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# Tissue Doppler Imaging and Two-Dimensional Speckle Tracking of Left Ventricular Function in Healthy Horses After Clenbuterol Application

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The cardiac effects of high dosages of the ß$^2$-adrenergic agent clenbuterol have been the focus of several histological, biochemical and echocardiographic studies in the past. Possible effects of a therapeutic dosage on myocardial contractility and velocities have not been evaluated using tissue Doppler imaging (TDI) and two-dimensional speckle tracking (2DST) in equine medicine. Twenty-five healthy horses were treated over 14 days with clenbuterol in a normal dosage (0.8 μg/kg every 12 hours). Before and after the treatment, an echocardiographic examination was performed using B-mode, M-mode, color flow Doppler, and tissue Doppler imaging (TDI). In all horses, the radial and circumferential myocardial functions were recorded in the right parasternal short-axis view (SAX). Pulsed-wave (PW) and color TDI were used for evaluation of peak and mean myocardial velocities; myocardial deformation was documented in 2DST. An improvement of diastolic function after clenbuterol treatment was demonstrated by a significant increase of the early diastolic radial wall motion velocity ($E_m$) in all myocardial sections except the right ventricular free wall (RVFW) in TDI, as well as an increase of the E/A quotient in the left ventricular free wall (LVFW) and the interventricular septum (IVS). Shortened time intervals, in particular in the LVFW and a tendency of increase of all deformation parameters showed improved relaxation characteristics of the cardiac muscle after treatment. The results can be interpreted as beginning physiologic cardiac hypertrophy due to clenbuterol treatment. No signs of increased rigidity or reduced compliance of the heart muscle could be found at the applied dosage. This study demonstrates the sensitivity of TDI and 2DST in equine cardiology to detect myocardial remodeling before the appearance of obvious findings in conventional echocardiographic techniques. This technique can be used to detect pharmacologic effects on myocardial function.



## 1. Introduction

Developed in the early 1970s as a ß$_2$-selective adrenergic agent, clenbuterol is used for the treatment of chronic obstructive airway disease (recurrent airway obstruction [RAO]) and as a tocolytic agent in veterinary medicine [1,2]. Adrenergic drugs stimulate sympathetic nerves and are categorized according to their chemical structure and selectivity for different adrenoceptors [3]. By several structural changes of natural catecholamines, ß$_2$-selective agents like clenbuterol, a synthetic derivate of the endogenous catecholamine adrenaline, could be developed. At therapeutic dosage, their effect on ß1-receptors is minimal; therefore, producing side effects like tachycardia, arrhythmia, and angina pectoris occur only at high dosages [2,3].

Clenbuterol is used as a broncholytic agent in respiratory disease, causing bronchospasm, for example, in subacute and chronic bronchitis and bronchiolitis in RAO

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and as a supportive drug in acute bronchitis and bronchopneumonia [4]. According to the manufacturer, clenbuterol should be given twice daily orally over a treatment period of 10-14 days at a dose of 0.8 μg/kg. Nevertheless, higher doses up to 3.2 μg/kg over a longer period of up to 30 days have been supported by several studies [5]. Side-effects from stimulation of ß$_1$-receptors like sweating, muscle tremor, and tachycardia were found in only 7% of horses at a dose of 3.2 μg/kg [5]. Possible effects on echocardiographic (ECG) survey have not been the focus of this study.

Next to its bronchodilatory and tocolytic effects, clenbuterol is known for its anabolic properties on muscle tissue and fat repartitioning in cardiac and skeletal muscle [6-8]. Further effects on growing bone tissue and the endocrine and immune systems have been described in the past.

In particular, the effects of ß$_2$-adrenergic agents, in particular of clenbuterol, on skeletal muscle and cardiac function have been studied intensively [9,10]. In men, these effects are used by a combination of "ventricular assist devices" and clenbuterol for the treatment of congestive heart failure [11,12]. In the course of ventricular dysfunction, there are changes on molecular, cellular, biochemical, and structural levels in the myocardium which lead to changes in the myocardial geometry and architecture, the so-called remodeling [13-15]. This remodeling is characterized by hypertrophy of the cardiomyocytes leading to a dysfunction of these cells' contractility [16]. As cardiac remodeling is a bidirectional process, reverse remodeling from a pathologic state to a physiologic state is possible [17]. Physiologic cardiac hypertrophy is achieved by the use of clenbuterol in a dosage of up to 700 μg every 8 hours, comparable to the effects of intense exercise [18]. This increases ventricular function and decreases wall tension, allowing recovery of the cardiac system [19]. Application of a selective ß$_1$-blocker during clenbuterol treatment reduces side effects by ß$_1$-stimulation, including not only increased heart frequency and blood pressure but also pathologic cardiac hypertrophy characterized by fibrosis and apoptosis [20].

In contrast to this, several studies have found a reduction in cardiac function and a potential risk of cardiomyopathy after clenbuterol application [21-24]. Besides necrosis and apoptosis in the soleus, plantaris, and tibialis anterior muscles, necrosis particularly in the subendocardium of the left ventricle apex were found in rats [21,22]. Burniston et al. [22] reported a dose dependence, which allows separation of physiologic cardiac hypertrophy and pathologic myotoxic effects. Echocardiographic and histologic studies in mice found functional and structural improvements at a dosage of 2.4 μg/kg over 7 days [25]. In horses, the aortic diameter was increased by 24%-39% after 8 weeks of clenbuterol treatment at a dosage of 2.4 μg/kg every 12 hours, particularly after exercise. This may increase the risk of aortic root rupture.

In this study, the cardiac effects of clenbuterol at a therapeutic dose of 0.8μg/kg were evaluated using noninvasive tissue Doppler imaging (TDI) over 14 days. Clenbuterol induced cardial effects were studied by measurements of systolic and diastolic heart functions.

## 2. Materials and Methods

### 2.1. Animals

The study population consisted of 25 healthy German Warmbloods from the Bavarian stud farm Schwaiganger and the riding school of the University of Munich. The 11 geldings and 14 mares were between 7 and 11 years of age, with a mean body weight of 589 kg and a mean height of 168 cm.

### 2.2. Preparticipation Examination

After detailed clinical examination and questioning of the owners regarding the history of the horses, a specific examination of the cardiovascular system and a clinical examination of the respiratory apparatus including auscultation and percussion were performed. Horses were eligible for participation in the study provided the owner had given informed consent. Horses were ineligible for inclusion in the study if they had clinical signs of lung or heart disease.

### 2.3. Echocardiographic Examination and Off-Line Data Analysis

ECG examinations were performed by one observer throughout the entire study period, using a Vivid *i* echocardiograph[1] with continuous base-apex ECG. A 3.5-MHz annular, phased-array probe[2] with a maximum depth of 30 cm was used. Standardized right and left parasternal B-mode and color flow Doppler images were recorded for evaluation of cardiac dimensions and valvular functions. A right parasternal short axis (SAX) M-mode image of the left ventricle was acquired at the chord level for calculation of fractional shortening.

TDI was used to measure myocardial velocities in the left ventricular free wall (LVFW) and the interventricular septum (IVS).

For TDI and two-dimensional speckle tracking (2DST), a right parasternal SAX image of the left ventricle was acquired at the chord level immediately below the mitral valve. A depth of 25 cm and an image angle of 65° were chosen. Using these preset values, a frame rate of at least 63.3 frames per second was achieved with a sufficient image quality.

After identification of the correct ECG position the regions of interest (ROI) were positioned at the different myocardial segments, and measurements were taken in pulse wave (PW) and color TDI for evaluation of maximal and mean myocardial velocities. Still frames were stored during three following cardiac cycles, and afterwards, analyses of mean speed and time parameters were performed offline, following the examination, using Echopac software[3].

Color TDI was used to evaluate the mean myocardial speed in defined myocardial sections by encoding the

[1] Vivid *i* echocardiograph; medical Sy2DS (version 6.1.110 application software; Sy2DS version 1.36.18 software).

[2] Vivid *i*, 3s-RS probe; Medical Sy2DSTms; General Electric Healthcare.

[3] EchoPac Version 7.0 software Only; Firma, GE Healthcare, Horton, Norway.