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# Sum of effects of myocardial ischemia followed by electrically induced tachycardia on myocardial function

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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**Background:** The alteration of contractile function after tachyarrhythmia ceases is influenced by the type of prior ischemia (acute coronary syndrome or ischemia inherent in a coronary revascularization procedure). We aimed to analyze cardiac dysfunction in an acute experimental model of supraphysiological heart rate preceded by different durations and types of ischemia.

**Material/Methods:** Twenty-four pigs were included in: (S1) series of ventricular pacing; (S2, A and B) series with 10 or 20 min, respectively, of coronary occlusion previous to ventricular pacing; S3 with 20 brief, repeated ischemia/reperfusion processes prior to ventricular pacing and; (S4) control series. Overall cardiac function parameters and regional myocardial contractility at the apex and base of the left ventricle were recorded, as were oxidative stress markers (glutathione and lipid peroxide serum levels). Left ventricular pacing at 60% over baseline heart rate was performed for 2 h followed by 1 h of recovery.

**Results:** High ventricular pacing rates preceded by short, repeated periods of coronary ischemia/reperfusion resulted in worse impairment of overall cardiac and regional function that continued to be altered 1 h after tachycardia ceased. There was significant reduction of stroke volume (26.9±5.3 basal *vs.* 16±6.2 ml; p<0.05), LVP; dP/dt and LAD flow (13.1±1.5 basal *vs.* 8.4±1.6 ml/min; p<0.05); the base contractility remained altered when recovering compared to baseline (base SF: 5.6±2.8 *vs.* 2.2±0.7%; p<0.05); and LPO levels were higher than less aggressive series at the end of recovery.

**Conclusions:** Ischemia and tachycardia accumulate their effects, with increased cardiac involvement depending on the type of ischemia.

**Key words:** tachyarrhythmia • cardiac ischemia • cardiac dysfunction

**Full-text PDF:** http://www.basic.medscimonit.com/download/index/idArt/889115







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## Background

Ischemic heart disease and heart failure are diseases that have a tremendous impact on our society [1]. Experimental study of aspects of these processes is of great interest.

Tachycardia-induced cardiomyopathy is a condition of atrial or ventricular dysfunction due to the increased atrial or ventricular rates; the degree of dysfunction correlates with the duration and rate of tachycardia without structural heart disease and it represents as reversible dilated cardiomyopathy [2]; but tachycardia caused by atrial fibrillation has been linked with poor outcome after both percutaneous [3] and surgical coronary revascularization [4], the 2 processes being associated with periods of regional or global ischemia. In 25–50% of patients undergoing cardiac surgery, paroxysmal atrial fibrillation occurs with immediate repercussions such as hemodynamic instability and heart failure [5]. The alteration of the contractile function after the cessation of a tachyarrhythmia, either atrial or ventricular, is influenced by the type of prior ischemia (acute coronary syndrome or ischemia inherent in a coronary revascularization procedure) [6–11]. In patients with stable ischemic heart disease and left ventricular systolic dysfunction, decreased heart rate improves prognosis [12].

There are few studies on the hypothesis of "sum of stuns" to date in animal models. Most have examined the simultaneous effects of tachycardia on coronary stenosis (demand ischemia) [13–15] but few have explored the sequence of cardiac ischemia/reperfusion followed by tachycardia [16].

### Objective

The aim of the study was to analyze the physiopathology of cardiac dysfunction in an acute experimental model of a supraphysiological increase in heart rate preceded by ischemia, considering the influence of the type and duration of ischemia.

## Material and Methods

### Experimental series

A total of 24 young pigs of both sexes (7 males and 17 females) with a mean weight of 24.5±3 kg were used. The experiments were conducted respecting the Spanish legislation on "Protection of animals used for experimental and other scientific purposes" and the directives of the European Community (Royal Decree 1201/2005).

Four experimental series were formed by direct allocation of animals to different groups by a random method of the animal house staff, using no specific procedure to mask distribution. The series were: (S1), ventricular pacing was performed in 5 pigs following the protocol discussed later; (S2: A and B), series of a single ischemia and ventricular pacing, S2A consisted of 5 pigs that underwent complete occlusion of the left anterior descending coronary artery (LAD) for 10 min before applying the stimulation protocol, and S2B included 5 pigs with occlusion of LAD for 20 min prior to the stimulation protocol; S3, in 5 pigs, 20 processes of ischemia with a duration of 2 min followed by 3 min of reperfusion for each process were performed; (S4), control series in which the experimental preparation was performed in 4 pigs without causing ischemia or applying ventricular pacing.

### Experimental preparation

The animals were anesthetized with thiopental (15 mg/kg. weight) bolus IV followed by endotracheal intubation and channelling of the external jugular. Maintenance of anesthesia was achieved with sevoflurane (2.5%) in a mixture of 40% oxygen and 60% nitrous oxide. Analgesia and relaxation were obtained with vecuronium bromide (0.08 mg/kg. weight) and 20 mg of morphine chloride bolus IV and maintained at the same dose in 50 cc of physiological saline in an infusion pump at 12 ml/h. Gasometric tests were conducted to monitor and correct the respiration parameters; rectal temperature was monitored and maintained at 38–39°C with an electric blanket; and continuous peripheral ECG recording was performed with subcutaneous electrodes in the extremities.

Opening the thorax was performed through a sternotomy and pericardial opening to expose the heart. After dissection of the LAD at its upper and middle thirds, a flowmeter (Transonic Systems, NY, USA) was placed at the top, and in the middle third a reference was located to carry out coronary occlusions. A flowmeter was also implanted around the aortic root for calculation of stroke volume and cardiac output. Two pairs of ultrasonic microcrystals (Biopac Systems Santa Barbara, CA, USA) were introduced in both the subepicardium in the area of the left ventricular apex (subsidiary of LAD) and the subepicardium of the base of the left ventricle (subsidiary of the circumflex coronary artery [LCx]). A catheter was introduced through the free wall of the left ventricle to record ventricular pressure.

### Ischemic protocol

In series 2A and B, there was a single occlusion of the LAD in its middle third with a vascular clamp, in S2A for 10 min and in S2B for 20 min. In series 3, there were 20 occlusions of the LAD of 2 min each followed by 3 min of reperfusion.

### Stimulation protocol

An electro-catheter was fixed by 1 point of suture to the anterior face of the upper third of the left ventricular epicardium in all the





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**Table 1.** Series 1 ventricular pacing.

| Series 1 | Basal | 1 h pac | 2 h pac | 5 min rec | 30 min rec | 1 h rec |
|---|---|---|---|---|---|---|
| HR bpm | 98±12.5 | 162±18.7 | 162±18.7 | 122.2±7.5* | 116±21.9 | 116±20.3 |
| LVP mm Hg | 89.4±11.1 | 56.8±15.6* | 64.4±21.1* | 96.6±6.6 | 80.8±11.1 | 77.2±16.4* |
| EDLVP mmHg | 10±3.1 | 7.7±2.1 | 6.7±2.9 | 10.6±4.3 | 11.8±2 | 11±2 |
| S.V. ml | 30.1±2.2 | 19±4.8* | 19.6±6* | 31.8±9.1 | 31.5±7.6 | 29.5±6.7 |
| C.O. l/min | 2.9±0.5 | 3±0.7 | 3.1±0.8 | 4.2±1.7 | 3.8±1.7 | 3.5±1.5 |
| LAD flow ml/min | 12.1±4.8 | 7.4±4.6* | 7.5±2.2 | 14.8±1.3 | 9.8±2.5 | 8.9±2.3 |
| +dP/dt mmHg/s | 1730±159 | 1490±178* | 1430±171* | 1385±150* | 1320±152* | 1345±109* |
| –dP/dt mmHg/s | 1805±62 | 1105±289* | 1200±272* | 1868±123 | 1612±157* | 1736±202 |
| Apex SF% | 16.9±6.5 | 6.5±2.5* | 7.9±1.4* | 12.1±2.9 | 13.6±3.3 | 12.9±3.6 |
| Base SF% | 7.7±2.3 | 3.3±1.9* | 2.6±2.1* | 3.8±1.7* | 4.5±1.6 | 4.3±1.4* |

Pac – ventricular pacing; rec – recovery; HR – heart rate; LVP – left ventricular peak systolic pressure; EDLVP – end-diastolic left ventricular pressure; S.V. – stroke volume; C.O. – cardiac output; LAD flow – left anterior descending coronary artery flow; dP/dt – first temporal derivative of left ventricular pressure (+) maximum positive, (–) negative minimum; SF – shortening fraction. Statistical significance regarding basal values * $p<0.05$; values shown as mean and standard deviation.

animals. Electrical stimulation (Arrhythmia Investigation System Type 4279 DEVICES, Hertfordshire, UK.) was performed with an intensity twice the threshold calculated for each experiment and 60% above its baseline rate for 2 h. The 4 control animals of the S4 remained with the thorax open and instrumented for 5 h without performing coronary ischemia or ventricular pacing.

### Data collection

All parameters of cardiac function were digitized and stored in an electronic memory system (BIOPAC systems Inc., Santa Barbara, CA, USA): ECG (LII or LIII); left ventricular peak systolic pressure (LVP in mm Hg); end-diastolic left ventricular pressure (EDLVP in mm Hg); the first derivative of the pressure in relation to time (dP/dt of LVP in mm/sec.); aortic flow shown as stroke volume (ml) and LAD flow (ml/min). Segmental myocardial function parameters, ie, end-diastolic and end-systolic lengths (mm), and the shortening fraction (% over the end-diastolic length) of the apex and base of the left ventricle were recorded with a Sonometrics Corporation Digital Ultrasonic Measurement System (London, Ontario, Canada).

The following measures were selected for analysis: 1) baseline after experimental preparation; 2) at the end of ischemia in series 2A, 2B and 3; 3) before pacing; 4) during pacing at 30, 60, 90, and 120 min; and 5) at 5, 30, and 60 min after cessation of pacing. In the control series (S4), these parameters were recorded after preparation (1$^{st}$ h) and 4 more hourly monitorings.

The role that oxidative stress may play in this model of tachymyocardiopathy and ischemia was evaluated by quantification of serum levels of glutathione (GSH GSSG; Cayman Chemical Company, USA) as an antioxidant marker, and lipoperoxide (LPO) levels (Lipid hydroperoxide Assay Kit Cayman Chemicals Company) as an oxidative marker.

Blood samples for analysis of parameters of oxidative stress were obtained: 1) at baseline (before opening of thorax); 2) before pacing; 3) after 60 min of pacing; 4) immediately after cessation of pacing; and 5) 60 min after cessation of pacing. In S4, blood samples were drawn: 1) at baseline; 2) after instrumentation; and 3) at 60, 120, and 180 min after manipulation.

A total of 15 ml of blood was extracted each time in sterile tubes containing EDTA, centrifuged at 3000 rpm at 4°C for 10 min and the plasma obtained was stored at –80°C in 3 cryopreservation tubes of 2 ml each until their study.

### Statistical analysis

To determine the differences between means, sample size was calculated based on those used in other studies [14–16]. All data are represented as mean and standard deviation. Normal distribution was verified with the Kolmogorov-Smirnov test. To determine differences between mean values along the protocol with respect to baseline, ANOVA and Student's t test were used for paired and unpaired samples when comparisons were made between series. Mann-Whitney or Kruskal-Wallis tests were performed in the parameters whose distribution was not normal. It was assumed that differences between means were statistically significant when the p value was $<0.05$. Statistical analysis was performed using SPSS 9.0 for Windows.





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**Table 2.** Series 2A and 2B; 10 and 20 minutes of coronary occlusion, respectively, followed by ventricular pacing.

| Series 2 A | Basal | After isch | 1 h pac | 2 h pac | 30 min. rec | 1 h rec |
|---|---|---|---|---|---|---|
| HR bpm | 106.6±15.2 | 98.8±12.1 | 164±3.8 | 164±3.8 | 108.4±9.9 | 102.4±7.6 |
| LVP mm Hg | 81±18.1 | 75.6±18.9 | 62.4±15.2 | 65.2±14.6 | 73.7±14.9 | 69.6±7.8 |
| EDLVP mmHg | 9.7±4.7 | 9±6.3 | 6±1.4 | 9.4±3.6 | 12.2±5.6 | 9.8±3.3 |
| S.V. ml | 32.1±11.4 | 31.4±11.4 | 21.9±6.3* | 20.8±5.7* | 27.5±8.9* | 26.7±9.1* |
| C.O. l/min | 3.5±1.6 | 3.2±1.4 | 3.6±1 | 3.4±0.9 | 2.9±1.1* | 2.7±0.5* |
| LAD flow ml/min | 20.2±3.1 | 33.2±10.3* | 18.8±7.3 | 17.9±7.1 | 15.8±4.1* | 18.9±6.2 |
| +dP/dt mmHg/s | 1488±243 | 1840±485* | 1813±437* | 1535±413 | 1339±342 | 1289±318 |
| –dP/dt mmHg/s | 1510±595 | 1538±436 | 1386±307 | 1130±548* | 1338±379 | 1364±244 |
| Apex SF% | 14.6±5.4 | 11.6±6.6 | 7.9±2.1* | 8.6±3.4* | 10.2±4.2* | 12.6±4.5 |
| Base SF% | 6.3±4 | 6.3±4.1 | 3.1±4.5 | 2.7±3.4 | 4.5±1.7 | 5.1±1.9 |
| **Series 2 B** | **Basal** | **After isch** | **1 h pac** | **2 h pac** | **30 min rec** | **60 min rec** |
| HR bpm | 92±6.2 | 92±17.2 | 157±4.5 | 157±4.5 | 99.5±7.6 | 97.2±6.5 |
| LVP mm Hg | 73±14.8 | 71±6.5 | 59±18.5 | 64.4±13.2 | 73.7±6.3 | 86.7±16.1 |
| EDLVP mmHg | 8.8±3.2 | 8.8±3.8 | 7.2±1.7 | 7.8±2.1 | 8.4±1.5 | 7.6±1.3 |
| S.V. ml | 33.8±10.8 | 32.1±11.4 | 25.2±12.9* | 24.1±13.2* | 31.9±11.9 | 31.5±11.4 |
| C.O. l/min | 3.1±1.1 | 3±1.5 | 3.9±2.1 | 3.8±2.1 | 3.6±2 | 3.6±2.1 |
| LAD flow ml/min | 17.4±5.9 | 30.8±8.2* | 6±0.3* | 8±0.3* | 15.9±7.1 | 13.8±4.1 |
| +dP/dt mmHg/s | 1440±233 | 1580±485 | 1440±763 | 1570±615 | 1610±692 | 1490±327 |
| –dP/dt mmHg/s | 1255±346 | 1500±799 | 1090±835 | 1080±834 | 1490±838 | 1290±542 |
| Apex SF% | 13±5.2 | 11.9±2.9 | 6.5±4* | 7.2±4.4* | 10±3.3 | 8.1±3.1* |
| Base SF% | 6.4±1.8 | 4.5±1.6 | 0.7±4.7* | –0.1±3.7* | 4.6±2.2 | 4.3±3.3 |

Pac – ventricular pacing; Isch – coronary occlusion; rec – recovery; HR – heart rate; LVP – left ventricular peak systolic pressure; EDLVP – end-diastolic left ventricular pressure; S.V. – stroke volume; C.O. – cardiac output; LAD flow – left anterior descending coronary artery flow; dP/dt – first temporal derivative of left ventricular pressure (+) maximum positive, (–) minimum negative; SF – shortening fraction. Statistical significance regarding basal values * $p<0.05$; values shown as mean and standard deviation.

## Results

There were no differences in the basal situation among the series in any parameter considered. Tables 1–4 show the average values of each parameter and their variation in the different phases relative to baseline for all series.

### Overall function

Stroke volume did not differ significantly during ischemia and reperfusion (S2A-B), presenting similar values in all series. Ventricular pacing led to a decline in stroke volume and its recovery at the cessation of pacing. There were no significant differences among series in any of the phases of the study. S3 showed a significant decrease until the end of follow-up compared to baseline (26.9±5.3 *vs.* 16±6.2 ml; $p<0.05$) (Table 3).

Cardiac output did not differ among series in any phase of the study, although in S1 there was an elevation together with increased heart rate and stroke volume after ventricular pacing with respect to baseline (Table 1), and in S3 there was a significant decline after ventricular pacing with respect to baseline (2.7±0.6 *vs.* 1.5±0.9 l/min, $p<0.05$) (Table 3).

During ventricular pacing, LVP decreased significantly in all cases and recovered after cessation except in S3, which was already affected by the ischemia and did not recuperate after pacing, remaining significantly lower at the end of the study





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**Table 3.** Series 3. Brief and repeated coronary occlusions followed by ventricular pacing.

| Series 3 | Basal | After isch | 1 h pac | 2 h pac | 30 min rec | 1 h rec |
|---|---|---|---|---|---|---|
| HR bpm | 101±5.8 | 91±6.5* | 158±5.5 | 158±5.5 | 90±31.9 | 85±28.4 |
| LVP mm Hg | 70.6±6.6 | 60.8±12.9* | 48.6±6.1* | 39.8±8.9* | 52±11.9* | 46±8.4* |
| EDLVP mmHg | 7.8±3.1 | 9.2±6.3 | 5.6±1.3 | 4.8±1.8 | 7.5±3.8 | 7.2±3.3 |
| S.V. ml | 26.9±5.3 | 24.3±5.7* | 19.2±2.7* | 14.3±4.9* | 17.3±6.9* | 16±6.2* |
| C.O. l/min | 2.7±0.6 | 2.2±0.6* | 3±0.5 | 2.3±0.6 | 1.7±1.2 | 1.5±0.9* |
| LAD flow ml/min | 13.1±1.5 | 13.5±1.5 | 7.8±1.6* | 5.5±2.6* | 8±2.2* | 8.4±1.6* |
| +dP/dt mmHg/s | 1710±383 | 1375±245* | 1470±419 | 1110±475* | 1090±272* | 970±189* |
| –dP/dt mmHg/s | 1455±363 | 1080±250* | 870±301* | 650±154* | 800±61* | 665±89* |
| Apex SF% | 10.1±4.3 | 12±7 | 4.3±2.9* | 4.7±4.2* | 7.1±2.7 | 8.1±4.4 |
| Base SF% | 5.6±2.8 | 4.1±2.8* | 1.1±4.8* | –1.3±5.3* | 3±0.8 | 2.2±0.7* |

Pac – ventricular pacing; Isch: coronary occlusion; rec – recovery; HR: heart rate; LVP – left ventricular peak systolic pressure; EDLVP – end-diastolic left ventricular pressure; S.V. – stroke volume; C.O. – cardiac output; LAD flow – left anterior descending coronary artery flow; dP/dt – first temporal derivative of left ventricular pressure (+) maximum positive, (–) minimum negative; SF – shortening fraction. Statistical significance regarding basal values * p<0.05; values shown as mean and standard deviation.

**Table 4.** Series 4. Control.

| Series 4 | Basal | 60 min | 120 min | 180 min | 240 min |
|---|---|---|---|---|---|
| HR bpm | 110±7.2 | 101±13 | 102±16 | 109±15 | 112±15 |
| LVP mm Hg | 67±9 | 64±15 | 63±14 | 65±12 | 68±19 |
| EDLVP mmHg | 6.2±1.5 | 6.2±1.3 | 7±2.9 | 7.5±3 | 7±2.5 |
| S.V. ml | 24±8 | 17.1±2.7 | 22.5±8.8 | 17±2.9 | 25±8.6 |
| C.O. l/min | 2.5±0.9 | 2.3±1.3 | 2.5±1.2 | 2.6±1.3 | 2.9±1.7 |
| LAD flow ml/min | 17.4±1.8 | 16.7±2.5 | 16.5±0.8 | 15.5±0.6 | 15.6±0.7 |
| + dP/dt mmHg/s | 1537±320 | 1500±389 | 1425±405 | 1575±405 | 1700±422 |
| - dP/dt mmHg/s | 1262±377 | 1218±504 | 1300±438 | 1394±466 | 1525±510* |
| Apex SF% | 12.6±1.8 | 10.4±1.9* | 11.4±1.2 | 10.2±2.4* | 11.1±2.2* |
| Base SF% | 7.7±7 | 8±6.7 | 5.7±1.5 | 5.1±4.2 | 5.1±4.5 |

HR – heart rate; LVP – left ventricular peak systolic pressure; EDLVP – end-diastolic left ventricular pressure; S.V. – stroke volume; C.O. – cardiac output; LAD flow – left anterior descending coronary artery flow; dP/dt – first temporal derivative of left ventricular pressure (+) maximum positive, (–) minimum negative; SF – shortening fraction. Statistical significance regarding basal values * p<0.05; values shown as mean and standard deviation.

compared to baseline (70.6±6.6 *vs.* 46±8.4 mm Hg, p<0.05, Table 3) and other series (Figure 1).

The EDLVP showed no significant differences during the study from baseline in any series (Tables 1–4) or between series. Both maximum +dP/dt and minimum –dP/dt of the left ventricle in S3 showed a significant impairment relative to baseline (Table 3) and they were significantly reduced with respect to the other series at 60 min after pacing (p<0.05 S3 *vs.* S1, S2A, S2B and S4).

### Coronary flow

The flow of the LAD in S2A and B, in which continuous ischemia was induced, showed significant hyper flux after ischemia compared to baseline (Table 2) and to the other series (Figure 2). During ventricular pacing it decreased in all series and recovered in all except for S3, which remained significantly lower at the end of the study when compared to baseline (13.1±1.5 *vs.* 8.4±1.6 ml/min, p<0.05) (Table 3).





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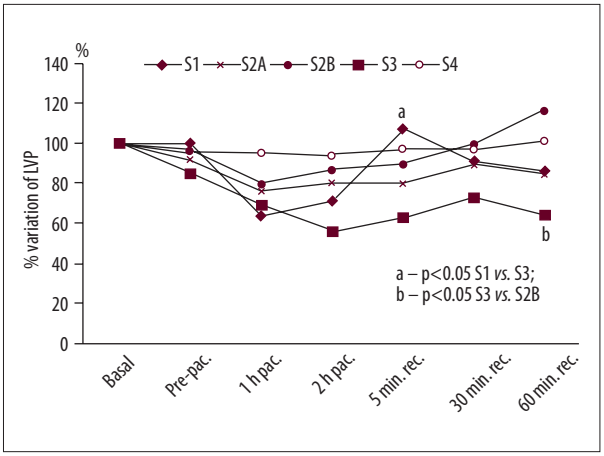


**Figure 1.** Percent variation of peak systolic left ventricular pressure (LVP) in all series. Results obtained under basal conditions, after preparation (pre-pac.) at 1 and 2 hours of pacing (1 h pac., 2 h pac.) and 5, 30 and 60 minutes after ventricular pacing ceased (5 min rec., 30 min rec, 60 min rec). Statistical significance comparing among series (S1 n=5; S2A n=5; S2B n=5; S3 n=5 and S4 n=4).

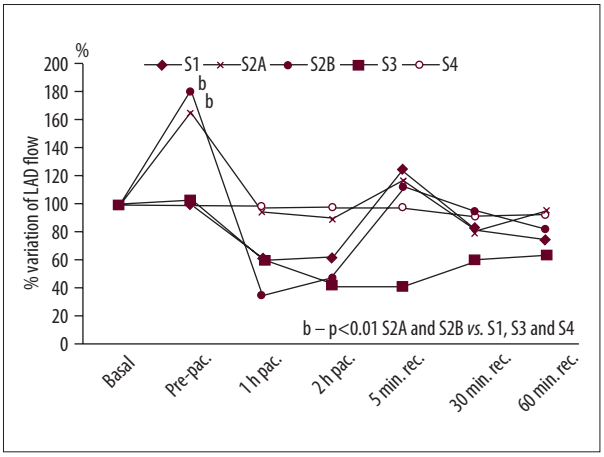


**Figure 2.** Percent variation of left anterior descending coronary artery (LAD) flow in all series. Results obtained under basal conditions, after preparation (pre-pac.) at 1 and 2 hours of pacing (1 h pac., 2 h pac.) and 5, 30 and 60 minutes after ventricular pacing ceased (5 min rec., 30 min rec, 60 min rec). Statistical significance comparing among series (S1 n=5; S2A n=5; S2B n=5; S3 n=5 and S4 n=4).

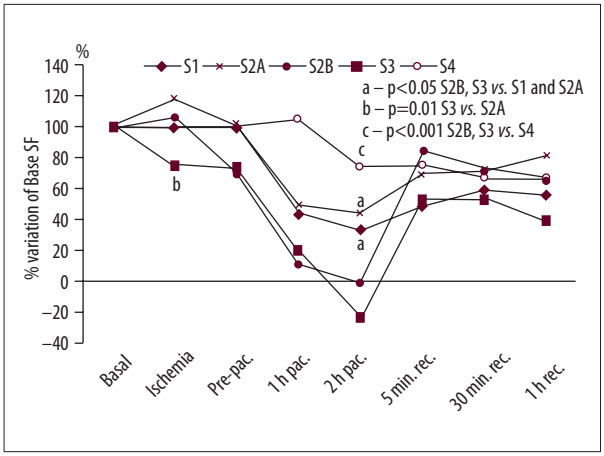


**Figure 3.** Percent variation of left ventricle base shortening fraction (Base SF) in all series. Results obtained under basal conditions, during ischemia; after preparation (pre-pac.) at 1 and 2 hours of pacing (1 h pac., 2 h pac.) and 5, 30 and 60 minutes after ventricular pacing ceased (5 min rec, 30 min rec, 60 min rec). Statistical significance comparing among series (S1 n=5; S2A n=5; S2B n=5; S3 n=5 and S4 n=4).

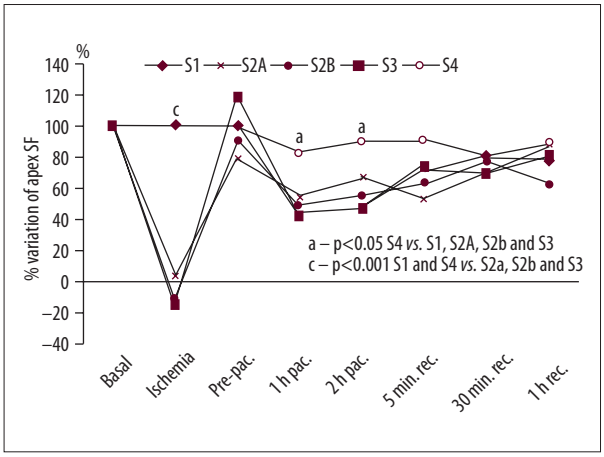


**Figure 4.** Percent variation of left ventricle apex shortening fraction (Apex SF) in all series. Results obtained under basal conditions, during ischemia; after preparation (pre-pac.) at 1 and 2 hours of pacing (1 h pac., 2 h pac.) and 5, 30 and 60 minutes after ventricular pacing ceased (5 min rec, 30 min rec, 60 min rec). Statistical significance comparing among series (S1 n=5; S2A n=5; S2B n=5; S3 n=5 and S4 n=4).

### Regional function

Tables 1–4 show the mean and standard deviation values of the myocardial-shortening fraction at the base and apex of the left ventricle in each series throughout the study. To compare regional function parameters among series, the percentage of change of shortening fraction compared to baseline has been used (Figures 3 and 4).

Left ventricle base shortening fraction (Base SF): During ischemia, significant differences between series were observed. In the series with short, repeated ischemia (S3), there was a reduction of the shortening fraction compared with an increase in the series with 10 min of ischemia (S2A) (p=0.01). In the 2 h of ventricular pacing, the shortening fraction was significantly decreased in S3 and S2B compared to S1 (p=0.04), S2A (p=0.04), and S4 (p<0.001). These differences disappeared in the recovery period, although there was a downward trend in





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**Table 5.** Oxidative stress parameters.

| LPO nmol/L | Basal | Pre-pac. | 1h pac. | 5 min rec | 1 h rec |
|---|---|---|---|---|---|
| S1 | 19±1.03 | 18.9±0.7 | 12.3±0.8** | 15.7±1.5* | 17.8±0.9 |
| S2A | 15.9±6.3 | 17.5±5.2 | 10.2±5.3 | 11.6±2.9 | 9.7±2.8* |
| S2B | 13.9±3.4 | 11.6±3.9 | 8.9±2.4 | 13.9±2.6 | 15.6±1.8 |
| S3 | 13.9±2.6 | 16.1±5.2 | 8.1±3.2* | 13.3±3.7 | 17.6±3.6* |
| | **Basal** | **60 min** | **120 min** | **180 min** | **240 min** |
| S4 | 20.1±1.5 | 19.3±2.8 | 6.5±0.9* | 15.8±3.9 | 19.4±5.1 |
| **GSH/GSSG** | | | | | |
| S1 | 15.5±3.3 | 6.6±1.4** | 14.6±9.7 | 21.7±3.1 | 12.0±3.8 |
| S2A | 20.1±4.6 | 7.7±4.6 | 6.5±4.2 | 8.7±1.5 | 5.5±3.1* |
| S2B | 20.5±5.3 | 3.4±0.6** | 23.4±6.3 | 5.1±3.8** | 10.1±4.5** |
| S3 | 13.3±4.3 | 8.7±6.2 | 6.7±6.1 | 4.3±2.0* | 4.4±1.3* |
| | **Basal** | **60 min** | **120 min** | **180 min** | **240 min** |
| S4 | 14.2±5.3 | 4.0±1.1* | 6.9±1.6* | 7.9±3.3* | 6.7±1.9* |

LPO – lipid hydroperoxide; GSH/GSSG – ratio of reduced and oxidised glutathione; Pac – ventricular pacing; rec – recovery. Statistical significance regarding basal values * p<0.05; ** p<0.01; values shown as mean and standard deviation.

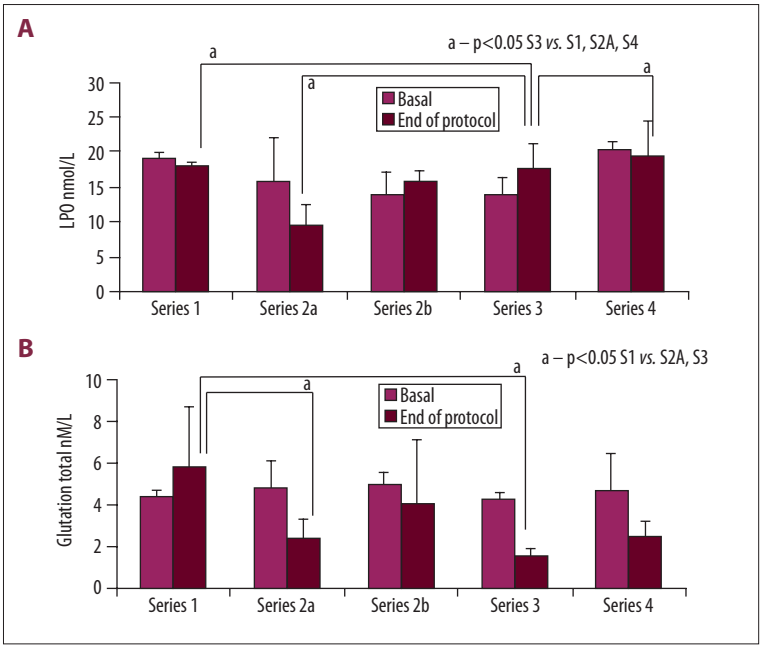


**Figure 5.** Parameters of oxidative stress. (**A**) Mean of LPO (Lipid hydroperoxide) levels in the basal and at the end of protocol (60 min after pacing ceased). Statistical analysis comparing among series. (**B**) Mean of total glutation levels in the basal and at the end of protocol (60 min after pacing ceased) in series. Statistical analysis comparing among series (S1 n=5; S2A n=5; S2B n=5; S3 n=5 and S4 n=4).

S3 (Figure 3) with significantly lower values when recovering with respect to baseline (5.6±2.8 *vs.* 2.2±0.7%, p<0.05, Table 3).

Left ventricle apex shortening fraction (SF apex): In the variable percentage of change in the shortening fraction, differences among series were statistically significant (p<0.001) during the course of ischemia. These differences were expected and were due to the fact that the series with ischemia showed a reduction in shortening fraction compared with control series (S4) and the series without ischemia (S1) in measurements made in the first hour, and before pacing, respectively. However, among the series submitted to ischemia, there were no differences during ischemia or reperfusion. During ventricular pacing, all series showed significantly lower values





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in the shortening fraction compared to S4, with no differences between them, but in the recovery, differences were lost (Figure 4). The 3 series with ischemia prior to pacing showed impairment of the shortening fraction of this region at the end of follow-up (Tables 2 and 3).

**Parameters of oxidative stress**

The measurements of LPO levels show that in the extraction performed prior to ventricular pacing there were no significant changes compared to baseline in any series or differences between series. Table 5 shows the average values of each series in each extraction. During ventricular pacing, all series reduced LPO levels, without significant differences between them. After cessation of pacing, LPO values were increased compared to values obtained during ventricular pacing in all series. After 1 h of recovery, S2B and S3 presented higher levels than the initial LPO (Figure 5A), with significantly higher levels in S3 than the less aggressive S1 ($p<0.05$), S4 ($p<0.05$), and S2A ($p=0.02$).

During the experimental protocol, the total glutathione (GSH + GSSG) activity and the GSH/GSSG ratio of the plasma underwent fluctuations that differed depending on the series. In the blood extraction conducted before ventricular pacing, it was observed that glutathione activity was reduced in all series when compared with the values prior to opening the thorax (Table 5), with values of GSH/GSSG below baseline values. Only series 1 showed recovery of the ratio during and after stimulation; the other series presented significantly lower endpoint values of the ratio GSH/GSSG and total glutathione compared to baseline (Figure 5), with significant differences in S1 compared to S2A ($p<0.05$) and S3 ($p<0.05$) series with previous ischemia.

## Discussion

Myocardial stunning has been defined as "prolonged, post-ischemic dysfunction of viable tissue salvaged by reperfusion" [17]. It is a common finding in different clinical settings when the sequence ischemia/reperfusion occurs: after reperfusion in ST segment elevation myocardial infarction (STEMI), following an unstable angina or non-STEMI or after coronary revascularization (percutaneous or surgical) [18]. Stunned cardiomyocytes may be prone to additive stunning if tachycardia happens soon after the ischemia, when reperfusion injury occurs. To test this hypothesis, this paper discusses the physiopathology of cardiac stunning that occurred in the case of processes of ventricular rate above physiological values preceded by short cardiac ischemia, and whether this effect would depend on the length and type of ischemia, as may occur in the clinical scenarios previously mentioned.

Ventricular pacing at supraphysiological rates affected regional cardiac function that was maintained for at least 60 min following its cessation. In agreement with other authors, previous ischemia, although short-term, increased the effect on global and regional cardiac function [17–20]. It produced myocardial stunning that had greater involvement in the ventricular base.

In the area of heart subjected to ischemia, regional myocardial function was affected first by the ischemia and then after reperfusion by tachycardia, and subsequently recovered with no differences between series.

The contractile response of the control region (base of left ventricle) was different in the series with brief, repeated ischemia compared to the 2 series with only 1 ischemia. In the latter, an increase was observed during the ischemia in the shortening fraction of the control zone, which contrasts with the reduction of contractility in this area in the series with brief and repeated ischemia. In this series, a protocol was followed in which the total time of ischemia was 40 min (20 occlusions of 2 min each) and 20 short reperfusion periods (3 min each). The post-ischemic ventricular dysfunction depends on the duration of the ischemia, reperfusion intervals (the longer the duration of reperfusion, the less important the dysfunction), and the number of reperfusions [21].

In the non-ischemic area of the base of the left ventricle, the series undergoing 10 min of ischemia normalized values of myocardial contractility during reperfusion, but the series with 20 min of ischemia and the series with brief, repeated ischemia reduced them. This response appears to have influenced what happened later during and after tachycardia, since the series of 20 min ischemia and particularly that of short repeated ischemia maintained the stunning in the area of the base of the left ventricle.

In experimental animal models, rapid sustained ventricular pacing produces increased metabolic demands with activation of the renin-angiotensin system [22,23], reduced blood flow, and stimulation of oxidative stress mechanisms [24,25]. Changes in the plasma concentration of lipo-hydroperoxides (an expression of pro-oxidant activity), and the reduced oxidized glutathione (an expression of the dynamic changes in antioxidant defences) have been analyzed. At the end of the experimental protocol, at 60 min of recovery after ventricular pacing, series 3 (brief, repeated ischemia prior to pacing) showed significantly higher LPO levels compared to baseline, and glutathione levels below baseline, which means that in this series the oxidative stress response underwent a more pronounced increase than in the other series. However, there was a total glutathione consumption and a reduction in the ratio GSH/GSSG in all series, regardless of the type of ischemia, even in S4 and control. This response of the activation of antioxidant





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defences was due to the great stress caused by the sternotomy and exposure of the heart to the air. Regarding this, Ramos and Guisasola [26] reported that after intense, prolonged surgical stress, such as a thoracotomy, there was a large activation of antioxidants, in particular systems for the synthesis of heat shock proteins, which reduce total glutathione and the ratio GSH/GSSG and increase lipid peroxidation [27,28].

Rapid pacing from the anterior wall of the left ventricle results in both intraventricular and interventricular dyssynchrony, altering the sequence of physiological contraction of the heart [29–31]. In the series without ischemia, after the cessation of ventricular pacing, although stroke volume recovered immediately and cardiac output remained normal, the positive dP/dt continued below the initial figures, and LVP had progressive reduction that was significant at the end of the observation, which supports the concept of post-tachycardia stunning.

In this experimental model in anesthetized pigs with open thorax subjected to different types of ischemia followed by ventricular pacing, we attempted to reproduce the clinical setting of coronary artery bypass surgery complicated by tachyarrhythmia in the immediate postoperative period. Indirectly, although the translation to humans is not easy, it may provide useful information about the study of the mechanisms of ventricular dysfunction in patients with acute coronary syndrome who, after the opening of the artery, suffer tachyarrhythmia and heart failure, or in the case of elderly and/or diabetic patients with silent ischemia processes that are complicated by increased heart rate.

### Limitations of the study

Since this experimental model involves opening the thorax, there is an increase in oxidative stress (see control series, S4) that adds to the oxidative stress due to ischemia and cardiac pacing. In spite of this, series with more aggressive ischemic protocol had higher oxidative activation.

## Conclusions

Ischemia and high cardiac rate occurring simultaneously have additive effects, resulting in increased cardiac involvement. The type of ischemia influenced the involvement of global and regional cardiac function; the model of brief, repeated ischemia and reperfusion followed by tachycardia was more likely to induce heart dysfunction than the models in which ischemia was continuous (at least 20 min). This could be useful for the development of new animal models that reproduce mechanisms of acute cardiac failure in the context of ischemic heart disease and tachyarrhythmia.

### Abbreviations

**ACS** – acute coronary syndrome; **LAD** – Left anterior descending coronary artery; **LCx** – left circumflex coronary artery; **LVP** – left ventricular peak systolic pressure; **EDLVP** – end-diastolic left ventricular pressure; **SF** – myocardial segmentary shortening fraction; **LPO** – lipid hydroperoxide; **GSH** – reduced glutathione; **GSSG** – oxidized glutathione.

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