# Original Article

# Experimental myocardial infarction and increased oxidative stress in the rat diaphragm*

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## ABSTRACT

**Objective:** To use an experimental model to evaluate the effect of heart failure on oxidative stress in the rat diaphragm. **Methods:** The model of myocardial infarction was developed through left coronary artery ligation. On day 42 after coronary artery ligation, the animals were killed, after which the diaphragms were collected and homogenized. Oxidative stress was evaluated in diaphragm homogenates through measurement of lipid peroxidation and assays of the activity of antioxidant enzymes, including catalase and glutathione peroxidase (enzymes that reduce hydrogen peroxide to water), as well as superoxide dismutase (an antioxidant enzyme that reduces superoxide anions to hydrogen peroxide). **Results:** The coronary artery ligation model was found to be effective in causing heart failure. In the animals submitted to coronary artery ligation, the mean infarcted area of the left ventricle was 39%. Lipid peroxidation was 217% greater in the diaphragms of ligated animals than in those of controls. The activity of catalase and glutathione peroxidase was 77% and 20% lower, respectively, in study rats than in control rats. Infarction did not modify superoxide dismutase activity. **Conclusion:** The results suggest that left coronary artery ligation results in oxidative stress in the diaphragm.

**Keywords:** Myocardial infarction; Oxidative stress; Congestive heart failure; Antioxidants; Diaphragm; Rats

## INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality worldwide.[1-2] Heart failure accounts for at least 20% of hospital admissions among patients over the age of 65, and this index has presented a trend toward the increase.[3]

The main symptoms of heart failure are dyspnea, fatigue and decreased muscle strength. These symptoms may limit exercise tolerance, leading to pulmonary congestion and peripheral edema. The mechanisms related to exercise intolerance in patients with heart failure have not been fully defined.[4-6] However, one of the working hypotheses for the decreased muscle strength is the increased oxidative damage caused by hypoxemia related to decreased blood supply.[7] That hypothesis is based on the fact the structure and function of the cell membranes are affected, resulting in cell dysfunction.[8-10]

Based on these data, it is possible to suppose that heart failure produces a reduction in the blood supply to the diaphragm, thereby increasing diaphragmatic oxidative stress.

In the present study, we developed an experimental rat model of myocardial infarction by which we induced heart failure. We examined the diaphragmatic oxidative profile as well as alterations in the activity of antioxidant enzymes.

## METHODS

Wistar rats (7 per group), provided by the animal facilities of the Federal University of Rio Grande do Sul Institute of Basic Health Science, were used in accordance with the guidelines established by the International Committee for the Care and Use of Laboratory Animals.[11-12] The rats had free access to rat chow and water, and were maintained on a 12-hour light-dark cycle in a temperature controlled environment (20-25°C). The animals were divided into two experimental groups: the infarcted group (animals submitted to left coronary artery ligation); and the control group (animals undergoing a sham operation). The method used to produce myocardial infarction was based on the Pfeffer model, with minor modifications.[13] The animals were anesthetized via intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg).

On day 42 after left coronary artery ligation, the animals were weighed and killed by cervical dislocation. Diaphragms, hearts, lungs and livers were immediately collected. Diaphragms were homogenized in an Ultra-Turrax tissue homogenizer (IKA, Labortechnik, Staufen, Germany) using 5 mL/g of tissue at 4°C with KC1 at 1.15% (p/v), 15 µL of 10% Triton X-100 and phenylmethylsulfonyl fluoride (100mmol/L), at a proportion of 10 µL of KC1 in order to inhibit protease activity in the sample. The mixture was centrifuged for 20 minutes (0-4°C) at 3000 rpm (Sorvall RC 5B-rotor SM24, DuPont Instruments, Wilmington, DE, USA). The supernatant was then collected and frozen at -80°C for later measurement of oxidative stress.[14]

Hearts were weighed and ventricles were dissected. Through macroscopic examination of the material, infarction was easily confirmed by identification of the scar in the anterolateral portion of the left ventricle. The infarcted area was calculated and is presented as the percentage of the endocardial surface of the left ventricle covered by scar.[15] Only hearts in which the infarcted area was greater than 25% of the left ventricular area were included in the study. The index of heart hypertrophy was obtained by calculating the ratio between heart mass and total body mass (mg/g of body weight). This ratio was also calculated regarding the mass of the right ventricle (right ventricle hypertrophy) and the mass of the left ventricle (left ventricle hypertrophy).

In order to obtain the wet weight/dry weight ratio of the lungs and the liver, the organs were collected and freed from adherent tissues. In each case, the tissues were weighed and maintained in an incubator at 70°C until reaching constant weight.

Lipid peroxidation was estimated using the thiobarbituric acid reactive substances method. Therefore, trichloroacetic acid (10%) and thiobarbituric acid (0.67%) were added to diaphragm homogenates - incubation at 100°C for 15 minutes - and absorbance at 535 nm was measured using a spectrometer. Results are expressed as nmols per mg of protein.[16]

The activity of superoxide dismutase, an antioxidant enzyme that reduces superoxide anions to hydrogen peroxide, was determined in diaphragm homogenates through the inhibition rate of the reaction of superoxide anion with pyrogallol. Enzyme activity is expressed as U/mg

of protein.[17] Glutathione peroxidase activity was determined using a spectrometer to measure the oxidation of NADPH at 340 nm in a reaction medium containing a phosphate-regulating solution. Enzyme activity is expressed as nmol/min/mg of protein.[18] Catalase activity was measured by calculating the decrease in the absorption of hydrogen peroxide at 240 nm and is expressed as pmol/mg of protein.[19]

Total protein concentration in the samples was measured through the method described by Lowry et al., using bovine albumin as a reference.[20]

Values are expressed as mean ± standard error of the mean. The Student's t-test was used for comparisons between the groups, and values of p < 0.05 were considered statistically significant.

## RESULTS

The mortality rate in the infarcted group during or immediately after the surgical procedure was 33%. Another 2% died in the second postoperative week. The mean infarcted area, promoted by the left coronary ligation, was 39% of the total ventricular area (confidence interval: 29%-44%).

There were statistically significant differences between the groups regarding the indices of heart hypertrophy and left and right ventricular hypertrophies. Table 1 shows these indices as well as the wet weight/dry weight ratios of the lungs and the liver, which were also higher in the infarcted group than in the control group.

The results regarding oxidative stress analyses are shown in Table 2. These results indicate that lipid peroxidation, estimated by the thiobarbituric acid reactive substances method, was significantly higher

### TABLE 1

Morphometric analysis of the two groups studied

| Parameter | Group | |
| --- | --- | --- |
| | Control | Infarcted |
| Heart hypertrophy (mg/g) | 2,70 ± 0,09 | 3,50 ± 0,08 * |
| LV hypertrophy (mg/g) | 2,20 ± 0,07 | 2,40 ± 0,06 * |
| RV hypertrophy (mg/g) | 0,50 ± 0,04 | 0,90 ± 0,10 * |
| Pulmonary congestion (mg/mg) | 1,60 ± 0,06 | 1,80 ± 0,02 * |
| Hepatic congestion (mg/mg) | 0,70 ± 0,03 | 0,90 ± 0,04 * |

Values represent means ± standard error of 7 animals per group; *Significant difference in comparison to controls (p < 0.05); LV: left ventricle; RV: right ventricle

### TABLE 2

Oxidative stress parameters in the two groups studied

| Parameter | Group | |
| --- | --- | --- |
| | Control | Infarcted |
| TBARS (nmoles/mg prot) | 0,29 ± 0,06 | 0,92 ± 0,08* |
| Antioxidant enzymes | | |
| SOD (U /mg prot) | 16 ± 0,80 | 15 ± 0,70 |
| CAT (pmoles /mg prot) | 158 ± 3 | 37 ± 6* |
| GPx (nmoles/min/mg prot) | 77,0 ± 4,7 | 62,0 ± 3,3* |

Values represent means ± standard error of 5-6 animals per group; *Significant difference in comparison to controls (p < 0.05); TBARS: thiobarbituric acid reactive substances; prot: protein; SOD: superoxide dismutase; CAT: catalase, GPx: glutathione peroxidase

in the infarcted group than in the control group (217%). In the infarcted group, glutathione peroxidase and catalase activity were 20% and 77% lower, respectively, than in the control group.

## DISCUSSION

After an ischemic event, such as acute myocardial infarction, the heart tissue begins to undergo modifications that result in a complex of changes involving not only the infarcted tissue but the whole heart. In order to adapt to this new condition, the ischemic region modifies itself morphologically and, after the local inflammatory reaction, the damaged fibers are gradually replaced by fibrous tissue, forming the scar.[1]

A study using a similar technique to induce infarction found decreased systolic pressure in the left ventricle and a lower cardiac index, data that corroborate our findings.[21] Therefore, the development of heart hypertrophy, as well as of pulmonary and hepatic congestion, may result from a change in the cardiac mechanics and from a lower cardiac index.

Pulmonary congestion increases the load on the respiratory musculature. In the present study, due to the lower cardiac index, the blood supply to the diaphragm was probably reduced. As a result, since diaphragmatic function is critically dependent on adequate blood flow to provide oxygen for its metabolic function and contractile activity, the respiratory musculature became overloaded.

In a study of rat diaphragm fibers, it was found

that production of active oxygen species increases when such fibers are subjected to hypoxia.[10] In a similar study, the diaphragm muscle fibers of 81 Wistar rats were analyzed.[22] The authors demonstrated that, under hypoxic conditions, contractile strength of the striated musculature was decreased, and production of active oxygen species was increased.

In the present study, we found that levels of thiobarbituric acid reactive substances in the diaphragm homogenates of the animals submitted to coronary artery ligation were significantly higher than those seen in the control group, suggesting that oxidative damage to the diaphragm was greater in the study animals. We also observed that catalase and glutathione peroxidase activity were lower in the diaphragm homogenates of study rats than in those of control rats. These findings (greater lipid peroxidation and lower antioxidant enzyme activity) are consistent with oxidative stress.

Antioxidant enzymes play a fundamental role in protecting the organism since they constitute the first line of defense against active oxygen species.[23] It has been demonstrated that enzyme activity is lower in tissues subjected to ischemia,[24-25] and that higher concentrations of some active oxygen species, such as superoxide radicals and hydrogen peroxide, can inhibit enzyme activity.(26)

In view of these facts, we suggest that the imbalance found in the enzyme activity of the diaphragm in this study results from the systemic conditions created by myocardial infarction.

Higher levels of thiobarbituric acid reactive substances denote greater lipid oxidative damage. Such damage promotes loss of the capacity to maintain homeostatic balance within cells and impairs the vital functions of the cells, among which is mitochondrial function. When the mitochondrial membrane is injured, there is a loss of oxygen transport capacity.[27] Under high demand, this mechanism leads to a metabolic adaptation to produce less energy, resulting in lower muscle contractility.[28]

Some authors have described physiological modifications in diaphragms under ischemic conditions. Under such conditions, the adaptation involves increased numbers of type IIb fibers, typically of the fast-twitch type, although oxidative capacity is decreased.[27,29]

Our results show that myocardial infarction increased oxidative damage, as well as decreasing antioxidant enzyme activity in the diaphragm. Strategies to modulate oxidative stress through adaptations of the antioxidant system, such as exercising with appropriate intensity, frequency and duration, may provide improvement in diaphragm performance under ischemic conditions.

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