Effects of experimental iatrogenic hypercortisolism on systemic and pulmonary artery pressure, left ventricular mass as well as left and right ventricular dimension and function in dogs - an echocardiographic study

Winkler, P
Effects of experimental iatrogenic hypercortisolism on systemic and pulmonary artery pressure, left ventricular mass as well as left and right ventricular dimension and function in dogs – an echocardiographic study

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vorgelegt von

Pascal Winkler

Tierarzt
von Schwerzenbach ZH, Schweiz

genehmigt auf Antrag von

PD Dr. med. vet. Tony Glaus, Referent
Prof. Dr. med. vet. Rico Thun, Co-Referent

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Summary

Background: In people, hypercortisolism (HC) causes systemic hypertension, specific secondary cardiac changes and increased morbidity and mortality. In dogs, HC is considered an important cause of systemic and possibly pulmonary hypertension (PH) potentially causing or complicating cardiac disease. Secondary cardiac changes have not been described.

Objectives: To induce systemic arterial hypertension by iatrogenic HC (IHC), to evaluate the development of PH, and to study the effects on cardiac hypertrophy and function.

Materials and methods: 6 Beagles received oral hydrocortisone and 6 Beagles placebo for 84 days. Left ventricular (LV) mass and morphology, LV and right ventricular (RV) systolic and diastolic function, and evidence of PH were assessed by repeated two-dimensional, Motion Mode, Flow and Tissue Doppler echocardiography.

Results: IHC led to a significant but only mild increase in systemic blood pressure (BP). LV hypertrophy and asymmetry could not be observed, and calculated LV mass index and eccentricity indices did not change significantly. Relevant significant changes in LV and RV systolic and diastolic function could not be observed with routine Teichholz and flow Doppler parameters and the newer Tei and modified Tei indices. Direct or indirect evidence of PH was not detectable.

Conclusions: IHC leads to a significant but only mild increase in BP, but not to relevant changes in LV and RV dimension or function and PH, detectable by echocardiography.
INTRODUCTION

Hypercortisolism (HC) has important effects on the cardiovascular system in people causing systemic arterial hypertension, atherosclerosis and an increased thromboembolic risk. The prevalence of hypertension in spontaneous hyperadrenocorticism (HAC) has been reported to range between 55 and 80%. In iatrogenic hypercortisolism (IHC), the prevalence of hypertension is much lower, with about 20% of patients who receive glucocorticoids chronically for various diseases being hypertensive; in these cases hypertension is dose-dependent. Patients with HC have a 4 to 5 times higher mortality mainly due to cardiovascular disease, typical causes of death being congestive heart failure, cardiac and cerebral stroke.

The exact mechanism of the hypertension is not clearly determined. Considered mechanism are abnormal renal vascular resistance and increased pressor responsiveness, decreased nitric oxide (NO) activity, and absence or lack of the enzyme 11β-hydroxysteroid dehydrogenase type 2 leading to increased local exposure to excess cortisol. Furthermore erythropoietin is discussed to change regional resistance in blood vessels by causing relative NO resistance. Little is known about the cardiovascular effects of HC in small animals. In dogs, HAC is considered an important cause of hypertension that may contribute to the development of left sided heart failure in association with underlying cardiac disease. Hypertension also has been recognized in close relationship to acute death in dogs shortly after treatment for HAC had been initiated. Also, severe pulmonary arterial hypertension (PH) causing right sided heart failure has been observed in association with HAC. In cats, the development of reversible congestive heart failure mimicking hypertrophic cardiomyopathy has been observed in association with glucocorticoid application, but this could not be reproduced experimentally.

Secondary morphologic cardiac changes induced by the increased afterload of hypertension have been described in people and cats, and include left ventricular (LV) hypertrophy and abnormalities in systolic and diastolic function. Interestingly, in cortisol-induced hypertension in people secondary morphologic cardiac changes do not simply reflect the degree of increased afterload. LV hypertrophy disproportionate to the degree of hypertension, asymmetrical septal hypertrophy, and changing eccentricity indicate cortisol by itself to induce LV remodeling.

In dogs, effects of HC or hypertension on LV and RV morphology and function have not been reported. Thus, the aims of this study were to induce IHC and associated hypertension, in order to evaluate echocardiographically the secondary effects on LV morphology and function.

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function, to evaluate for the development of PH and its effects on right ventricular (RV) function. Besides routine M-Mode and Doppler parameters of ventricular dimension and function, LV mass and eccentricity indices, LV and RV Tei and modified Tei indices were calculated and references values are provided.
MATERIALS AND METHODS
This randomized, placebo-controlled study was approved by the Cantonal Veterinary Office (Zurich, Canton of Zurich, Switzerland).

Dogs
Twelve 3.5-year old laboratory Beagles, 6 intact males and 6 intact females, with a body weight ranging from 9.6-14.8 kg (median 12.9 kg) were studied. Dogs were determined to be healthy on the basis of physical examination, complete blood count, serum biochemistry profile, urinalysis and indirect systemic arterial blood pressure (BP) measurement.

Study Design
In order to mimic the natural disease as closely as possible the natural glucocorticoid hydrocortisone was used to induce HC. The dosage was chosen extrapolating from previous studies inducing IHC in dogs. Dogs were randomly allocated to two groups of 6 dogs. Dogs in the treatment group received hydrocortisone at a median dose of 8.5 mg/kg (range of 7.5-9.6 mg/kg) per os q12h for 84 days (IHC group), while dogs in the control group received a placebo gelatin capsule per os q12h. BP measurements were performed before (d0), on day (d) 1, d5 d28, d56, d84 of treatment and on post drug withdrawal days d1p, d5p, d28p, d56p and d84p. Echocardiographic examinations were performed on d0, d28, d56, d84 and on d28p, d56p and d84p.

Blood pressure measurement
Before the beginning of the study, dogs were acclimated to the BP measurement procedure on 12 different days, to minimize excitement and anxiety during the study. Systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressure were measured using an indirect oscillometric device. An inflatable cuff of approximately 40% of the tail circumference was placed directly around the base of the tail without clipping hair. Before recording BP, dogs were placed in left lateral recumbency and allowed to acclimate to the surroundings for at least 10 minutes and the first BP readings were discarded. For data analysis the arithmetic mean of 10 measurements was used.
Echocardiographic measurements
Images for Two-dimensional (2D), Motion (M)-mode, Color, Pulse wave (PW), Continuous wave (CW) and PW Tissue Doppler (TDI) echocardiography were performed by one operator blinded to the treatment with an Acuson Sequoia 512. Echocardiographic images were obtained from the dogs placed in right and left lateral recumbency on a cardiac table according to published standards. 2-D and M-mode echocardiographic images were obtained using a 7 MHz transducer, and Doppler images were obtained using a 3.5 MHz transducer. All data were stored digitally and measured offline.

Echocardiographic parameters
Quantitative 2-D parameters and views included LV systolic and diastolic eccentricity indices, shortening fraction (%FS) and LV mass index. Systolic and diastolic eccentricity indices (LVEID, LVEIS) were calculated by dividing the LV septo-lateral diameter by the perpendicular diameter as described. Briefly, in right parasternal short axis the maximum diastolic septo-lateral diameter was obtained from between the papillary muscles to the septum on a frozen image just below the mitral valve level at the beginning of the QRS complex using the trailing edge to leading edge method. The maximum cranio-caudal diameter was measured on the same image on the perpendicular axis (Fig. 1).

Fig. 1. Two-dimensional echocardiographic image to obtain LV perpendicular and septo-lateral diameters to calculate LV diastolic eccentricity index.
The minimum systolic diameters were analogously obtained at the moment of maximal systolic contraction. These diameters were also used to calculate 2D %FS. For each parameter, the mean of 5 beats was calculated.

LV mass was calculated by the area length method and the formula LV mass = 1.05 \{[\frac{5}{6} A_1 \ (a+d+t)] - [\frac{5}{6} A_2 \ (a+d)]\}, and indexed to the body surface area. For the calculations, LV area was obtained at end diastole along the outer surface of the left ventricle and right sided surface of the septum (A_1) and along the subendocardial surface excluding the papillary muscles (A_2) just below the mitral valve in right parasternal short axis, maximum LV length was obtained at end diastole form the endocardial apex to the anterior mitral leaflet insertion in right parasternal long axis. The papillary muscles were excluded because they contribute only little to the total wall volume or mass, and inclusion would lead to overestimation. For each parameter, the mean of 5 measurements was calculated. Routine M-Mode Teichholz parameters were obtained from the right parasternal short axis view. Systolic time intervals (STIs, pre-ejection period PEP, ejection time ET and PEP/ET) were obtained from the right parasternal LV outflow tract view.

PW and CW Doppler images from all valves were obtained from right parasternal short axis, left caudal and left cranial views, using Color Doppler guidance and modifying the position of the probe to obtain optimal alignment with flow. Parameters measured at the semilunar valves were peak velocities (V_{max}), velocity time integrals (VTI) and STIs including PEP, ET, acceleration (AT) and deceleration time (DT). Parameters measured at the atrioventricular (AV) valves were peak E and A velocities, E/A ratio, E wave deceleration time (DTE) and the duration of the A wave (T_A). LV and RV Tei indices of global myocardial performance were calculated by the formula (a-b)/b where a is the time interval between the end of the A-wave and the onset of the next E-wave and b is the ET^{32,33} (Fig. 2). AV-valve inflow and ET time were not obtained from the same cardiac cycle (Fig. 3), and ET for RV Tei index was measured from the right parasternal short axis as well as the left cranial view. For Tei index calculations, the mean of 10 measurements was obtained.
Fig. 2. Schematic of Doppler Intervals to calculate the LV Tei-index (index myocardial performance, IMP)*

\[
\text{Index} = \frac{a \cdot b}{b}
\]

- **a** = Interval between cessation and onset of mitral inflow
- **b** = Ejection time of LV outflow

Other intervals depicted in schematic are:
- ICT = Isovolumic contraction time
- IRT = Isovolumic relaxation time


Fig. 3. Echocardiographic images to demonstrate the measurements for calculating the RV Tei-index

The left image is obtained from the left parasternal apical 4-chamber view. The sample volume is placed at the tip of the tricuspid valve to obtain right ventricular inflow. The time period between the two white lines represents the end of atrial filling (end of A-wave) till the beginning of the fast filling phase (beginning of E-wave).

The right image is obtained from the right parasternal short axis view. The sample volume is placed at the tip of the pulmonic valve to obtain right ventricular outflow. The time period between the two white lines represents the right ventricular ejection time.
PW TDI for calculating the modified Tei indices were obtained from the right parasternal short axis view between the papillary muscles for the LV free wall motion, from the left caudal view below the mitral valve anulus for the interventricular septal motion and from the left cranial view below the tricuspid valve anulus for the RV free wall motion (Fig. 4). The sample volume was put at 2 mm, high velocity low intensity signals were filtered out, and the resulting velocities were recorded at a sweep speed of 100 mm/s. The modified Tei index was calculated by the formula \((a_m-b_m)/b_m\) where \(a_m\) is the time interval between the end of the a-wave and the onset of the next e-wave and \(b_m\) is the ET.\(^{36,37}\)

**Fig. 4. Echocardiographic image to obtain measurements for calculating the RV modified Tei-index**

The image is obtained from the left parasternal apical 4-chamber view. The sample volume is placed at the anulus of the tricuspid valve on the right ventricular free wall to obtain right ventricular myocardial motion. The time period between the upper two white lines represents the right ventricular ejection time. The time period between the lower two white lines represents the sum of isovolumic contraction time + ejection time + isovolumic relaxation time.

**Statistical analysis**

Results were analyzed using nonparametric statistical methods\(^{e,f}\) and are reported as median and ranges. Differences within groups over time were tested by the Friedman's repeated measures test followed by Dunn's post-tests. Differences between groups were tested using the Mann-Whitney U test and considered significant at \(p \leq 0.05\). Intraoperator interday variability as index of reproducibility was assessed by calculating the coefficient of variation (CV) using the data in the control dogs at each examination time.\(^{38}\)
RESULTS

Induction of hypercortisolism
All dogs in the I-HC group developed typical clinical signs of cortisol excess including polyuria, polydipsia, no regrowth of clipped hair within 1 month, and thinning of the ventral abdominal skin with prominent subcutaneous veins. They also developed typical laboratory abnormalities, i.e. stress leukogramm, increased alkaline phosphatase activity, isosthenuria. Finally, plasma cortisol concentrations were significantly higher in dogs in the IHC group.

Blood Pressure
Systolic BP significantly increased from a baseline of 123 mmHg (114-136 mmHg) peaking at 143 mmHg (128-148 mmHg, p < 0.01) on d28. The highest systolic BP obtained in any individual IHC dog was 148 mmHg on d28. Diastolic BP increased from 69 mmHg (53-78 mmHg) peaking at 82 mmHg (74-82 mmHg, p < 0.05) on d56. The highest diastolic BP obtained in any individual IHC dog was 90 mmHg on d28. Mean BP increased from 90 mmHg (81 – 101 mmHg) peaking at 103 mmHg (96 – 105 mmHg, p < 0.05) on d56. On d84 diastolic and mean but not systolic BP were still significantly higher than at baseline (Fig. 5).

LV morphology and mass
No development of left ventricular hypertrophy or asymmetry under hydrocortisone treatment was evident with 2D or M-Mode parameters. During the 6-month study period in IHC dogs, LV eccentricity indices in diastole varied between 1.12 (1.05-1.24) and 1.24 (1.19-1.34 ), in systole between 0.98 (0.80-1.01) and 1.04 (0.98-1.08) without significant change or even a pattern. LV mass index did not increase, but rather decreased at 2 and 3 months post initiating hydrocortisone treatment, however, changes were not change (Fig. 6). There was not either evidence of LV volume overload as assessed by LV diastolic diameter, measured in 2D septo-laterally and perpendicularly, measured in M-Mode, and assessed by LV diastolic area. The 2D septo-lateral LV diameter only poorly correlated with the routine M-Mode LV diameter. Selective results of 2D parameters of all 12 dogs at baseline and the CVs are shown in table 1.
Fig. 5. Systolic systemic arterial blood pressure in six healthy control beagles (left) and six Beagles with iatrogenic hypercortisolism (right)*


d0 = baseline, d1-d56 = hydrocortisone respectively placebo administration, d1p-d84p = follow-up examinations after hydrocortisone respectively placebo withdrawal.
There was no increase in LV mass index during hydrocortisone treatment. Rather, LV mass index decreased during hydrocortisone therapy, however, changes were not significant.

x-axis represents measurements time points at:
1 = one month before starting oral hydrocortisone application
2 = one day before starting oral hydrocortisone application
3, 4, 5 = 28 days, 56 days and 84 days after starting oral hydrocortisone application
6, 7, 8 = 28 days, 56 days and 84 days after discontinuing oral hydrocortisone application.
Table 1: Selective quantitative 2-dimensional LV parameters in 12 healthy Beagles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (range)</th>
<th>Unit</th>
<th>CV* (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EccD</td>
<td>1-15 (1.10-1.25)</td>
<td>-</td>
<td>3.80 (2.87-6.64)</td>
</tr>
<tr>
<td>EccS</td>
<td>1.00 (0.84-1.08)</td>
<td>-</td>
<td>6.46 (4.63-8.62)</td>
</tr>
<tr>
<td>%FSsl</td>
<td>0.34 (0.20-0.40)</td>
<td>-</td>
<td>11.17 (8.77-16.20)</td>
</tr>
<tr>
<td>%FScc</td>
<td>0.41 (0.33-0.50)</td>
<td>-</td>
<td>6.63 (3.65-12.38)</td>
</tr>
<tr>
<td>LVMI</td>
<td>115.4 (100.0-138.4)</td>
<td>g/m²</td>
<td>6.85 (4.18-10.47)</td>
</tr>
</tbody>
</table>

* Coefficient of variation, calculated on results of 7 measurements at monthly intervals in 6 healthy Beagles

EccD, EccS = LV eccentricity index in diastole, systole; %FSsl, %FScc = 2D short axis LV shortening fraction, septolateral, craniocaudal; LVMI = LV mass index, calculated by the area-length method

LV function

With 2D, routine Teichholz and M-Mode STI parameters neither significant functional changes nor any tendencies were discernible. Concerning the not routinely calculated parameters, in IHC dogs 2D %FS septolateral varied between 0.29 (0.19-0.40) and 0.36 (0.20-0.39), 2D %FS craniocaudal varied between 0.36 (0.34-0.52) and 0.45 (0.37-0.52), and both only poorly correlated with routine M-Mode %FS.

Flow Doppler evaluation at the mitral inflow as well as aortic outflow did not reveal differences induced by hydrocortisone, with few exceptions. Vmax of the A-wave significantly increased from 0.45 m/s (0.36-0.50 m/s, d0) to 0.58 m/s (0.47-0.61 m/s, d56, p=0.017) and decreased again to 0.42 m/s (0.39-0.58 m/s, d84p, p=0.095), without associated significant change of the E/A ratio. VmaxAo increased steadily and significantly from 1.3 m/s (1.1-1.3 m/s, d0) to 1.4 m/s (1.3-1.8 m/s, d56, p= 0.026) and decreased again to 1.2 m/s (1.1-1.4 m/s, d56p). LVAT decreased from 41.2 ms (36.0-51.7 ms, d0) to 38.6 ms (34.0-48.6 ms, d56, p=0.24) and increased again to 43.4 ms (35.0-51.7 ms, d84p), however, changes were not significant. LVET steadily decreased from 178 ms (161-193 ms, d0) to 162 ms (137-178 ms, d 84, p = 0.041). LVPEP decreased from 53 ms (48-62 ms, d0) to 48 ms (44-60, d 84, p = 0.39.) After withdrawal of hydrocortisone, both LVET and LVPEP again increased.
The LV Tei-index fluctuated between 0.37 (0.33-0.68) and 0.52 (0.45-0.65), the modified LV Tei-indices obtained at the left ventricular free wall (Fig. 7) varied between 0.68 (0.65-0.92) and 0.81 (0.69-1.17), at the interventricular septum between 0.70 (0.65-0.85) and 0.81 (0.64-1.08), all without any significant change or even tendency during hydrocortisone application. The CV for the reproducibility of the LV Tei-index was 18.4%, and of the modified Tei-indices were 10.6 and 7.4%, respectively. The results of LV flow and tissue Doppler parameters of all 12 dogs at baseline, and the CVs are summarized in table 2.

**Fig. 7. LV modified Tei-index in six healthy control beagles (left) and six Beagles with iatrogenic hypercortisolism (right)**

- Placebo
- I-HC

\[ \text{d0} = \text{baseline, d1-d56 = hydrocortisone respectively placebo administration,} \\
\text{d1p-d84p = follow-up examinations after hydrocortisone respectively placebo withdrawal.} \]
Table 2: LV Flow and Tissue Doppler parameters in 12 healthy Beagles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (range)</th>
<th>Unit</th>
<th>CV* (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax Ao</td>
<td>1.27 (1.04-1.35)</td>
<td>ms</td>
<td>8.2 (5.9-9-9)</td>
</tr>
<tr>
<td>VTI Ao</td>
<td>0.12 (0.10-0.15)</td>
<td>m</td>
<td>6.0 (3.8-11.1)</td>
</tr>
<tr>
<td>PEP</td>
<td>52.0 (48.0-62.0)</td>
<td>ms</td>
<td>9.8 (7.6-12.5)</td>
</tr>
<tr>
<td>ET</td>
<td>176.7 (160.6-193.2)</td>
<td>m/s</td>
<td>4.2 (3.9-7.6)</td>
</tr>
<tr>
<td>PEP/ET</td>
<td>0.30 (0.26-0.33)</td>
<td>-</td>
<td>11.6 (8.8-15.5)</td>
</tr>
<tr>
<td>AT</td>
<td>41.6 (36.0-53.8)</td>
<td>ms</td>
<td>9.7 (6.4-13.4)</td>
</tr>
<tr>
<td>AT/ET</td>
<td>0.24 (0.21-0.28)</td>
<td>-</td>
<td>8.9 (5.7-10.7)</td>
</tr>
<tr>
<td>MVE</td>
<td>0.66 (0.53-0.82)</td>
<td>m/s</td>
<td>7.1 (5.4-11.1)</td>
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<tr>
<td>MVA</td>
<td>0.47 (0.36-0.66)</td>
<td>m/s</td>
<td>15.5 (9.8-20.9)</td>
</tr>
<tr>
<td>MVE/A</td>
<td>1.34 (0.97-1.85)</td>
<td>-</td>
<td>15.1 (10.4-15.6)</td>
</tr>
<tr>
<td>MVDTE</td>
<td>63.0 (48.2-71.8)</td>
<td>ms</td>
<td>16.4 (11.0-25.2)</td>
</tr>
<tr>
<td>MVTA</td>
<td>78.0 (64.4-84.0)</td>
<td>ms</td>
<td>9.6 (8.1-17.7)</td>
</tr>
<tr>
<td>Tei</td>
<td>0.51 (0.38-0.60)</td>
<td>-</td>
<td>18.8 (9.8-21.5)</td>
</tr>
<tr>
<td>TDI Tei LVW</td>
<td>0.80 (0.64-1.17)</td>
<td>-</td>
<td>10.6 (7.6-13.0)</td>
</tr>
<tr>
<td>TDI Tei IVS</td>
<td>0.78 (0.65-0.91)</td>
<td>-</td>
<td>11.0 (6.2-12.7)</td>
</tr>
</tbody>
</table>

* Coefficient of variation, calculated on results of 7 measurements at monthly intervals in 6 healthy Beagles

V\text{max}, VTI = blood flow peak velocity, velocity time integral across aortic valve; PEP = pre-ejection period; ET = ejection time; PEP/ET = pre-ejection to ejection time ratio; AT = acceleration time; AT/ET = acceleration time to ejection time ratio; MVE, MVA = mitral valve peak E and A velocity; MVE/A = mitral valve peak E to A ratio; MVDTE = mitral valve E wave deceleration time; MVTA = mitral valve A wave duration; Tei = LV Tei index; TDI Tei LVW = LV modified Tei index of the LV free wall; TDI Tei IVS = LV modified Tei index of the interventricular septum.
Right ventricular size and function and evidence of pulmonary hypertension

Right ventricular dimensions (RVWd and RVDd) obtained by M-Mode did not change during hydrocortisone treatment. These 2 parameters had poor reproducibility with CVs above 20%. Flow Doppler examination at the pulmonic outflow as well as the tricuspid inflow revealed only a few differences induced by hydrocortisone. RVET obtained from the right short axis steadily decreased from 193 ms (178-218 ms, d0) to 177 ms (153-203 ms, d84, p = 0.093), RVET from the left cranial view decreased from 197 ms (179-201 ms, d0) to 186 ms (164-195 ms, d84, p = 0.065), and both increased again after hydrocortisone withdrawal. AT/ET increased from 0.36 (0.32-0.42, d0) to 0.39 (0.38-0.47, d84, p = 0.180) and decreased again after hydrocortisone withdrawal. Vmax of the A-wave significantly increased from 0.37 m/s (0.31-0.45 m/s, d0) to 0.46 m/s (0.42-0.60 m/s, d56, p=0.009), with associated significant decrease of the E/A ratio from 1.64 (1.29-1.89, d0) to 1.43 (1.24-1.51, d56, p=0.041). On Color Doppler examination, only trivial degrees of pulmonic and tricuspid regurgitation were detectable, insufficient to accurately calculate PA-to-RV- or RV-to-RA-gradients.

The RV Tei-index with ET obtained from the right side varied between 0.11 (0.08-0.27) and 0.22 (0.10-0.37), with ET obtained from the left side between 0.12 (0.09-0.22)) and 0.22 (0.01-0.31) without a significant change or a tendency. Reproducibility was poor for both with median CVs of 23% and 25 %. The modified RV Tei-index showed a tendency to increase from 0.63 (0.57-0.99, d0) to 0.73 (0.66-0.81, d84, p = 0.18, and again decrease after hydrocortisone withdrawal (Fig. 8), the median CV was 9.43%. The results of RV flow and tissue Doppler parameters of all 12 dogs at baseline, and the CVs calculated in the control dogs are summarized in table 3.
Fig. 8. RV modified Tei-index in six healthy control beagles (left) and six Beagles with iatrogenic hypercortisolism (right)

\[ \text{RV modified Tei index} \]

\[ d0 = \text{baseline, } d1-d56 = \text{hydrocortisone respectively placebo administration,} \]
\[ d1p-d84p = \text{follow-up examinations after hydrocortisone respectively placebo withdrawal.} \]
Table 3: RV Flow and Tissue Doppler parameters in 12 healthy Beagles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (range)</th>
<th>Unit</th>
<th>CV* (median and range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>0.90 (0.64-1.12)</td>
<td>m/s</td>
<td>9.97 (3.92-13.38)</td>
</tr>
<tr>
<td>VTI PO</td>
<td>0.11 (0.08-0.13)</td>
<td>m</td>
<td>8.77 (7.68-16.05)</td>
</tr>
<tr>
<td>PEP</td>
<td>44.4 (36.4-56.6)</td>
<td>ms</td>
<td>8.59 (3.61-10.33)</td>
</tr>
<tr>
<td>ET</td>
<td>192.0 (178.0-218.2)</td>
<td>ms</td>
<td>4.01 (3.15-4.79)</td>
</tr>
<tr>
<td>PEP/ET</td>
<td>0.23 (0.19-0.29)</td>
<td>-</td>
<td>9.23 (3.84-14.12)</td>
</tr>
<tr>
<td>AT</td>
<td>76.3 (59.2-86.6)</td>
<td>ms</td>
<td>8.14 (7.21-11.73)</td>
</tr>
<tr>
<td>AT/ET</td>
<td>0.39 (0.32-0.45)</td>
<td>-</td>
<td>8.6 (7.68-10.15)</td>
</tr>
<tr>
<td>DT</td>
<td>117.8 (104.0-138.2)</td>
<td>ms</td>
<td>7.04 (5.56-8.92)</td>
</tr>
<tr>
<td>VTI left</td>
<td>0.13 (0.09-0.14)</td>
<td>m</td>
<td>9.61 (8.19-13.03)</td>
</tr>
<tr>
<td>$V_{\text{max}}$ left</td>
<td>1.08 (0.74-1.24)</td>
<td>m/s</td>
<td>11.1 (6.31-15.75)</td>
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<tr>
<td>ET left</td>
<td>196.0 (179.0-201.3)</td>
<td>ms</td>
<td>4.1 (2.38-6.09)</td>
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<tr>
<td>TVE</td>
<td>0.61 (0.47-0.70)</td>
<td>m/s</td>
<td>12.47 (8.79-16.00)</td>
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<tr>
<td>TVA</td>
<td>0.38 (0.31-0.48)</td>
<td>m/s</td>
<td>17.19 (13.28-32.96)</td>
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<tr>
<td>TVE/A</td>
<td>1.55 (1.11-2.09)</td>
<td>-</td>
<td>17.52 (14.71-29.77)</td>
</tr>
<tr>
<td>Tei</td>
<td>0.17 (0.07-0.31)</td>
<td>-</td>
<td>22.95 (20.29-52.74)</td>
</tr>
<tr>
<td>TDI Tei RVW</td>
<td>0.60 (0.55-0.99)</td>
<td>-</td>
<td>9.43 (6.41-13.84)</td>
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* Coefficient of variation, calculated on results of 7 measurements at monthly intervals in 6 healthy Beagles

$V_{\text{max}}$, VTI = blood flow peak velocity, velocity time integral across pulmonic valve; PEP = pre-ejection period; ET = ejection time; PEP/ET = pre-ejection to ejection time ratio; AT, DT = acceleration, deceleration time; AT/ET = acceleration time to ejection time ratio; VTI left, $V_{\text{max}}$ left, ET left = velocity time integral, peak velocity, ejection time obtained from the left cranial view; TVE, TVA, TVE/A = tricuspid valve peak E and A velocity, and peak E to A ratio; Tei = RV Tei index; TDI Tei RVW = RV modified Tei index of the RV free wall.
DISCUSSION

Reports from human patients with natural HAC indicate that glucocorticoids have an important effect on LV mass and morphology.\textsuperscript{1,5,10,16,20} Reports from veterinary medicine indicate that natural HAC is an important cause of systemic arterial hypertension which then would be expected to affect cardiac mass, and extrapolating from human medicine potentially also heart shape.\textsuperscript{10,21} The goals of this study were to induce IHC, to effect secondary systemic hypertension, to measure the degree of resulting LV hypertrophy by echocardiography, to assess the effects on left ventricular systolic and diastolic function, and finally to assess development and degree of HC-induced PH and its effects on RV systolic and diastolic function. Besides the attempts to answer these questions using routine quantitative M-Mode and flow Doppler echocardiographic parameters, a specific focus in the examinations was placed on more sophisticated, not routinely obtained parameters of 2D, M-Mode, flow Doppler as well as TDI echocardiography.

Even though high dose oral hydrocortisone over 3 months, indeed, resulted in clinical HC and a significant increase in systemic BP, this increase did not result in any discernible changes in LV mass or morphology. Whereas a mild increase in muscle mass may be missed by only measuring wall diameters using M-Mode echocardiography, our attempt to detect subtle LV hypertrophy by calculating LV mass was not successful, either. Furthermore, we were unable to detect the particular pattern of predominant septal hypertrophy described in humans with HC, which has been presumed to be a direct effect of excess hydrocortisone on ventricular remodeling.\textsuperscript{38} Finally, the mild increase in BP respectively potential mild increase in pulmonary artery pressure did not result in any significant changes in the LV eccentricity indices, normal reportedly being around 1.0 in people.\textsuperscript{28} Relevant changes in cardiac function secondary to HC and mild BP elevation were not discernible, either. AO Vmax significantly increased and LVAT decreased, both changes that can be explained with increasing BP. MVA (p=0.017) and MVE/A (p = 0.082) showed a significant change or a tendency, respectively, suggesting some diastolic dysfunction. However, MVE/A remained above 1 which is considered normal relaxation.\textsuperscript{39} In addition, there was a large overlap of the results, and therefore these statistically significant changes do not translate into a clinically relevant change. Newer echocardiographic parameters like STIs, Tei-index or modified Tei-index were not more sensitive than routine parameters. Recently, TDI measurements of myocardial wall motion velocities were found to be more sensitive for detecting early cardiac changes in dogs with Duchenne’s myopathy.\textsuperscript{40} We did not attempt to measure and calculate changes in wall motion velocities, because for one thing meaningful
results were not obtainable with our equipment. Furthermore, the clinical value and appropriateness of TDI had recently been questioned for reasons like its inability to detect the clinically significant peak velocity, its unsatisfactory discriminatory power within the low velocity range and its directional bias. Nevertheless, it has recently been published that reproducible results are obtainable. The primary explanation for our negative echocardiographic findings is the unexpected fact that BP elevation was only mild in our dogs. Even though increase in blood pressure was consistent and significant, in none of the dogs did BP rise to a level considered to be systemic hypertension. Second, the duration of IHC and associated mild BP elevation was 3 months, and natural HAC may be present for months to years before diagnosis. However, it is unlikely that longer treatment would have fundamentally changed the BP, because it actually tended to again decrease after 2 months of hydrocortisone treatment. Third, duration of hypercortisolism rather than duration and degree of hypertension may have been of too short duration. Indeed, Fallo et al. suggested long-lasting exposure to increased cortisol, rather than hormone or BP levels to be the most relevant determinant of LV concentric remodeling in patients with HAC. Fourth, our dogs were only 3.5 years old while HAC is more likely a disease of older dogs where other factors such as obesity, subnormal thyroid function, latent atherosclerosis or diabetes mellitus may influence the BP. Fifth, the dosage chosen is highly supraphysiological and did, indeed, result in clinical signs of HC, however, in the natural disease there are probably not just 2 daily peaks of only hydrocortisone. Sixth, there may also be some kind of adaptation to the exogenous cortisol excess. Therefore, our model of IHC that leads to the usual clinical and clinico-pathological changes of HC may only poorly reflect the natural disease. Finally, the cardiovascular effects of HC may be overestimated in dogs. Systemic hypertension may have been postulated without confirming elevated BP by repeated measurements, and adaptation to the measurement procedure itself is of crucial importance.

PH is an entity that has been receiving much attention in recent years. Several causes are established in veterinary medicine, e.g. chronic left ventricular congestive heart failure, pulmonary thromboembolism associated with various underlying diseases, and a variety of interstitial lung diseases. Natural HAC is one risk factor for pulmonary thromboembolism in dogs. Furthermore, severe pulmonary hypertension has been described in association with HAC, where no pulmonary thromboembolism and no other plausible explanation for PH were found on necropsy. Therefore, one declared goal of this study was to evaluate the effect of IHC on pulmonary artery pressure by echocardiography. The gold standard to obtain pulmonary artery pressure is invasive catheterization under anesthesia.
Pulmonary artery pressure can also reproducibly and quite exactly be estimated by flow Doppler echocardiography, when a measurable jet of tricuspid or pulmonic regurgitation can be visualized by color Doppler echocardiography.\textsuperscript{51} When regurgitation can not be documented, indirect parameters for estimating RV pressure are RV STIs, including PEP, RV AT and RV AT/ET.\textsuperscript{46,49} In this study, only trivial regurgitation jets were found, which did not allow the calculation of RV pressure and therefore potential changes in RV pressure, i.e. the development of PH. However, there were not either relevant changes in indirect parameters of PH visible. Even though $V_{\text{max}}$ across the pulmonic valve showed a tendency to increase and RVET showed a tendency to decrease, both changes associated with developing PH\textsuperscript{46}, these changes were not significant. Furthermore, RVAT/ET showed a tendency to increase which is opposite to what is expected in developing PH.\textsuperscript{46,49} Obtaining these Doppler flow parameters from the left cranial view including VTI, Vmax and RVET did not yield different results. None of the flow profiles across the pulmonic valve showed a shift towards shape with shorter AT, higher Vmax, shorter ET, and smaller AT/ET, echocardiographic signs of increasing afterload. The observation of decreasing TVE and TV E/A imply some RV diastolic dysfunction, but median TV E/A remained above 1 which is considered normal relaxation. Finally, only the modified Tei index calculated from the RVW increased and not even significantly. Therefore, under the presumption that pulmonary artery pressure did mildly increase like systemic BP, these findings would suggest that the modified Tei indices are not more sensitive than more established parameters to discern subtle changes in myocardial function.

In conclusion, based on the results of this experimental echocardiographic study, we have to dismiss our working hypothesis that IHC in dogs would cause important morphological and functional left and right ventricular cardiac changes associated with systemic and pulmonary hypertension. Rather, the study allows the only conclusion that 3 months of IHC does not measurably affect cardiac dimensions or function. Even though our model did not allow us to document the value of echocardiography to detect clinically relevant changes in cardiac mass, shape or function, this study allowed us to evaluate the reproducibility of and to provide reference values for several echocardiographic parameters that previously have not or only rarely been used in veterinary medicine. The 2D eccentricity index, %FS and LV mass index showed good reproducibility with CVs below or around 10%. RVWDd and RVDd had the worst CVs among all parameters. Reasons may be the difficulty to obtain high quality images at this level in short axis view and the small dimensions which tend more to vary. Most flow Doppler parameters had similarly good
reproducibility, except mitral and tricuspid valve inflow parameters, as well as LV and RV Tei indices with CVs around 15-20%. LV and RV modified Tei indices again had good reproducibility with CVs around 10%. The rather poor reproducibility of LV and RV Tei-indices deserves additional discussion. As a matter of fact, in individual dogs the calculated Tei-index gave a negative result. Mathematically simple, a negative result is obtained when the sum of the durations of isovolumic contraction time, ET and isovolumic relaxation time is shorter than the ET, something that is physiologically impossible. The explanation lies in the methodology that these time intervals are not obtained from the same cardiac cycles. Thus, the Tei-indices not only were not more sensitive to detect functional cardiac changes but also were only poorly reproducible. We therefore question the utility of this parameter for assessing global cardiac function. In contrast, the time intervals to calculate the modified Tei-indices are obtained from the same cardiac cycle and showed good reproducibility. In conclusion, high dose oral hydrocortisone did induce HC in healthy Beagles with associated mild increase in systolic BP, however, no measurable or relevant effects on cardiac morphology, mass or function were detectable.
FOOTNOTES

a Watson P. and Herrtage M., Round Table Discussion on treatment of HC with trilostane, 12. ECVIM-CA Conference Munich, 2002.
c Hydrocortisone tablets, Hotz Pharmacy, CH-Kusnacht.
d SDI, Vet/BP 6000; SDI, Waukesha, WI, USA.
e SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA.
f GraphPad Prism 4, San Diego, CA, USA.
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14. Glaus T. Studies on diagnosis, characterisation and pathophysiological aspects of pulmonary hypertension in the dog. Habilitation, Vetsuisse Faculty University of Zurich 2004;pp 4-16.


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## Curriculum Vitae

<table>
<thead>
<tr>
<th>Name, Vorname</th>
<th>Winkler Pascal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geburtsdatum</td>
<td>20.05.1978</td>
</tr>
<tr>
<td>Geburtsort</td>
<td>Zürich</td>
</tr>
<tr>
<td>Nationalität</td>
<td>Schweizer</td>
</tr>
<tr>
<td>Heimatort</td>
<td>Schwerzenbach</td>
</tr>
<tr>
<td>Aug. 1994-Jul.1999</td>
<td>Matura, Kantonsschule Oerlikon, Zürich, Schweiz</td>
</tr>
<tr>
<td>Okt. 2000-Nov.2006</td>
<td>Studium der Veterinärmedizin, Universität Zürich, Schweiz</td>
</tr>
<tr>
<td>Dez. 2006-July 2009</td>
<td>Anfertigung der Dissertation unter der Leitung von PD Dr. med. vet. Tony Glaus in der Abteilung für Kardiologie der Klinik für Kleintiermedizin der Vetsuisse Fakultät der Universität Zürich</td>
</tr>
<tr>
<td>Mär. 2008-Nov. 2008</td>
<td>Assistenztierarzt, Achilles Vetclinic AG, 9512 Rossrüti, Schweiz</td>
</tr>
<tr>
<td>Jan. 2009- jetzt</td>
<td>Assistenztierarzt, Tierarztpraxis Bachtelwald, 8636 Wald, Schweiz</td>
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