

國立臺灣大學獸醫專業學院臨床動物醫學研究所

碩士論文

Graduate Institute of Veterinary Clinical Science

School of Veterinary Medicine

National Taiwan University

Master Thesis

以超音波評估腎上腺功能亢進犬隻心臟及

腎臟功能

Evaluation of Cardiac and Renal Function in Dogs
Affected with Hyperadrenocorticism by Ultrasonography

陳虹吟

Hung-Yin Chen

指導教授：黃慧璧 博士

Advisor: Hui-Pi Huang, DVM, Ph.D.

中華民國 101 年 7 月

July 2012

國立臺灣大學 (碩) 博士學位論文
口試委員會審定書

以超音波評估腎上腺功能亢進犬隻心臟及
腎臟功能

Evaluation of Cardiac and Renal Function in Dogs
Affected with Hyperadrenocorticism by
Ultrasonography

本論文係陳虹吟君 (R98643011) 在國立臺灣大學獸醫學系、所完成之碩士學位論文，於民國 101 年 07 月 18 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

黃翹壁

(簽名)

吳文亭 (指導教授)

林菊龍

系主任、所長

吳文亭

(簽名)

Acknowledgement

回首這三年的研究時光，從懵懂無知到學有專精，從瑟瑟縮縮到獨當一面，對我來說是改變相當大的三年，除了在學術上的精進外，在待人處事及人生態度上也有了不小的轉變。其中最感謝的就是我的指導教授黃慧璧博士，老師對於學術的堅持及專業在這三年中深深影響著我，獸醫不該只是獨善其身的行業，研究的成果可以對實證醫學提供貢獻才是最重要的。此外，老師樂觀開朗的性格與自由開放的教學態度，也讓我學習到如何正面思考及自我管理。

本論文的完成另外亦得感謝口試委員吳應寧教授與林荀龍副教授的協助，因為有您們的幫忙與指教，使得本論文能夠更完整而嚴謹。

與我一起天天待在 402 的同學，悅倫與子綺，感謝妳們的幫助與陪伴，一起處理不同的病例，一起開會檢討門診的大小事，一起接電話，一起在 402 待到天亮，一起歡笑一起崩潰，最後，能夠一起順利畢業真是太好了，能與你們共事我感到非常幸運。

感謝于欣學姊與小向學長，謝謝你們在臨床上的指導與幫助。謝謝阿花與彥貞學姐，謝謝你們在我們慌亂無助的時候，第一線的提供幫助，也謝謝你們的傳承讓我們學習到實驗室的技術。謝謝小安學長、佩盈學姐與士琬學姐，雖然相處的時間不長，但大五時候在你們的門診獲益良多，你們是我進這個實驗室的契機。唯一的小學妹秉燁，有你真好，非常感謝你總是沒有任何推辭的就幫我們收集及處理病例，相信你的大方與優秀的外語能力能让你未來一路順遂。

謝謝台大內科的同仁與助理以及范先生，謝謝你們的幫助讓我病例的收集如此順利。感謝我最要好的朋友妮妮，雖然你早我一年離開，但研究生生活中與你一起出外玩耍與互吐苦水是忙亂的研究生活中最快樂的時光。感謝這三年中一直陪在我身邊的阿杜哥，謝謝你的體諒與包容，你的體貼與陪伴是支持我完成論文的最大動力。

最後要感謝我的摯愛的家人，謝謝你們當我最大的靠山，讓我在學習的路上無後顧之憂，這篇論文是獻給你們的。

Abstract

Hyperadrenocorticism (HAC) is a common endocrine disorder in middle-aged and older dogs. Long-term exposure to high levels of glucocorticoids causes several complications, such as hypertension, left ventricular hypertrophy, myocardial fibrosis, glomerulosclerosis and elevated renal resistance. Meanwhile, HAC also contributed both cardiac and renal dysfunction. Left ventricular myocardial function was investigated using two-dimensional speckle tracking echocardiography (2D-STE). Resistive index (RI) and pulsatility index (PI) of the renal artery and aorta, as well as the presence of microalbuminuria were also evaluated. In the present study, the prevalence of hypertension, hypertrophic cardiomyopathy (HCM), renal vascular resistance and albuminuria were significantly increased in dogs with HAC compared with age-matched non-HAC group. In dogs with HCM, dogs with adrenal-dependent hyperadrenocorticism had significantly decreased longitudinal systolic and diastolic function despite systolic performance of left ventricular were normal when evaluated using parameters derived from the conventional echocardiography. The presence of albuminuria was positive associated with increased renal RI, suggesting that albuminuria might be associated with increased vascular resistance within the kidneys.

中文摘要

腎上腺功能亢進為中老年犬隻常見的內分泌疾病，長期暴露於高濃度的糖皮質醇會導致許多併發症，包括高血壓，左心室壁肥厚，心肌纖維化，絲球體硬化及血管阻力提高。除了結構改變，腎上腺功能亢進同時也會影響心臟及腎臟的功能，造成心臟舒張不良，尿液濃縮能力下降及蛋白尿比例提升。此論文包含兩個部份：(1) 以腹腔超音波評估腎上腺功能亢進犬隻腎臟間動脈及後大動脈血管阻力改變，與蛋白尿與微蛋白尿的發生率 (2) 以新式的二維斑點追蹤超音波技術評估腎上腺功能亢進犬隻心肌運動性的改變。整體而言，腎上腺功能亢進犬隻顯著出現高血壓、心肌增厚、腎臟血管阻力增加與增加蛋白尿的發生率。此外本研究中，尿蛋白與腎臟阻抗係數成正相關，顯示尿蛋白的增加與腎臟血管阻力增加有關。在心臟評估方面，儘管傳統超音波用來計算心臟收縮功能的數值在所有犬隻都是正常且沒有差異的，但心肌厚的犬隻在斑點追蹤超音波結果顯示較正常犬隻來的低，其中又以腎上腺型腎上腺功能亢進的犬隻最為顯著。顯示心肌厚會同時影響舒張其收縮功能，而新式的二維斑點追蹤超音波可做為一敏感性較高的診斷工具。

Table of Contents

	口試委員審定書	i
	Acknowledgement	ii
	Abstract	iii
	中文摘要	iv
	Table of Contents	v
	List of Tables	vi
	List of Figures	vii
Chapter 1	General Introduction	1
	References	13
Chapter 2	Vascular Resistance and Albuminuria in Dogs Affected with Chronic Hyperadrenocorticism and Chronic Degenerative Mitral Valvular Disease	19
	References	32
Chapter 3	Assessment of Hypertrophic Cardiomyopathy in Small Breed Dogs Affected with Hyperadrenocorticism Using 2D Speckle-Tracking Echocardiography	39
	References	54
Chapter 4	Summarized Discussion	68
	References	72

List of Tables

Chapter 2

- | | | |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. | Baseline characteristics of healthy controls, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group) | 36 |
| Table 2. | The resistive index (RI), and pulsatility index (PI) of renal artery and aorta in healthy controls, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group) | 37 |

Chapter 3

- | | | |
|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. | Baseline characteristics of groups of the healthy controls, age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH) | 58 |
| Table 2. | Baseline characteristics of dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH) | 59 |
| Table 3. | Global strains and strain rates derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH) | 60 |
| Table 4. | Indices of left ventricle longitudinal tissue velocities (LTV) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH) | 61 |
| Table 5. | Indices of left ventricle radial tissue velocities (RTV) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) | 62 |

	and adrenal-dependent hyperadrenocorticism (ADH)	
Table 6.	Indices of left ventricle longitudinal strain rates (LSR) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH)	63
Table 7.	Indices of left ventricle circumferential strain rates (CSR) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH)	64
Table 8.	Indices of left ventricular longitudinal (LS) and circumferential (CS) peak strain derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH)	65
Table 9.	The intra-observer coefficient of variance (CV) of 2D-STE variables	66

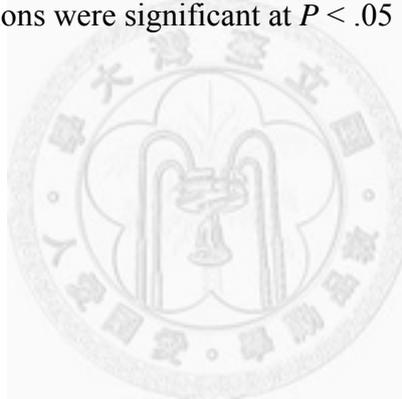
List of Figures

Chapter 2

- Figure 1. Box-and-whiskers plot of the (a) mean renal resistive index (RI) and (b) mean renal pulsatility index (PI) in clinical healthy control, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group). For each plot, the box represents the inter quartile range (IQR), the horizontal line in the middle of the box represents the median, and the whiskers denote the range. Outliers between 1.5 and 3 times the IQR are indicated by a circle

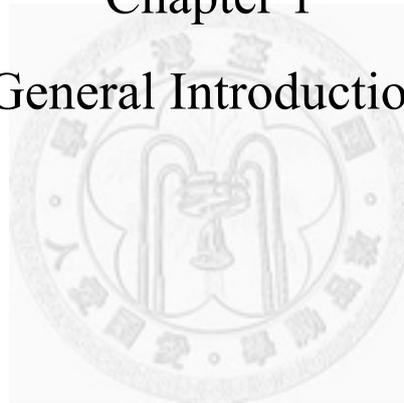
Chapter 3

- Figure 1. Variables correlated to interventricular septal thickness: (a) systolic longitudinal strain rate, (b) peak early diastolic longitudinal strain rate, and (c) peak systolic longitudinal strain. All correlations were significant at $P < .05$



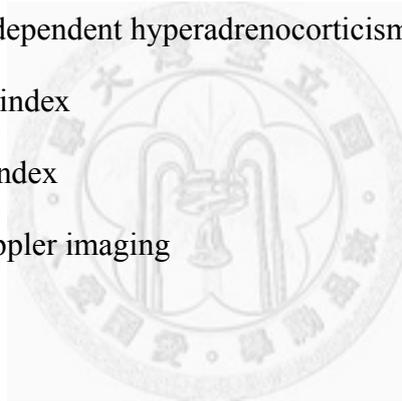
Chapter 1

General Introduction



Key to Abbreviations

2D-STE	Two-dimensional speckle-tracking echocardiography
A wave	Maximal late diastolic mitral flow velocity
ACTH	Adrenocorticotrophic hormone
ADH	Adrenal-dependent hyperadrenocorticism
E wave	Maximal early diastolic mitral flow velocity
FS	Fractional shortening
GFR	Glomerular filtration rate
HAC	Hyperadrenocorticism
HCM	Hypertrophic cardiomyopathy
PDH	Pituitary- dependent hyperadrenocorticism
PI	Pulsatility index
RI	Resistive index
TDI	Tissue Doppler imaging



HYPERADRENOCORTICISM

Hyperadrenocorticism (HAC) is often called Cushing's syndrome, after Harvey Cushing, the neurosurgeon who first described the syndrome in humans in 1932.¹ This disorder is characterized by physical and biochemical changes resulting from chronic exposure to elevated circulating glucocorticoid concentrations.² The most common clinical signs of HAC are polydipsia, polyuria, polyphagia, lethargy, abdominal enlargement or potbelly, panting, obesity, muscle weakness, and recurrent urinary tract infections. The mean age at diagnosis is 11 years, and almost all dogs with HAC are older than 9 years of age.³ The main three types of HAC are pituitary-dependent hyperadrenocorticism (PDH), adrenal-dependent hyperadrenocorticism (ADH) and iatrogenic causes. Fifty-five percent to 60% of dogs affected with PDH and 60% to 65% of dogs affected with ADH are female.³

Pathophysiology

PDH is a common endocrine disease in dogs that accounts for 80–85% of spontaneous HAC^{2,4} and is associated with excessive adrenocorticotropin hormone (ACTH) secretion which induces bilateral adrenocortical hyperplasia and excess production of cortisol. ACTH is secreted by a microscopic tumor (microadenoma) of the pars distalis or the pars intermedia in most dogs with PDH.^{5,6} Grossly visible pituitary tumors (macroadenomas) account for only 10–30% of cases of PDH in dogs.^{7,8} In one study of 33 dogs, approximately 60% has noninvasive adenomas, whereas 35% had invasive adenomas and about 5% had pituitary adenocarcinoma.⁹

The remaining 15% to 20% of dogs of spontaneous hypercortisolism are ACTH-independent and result from autonomous hypersecretion of cortisol by an adrenocortical adenoma or adenocarcinoma (ADH).² Cortisol production by the adrenal gland tumor lowers corticotropin-releasing hormone and ACTH secretion via

a negative feedback mechanism, resulting in atrophy of the unaffected adrenal gland and the normal cells of the involved adrenal gland.¹⁰ Most dogs of ADH are unilateral adrenal gland involved, the two glands being affected about equally. Bilateral adrenal tumors occur in about 10% of cases.¹¹⁻¹⁴ Differentiation between adrenocortical adenoma and carcinomas on clinical is often difficult, but dogs with an adrenal diameter greater than 2 cm are more likely to have an adrenocortical carcinoma.¹⁵

The effect on blood pressure

Hypertension is one of the most distinguishing features of spontaneous HAC, as it is present in 45-85% of adult patients¹⁶⁻¹⁸ and in 47% of children and adolescents patients.^{19,20} Likewise, systemic hypertension occurs in 32-70% of dogs with HAC.²¹⁻²³ The pathophysiologic mechanism of hypertension in Cushing's disease is complex and multifactorial affect, is characterized by: (1) activation of the mineralocorticoid receptor (2) activation of the renin-angiotensin system (3) enhanced activity of vasoactive substances (vasopressin and angiotensin II) and reduced activity of depressor systems (nitric oxide synthase, prostacyclin, and kinin-kallikrein systems) (4) enhanced pressor responses to catecholamines.²⁴⁻²⁸

The effect on cardiovascular system

In patient with activate Cushing's syndrome cardiovascular complications cause a mortality rate 4-fold higher than the age- and gender-matched general population.²⁹ It also contributes to cardiac structural changes which were characterized by left ventricular hypertrophy and concentric remodeling.³⁰⁻³⁵ All the human patients with HAC had evidence of asymmetric septal hypertrophy.³² In addition to cardiac structural change, cardiac diastolic dysfunction (e.g. reduced E/A ratio, prolonged E deceleration time) and systolic dysfunction (e.g. mid-wall FS) were also detected by

conventional echocardiography.^{30,31,33-35} But using tissue Doppler imaging could detect myocardial dysfunction in early stage.³³ The study of Pereira et al., which evaluate cardiac structural and functional changes in HAC patient with two-dimensional speckle tracking (2D-STE) image, demonstrated that left ventricular shortening in the circumferential and longitudinal directions was significantly impaired in Cushing patients compared to controls at short-term follow-up after transsphenoidal adenomectomy, indicated 2D-STE enabled an earlier detection of subtle changes in left ventricular systolic performance.³⁴

The reasons why left ventricular hypertrophy in HAC is remain unknown. Hypertension was thought to be the major factor in previous studies.^{36,37} But the increased blood pressure seems not to be essential for the development of initial structural changes in left ventricular wall, because an increase in left ventricular wall thickness could also be demonstrated in normotensive patients compared with controls.^{33,35} However, hypertensive patients with Cushing's syndrome presented a more severe alteration of cardiac mass parameters compared with both normotensive patients and hypertensive controls, and proven to worsen cardiac parameters in patients with Cushing's syndrome, even after remission of HAC. This finding suggests that hypertension is one of the main determinants of cardiac hypertrophy.³¹ Long-lasting exposure to excess circulating cortisol maybe another factor contribute to left ventricular hypertrophy in patients with Cushing's syndrome. Several ventricular structural changes occurred also in normotensive patients with Cushing's syndrome, upon remission of HAC, most abnormalities in left ventricular mass measurements reversed, and the prevalence of abnormal measurements was markedly reduced, suggesting a direct role of cortisol on myocardial tissue.³¹ Tissue effect of cortisol could also include potentiation of cardiac angiotensin II and noradrenaline responsiveness or stimulation of the local renin-angiotensin system.³⁸⁻⁴⁰ Hypokalemia,

diabetes mellitus, and increased serum cortisol levels are important factors in the development of cardiac dysfunction in patients with adrenal Cushing's syndrome.¹⁸

The effect on kidney

Glucocorticoids influence glomerular and tubular function in kidneys. Excess of glucocorticoids can cause alterations in renal parameters such as glomerular filtration rate (GFR) and urinary protein excretion. Otherwise, renal parenchyma is may influenced by excess glucocorticoids, too.

(1) Glomerular filtration rate

Short term administration of ACTH or Glucocorticoids increases GFR in humans, rats and dogs.⁴¹⁻⁴³ Glucocorticoid-induced rise in GFR by several mechanisms, including plasma volume expansion due to sodium retention, increased plasma level of atrial natriuretic peptide and amino acid ,as well as increased glomerular ultrafiltration coefficient.^{41,44,45} Long-term effects of Cushing's syndrome in humans may decrease GFR, and the researcher also indicated that disease duration was the strongest predictor for a worse GFR.⁴⁶ The same situation was noted on dogs, which decrease post-treatment GFR, urinary protein-to-creatinine ratio, urinary albumin to-creatinine ratio, urinary immunoglobulin G to-creatinine ratio, and urinary retinol-binding protein to-creatinine ratio.⁴⁷

(2) Proteinuria/ microalbuminuria

The value of proteinuria as a marker of clinically important events in the kidney arises because it can occur and subsequently vary in magnitude because of altered vascular permeability of glomerular capillary walls (possibly marking the presence of immune complexes, vascular inflammation, or intra-glomerular hypertension) or impaired tubular handling of filtered proteins (possibly marking the presence of tubulointerstitial dysfunction) or both.⁴⁸ In HAC, the increased urinary albumin

excretion was associated with several machines, including elevated blood pressure and plasma glucose levels, and abnormalities in lipid metabolism which result of excessive free fatty acids binding to the protein.^{49,50} With a reported incidence of 44 to 75%, pathological proteinuria is common in dogs affected with HAC.^{22,51,52} Presence of microalbuminuria and albuminuria in clinically healthy dogs and dogs affected with HAC was significantly different. The incidences of albuminuria was highest in dogs with ADH, followed by dogs with PDH, with the lowest levels in clinically healthy dogs; but microalbuminuria showed a different pattern which highest incidence in clinically healthy dogs, and lowest level in dogs with ADH.²² In human patients with HAC, urinary albumin excretion also increased before treatment but it was almost completely reversed after successful treatment of HAC.⁴⁹ This finding was corroborated in a second report, which mainly involved cured subjects without an increase in microalbuminuria.⁴⁶

(3) Renal parenchyma

In human patient, kidney biopsy was performed in three ADH patients with microalbuminuria showed normal glomeruli and mild, nonspecific tubular atrophy.⁴⁹ Other renal histopathological findings in humans with Cushing's syndrome included focal segmental glomerulosclerosis and membranoproliferative glomerulonephritis.^{53,54} Glomerulonephritis and amyloidosis have been described in dogs with HAC.^{52,55} But a cause-effect relationship between Cushing's syndrome and these renal lesions remains uncertain.

SPECKLE TRACKING ECHOCARDIOGRAPHY

Speckle tracking echocardiography (STE) is a new, non-invasive method for the assessment of left ventricular global and regional function. Similar to tissue Doppler

imaging (TDI), it permits offline calculation of myocardial velocities and deformation parameters such as strain and strain rate. However, in comparison to TDI, STE is insonation angle independent and does not require such high frame rates, and is not influenced by passive traction of scar tissue by adjacent vital myocardium. Speckle tracking is an echocardiographic method based on tracking of characteristic speckle patterns created by interference of ultrasound beams in the myocardium.⁵⁶

The image-processing algorithm tracks user-defined regions of interest which are comprised of blocks of approximately 20 to 40 pixels containing stable patterns that are described as “speckles”. Single speckles are merged in functional units (kernels) and each kernel constitutes a sort of ultrasound fingerprint that can be tracked by software during the entire cardiac cycle. Through analysis of the motion of each kernel that composes a routine two dimensional gray scale image, the system can calculate displacement, the rate of displacement (velocity), deformation (strain), and the rate of deformation (strain rate) of the selected myocardial segments and left ventricle rotation.⁵⁷ Strain is a dimensionless quantity of myocardial deformation. The so-called Lagrangian strain is mathematically defined as the change of myocardial fiber length during stress at end-systole compared to its original length in a relaxed state at end-diastole $(L-L_0)/L_0$.⁵⁸ It is expressed as a percentage (%). The change of strain per unit of time is referred to as strain rate. The strain rate represents the myocardial deformation rate. It is expressed as seconds^{-1} (1/s). As strain rate (1/s) is the spatial derivative of tissue velocity (cm/s), and strain (%) is the temporal integral of strain rate, all of these three parameters are mathematically linked to each other. Otherwise, speckle analysis is the ability to examine several components or planes in a single data set. Three different components of contraction have been defined: longitudinal, radial, and circumferential.⁵⁹

(1) Longitudinal deformation represents motion from the base to the apex.

- (2) Radial deformation in the short axis is perpendicular to both long axis and epicardium. Thus, radial strain represents myocardial thickening and thinning.
- (3) Circumferential strain is defined as the change of the radius in the short axis, perpendicular to the radial and long axes.

Longitudinal deformation is assessed from the apical 4-chamber views while circumferential and radial deformations are assessed from short axis views of the left ventricle. The description of these three aforementioned 'normal strains' - which can be measure using current STE technology- allows a good approximation of active cardiac motion. However, they still represent a simplification. When considering myocardial deformation during contraction in three dimensional space, six more 'shear strains' can be defined in addition to the normal strains.⁶⁰

Clinical applications on left ventricular hypertrophy

In hypertrophic cardiomyopathy (HCM), malfunctioning sarcomeric protein, myocyte hypertrophy, fiber disarray, and interstitial fibrosis which result in left ventricular systolic and diastolic dysfunction.^{61,62} STE has been used in distinguishing the different causes of left ventricular hypertrophy. Investigations with tissue Doppler-derived strains previously reported that longitudinal myocardial deformation is markedly attenuated in HCM, and the extent and pattern of regional abnormalities show wide regional heterogeneity⁶³ which helps distinguish HCM from athlete's left ventricular hypertrophy.⁶⁴ The ability of strain rate imaging derived from TDI to discriminate HCM from hypertensive left ventricular hypertrophy. The mean values of systolic strain cutoff value of -10.6% was associated with a sensitivity of 85.0%, specificity of 100%, and predictive accuracy of 91.2% for discrimination between these 2 conditions.⁶⁵ Subclinical myocardial changes in left ventricular hypertrophy also could detect by STE. Despite an apparently normal left ventricular systolic

function based on conventional echocardiography, all strain, longitudinal, radial and circumferential components were significantly reduced in patients with HCM.⁶⁶ The amount and location of left ventricular fibrosis and end-diastolic wall thickness are independent predictors of end-systolic longitudinal strain.⁶⁷ Similarly, patients with active Cushing's syndrome showed impaired left ventricular shortening in the circumferential and longitudinal directions. After normalization of corticosteroid excess, left ventricular circumferential and longitudinal shortening occurred, suggest STE is a valuable tool to detect subtle left ventricular dysfunction.³⁴ The prospective study which evaluates longitudinal myocardial strain to assess systolic function in healthy cats and in various stages of feline HCM demonstrate that systolic myocardial strain is already decreased in mild forms of HCM and decreases more in more severe HCM stages. Hence, strain analysis appears to be a more sensitive index of myocardial function than standard left ventricular function assessment.⁶⁸

RESISTIVE INDEX AND PULSATILITY INDEX

Doppler ultrasound can be used to record the velocity profile non-invasively from most peripheral vessels. During ultrasound examination, the wave is sent from a stationary transducer and the moving red blood cells reflected wave towards the transducer, which result in the frequency shift (Δf) could indirectly measure the red blood cell velocity (V). The relationship between Δf and V is:

$$\Delta f = \frac{2f_0 V \cos\theta}{c}$$

Where Δf is the frequency shift, f_0 is the transmitted frequency, V is the blood velocity, $\cos\theta$ is the insonation angle, between the ultrasound beam and the direction of motion (blood flow vector) and c is the sound velocity in tissue.

Flow measurements providing indirect information of peripheral vascular resistance can be obtained from this spectral display. Some indices have been developed to estimate vascular resistance and to evaluate and compare Doppler waveforms. These indices can be calculated from ratios including peak systolic velocity, end diastolic velocity, and mean velocity through the cardiac cycle.⁶⁹

The two primary indexes used clinically are resistive index (RI) and pulsatility index (PI). RI and PI were calculated by the following equation:

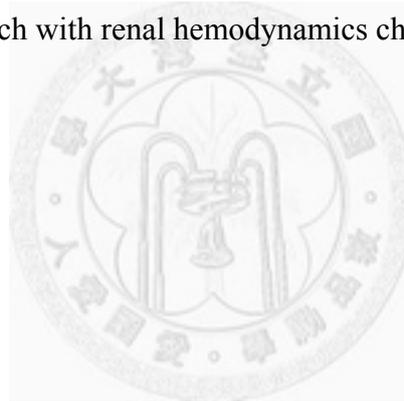
$$\text{RI} = \frac{\text{peak systolic velocity} - \text{peak end diastolic velocity}}{\text{peak systolic velocity}}$$
$$\text{PI} = \frac{\text{peak systolic velocity} - \text{peak end diastolic velocity}}{\text{mean velocity through the cardiac cycle}}$$

These indices are advanced as a useful parameter for quantifying the changes in vascular resistance from many diseases. For instance, alteration in hepatic RI have been identified with hepatic disorders,⁷⁰ higher splanchnic vascular resistance detected on acute pancreatitis,⁷¹ and higher RI and PI on metastatic lymphoma nodes.⁷² Several studies have also evaluated the benefit of using renal RI and PI in renal and urinary abnormalities.⁷³

The kidney is a well-vascularized organ which receiving on average approximately 20–25% of the total cardiac output. Many renal diseases have an important vascular component and some systemic diseases, such as hypertension, are mediated by the vascular control system of the juxtaglomerular apparatus. This fact makes the kidney a suitable organ to be evaluated by Doppler.

Renal RI and PI are measured on the blood velocity waveform obtained by placing the sample on the renal interlobar arteries or arcuate arteries. Waveforms should be optimized for measurement using the highest frequency probe, the lowest pulse repetition frequency without aliasing, the highest gain without obscuring background noise, and the lowest wall filter.⁷⁴ Several factors can produce increased renal RI and

PI, not only dependent on renal vascular resistance but also affected by other confounding factors, including patient characteristics (age, renal disease, arterial disease),^{73,75} central hemodynamics (heart rate, pulse pressure),^{76,77} intra-abdominal pressure,⁷⁸ and renal vascular compliance.^{77,79} In clinical application, measurement of renal RI and PI are widely used in the evaluation of renal parenchymal and vascular damage, renal failure, and transplantation.^{73,80} Furthermore, RI of intrarenal arteries has been shown to be associated with the severity of target organ damage in patients with hypertension⁸¹ and diabetes mellitus,⁸² as well as in dogs with hepatic disorders,⁸³ HAC, diabetes mellitus,²¹ and degenerative mitral valve disease.⁸⁴ In summary, renal RI and PI is useful for the evaluation of renal parenchymal injury and other systemic disease which with renal hemodynamics changes.



Reference:

1. Cushing H. The basophil adenomas of the pituitary body and their clinical manifestations (Pituitary Basophilism). *Bull Johns Hopkins Hosp* 1932;50:137-195.
2. Feldman EC, Nelson RW. Hyperadrenocorticism (Cushing's syndrome). In: Feldman EC, Nelson RW, eds. *Canine and Feline Endocrinology and Reproduction*. Philadelphia: WB Saunders; 2004:252-357.
3. Reusch CE, Feldman EC. Canine hyperadrenocorticism due to adrenocortical neoplasia. Pretreatment evaluation of 41 dogs. *J Vet Intern Med* 1991;5:3-10.
4. Feldman EC. Distinguishing dogs with functioning adrenocortical tumors from dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1983;183:195-200.
5. Feldman EC, Nelson RW. Neoplasms in the adeno-hypophysis of dogs. A clinical and pathologic study. *Vet Pathol* 1967;4:301-325.
6. Peterson ME, Krieger DT, Drucker WD, et al. Immunocytochemical study of the hypophysis in 25 dogs with pituitary-dependent hyperadrenocorticism. *Acta Endocrinol* 1982;101:15-24.
7. Pollard RE, Reilly CM, Uerling MR, et al. Neurologic, endocrinologic, and pathologic findings associated with large pituitary tumors in dogs: eight cases. *J Am Vet Med Assoc* 2010;24:160-165.
8. Nelson RW, Ihle SL, Feldman EC. Pituitary macroadenomas and macroadenocarcinomas in dogs treated with mitotane for pituitary-dependent hyperadrenocorticism: 13 cases (1981-1986). *J Am Vet Med Assoc* 1989;194:1612-1617.
9. Pollard RE, Reilly CM, Uerling MR, et al. Cross-Sectional Imaging Characteristics of Pituitary Adenomas, Invasive Adenomas and Adenocarcinomas in Dogs: 33 Cases (1988 –2006). *J Vet Intern Med* 2010;24:160-165.
10. Ettinger SJ, Feldman EC. *Textbook of veterinary internal medicine : diseases of the dog and the cat*. St. Louis, Mo.: Elsevier Saunders; 2010.
11. Hoerauf A, Reusch C. Ultrasonographic characteristics of both adrenal glands in 15 dogs with functional adrenocortical tumors. *J Am Anim Hosp Assoc* 1999;35:193-199.
12. Anderson CR, Birchard SJ, Powers BE, et al. Surgical treatment of adrenocortical tumors: 21 cases (1990-1996). *J Am Anim Hosp Assoc* 2001;37:93-97.
13. Ford SL, Feldman EC, Nelson RW. Hyperadrenocorticism caused by bilateral adrenocortical neoplasia in dogs: four cases (1983-1988). *J Am Vet Med Assoc* 1993;202:789-792.
14. Kyles AE, Feldman EC, De Cock HE, et al. Surgical management of adrenal gland tumors with and without associated tumor thrombi in dogs: 40 cases

- (1994-2001). *J Am Vet Med Assoc* 2003;223:654-662.
15. Labelle P, Kyles AE, Farver TB, et al. Indicators of malignancy of canine adrenocortical tumors: histopathology and proliferation index. *Vet Pathol* 2004;41:490-497.
 16. Colao A, Pivonello R, Spiezia S, et al. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab* 1999;84:2664-2672.
 17. Mancini T, Kola B, Mantero F, et al. High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. *Clin Endocrinol (Oxf)* 2004;61:768-777.
 18. Tsuiki M, Tanabe A, Takagi S, et al. Cardiovascular risks and their long-term clinical outcome in patients with subclinical Cushing's syndrome. *Endocr J* 2008;55:737-745.
 19. Magiakou MA, Mastorakos G, Oldfield EH, et al. Cushing's syndrome in children and adolescents. Presentation, diagnosis, and therapy. *N Engl J Med* 1994;331:629-636.
 20. Magiakou MA, Mastorakos G, Zachman K, et al. Blood pressure in children and adolescents with Cushing's syndrome before and after surgical care. *J Clin Endocrinol Metab* 1997;82:1734-1738.
 21. Novellas R, de Gopegui RR, Espada Y. Determination of renal vascular resistance in dogs with diabetes mellitus and hyperadrenocorticism. *Vet Rec* 2008;163:592-596.
 22. Lien YH, Hsiang TY, Huang HP. Associations among systemic blood pressure, microalbuminuria and albuminuria in dogs affected with pituitary- and adrenal-dependent hyperadrenocorticism. *Acta Vet Scand* 2010;52:61.
 23. Goy-Thollot I, Péchereau D, Kéroack S, et al. Investigation of the role of aldosterone in hypertension associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. *J Small Anim Pract* 2002;43:489-492.
 24. Ulick S, Wang JZ, Blumenfeld JD, et al. Cortisol inactivation overload: a mechanism of mineralocorticoid hypertension in the ectopic adrenocorticotropin syndrome. *J Clin Endocrinol Metab* 1992;74:963-967.
 25. Saruta T, Suzuki H, Handa M, et al. Multiple factors contribute to the pathogenesis of hypertension in Cushing's syndrome. *J Clin Endocrinol Metab* 1986;62:275-279.
 26. Saruta T. Mechanism of glucocorticoid-induced hypertension. *Hypertens Res* 1996;19:1-8.
 27. Kirilov G, Tomova A, Dakovska L, et al. Elevated plasma endothelin as an additional cardiovascular risk factor in patients with Cushing's syndrome. *Eur J*

Endocrinol 2003;149:549-553.

28. Sakaue M, Hoffman BB. Glucocorticoids induce transcription and expression of the alpha 1B adrenergic receptor gene in DTT1 MF-2 smooth muscle cells. *J Clin Invest* 1991;88:385-389.
29. Boscaro M, Barzon L, Fallo F, et al. Cushing's syndrome. *Lancet* 2001;357:783-791.
30. Baykan M, Erem C, Gedikli O, et al. Assessment of left ventricular diastolic function and Tei index by tissue Doppler imaging in patients with Cushing's Syndrome. *Echocardiography* 2008;25:182-190.
31. Toja PM, Branzi G, Ciambellotti F, et al. Clinical relevance of cardiac structure and function abnormalities in patients with Cushing's syndrome before and after cure. *Clin Endocrinol (Oxf)* 2012;76:332-338.
32. Sugihara N, Shimizu M, Kita Y, et al. Cardiac characteristics and postoperative courses in Cushing's syndrome. *Am J Cardiol* 1992;69:1475-1480.
33. Bayram NA, Ersoy R, Aydin C, et al. Assessment of left ventricular functions by tissue Doppler echocardiography in patients with Cushing's disease. *J Endocrinol Invest* 2009;32:248-252.
34. Pereira AM, Delgado V, Romijn JA, et al. Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome. *Eur J Endocrinol* 2010;162:331-340.
35. Muiesan ML, Lupia M, Salvetti M, et al. Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003;41:2275-2279.
36. Cushing H. The basophil adenomas of the pituitary body and their clinical manifestations. *Bull Johns Hopkins Hasp* 1932;50:137-195.
37. Scholz DA, Sprague RG, Kernohan JW. Cardiovascular and renal complications of Cushing's syndrome. *N Engl J Med* 1957;256:833-837.
38. Sudhir K, Jennings GL, Esler MD, et al. Hydrocortisone-induced hypertension in humans: pressor responsiveness and sympathetic function. *Hypertension* 1989;13:416-421.
39. Dzau VJ. Cardiac renin-angiotensin system. Molecular and functional aspects. *Am J Med* 1988;84:22-27.
40. Ritchie CM, Sheridan B, Fraser R, et al. Studies on the pathogenesis of hypertension in Cushing's disease and acromegaly. *Q J Med* 1990;76:855-867.
41. Connell JM, Whitworth JA, Davies DL, et al. Effects of ACTH and cortisol administration on blood pressure, electrolyte metabolism, atrial natriuretic peptide and renal function in normal man. *J Hypertens* 1987;5:425-433.
42. Davis JO, Howell DS. Comparative effect of ACTH, cortisone and DCA on renal function, electrolyte excretion and water exchange in normal dogs.

Endocrinology 1953;52:245-255.

43. Wen C, Fraser T, Li M, et al. Hemodynamic profile of corticotropin-induced hypertension in the rat. *J Hypertens* 1998;16:187-194.
44. Hall JE, Morse CL, Smith MJ, Jr., et al. Control of arterial pressure and renal function during glucocorticoid excess in dogs. *Hypertension* 1980;2:139-148.
45. Manning RD, Jr. Renal hemodynamic, fluid volume, and arterial pressure changes during hyperproteinemia. *Am J Physiol* 1987;252:F403-411.
46. Haentjens P, De Meirleir L, Abs R, et al. Glomerular filtration rate in patients with Cushing's disease: a matched case-control study. *Eur J Endocrinol* 2005;153:819-829.
47. Smets PM, Lefebvre HP, Meij BP, et al. Long-Term Follow-Up of Renal Function in Dogs after Treatment for ACTH-Dependent Hyperadrenocorticism. *J Vet Intern Med* 2012;26:565-574.
48. Lees GE, Brown SA, Elliott J, et al. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). *J Vet Intern Med* 2005;19:377-385.
49. Koh JM, Kim JY, Chung YE, et al. Increased urinary albumin excretion in Cushing's syndrome: remission after correction of hypercortisolaemia. *Clin Endocrinol (Oxf)* 2000;52:349-353.
50. Hayashi Y, Morikawa A, Makino M. Heterogeneity of urinary albumin from diabetic patients. *Clin Chim Acta* 1990;190:93-103.
51. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1998;212:369-373.
52. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *Journal of the American Veterinary Medical Association* 1996;209:1724-1729.
53. Hsieh Ck Fau - Hsieh YP, Hsieh Yp Fau - Wen YK, Wen Yk Fau - Chen ML, et al. Focal segmental glomerulosclerosis in association with Cushing's disease. 2007.
54. Tatsumi T, Morishima T, Watarai T, et al. Recurrent Cushing's disease associated with nephrotic syndrome. *Intern Med* 1995;34:10-14.
55. Center SA, Smith CA, Wilkinson E, et al. Clinicopathologic, renal immunofluorescent, and light microscopic features of glomerulonephritis in the dog: 41 cases (1975-1985). *J Am Vet Med Assoc* 1987;190:81-90.
56. Bohs LN, Trahey GE. A novel method for angle independent ultrasonic imaging of blood flow and tissue motion. *IEEE Trans Biomed Eng* 1991;38:280-286.
57. Perk G, Tunick PA, Kronzon I. Non-Doppler two-dimensional strain imaging by echocardiography--from technical considerations to clinical applications. *J Am Soc*

Echocardiogr 2007;20:234-243.

58. Urheim S, Edvardsen T, Torp H, et al. Myocardial strain by Doppler echocardiography. Validation of a new method to quantify regional myocardial function. *Circulation* 2000;102:1158-1164.
59. Blessberger H, Binder T. Two dimensional speckle tracking echocardiography: clinical applications. *Heart* 2010;96:2032-2040.
60. Pavlopoulos H, Nihoyannopoulos P. Strain and strain rate deformation parameters: from tissue Doppler to 2D speckle tracking. *Int J Cardiovasc Imaging* 2008;24:479-491.
61. Unverferth DV, Baker PB, Pearce LI, et al. Regional myocyte hypertrophy and increased interstitial myocardial fibrosis in hypertrophic cardiomyopathy. *Am J Cardiol* 1987;59:932-936.
62. Varnava AM, Elliott PM, Sharma S, et al. Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. *Heart* 2000;84:476-482.
63. Sengupta PP, Mehta V, Arora R, et al. Quantification of regional nonuniformity and paradoxical intramural mechanics in hypertrophic cardiomyopathy by high frame rate ultrasound myocardial strain mapping. *J Am Soc Echocardiogr* 2005;18:737-742.
64. Rajiv C, Vinereanu D, Fraser AG. Tissue Doppler imaging for the evaluation of patients with hypertrophic cardiomyopathy. *Curr Opin Cardiol* 2004;19:430-436.
65. Kato TS, Noda A, Izawa H, et al. Discrimination of nonobstructive hypertrophic cardiomyopathy from hypertensive left ventricular hypertrophy on the basis of strain rate imaging by tissue Doppler ultrasonography. *Circulation* 2004;110:3808-3814.
66. Serri K, Reant P, Lafitte M, et al. Global and regional myocardial function quantification by two-dimensional strain: application in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006;47:1175-1181.
67. Popović ZB, Kwon DH, Mishra M, et al. Association between regional ventricular function and myocardial fibrosis in hypertrophic cardiomyopathy assessed by speckle tracking echocardiography and delayed hyperenhancement magnetic resonance imaging. *J Am Soc Echocardiogr* 2008;21:1299-1305.
68. Wess G, Sarkar R, Hartmann K. Assessment of left ventricular systolic function by strain imaging echocardiography in various stages of feline hypertrophic cardiomyopathy. *J Vet Intern Med* 2010;24:1375-1382.
69. Nelson TR, Pretorius DH. The Doppler signal: where does it come from and what does it mean? *AJR Am J Roentgenol* 1988;151:439-447.
70. García-Criado A, Gilabert R, Salmerón JM, et al. Significance of and contributing factors for a high resistive index on Doppler sonography of the hepatic artery immediately after surgery: prognostic implications for liver transplant recipients. *AJR Am J Roentgenol* 2003;181:831-838.

71. Topal NB, Kaya E, Ercan I, et al. The role of Doppler sonography in predicting severity of acute pancreatitis. *J Clin Ultrasound* 2008;36:141-147.
72. Nyman HT, Kristensen AT, Skovgaard IM, et al. Characterization of normal and abnormal canine superficial lymph nodes using gray-scale B-mode, color flow mapping, power, and spectral Doppler ultrasonography: a multivariate study. *Vet Radiol Ultrasound* 2005;46:404-410.
73. Rivers BJ, Walter PA, Polzin DJ, et al. Duplex doppler estimation of intrarenal pourcelot resistive index in dogs and cats with renal disease. *J Vet Intern Med* 1997;11:250-260.
74. Tublin ME, Bude RO, Platt JF. Review. The resistive index in renal Doppler sonography: where do we stand? *AJR Am J Roentgenol* 2003;180:885-892.
75. Chang YJ, Chan IP, Cheng FP, et al. Relationship between age, plasma renin activity, and renal resistive index in dogs. *Vet Radiol Ultrasound* 2010;51:335-337.
76. Mostbeck GH, Gossinger HD, Mallek R, et al. Effect of heart rate on Doppler measurements of resistive index in renal arteries. *Radiology* 1990;175:511-513.
77. Tublin ME, Tessler FN, Murphy ME. Correlation between renal vascular resistance, pulse pressure, and the resistive index in isolated perfused rabbit kidneys. *Radiology* 1999;213:258-264.
78. Umgelter A, Reindl W, Franzen M, et al. Renal resistive index and renal function before and after paracentesis in patients with hepatorenal syndrome and tense ascites. *Intensive Care Med* 2009;35:152-156.
79. Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. *Radiology* 1999;211:411-417.
80. Novellas R, Ruiz de Gopegui R, Espada Y. Assessment of renal vascular resistance and blood pressure in dogs and cats with renal disease. *Vet Rec* 2010;166:618-623.
81. Raff U, Schmidt BM, Schwab J, et al. Renal resistive index in addition to low-grade albuminuria complements screening for target organ damage in therapy-resistant hypertension. *J Hypertens* 2010;28:608-614.
82. Ohta Y, Fujii K, Arima H, et al. Increased renal resistive index in atherosclerosis and diabetic nephropathy assessed by Doppler sonography. *J Hypertens* 2005;23:1905-1911.
83. Novellas R, de Gopegui RR, Espada Y. Increased renal vascular resistance in dogs with hepatic disease. *Vet J* 2008;178:257-262.
84. Chetboul V, Daste T, Gouni V, et al. Renal resistive index in 55 dogs with degenerative mitral valve disease. *J Vet Intern Med* 2012;26:101-108.

Chapter 2

Vascular Resistance and Albuminuria in Dogs
Affected with Chronic Hyperadrenocorticism and
Chronic Degenerative Mitral Valvular Disease

Key to Abbreviations

ACTH	Adrenocorticotropic hormone
ADH	Adrenal-dependent hyperadrenocorticism
ALKP	Alkaline phosphatase
ALT	Alanine transaminase
BUN	Blood urea nitrogen
CDMD	Chronic degenerative mitral valvular disease
CV	Coefficients of variations
HAC	Hyperadrenocorticism
HPF	High power of field
ISACHC	International Small Animal Cardiac Health Council
PDH	Pituitary- dependent hyperadrenocorticism
PI	Pulsatility index
RI	Resistive index
SD	Standard deviation
UACR	Urinary albumin:creatinine ratio

Vascular Resistance and Albuminuria in Dogs Affected with Chronic Hyperadrenocorticism and Chronic Degenerative Mitral Valvular Disease

Hung-Yin Chen, Hui-Pi Huang

Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

Background: Hypertension and increased peripheral vascular resistance is associated with hyperadrenocorticism (HAC).

Hypothesis/Objectives: Microalbuminuria is associated with increased systemic blood pressure, resistive index (RI) and pulsatility index (PI) of the renal artery and aorta in dogs affected with chronic degenerative mitral valvular disease (CDMD) with and without HAC in dogs.

Animals: 25 clinical healthy controls, 27 age-matched dogs affected with CDMD, and 20 dogs with spontaneous HAC and concurrent CDMD

Methods: A non-invasive blood pressure measurement, routine blood workups, echocardiography, abdominal Doppler sonography, and urine albumin analysis.

Results: Systolic blood pressure, mean renal RI, and mean renal PI were significant higher in dogs with spontaneous HAC and concurrent CDMD compared with other 2 groups ($P = .002$, $P < .001$, and $P < .001$, respectively). The prevalence of increased renal vascular resistance and albuminuria were higher in dogs with HAC and concurrent CDMD compared with other 2 groups ($P < .001$ and $P < .001$, respectively). No significant differences in aortal RI ($P = .124$) or aortal PI ($P = .067$) were found among all groups. In general, the presence of albuminuria was associated with increased renal RI ($r = .421$, $P < .001$). No correlation among blood pressure, renal RI, PI and albuminuria was found.

Conclusion and Clinical Importance: Presence of microalbuminuria and albuminuria might be associated with increased vascular resistance within the kidneys of dogs with HAC, but was not associated with increased systemic pressure, and concurrent CDMD.

Key words: canine, hyperadrenocorticism, resistive index, pulsatility index, microalbuminuria

Part of the study is going to present as an abstract in the 22nd European College of Veterinary Internal Medicine Congress, Maastricht, the Netherlands. Sep. 6-8, 2012.

Introduction

Hyperadrenocorticism (HAC) is a frequent endocrine disorder in middle-aged and older dogs. Up to 86% of dogs with untreated HAC have systemic hypertension and up to 75% have proteinuria.^{1,2} Glucocorticoid-induced hypertension results from several pathophysiological mechanisms, including increased cardiac output, total peripheral resistance and renal vascular resistance.³ Systemic hypertension leads to an increased capillary hydrostatic pressure and hyperfiltration, may eventually result in glomerulosclerosis and proteinuria. Proteinuria and systemic hypertension are major complications in development and progression of chronic kidney disease in dogs with chronic renal failure.^{4,5} Several studies have demonstrated that a correlation between human patients affected with HAC and renal failure. One case-control study reported patients with Cushing's disease have a decreased glomerular filtration rate.⁶ Creatinine clearance was lower in patients with active and cured Cushing's disease compared with controls.⁷ In addition, a highly significant correlation between endogenous glucocorticoid production and the progression rate of chronic renal failure has been described.⁸ In dogs, glomerular markers (urinary protein-to-creatinine ratio, urinary albumin and urinary immunoglobulin) and tubular markers (urinary retinol-binding protein and urinary N-acetyl-b-D-glucosaminidase) were significantly higher in the dogs with HAC compared with control dogs, which indicated glomerular and tubular dysfunction.⁹ Research regarding renal vascular resistance of dogs affected with HAC also indicated that the mean values of resistive index (RI) and pulsatility index (PI) in the dogs with HAC were significant higher than health control dogs, suggesting that the dogs with HAC had a higher vascular resistance.¹⁰

Renal RI and PI were a useful parameter for quantifying the alterations in renal blood flow that occur with renal or systemic disease. By analysis of the blood flow

velocity waveform these indices are widely accepted as indicators of vascular resistance. In human medicine, the renal RI and PI are widely used to assess renal vascular resistance in diseases involving essential hypertension, acute and chronic renal failure and, diabetes mellitus.¹¹⁻¹⁶ Not only renal vascular, RI and PI also used in different major abdominal blood vessels to predicting the severity of disease in the early stage or organ perfusion changes after transplantation^{17,18}

The aim of the study was to report systemic blood pressure, RI and PI of the renal artery and aorta, as well as the presence of microalbuminuria in dogs affected with HAC and chronic degenerative mitral valvular disease (CDMD).

Materials and Methods

Animals

Seventy-two client-owned dogs that presented to the National Taiwan University Veterinary Hospital since November 2010 to April 2012 were enrolled in this study. These dogs were categorized into clinical healthy controls, dogs affected with CDMD, dogs with CDMD and concurrent spontaneous HAC. All dogs underwent physical examination, blood pressure measurements, routine blood work (complete blood counts and biochemical profiles) examinations, conventional echocardiography, renal and aorta ultrasonography and freshly voided urine samples collected.

Dogs that had acute renal failure, hydronephrosis and nephrolithiasis were excluded from the study.

Clinically healthy dogs (control)

Twenty-five were included as healthy controls, 14 were males and 11 were females. The breeds represented in this groups including 13 miniature Dachshunds, 5 Maltese terriers, 2 each of Chihuahuas, Mongrels and toy Poodles and 1 miniature Schnauzer.

The median age of these dogs was 3.2 ± 1.9 years (range 1 to 7 years) and the mean body weight was 5.5 ± 2.3 kg (range 2.2 to 13 kg).

Dogs affected with CDMD along (CDMD group)

Twenty-seven dogs included in this group, 11 were males and 16 were females. The mean age was 11.7 ± 2.6 years (range 7 to 17 years) and the mean body weight was 9.3 ± 8.1 kg (range 2.4 to 32 kg). The following breeds were represented: 11 Maltese terriers, 7 Mongrels, 3 miniature Schnauzers, 2 Yorkshire terriers, 1 each of English Cocker Spaniel, Labrador Retriever, Pomeranian, and Shih-Tzu.

Diagnosis of CDMD was based on echocardiographic evidence of degenerative lesions of mitral valves and mitral regurgitation. Dogs with CDMD were classified by International Small Animal Cardiac Health Council (ISACHC) classification system: 13 dogs were into ISACHC class 1, 11 were into class 2, and 3 dogs were into class 3.

Dogs affected with CDMD concurrent with HAC (HAC group)

Twenty dogs affected with HAC were included in this group; 15 dogs affected with pituitary-dependent hyperadrenocorticism (PDH) and 5 dogs affected with adrenal-dependent hyperadrenocorticism (ADH). The mean age of the 20 dogs with HAC was 12.3 ± 2.5 years (range 8 to 17 years), with a mean body weight of 7.0 ± 4.2 kg (range 3.5 to 15.3 kg), 17 were females and 3 were males. Breeds represented in this group were 6 Maltese terriers, 4 Mongrels, 3 Pomeranians, 2 each of Shih-Tzus and Yorkshire terriers, 1 each of miniature Dachshund, miniature Schnauzer and Whippet.

Diagnosis of CDMD was used the same criteria as described above. Nine was classified as ISACHC class 1, 11 were in the ISACHC class 2, and none in the ISACHC class 3. Diagnosis of HAC was based on clinical signs (e.g., polydipsia,

polyuria, polyphagia, alopecia, heat intolerance, lethargy, and abdominal enlargement), increased plasma hepatic enzyme activities, and affirmative results of adrenocorticotrophic hormone (ACTH) stimulation test of post-ACTH cortisol concentration > greater than 20 µg/dl one hour after intramuscular administration of 0.25 mg synthetic ACTH (Cortrosyn, Organon, The Netherlands).¹⁹ Differentiation of PDH and ADH was based on adrenal morphological assessment.^{20,21} Bilaterally symmetrical maintenance of the normal adrenal shape and contour, and homogeneous echogenicity were classified as PDH; whereas adrenal glands with an irregular, rounded shape and mixed echogenicity were classified as ADH.^{20,21}

Systemic blood pressure measurement

Systolic blood pressure was evaluated using the indirect method of Doppler flow detector with a 9.5 MHz transducer.^a Dogs were considered as hypertensive if the average systemic blood pressure exceeded 160 mmHg.²²

Renal Doppler Ultrasonography

Hairs of the ventral abdomen and left flank areas were clipped and cleaned, and then acoustic coupling gel was applied onto the skin surface. The dogs were in right or left lateral recumbency to scan the uppermost kidney and no sedation was required. Triplex Doppler ultrasonography was performed by a single operator (HYC) using an ultrasound machine^b with a high-resolution multi-frequency Convex probe (4-8 MHz). Using color Doppler to visualize the intrarenal vasculature and then detecting renal blood flow on one of the interlobar or arcuate arteries by pulsed Doppler. The smallest scale that displayed the flow without aliasing was selected. The mean RI and PI for each kidney were determined by averaging a total of nine Doppler waveforms at the arteries at three separate locations.^{23,24} RI and PI were calculated by the following

equation:

$$RI = \frac{\text{peak systolic velocity} - \text{peak end diastolic velocity}}{\text{peak systolic velocity}}$$

$$PI = \frac{\text{peak systolic velocity} - \text{peak end diastolic velocity}}{\text{mean velocity through the cardiac cycle}}$$

Aorta Doppler ultrasonography

Aorta Doppler ultrasonography was obtained with the animal in right lateral recumbency. Aorta waveforms were obtained with pulsed Doppler ultrasound and aorta RI and PI were calculated by mean of six Doppler waveforms by the machine software applications.

Microalbuminuria assay and urinary albumin:creatinine ratio

The freshly voided urine samples were collected. Samples of hematuria (≥ 10 RBC/high power of field), pyuria (≥ 5 WBC/HPF), and bacteriuria were excluded from the study. The urine albumin and creatinine concentrations were semi-quantitated^c and urinary albumin:creatinine ratio (UACR) was calculated accordingly. In this study, no microalbuminuria, microalbuminuria and albuminuria were defined as UACR < 30 mg/g (0.03), 30-300 mg/g (0.03-0.3), and > 300 mg/g (0.3), respectively.

Statistical analysis

The results of data were expressed as mean \pm standard deviation (SD). The Kolomogorov-Smirnov test was used for testing normal distribution of all data with commercial computer statistics software.^d

One-way ANOVA with Bonferroni correction was used for the normally distributed data to make comparison between groups, while the non-normally distributed data

were analyzed by using Kruskal-Wallis test. Chi-square statistic was used to assess differences of categorical variables between groups. Left and right interlobar RI values were compared in each class by a paired *t*-test. The relationship between RI, PI, age and blood pressure was assessed with Pearson's correlation coefficient. The relationships between RI, PI and UACR were assessed with Spearman's correlation coefficient.

The intra-observer coefficients of variations (CV) of the RI and PI were calculated by a variance component analysis. The CV was determined by dividing the SD value by the overall mean. Six clinical healthy dogs were scanned on three nonconsecutive occasions within one day by the same examiner (HYC), who was unaware of values from the first examination.

All tests were two-tailed and the *P* value < .05 was considered statistically significant.

Results

Baseline characteristics of the study population are shown in Table 1. The age of clinical healthy control was significant younger than CDMD and HAC groups (*P* < .001). No significant differences in body weight (*P* = .055) were found among all groups. The systemic blood pressure and body condition score was significant higher in HAC group compared with control group (*P* = .002 and *P* = .018, respectively). Prevalence of hypertension among control, CDMD and HAC groups were 4% (1/25), 4% (1/27) and 30% (6/20), respectively. The prevalence of hypertension was also significantly different among groups (*P* = .007). Plasma ALKP concentration was significantly higher in HAC group compared with control and CDMD group (*P* = .002). Plasma urea and creatinine concentrations were significantly lower in control group than CDMD group (*P* = .013 and *P* = .001, respectively). No significances in

plasma albumin, ALT, glucose and total protein concentrations were found among all groups ($P = .172$, $P = .067$, $P = .403$ and $P = .869$, respectively). (Table 1)

The RI and PI of right and left kidneys were not different in all dogs ($P = .103$ and $P = .175$, respectively). Both right and left renal RI of the HAC group were significantly higher than the indices of the control and CDMD group ($P < .001$ and $P < .001$, respectively). Similarly, both right and left renal PI were significantly higher than the indices of the control and CDMD group ($P < .001$ and $P < .001$, respectively, Table 2). The means of renal RI and PI are also presented graphically in Figure 1. The upper cut-off values for renal RI and PI were calculated as the mean + 2 SD of the indices of the clinical healthy controls and were 0.70 for renal RI and 1.45 for renal PI. Based on these upper limits, the prevalence of increased renal vascular resistance of the dogs in control group (1/25, 4%), CDMD group (2/27, 7%) and HAC group (10/20, 50%) were significantly different ($P < .001$). However, no significant differences in aortal RI and aortal PI values were found between all groups ($P = .124$ and $P = .067$, respectively, Table 2).

In control group, 68% (17/25) did not have microalbuminuria, 32% (8/25) had microalbuminuria, and 0% (0/25) had albuminuria. In CDMD group, 44% (12/27) did not have microalbuminuria, 56% (15/27) had microalbuminuria, and 0% (0/27) had albuminuria. In HAC group, 30% (6/20) did not have microalbuminuria, 30% (6/20) had microalbuminuria, and 40% (8/20) had albuminuria. Presence of microalbuminuria and albuminuria in dogs among three groups were significantly different ($P < .001$).

In general, the presence of albuminuria was positive associated with increased mean renal RI ($r = .421$, $P < .001$). Age was mild but positively correlated with mean renal RI ($r = .296$, $P = .012$). No correlation among systemic blood pressure, renal RI ($P = .103$), renal PI ($P = .161$) and albuminuria ($P = .452$) were found.

CV (%) of renal RI, renal PI, aorta RI and aorta PI were 2.8%, 4.5%, 7.1% and 9.1%, respectively.

Discussion

The RI and the PI provide information on expression of the resistance to blood flow within an artery by means of a ratio between peak systolic, end diastolic, and mean velocities of spectral Doppler vascular flow waveform. When an increase in vascular resistance occurs, diastolic blood flow is reduced to a greater degree than systolic blood flow. Then, the relatively greater decrease in end diastolic velocity than in peak systolic velocity causes an elevation in RI and PI.²⁵ Increased RI and PI has been reported in dogs with ureteral obstruction,²³ acute or chronic kidney disease,^{15,16} liver disease,²⁶ CDMD,²⁷ diabetes mellitus and HAC.¹⁰ In the present study, higher renal RI and PI were recorded in HAC group compared with control and CDMD groups, suggesting that the dogs affected with HAC had a higher vascular resistance. These findings agreed with the results of previous study.¹⁰ However, there was no statistically significant in renal RI and PI values between control and CDMD groups, although both values of CDMD group were higher than control group. Although a positive association between increased interlobar RI and severity of heart failure class has been reported in dogs with CDMD, interlobar RI values in ISACHC class 1 dogs and normal dogs were not different.²⁷ In this study, 48% of dogs in CDMD group was classified as ISACHC class 1, this might explain that the difference of RI of controls and CDMD was not significant.

The renal RI can be affected by age. The renal RI were significantly higher in the young dogs (< 4 months old) and children (< 4 years old).^{28,29} In elderly patients without renal insufficiency, the normal renal RI can also exceed 0.70. In this study, a positive correlation was found between age and mean renal RI. This finding was

based on a pool of clinically healthy dogs, dogs with CDMD with and without HAC. However, control group was significant younger than groups of both CDMD and HAC. Most dogs with higher renal RI were affected by diseases. The effect of age on renal arterial resistance in dogs remains unclear.

No significant different was found between aorta RI and PI among all groups. Some studies in animals suggested that several mechanisms may cause marked alterations in blood flow resistance in more distal arterial branches of the kidney.³⁰ Excess glucocorticoids have many detrimental effects on kidney function. High levels of glucocorticoids accelerate the progression of chronic renal failure.⁸ The glomerular and tubular dysfunction has also been indicated in dogs with HAC.⁹ In this study increased RI and PI was detected in renal artery instead of aorta, suggesting that kidney was sensitive to evaluate vascular resistance than aorta.

Microalbuminuria, is an early and sensitive marker which permits timely and effective therapy in the early phase of renal damage, and is independently associated with increased cardiovascular risk factors and cardiovascular morbidity in human patients.³¹ Microalbuminuria is also associated with various systemic diseases such as neoplasia, infectious diseases, and immune-mediated, or inflammatory disease and urinary disorders.³² Several machines contribute to increased urinary albumin excretion in patient affected with HAC, such as hypertension, elevated plasma glucose levels, and abnormalities in lipid metabolism.³³⁻³⁵ In the present study, the prevalence of microalbuminuria in control group was similar to previous studies which ranged from 19 to 52%.^{36,37} And the prevalence of microalbuminuria was higher in CDMD group than HAC group, and the prevalence of albuminuria was higher in HAC group than CDMD group. In this study all dogs in HAC group had concurrent CDMD. Presence of albuminuria was positively associated with increased mean renal RI. This finding suggested that the kidney injury resulted from excessive

circulating glucocorticoids might be responsible for a greater degree of albuminuria secretion in HAC group.

Systemic hypertension is commonly found in dogs with HAC.^{1,2,10,38} Activation of the renin-angiotensin system and mineralocorticoid receptor, enhanced activity of vasoactive substances (catecholamines and/or vasopressin and angiotensin II), and reduced activity of depressor systems (nitric oxide synthase, prostacyclin, and kinin-kallikrein systems) may be involved in glucocorticoid associated hypertension.³⁹⁻⁴³ In this study, both the mean systolic blood pressure and the prevalence of hypertension of HAC group were significantly higher than control and CDMD group. Although hypertension can be a cause and a consequence of renal failure.^{4,44} Significantly higher systemic blood pressure, increased renal RI, PI and prevalence of albuminuria was found in dogs with HAC, our study failed to demonstrate the correlation between hypertension and albuminuria.

Conclusion

Increased systemic hypertension, renal vascular resistance and prevalence of albuminuria were found in dogs with HAC. The presence of microalbuminuria and albuminuria might be associated with increased vascular resistance within the kidneys, but was not associated with increased systemic pressure or concurrent CDMD.

Footnotes

- a. Model 811-B, Parks Medical Electronics Inc., U.S.A.
 - b. MyLab™ 50 Family, Esaote, Genova, Italy
 - c. Clinitek Microalbumin Reagent Strips and Clinitek Status Analyser, Bayer Diagnostic Mfg. Ltd., UK
 - d. SPSS Version 12.0.1, Lead Technologies Inc., Charlotte, North Carolina, U.S.A.
-

Reference:

1. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1998;212:369-373.
2. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *J Am Vet Med Assoc* 1996;209:1724-1729.
3. Magiakou MA, Smyrnaki P, Chrousos GP. Hypertension in Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab* 2006;20:467-482.
4. Jacob F, Polzin DJ, Osborne CA, et al. Association between initial systolic blood pressure and risk of developing a uremic crisis or of dying in dogs with chronic renal failure. *J Am Vet Med Assoc* 2003;222:322-329.
5. Jacob F, Polzin DJ, Osborne CA, et al. Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. *J Am Vet Med Assoc* 2005;226:393-400.
6. Haentjens P, De Meirleir L, Abs R, et al. Glomerular filtration rate in patients with Cushing's disease: a matched case-control study. *Eur J Endocrinol* 2005;153:819-829.
7. Faggiano A, Pivonello R, Spiezia S, et al. Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission. *J Clin Endocrinol Metab* 2003;88:2527-2533.
8. Walser M, Ward L. Progression of chronic renal failure is related to glucocorticoid production. *Kidney Int* 1988;34:859-866.
9. Smets PM, Lefebvre HP, Meij BP, et al. Long-Term Follow-Up of Renal Function in Dogs after Treatment for ACTH-Dependent Hyperadrenocorticism. *J Vet Intern Med* 2012;26:565-574.
10. Novellas R, de Gopegui RR, Espada Y. Determination of renal vascular resistance in dogs with diabetes mellitus and hyperadrenocorticism. *Vet Rec* 2008;163:592-596.
11. Sugiura T, Wada A. Resistive index predicts renal prognosis in chronic kidney disease. *Nephrol Dial Transplant* 2009;24:2780-2785.
12. Ohta Y, Fujii K, Arima H, et al. Increased renal resistive index in atherosclerosis and diabetic nephropathy assessed by Doppler sonography. *J Hypertens* 2005;23:1905-1911.
13. Petersen LJ, Petersen JR, Talleruphuus U, et al. The pulsatility index and the resistive index in renal arteries. Associations with long-term progression in chronic renal failure. *Nephrol Dial Transplant* 1997;12:1376-1380.
14. Stevens PE, Gwyther SJ, Hanson ME, et al. Noninvasive monitoring of renal

- blood flow characteristics during acute renal failure in man. *Intensive Care Med* 1990;16:153-158.
15. Rivers BJ, Walter PA, Polzin DJ, et al. Duplex doppler estimation of intrarenal pourcelot resistive index in dogs and cats with renal disease. *J Vet Intern Med* 1997;11:250-260.
 16. Novellas R, Ruiz de Gopegui R, Espada Y. Assessment of renal vascular resistance and blood pressure in dogs and cats with renal disease. *Vet Rec* 2010;166:618-623.
 17. Garcia-Criado A, Gilabert R, Berzigotti A, et al. Doppler ultrasound findings in the hepatic artery shortly after liver transplantation. *AJR Am J Roentgenol* 2009;193:128-135.
 18. Topal NB, Kaya E, Ercan I, et al. The role of Doppler sonography in predicting severity of acute pancreatitis. *J Clin Ultrasound* 2008;36:141-147.
 19. Lien Y-H, Huang H-P. Use of ketoconazole to treat dogs with pituitary-dependent hyperadrenocorticism: 48 cases (1994-2007). *J Am Vet Med Assoc* 2008;233:1896-1901.
 20. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in dogs. *J Am Vet Med Assoc* 1995;207:1180-1183.
 21. Hoerauf A, Reusch C. Ultrasonographic characteristics of both adrenal glands in 15 dogs with functional adrenocortical tumors. *J Am Anim Hosp Assoc* 1999;35:193-199.
 22. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med* 2007;21:542-558.
 23. Nyland TG, Fisher PE, Doverspike M, et al. Diagnosis of urinary tract obstruction in dogs using duplex Doppler ultrasonography. *Vet Radiol Ultrasound* 1993;34:348-352.
 24. Pollard R, Nyland TG, Bernsteen L, et al. Ultrasonographic evaluation of renal autografts in normal cats. *Vet Radiol Ultrasound* 1999;40:380-385.
 25. Rifkin MD, Needleman L, Pasto ME, et al. Evaluation of renal transplant rejection by duplex Doppler examination: value of the resistive index. *AJR Am J Roentgenol* 1987;148:759-762.
 26. Novellas R, de Gopegui RR, Espada Y. Increased renal vascular resistance in dogs with hepatic disease. *Vet J* 2008;178:257-262.
 27. Chetboul V, Daste T, Gouni V, et al. Renal resistive index in 55 dogs with degenerative mitral valve disease. *J Vet Intern Med* 2012;26:101-108.
 28. Chang YJ, Chan IP, Cheng FP, et al. Relationship between age, plasma renin activity, and renal resistive index in dogs. *Vet Radiol Ultrasound* 2010;51:335-337.

29. Bude RO, DiPietro MA, Platt JF, et al. Age dependency of the renal resistive index in healthy children. *Radiology* 1992;184:469-473.
30. Platt JF. Duplex Doppler evaluation of native kidney dysfunction: obstructive and nonobstructive disease. *AJR Am J Roentgenol* 1992;158:1035-1042.
31. Hillege HL, Janssen WM, Bak AA, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med* 2001;249:519-526.
32. Whittemore JC, Gill VL, Jensen WA, et al. Evaluation of the association between microalbuminuria and the urine albumin-creatinine ratio and systemic disease in dogs. *J Am Vet Med Assoc* 2006;229:958-963.
33. Koh JM, Kim JY, Chung YE, et al. Increased urinary albumin excretion in Cushing's syndrome: remission after correction of hypercortisolaemia. *Clin Endocrinol (Oxf)* 2000;52:349-353.
34. Hayashi Y, Morikawa A, Makino M. Heterogeneity of urinary albumin from diabetic patients. *Clin Chim Acta* 1990;190:93-103.
35. Kubota E, Hayashi K, Matsuda H, et al. Role of intrarenal angiotensin II in glucocorticoid-induced renal vasodilation. *Clin Exp Nephrol* 2001;5:186-192.
36. Jensen WA, Grauer GF, Andrew J, et al. Prevalence of microalbuminuria in dogs. *J Vet Intern Med* 2001;15:300.
37. Pressler BM, Vaden SL, Jensen WA, et al. Prevalence of microalbuminuria in dogs evaluated at a referral veterinary hospital. *J Vet Intern Med* 2001;15.
38. Goy-Thollot I, Péchereau D, Kéroack S, et al. Investigation of the role of aldosterone in hypertension associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. *J Small Anim Pract* 2002;43:489-492.
39. Ulick S, Wang JZ, Blumenfeld JD, et al. Cortisol inactivation overload: a mechanism of mineralocorticoid hypertension in the ectopic adrenocorticotropin syndrome. *J Clin Endocrinol Metab* 1992;74:963-967.
40. Saruta T, Suzuki H, Handa M, et al. Multiple factors contribute to the pathogenesis of hypertension in Cushing's syndrome. *J Clin Endocrinol Metab* 1986;62:275-279.
41. Saruta T. Mechanism of glucocorticoid-induced hypertension. *Hypertens Res* 1996;19:1-8.
42. Kirilov G, Tomova A, Dakovska L, et al. Elevated plasma endothelin as an additional cardiovascular risk factor in patients with Cushing's syndrome. *Eur J Endocrinol* 2003;149:549-553.
43. Sakaue M, Hoffman BB. Glucocorticoids induce transcription and expression of the alpha 1B adrenergic receptor gene in DTT1 MF-2 smooth muscle cells. *J Clin*

Invest 1991;88:385-389.

44. Finco DR. Association of systemic hypertension with renal injury in dogs with induced renal failure. J Vet Intern Med 2004;18:289-294.



Table 1. Baseline characteristics of healthy controls, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group).

Variables	Control (n=21)	CDMD group (n=27)	HAC group (n=20)
Age (y/o)	3.2 ± 1.9	11.7 ± 2.6 [‡]	12.3 ± 2.4 [‡]
Heart rate (bpm)	122.3 ± 14.4	118.6 ± 18.6	127.1 ± 20.3
Blood pressure (mmHg)	133.9 ± 13.2	133.7 ± 16.8	148.8 ± 13.9 ^{‡¶}
Body weight (kg)	5.5 ± 2.3	9.3 ± 8.1	7.0 ± 4.2
Body condition scores	3.0 ± 0.5	3.2 ± 0.4	3.5 ± 0.8 [‡]
Albumin (g/dL)	3.3 ± 0.4	3.2 ± 0.3	3.4 ± 0.4
ALKP (U/L)	70.8 ± 23.8	176.5 ± 221.0 [‡]	950.0 ± 1147.9 ^{‡¶}
ALT (U/L)	27.2 ± 11.4	116.0 ± 178.4	235.9 ± 277.8
BUN (mg/dL)	13.3 ± 2.8	29.8 ± 23.7 [‡]	23.7 ± 12.1
Creatinine (mg/dL)	0.6 ± 0.2	1.0 ± 0.4 [‡]	0.8 ± 0.3
Glucose (mg/dL)	111.2 ± 16.5	109.5 ± 40.1	141.7 ± 123.1
Total protein (g/dL)	6.8 ± 0.2	6.8 ± 0.5	6.9 ± 0.6

Indices are presented as mean ± SD.

ALKP, alkaline phosphatase; ALT, alanine transaminase; BUN, blood urea nitrogen.

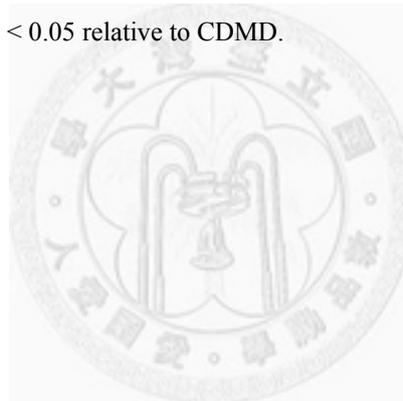
[‡]*P* < 0.05 relative to control; [¶]*P* < 0.05 relative to CDMD.

Table 2. The resistive index (RI), and pulsatility index (PI) of renal artery and aorta in healthy controls, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group).

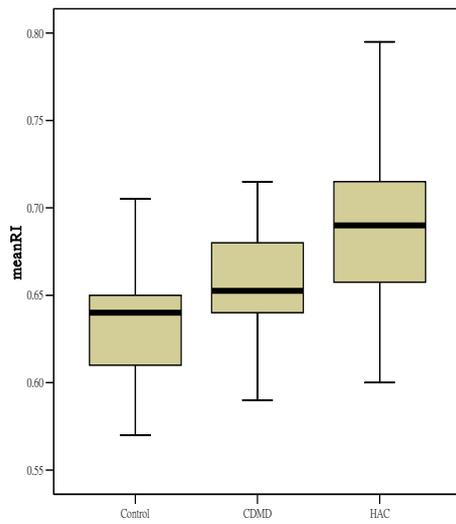
Variables	Control (n=21)	CDMD group (n=27)	HAC group (n=20)
Right renal RI	0.63 ± 0.04	0.65 ± 0.03	0.69 ± 0.05 ^{‡¶}
Right renal PI	1.15 ± 0.16	1.20 ± 0.11	1.39 ± 0.27 ^{‡¶}
Left renal RI	0.63 ± 0.03	0.66 ± 0.03	0.70 ± 0.06 ^{‡¶}
Left renal PI	1.16 ± 0.14	1.26 ± 0.13	1.40 ± 0.27 ^{‡¶}
Mean renal RI	0.64 ± 0.03	0.66 ± 0.03	0.69 ± 0.06 ^{‡¶}
Mean renal PI	1.17 ± 0.11	1.22 ± 0.11	1.39 ± 0.27 ^{‡¶}
Aorta RI	0.93 ± 0.04	0.90 ± 0.04	0.91 ± 0.07
Aorta PI	3.33 ± 0.62	2.94 ± 0.85	2.87 ± 0.50

Indices are presented as mean ± SD.

[‡]*P* < 0.05 relative to control; [¶]*P* < 0.05 relative to CDMD.



(a)



(b)

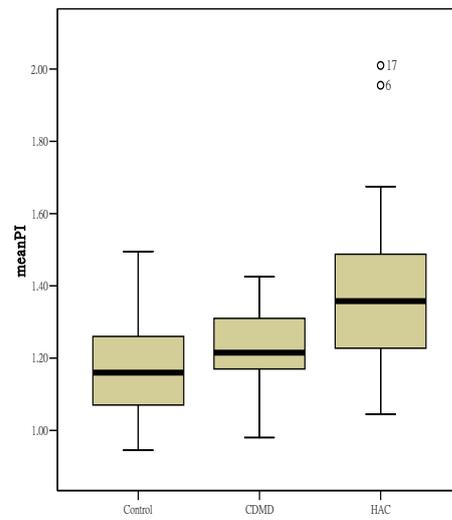


Figure 1: Box-and-whiskers plot of the (a) mean renal resistive index (RI) and (b) mean renal pulsatility index (PI) in clinical healthy control, dogs affected with chronic degenerative mitral valvular disease (CDMD group) along and dogs affected with CDMD and concurrent hyperadrenocorticism (HAC group). For each plot, the box represents the inter quartile range (IQR), the horizontal line in the middle of the box represents the median, and the whiskers denote the range. Outliers between 1.5 and 3 times the IQR are indicated by a circle.

Chapter 3

Assessment of Hypertrophic Cardiomyopathy in Small Breed Dogs Affected with Hyperadrenocorticism Using 2D Speckle-Tracking Echocardiography

Key to Abbreviations

2D-STE	Two-dimensional speckle-tracking echocardiography
A wave	Maximal late diastolic mitral flow velocity
ACTH	Adrenocorticotrophic hormone
ADH	Adrenal-dependent hyperadrenocorticism
Ao	Aorta
CS	Circumferential strain
CSR	Circumferential strain rate
CV	Coefficients of variations
E wave	Maximal early diastolic mitral flow velocity
EF	Ejection fraction
EPSS	E-point to septal separation
FS	Fractional shortening
HAC	Hyperadrenocorticism
HCM	Hypertrophic cardiomyopathy
IVRT	Isovolumic relaxation time
IVSd	Interventricular septal thickness
LA	Left atrium
LS	Longitudinal strain
LSR	Longitudinal strain rate
LTV	Longitudinal tissue velocity
LVEDD	Left ventricular end-diastolic diameters
LVESD	Left ventricular end-systolic diameter
LVFWd	Left ventricular free-wall thickness
PDH	Pituitary- dependent hyperadrenocorticism
RTV	Radial tissue velocity

SD

Standard deviation



Assessment of Hypertrophic Cardiomyopathy in Small Breed Dogs Affected with Hyperadrenocorticism Using 2D Speckle-Tracking Echocardiography

Hung-Yin Chen, Hui-Pi Huang

Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

Background: Chronic excessive cortisol concentrations cause hypertension, subsequent left ventricular hypertrophy and altered diastolic function. Patients with hyperadrenocorticism (HAC) were increased prevalence of left ventricular hypertrophy and affect systolic and diastolic function.

Hypothesis/Objectives: HAC is associated with increased prevalence of hypertension and hypertrophic cardiomyopathy (HCM).

Animals: Thirteen dogs with pituitary-dependent hyperadrenocorticism (PDH), 12 dogs with adrenal-dependent hyperadrenocorticism (ADH), 53 age-matched non-HAC dogs with HCM and 23 healthy control dogs.

Methods: Left ventricular systolic and diastolic was assessed using conventional echocardiography and two-dimensional speckle-tracking echocardiography (2D-STE).

Results: In conventional echocardiography, 6 dogs with PDH (46%), 8 dogs with ADH (67%) and 6 non-HAC dogs (11%) were diagnosed as HCM ($P < .001$). In these dogs, either the systolic or diastolic parameters were not different. In 2D-STE analysis, dogs with ADH exhibited significantly decreased longitudinal peak systolic and early diastolic strain rates ($P = .001$ and $P < .001$, respectively), and longitudinal peak systolic strain ($P < .001$) compared to non-HAC and control groups.

Conclusion and Clinical Importance: HCM was prevalent in HAC comparing to age-matched non-HAC small breed dogs. In dogs with HCM, 2D-STE revealed significant decreases in both diastolic and systolic functions those were not detected using conventional echocardiography in ADH comparing to non-HAC and control groups.

Key words: canine, 2D-STE, hypertrophic cardiomyopathy, hyperadrenocorticism, hypertension

Part of the study was presented as an abstract in the 2012 American College of Veterinary Internal Medicine Forum, New Orleans, Louisiana. May 30 - June 2, 2012.

Introduction

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterized by left ventricular hypertrophy.¹ Histopathologically it is associated with myocardial hypertrophy, disarray, interstitial fibrosis, increased loose connective tissue and abnormal small intramural coronary arteries.²⁻⁴ HCM is the most common cardiomyopathy of cats, comprise approximately 58% to 68 % of feline cardiomyopathies.⁵ However, it is an infrequent form of heart disease in dogs. In dogs, the prevalence of HCM is accounting for 5% of canine cardiac disease in one study.² However, patients with hyperadrenocorticism (HAC) which chronic exposure to elevated circulating glucocorticoid concentrations were significantly increased prevalence of left ventricular hypertrophy and concentric remodeling.⁶⁻⁸ These structural abnormalities can be partially explained by the rise in blood pressure. Toja et al demonstrated hypertensive patients with Cushing's syndrome had twofold higher risk of detection of cardiac mass abnormality compared with normotensive patients and hypertensive controls.⁹ Otherwise, another important factor is attributed to glucocorticoid excess, which directly activates some of the components of the rennin-angiotensin system^{10,11} and in particular enhances angiotensin-II responsiveness of the myocytes.¹² More importantly, control of both the mineralocorticoid and the glucocorticoid receptors appears to be critical in the regulation and development of myocardial fibrosis.¹³ In clinical research, the study by Yiu et al also demonstrated that patients with active Cushing's syndrome have increased myocardial fibrosis as assessed by calibrated integrated backscatter, when compared with both normal subjects and hypertension patients with similar left ventricular wall thickness and mass index.¹⁴ Another study researched by Toja et al demonstrated the left ventricular wall thickness was significantly increased in hypertensive patients with Cushing's syndrome compared with hypertensive controls,

increase ventricular wall thickness was also detected on normotensive patients with Cushing's syndrome compared with age-, sex- and body mass index-matched controls.⁹ These findings appear that hypercortisolism per se worsens ventricular mass changes.

Speckle tracking echocardiography is a more recent ultrasound technique, which has already been used for investigating myocardial function in humans and in dogs and cats.¹⁵⁻¹⁷ By tracking of characteristic speckle patterns created by interference of ultrasound beams in the myocardium, it offers sensitive noninvasive and quantitative in vivo analysis of left ventricular global and regional function.¹⁸ It permits offline calculation of myocardial velocities and deformation parameters such as strain and strain rate. Basically, strain measures the magnitude of myocardial fiber deformation, and is expressed as the % of change from its original dimension. Strain rate measures the rate of deformation of a tissue segment and is measured in s^{-1} .¹⁹ It is well accepted that these parameters provide important insights into systolic and diastolic function, ischaemia, myocardial mechanics and many other pathophysiological processes of the heart.²⁰

The aim of the study was to assess left ventricular systolic and diastolic function using 2D speckle-tracking echocardiography (2D-STE) in small breed dogs affected with HCM in pituitary- and adrenal-dependent hyperadrenocorticism (PDH and ADH, respectively) and age-matched non-HAC dogs.

Subjects and Methods

Animals

One hundred and one client-owned small breed dogs (body weight < 10 kg) that presented to the National Taiwan University Veterinary Hospital since August 2010 to April 2012 were included in this perspective study. These dogs were categorized into

clinical healthy controls, dogs affected with HAC, and age-matched non-HAC dogs. All dogs underwent physical examination, blood pressure measurements, routine blood workups (complete blood counts and biochemical profiles), echocardiographic examination and 2D-STE.

Clinically healthy controls

Twenty-three were included as healthy controls, 14 were males and 9 were females. The breeds represented in this groups including 7 miniature Dachshunds, 6 Maltese terriers, 5 Chihuahuas, 2 each of miniature Schnauzers and toy Poodles and 1 Beagle. The median age of these dogs was 3.6 ± 2.1 years (range 1 to 7 years) and the mean body weight was 5.2 ± 2.3 kg (range 2.6 to 9 kg).

Age-matched non-hyperadrenocorticism dogs (non-HAC group)

Fifty-three dogs included in this group, 26 were males and 27 were females. The mean age was 11.6 ± 3.0 years (range 4 to 18 years) and the mean body weight was 4.5 ± 2.3 kg (range 2.1 to 9.8 kg). The following breeds were represented: 17 Maltese terriers, 9 Pomeranians, 7 Shih-Tzus, 5 Yorkshire terriers, 4 miniature Schnauzers, 4 toy Poodles, 3 Chihuahuas, 2 Mongrels, 1 each of Welsh corgi and Pug.

Dogs affected with hyperadrenocorticism (HAC group)

Twenty-five dogs affected with HAC were included in this group; 13 dogs affected with PDH and 12 dogs affected with ADH. The mean age of the 13 dogs with PDH was 12.8 ± 2.4 years (range 9 to 17 years), with a mean body weight of 4.4 ± 1.2 kg (range 2.7 to 6.6 kg), 6 were males and 7 were females. The mean age of the dogs affected with ADH was 12.7 ± 2.3 years (range 9 to 15 years), mean body weight was 6.1 ± 2.7 kg (range 2.1 to 10.0 kg), 4 were males and 9 were females. Breeds of 25

dogs affected with HAC are 9 Maltese terriers, 5 Shih-Tzus, 3 miniature Schnauzers, 2 Yorkshire terriers, 2 Chihuahuas, 1 each of Dachshund, West Highland White terrier, Pomeranian and toy poodle. The diagnosis of HAC was based on clinical signs (e.g., polydipsia, polyuria, polyphagia, alopecia, heat intolerance, lethargy, and abdominal enlargement), increased plasma hepatic enzyme activities, and affirmative results of Adrenocorticotrophic hormone (ACTH) stimulation test of post-ACTH cortisol concentration > greater than 20 µg/dl one hour after intramuscular administration of 0.25 mg synthetic ACTH (Cortrosyn, Organon, The Netherlands).²¹ Differentiation of PDH and ADH was based on adrenal morphological assessment.^{22,23} Bilaterally symmetrical maintenance of the normal adrenal shape and contour, and homogeneous echogenicity were classified as PDH; whereas adrenal glands with an irregular, rounded shape and mixed echogenicity were classified as ADH.^{22,23}

Systemic blood pressure measurement

Systolic blood pressure was evaluated using the indirect method of Doppler flow detector with a 9.5 MHz transducer^a. Dogs were considered as hypertensive if the average systemic blood pressure exceeded 160 mmHg.²⁴

Conventional echocardiography

Conventional echocardiography was performed without sedation in gently restrained recumbent position. Standard echocardiographic views were obtained in right and left lateral recumbency using the 2D-guided M mode with ultrasound units equipped with 2-5 and 5-7.5 MHz transducers.^b Left ventricular end-diastolic diameters (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular free-wall thickness (LVFWd) and interventricular septal thickness (IVSd) at end-diastole, E-point to septal separation (EPSS), ejection fraction (EF), fractional

shortening (FS) were measured in the right parasternal long-axis view. The left atrium to aorta ratio (LA/AO) was measured in the short-axis view. The inflow of the maximal early (E) and late (A) diastolic mitral and tricuspid flow velocities, E/A ratio and isovolumic relaxation time (IVRT) were determined by pulsed-wave Doppler from the left apical 4- or 5-chamber view.

HCM was defined as regional or generalized concentric hypertrophy with a diastolic wall thickness ≥ 6 mm of the LVFWd or of the IVSd in body weight < 5 kg dogs, and ≥ 7 mm of the LVFWd or of the IVSd in body weight 5–11 kg dogs.

Two-dimensional speckle-tracking echocardiography

2D-STE examinations were performed at the end conventional echocardiography, the standard left apical long-axis 4-chamber and the right parasternal short-axis views at the level of papillary muscles were obtained with sampling rates from 45-96 Hz. For each dog, 3 consecutive cardiac cycles were recorded using 1 single loop, and digitally stored in a hard disk for offline analysis on a workstation.^c The STE variables included myocardial tissue velocity, strain, and strain rate. Peak systolic, early diastolic, and late diastolic radial tissue velocity (RTV), circumferential strain rate (CSR) values and peak circumferential strain (CS) values were measured at six segments (cranioseptal, cranial, lateral, caudal, ventral, and septal) from the right parasternal short-axis view at the level of papillary muscles. Peak systolic, early diastolic, and late diastolic longitudinal tissue velocity (LTV), strain rate (LSR) values and peak longitudinal strain (LS) values were measured at three segments (basal, middle, and apical) of LVFWd and IVSd from the left apical long-axis 4-chamber view, and also at septal and lateral corners of mitral annulus. Global strain and strain rate were calculated by averaging values of all segmental longitudinal or circumferential strain and strain rate. All 2D-STE variables were recorded in dogs

with HCM.

Statistical analysis

The results of data were expressed as mean \pm standard deviation (SD). The Kolomogorov-Smirnov test was used for testing normal distribution of all data with commercial computer statistics software.^d

One-way ANOVA with Bonferroni correction was used for the normally distributed data to make comparison between groups, while the non-normally distributed data were analyzed by using Kruskal-Wallis test. Chi-square statistic was used to assess differences of categorical variables between groups. The relationship between strain and strain rate components and left ventricular wall thickness (LVFWd and IVSd) was assessed with Pearson's correlation coefficient.

The intra-observer coefficients of variations (CV) of the conventional echocardiographic and 2D-STE variables were calculated by a variance component analysis. The CV was determined by dividing the SD value by the overall mean. Six clinical healthy dogs were scanned on three nonconsecutive occasions within one day by the same examiner (HYC), who was unaware of values from the initial examination.

All tests were two-tailed and the *P* value $< .05$ was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics of the study population are shown in Table 1. Control group was significantly younger comparing with groups of non-HAC, PDH and ADH ($P < .001$). No difference in heart rate, LVEDD, LVESD, EF, FS and tricuspid E wave,

and tricuspid E/A ratio were found among these four groups.

Blood pressure was significantly higher in ADH group compared with non-HAC and control groups ($P = .002$). Prevalence of hypertension among ADH, PDH and non-HAC groups were 58% (7/12), 15% (2/13) and 6% (3/53), respectively. The prevalence of hypertension was also significantly difference among three groups ($P < .001$).

IVSd were significantly higher in groups of ADH and PDH compared with non-HAC groups, and IVSd was significant higher in ADH than in controls ($P < .001$). LVFWd was significantly higher in ADH compared with non-HAC ($P = .028$). Mitral E wave was significantly higher in non-HAC compared with groups of PDH and ADH ($P = .001$). Mitral E/A ratio was significantly lower in ADH compared with non-HAC and control groups, and significantly lower in PDH group than control group ($P < .001$).

HCM was diagnosed in 11% (6/35) non-HAC dogs, 46% (6/13) dogs with PDH, and 67% (8/12) dogs with ADH ($P < .001$). In these dogs presented with HCM, blood pressure was significantly higher in ADH compared with non-HAC ($P = .025$).

However, FS, EF, E/A ratio, IVRT were not different among these 3 groups (Table 2).

The IVSd and LA/Ao ratio were significant higher in dogs affected with HCM (total n = 20) compared to control group ($P < .001$ and $P = .025$, respectively). However, no significant difference FS or EF was found between these 2 groups. In diastolic function, mitral and tricuspid E/A ratio were significantly decreased and IVRT were significantly increased in dogs affected with HCM compared to control group ($P = .002$, $P = .001$ and $P = .036$, respectively).

Coefficients of variation (%) of IVSd, LEFWd, LVEDD and LVESD were 6.0%, 12.5%, 2.5% and 7.0% respectively.

Two-dimensional speckle-tracking echocardiography

Among the dogs affected with HCM, the global peak systolic LSR, peak early diastolic LSR and peak LS values in dogs of ADH was significantly lower compared to non-HAC and control groups ($P = .001$, $P < .001$ and $P < .001$, respectively, Table 3). The IVSd was negatively correlated to global peak systolic LSR, peak early diastolic LSR and peak LS values ($r = -.409$, $P = .004$; $r = -.455$, $P = .001$; and $r = -.399$, $P = .005$, respectively, Figure 1). No correlation between LVFWd and STE values were found.

In regional long-axis and short-axis deformation, 28 of 98 (29%) STE variables differed significantly among non-HAC, PDH, ADH and control groups, including 4 peak diastolic LTV, all peak systolic and early diastolic LSRs and all LSs. No significant difference in peak systolic, early diastolic, and late diastolic RTV, CSR values and CS values were found among these controls, non-HAC, PDH, ADH (Table 4-8).

Coefficients of variation (%) of 2D-STE variables were all less than 25%. (Table 9)

Discussion

It is well-documented that human patients with Cushing's syndrome are at an increased risk of cardiovascular morbidity and mortality.²⁵⁻²⁷ Chronic excessive cortisol concentrations cause hypertension, subsequent left ventricular remodeling, hypertrophy, and altered diastolic function are the most important risk factors of mortality.²⁵⁻²⁷

Approximately 70-90% of human patients with HAC present with hypertension that develops in consequence of the dyslipidemia, vascular remodeling, and increased vascular resistance associated with chronic hypercortisolism.²⁸⁻³⁰ Hypertension secondary to water retention and volume overload is caused by increased

mineralocorticoid hormone levels in human patients with adenomatous adrenals.³¹ Likewise, systemic hypertension occurs in 47 to 86% of HAC in dogs.³²⁻³⁵ In this study, the prevalence of hypertension in dogs with HAC was 36%. The prevalence of hypertension was low compared to the previous studies, the prevalence of hypertension in dog with HAC was significant higher than age-matched non-HAC dogs. This finding supported that dogs affected with HAC might be prone to develop hypertension. Different methods of blood pressure measurements, definition of hypertension, and case numbers may explain lower incidence in this study.³⁶ In this study, the condition of HAC was newly diagnosed. Systemic blood pressure was measured at the initial diagnosis. A higher incidence of hypertension might be seen at later stages of the condition.

In this study, the left ventricular wall thickness, IVSd and LVFWd, was significantly increased in ADH group compared with age-matched non-HAC group. The prevalence of HCM was significantly higher in dogs with PDH and ADH compared with age-matched non-HAC group. Cardiac hypertrophy after hypertension, and chronic exposure to excess circulating cortisol may also contribute directly to left ventricular concentric remodeling in human patients with HAC.^{37,38} Subsequently functional abnormality, such as diastolic dysfunction (reduced E/A values and/or E velocity) has also been reported in human patients with HAC.^{31,39-41} The E/A ratio was significant decreased in PDH and ADH groups than control group. The mitral E wave value was significant decreased in ADH and PDH groups compared with age-matched non-HAC group.

Based on the 2D-STE analysis, global peak systolic and early diastolic LSR and global LS were significant decreased in dogs with HCM compared with control group. Among the dogs with HCM, decreased global peak systolic and early diastolic LSR and global LS were found in ADH, followed by PDH, then age-matched non-HAC

group. However, the parameters of left ventricular systolic function derived from conventional echocardiography, FS and EF, were not significantly different among these dogs with HCM compared to the non-HCM. This finding suggested that dog with HAC might have subclinical systolic dysfunction, and dog with ADH exhibited significantly decreased systolic function. Similar findings are also reported in human patients with HCM associated with Cushing's syndrome, subclinical left ventricular systolic dysfunction is indicated using STE, and nonetheless, the assessment of FS and EF is usually normal or supernormal.^{15,39,42-47} Both systemic hypertension and elevated cortisol concentration would deteriorate myocardial structure and function.^{15,45} In this study, the blood pressure of ADH group was significant increased than that of PDH and non-HAC groups, elevated blood pressure might cause decreased cardiac function in ADH group. However, no significant difference in circumferential strain and strain rate were noted in this study. Circumferential strain and strain rate changes in human patients with HCM are less consistent.^{15,44,46,47} The left ventricular myocardium consists of circumferential fibers in the mid-wall layer and longitudinal fibers in the endocardial layers.⁴⁸ In general, subendocardial longitudinal fibers are vulnerable in the presence of myocardial ischemia, hemodynamic overload, or age-related changes.^{49,50} Therefore, left ventricular systolic dysfunction may begin with a reduction in longitudinal shortening in the early stage, and circumferential strain may remain normal or show exaggerated compensation for preserving left ventricular systolic performance. However, progression of disease results in concomitant midmyocardial dysfunction, leading to a reduction in left ventricular circumferential mechanics.⁴⁸

Based on the findings of this study, dogs with HAC have significant increase prevalence of HCM compared to the age-matched non-HAC dogs. Further 2D-STE revealed left ventricular mechanics alterations despite a preserved systolic function

detected by conventional echocardiography. Systolic dysfunction was found among the dogs with HCM, and significantly decreased systolic function was ADH associated HCM, followed by PDH associated HCM, and age-matched non-HAC.

Footnotes

- a. Model 811-B, Parks Medical Electronics Inc., U.S.A.
 - b. MyLab™ 50 Family, Esaote, Genova, Italy
 - c. XStrain™ software for MyLab™ 50 XVision, Esaote, Genova, Italy.
 - d. SPSS Version 12.0.1, Lead Technologies Inc., Charlotte, North Carolina, USA.
-



Reference:

1. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006;113:1807-1816.
2. Liu SK, Roberts WC, Maron BJ. Comparison of morphologic findings in spontaneously occurring hypertrophic cardiomyopathy in humans, cats and dogs. *Am J Cardiol* 1993;72:944-951.
3. Varnava AM, Elliott PM, Sharma S, et al. Hypertrophic cardiomyopathy: the interrelation of disarray, fibrosis, and small vessel disease. *Heart* 2000;84:476-482.
4. Unverferth DV, Baker PB, Pearce LI, et al. Regional myocyte hypertrophy and increased interstitial myocardial fibrosis in hypertrophic cardiomyopathy. *Am J Cardiol* 1987;59:932-936.
5. Ferasin L, Sturgess CP, Cannon MJ, et al. Feline idiopathic cardiomyopathy: a retrospective study of 106 cats (1994-2001). *Journal of feline medicine and surgery* 2003;5:151-159.
6. Lindholm J, Juul S, Jørgensen JO, et al. Incidence and late prognosis of cushing's syndrome: a population-based study. *J Clin Endocrinol Metab* 2001;86:117-123.
7. Colao A, Pivonello R, Spiezia S, et al. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab* 1999;84:2664-2672.
8. Whitworth JA, Mangos GJ, Kelly JJ. Cushing, cortisol, and cardiovascular disease. *Hypertension* 2000;36:912-916.
9. Toja PM, Branzi G, Ciambellotti F, et al. Clinical relevance of cardiac structure and function abnormalities in patients with Cushing's syndrome before and after cure. *Clin Endocrinol (Oxf)* 2012;76:332-338.
10. Ainscough JF, Drinkhill MJ, Sedo A, et al. Angiotensin II type-1 receptor activation in the adult heart causes blood pressure-independent hypertrophy and cardiac dysfunction. *Cardiovasc Res* 2009;81:592-600.
11. Connell JM, MacKenzie SM, Freel EM, et al. A lifetime of aldosterone excess: long-term consequences of altered regulation of aldosterone production for cardiovascular function. *Endocr Rev* 2008;29:133-154.
12. Burns AT, La Gerche A, D'Hooge J, et al. Left ventricular strain and strain rate: characterization of the effect of load in human subjects. *Eur J Echocardiogr* 2010;11:283-289.
13. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium, and myocardial

fibrosis. *J Lab Clin Med* 1992;120:893-901.

14. Yiu KH, Marsan NA, Delgado V, et al. Increased myocardial fibrosis and left ventricular dysfunction in Cushing's syndrome. *Eur J Endocrinol* 2012;166:27-34.
15. Imbalzano E, Zito C, Carerj S, et al. Left ventricular function in hypertension: new insight by speckle tracking echocardiography. *Echocardiography* 2011;28:649-657.
16. Amundsen BH, Helle-Valle T, Edvardsen T, et al. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol* 2006;47:789-793.
17. Wess G, Sarkar R, Hartmann K. Assessment of left ventricular systolic function by strain imaging echocardiography in various stages of feline hypertrophic cardiomyopathy. *J Vet Intern Med* 2010;24:1375-1382.
18. Bohs LN, Trahey GE. A novel method for angle independent ultrasonic imaging of blood flow and tissue motion. *IEEE Trans Biomed Eng* 1991;38:280-286.
19. Pellerin D, Sharma R, Elliott P, et al. Tissue Doppler, strain, and strain rate echocardiography for the assessment of left and right systolic ventricular function. *Heart* 2003;89 Suppl 3:iii9-17.
20. Blessberger H, Binder T. Two dimensional speckle tracking echocardiography: basic principles. *Heart* 2010;96:716-722.
21. Lien YH, Huang HP. Use of ketoconazole to treat dogs with pituitary-dependent hyperadrenocorticism: 48 cases (1994-2007). *J Am Vet Med Assoc* 2008;233:1896-1901.
22. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in dogs. *J Am Vet Med Assoc* 1995;207:1180-1183.
23. Hoerauf A, Reusch C. Ultrasonographic characteristics of both adrenal glands in 15 dogs with functional adrenocortical tumors. *J Am Anim Hosp Assoc* 1999;35:193-199.
24. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med* 2007;21:542-558.
25. Etxabe J, Vazquez JA. Morbidity and mortality in Cushing's disease: an epidemiological approach. *Clin Endocrinol (Oxf)* 1994;40:479-484.
26. Arnaldi G. Diagnosis and Complications of Cushing's Syndrome: A Consensus Statement. *J Clin Endocrinol Metab* 2003;88:5593-5602.
27. Boscaro M, Sonino N, Scarda A, et al. Anticoagulant prophylaxis markedly reduces thromboembolic complications in Cushing's syndrome. *J Clin Endocrinol Metab* 2002;87:3662-3666.

28. Mancini T, Kola B, Mantero F, et al. High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. *Clin Endocrinol (Oxf)* 2004;61:768-777.
29. Boscaro M, Barzon L, Fallo F, et al. Cushing's syndrome. *Lancet* 2001;357:783-791.
30. Magiakou MA, Mastorakos G, Zachman K, et al. Blood pressure in children and adolescents with Cushing's syndrome before and after surgical care. *J Clin Endocrinol Metab* 1997;82:1734-1738.
31. Baykan M, Erem C, Gedikli O, et al. Assessment of left ventricular diastolic function and Tei index by tissue Doppler imaging in patients with Cushing's Syndrome. *Echocardiography* 2008;25:182-190.
32. Novellas R, de Gopegui RR, Espada Y. Determination of renal vascular resistance in dogs with diabetes mellitus and hyperadrenocorticism. *Vet Rec* 2008;163:592-596.
33. Goy-Thollot I, Péchereau D, Kéroack S, et al. Investigation of the role of aldosterone in hypertension associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. *J Small Anim Pract* 2002;43:489-492.
34. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1998;212:369-373.
35. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *J Am Vet Med Assoc* 1996;209:1724-1729.
36. Brown SA, Henik RA. Diagnosis and treatment of systemic hypertension. *Vet Clin North Am Small Anim Pract* 1998;28:1481-1494.
37. Fallo F, Budano S, Sonino N, et al. Left ventricular structural characteristics in Cushing's syndrome. *J Hum Hypertens* 1994;8:509-513.
38. Salcedo EE, Gockowski K, Tarazi RC. Left ventricular mass and wall thickness in hypertension. Comparison of M mode and two dimensional echocardiography in two experimental models. *Am J Cardiol* 1979;44:936-940.
39. Muiesan ML, Lupia M, Salvetti M, et al. Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003;41:2275-2279.
40. Bayram NA, Ersoy R, Aydin C, et al. Assessment of left ventricular functions by tissue Doppler echocardiography in patients with Cushing's disease. *J Endocrinol Invest* 2009;32:248-252.
41. Takagi S, Tanabe A, Tsuiki M, et al. Hypokalemia, diabetes mellitus, and hypercortisolemia are the major contributing factors to cardiac dysfunction in adrenal Cushing's syndrome. *Endocr J* 2009;56:1009-1018.

42. Hartford M, Wikstrand JC, Wallentin I, et al. Left ventricular wall stress and systolic function in untreated primary hypertension. *Hypertension* 1985;7:97-104.
43. Lutas EM, Devereux RB, Reis G, et al. Increased cardiac performance in mild essential hypertension. Left ventricular mechanics. *Hypertension* 1985;7:979-988.
44. Carasso S, Yang H, Woo A, et al. Systolic myocardial mechanics in hypertrophic cardiomyopathy: novel concepts and implications for clinical status. *J Am Soc Echocardiogr* 2008;21:675-683.
45. Pereira AM, Delgado V, Romijn JA, et al. Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome. *Eur J Endocrinol* 2010;162:331-340.
46. Serri K, Reant P, Lafitte M, et al. Global and regional myocardial function quantification by two-dimensional strain: application in hypertrophic cardiomyopathy. *J Am Coll Cardiol* 2006;47:1175-1181.
47. Popović ZB, Kwon DH, Mishra M, et al. Association between regional ventricular function and myocardial fibrosis in hypertrophic cardiomyopathy assessed by speckle tracking echocardiography and delayed hyperenhancement magnetic resonance imaging. *J Am Soc Echocardiogr* 2008;21:1299-1305.
48. Geyer H, Caracciolo G, Abe H, et al. Assessment of myocardial mechanics using speckle tracking echocardiography: fundamentals and clinical applications. *J Am Soc Echocardiogr* 2010;23:351-369; quiz 453-355.
49. Bolognesi R, Tsialtas D, Barilli AL, et al. Detection of early abnormalities of left ventricular function by hemodynamic, echo-tissue Doppler imaging, and mitral Doppler flow techniques in patients with coronary artery disease and normal ejection fraction. *J Am Soc Echocardiogr* 2001;14:764-772.
50. Vinereanu D, Khokhar A, Tweddel AC, et al. Estimation of global left ventricular function from the velocity of longitudinal shortening. *Echocardiography* 2002;19:177-185.

Table 1. Baseline characteristics of groups of the healthy controls, age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables	Control (n=23)	Non-HAC (n=53)	PDH (n=13)	ADH (n=12)
Age (y/o)	3.5 ± 2.1	11.7 ± 2.9 [†]	11.7 ± 2.5 [†]	12.7 ± 2.4 [†]
Heart rate (bpm)	119.8 ± 20.0	119.4 ± 24.6	124.8 ± 15.5	129.5 ± 13.9
Blood pressure (mmHg)	131.3 ± 16.5	132.8 ± 20.7	138.7 ± 12.7	158.8 ± 18.5 ^{†‡}
Incidence of hypertension (%)	0	6	15	58 ^{†‡¶}
LVEDD (mm)	22.3 ± 4.0	23.7 ± 4.8	23.7 ± 6.0	23.1 ± 4.9
LVESD (mm)	12.6 ± 2.7	14.5 ± 9.7	12.9 ± 2.8	12.6 ± 2.6
LVPWd (mm)	5.9 ± 1.4	5.2 ± 1.5	6.3 ± 1.3	6.3 ± 1.8 [‡]
IVSd (mm)	5.8 ± 1.0	5.6 ± 1.4	7.0 ± 1.6 [‡]	7.2 ± 1.6 ^{†‡}
LA/Ao ratio	1.3 ± 0.2	1.7 ± 0.4 [†]	1.7 ± 0.3 [†]	1.5 ± 0.3
EPSS (mm)	1.4 ± 0.5	1.5 ± 0.7	1.7 ± 0.6	1.8 ± 0.7
EF (%)	77.3 ± 5.7	76.5 ± 11.3	78.1 ± 8.1	78.1 ± 7.3
FS (%)	44.7 ± 4.9	44.12 ± 7.5	45.2 ± 7.2	44.9 ± 7.1
Mitral peak E wave (m/s)	0.67 ± 0.09	0.82 ± 0.28	0.62 ± 0.14 [‡]	0.57 ± 0.20 [‡]
Mitral E/A ratio	1.32 ± 0.32	1.11 ± 0.42	0.91 ± 0.32 [†]	0.74 ± 0.33 ^{†‡}
Tricuspid peak E wave (m/s)	0.53 ± 0.12	0.52 ± 0.33	0.42 ± 0.11	0.45 ± 0.21
Tricuspid E/A ratio	1.24 ± 0.31	1.00 ± 0.42	0.90 ± 0.24	0.88 ± 0.41
IVRT (mm)	45.9 ± 11.0	58.7 ± 19.0	61.5 ± 20.2	51.2 ± 17.9

Indices are presented as mean ± SD.

E/A ratio, early diastolic velocity/late diastolic velocity ratio; EF, ejection fraction; EPSS, E-point to septal separation; FS, fractional shortening; IVRT, isovolumetric relaxation time; IVSd, thickness of interventricular septum at end-diastole; LA/Ao, left atrial/aortic root ratio; LVEDD, left ventricular dimension at end-diastole; LVESD, left ventricular dimension at end-systole; LVPWd, thickness of left ventricular free wall at end-diastole.

[†] $P < 0.05$ relative to controls; [‡] $P < 0.05$ relative to non-HAC; [¶] $P < 0.05$ relative to PDH.

Table 2. Baseline characteristics of dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables	Non-HAC (n=6)	PDH (n=6)	ADH (n=8)
Age (y/o)	11.0 ± 3.7	11.2 ± 2.8	12.9 ± 2.7
Heart rate (bpm)	127.0 ± 42	124.8 ± 19.2	129.8 ± 12.4
Blood pressure (mmHg)	129.7 ± 20.6	136.8 ± 8.1	159.3 ± 21.7 [‡]
LVEDD (mm)	24.6 ± 5.2	23.6 ± 4.7	22.3 ± 5.0
LVESD (mm)	14.9 ± 3.7	12.4 ± 2.3	12.3 ± 2.2
LVFWd (mm)	7.3 ± 2.5	6.7 ± 1.6	6.4 ± 2.1
IVSd (mm)	6.6 ± 0.7	7.6 ± 0.8	7.6 ± 1.4
LA/Ao ratio	1.7 ± 0.3	1.5 ± 0.2	1.4 ± 0.3
EPSS (mm)	1.7 ± 1.1	1.5 ± 0.8	1.8 ± 0.7
EF (%)	73.5 ± 4.4	80.7 ± 4.9	77.0 ± 8.3
FS (%)	39.0 ± 3.5	47.5 ± 5.1	43.9 ± 7.7
Mitral peak E wave (m/s)	0.79 ± 0.51	0.66 ± 0.16	0.51 ± 0.22
Mitral E/A ratio	0.97 ± 0.47	1.07 ± 0.26	0.62 ± 0.29
Tricuspid peak E wave (m/s)	0.48 ± 0.10	0.37 ± 0.79	0.49 ± 0.24
Tricuspid E/A ratio	0.92 ± 0.29	0.87 ± 0.25	0.83 ± 0.42
IVRT (mm)	53.2 ± 18.4	57.0 ± 19.7	59.6 ± 16.1

Indices are presented as mean ± SD.

E/A ratio, early diastolic velocity/late diastolic velocity ratio; EF, ejection fraction; EPSS, E-point to septal separation; FS, fractional shortening; IVRT, isovolumetric relaxation time; IVSd, thickness of interventricular septum at end-diastole; LA/Ao, left atrial/aortic root ratio; LVEDD, left ventricular dimension at end-diastole; LVESD, left ventricular dimension at end-systole; LVFWd, thickness of left ventricular free wall at end-diastole.

[‡]*P* < 0.05 relative to non-HAC.

Table 3. Global strains and strain rates derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables	Control (n=23)	Non-HAC (n=6)	PDH (n=6)	ADH (n=8)
Global peak systolic LSR (S⁻¹)	-1.60 ± 0.29	-1.51 ± 0.53	-1.48 ± 0.47	-0.95 ± 0.23 ^{†‡}
Global peak E LSR (S⁻¹)	1.40 ± 0.40	1.14 ± 0.14	1.06 ± 0.49	0.56 ± 0.29 ^{†‡}
Global peak A LSR (S⁻¹)	0.67 ± 0.18	0.88 ± 0.49	0.83 ± 0.31	0.71 ± 0.49
Global peak systolic CSR (S⁻¹)	-1.90 ± 0.81	-1.54 ± 0.59	-2.06 ± 1.65	-1.48 ± 0.90
Global peak E CSR (S⁻¹)	1.58 ± 0.94	1.34 ± 0.57	1.37 ± 0.99	1.24 ± 0.82
Global peak A CSR (S⁻¹)	0.68 ± 0.28	0.84 ± 0.53	0.87 ± 0.52	0.68 ± 0.43
Global LS (%)	-16.27 ± 3.12	-14.74 ± 5.07	-13.89 ± 6.69	-7.87 ± 3.62 ^{†‡}
Global CS (%)	-17.34 ± 8.12	-15.53 ± 6.13	-14.26 ± 12.87	-14.44 ± 8.54

Indices are presented as mean ± SD.

A, late diastolic; CS, circumferential strain; CSR, circumferential strain rate; E, early diastolic; LS, longitudinal strain; LSR, longitudinal strain rate.

[†]*P* < 0.05 relative to controls; [‡]*P* < 0.05 relative to non-HAC.

Table 4. Indices of left ventricle longitudinal tissue velocities (LTV) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables		Peak systolic LTV (cm/sec)	Peak E LTV (cm/sec)	Peak A LTV (cm/sec)
Basal septal	Control	2.37 ± 0.71	-1.74 ± 0.79	-1.33 ± 0.63
	Non-HAC	2.06 ± 1.30	-1.35 ± 0.52	-1.36 ± 1.11
	PDH	2.27 ± 0.93	-1.38 ± 0.69	-1.72 ± 0.85
	ADH	1.77 ± 0.58	-1.06 ± 0.60	-1.29 ± 0.96
Mid septal	Control	1.44 ± 0.71	-1.05 ± 0.70	-0.81 ± 0.46
	Non-HAC	1.29 ± 0.82	-0.95 ± 0.56	-1.11 ± 0.80
	PDH	1.59 ± 0.90	-0.97 ± 0.59	-1.20 ± 0.69
	ADH	1.16 ± 0.43	-0.87 ± 0.48	-0.76 ± 0.64
Apical septal	Control	0.88 ± 0.54	-0.58 ± 0.40	-0.43 ± 0.27
	Non-HAC	1.01 ± 0.54	-0.61 ± 0.33	-0.88 ± 0.86
	PDH	1.10 ± 0.61	-0.50 ± 0.40	-0.78 ± 0.50
	ADH	0.71 ± 0.37	-0.48 ± 0.27	-0.58 ± 0.34
Apical lateral	Control	1.17 ± 0.81	-0.76 ± 0.50	-0.54 ± 0.34
	Non-HAC	1.20 ± 0.46	-1.50 ± 0.92	-0.95 ± 0.66
	PDH	0.96 ± 0.57	-0.83 ± 0.47	-0.77 ± 0.28
	ADH	0.90 ± 0.55	-0.69 ± 0.36	-0.43 ± 0.33
Mid lateral	Control	1.79 ± 0.99	-1.55 ± 1.11	-1.00 ± 0.55
	Non-HAC	1.20 ± 1.19	-1.61 ± 1.20 [†]	-0.77 ± 0.43
	PDH	1.59 ± 1.09	-0.94 ± 0.76	-1.08 ± 0.42
	ADH	1.09 ± 0.53	-0.83 ± 0.68	-0.70 ± 0.45
Basal lateral	Control	1.93 ± 1.00	-1.63 ± 0.88	-1.07 ± 0.71
	Non-HAC	1.62 ± 1.11	-0.93 ± 0.71	-0.97 ± 0.71
	PDH	1.66 ± 0.76	-1.17 ± 0.89	-1.10 ± 0.85
	ADH	1.32 ± 0.60	-0.89 ± 0.80	-1.26 ± 0.82

Indices are presented as mean ± SD.

A, late diastolic; E, early diastolic.

[†]*P* < 0.05 relative to controls.

Table 5. Indices of left ventricle radial tissue velocities (RTV) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables		Peak systolic RTV (cm/sec)	Peak E RTV (cm/sec)	Peak A RTV (cm/sec)
Cranioseptal	Control	2.31 ± 0.97	-1.84 ± 0.99	-0.92 ± 0.42
	Non-HAC	2.38 ± 0.79	-1.82 ± 0.72	-1.12 ± 0.60
	PDH	2.68 ± 1.19	-1.60 ± 0.88	-1.05 ± 0.27
	ADH	1.79 ± 0.83	-1.41 ± 0.66	-0.94 ± 0.40
Cranial	Control	2.32 ± 0.94	-1.72 ± 0.97	-0.82 ± 0.39
	Non-HAC	1.98 ± 0.70	-1.69 ± 0.82	-1.01 ± 0.34
	PDH	1.82 ± 1.12	-1.18 ± 0.57	-0.87 ± 0.47
	ADH	1.52 ± 0.81	-1.21 ± 0.67	-0.81 ± 0.44
Lateral	Control	2.11 ± 1.06	-1.59 ± 0.98	-0.81 ± 0.34
	Non-HAC	1.94 ± 0.85	-1.59 ± 0.78	-1.13 ± 0.55
	PDH	1.80 ± 1.00	-1.26 ± 0.49	-1.14 ± 0.64
	ADH	1.58 ± 0.59	-1.42 ± 0.83	-0.77 ± 0.28
Caudal	Control	2.36 ± 1.29	-1.78 ± 1.19	-0.87 ± 0.37
	Non-HAC	2.14 ± 0.73	-1.75 ± 0.73	-1.21 ± 0.64
	PDH	2.73 ± 1.02	-1.71 ± 0.82	-1.31 ± 0.81
	ADH	1.47 ± 0.63	-1.36 ± 0.69	-0.69 ± 0.35
Ventral	Control	2.25 ± 1.17	-1.79 ± 1.15	-0.85 ± 0.44
	Non-HAC	1.65 ± 0.78	-1.39 ± 0.69	-1.01 ± 0.63
	PDH	2.30 ± 0.96	-1.54 ± 0.63	-1.18 ± 0.54
	ADH	1.50 ± 0.58	-1.22 ± 0.51	-0.63 ± 0.30
Septal	Control	2.19 ± 1.09	-1.71 ± 1.01	-0.88 ± 0.48
	Non-HAC	2.08 ± 0.85	-1.71 ± 0.75	-1.09 ± 0.65
	PDH	1.85 ± 1.28	-1.30 ± 0.71	-0.95 ± 0.49
	ADH	1.60 ± 0.61	-1.30 ± 0.53	-0.81 ± 0.41

Indices are presented as mean ± SD.

A, late diastolic; E, early diastolic.

Table 6. Indices of left ventricle longitudinal strain rates (LSR) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables		Peak systolic LSR (s ⁻¹)	Peak E LSR (s ⁻¹)	Peak A LSR (s ⁻¹)
Basal septal	Control	-1.60 ± 0.44	1.42 ± 0.54	0.78 ± 0.26
	Non-HAC	-1.65 ± 0.46	1.03 ± 0.27	1.02 ± 0.68
	PDH	-1.43 ± 0.41	0.96 ± 0.45	0.90 ± 0.21
	ADH	-0.97 ± 0.23 ^{†‡}	0.61 ± 0.47 [†]	0.76 ± 0.60
Mid septal	Control	-1.70 ± 0.42	1.48 ± 0.48	0.80 ± 0.26
	Non-HAC	-1.39 ± 0.34	1.14 ± 0.20	0.90 ± 0.68
	PDH	-1.61 ± 0.57	1.13 ± 0.55	0.88 ± 0.33
	ADH	-0.91 ± 0.24 [†]	0.56 ± 0.38 [†]	0.75 ± 0.62
Apical septal	Control	-1.70 ± 0.39	1.39 ± 0.43	0.67 ± 0.22
	Non-HAC	-1.55 ± 0.88	1.18 ± 0.59	0.77 ± 0.44
	PDH	-1.67 ± 0.73	1.28 ± 0.63	0.79 ± 0.49
	ADH	-0.89 ± 0.37 ^{†¶}	0.50 ± 0.31 ^{†¶}	0.74 ± 0.61
Apical lateral	Control	-1.51 ± 0.39	1.36 ± 0.45	0.49 ± 0.21
	Non-HAC	-1.39 ± 0.53	1.33 ± 0.38	0.81 ± 0.49
	PDH	-1.42 ± 0.56	1.20 ± 0.54	0.66 ± 0.49
	ADH	-0.89 ± 0.39 [†]	0.40 ± 0.26 ^{†¶¶}	0.59 ± 0.39
Mid lateral	Control	-1.52 ± 0.38	1.40 ± 0.63	0.61 ± 0.28
	Non-HAC	-1.36 ± 0.20	1.08 ± 0.51	0.92 ± 0.66
	PDH	-1.35 ± 0.32	1.04 ± 0.51	0.71 ± 0.47
	ADH	-0.89 ± 0.29 [†]	0.50 ± 0.24 [†]	0.68 ± 0.48
Basal lateral	Control	-1.57 ± 0.40	1.27 ± 0.47	0.64 ± 0.26
	Non-HAC	-1.55 ± 0.97	1.09 ± 0.55	0.68 ± 0.55
	PDH	-1.39 ± 0.41	0.99 ± 0.41	0.67 ± 0.52
	ADH	-0.92 ± 0.28 [†]	0.53 ± 0.25 [†]	0.76 ± 0.56

Indices are presented as mean ± SD.

A, late diastolic; E, early diastolic.

[†]*P* < 0.05 relative to controls; [‡]*P* < 0.05 relative to non-HAC; [¶]*P* < 0.05 relative to PDH.

Table 7. Indices of left ventricle circumferential strain rates (CSR) derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables		Peak systolic CSR (s ⁻¹)	Peak E CSR (s ⁻¹)	Peak A CSR (s ⁻¹)
Basal septal	Control	-1.89 ± 0.80	1.44 ± 0.74	0.70 ± 0.38
	Non-HAC	-1.69 ± 0.83	1.39 ± 0.61	0.83 ± 0.53
	PDH	-2.06 ± 1.70	1.29 ± 1.11	0.67 ± 0.43
	ADH	-1.48 ± 0.97	1.22 ± 0.81	0.76 ± 0.49
Mid septal	Control	-1.89 ± 0.78	1.22 ± 0.53	0.65 ± 0.30
	Non-HAC	-1.62 ± 0.55	1.39 ± 0.57	0.80 ± 0.47
	PDH	-1.89 ± 1.76	1.29 ± 1.03	0.79 ± 0.55
	ADH	-1.57 ± 1.15	1.33 ± 1.00	0.69 ± 0.45
Apical septal	Control	-1.84 ± 0.77	1.43 ± 0.84	0.67 ± 0.30
	Non-HAC	-1.48 ± 0.49	1.31 ± 0.62	0.83 ± 0.51
	PDH	-1.88 ± 1.83	1.27 ± 1.00	0.95 ± 0.63
	ADH	-1.57 ± 0.87	1.32 ± 1.01	0.62 ± 0.40
Apical lateral	Control	-1.64 ± 0.66	1.34 ± 0.83	0.61 ± 0.49
	Non-HAC	-1.39 ± 0.55	1.31 ± 0.58	0.87 ± 0.56
	PDH	-2.07 ± 1.77	1.41 ± 0.97	1.01 ± 0.64
	ADH	-1.45 ± 0.81	1.21 ± 0.87	0.50 ± 0.28
Mid lateral	Control	-1.81 ± 0.87	1.40 ± 0.86	0.60 ± 0.27
	Non-HAC	-1.46 ± 0.64	1.29 ± 0.58	0.87 ± 0.60
	PDH	-2.24 ± 1.58	1.52 ± 0.94	0.97 ± 0.52
	ADH	-1.47 ± 0.84	1.01 ± 0.50	0.66 ± 0.49
Basal lateral	Control	-1.87 ± 0.88	1.58 ± 0.95	0.60 ± 0.29
	Non-HAC	-1.61 ± 0.81	1.34 ± 0.62	0.87 ± 0.61
	PDH	-2.24 ± 1.59	1.46 ± 1.00	0.79 ± 0.43
	ADH	-1.46 ± 0.91	1.00 ± 0.55	0.71 ± 0.53

Indices are presented as mean ± SD.

A, late diastolic; E, early diastolic.

Table 8. Indices of left ventricular longitudinal (LS) and circumferential (CS) peak strain derived from two-dimensional speckle-tracking echocardiography in healthy controls and dogs affected with hypertrophic cardiomyopathy in age-matched non-hyperadrenocorticism (non-HAC), pituitary-dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH).

Variables	Peak LS (%)	Variables	Peak CS (%)
Basal septal		Cranioseptal	
Control	-17.22 ± 3.98	Control	-18.10 ± 8.56
Non-HAC	-15.72 ± 4.15	Non-HAC	-16.05 ± 7.70
PDH	-13.53 ± 6.09	PDH	-13.25 ± 13.43
ADH	-9.22 ± 4.17 [†]	ADH	-14.74 ± 9.39
Mid septal		Cranial	
Control	-18.05 ± 3.96	Control	-18.10 ± 8.56
Non-HAC	-15.76 ± 7.08	Non-HAC	-16.05 ± 7.70
PDH	-17.64 ± 3.39	PDH	-13.14 ± 13.70
ADH	-9.07 ± 4.23 ^{†¶}	ADH	-14.73 ± 9.38
Apical septal		Lateral	
Control	-16.89 ± 4.00	Control	-16.91 ± 8.12
Non-HAC	-14.53 ± 8.51	Non-HAC	-16.03 ± 6.64
PDH	-15.23 ± 8.52	PDH	-13.97 ± 13.88
ADH	-8.58 ± 4.40 [†]	ADH	-14.72 ± 9.74
Apical lateral		Caudal	
Control	-14.70 ± 3.49	Control	-16.75 ± 7.99
Non-HAC	-14.63 ± 4.97	Non-HAC	-15.26 ± 5.88
PDH	-13.60 ± 8.06	PDH	-15.21 ± 13.41
ADH	-7.34 ± 3.56 ^{†‡}	ADH	-14.44 ± 7.80
Mid lateral		Ventral	
Control	-15.66 ± 4.27	Control	-16.74 ± 8.21
Non-HAC	-14.37 ± 1.85	Non-HAC	-14.41 ± 5.61
PDH	-13.35 ± 6.92	PDH	-15.55 ± 12.18
ADH	-7.03 ± 3.92 ^{†‡}	ADH	-14.12 ± 7.80
Basal lateral		Septal	
Control	-15.25 ± 3.93	Control	-17.90 ± 8.64
Non-HAC	-14.36 ± 8.75	Non-HAC	-16.59 ± 7.87
PDH	-12.78 ± 6.16	PDH	-14.41 ± 12.33
ADH	-6.88 ± 4.64 [†]	ADH	-14.41 ± 8.46

Indices are presented as mean ± SD.

[†]*P* < 0.05 relative to controls; [‡]*P* < 0.05 relative to non-HAC; [¶]*P* < 0.05 relative to PDH.

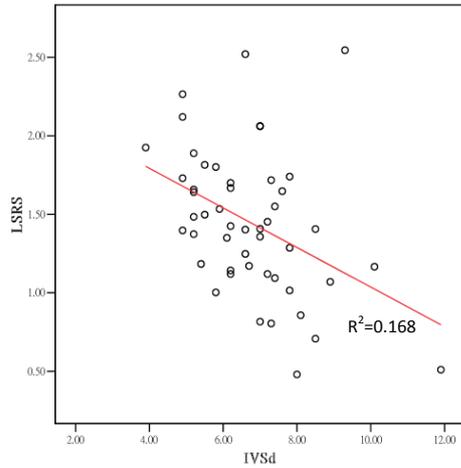
Table 9. The intra-observer coefficient of variance (CV) of 2D-STE variables.

Variables	CV (%)	Variables	CV (%)
Global peak systolic LTV	6.5	Global peak systolic RTV	12.0
Global peak E LTV	13.0	Global peak E RTV	11.0
Global peak A LSR	12.5	Global peak A RTV	18.5
Global peak systolic LSR	6.0	Global peak systolic CSR	10.5
Global peak E LSR	12.0	Global peak E CSR	16.5
Global peak A LSR	16.5	Global peak A CSR	18.0
Global LS	10.5	Global CS	8.0

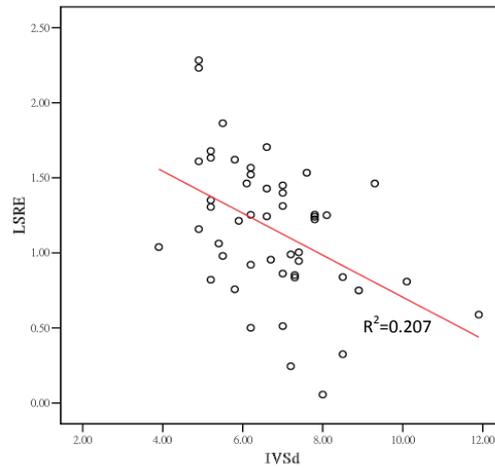
A, late diastolic; CS, circumferential strain; CSR, circumferential strain rate; E, early diastolic; LS, longitudinal strain; LSR, longitudinal strain rate.



(a)



(b)



(c)

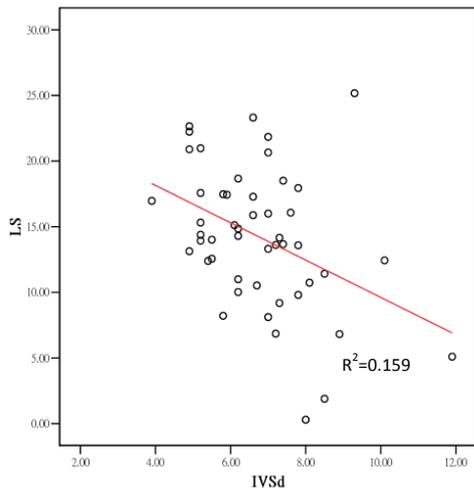


Figure 1. Variables correlated to interventricular septal thickness: (a) peak systolic longitudinal strain rate, (b) peak early diastolic longitudinal strain rate, and (c) peak systolic longitudinal strain. All correlations were significant at $P < .05$.

IVSd, interventricular septal thickness; LSRS, peak systolic longitudinal strain rate; LSRE, peak early diastolic longitudinal strain rate; LS, peak systolic longitudinal strain.

Chapter 4

Summarized Discussion



Key to Abbreviations

2D-STE	Two-dimensional speckle-tracking echocardiography
ADH	Adrenal-dependent hyperadrenocorticism
CDMD	Chronic degenerative mitral valvular disease
HAC	Hyperadrenocorticism
HCM	Hypertrophic cardiomyopathy
PDH	Pituitary- dependent hyperadrenocorticism
PI	Pulsatility index
RI	Resistive index



Chronic Hyperadrenocorticism (HAC) causes hypertension, insulin resistance, central obesity, hyperlipidemia, coagulopathy and target organ damage (left ventricular hypertrophy, proteinuria, etc). These abnormalities enhance the global cardiovascular risk that is responsible for the increased mortality of human patients with HAC.^{1,2}

The resistive index (RI) and pulsatility index (PI) of renal vessel and aorta, and the prevalence of albuminuria and microalbuminuria among the healthy controls, dogs with chronic degenerative mitral valvular disease (CDMD), dogs with HAC and concurrent CDMD were investigated in this study. Dogs with HAC and concurrent CDMD had significantly increased renal vascular resistance and prevalence of albuminuria than dogs with CDMD along. Increased urinary protein secretion had been reported in dogs which long-term administration of glucocorticoids and affected with HAC.¹⁴⁻¹⁷ Human patients with HAC had a lower glomerular filtration rate and creatinine clearance.^{18,19} In addition, a highly significant correlation between endogenous glucocorticoid production and the progression rate of chronic renal failure has been reported.²⁰ Similarly, both glomerular and tubular function are altered in dogs with HAC.¹⁷ Increased renal vascular resistance was also been found in dogs affected with HAC.²¹ The present study is in accord with the results of the previous studies, suggesting that dogs with HAC would have increased risk of renal injury. Future research with longer follow-up and kidney histopathology of dogs with persistent proteinuria or higher RI/PI is needed to establish the link and assess the outcome.

Changes of left ventricular structural morphology and function in dogs affected with HAC were demonstrated by noninvasive Two-dimensional speckle-tracking echocardiography (2D-STE) technique, and changes between pituitary- dependent hyperadrenocorticism (PDH) and adrenal-dependent hyperadrenocorticism (ADH)

were also revealed. Based on the results of this study, dogs with HAC were significant increased the prevalence of hypertension and hypertrophic cardiomyopathy (HCM) compared with age-matched non-HAC group. In dogs with HCM, dogs with ADH had significantly decreased longitudinal systolic and diastolic function, followed by dogs with PDH, then age-matched non-HAC group. This finding was similar to the studies that significantly increased prevalence of left ventricular hypertrophy and concentric remodeling was found in human patients with HAC.³⁻⁵ Subsequently functional abnormality, such as diastolic dysfunction has also been reported in human patients with HAC.⁶⁻⁹ According to previous studies, many factors have been found to be involved in development of HCM and myocardial dysfunction, including the duration of HAC, hypertension and extent of myocardial fibrosis.¹⁰⁻¹³ In this study, dogs with ADH had higher blood pressure than PDH, these factor might partially explain the difference.

In the present study, injury of heart and kidney associated with HAC was demonstrated by ultrasound. While further studies are needed to clarify the exact pathophysiological mechanisms underlying increased renal RI, PI and left ventricular hypertrophy.

Reference:

1. Arnaldi G. Diagnosis and Complications of Cushing's Syndrome: A Consensus Statement. *J Clin Endocrinol Metab* 2003;88:5593-5602.
2. Mancini T, Kola B, Mantero F, et al. High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. *Clin Endocrinol (Oxf)* 2004;61:768-777.
3. Lindholm J, Juul S, Jørgensen JO, et al. Incidence and late prognosis of Cushing's syndrome: a population-based study. *J Clin Endocrinol Metab* 2001;86:117-123.
4. Colao A, Pivonello R, Spiezia S, et al. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab* 1999;84:2664-2672.
5. Whitworth JA, Mangos GJ, Kelly JJ. Cushing, cortisol, and cardiovascular disease. *Hypertension* 2000;36:912-916.
6. Muiesan ML, Lupia M, Salvetti M, et al. Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003;41:2275-2279.
7. Baykan M, Erem C, Gedikli O, et al. Assessment of left ventricular diastolic function and Tei index by tissue Doppler imaging in patients with Cushing's Syndrome. *Echocardiography* 2008;25:182-190.
8. Bayram NA, Ersoy R, Aydin C, et al. Assessment of left ventricular functions by tissue Doppler echocardiography in patients with Cushing's disease. *J Endocrinol Invest* 2009;32:248-252.
9. Takagi S, Tanabe A, Tsuiki M, et al. Hypokalemia, diabetes mellitus, and hypercortisolemia are the major contributing factors to cardiac dysfunction in adrenal Cushing's syndrome. *Endocr J* 2009;56:1009-1018.
10. Hoerauf A, Reusch C. Ultrasonographic characteristics of both adrenal glands in 15 dogs with functional adrenocortical tumors. *J Am Anim Hosp Assoc* 1999;35:193-199.
11. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in dogs. *J Am Vet Med Assoc* 1995;207:1180-1183.
12. Hsiang TY, Lien YH, Huang HP. Indirect measurement of systemic blood pressure in conscious dogs in a clinical setting. *J Vet Med Sci* 2008;70:449-453.
13. Sato A, Suzuki H, Murakami M, et al. Glucocorticoid increases angiotensin II type 1 receptor and its gene expression. *Hypertension* 1994;23:25-30.
14. Waters CB, Adams LG, Scott-Moncrieff JC, et al. Effects of glucocorticoid therapy on urine protein-to-creatinine ratios and renal morphology in dogs. *J Vet Intern Med* 1997;11:172-177.
15. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1998;212:369-373.

16. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *J Am Vet Med Assoc* 1996;209:1724-1729.
17. Smets PM, Lefebvre HP, Meij BP, et al. Long-Term Follow-Up of Renal Function in Dogs after Treatment for ACTH-Dependent Hyperadrenocorticism. *J Vet Intern Med* 2012;26:565-574.
18. Haentjens P, De Meirleir L, Abs R, et al. Glomerular filtration rate in patients with Cushing's disease: a matched case-control study. *Eur J Endocrinol* 2005;153:819-829.
19. Faggiano A, Pivonello R, Spiezia S, et al. Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission. *J Clin Endocrinol Metab* 2003;88:2527-2533.
20. Walser M, Ward L. Progression of chronic renal failure is related to glucocorticoid production. *Kidney Int* 1988;34:859-866.
21. Novellas R, de Gopegui RR, Espada Y. Determination of renal vascular resistance in dogs with diabetes mellitus and hyperadrenocorticism. *Vet Rec* 2008;163:592-596.

