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Reversible pulmonary trunk banding. VI: Glucose-6-phosphate dehydrogenase activity in rapid ventricular hypertrophy in young goats

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Objective: Increased myocardial glucose-6-phosphate dehydrogenase (G6PD) activity occurs in heart failure. This study compared G6PD activity in 2 protocols of right ventricle (RV) systolic overload in young goats.

Methods: Twenty-seven goats were separated into 3 groups: sham (no overload), continuous (continuous systolic overload), and intermittent (four 12-hour periods of systolic overload paired with a 12-hour resting period). During a 96-hour protocol, systolic overload was adjusted to achieve a 0.7 RV/aortic pressure ratio. Echocardiographic and hemodynamic evaluations were performed before and after systolic overload every day postoperatively. After the study period, the animals were humanely killed for morphologic and G6PD tissue activity assessment.

Results: A 92.1% and 46.5% increase occurred in RV and septal mass, respectively, in the intermittent group compared with the sham group; continuous systolic overload resulted in a 37.2% increase in septal mass. A worsening RV myocardial performance index occurred in the continuous group at 72 hours and 96 hours, compared with the sham ($P < .039$) and intermittent groups at the end of the protocol ($P < .001$). Compared with the sham group, RV G6PD activity was elevated 130.1% in the continuous group ($P = .012$) and 39.8% in the intermittent group ($P = .764$).

Conclusions: Continuous systolic overload for ventricle retraining causes RV dysfunction and upregulation of myocardial G6PD activity, which can elevate levels of free radicals by NADPH oxidase, an important mechanism in the pathophysiology of heart failure. Intermittent systolic overload promotes a more efficient RV hypertrophy, with better preservation of myocardial performance and and less exposure to hypertrophic triggers. (J Thorac Cardiovasc Surg 2011;142:1108-13)

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Traditional pulmonary artery banding (PAB) aimed at ventricular retraining causes an abrupt and fixed systolic overload. Although clinical studies have proved that PAB induces myocardial hypertrophy, it is frequently preceded by ventricular dysfunction. Therefore, an adaptation period with inotropic support is generally required. Most important, previous studies have demonstrated myocardial edema and necrosis in hearts that experience abrupt systolic overload, followed by late ventricular failure.[1]

However, it is essential to understand the molecular mechanisms involved in PAB-induced myocardial hypertrophy to establish training protocols that lead to a desirable ''physiologic hypertrophy'' versus a deleterious ''pathologic hypertrophy.'' Because a known shift occurs in energy substrate use in favor of glucose in pathologic conditions, energy metabolism might be altered in PAB ventricular retraining protocols.[2] In addition, recent experimental studies have linked an unbalanced oxidative and reductive process to a variety of diseases, such as atherosclerosis and heart failure.[3]

Glucose 6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme that commits glucose to the pentose phosphate pathway, is mainly responsible for the generation of nicotinamide adenine dinucleotide phosphate (NADPH) and ribose 5-phosphate, an essential precursor of the de novo synthesis of RNA and DNA. G6PD-derived NADPH, a cofactor for glutathione and thioredoxin reductase, preserves reducing potentials and protects the cell from

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Abbreviations and Acronyms

G6P = glucose-6-phosphate
G6PD = glucose 6-phosphate dehydrogenase
LV = left ventricle
$NADP^+$ = oxidized nicotinamide adenine dinucleotide phosphate
NADPH = nicotinamide adenine dinucleotide phosphate
PA = pulmonary artery
PAB = pulmonary artery banding
RV = right ventricle

oxidative stress in normal conditions.[4] In human diseases, G6PD can be either activated or inhibited; however, evidence has emerged that the overexpression and activation of G6PD enhances NADPH oxidase–derived superoxide generation and increases oxidative stress in diseases like diabetes, heart failure, and hypertension.[5]

In regard to rapid ventricular training, it would be of great interest to study myocardial energy metabolism in response to different cardiac hypertrophy models and its relationship to heart function.[6] The main objective of this study was to compare the G6PD activity in 2 right ventricle (RV) training protocols through an adjustable PAB system.

METHODS

Twenty-seven young goats, aged between 30 and 60 days and of comparable weight ($P = .38$), were split into 3 groups: sham (n = 7; weight, 11.93 ± 2.67 kg), continuous (n = 9; weight, 10.74 ± 2.62 kg), and intermittent (n = 11; weight, 10.25 ± 2.20 kg). All animals received humane care in compliance with the guidelines established by the Brazilian regulations for animal experimentation. The protocol was reviewed and approved by the Ethics Committee for Research Protocols at the University of São Paulo Medical School.

Surgical Procedure

All operations were performed with the goats under general anesthesia (pentobarbital sodium 5 mg/kg intravenously and ketamine 20 mg/kg intramuscularly) and through a left lateral thoracotomy. The lung was retracted laterally to allow exposure of the right ventricular outflow tract, pulmonary trunk, and descending aorta. A 17-gauge heparinized catheter was inserted into each of these structures, and its corresponding pressures were measured at specific time intervals during the entire study. The adjustable PAB system (SILIMED; Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda, Rio de Janeiro, Brazil) was implanted just beyond the pulmonary valve and sutured at the adventitia of the pulmonary trunk, as previously described (Figure E1).[7] Antibiotics (cefazolin 500 mg and gentamicin 40 mg) were administered daily during the study, as were digoxin (0.04 mg/kg) and subcutaneous heparin (5000 U).

Training Protocol

RV systolic overload was initiated 72 hours postoperatively. Baseline hemodynamic data (RV, pulmonary artery [PA], and aortic pressures) were collected in a conscious, immobilized animal with the adjustable banding system deflated. Blood pressure measurements were obtained through computer software (ACQknowledge 3.01; Biopac Systems, Inc, Goleta, Calif). Then, the banding system was adjusted to achieve an RV/aortic pressure ratio of 0.7, limited by a 10% drop in systolic blood pressure. Adjustments were made just once, every morning throughout the protocol, by percutaneous injection of saline solution with a 3-mL syringe, under sterile conditions. That rule was violated in case of the latter occurring or if there were agitation, dyspnea, arrhythmia, or a combination of these. The banding system was then deflated up to a tolerable point.

Continuous Group Protocol

The animals remained with continuous systolic overload for 96 hours, with daily assessment to keep the RV/aortic pressure ratio at 0.7. Hemodynamic data were collected once a day (mornings) during PAB readjustments.

Intermittent Group Protocol

The animals underwent 4 daytime periods of RV 12-hour systolic overload, alternating with a 12-hour nighttime resting period. Hemodynamic data were collected twice a day (every 12 hours) during PAB readjustments.

Sham Group Protocol

The PAB system was maintained deflated during the entire protocol. Hemodynamic data were collected daily (mornings).

Echocardiography

A single experienced observer conducted the echocardiographic examination with the animals under light sedation (ketamine 15 mg intramuscularly) approximately 120 hours before the beginning of the protocol and daily thereafter until the end of the protocol. Image acquisition was obtained through 7.5-MHz and 2.5-MHz multifrequency transducers (Acuson Cypress Echocardiology System, Siemens, Erlagen, Germany). The following echocardiographic parameters were studied: left ventricle (LV), RV, and septal wall thicknesses, RV end-diastolic volume, and myocardial performance index.

Morphology

Animals were humanely killed after 96 hours of the study protocol. Cardiac samples were drawn from the RV, LV, and ventricular septum just before cardiac arrest. These samples were immediately frozen at –80°C (Forma Scientific Inc, Marietta, Ohio) to be subsequently analyzed for G6PD activity. The pericardial fat, both atria, and semilunar valves were dissected from the heart; RV, LV, and ventricular septum were separated by the Fulton technique, individually weighed (METTLER AE-200; Mettler-Toledo AG, Greifensee, Switzerland), and indexed to each animal's body weight.[8]

Water content was obtained individually in each cardiac chamber by subtracting the collected sample weight at autopsy from the weight of the dehydrated chamber (70 hours at 60°C). Values were obtained as a percentage of weight change.

G6PD Activity

Tissue samples were homogenized in extraction buffer (proportion 1:5 weight/volume). The material was stored in ice and homogenized for 30 seconds using Polytron (PT 3100; Kinematica AG, Littau-Lucerne, Switzerland) at maximum speed and centrifuging (15 kg, 15 minutes, 4°C) to separate from cell remnants. Enzymatic activity was analyzed using the supernatant of the last centrifugation. Proteins were quantified with the protein assay kit BCA (PIERCE Biotechnology, Rockford, Ill). Results are expressed as nmol min^{-1} mg^{-1} of protein. The extraction buffer for G6PD contained Tris-HCl (50 mmol/L) and ethylenediaminetetraacetic acid (1 mmol/L), with a pH of 8. The assay buffer (270 μL/sample) was Tris-HCl (8.6 mmol/L), $MgCl_2$ (6.9 mmol/L), (oxidized nicotinamide