF), 8 dogs with acquired RVH and CHF due to Dirofilaria immitis, and 13 controls. Disc gel electrophoresis revealed that muscle-type (anaerobic) lactate dehydrogenase (M-LDH) was significantly increased in the hypertrophied right ventricles, and there was a lesser, but significant, increase of M-LDH in the left ventricle. Lactate production was significantly increased after 30-min anaerobic incubation of hypertrophied right ventricular homogenates compared to control right ventricular homogenates. Left ventricular homogenates from the D. immitis group produced more lactate than control group left ventricles. Lactate production by left ventricles from dogs with experimentally produced RVH and CHF was not significantly different from control values. Glucose utilization by anaerobic homogenates was increased for both ventricles in the D. immitis group. These findings are interpreted as evidence for increased potential of the glycolytic pathways for energy production in the hypertrophied failing myocardium.

METHODS

Animal preparation. Healthy 1- to 3-year-old dogs weighing 14-23 kg were used for experimental procedures. The animals were fed a cereal type dog food (Ken-L-Ration, Quaker Oats Company), and their weight did not change appreciably during the course of the experiment except for an increase associated with the development of ascites.

Progressive pulmonary artery stenosis was produced in 11 dogs by means of a rubber Jacobson cuff surgically implanted around the main pulmonary artery (6). With the animal under methoxyflurane anesthesia, the pulmonary artery was progressively narrowed at biweekly intervals by percutaneous injection of fluid into a connecting bulb located subcutaneously. Right ventricular pressure was monitored during this procedure using a 15-gauge polyethylene catheter attached to a Statham P23AA transducer and displayed on a direct-writing oscillograph. The right ventricle progressively increased in size, as determined by a qualitative increase in heart size and wall thickness on thoracic radiographs and cineangiograms, the appearance of a right ventricular hypertrophy (RVH) pattern on the electrocardiogram (13), and increased right ventricular mass determined at necropsy. The experiment was terminated 55-80 days following surgery, when the dogs were in advanced congestive heart failure as determined by the presence of the following signs: venous distention, palpable liver enlargement, ascites, an observed decrease in exercise tolerance, and an elevation of right ventricular end diastolic pressure above 7 mm Hg.

The control group consisted of nine normal dogs without thoracic surgery and four dogs that had undergone a sham thoracotomy and pulmonary artery dissection 6-10 weeks previously. Since no functional or biochemical differences were found between the unoperated and sham-operated animals, they were considered as one group.

The third group consisted of eight dogs with spontaneously acquired RVH and CHF secondary to infestation with the canine heartworm Dirofilaria immitis. Animals in this group had signs of cardiopulmonary insufficiency for 2 months to over 2 years. The diagnosis was confirmed by the demonstration of typically dilated pulmonary arteries by cineangiogram, a right ventricular hypertrophy pattern on the electrocardiogram (13), elevated mean pulmonary artery and right ventricular systolic and end-diastolic pressure, presence of D. immitis microfilaria in the peripheral blood, presence of adult parasites in the right ventricle and pulmonary artery at necropsy, and micro-
scopic demonstration of obstructing vascular lesions in the pulmonary arteries and arterioles.

The dogs were killed by a 15-sec application of 110-v, 60-cycle electricity via clip electrodes attached to the head and base of the tail of the unanesthetized dog. Within 1 min the nonbeating heart was removed and washed in ice-cold 0.25 M sucrose. The heart was dissected and total weight, right ventricular free-wall weight, and combined left ventricle and septum weight were recorded. Necropsy examination was performed on all dogs, and tissues from each body organ were fixed in 10 % buffered formalin. Representative samples of major body organs were embedded in paraffin, sectioned at 6 µ, and stained with hematoxylin and cosin and Gomori aldehyde-fuchsin-trichrome.

Preparation of homogenates. Immediately after death, approximately 3 g of a full-thickness sample of right and left ventricular free wall were freed of epicardium, endocardium, visible blood vessels, connective tissue, and adipose tissue. The tissues were then weighed, minced, and forced through a chilled stainless steel tissue press. The tissue minces were homogenized with 9 volumes of iced 0.154 M KCl using 15 strokes of a motor-driven Teflon pestle in a Potter-Elvehjem glass homogenizer.

In some early experiments additional samples of right and left ventricular free wall were homogenized in pH 7.4, 0.1 M phosphate buffer 0.25 M sucrose, and these homogenates were used for determination of lactate dehydrogenase isozymes. A comparison study revealed no differences between isozyme patterns prepared from sucrose or KCl homogenates, and in later experiments all homogenates were prepared with 0.154 M KCl.

Determination of glycolytic activity. Glycolytic activity was measured by injecting 1.5 ml right or left ventricular homogenate into stoppered bottles containing 6 ml modified LePage buffer (29), pH 7.6, which had been equilibrated at 37 °C with 95 % N2, 5 % CO2. Final concentration of the incubation mixture in millimoles per liter was K2HPO4, 3; nicotinamide, 50; MgCl2, 8.3; NAD, 0.25; KCl, 60; ADP, 4.2; glucose, 12.5; and 0.1 mg/ml type III yeast hexokinase (Sigma).

Samples were withdrawn at 0, 15, and 30 min of incubation and immediately deproteinized with an equal volume of chilled 0.6 M perechloric acid. Lactate was determined by the method of Scholz et al. (30). Glucose concentration of the homogenates was determined by a glucose oxidase method (Glucostat, Worthington Biochemical Corp.).

Determination of lactate dehydrogenase (LDH) isozymes. The 10 % homogenate was centrifuged at 100,000 × g for 1 hr and the clear supernatant used for total LDH enzyme activity and electrophoretic separation of the isozymes within 6 hr. Neutralized reduced glutathione, 10 mg/ml, was added to the supernatant to increase stability of the enzyme (12). Protein was determined by the biuret method and total lactate dehydrogenase activity by the method of Wroblewski and LaDue (41). The reaction was started by the addition of pyruvate (3.3 × 10⁻⁴ M final concentration) to the cuvette at 30 °C and the oxidation of NADH followed at 340 nm with either a Cary 15 or Beckman DU spectrophotometer with Gilford recorder.

Electrophoresis was performed at 4 °C using a discontinuous polyacrylamide gel system according to a modified method of Davis (11) with a constant voltage supply power source (Canalco) at 5 ma. An amount of sample containing 20 Wroblewski-LaDue units of enzyme was placed in 0.2 ml of a large-pore 2.5 % polyacrylamide sample gel, pH 6.8. This was polymerized on top of a column of 5 % polyacrylamide running gel, pH 8.9. A sample of liver was run concurrently to ensure that slower moving heat-sensitive isozymes were retained.

After the completion of the electrophoretic separation, usually 40-80 min, the gels were removed from the glass columns and stained according to the tetrazolium method of Goldberg and Cather (15). Gels were scanned with a Densitord recording densitometer with integrator attachment (Photovolt Corporation) using a 595-nm filter. The percentage of each isozyme was determined directly from the integrator marks. The relative amounts of heart-type (H-LDH) and muscle-type (M-LDH) isozyme were calculated from the isozyme percentages using the ratio of H-LDH and M-LDH subunits present in each isozyme as previously described (3, 8, 24). Values reported are the mean of two or more gels for each sample.

Statistical analysis was made by the Student t test utilizing a 5 % level of significance.

RESULTS

Animal characterization. The increase in right ventricular free-wall weight of dogs with both experimental and naturally occurring RVH is compared with the control animals in Fig. 1. The mean value for the experimental animals is similar to that for dogs with naturally acquired RVH, although there is more variability in the latter group. Combined left ventricle and septum-to-body weight ratios were not altered. Right ventricular systolic and end-diastolic
pressures are presented in Fig. 2; mean values are similar for both groups with RVH, with more variability in the group infested with heartworms.

Histologic examination revealed chronic passive congestion of the liver in all dogs with RVH and CHF. Mesenteric lymph nodes were also congested, and congestion of the renal medulla was occasionally found. The lungs of the dogs infected with D. immitis had proliferative and degenerative lesions of the pulmonary vessels and parenchyma similar to those which have been described previously (1). In the hypertrophied right ventricles a slight increase in interstitial connective tissue was sometimes found; this was evident in trichrome-stained sections as a barely perceptible diffuse thickening of the interstitium. Occasionally, focal areas of fibrosis up to 1 mm diameter were found. In others, no increase in fibrous connective tissue was detected histologically. No increase in connective tissue was evident in left ventricles.

**LDH isozyme activity.** Total LDH activity and LDH isozyme distribution in the left and right ventricles are shown in Table 1. Although total LDH activity varied considerably from one animal to another, left and right ventricles contained similar amounts in any individual dog, and there were no statistically significant differences between the experimental groups and the control group. The results for total LDH activity were similar whether related to wet weight of tissue or to protein in the supernatant.

Distribution of LDH isozymes in control dog left and right ventricles was similar (Table 1 and Fig. 3). LDH-1, the most rapidly migrating isozyme, exhibited the greatest enzyme activity, LDH-2 somewhat less, and LDH-3 was very weak. LDH-4 and -5 were not found in normal dog hearts. In both groups of dogs with RVH and CHF there was a relative decrease in LDH-1 activity and an increase in the activity of LDH-2 and -3, with the occasional appearance of LDH-4 in the right ventricle (Table 1 and Fig. 1). The LDH isozyme distribution in the left ventricle from dogs with RVH and CHF was more like the control dog left ventricle, but nevertheless had a significant isozyme shift toward those isozymes containing M-LDH.

The amount of M-LDH in the control dogs, when calculated as a percentage of total LDH II activity, averaged 11.4 ± 0.56 (SE) % and 10.6 ± 0.54 % in the right and left ventricles, respectively (Fig. 5). In both experimental and naturally occurring RVH and CHF there was a significant increase to 19.2 ± 0.92 % and 19.1 ± 1.44 % M-LDH (P < .001), respectively, in the right ventricles. The left ventricles had a lesser increase to 13.6 ± 0.76 and 13.6 ± 1.43 % M-LDH (P < .01).

**Anaerobic glycolytic metabolism.** The production of lactate during 30-min anaerobic incubation by the right ventricular homogenates was significantly increased from a control group mean of 2.53 ± 0.58 mM/liter to 4.05 ± 0.54 and 5.26 ± 0.90 mM/liter in the experimental and heartworm RVH groups, respectively (Fig. 6). Left ventricular homogenates from control dogs consistently produced more lactate than the corresponding right ventricular homogenate. Although lactate production by left ventricular homogenates from dogs with experimental RVH and CHF was not different from that produced by control dog left ventricles.

### Table 1. Total lactate dehydrogenase activity and isozyme distribution in ventricles of dogs with right ventricular hypertrophy and failure

<table>
<thead>
<tr>
<th>Units LDH/g Tissue, ×10⁶</th>
<th>Isozyme, %</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Control (14)</td>
<td></td>
<td>247</td>
<td>±21</td>
<td>56.6</td>
<td>41.4</td>
<td>2.0</td>
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<tr>
<td></td>
<td></td>
<td>275</td>
<td>±25</td>
<td>59.0</td>
<td>39.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Experimental RVH and CHF (11)</td>
<td></td>
<td>968</td>
<td>±20</td>
<td>52.4</td>
<td>58.3</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>951</td>
<td>±25</td>
<td>52.4</td>
<td>58.3</td>
<td>9.3</td>
</tr>
<tr>
<td>P &gt; .05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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<tr>
<td>Ditotilariosis RVH and CHF (8)</td>
<td></td>
<td>245</td>
<td>±27</td>
<td>53.5</td>
<td>58.5</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>257</td>
<td>±29</td>
<td>44.6</td>
<td>47.8</td>
<td>7.6</td>
</tr>
<tr>
<td>P &gt; .05</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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Values are means ± se. Numbers in parentheses are numbers of animals in each group.
ventricular homogenates, left ventricular homogenates from dogs with heartworm RVH and CHF produced 6.25 ± 0.68 mm/liter lactate compared to 3.97 ± 0.39/liter for the controls (P < .05).

The utilization of glucose by left and right ventricular homogenates under anaerobic conditions is shown in Table 2. Glucose utilization was slightly, but not significantly, increased in the experimentally hypertrophied right ventricle. Utilization was significantly increased compared to controls in both ventricles of the dogs with the more chronic, naturally occurring RVH and CHF secondary to infestation with *D. immitis*.

**FIG. 3.** Lactate dehydrogenase isozyme distribution in left and right ventricles of a normal control dog. Denstometer recordings and polyacrylamide gels stained for lactate dehydrogenase. Anodal end of gels is at bottom of tubes.

**FIG. 4.** Lactate dehydrogenase isozyme distribution in left and right ventricles of a dog with experimental RVH and CHF. In right ventricle there is a relative increase in LDH-2 and -3

**FIG. 5.** Relative percentage of muscle-type lactate dehydrogenase in right and left ventricles of control dogs and those with experimental and naturally acquired RVH and CHF.

**FIG. 6.** Lactate production by anaerobically incubated left and right ventricular homogenates of control dogs and those with experimental and naturally acquired RVH and CHF.

**TABLE 2.** Glucose utilization during 30 min anaerobic incubation of heart homogenates

<table>
<thead>
<tr>
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<th>Right Ventricle, mm/liter</th>
<th>Left Ventricle, mm/liter</th>
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<tbody>
<tr>
<td>Control</td>
<td>4.9 ± 0.57</td>
<td>6.5 ± 0.51</td>
</tr>
<tr>
<td>Experimental RVH</td>
<td>5.9 ± 0.55</td>
<td>5.6 ± 0.94</td>
</tr>
<tr>
<td>Heartworm RVH</td>
<td>7.1 ± 0.25*</td>
<td>8.3 ± 0.63*</td>
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*P < .05 when compared to control.

**DISCUSSION**

The occurrence of an increased proportion of M-LDH in the LDH isozyme pattern of hypertrophied ventricles as shown in this study is indirect evidence in support of the hypothesis that cellular hypoxia may be present in hypertrophied myocardium. The association of M-LDH with anaerobic metabolism is based on several observations. Tissues which are highly dependent on aerobic metabolism such as heart and brain have a preponderance of those
LDH isozymes composed primarily of the heart-type subunit (37). Tissues such as white skeletal muscle and uterine smooth muscle which function well under anaerobic conditions contain the electrophoretically slower moving LDH isozymes composed of muscle-type subunit (8, 37). Decreasing the oxygen availability in cultured liver cells (17), chick embryos (22), or in rabbit renal cortex (35) results in a redistribution of LDH isozymes toward those isozymes containing M-LDH. These findings suggest that cells are capable of adapting their LDH isozyme content in response to environmental variations in oxygen availability. Based on this experimental evidence in other tissues, it is attractive to suggest that the increased proportion of M-LDH found in hypertrophied myocardium is a consequence of decreased oxygen in the enlarged myocardial cell. It should be pointed out, however, that certain mammalian tissues utilizing anaerobic metabolism such as erythrocytes, platelets, and bovine lens fibers contain isozymes with predominantly the heart-type subunit (36). In addition, certain fish are able to function with only one isozyme in both heart and skeletal muscle that appears to be homologous to LDH-5 of mammals (25). These discrepancies with the theory relating LDH isozyme function with a metabolic role have yet to be explained.

Due to the difficulty in measuring cellular levels of oxygen in the working myocardium, there is no direct experimental evidence concerning the availability of oxygen at the cellular level in myocardial hypertrophy. Attempts to produce LDH isozyme redistribution by altering the oxygen and blood supply to the myocardium have yielded conflicting results. Animals made hypoxic by exposure to simulated high altitudes have an increase in myocardial M-LDH (23), whereas coronary artery stenosis to produce myocardial ischemia does not result in a shift in myocardial LDH isozymes in the absence of myocardial fibrosis (14). Although reported studies suggest that a decreased level of oxygen is present in the subendocardial layers of the normal and stressed left ventricle (19, 26), no difference was found in LDH isozyme distribution in either left or right ventricular subendocardial or subepicardial myocardium of dogs with right ventricular hypertrophy (14). In contrast to the present study, Schwartz and Lee (39) found decreased anaerobic glyceraldehyde phosphate dehydrogenase in the myocardium of patients who died with congestive heart failure, lending further support for increased activity of the glycolytic pathway in this condition. In contrast to the present study, Schwartz and Lee (31) found decreased anaerobic evidence for increased glycolytic metabolism in the hypertrophied failing myocardium because of changes observed in the nonhypertrophied left ventricle of both groups of dogs with RVH and CHF. The change in isozyme distribution in the nonhypertrophied left ventricle found in our study differs from a recent report in which no significant change in LDH isozyme distribution was found in the left ventricle of dogs with experimental RVH (14). The increased proportion of M-LDH in the nonhypertrophied left ventricles found in all dogs with RVH in the present study and increased glucose utilization and lactate production by left ventricular homogenates from dogs with the more chronic RVH resulting from D. immitis infestation indicate that the nonhypertrophied ventricle is affected by hypertrophy in the opposite ventricle. This phenomenon has also been noted with respect to decreased catecholamines (33), decreased myofibrillar adenosine triphosphatase activity (9), morphologically altered Z bands (38), increased hydroxyproline content (7), and increased cell length (20) in nonstressed left ventricles from animals with right ventricular hypertrophy. The increased proportion of M-LDH in nonhypertrophied left ventricles in this study suggests that factors other than local cellular hypoxia may be responsible for isozyme redistribution. Hydroxyproline content is elevated in both ventricles of cats with experimental RVH (9), presumably the result of increased fibroblastic activity in the myocardium. Although the possibility that increased fibroblasts may be responsible for the increased proportion of M-LDH in hypertrophied hearts cannot be completely discounted, on the basis of histologic examination this appears unlikely. Only small amounts of increased connective tissue were found in hypertrophied right ventricles. There was no apparent correlation between those hearts with histologically evident increased connective tissue and LDH isozyme redistribution. The left ventricles did not have histologically demonstrable increased connective tissue.

The finding of an increased rate of lactate production and glucose uptake by anaerobically incubated homogenates of hypertrophied ventricles is additional evidence suggesting that hypertrophied myocardium utilizes anaerobic glycolytic metabolism to a greater degree than normal myocardium. Although there is no general agreement, several studies have suggested that mitochondria isolated from hypertrophied failing myocardium have normal respiratory control and oxidative phosphorylation (10, 27, 32, 34) and that normal levels of high-energy phosphates are present (10, 26). In the afterloaded heart, mitochondrial energy-producing mechanisms may nevertheless be inadequate to meet increased energy requirements. Our findings suggest that in the hypertrophied myocardium the oxidative mechanisms of energy production are augmented through utilization of the more inefficient glycolytic pathway.

Few studies have been concerned with glycolytic metabolism in cardiac hypertrophy and congestive failure. Gudbjarnason and associates (16) found increased activity of glyceraldehyde phosphate dehydrogenase in the myocardium of patients who died with congestive heart failure, lending further support for increased activity of the glycolytic pathway in this condition. In contrast to the present study, Schwartz and Lee (31) found decreased anaerobic
lactate production and glucose utilization in the cell-free supernatant of homogenized failing guinea pig ventricles. A possible explanation for this discrepancy resides in the fact that different systems were employed to measure anaerobic glycolysis. Schwartz and Lee incubated the homogenates with ATP and glucose, whereas ADP, glucose, and hexokinase were used in the present study. Only the reactions subsequent to the formation of glucose 6-phosphate are dependent on the activity of heart homogenate enzymes when ADP, glucose, and exogenous hexokinase are present. With this system as used in the present study, ADP is continuously regenerated, ATP concentrations are maintained at a low level, and the inhibition of phosphofructokinase by high concentrations of ATP is avoided.

Glucose phosphorylation is not rate limiting in this system, as shown by the fact that glucose 6-phosphate accumulates during incubation. Following 15 min of incubation the glucose 6-phosphate concentration averaged 2.47 mm compared to 4.31 mm after 30 min of incubation. There were no significant differences between control and hypertrophied myocardium. The ATP necessary for phosphorylation of glucose is generated initially via the myokinase reaction (2 ADP + ATP + AMP) and subsequently from the myokinase activity and as a product of anaerobic glycolysis. 

Further evidence in support of using glucose, ADP, and hexokinase rather than glucose and ATP to measure anaerobic glycolysis has recently been presented (2). Lactic acid production by heart homogenates incubated with glucose and hexokinase was greater than that obtained by incubating with an equal amount of glucose 6-phosphate. Moreover, lactic acid production from fructose-1,6-diphosphate was less than that from glucose and hexokinase or glucose 6-phosphate. The inclusion of an ATP consuming system (2-deoxyglucose plus hexokinase) greatly enhanced lactic acid production from glucose 6-phosphate and fructose-1,6-diphosphate. It would appear that continual removal of ATP is necessary for a maximal rate of anaerobic glycolysis.

Wittels and Spann (39) recently reported that in cardiac hypertrophy and congegative failure in the guinea pig there is defective oxidation of fatty acids associated with decreased levels of carnitine necessary for transport of fatty acids into mitochondria. They found that although fatty acid oxidation was decreased, glucose oxidation was not impaired in homogenates of hypertrophied failing guinea pig ventricles. They suggested, in agreement with the present study, that the failing heart may be forced to utilize pathways other than fatty acid oxidation for ATP production. Further experimental evidence is required to define the mechanisms involved in the LDH isozyme redistribution and increased capacity for in vitro glycolytic metabolism demonstrated in the hypertrophied dog heart.

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