Effect of Atropine-Dobutamine Stress Test on Left Ventricular Echocardiographic Parameters in Untrained Warmblood Horses

Charlotte F. Sandersen, Johanne Detilleux, Brieuc de Moffarts, Gunther Van Loon, and Hélène Amory

The aim of this study was to investigate the effect of combined atropine low-dose dobutamine stress test on left ventricular parameters in adult warmblood horses, to establish a potential protocol for pharmacological stress echocardiography. Seven healthy untrained warmblood horses aged 9 to 22 years were used. Heart rate (HR) and left ventricular B- and M-mode dimensions were recorded at baseline and during stress testing with 35 μg/kg atrope IV followed by incremental dobutamine infusion of 2 to 6 μg/kg/min. HR increased significantly (P < .05) during the pharmacological challenge, and a maximal HR of 156.6 ± 12.5 bpm was reached at maximal dobutamine infusion rate. Systolic and diastolic interventricular septum thickness, systolic and diastolic left ventricular free wall thickness, and fractional shortening increased significantly and reached a maximum at the highest infusion rate (mean ± SD: 4.51 ± 0.27 versus 5.65 ± 0.31 cm, 2.89 ± 0.19 versus 3.78 ± 0.10 cm, 3.72 ± 0.34 versus 4.77 ± 0.18 cm, 2.44 ± 0.28 versus 3.11 ± 0.34 cm, 34.98 ± 3.82 versus 50.56 ± 3.42%, respectively). Systolic and diastolic left ventricular internal diameter decreased significantly during dobutamine infusion. Left ventricular external and internal area were significantly lower at a dobutamine infusion rate of 2 μg/kg/min but no further decrease was observed during the subsequent steps. Systolic and diastolic myocardial area was significantly lower after the administration of dobutamine but not significantly different during dobutamine infusion, when compared to baseline values. This pharmacological stress test induced significant changes in left ventricular echocardiographic parameters in adult warmblood horses. Additional research should evaluate the value of this stress test in horses suffering from cardiac disease.

**Key words:** Cardiac stress test; Echocardiography; Exercise intolerance.

Cardiovascular disease is the 3rd most commonly diagnosed cause of poor performance in horses, after diseases of the musculoskeletal and respiratory systems. Most frequently, cardiovascular problems associated with poor performance (eg, cardiac arrhythmias, cardiac murmurs) are detected at rest, but occasionally cardiac disease may be subclinical or not obvious at rest and therefore may go undiagnosed. Such is the case in exercise-induced myocardial dysfunction, which should be suspected in horses with poor performance in which no other cause of exercise intolerance is identified. In these horses, stress echocardiography may be of diagnostic value.

Exercise stress echocardiography in horses consists of an echocardiographic examination performed before and immediately after a near-maximal to maximal treadmill test. Equine exercise stress echocardiography is hampered by technical difficulties, such as the fast decline in heart rate (HR) immediately after the end of the exercise and the restlessness of the horses when the treadmill stops. To overcome these problems, dobutamine stress echocardiography has been investigated in horses. Although chronotropic and inotropic changes induced by dobutamine at an infusion rate of 50 μg/kg/min appeared to be similar to those induced by exercise, the authors of this study did not recommend the use of dobutamine as a cardiac stressor in the horse because of its cardiomyotoxic and arrhythmogenic effects.

An alternative pharmacological stress, which consists of premedication with 50 μg/kg atropine IV followed by an incremental challenge of low-dose dobutamine, has recently been described. It allows a reduction in the required dobutamine dosage by almost a factor of ten to obtain a cardiac stimulation comparable to that achieved using high dosages of dobutamine alone without inducing adverse effects such as cardiac arrhythmias, trembling, coughing, and restlessness.

The aim of the study was to test the feasibility of this pharmacological stress protocol and the effect of an atropine and low-dose dobutamine stress test on left ventricular echocardiographic parameters in untrained warmblood horses.

**Material and Methods**

Seven healthy untrained warmblood horses ranging from 9 to 22 years old (18.5 ± 6.0 years, mean ± SD) and weighing between 440 and 560 kg (506 ± 51 kg, mean ± SD) were used in this study. They had been selected from the University of Liège Faculty of Veterinary Medicine teaching herd and were treated according to the principles of the guidelines of the National Institute of Health for the care and use of laboratory animals. The protocol was approved by the ethical committee of the University of Liège.

The horses were considered healthy based on history and clinical examination and were free of cardiac disease based on clinical examination, cardiac auscultation, and echocardiographic examination. Before the protocol, a 16G catheter was inserted into the left jugular vein under local anesthesia and in aseptic conditions.

After baseline echocardiographic recordings of the left ventricle, a single dose (35 μg/kg) of atropine (crystalline powder in 1% aqueous solution) was administered IV. Five minutes after the injection of atropine, dobutamine was infused by means of an infusion pump at a rate of 2 μg/kg/min and then increased every 5 minutes in incremental steps of 1 μg/kg/min until an infusion rate of 6 μg/kg/min was reached. Echocardiographic recordings were performed 3 minutes after the injection of atropine, as well as...
3 minutes after the onset of each incremental step of dobutamine infusion.

Echocardiographic examination was performed with a 2.5 MHz phased array sector transducer. All examinations were recorded on VHS tape for later analysis. Simultaneously, the integrated apex-base ECG was recorded for later calculation of HR. For this purpose the duration of 3 consecutive RR intervals was measured and extrapolated to 60 seconds. For each parameter, the mean of 3 cardiac cycles was calculated. Left ventricular internal area (LVIA) and left ventricular external area (LVEA) were measured at end-systole (s) and end-diastole (d) from a B-mode echocardiogram of a standardized right parasternal short axis-view of the left ventricle at the chordal level. From these measurements, the following indices were calculated:

Myocardial area at end-systole (MYAs) =

\[ \text{LVEAs} - \text{LVIA}s \]

Myocardial area at end-diastole (MYAd) =

\[ \text{LVEAd} - \text{LVIA}d \]

Fractional area change (FAC) =

\[ \frac{\text{MYAd} - \text{LVIA}d}{\text{LVIA}d} \times 100. \]

From the same view, an M-mode echogram was obtained by placing the M-mode cursor across the left ventricle so that the interventricular septum and the left ventricular free wall were intersected at right angles. The following measurements were made from this M-mode echogram at end-systole (s) and end-diastole (d): interventricular septal thickness (IVS), left ventricular internal diameter (LVID), and left ventricular free wall thickness (LVFW). These parameters were used for the calculation of the following index:

Fractional shortening (FS) =

\[ \frac{\text{LVID}s - \text{LVID}d}{\text{LVID}d} \times 100. \]

During the procedure, ECG was monitored on a separate machine for the detection of arrhythmias. Each variable was analyzed separately with a repeated measures model with an auto-regressive variance-covariance matrix for the repeated measures. Least-square means were computed for each dose. Differences were considered significant at \( P < 0.05 \). The repeated mixed model included the fixed group and dose effects and the random animal and time effects. The autoregressive AR(1) covariance structure among repeated measurements gave the best fit, as measured by the largest Akaike and Schwarz values.

**Results**

All horses tolerated atropine and dobutamine administration without any adverse reactions. None of the horses developed cardiac arrhythmias during or after the pharmacological stress test.

Mean HR increased significantly after the injection of atropine (Table 1). Mean HR at dobutamine infusion rates of 2 to 6 \( \mu g/kg/min \) were significantly higher than after the administration of atropine. Mean HR obtained at dobutamine infusion rates of 5 and 6 \( \mu g/kg/min \) were significantly higher than at infusion rates of 2 \( \mu g/kg/min \) and mean HR obtained at an infusion rate of 7 \( \mu g/kg/min \) was significantly higher than at 3 \( \mu g/kg/min \). A maximal HR of 156.6 ± 12.5 bpm (mean ± SD) was reached at the rate of 6 \( \mu g/kg/min \). Results of B-mode measurements are given in Table 1. Mean values for

<table>
<thead>
<tr>
<th>Table 1. Heart rate and left ventricular echocardiographic B-mode parameters measured during pharmacological stress echocardiography in 7 healthy horses.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Atropine 35 ( \mu g/kg )</td>
</tr>
<tr>
<td>Dobutamine 2 ( \mu g/kg/min )</td>
</tr>
<tr>
<td>Dobutamine 3 ( \mu g/kg/min )</td>
</tr>
<tr>
<td>Dobutamine 4 ( \mu g/kg/min )</td>
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<tr>
<td>Dobutamine 5 ( \mu g/kg/min )</td>
</tr>
<tr>
<td>Dobutamine 6 ( \mu g/kg/min )</td>
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</tbody>
</table>

- Significantly different from baseline.  
- Significantly different from atropine.  
- Significantly different from 2 \( \mu g/kg/min \) dobutamine rate.  
- Significantly different from 3 \( \mu g/kg/min \) dobutamine rate.

\( P < 0.05 \).
Table 2. Left ventricular echocardiographic M-mode parameters measured during pharmacological stress echocardiography in 7 healthy horses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Atropine</th>
<th>Dobutamine 2 mg/kg/min</th>
<th>Dobutamine 4 mg/kg/min</th>
<th>Dobutamine 5 mg/kg/min</th>
<th>Dobutamine 6 mg/kg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVIDd (cm)</td>
<td>10.80±0.69</td>
<td>10.06±0.66</td>
<td>9.19±0.51</td>
<td>8.90±0.51</td>
<td>9.14±0.67</td>
<td>7.9±0.7</td>
</tr>
<tr>
<td>LVIDs (cm)</td>
<td>7.02±0.65</td>
<td>6.56±0.55</td>
<td>6.31±0.59</td>
<td>6.85±0.59</td>
<td>6.38±0.59</td>
<td>7.02±0.65</td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>1.89±0.19</td>
<td>2.02±0.17</td>
<td>2.10±0.20</td>
<td>2.08±0.19</td>
<td>2.02±0.13</td>
<td>2.02±0.17</td>
</tr>
<tr>
<td>IVSs (cm)</td>
<td>1.00±0.14</td>
<td>1.00±0.14</td>
<td>1.00±0.14</td>
<td>1.00±0.14</td>
<td>1.00±0.14</td>
<td>1.00±0.14</td>
</tr>
<tr>
<td>LVFWd (cm)</td>
<td>9.91±0.43</td>
<td>9.14±0.60</td>
<td>8.84±0.59</td>
<td>9.38±0.59</td>
<td>9.64±0.55</td>
<td>9.46±0.55</td>
</tr>
<tr>
<td>LVFWs (cm)</td>
<td>5.04±0.35</td>
<td>5.40±0.32</td>
<td>5.40±0.32</td>
<td>5.40±0.32</td>
<td>5.40±0.32</td>
<td>5.40±0.32</td>
</tr>
<tr>
<td>FS (%)</td>
<td>38.9±11.0</td>
<td>36.7±10.0</td>
<td>36.7±10.0</td>
<td>36.7±10.0</td>
<td>36.7±10.0</td>
<td>36.7±10.0</td>
</tr>
</tbody>
</table>

IVS, interventricular septum; LVFW, left ventricular free wall; LVID, left ventricular internal diameter; FS, fractional shortening; s, end-systole; d, end-diastole. All values expressed as mean ± SD.

For all parameters, the values at the highest dose (6 mg/kg/min) were significantly lower than at the lower doses, with the exception of LVIDd at 6 mg/kg/min, which was not significantly different from baseline values. There were no significant differences between mean values at 2, 5, and 6 mg/kg/min.

Atropine was significantly lower at dobutamine infusion rates of 2 and 4 mg/kg/min compared to values obtained at baseline. Furthermore, LVIDd was significantly lower at all steps of dobutamine infusion than at baseline, but there were no significant differences between mean values obtained at all steps of dobutamine infusion and mean values obtained at baseline. Mean values of MYAd at all steps of dobutamine infusion were significantly higher than values obtained after administration of atropine.

FAC was highest at a dobutamine infusion rate of 5 mg/kg/min with a mean value of 63.4 ± 6.3% compared to 56.2 ± 8.6% obtained at baseline. At this step, it was significantly higher than mean values obtained at baseline, mean values obtained after the administration of atropine, and mean values obtained at all steps of dobutamine infusion rates from 2 to 6 mg/kg/min. The mean value of FAC obtained at a dobutamine infusion rate of 6 mg/kg/min was significantly different from mean values obtained after the administration of atropine and mean values obtained at an infusion rate of 2 mg/kg/min.

Results of M-mode measurements of the pharmacological stress test are given in Table 2. Mean LVIDd and mean LVIDs were significantly lower at all steps of pharmacological challenge than mean values at baseline. Furthermore, mean LVIDd and mean LVIDs obtained at all steps of dobutamine infusion were significantly lower than mean values obtained after the administration of atropine, with the exception of LVIDd at 6 mg/kg/min, which was not significantly different from mean values obtained after the administration of atropine. The lowest mean value of LVIDd of 8.90 ± 0.43 cm was obtained at a dobutamine infusion rate of 3 mg/kg/min, compared to a mean value of 10.80 ± 0.69 cm obtained at baseline. The lowest mean value of LVIDs of 4.65 ± 0.15 cm was obtained at a dobutamine infusion rate of 6 μg/kg/min, compared to a mean value of 7.02 ± 0.65 cm obtained at baseline. Mean LVIDs at dobutamine infusion rates of 3 and 5 μg/kg/min were significantly lower than at an infusion rate of 2 μg/kg/min.
Mean IVSd was significantly higher at all rates of dobutamine infusion than mean values obtained at baseline. Furthermore, mean values obtained at 3, 5, and 6 μg/kg/min were significantly higher than mean values obtained at an infusion rate of 2 μg/kg/min and mean values obtained at 5 and 6 μg/kg/min were significantly higher than at 4 μg/kg/min. The highest mean value of 3.78 ± 0.10 cm was obtained at an infusion rate of 6 μg/kg/min, compared to 2.89 ± 0.19 cm obtained at baseline.

Mean IVSS was significantly higher at all rates of pharmacological challenge than mean values obtained at baseline. Mean IVSS was significantly higher at dobutamine infusion rates of 3 to 6 μg/kg/min than mean values obtained at 2 μg/kg/min. Furthermore, mean values obtained at 6 μg/kg/min were significantly higher than mean values obtained at an infusion rate of 3 μg/kg/min. The highest IVSS mean value of 5.65 ± 0.31 cm was obtained at a dobutamine infusion rate of 6 μg/kg/min, compared to 4.51 ± 0.27 cm obtained at baseline.

Mean LVFWd and mean LWFWs obtained at all rates of dobutamine infusion were significantly higher than those obtained at baseline or after the administration of atropine. Mean LVFWd obtained at dobutamine infusion rates of 3 and 6 of μg/kg/min were significantly higher than those obtained at an infusion rate of 2 μg/kg/min. Furthermore, the mean value obtained at a rate of 6 μg/kg/min was higher than at rates of 4 and 5 μg/kg/min.

Mean LVFWs values obtained at dobutamine infusion rates of 3, 5, and 6 μg/kg/min were significantly higher than those obtained at an infusion rate of 2 μg/kg/min. Furthermore, the mean value obtained at a rate 6 μg/kg/min was higher than that obtained at rates of 3, 4, and 5 μg/kg/min. The highest mean values for LVFWd and LVFWs of 3.11 ± 0.34 cm and 4.77 ± 0.18 cm, respectively, were obtained at an infusion rate of 6 μg/kg/min.

Mean values of FS at all rates of dobutamine infusion were significantly higher than at baseline or after the administration of atropine. Mean values obtained at 4, 5, and 6 μg/kg/min of dobutamine infusion were significantly higher than at 2 μg/kg/min. Mean values obtained at 6 μg/kg/min of dobutamine infusion were significantly higher than at 3 μg/kg/min. Mean FS was highest at 6 μg/kg/min with a value of 50.56 ± 3.42%.

**Discussion**

The pharmacological stress test with atropine and low-dose dobutamine used in the present study was well tolerated in all horses. In contrast to high-dose dobutamine infusion, no cardiac arrhythmias or adverse reactions such as restlessness were observed during or after the pharmacological stress test. These findings confirm an earlier study demonstrating that premedication with atropine allows reduction of dobutamine dosage almost 10-fold, reaching HR comparable to those obtained after high doses of dobutamine alone. However, in contrast to the previously reported atropine/low-dose dobutamine protocol atropine was used at a dosage of 35 μg/kg instead of 50 μg/kg to decrease the risk of abdominal discomfort. Although in the previous study using atropine at a dosage of 50 μg/kg did not result in abdominal discomfort, other studies reported abdominal discomfort after even lower doses of atropine. One study reported abdominal pain in 1 out of 5 ponies that had received 44 μg/kg of atropine IV. Another study described signs of colic in 4 of 6 horses that received cumulative doses of 11 to 22 mg of atropine as topical treatment into the ventral conjunctival sac and in 1 of 6 horses that received as little as 3 mg of atropine as a subconjunctival injection. These data indicate that abdominal discomfort may be induced in horses by much lower doses of atropine than the doses used in the pharmacological stress test. The aim was to maximally reduce the risk of abdominal discomfort while still reaching maximal HR that are similar to those observed in the previous study. The reduced dose of atropine premedication led to a slightly lower mean maximal HR than in the previous study (157 ± 7 versus 168 ± 12 bpm). However, in the previous study, young Shetland ponies were tested, and their response to atropine and dobutamine might be different than in older warmblood horses.

The ability of the dobutamine stress test to detect myocardial ischemia is dependent on an adequate increase in myocardial oxygen consumption, which is directly related to the HR achieved during stress. Achievement of a high HR seems to be essential for the ability of the stress test to detect cardiac dysfunction. In the study of Frye et al., mean postexercise HR reached 160 bpm, whereas in the studies of Marr et al. and Sampson et al., mean postexercise HR at the time of exercise stress echocardiography was 97 bpm and 112 bpm, respectively. In horses, HR declines rapidly within the first minute after exercise, which makes postexercise echocardiographic examination at high HR difficult. The protocol of the present study seems to be a good alternative to exercise stress echocardiography and to high-dose dobutamine stress echocardiography, as it allows performance of echocardiography at high HR and avoids adverse effects of high doses of dobutamine. In human medicine, the target HR of stress echocardiography is 85% of the age-predicted maximal HR, which would correspond to 187–204 bpm in these horses. Although the mean maximal HR achieved in the present study was below this value, the mean maximal HR was still higher than those that can be routinely obtained in postexercise stress echocardiography. Furthermore, in horses as well as in humans, the maximal HR decreases with increasing age. Given the high mean age of the horses of this study (18.5 years), slightly higher values can be expected in younger performance horses.

The increase in HR and the decrease in LVIDd observed in the horses of this study after the administration of atropine are in accordance with previous studies. Atropine-induced tachycardia is a consequence of the inhibition of the predominantly parasympathetic control of HR in the resting horse. Increases in HR decrease preload. In the present study, the administra-
tion of atropine led to a decrease in LVIDd, which is assumed to reflect preload. Preload is mainly defined by venous return and atrial activity. Because atropine does not influence venous return, the observed decrease LVIDd may have resulted from a reduction in diastolic filling time coupled with unchanged venous return.

Additionally, reduced venous return seems to be responsible for the changes observed during the dobutamine infusion. Dobutamine administration resulted in significant increases in IVSs, IVSd, LVFWs, and LVFWd and significant decreases in LVIDs and LVIDd. These results are similar to those obtained in a study in humans in whom dobutamine combined with atropine resulted in a gradual decrease in LVIDd and LVIDs and a gradual increase in LVFWs, LVFWd, IVSd, and IVSs during increasing pharmacological stimulation. These findings reflect the positive inotropic effect of dobutamine and are in contrast to exercise stress tests in horses in which the same parameters were not significantly altered immediately after treadmill exercise. During exercise, venous return increases as mean systemic filling pressure rises because of sympathetic stimulation of the veins and because tensing of abdominal and other muscles of the body compresses capacitance vessels. In contrast, the muscular pump function that leads to increased venous return is missing during the dobutamine stress test. The finding that the results of dobutamine stress test are related to decreased preload is suggested by studies performed in humans, in whom LVIDd was significantly lower during dobutamine than during physical heart stimulation. Furthermore, in humans as well as in horses, LVIDd is unchanged in the immediate postexercise period, but decreases thereafter because venous return is no longer maintained by sympathetic stimulation and the compressive effect of muscles, while vasodilatation of the peripheral vascular beds continues.

The addition of atropine, a cholinergic antagonist, potentiates both the positive chronotropic and positive inotropic effects of dobutamine but prolongs the relative duration of systolic emptying and shortens the diastolic filling phase of the cardiac cycle. The progressive decrease in left ventricular chamber diameter and progressive increase in wall thickness during increasing dobutamine infusion rates demonstrated in the study of Carstensen et al are in accordance with the properties of the drug and may be explained by a combined effect of increased contractility, decreased afterload, shortening of diastole, and a relative reduction in venous return. A similar mechanism could be responsible for the changes observed in the present study.

The changes in B-mode parameters obtained in the present study were in agreement with the changes in the M-mode parameters and were most significant for the LVIAs and LVIAd. These parameters also decrease in humans undergoing dobutamine or dobutamine/atropine stress echocardiography. Changes in B-mode parameters were observed after the administration of atropine and at a dobutamine infusion rate of 2 μg/kg/min, whereas no more significant change was observed in the subsequent infusion rates. Consequently, measurements of B-mode parameters at higher infusion rates can be omitted.

None of the horses in the study were in training, and therefore conclusions about their physical fitness or normal myocardial function cannot be made. However, the consistency of the response within the group suggests that these animals are representative of their breed and fitness. Additional studies using this protocol are required with animals in training so that recommendations for detecting myocardial dysfunction can be identified as a cause of poor performance.

In conclusion, the current pharmacological stress test induced the most prominent changes in the M-mode parameters LVID, FS, IVS, and LVFW. The B-mode parameters changed significantly after atropine administration and at a dobutamine infusion rate of 2 μg/kg/min, whereas there was no significant change in the subsequent steps of dobutamine infusion. This study reveals that the combination of atropine and low-dose dobutamine is a practical method to induce cardiac stimulation in the horses. It allows examination of the heart at high HR, which can be difficult to achieve in postexercise examinations, and decrease the occurrences of dobutamine-induced adverse effects, which can be observed when high doses of dobutamine are administered.

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### Footnotes

1. Intralpon2, Vygon, Ecouen, France
2. Atropine, Sigma, Bornem, Belgium
3. Dobutrex, Lilly, Brussels, Belgium
4. Model 960, Imed, San Diego, CA
5. Vingmed CFM 800, GE medical systems, Brussels, Belgium
6. Cardiofax GEM, Nihon-Kondem, Tokyo, Japan

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### References


