การตรวจค่าพลาสมาคาร์ดิแอกโทรโปนินไอ กาแลกติน 3 และ เอ็นทีโปรบีเอ็นพี สัมพันธ์กับค่าคลื่นเสียงสะท้อนความถี่สูงในสุนัขที่ป่วยด้วยโรคเบาหวาน

นางสาวพฤนท์สรวง วิจิตร

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MEASUREMENT OF PLASMA CARDIAC TROPONIN I, GALECTIN-3, AND NT-proBNP CORRELATED WITH ECHOCARDIOGRAPHIC VALUES IN DIABETIC DOGS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	MEASUREMENT OF PLASMA CARDIAC TROPONIN I, GALECTIN-3, AND NT-proBNP CORRELATED WITH ECHOCARDIOGRAPHIC VALUES IN DIABETIC DOGS
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ภาวะกล้ามเนื้อหัวใจผิดปกติจากเบาหวานเป็นความผิดปกติของของโครงสร้างและการทำงานของกล้ามเนื้อหัวใจแบบปฐมภูมิที่ถูกเหนี่ยวนำมาจากเบาหวาน โดยที่ไม่มีโรคหลอดเลือดหัวใจ ภาวะความดันเลือดสูง และโรคหัวใจอื่นๆ เข้ามาเกี่ยวข้อง การทำงานของหัวใจที่ผิดปกติทั้งในช่วงหดตัวและคลายตัวในผู้ป่วยเบาหวานมักเป็นผลมาจากความเสียหายของเซลล์กล้ามเนื้อหัวใจ ภาวะไฟโบรซิสที่กล้ามเนื้อหัวใจ และเซลล์หัวใจที่มีขนาดใหญ่กว่าปกติ ในปัจจุบันยังไม่มีการศึกษาเกี่ยวกับการเปลี่ยนแปลงการทำงานหัวใจและระดับตัวชี้วัดทางชีวภาพของหัวใจในสุนัขที่ป่วยเป็นโรคเบาหวาน การศึกษานี้มีวัตถุประสงค์เพื่อประเมินการทำงานของหัวใจและตรวจวัดระดับตัวชี้วัดทางชีวภาพหัวใจ ได้แก่ พลาสมาคาร์ดิแอกโทรโปนินไอ กาแลกติน 3 และ เอ็นทีโปรบีเอ็นพี ในสุนัขที่ป่วยเป็นโรคเบาหวานเทียบกับสุนัขปกติ และเพื่อหาความสัมพันธ์ระหว่างตัวชี้วัดทางชีวภาพหัวใจกับค่าที่ได้จากการตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูง การศึกษาครั้งนี้ประกอบด้วยสุนัขที่ป่วยเป็นโรคเบาหวานจำนวน 19 ตัว และสุนัขปกติจำนวน 20 ตัว ที่มีอายุ และขนาดที่ใกล้เคียงกัน สุนัขที่ป่วยเป็นโรคเบาหวานพบความชุกของการทำงานของหัวใจที่ผิดปกติในช่วงคลายตัว (57.88 %) มากกว่าสุนัขปกติ (15.00%) สุนัขที่ป่วยเป็นโรคเบาหวานมี E wave decelaration time และ Peak PVar duration รวมทั้ง peak A' velocity ที่มากกว่าสุนัขปกติอย่างมีนัยสำคัญทางสถิติ และ E'/A' ratio น้อยกว่าสุนัขปกติอย่างมีนัยสำคัญทางสถิติ โดยที่มีการทำงานของหัวใจในช่วงหดตัวอยู่ในเกณฑ์ปกติ ระดับพลาสมาคาร์ดิแอกโทรโปนินไอและกาแลกติน 3 ระหว่างสุนัขที่ป่วยเป็นโรคเบาหวานและสุนัขปกติไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ระดับพลาสมาเอ็นทีโปรบีเอ็นพีมีความสัมพันธ์อย่างมีนัยสำคัญกับค่าการทำงานของหัวใจในช่วงหัวใจคลายตัวที่ได้จากการตรวจด้วยวิธีคลื่นเสียงสะท้อนความถี่สูง กล่าวโดยสรุปประชากรของสุนัขที่ป่วยเป็นโรคเบาหวานโดยธรรมชาติในการศึกษานี้มีการทำงานของหัวใจในช่วงหดตัวปกติ มีความชุกของการทำงานของหัวใจในช่วงคลายตัวที่ผิดปกติมากกว่าสุนัขที่มีอายุ และขนาดที่ใกล้เคียงกัน

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สาขาวิชา	อายุรศาสตร์สัตวแพทย์	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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PLEANSAUNG VICHIT: MEASUREMENT OF PLASMA CARDIAC TROPONIN I, GALECTIN-3, AND NT-proBNP CORRELATED WITH ECHOCARDIOGRAPHIC VALUES IN DIABETIC DOGS. ADVISOR: ASST. PROF. SIRILAK SURACHETPONG, D.V.M., M.S., Ph.D., CO-ADVISOR: ASSOC. PROF. ANUDEP RUNGSIPIPAT, D.V.M., Ph.D., 74 pp.

Diabetic cardiomyopathy is a primary abnormality of cardiac structure and function induced by diabetes independent of coronary artery disease, hypertension, and other cardiac diseases. Systolic and diastolic dysfunction in diabetic human patients occur secondary to cardiomyocyte loss, interstitial fibrosis, and hypertrophy of cardiac cell. Currently, there is no study determining changes of cardiac function and cardiac biomarker levels in diabetic dogs. This study aimed to evaluate cardiac function and measure cardiac biomarker concentrations including cardiac troponin I (cTnI), galectin 3 (Gal-3), and N-terminal pro B-type natriuretic peptides (NT-proBNP) in diabetic dogs compared to healthy control dogs and to determine correlations between cardiac biomarkers and echocardiographic parameters in diabetic dogs. Nineteen diabetic and 20 age and size matched control dogs were included in the study. Diabetic dogs had a higher prevalence of diastolic dysfunction (57.88%) than control dogs (15.00 %). Diabetic dogs had E-wave deceleration time, Peak PVar duration, and Peak A' velocity significantly higher than control dogs and E'/A' ratio significantly lower than control dogs. No significant difference of plasma cTnI and Gal-3 concentrations was found between two groups. There were correlations between plasma NT-proBNP concentrations and echocardiographic diastolic parameters. In conclusion, diabetic dogs in this study had a preserved systolic function with a higher prevalence of left ventricular diastolic dysfunction than control dogs.

Department:	Veterinary Medicine	Student's Signature .....
Field of Study:	Veterinary Medicine	Advisor's Signature .....
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CHAPTER I
INTRODUCTION

Diabetic mellitus is one of the most frequent endocrinopathy in dogs. The prevalence of diabetes has increased dramatically over the past 30 years in veterinary medicine. According to the study of Guptill et al. (2003), the result showed a continuously increasing prevalence of diabetes in dogs from 19 per 10,000 cases per year in 1970 to 64 per 10,000 cases per year in 1999.

Diabetic mellitus has been established as a major risk factor for cardiovascular mortality and morbidity in humans (Kannel and McGee, 1979b). Several studies in both humans and experimental animals showed an association between diabetic mellitus and specific cardiomyopathy independent of coronary vascular disease and hypertension (Regan et al., 1977; Cosson and Kevorkian, 2003; Mochizuki et al., 2015)

The term “diabetic cardiomyopathy” is a primary abnormality of cardiac structure and function induced by diabetes independent of coronary artery disease, hypertension, and other cardiac diseases (Rubler et al., 1972). Diabetic cardiomyopathy can lead to left ventricular dysfunction in parallel with structural changes leading to heart failure and eventually cardiac death in humans. The pathophysiology of diabetic-induced cardiac damage in humans is complicated and multifactorial. There are several proposed mechanisms implicating diabetic cardiomyopathy including elevation of oxidative stress (Huynh et al., 2014), metabolic disturbances, insulin resistance (Sasso et al., 2010), and remodeling of the extracellular matrix (Miki et al., 2013). Systolic and diastolic dysfunction secondary to cardiomyocyte loss, interstitial fibrosis, and hypertrophy of cardiac cells are the prominent characteristics of diabetic cardiomyopathy (Aragno et al., 2008). However, there are few studies about cardiac structural and functional changes in diabetic dogs. Changes in cardiac function detected from a non-invasive method such as echocardiography have been widely reported in both experimental animals and human diabetic patients (Shimizu et al., 1993; Mizushige et al., 2000; Rajan and Gokhale, 2002). Left ventricular fibrosis can lead to an alteration in cardiac function

(Mizushige et al., 2000). Early changes in cardiac function of human diabetic patients are generally manifested as diastolic dysfunction preceeding systolic dysfunction (Cosson and Kevorkian, 2003). The histopathologic study of Regan et al. (1974) in experimental alloxan-induced diabetic dogs showed an increased stiffness of the left ventricle associated with an accumulation of glycoprotein in the interstitium of the heart.

There is no single diagnostic method for detection of diabetic cardiomyopathy. Non-invasive imaging modalities such as echocardiography and cardiac biomarker measurements provide a valuable information to detect diabetic cardiomyopathy (Ihm et al., 2007; Maya and Villarreal, 2010).

Cardiac troponin I (cTnI) is an intracellular protein regulating the interaction between actin and myosin within the myocardium. An increase of serum cTnI concentration indicates myocardial damage or death (Adams et al., 1993). An elevated cTnI has a strong correlation with a subsequent cardiovascular event in human diabetic patients. In humans, cTnI can be used as an indicator to identify diabetic patients at risk for developing poor long-term outcome secondary to cardiac injury (Eubanks et al., 2012).

Galectin-3 (Gal-3) is a complex protein of the galectin family. Galectin-3 has been shown an important role implicating pathophysiology of heart failure (Milting et al., 2008). In humans, Gal-3 is used as a prognostic biomarker in human patients with heart failure (Ho et al., 2012). Recently, there are several experimental studies suggesting that Gal-3 is an important mediator for cardiac fibrosis (Sharma et al., 2004; Liu et al., 2009; de Boer et al., 2010) The up-regulation of myocardial Gal-3 has been detected in the heart of diabetic rats (Aragno et al., 2008; Thandavarayan et al., 2008). However, an expression of Gal-3 in cardiac tissues and the blood concentration of Gal-3 in diabetic dogs have not been studied.

N-terminal-pro natriureticpeptide (NT-proBNP) is a neurohormonal substance synthesized and secreted from atrium and ventricle in response to stretch of the myocardium (Kinnunen et al., 1993). An elevation of these peptides has been shown a strong association with an increase of pressure and volume load on the left ventricle and the severity of systolic dysfunction (Kouloubinis et al., 2007). In human

medicine, natriuretic peptides have been proved to use as a reliable marker of heart disease and heart failure (Maisel et al., 2002). In veterinary medicine, natriuretic peptides are elevated in dogs with heart disease including myxomatous mitral valve disease, congestive heart failure, and occult cardiomyopathy (MacDonald et al., 2003; Oyama et al., 2007).

An early detection of myocardial dysfunction in diabetic dogs is a primary goal for both cardiologists and endocrinologists to improve a prognosis. Recently, the study determining changes of cardiac function and cardiac biomarker levels in blood circulation has not been performed in diabetic dogs. Therefore, this study was created to evaluate cardiac function assessed by echocardiography and to measure levels of cardiac biomarkers including cTnI, Gal-3, and NT-proBNP in diabetic dogs compared to healthy control dogs. The goal of this study was to investigate an evidence of cardiomyopathy in diabetic dogs by using echocardiography and measuring cardiac biomarkers including cTnI, Gal-3, and NT-proBNP.

Objectives of this study

1. To measure plasma cTnI, Gal-3, and NT-proBNP concentrations in diabetic dogs compared to healthy control dogs.
2. To evaluate cardiac function during systole and diastole assessed by echocardiography in diabetic dogs compared to healthy control dogs.
3. To determine correlations between cTnI, Gal-3, and NT-proBNP concentrations with echocardiographic values.

Hypothesis

1. Plasma cTnI, Gal-3, and NT-proBNP concentrations are increased in diabetic dogs compared to healthy control dogs.
2. Cardiac function changes either in diastolic or systolic phases assessed by echocardiography in diabetic dogs compared to healthy control dogs.
3. There are correlations between cTnI, Gal-3, and NT-proBNP concentrations and echocardiographic values.

Keywords (Thai):

การทำงานของหัวใจ เบาหวาน สุนัข กาแลกติน 3 โทรโปนินไอ เอ็นทีโปรบีเอ็นพี

Keywords (English):

Cardiac function, Diabetic mellitus, Dog, Galectin-3, Troponin I, NT-proBNP

Advantages of the study

Advantages of this study are to prove a role of diabetic in inducing cardiomyopathy in dogs and to develop a protocol to diagnose diabetic induced cardiomyopathy by determining cardiac biomarker levels accompany with evaluating cardiac function assessed by echocardiography.

CHAPTER II
LITERATURE REVIEWS

Diabetic mellitus

Diabetic mellitus is one of the most common endocrinopathy in dogs. Age of dogs suffering from diabetic mellitus usually ranges from 4-14 years with a peak incidence at 7-9 years (Nelson, 1995). The prevalence of diabetic dogs in the United States increased from < 0.02% to 0.064% between 1970 and 1999 (Guptill et al., 2003). Poodles, Samoyeds, Miniature Schnauzers, and Pugs are prone to develop diabetes (Hess et al., 2000a).

The most common clinically recognized form of diabetic mellitus in dogs is insulin-dependent diabetic mellitus (IDDM), characterized by pronounced hyperglycemia and a decrease in insulin levels (Greco, 2001). A decrease in insulin levels may result from beta cell loss due to autoimmune processes, beta cell hypoplasia, atrophy exocrine pancreatic disease, or idiopathic causes (Catchpole et al., 2008).

Diagnosis of diabetes is based on clinical signs, history, physical examination, and blood results. Diabetic mellitus is defined as an evidence of a high fasting blood glucose level (> 200 mg/dL in dog) and glucosuria (Rucinsky et al., 2010). The most classical clinical signs of diabetic dogs are polyuria and polydipsia. Polydipsia is the most common clinical sign of diabetes in dogs presenting around 93%. Seventy-seven percent of diabetic dogs present with polyuria (Greco, 2001). The most common physical examination findings in diabetic dogs are dehydration (48%) and loss of muscle mass (44%) (Greco, 2001). Moreover, hypercholesterolemia, elevated liver enzymes (alkaline phosphatase and alanine aminotransferase), neutrophilic leukocytosis, proteinuria, increased urine specific gravity, and glucosuria can be found in diabetic dogs (Greco, 2001). Diet modification and insulin treatment are the treatment protocol for diabetic dogs. Clinical diabetic dogs usually require an

exogenous insulin therapy. The first choice recommendation of insulin in diabetic dogs is porcine zinc insulin suspension (Rucinsky et al., 2010).

Diabetic cardiomyopathy

Diabetic mellitus is known to be associated with an increase in morbidity and mortality rate from the cardiac abnormality (Koltai et al., 2002). Several studies in both humans and experimental animals showed that diabetes has been associated with a specific cardiomyopathy and an abnormality of cardiac function independent of coronary vascular diseases (Regan et al., 1977; Cosson and Kevorkian, 2003; Mochizuki et al., 2015).

Primary abnormality of myocardium induced by diabetes was first introduced by Rubler et al. (1972) in four decades ago. Diabetic cardiomyopathy is defined as a disease that directly affects the structure and the function of the myocardium in the absence of other abnormalities such as coronary artery disease, valvular disease , or hypertension. The alteration of myocardium induced by diabetes can finally lead to heart failure and cardiac death (Boudina and Abel, 2010).

The pathophysiology of diabetic-induced cardiac damage in humans is complicated and multifactorial, but the exact mechanism remains unknown. There are several proposed mechanisms implicating diabetic cardiomyopathy including elevation of oxidative stress (Cai and Kang, 2001; Huynh et al., 2014), metabolic disturbances, insulin resistance (Sasso et al., 2010), and remodeling of the extracellular matrix (Miki et al., 2013). However, the exact pathology of diabetic-induced cardiac damage in dogs is not clearly understood.

Diabetic cardiomyopathy can affect both structural and functional changes in the myocardium. Cardiac cell death, interstitial fibrosis, and cardiomyocyte hypertrophy are the most prominent structural changes found in diabetic cardiomyopathy. Changes in the cardiac structure can lead to functional changes defined as diastolic and systolic dysfunction and overt heart failure (Huynh et al., 2014). In human diabetic patients, changes in cardiac structure and function can be

identified by using various diagnostic methods. Presently, there is no single diagnostic method for detecting diabetic cardiomyopathy in humans (Maya and Villarreal, 2010).

Echocardiography is a useful and non-invasive method for assessing systolic and diastolic function in the heart (Mihm et al., 2001; Maya and Villarreal, 2010). Transmitral pulsed-wave Doppler echocardiography is a commonly used technique to assess cardiac diastolic function by measuring early ventricular filling velocity (E-wave), the late ventricular filling velocity (A-wave), and E-wave deceleration time (Dec time) (Danzmann et al., 2008). Reduced E-wave and prolonged dec time represent early diastolic dysfunction in diabetic rats (Mihm et al., 2001).

Moreover, a combined use of echocardiography with cardiac biomarkers can provide valuable information for identifying diabetic cardiomyopathy in humans (Ihm et al., 2007).

Structural abnormalities

Cardiac fibrosis

Cardiac fibrosis was first described in 1972 by Rubler et al. Several histological studies in both humans and experimental animals confirmed the existence of cardiac fibrosis in diabetic hearts (van Hoeven and Factor, 1990; Shimizu et al., 1993; Mizushige et al., 2000). Perivascular and interstitial fibrosis are characterized by microscopic alterations in diabetic patients (Fang et al., 2004).

The histopathological study of Regan et al. (1974) in experimental alloxan-induced diabetic dogs showed an increased stiffness of the left ventricle associated with the accumulation of glycoprotein in the interstitium of the heart. The study in human diabetic patients also showed a significant increase in the deposition of collagen around the vessels and between the myofibers in the myocardium (Regan et al., 1977).

An immunohistochemical study of the myocardium in 21 human diabetic patients without hypertension and coronary heart disease revealed a significantly higher percentage of interstitial fibrosis in the diabetic group compared with the control group (Shimizu et al., 1993). The percentage of collagen type III was

significantly increased in human diabetic patients resulting in collagen remodeling in the myocardium (Shimizu et al., 1993). The diabetic rat model also showed an increased extracellular fibrosis and abundant transforming growth factor beta 1 (TGF-β1), a cytokine playing a role in fibrous tissue formation (Mizushige et al., 2000).

Cardiac hypertrophy

Left ventricular hypertrophy is one of the most morphological abnormalities found in diabetic cardiomyopathy. The histopathological study in human diabetic patients showed a significant increase of mean myocardial cell diameter in the diabetic group compared with the control group (Nunoda et al., 1985). Additionally, the clinical studies revealed significantly increased LV thickness and LV mass in human diabetic patients independent of other conventional risk factors including age, hypertension, and obesity (Kannel and McGee, 1979a; Galderisi et al., 1991; Devereux et al., 2000).

Functional abnormalities

Diastolic dysfunction in diabetic cardiomyopathy

The normal diastolic function allows blood to fill in the ventricle at normal filling pressure. An increase left ventricular pressure can lead to diastolic failure. Diastole starts from a closure of the semilunar valves to a closure of atrioventricular valves (a period from the T wave on electrocardiography to the beginning of QRS complex). This period includes a rapid ventricular filling phase, a slow ventricular filling phase, and atrial contraction (Brutsaert et al., 1993). The gold standard for assessment of left ventricular function is a cardiac catheterization (Mirsky, 1984); however, this diagnostic method is invasive and not suitable to perform in all diabetic dogs. Echocardiography is a non-invasive method for assessing diastolic dysfunction (Cosson and Kevorkian, 2003). Assessment of diastolic dysfunction requires several echocardiographic techniques including transmitral flow pulsed-wave Doppler, pulmonary vein flow, and tissue Doppler imaging. Parameters of transmitral flow pulsed-wave Doppler include the peak early diastolic filling velocity (E wave),

late diastolic filling velocity (A wave), E/A ratio, deceleration time of E wave, and isovolumic relaxation time (IVRT). The transmitral peak E and A velocities reflect the pressure gradient between left ventricle and left atrium during early and late diastolic periods, respectively. In the normal heart at the beginning of the diastolic period, left ventricular pressure is less than left atrial pressure creating a rapid filling of blood flow into the left ventricle. Then, left ventricular pressure is increased by the blood filling in the left ventricle. As left ventricular pressure equals to left atrium pressure, blood flow velocity is decelerated. The period for generating left ventricular pressure to equal left atrial pressure is determined as deceleration time of E wave. On the second phase of the diastolic period, atrial contraction occurs and generates blood flow to the left ventricle; however, blood flow velocity from atrial contraction is not high as the degree of rapid ventricular filling phase (Boon, 2011).

E/A ratio is higher than 1.0 (E/A>1) in the normal heart. Diastolic dysfunction can be divided into three patterns according to E/A ratio. Impaired relaxation pattern is characterized by reversed E/A ratio (E/A <1), prolonged deceleration time, and prolonged isovolumic relaxation time. Pseudonormalization pattern represents the abnormalities of both relaxation and compliance. As left atrial pressure increases from the compensation process, A wave velocity increases resulting in an increase of E/A ratio more than 1 (Boon, 2011). The restrictive filling pattern represents severely impaired relaxation, severe decrease compliance, and high left atrial pressure. The restrictive filling pattern is characterized by E/A ratio more than 2.0, shorten isovolumic relaxation time and, rapid E wave deceleration time (Cosson and Kevorkian, 2003) (Figure 1).

Left ventricular diastolic dysfunction is usually recognized as the earliest detectable cardiac functional abnormality in diabetic patients and can be used as a prognostic parameter in diabetic cardiomyopathy (Poanta et al., 2010). Diastolic dysfunction is reported in both diabetic rats and human diabetic patients without other concurrent cardiac diseases (Dent et al., 2001; Schannwell et al., 2002; Fontes et al., 2015) According to the previous studies in human diabetic patients, the prevalence of diastolic dysfunction ranged from 16%-75% (Poirier et al., 2001; Zabalgoitia et al., 2001; Liu et al., 2003; Boyer et al., 2004).

Transmitral flow pulsed-wave Doppler is known to be affected by various factors including age, heart rate, and loading condition. Early diastolic dysfunction can be identified by tissue Doppler imaging in human diabetic patients even a normal diastolic function is detected by conventional echocardiography (Di Bonito, 2005). This finding reflects a poor sensitivity of conventional echocardiography to identify subtle changes in diastolic function (Di Bonito et al., 2005). Thus, tissue Doppler imaging should be performed accompany with conventional echocardiography to provide a reliable information of diastolic function (Boyer et al., 2004).

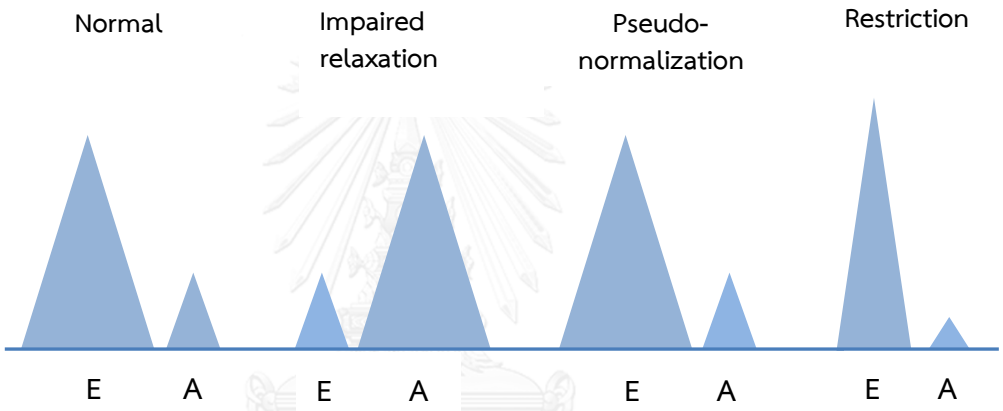

Figure 1: Transmitral flow patterns

Systolic dysfunction in diabetic cardiomyopathy

The normal heart must pump an adequate amount of blood to meet the metabolic need of the body. Systolic dysfunction is defined as an impaired pumping ability of blood out of the heart (Federmann and Hess, 1994).

The most common echocardiographic assessment of left ventricular (LV) systolic function is left ventricular fractional shortening (FS). Percent FS is calculated by subtracting systolic from diastolic dimensions and dividing by diastolic dimension. Factors affecting FS are preload, afterload, and contractility. Reduction of FS may be secondary to decreased contractility, poor preload, or increased afterload (Boon, 2011).

Ejection fraction (EF) is a measurement of blood volume out of the left ventricle whether through the aorta, mitral valve, or shunt. The ejection fraction is

calculated from the difference between end-diastolic and end-systolic LV volume, divided by the end-diastolic LV volume. The most common method of EF measurements is modified Simpson's method (Schiller, 1991). Modified Simpson's method provides accurate LV ventricular volume that relatively unaffects by geometric changes of the heart (Lang et al., 2015).

However, %FS and %EF are not sensitive enough to detect a subtle change in myocardial contractility. A more sensitive method such as tissue Doppler imaging should be performed to assess the systolic function (Zhang et al., 2012). Several studies reported systolic dysfunction in diabetic patients and experimental animals. Systolic dysfunction usually occurs after significant diastolic dysfunction has already developed (Mihm et al., 2001; Boudina, 2009; Patil and Burji, 2012).

Cardiac biomarkers in diabetic cardiomyopathy

Biomarkers have been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." (Biomarkers Definitions Working, 2001). The usefulness of biomarkers in both human and veterinary medicine including diagnosis of subclinical and clinical diseases, risk stratification, monitoring of disease progression, and evaluating response to therapy (Boswood, 2009).

Cardiac troponin I (cTnI)

Troponin complex is composed of three subunits including cTnI, cTnT, and cTnC that play an important role in the excitation-contraction coupling of cardiac sarcomere. Cardiac TnI is composed of two populations. The main population is structurally bound to the thin filament. The small amount of cTnI (2-4%) remains free in the cytosol (Katus et al., 1991). An increased serum cTnI concentration indicates myocardial damage or death (Adams et al., 1993). The use of cTnI has been widely studied in human and veterinary medicine because of its high sensitivity and specificity in detecting cardiac damage (Oyama and Sisson, 2004). The primary

indication for measurement of cTnI in dogs and cats is to detect cardiac damage from both extracardiac and cardiac diseases (Boswood, 2009).

The half-life of troponin in the blood stream is approximately 2 hours. Cardiac TnI increases and gradually returns to baseline levels within 7-10 days after the onset of the myocardial damage. The homology of cTnI protein structure between humans and dogs is 95%. The close homology of cTnI structure between humans and dogs allows using human immunoassays technique for measuring of canine cTnI concentration (Rishniw et al., 2004).

In healthy dogs, cTnI concentration is typically low because cTnI is tightly bound to the actin filament. (Adams et al., 1993). Normal value of cTnI is < 0.03-0.07 ng/ml with a median value of 0.02 ng/ml (Sleeper et al., 2001).

Galectin-3 (Gal-3)

Galectin-3 (Gal-3) is a beta-galactoside-binding lectin that involved in several pathological processes including tumor growth, tissue repair, inflammation, cardiac remodeling, and fibrosis (Dumic et al., 2006). Galectin-3 is predominantly synthesized and secreted by activated macrophages, eosinophils, and mast cells (Yang et al., 2008). In human patients, Gal-3 is a novel biomarker of fibrosis (van Kimmenade et al., 2006). Up-regulation of gal-3 has been found in fibrotic conditions in various organs including the heart, liver, and kidney (Henderson et al., 2006; Henderson et al., 2008). Aragno et al. (2008) showed an increased expression of Gal-3 in the myocardium of diabetic rats. The recent clinical study in human patients revealed a significant increase of serum Gal-3 levels in the diabetic group compared to the control group (Yilmaz et al., 2015). Recently, the concentration of circulating Gal-3 in diabetic dogs has not yet been studied.

N-terminal-proB-type natriuretic peptide (NT-proBNP)

N-terminal-pro B-type natriuretic peptide (NT-proBNP) is a neurohormonal substance that synthesized and secreted from the atrium and ventricle in response to stretch of the myocardium (Kinnunen et al., 1993). proBNP is a prohormone cleaved into a biologically C- terminal active molecule (BNP) and a biologically

inactive N-terminal fragment (NT-proBNP). BNP and NT-proBNP release into blood circulation in the same ratio (Hall, 2004). The half-life of canine BNP in the circulation is short as 1.57 minutes (Thomas and Woods, 2003). NT-proBNP has a significantly longer half-life than BNP. Moreover, NT-proBNP is more stable from storage and has a significant higher plasma concentration (Pemberton et al., 2000). These properties make NT-proBNP more suitable than BNP for clinical use.

In human medicine, NT-proBNP helps in the diagnosis of congestive heart failure, screening of occult disease, risk stratification, as well as treatment and prognosis of heart disease. A previous study showed elevated BNP levels and left ventricular dysfunction in human diabetic patients (Maisel et al., 2002). The cohort study in 253 human patients with type II diabetes showed a significant increase of NT-proBNP concentrations in the diabetic group compared to the non-diabetic group (Magnusson et al., 2004).

In veterinary medicine, several studies have shown a correlation between NT-proBNP levels and severity of degenerative mitral valve disease in dogs (Haggstrom et al., 2000; Moonarmart et al., 2010; Reynolds et al., 2012). NT-proBNP assay is useful for differentiation respiratory distress dogs with the cardiac and non-cardiac origin. Dogs with congestive heart failure showed a significant higher NT-proBNP concentration (greater than 1400 pmol/L) than dogs with respiratory disease (less than 800 pmol/L) (Fine et al., 2008). A role of natriuretic peptides in identifying occult cardiomyopathy in dogs has been proposed (Chetboul et al., 2004). The study in 71 Doberman pinschers suggested the usefulness of the diagnostic combination between NT-proBNP assay together with Holter monitoring to identify occult cardiomyopathy (Oyama et al., 2007).

Recently, a sandwich enzyme immunoassay is commercially available to measure plasma NT-proBNP concentrations in dogs. The median value of NT-proBNP concentrations in normal dogs is 240 pmol/L (range from 131-546 pmol/L) (Hassdenteufel et al., 2012). However , the reference ranges and cut off values were differently reported due to a variation of sample handling and assay performance (Oyama and Singletary, 2010).

As described above, diabetes can induce cardiomyopathy in humans. However, it has been unproved whether diabetic cardiomyopathy also occurs in dogs. This study was created to prove the hypothesis that there is an alteration of cardiac function and plasma cardiac biomarker levels in diabetic dogs compared to normal dogs.

CHAPTER III
MATERIALS AND METHODS

Clinical study in diabetic affected dogs

Thirty-nine dogs included in the study were small to middle sized breed dogs, age more than 7 years old presented at Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. All dogs used in this study were permitted by the owner. The study protocol was subjected to approve by the Ethic Committee for the Animal Experimentation, Faculty of Veterinary Science, Chulalongkorn University. In the study, dogs were divided into diabetic and control groups according to history, physical examination, and blood results. Plasma cTnI, Gal-3, and NT-proBNP concentrations were measured in diabetic and control groups. Echocardiography was performed in both groups of dogs to evaluate cardiac function.

Clinical procedure

Comprehensive data from history taking, physical examination, blood profile values, electrocardiography, blood pressure measurement, and echocardiography were recorded.

1. Signalment and history taking including clinical signs, insulin in use, duration of treatment were recorded.
2. The complete physical examination was performed including temperature, hydration status, color of mucous membrane, capillary refilling time, heart sound, lung sound, pulse quality, heart rate, respiratory rate, and body condition score.
3. Blood collection for complete blood count, blood chemistry profiles, fructosamine concentration, and fasting blood glucose were performed to assess health status.
4. Blood pressure measurements were performed to evaluate systolic blood pressure.

5. Five minute electrocardiography was performed to evaluate cardiac electrical abnormalities.
6. Echocardiography was performed to evaluate cardiac structure and function.

Animals

After the clinical procedure, dogs were divided into diabetic and control groups. Dogs were excluded from the study if they had an evidence of other cardiac diseases, high blood pressure levels, and serious systemic illness. Two groups of dogs included:

- Diabetic group: 19 diabetic dogs were included in this group.

Inclusion criteria: Diabetic dogs diagnosed by fasting hyperglycemia and high fructosamine levels (defined as fasting blood glucose (FBG) > 200 mg/dl and fructosamine level > 350 mmol/L, respectively) were included.

Excluded criteria: Diabetic dogs with concurrent cardiac abnormalities such as degenerative mitral valve disease (DMVD), dilated cardiomyopathy (DCM), congenital cardiac diseases, and/or significant cardiac arrhythmias were excluded from the study. Dogs were excluded if they had other serious systemic illnesses such as cancer, kidney disease defined as blood urea nitrogen (BUN) greater than 27 mg% and creatinine greater than 1.4 mg% (International Renal Interest Society (IRIS), 2013), hepatic diseases defined as alkaline phosphatase (ALP) greater than 5 times the upper limit of normal range and alanine aminotransferase (ALT) more than 3 times the upper limit of normal range (Alvarez and Whittemore, 2009), and hypertension defined as systolic blood pressure more than 160 mmHg (Brown et al., 2007).

- Control group**:** 20 healthy dogs were selected into this group.

Inclusion criteria: Dogs with normal physical examination findings and blood profile values were included.

Excluded criteria: Dogs with abnormal echocardiographic findings were excluded.

Measurements of plasma cTnI, Gal-3, and NT-proBNP concentrations

Blood collection

Blood samples were collected from diabetic and control groups to measure cTnI, Gal-3, and NT-proBNP concentrations. Three ml of whole blood samples were collected by venipuncture from the cephalic or saphenous vein. Whole blood was placed into test tubes containing heparin to measure plasma cTnI and EDTA to measure plasma Gal-3 and NT-proBNP. Whole blood was centrifuged to separate plasma with 1000g for 15 minutes at 2-8 $^{\circ}$C within 30 minutes of collection. Plasma was stored at -20 $^{\circ}$C until measurement.

Measurement of plasma cTnI

Plasma cTnI was quantitatively measured by in-house laboratory unit of Small Animal Hospital, Chulalongkorn University by an automating system (AIA360) (TOSOH Corporation CO, LTD). The principle of the test kit is the sandwich-enzyme immunoassay (EIA) technique. All procedures were performed according to the machine direction. Briefly, samples were added to the reagent cups. After antigen-antibody reaction time (10 minutes), the substrate was added and fluorescence intensity was measured.

Measurement of plasma Gal-3

Plasma Gal-3 was quantitatively measured by a commercial ELISA test kit (BlueGene Biotech, Shanghai, China). The principle of the test kit is the competitive enzyme immunoassay technique utilizing a monoclonal anti-Gal-3 antibody and Gal-3- Horseradish Peroxidase (HRP) conjugate. All procedures were performed according to the test kit direction. Briefly, the sample and buffer were incubated with Gal-3-HRP conjugate in a pre-coated plate for one hour. After the incubation period, the wells were decanted and washed five times. After this process, the wells were incubated with a substrate for HRP enzyme. Finally, a stop solution was added to stop the reaction. The intensity of color was measured by spectrophotometry at 450 nm in a microplate reader. The intensity of the color was inverse to the

concentration of Gal-3. The quantity of Gal-3 concentration was determined by a standard curve plotted from the intensity of color and concentration from standard solutions (BlueGene Biotech).

Measurement of plasma NT-proBNP

Plasma NT-proBNP was measured by a commercial ELISA test kit (MyBioSource, California, USA). The principle of the test kit is a sandwich enzyme immunoassay. The minimum detectable level of NT-proBNP is less than 5.0 pg/mL. All procedures were performed according to the test kit direction. Briefly, standards or samples were added to the microtiter plate wells coated with antibody specific to NT-proBNP. Next, HRP-conjugate reagent was added to each microplate well and incubated. After tetramethylbenzidine (TMB) substrate solution was added. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450nm. The concentration of NT-proBNP in the samples was determined by comparing the optical density (O.D.) of the samples to the standard curve.

Echocardiography

Echocardiography was performed to evaluate cardiac function in diabetic and control dogs by using an ultrasound machine (Eko7, Samsung Medison) with 2-4 and 4-10 multifrequency MHz array transducers. Echocardiography was performed by an investigator who blinded to the group of dog. Three consecutive measurements were performed for each echocardiographic parameters.

Two-dimensional echocardiography

Two-dimensional echocardiography was performed on the right parasternal long and short axis views to determine the cardiac structure.

Left ventricular systolic function evaluation

M-mode echocardiography was performed on right parasternal long axis view to measure left ventricular dimension during systole and diastole, interventricular septal thickness during systole and diastole, and left ventricular free wall during systole and diastole. Echocardiographic ratio indices (ERIs) were calculated. All M-mode parameters were divided by a weight-based aortic value (AO_w). The AO_w was calculated as follow (Boon, 2011):

$$Ao_w = kW^{1/3}$$

Where W = body weight in kilograms and k = a constant value for dogs (0.795)

Percent fractional shortening (FS) was calculated from:

$$FS\ (\%) = [(LVEDD-LVESD)/LVEDD)] \times 100$$

Left ventricular ejection fraction (%EF) was assessed by modified Simpson's rule on left apical four chamber view (Boon, 2011).

Left ventricular diastolic function evaluation

Transmitral flow pulsed-wave Doppler was performed to evaluate the peak E and A flow patterns and velocities. The transmitral flow was recorded from left apical four chamber views. The gate was placed at the point proximal to the opening of the mitral leaflets (Boon, 2011). Isovolumic relaxation time (IVRT) was recorded from left apical five- chamber view by placing the gate in the left ventricular outflow tract near the mitral valve to reveal both aortic ejection flow and left ventricular inflow (Boon, 2011). Pulsed-wave Doppler-derived parameters including maximum velocities of E (peak E) and A (peak A) waves, E/A ratio (E/A), E-wave deceleration time (Dec time) and isovolumic relaxation time (IVRT) were recorded. Pulmonary vein flow pulsed-wave Doppler was performed to evaluate pulmonary vein flow during systole (Peak pul S), diastole (Peak pul D), peak velocity of pulmonary vein systolic flow to peak velocity of pulmonary vein diastolic flow ratio (S/D), pulmonary vein reversal flow during atrial contraction (Peak pul A) and pulmonary vein atrial reversal flow duration (PVar dur) on right parasternal short axis view (Boon, 2011).

Tissue Doppler imaging (TDI) was performed to evaluate myocardial velocity. Gate was placed at the left lateral annulus positions on left apical four chamber view to evaluate left ventricular myocardial motion (Boon, 2011). TDI parameters including positive systolic motion (Peak S'), early diastolic motion (Peak E'), late diastolic motion (Peak A') and E'/A' ratio were evaluated.

Diastolic function was categorized into three grades according to the alteration in echocardiographic parameters including transmitral flow, pulmonary venous flow, isovolumic relaxation time, and tissue Doppler velocity. The following criteria were used to classify all dogs into four groups (modified from Boon, 2011).

1. Normal diastolic function:
   - E/A >1
   - E-wave deceleration time = 52-108 msec
   - Isovolumic relaxation time = 38-54 msec
2. Impaired relaxation (Grade I):
   - E/A < 1
   - E-wave deceleration time > 108 msec
   - Isovolumic relaxation time > 54 msec
3. Pseudonormal pattern (Grade II): characterized by at least one of the following

   3.1 Normal E/A ratio (ranging from 1.0-2.0)

   Normal deceleration time (52-108 msec)

   Normal isovolumic relaxation time (38-54 msec)

   Pulmonary vein atrial reversal flow duration > 64 msec

   3.2 E'/A' < 1
4. Decrease compliance (Grade III): characterized by least three of the following
   - E/A > 2
   - E-wave deceleration time < 52 msec
   - Isovolumic relaxation time < 38 msec
   - Pulmonary vein atrial reversal flow duration > 64 msec
   - S:D < 0.32

Statistical analysis

Statistical analysis was performed by computer-based software, SPSS program. The Shapiro-Wilk test was used for a normality testing of all data before statistical analysis. Descriptive statistical analysis was applied to describe signalment of diabetic and control groups including sex and breeds. Mean ± SD (Standard deviation) was used to report signalment including age and body weight. Independent t-test was applied to assess mean of age and body weight between diabetic and control groups. Descriptive statistical analysis was used to report information of the history and physical examination findings. Complete blood count, blood chemistry, and echocardiographic parameter values were presented as mean ± SD. Independent t-test was used to compare the mean of CBC, blood chemistry, and echocardiographic parameter values between diabetic and control groups. Plasma cTnI, Gal-3, and NT-proBNP concentrations were non-parametric data and reported as median; 25^{th} and 75^{th} percentile. Mann-Whitney U test was applied to compare plasma cTnI, Gal-3, and NT-proBNP concentrations. Fisher's exact test was used to compare percent of sex, physical examination findings, and echocardiographic parameters between diabetic and control groups. Spearman's rank correlation was used to evaluate correlations between cTnI, Gal-3, and NT-proBNP and echocardiographic parameter values. A probability value < 0.05 was considered as statistical significance.

CHAPTER IV
RESULT

Part I: General information

1.1 Signalment

Thirty-nine dogs presented at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University were included in this study. All dogs were divided into diabetic and control groups. The diabetic group consisted of nineteen dogs included thirteen females (intact=3; sprayed=10) and six males (intact=4; castrated = 2). Breeds of dogs in the diabetic group were Poodle (n=8), Mixed breed (n=4), Pug (n=2), Shih-Tzu (n=2), Beagle (n=1), Pomeranian (n=1), and Chihuahua (n=1). The control group consisted of twenty dogs included eleven females (intact= 2; sprayed= 9) and nine males (intact=6; castrated=3). Breeds of dogs in the control group were Shih-Tzu (n=8), Chihuahua (n=6), Pomeranian (n=3), Mixed breed (n=1), Schnauzer (n=1), and Yorkshire terrier (n=1). Percent of male and female dogs between the two groups were not significant difference (p=0.514). In the diabetic group, 68.42% were female, and the remaining 31.58% were male. Whereas 55% of control dogs were female, and the remaining 45% were male. The mean body weight of dogs was significantly higher in the diabetic group than that of the control group (Table 1).

Table 1: Clinical characteristics of diabetic and control groups (mean ± SD)

Parameters	Diabetic group (n=19)	Control group (n=20)	*p*- value
Age (years)	9.7±0.6	9.1±0.4	0.43[a]
Body weight (kg)	7.73±0.62	5.03±0.52	0.002[*]

[*] Indicate statistical difference at $p < 0.05$ between diabetic and control groups.

The significant difference was assessed by independent t-test

1.2 History and physical examination findings

The abnormalities found in diabetic dogs including polyuria and polydipsia (7/19; 36.84%), dehydration (4/19; 21.05%), abdominal enlargement (a subjective assessment) (4/19; 21.05%), hair loss (3/19; 15.79), and cataract (4/19; 21.05%). In the diabetic group, 15 dogs (78.95%) and 4 dogs (21.05%) had respiratory sinus arrhythmia and normal sinus rhythm, respectively. In the control group, 12 dogs (60.00%) and 8 dogs (40.00%) had respiratory sinus arrhythmia and normal sinus rhythm, respectively. There was no significant difference in systolic blood pressure between diabetic group (125.1±24.9 mmHg) and control group (129.9±17.4 mmHg). There was no significant difference in heart rate between diabetic group (124.0±21.9 beats/min) and control group (130.3±21.4 beats/min).

All diabetic dogs received insulin therapy twice a day in the dose range from 0.33-1.0 unit/kg (mean=0.61 unit/kg). Of the 19 diabetic dogs, 4 dogs (21.05%) received neutral protamine Hagedorn insulin (Humulin N®) and 15 dogs (78.95%) received porcine insulin (Caninsulin®). The duration of diabetes ranged from 7-1,797 days (mean=674 days).

Table 2: Physical examination and history data of diabetic and control groups (percent)

Abnormalities	**Diabetic group (n = 19)**	**Control group (n=20)**	***p*- Value**
Polyuria and polydipsia	7(36.84%)	-	-
Dehydration	4(21.05%)	2(10%)	0.407
Abdominal enlargement	4(21.05%)	-	-
Hair loss	3(15.79%)	-	-
Cataract	4(21.05%)	-	-
Respiratory sinus	15(78.95%)	12(60.00%)	0.301
Normal sinus rhythm	4(21.05%)	8(40.00%)	0.301

1.3 Complete blood count and blood chemistry profile values

Complete blood count profile values

The mean complete blood count profile values of diabetic and control groups were shown as mean ± SD in Table 3. White blood cell and neutrophil numbers were significantly higher in the diabetic group than those in the control group. Hematocrit was significantly higher in the control group than that in the diabetic group. There was no significant difference in red blood cell, hemoglobin, platelet, eosinophil, lymphocyte, and monocyte numbers between diabetic and control groups. All mean ± SD of complete blood count profile values were within the normal limit.

Table 3: Complete blood count profile values of diabetic and control groups (mean ± SD)

Parameter	Unit	Normal value[a]	Diabetic group (n=19)	Control group (n=20)	*p*-value
RBC	$x10^6$cell/μl	5.2 - 8.06	6.3±1.3	7.0±1.0	0.051
Hematocrit	%	29.8 - 57.5	44.1±8.7	49.3±6.3	0.037*
Hemoglobin	g/dL	12.0-18.0	14.8±3.0	16.4±2.6	0.090
Platelet	$x10^3$cell/μl	160 - 525	445.9±222.8	331.0±127.9	0.054
WBC	$x10^3$cell/μl	5.4 -15.3	11.6±2.3	9.6±3.0	0.025*
Neutrophil	cell/μl	3,000-12,000	8,696.5±2,265.7	6,302.0±2,609.1	0.004*
Eosinophil	cell/μl	0-1,900	330.7±366.4	311.6±427.3	0.882
Lymphocyte	cell/μl	530-4,800	1,489.4±865.9	2,279.6±2,197.5	0.152
Monocyte	cell/μl	100-1,800	917.7±383.6	646.1±198.6	0.356

*Indicate statistical difference at $p < 0.05$ between diabetic and control groups.

The significant difference was assessed by independent t -test.

[a] Normal reference values from: Ettinger JS and Feldman EC. 2010. Canine diabetic mellitus.7 th ed. In: text book of Veterinary Internal Medicine. Elsevier Saunders, Davis, California. 1,461-1,462.

Abbreviations: RBC= Red blood cell; WBC = White blood cell.

Blood chemistry profile value

The mean complete blood count profile values of diabetic and control groups were presented as mean ± SD in Table 4. Plasma ALP and FBG were significantly higher in the diabetic group than those in the control group. Plasma ALT, BUN, and creatinine were not significantly different between diabetic and control groups. All mean ± SD of complete blood count profile values were within the normal limit.

Table 4: Blood chemistry profile values of diabetic and control groups (mean ± SD)

Parameter	**Unit**	**Normal value**[a]	**Diabetic group** (n= 19)	**Control group** (n = 20)	***p*- value**
ALT	IU/L	4 – 91	62.2±6.8	66.9±12.2	0.737
ALP	IU/L	3 – 60	150.8±14.6	75.3±16.1	0.001*
BUN	mg/dl	7 – 26	18.3±2.8	13.2±0.9	0.092
Creatinine	mg/dl	0.6 – 1.4	0.83±0.06	0.74±0.04	0.188
FBG	mg/dl	80-120	311.6±146.8	80.5±13.8	<0.001*
Fructosamine	µmol/L	225-365[b]	461.7±170.9	-	-

* Indicate statistical difference at $p < 0.05$ between diabetic and control groups.

The significant difference was assessed by independent t-test.

[a] Normal reference value from: Ettinger JS and Feldman EC. 2010. Canine diabetic mellitus.7 th ed. In: text book of Veterinary Internal Medicine. Elsevier Saunders, Davis, California.1,461-1,462

[b] Normal reference value from: Douglass KM, Kenneth JD, Steven CH and William DS. 2005. Normal value of oxygen and hemodynamic parameters. In: Manual of Small Animal Emergency and Critical Care Medicine. 1 st ed. BT David (ed). Philadelphia: Lippincott Williama and Wilkins. 429-431.

Abbreviations: ALT = Alanine amino transferase; ALP = Alkaline phosphatase; BUN = Blood urea nitrogen; FBG = Fasting blood glucose.

Part II: Cardiac biomarker concentrations

The data of cardiac biomarker levels were non-parametric and reported as median; 25^{th}-75^{th} percentile (Table 5).

Table 5: Cardiac biomarker concentrations of diabetic and control groups (median; 25^{th} and 75^{th} percentile)

Cardiac biomarkers	Diabetic group (n=19)	Control group (n=20)	*p- value*
cTnI (ng/ml)	0.019; 0.014-0.021	0.021; 0.013-0.027	0.430
Gal-3 (ng/ml)	0.68; 0.37-1.12	0.51; 0.36-0.57	0.249
NT-proBNP (pg/ml)	73.89; 46.42-131.92	116.04; 80.04-158.80	0.048*

* Indicate statistical difference at $p < 0.05$ between diabetic and control groups. The significant difference was assessed by Mann-Whitney U test.

2.1 Plasma cTnI concentration

There was no significant difference of median plasma cTnI concentrations between diabetic and control groups (Table 5). The scatter plot of plasma cTnI concentrations in diabetic and control groups was presented in Figure 2.

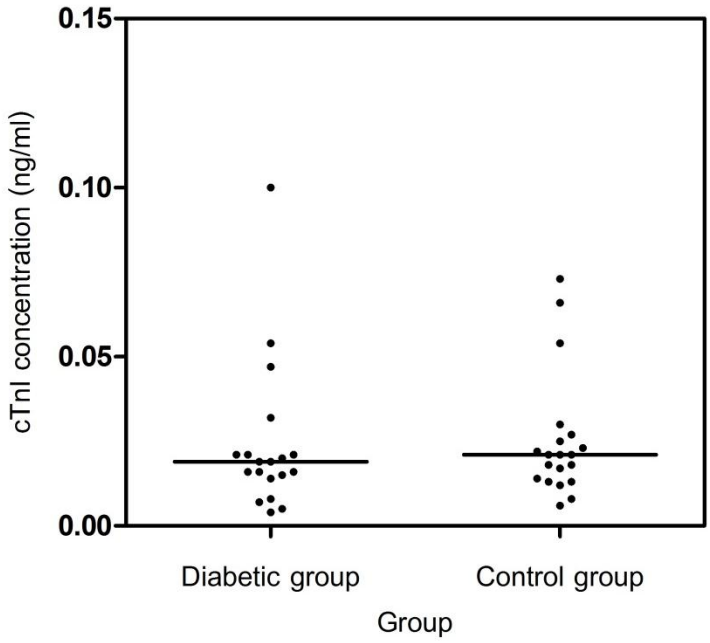

Figure 2: Scatter plot of plasma cTnI concentrations in diabetic and control groups. Median values were presented as lines.

2.2 Plasma Gal-3 concentration

Canine Gal-3 ELISA test kits were validated by assessment of an accuracy and precision. Five spiked plasma samples and 5 different standard concentrations were used to assess the average of percent recovery. The average of percent recovery for canine Gal-3 ELISA test was 87%. The coefficient of variance for intra- and inter-variability was 3.01% and 11.30%, respectively. There was no significant difference of median plasma Gal-3 concentrations between diabetic and control groups. The scatter plot of plasma Gal-3 concentrations in diabetic and control groups was shown in Figure 3.

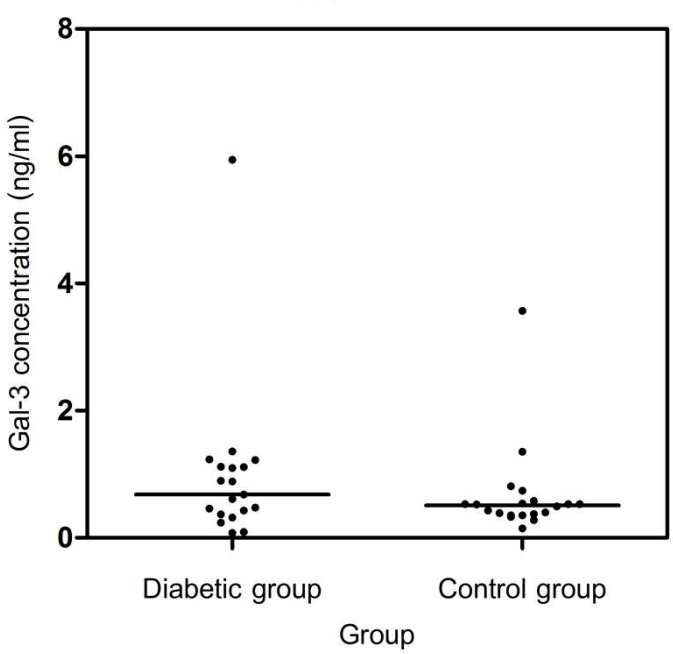

Figure 3: Scatter plot of plasma Gal-3 concentrations in diabetic and control groups. Median values were presented as lines.

2.3 Plasma NT-proBNP concentration

Canine NT-proBNP ELISA test kits were validated by assessment of an accuracy and precision. Five spiked plasma samples and 5 different standard concentrations were used to assess the average of percent recovery. The average of percent recovery for canine NT-proBNP ELISA test was 91.3%. The coefficient of variance for intra- and inter- variability were 2.98% and 14.50%, respectively. The median of plasma NT-proBNP concentrations in the control group was significantly

higher than that of the diabetic group. The scatter plot of plasma NT-proBNP concentrations in diabetic and control groups was presented in Figure 4.

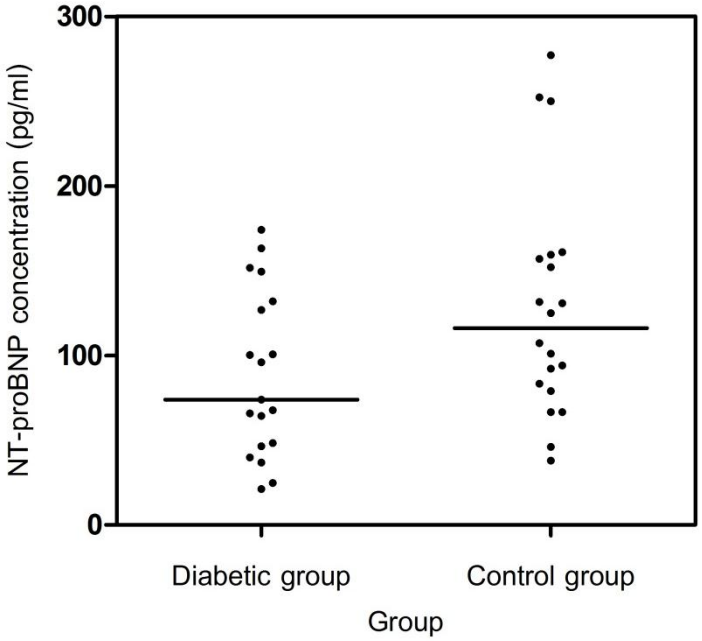

Figure 4: Scatter plot of plasma NT-proBNP concentrations in diabetic and control groups. Median values were presented as lines.

According to the subgroup analysis in the diabetic group, diastolic dysfunction dogs had a 2 fold increase of plasma cTnI and Gal-3 levels compared to dogs with normal diastolic function; however, an increase did not meet the statistical difference (p=0.35 and p=0.39, respectively).

Part III: Echocardiography

The diabetic group had significantly thinner inter ventricular septal thickness (IVSs) and significantly larger internal dimension during systolic period (LVIDs) than the control group (Table 6). According to conventional Doppler echocardiography (CE), E-wave deceleration time (Dec time) and pulmonary vein atrial reversal flow duration (PVar dur) were significantly longer in the diabetic group than those in the control group. Percent of peak velocity of early diastolic filling to peak velocity of late diastolic filling ratio less than 1 was significantly higher in the diabetic group than those in the control group. Peak velocity of late diastolic myocardial motion (Peak A') and peak velocity of myocardial systolic motion (Peak S') were significantly higher

in the diabetic group than those in the control group. Peak velocity of early diastolic myocardial motion to peak velocity of late diastolic myocardial motion ratio (E'/A') was significantly lower in the diabetic group than that in the control group. Percent of E'/A' less than 1 was significantly higher in the diabetic group than those in the control group (Table 7). Diastolic dysfunction was detected in 57.88% of diabetic dogs (26.31% with grade I and 31.57% with grade II diastolic dysfunction) and 15 % of control dogs (5% with grade I and 10% with grade II diastolic dysfunction) assessed by CE and TDI. Percent of diastolic dysfunction was significantly higher in diabetic group (57.88%) than those in control group (15%) (p=0.006). Abnormal transmitral flow patterns and abnormal TDI patterns were shown in Figure 5 and Figure 6, respectively.

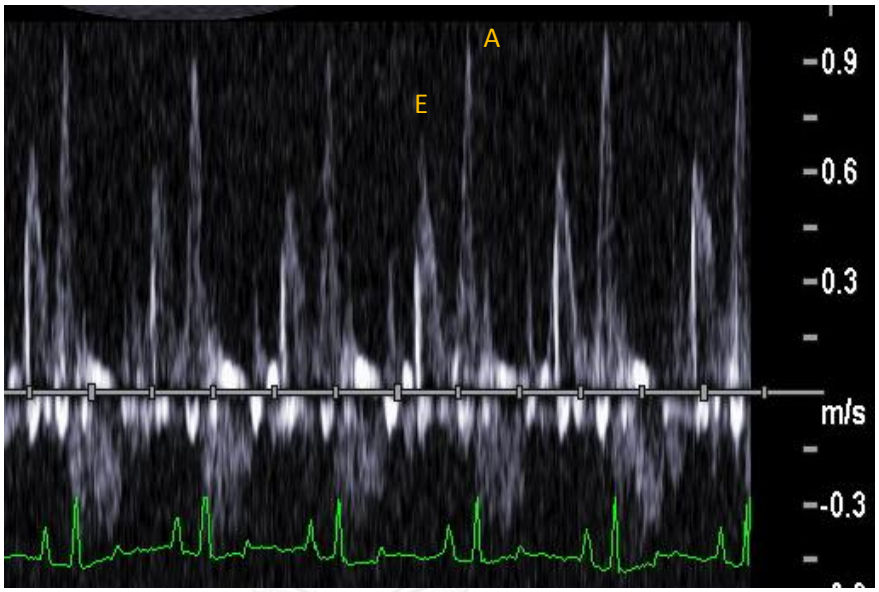

Figure 5: Transmitral flow Doppler echocardiography showed early diastolic dysfunction of left ventricle defined as E/A < 1 and prolonged E-wave deceleration time in a diabetic dog.

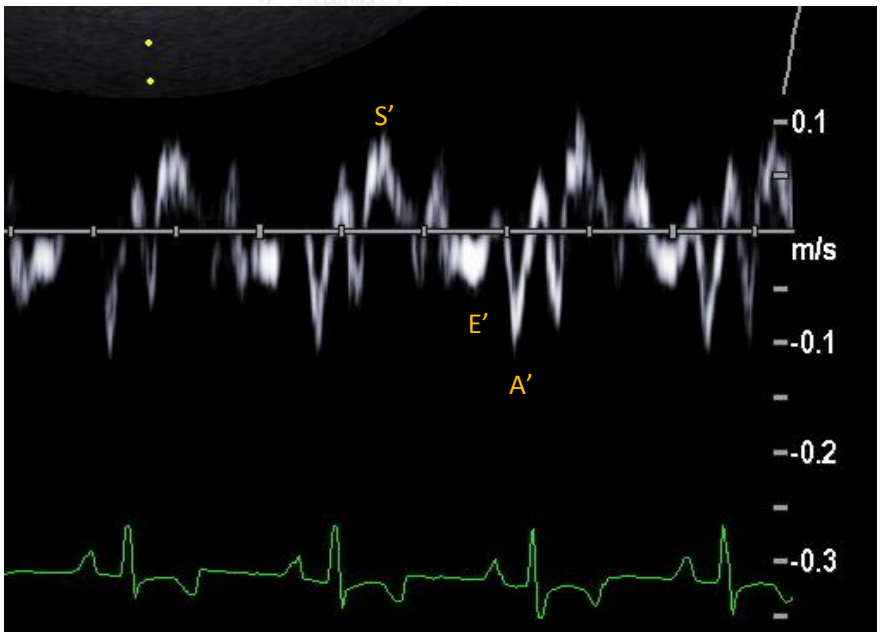

Figure 6: An abnormal TDI pattern defined as E'/A' less than 1 in a diabetic dogs.

Table 6: M-mode echocardiographic parameter values of diabetic and control groups (mean ± SD)

Parameters	Diabetic group (n=19)	Control group (n=20)	*p-value*
IVSd (cm/kg)	0.58±0.10	0.62±0.15	0.314
LVIDd (cm/kg)	1.55±0.18	1.46±0.121	0.175
LVPWd (cm/kg)	0.47±0.08	0.49±0.11	0.536
IVSs (cm/kg)	0.70±0.11	0.79±0.15	0.043*
LVIDs (cm/kg)	1.00±0.15	0.83±0.20	0.004*
LVPWs (cm/kg)	0.68±0.09	0.72±0.14	0.217
LA (cm/kg)	1.23±0.17	1.18±0.12	0.268
LA/Ao	1.28±0.16	1.28±0.17	0.922
%FS	35.20±5.81	38.21±8.01	0.189
%EF	66.73±8.37	70.82±9.53	0.164

The significant difference was assessed by independent t- test.

* Indicate statistical difference at $p < 0.05$ between diabetic and control groups

Abbreviations: d = diastole, s = systole, IVS = interventricular septal thickness, LVID = left ventricular internal dimension, LVPW = left ventricular free wall thickness, LA = left atrium, LA/Ao = left atrium to aorta ratio, FS = fractional shortening, EF = ejection fraction

Table 7: Conventional Doppler echocardiographic parameter and TDI values of diabetic and control groups (mean ± SD)

Parameters	Diabetic group (n=19)	Control group (n=20)	*p-value*
Transmitral flow			
Peak E (m/sec)	0.70±0.18	0.68±0.0.13	0.749
Peak A (m/sec)	0.60±0.13	0.57±0.16	0.538
E/A	1.20±0.36	1.26±0.29	0.596
Dec time (msec)	125.68±38.89	88.05±20.92	0.001*

IVRT (msec)	67.74±11.47	63.45±10.83	0.238
E/A < 1	5(26.31%)	1(5%)	0.044*,[a]
Pulmonary vein flow			
Peak pul S (m/s)	0.53±0.15	0.49±0.11	0.413
Peak pul D (m/s)	0.48±0.10	0.45±0.11	0.326
Peak pul A (m/s)	0.22±0.06	0.19±0.03	0.068
S/D	1.11±0.25	1.12±0.23	0.848
PVar dur (msec)	71.16±9.20	61.30±8.23	0.001*
Aortic flow			
AV max (m/s)	1.30±0.23	1.26±0.17	0.543
AVPG (mmHg)	6.96±2.27	6.46±1.71	0.450
Tissue Doppler imaging (TDI)			
Peak E'(m/s)	0.10±0.02	0.11±0.03	0.218
Peak A'(m/s)	0.11±0.02	0.09±0.03	0.009*
E'/A'	0.93±0.19	1.45±0.79	0.010*
E'/A' < 1	5(26.31%)	1(5%)	0.044*,[a]
Peak S'(m/s)	0.12±0.03	0.10±0.03	0.040*

The significant difference was assessed by independent t-test.

[a] The significant difference was assessed by Fisher's exact test.

* Indicate statistical difference at $p < 0.05$ between diabetic and control groups

Abbreviations: Peak E = peak velocity of early diastolic filling, Peak A = peak velocity of late diastolic filling, E/A = peak velocity of early diastolic filling to peak velocity of late diastolic filling ratio, Dec time = deceleration time, IVRT = isovolumic relaxation time, Peak pul S = peak velocity of pulmonary vein systolic flow, Peak pul D = peak velocity of pulmonary vein diastolic flow, Peak pul A = peak velocity of pulmonary vein reversal flow during atrial contraction, S/D = peak velocity of pulmonary vein systolic flow to peak velocity of pulmonary vein diastolic flow ratio, Pvar dur = pulmonary vein atrial reversal flow duration, AV max = maximal aortic pressure, AVPG = aortic pressure gradient, Peak E' = peak velocity of early diastolic myocardial motion, Peak A′ = peak velocity of late diastolic myocardial motion, E'/A'= peak velocity of early diastolic myocardial motion to peak velocity of late diastolic myocardial motion ratio. Peak S′ = peak velocity of myocardial systolic motion

Part IV: Correlation

4.1 The correlations between duration of diabetes with echocardiographic parameter values and cardiac biomarkers

There were moderate negative correlations between duration of diabetes with peak velocity of pulmonary vein systolic flow (Peak pul S), left ventricular internal dimension during diastole and systole (LVIDd and LVIDs), and weak negative correlation with peak velocity of pulmonary vein diastolic flow (Peak pul D). The moderate positive correlations between duration of diabetes with isovolumic relaxation time (IVRT) and left ventricular free wall thickness during diastole and systole (LVPWd and LVPWs) were demonstrated (Table 8).

Table 8: The correlations between duration of diabetes and echocardiographic values and cardiac biomarker concentrations of the diabetic group

Parameters	**r**	***p-value***
Cardiac biomarkers		
cTnI	0.22	0.929
Gal-3	-0.089	0.717
NT-proBNP	-0.475	0.40
Echocardiographic parameters		
Peak E (m/sec)	-0.112	0.648
Peak A (m/sec)	0.449	0.054
E/A	-0.321	0.180
Dec time (msec)	-0.051	0.836
IVRT (msec)	0.644	0.003*
Peak pul S (m/s)	-0.696	0.001*
Peak pul D (m/s)	-0.477	0.039*
Peak pul A (m/s)	0.438	0.061
S/D	-0.407	0.084

PVar dur (msec)	-0.213	0.380
AV max (m/s)	0.449	0.054
AVPG (mmHg)	-0.149	0.544
Peak E'(m/s)	0.307	0.202
Peak A'(m/s)	0.378	0.110
E'/A'	-0.034	0.889
Peak S'(m/s)	-0.334	0.162
IVSd (cm/kg)	0.319	0.183
LVIDd (cm/kg)	-0.512	0.025*
LVPWd (cm/kg)	0.617	0.005*
IVSs (cm/kg)	0.253	0.296
LVIDs (cm/kg)	-0.565	0.012*
LVPWs (cm/kg)	0.579	0.009*
LA (cm/kg)	0.009	0.971
LA/Ao	-0.056	0.821
%FS	0.286	0.236
%EF	0.278	0.249

The significant correlation was assessed by Spearman's rank correlation.

* Indicate statistical correlation at $p < 0.05$

4.2 The correlations between fructosamine concentration with echocardiographic parameters and cardiac biomarkes

According to the Table 9, the weak positive correlations between fructosamine concentrations and NT-proBNP concentrations were observed. The inverse correlations between fructosamine concentrations and peak velocity of early diastolic filling (Peak E) and peak velocity of late diastolic myocardial motion (Peak A') were found.

Table 9: The correlations between fructosamine concentrations with echocardiographic parameter values and cardiac biomarker concentrations of the diabetic group

Parameters	**r**	***p-value***
Cardiac biomarker concentrations		
cTnI	-0.251	0.300
Gal-3	-0.420	0.058
NT-proBNP	0.457	0.049*
Echocardiographic parameter values		
Peak E (m/sec)	-0.457	0.049*
Peak A (m/sec)	-0.198	0.417
E/A	-0.254	0.294
Dec time (msec)	-0.198	0.418
IVRT (msec)	-0.141	0.566
Peak pul S (m/s)	0.224	0.357
Peak pul D (m/s)	0.110	0.653
Peak pul A (m/s)	-0.205	0.399
S/D	0.241	0.319
PVar dur (msec)	-0.223	0.358
AV max (m/s)	-0.025	0.919
AVPG (mmHg)	0.024	0.924
Peak E'(m/s)	-0.392	0.097
Peak A'(m/s)	-0.489	0.038*
E'/A'	0.062	0.801
Peak S'(m/s)	0.089	0.717
IVSd (cm/kg)	-0.231	0.342
LVIDd (cm/kg)	0.181	0.458
LVPWd (cm/kg)	0.125	0.609
IVSs (cm/kg)	-0.101	0.680

LVIDs (cm/kg)	0.228	0.347
LVPWs (cm/kg)	0.004	0.988
LA (cm/kg)	0.163	0.504
LA/Ao	0.206	0.398
%FS	-0.150	0.539
%EF	-0.143	0.560

The significant correlation was assessed by Spearman's rank correlation.
*Indicate statistical correlation at $p < 0.05$

4.3 The correlations among cardiac biomarkers and the correlations between cardiac biomarkers and echocardiographic parameter values of entire population

There was no correlation between concentrations of each cardiac biomarker. There was no correlation between concentrations of cTnI and Gal-3 and echocardiographic parameter values. The moderate positive correlations were found between NT-BNP concentrations with E'/A' ratio. Weak negative correlations were found between NT-proBNP concentrations with left atrium diameter, Dec time, IVRT, and Peak A'.

Table 10: Correlations between each cardiac biomarker concentrations and Correlations between cardiac biomarker concentrations and echocardiographic parameter values of the entire population

Parameters	**r**	***p-value***
Cardiac biomarkers		
cTnI and Gal-3	0.026	0.875
cTnI and NT-proBNP	0.092	0.577
Gal-3 and NT-proBNP	-0.173	0.291
Cardiac biomarker concentrations and echocardiographic parameter values		
cTnI		

Peak E (m/sec)	0.172	0.295
Peak A (m/sec)	0.418	0.058
E/A	-0.168	0.307
Dec time (msec)	-0.134	0.415
IVRT (msec)	0.055	0.741
Peak pul S (m/s)	0.019	0.910
Peak pul D (m/s)	-0.079	0.632
Peak pul A (m/s)	0.034	0.838
S/D	0.104	0.530
PVar dur (msec)	-0.124	0.415
AV max (m/s)	0.106	0.521
AVPG (mmHg)	-0.104	0.529
Peak E'(m/s)	0.221	0.176
Peak A'(m/s)	0.149	0.364
E'/A'	-0.085	0.606
Peak S'(m/s)	-0.299	0.065
IVSd (cm/kg)	0.042	0.801
LVIDd (cm/kg)	-0.206	0.208
LVPWd (cm/kg)	0.007	0.967
IVSs (cm/kg)	0.041	0.804
LVIDs (cm/kg)	-0.177	0.282
LVPWs (cm/kg)	0.014	0.933
LA (cm/kg)	-0.199	0.223
LA/Ao	0.047	0.778
%FS	0.106	0.519
%EF	0.142	0.389
Gal-3		
Peak E (m/sec)	0.267	0.100
Peak A (m/sec)	-0.055	0.739

E/A	0.266	0.102
Dec time (msec)	0.127	0.422
IVRT (msec)	0.101	0.542
Peak pul S (m/s)	0.167	0.310
Peak pul D (m/s)	0.195	0.234
Peak pul A (m/s)	-0.231	0.158
S/D	-0.037	0.825
PVar dur (msec)	0.232	0.155
AV max (m/s)	-0.253	0.120
AVPG (mmHg)	0.291	0.072
Peak E'(m/s)	0.065	0.693
Peak A'(m/s)	-0.026	0.875
E'/A'	-0.115	0.487
Peak S'(m/s)	0.180	0.273
IVSd (cm/kg)	0.136	0.411
LVIDd (cm/kg)	0.061	0.711
LVPWd (cm/kg)	-0.086	0.605
IVSs (cm/kg)	0.069	0.677
LVIDs (cm/kg)	0.114	0.490
LVPWs (cm/kg)	-0.093	0.517
LA (cm/kg)	-0.208	0.204
LA/Ao	-0.515	0.090
%FS	0.015	0.930
%EF	0.007	0.966
NT-proBNP		
Peak E (m/sec)	0.106	0.519
Peak A (m/sec)	0.013	0.937
E/A	0.052	0.754
Dec time (msec)	-0.326	0.043*

IVRT (msec)	-0.413	0.009*
Peak pul S (m/s)	0.018	0.911
Peak pul D (m/s)	0.106	0.519
Peak pul A (m/s)	0.071	0.666
S/D	-0.155	0.345
PVar dur (msec)	-0.120	0.466
AV max (m/s)	-0.072	0.663
AVPG (mmHg)	0.090	0.588
Peak E'(m/s)	-0.254	0.119
Peak A'(m/s)	0.356	0.026*
E'/A'	0.620	0.001*
Peak S'(m/s)	-0.105	0.527
IVSd (cm/kg)	-0.053	0.749
LVIDd (cm/kg)	-0.053	0.749
LVPWd (cm/kg)	-0.05	0.764
IVSs (cm/kg)	0.070	0.674
LVIDs (cm/kg)	-0.183	0.264
LVPWs (cm/kg)	0.111	0.503
LA (cm/kg)	-0.377	0.018*
LA/Ao	-0.081	0.625
%FS	0.181	0.269
%EF	0.160	0.272

The significant correlation was assessed by Spearman's rank correlation.

* Indicate statistical correlation at $p < 0.05$

CHAPTER V
DISCUSSION

Part I: General information

1.1 Signalment

Thirty-nine dogs were enrolled in this study including 19 dogs with diabetes and 20 healthy control dogs. Only small to middle-sized breeds were included into the study to reduce the effect of breed variation. The breeds of diabetic dogs included Poodle, Mixed breed, Pug, Shih Tzu, Beagle, Pomeranian, and Chihuahua. The most predominant breeds were Poodle and Mixed breeds. This finding was consistent with the previous survey in the United Kingdom that Poodles and Mixed breeds had a higher risk to develop diabetes (Foster, 1975). However, this finding was different from studies in the USA and Canada finding that Australian Terrier, Standard Schnauzer, and Miniature Schnauzer were more prone to develop diabetes (Guptill et al., 2003). It may because Australian Terrier, Standard Schnauzer, and Miniature Schnauzer are less popular breeds in Thailand compared with Poodle and Mixed breeds. Although only small to middle-sized breed dogs were included in the study, average of body weight was significantly higher in diabetic dogs than that of control dogs. However, no obese dog was included in the study.

In the diabetic group, the percent of female dogs (68.42%) were higher than that of male dogs (31.58%). The proportion of female dogs in this study was consistent with a previous study of 6,860 dogs in which the percent of female and male diabetic dogs were 62.35% and 37.65%, respectively. This finding suggested that female dogs had a higher risk around 2 times to develop diabetes than male dogs. Female dogs may be more prone to develop diabetes due to an influence of sex hormone such as progesterone (Selman et al., 1994).

1.2 History and physical examination findings

The majority of diabetic dogs in this study had polyuria and polydipsia. Polyuria and polydipsia are the most common clinical signs in diabetic dogs presenting about 77%, and 93% respectively (Greco, 2001). Blood glucose concentration in diabetic dogs usually exceeds the renal threshold resulting in glucose excretion into urine; therefore, diabetic dogs often show a sign of polyuria with a compensatory polydipsia (Ettinger and Fledman, 2010).

The abnormality findings from physical examination in diabetic dogs included dehydration, abdominal enlargement, hair loss, and cataract. These findings were similar to a previous study showing that dehydration was the most common finding in diabetic dogs (Greco, 2001). Abdominal enlargement in diabetic dogs may be a result of hepatomegaly secondary to diabetes-induced hepatic lipidosis (Ettinger and Fledman, 2010). However, none of the diabetic dogs in this study had severe hepatomegaly or other hepatic diseases evaluated by radiography and ultrasound.

1.3 Complete blood count and blood chemistry profile values

Complete blood count values

In this study, complete blood count and blood chemistry profile values were evaluated to rule out other systemic diseases. The means of all complete blood count profile values in diabetic and control groups were within the normal limit. However, the means of white blood cell number and neutrophils were significantly higher in the diabetic group compared to the control group. An increase in white blood cells and the neutrophil numbers might be secondary to a stressful condition from a chronic hyperglycemic condition (Nelson, 2006).

Blood chemistry profile values

The mean of plasma ALP was significantly higher in the diabetic group than that of the control group. The means of ALP in diabetic and control groups were higher than normal limit for 2.5 folds and 1.2 folds, respectively. This result was similar to the study by Hess et al. (2000b) showing that 90% of diabetic dogs had an

increased plasma ALP concentration. Plasma ALP concentration in diabetic dogs may increase secondary to hepatic lipidosis or glycogen accumulation (James and Day, 1999; Alvarez and Whittemore, 2009).

Measurements of plasma fructosamine concentration were performed to evaluate the status of glycemic control and confirm the diagnosis of diabetes. Fructosamine does not effect by anxiety and concomitant disorders as blood glucose does (Reusch et al., 1993). Fructosamine measurements can provide much more information on glycemic control than fasting blood glucose that indicates only the glycemic status at a given point of time (Reusch et al., 1993). Half of diabetic dogs in this study (52.63%) had plasma fructosamine concentration higher than 450 µmol/L indicating a fair to poor glycemic control (Ettinger and Fledman, 2010). There was no significant correlation between fructosamine concentrations, fasting blood glucose concentrations, and duration of diabetes in this study. The inconsistency of fasting blood glucose and fructosamine concentrations in diabetic dogs might result from an abrupt change of blood glucose from anxiety or poor diet control (Reusch et al., 1993).

Part II: Echocardiography

2.1 Diastolic function

Several studies have reported the association between diastolic dysfunction and diabetic mellitus in human patients (Boyer et al., 2004; Fang et al., 2005; Saraiva et al., 2005). The prevalence of diastolic dysfunction in human diabetic patients is range from 27%-75% (Brooks et al., 2008). However, not all study confirmed the existence of diastolic dysfunction in human diabetic patients. Some studies failed to show the association between diabetes and diastolic dysfunction (Mathew et al., 1992; Romanens et al., 1999; Cosson et al., 2007; Konduracka et al., 2007).

Percent of diastolic dysfunction was significantly higher in diabetic group (57.88%) than those in control group (15%). Several abnormalities in diastolic parameter values including prolonged E-wave deceleration time, prolonged pulmonary vein atrial reversal flow duration (PVar dur), increased peak velocity of

late diastolic myocardial motion (Peak A') and decreased peak velocity of early diastolic myocardial motion to peak velocity of late diastolic myocardial motion ratio (E'/A') were shown in the diabetic group.

According to conventional Doppler echocardiography, the mean of E-wave deceleration time was significantly longer in the diabetic group than that in the control group. A prolong of E-wave deceleration time in diabetic dogs reflected an increase of a time to equilibrate pressure between left ventricle and left atrium during the early diastolic period secondary to an impaired myocardial relaxation (Boon, 2011). Consistent findings were observed in pulmonary vein flow profile values. In the diabetic group, PVar dur and peak velocity of pulmonary vein reverse flow during atrial contraction (Peak pul A) were increased indicating an increase of backward flow to pulmonary vein during atrial contraction period secondary to an impaired left ventricular relaxation (Nishimura and Tajik, 1997).

In the present study, a quarter of diabetic dogs had an impaired ventricular relaxation (diastolic dysfunction grade I). A few number of normal dogs also showed an impaired ventricular relaxation. It is known that impaired myocardial relaxation can be observed in aged dogs (Schober and Fuentes, 2001). Our study was performed in aged matched dogs; therefore, the higher prevalence of impaired diastolic dysfunction in diabetic dogs might result from changes of myocardial function rather than an effect by age.

Conventional Doppler echocardiography is known to be affected by loading condition and other factors including age and heart rate (Poirier et al., 2001). According to this consideration, tissue Doppler imaging (TDI), a relative loading independent technique (Thuesen et al., 1985) was also performed in this study.

There was a significant increase of peak A' velocity a non -significant decrease of Peak E' velocity and a significant decrease of E'/A' ratio in the diabetic group compared to the control group indicating an impaired left ventricular diastolic function in diabetic dogs. Interestingly, 26.31% of diabetic dogs with normal diastolic function assessed by conventional Doppler echocardiography had E'/A' < 1 assessed by TDI. With TDI assessment, the percent of diastolic dysfunction increased from 31.57% to 57.88% in the diabetic group and 10% to 15% in the control group

suggesting a more sensitivity of TDI in assessment of diastolic dysfunction. This finding coincided with the previous study in human diabetic patients that suggested the usefulness of TDI for detecting early diastolic dysfunction even in the early stage of diabetes (Boyer et al., 2004; Di Bonito, 2005).

In this study, there were negative correlations between Peak pul S and Peak pul D indicating that duration of diabetes might play a part in the progression of diastolic dysfunction. The association between diastolic dysfunction and glycemic control is still debated in human diabetic patients. In the present study, Peak E and Peak A' velocities showed negative correlations with fructosamine concentration. These findings were consistent with a previous study in human diabetic patients that suggested the effect of glycemic condition on diastolic dysfunction (Grandi et al., 2006).

Hyperglycemia can induce mechanisms implicating the progression of diabetic cardiomyopathy in humans including increased generation of reactive oxygen species (Yao and Brownlee, 2010) and activation of renin angiotensin aldosterone system (Putnam et al., 2012). An effective glycemic control may play an important role in prevention and slow progression of cardiovascular diseases in diabetic patients (Huynh et al., 2014).

2.2 Systolic dysfunction

Whether diabetes can induce systolic dysfunction is still a controversial topic. Several studies in humans and rats with diabetes have reported that systolic dysfunction usually occurs after significant diastolic dysfunction has already developed (Mihm et al., 2001; Boudina, 2009; Patil and Burji, 2012). Whereas several studies failed to report the existence of systolic dysfunction in human diabetic patients (Poirier et al., 2001; Schannwell et al., 2002; El Dayem and Battah, 2012).

Percent fractional shortening (%FS), percent ejection fraction (%EF), left ventricular internal dimension during systolic period (LVIDs), and peak velocity of myocardial systolic motion (Peak S') were recorded to evaluate systolic function in the present study. Systolic function parameter values in all dogs were within the normal limit. The diabetic group showed a significant increase of LVIDs and Peak S'

velocity compared to the control group. No significant difference of %FS and %EF was observed between the two groups. These findings were similar to a previous study that not found systolic dysfunction in human diabetic patients (Poirier et al., 2001; Schannwell et al., 2002; El Dayem and Battah, 2012).

The systolic function was preserved in the population of diabetic dogs in the present study. Systolic dysfunction may develop later after the significant diastolic dysfunction has presented same as in human patients (Huynh et al., 2014) or diabetes may not induce systolic dysfunction in the heart of diabetic dogs. The serial echocardiographic examination should be performed to monitor the changes in systolic function in diabetic dogs.

2.3 Two-dimensional echocardiography

No evidence of cardiac structural changes was seen in diabetic dogs. However, there were positive correlations between wall thickness with duration of diabetes. Additionally, there were negative correlations between chamber dimension and duration of diabetes. These correlations indicated the development of concentric remodeling of left ventricular chamber (Lang et al., 2006) which might relate to the development of diastolic dysfunction.

Part III: Cardiac biomarkers

There was no significant difference of plasma cTnI and Gal-3 concentrations between the two groups; whereas, plasma NT-proBNP concentration was significantly higher in the control group than the diabetic group.

These findings were inconsistent with a previous study that showed a significant increase of cTnI in alloxan diabetic induced dogs compared with normal dogs (Azimzadeh, 2014). An inconsistent result may be secondary to the different nature of experimentally induced diabetes and naturally occurring diabetes. According to release kinetics of troponins, cTnI is increased and gradually returns to baseline level within 7-10 days from the onset of myocardial damage (Oyama and Sisson, 2004). In case of the late diagnosis of acute or short-term myocardial damage,

an elevation of cTnI levels may not be detected. Therefore, serial measurements of cTnI should be performed in diabetic dogs.

A previous study in degenerative mitral valve disease (DMVD) dogs showed a correlation between the area of cardiac fibrosis and the expression of Gal-3 in the myocardium. Moreover, plasma Gal-3 concentration was significantly higher in DMVD dogs than control dogs suggesting the important role of Gal-3 as a marker of cardiac fibrosis in DMVD dogs (Sakarin et al., in press). Fasting blood glucose levels correlated well with plasma Gal-3 concentrations in human diabetic patients (Yilmaz et al., 2015). As our knowledge, the study about the association between circulating Gal-3 concentration and the expression of cardiac fibrosis has not been performed in diabetic dogs. In the present study, plasma Gal-3 concentration was higher in diabetic dogs than control dogs but not significantly difference. Further larger clinical studies accompany with histopathological studies of the myocardium should be performed to determine a role of Gal-3 as a fibrosis marker in diabetic dogs.

NT-proBNP concentration was higher in the control group than that in the diabetic group; however, the values from both groups were within the normal limit that was considered clinically insignificant. This finding coincided with the study in human diabetic patients that NT-proBNP concentrations remained within the normal range in both diabetic and control groups (Konduracka et al., 2007).

This study showed that NT-proBNP levels correlated with diastolic echocardiographic parameter values. A larger study may be performed to determine a role of NT-proBNP as a marker of diastolic function in dogs.

The limitation of this study is a small sample size. Only 39 dogs were included in the study. The sample size at least 18 dogs in each group made the power of the study to reach 80% assessed by G*Power test; thus, it was sufficient to describe the difference between diabetic and control groups with this number of sample size. Secondly, coronary blood flow was not measured in this study; thus, the possibility of coronary artery diseases could not be excluded. Thirdly, specific blood tests for other endocrine diseases were not performed; therefore, the possible concurrence of other endocrine diseases including Cushing's disease and hypothyroidism cannot be excluded. Lastly, the diabetic myocardium was not

studied histopathologically; therefore structural myocardial changes have not been evaluated.

In conclusion, a population of naturally occurring diabetic dogs in this study had a preserved systolic function with a higher prevalence of left ventricular diastolic dysfunction than the age and size matched control dogs. An early diastolic dysfunction in diabetic dogs should be identified by TDI even the presence of normal diastolic function is detected by conventional Doppler echocardiography. There was no clinical evidence implicating the usefulness of cTnI, Gal-3, and NT-proBNP to detect the difference between normal and diabetic dogs. Further histopathological studies should be performed to evaluate structural changes of the myocardium in naturally occurring diabetic dogs.

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APPENDIX

Appendix A: Signalment of the diabetic group

No.	Name	Breed	Sex	Age (year)	Weight (kg)
1	ปั๊กกี้	Pug	Fs	7	7.9
2	เจ้าหญิง	Shitzu	F	12	5.0
3	คุณนาย	Mixed	F	12	14.7
4	ซูชิ	Shitzu	M	9	6.5
5	ลูกหมี	Poodle	M	10	7.1
6	ปุ๊กกี้	Poodle	Fs	10	8.0
7	รถถัง	Poodle	Fs	13	5.7
8	คาเมล	Pug	Fs	14	12.0
9	สโนว์	Mixed	Mc	7	6.4
10	ดุ๊กดิ๊ก	Poodle	Fs	14	6.6
11	ป๊อปคอร์น	Beagle	Fs	12	8.8
12	ขนมครก	Mixed	Fs	8	10.5
13	โคล่า	Poodle	Fs	7	6.0
14	อิ๋ง อิ๋ง	Poodle	Fs	7	7.7
15	มอมแมม	Pom	M	9	4.8
16	บอสซี่	Chihuahua	M	8	4.7
17	แมทธิว	Poodle	Mc	12	8.5
18	ถุงทอง	Poodle	F	7	4.9
19	เก็บตก	Mixed	Fs	10	11.0

Appendix B: Signalment of the the control group

No.	Name	Breed	Sex	Age (year)	Weight (kg)
1	ชิโร่	Schnauzer	M	7	7.0
2	ปุ๊กกี้	Shitzu	M	7	8.4
3	ดุ๊กดิ๊ก	Shitzu	Fs	12	5.7
4	วิงกี้	Yorkshire terrier	Fs	9	2.4
5	โบนัส	Chihuahua	Fs	8	2.7
6	บราวนี่	Chihuahua	Fs	8	4.0
7	ตัวเล็ก	Chihuahua	Fs	8	6.0
8	ฮาชิ	Chihuahua	Fs	7	3.1
9	ออสการ์	Chihuahua	F	8	2.7
10	โมจิ	Pom	M	9	3.0
11	น้ำหวาน	Pom	Fs	10	3.5
12	ขนม	Pom	Fs	7	2.0
13	ชารอน	Chihuahua	F	7	2.7
14	เต็งหนึ่ง	Shitzu	Mc	11	7.1
15	มาเฟีย	Shitzu	M	10	6.0
16	ชีโตส	Shitzu	Mc	11	5.5
17	อาร์ตี้	Shitzu	Mc	7	5.5
18	ซูชิ	Shitzu	Fs	11	8.3
19	ข้าวปุ้น	Mixed	M	10	5.0
20	โอเลี้ยง	Shitzu	M	15	10.0

Appendix C: Clinical characteristic of the diabetic group

No.	Name	FBG mg/dl	Fructosamine µmol/L	Duration (day)	Type of insulin	Dose of insulin (unit/kg)
1	ปั๊กกี้	244	350	425	Caninsulin®	0.56
2	เจ้าหญิง	406	352	1077	Caninsulin®	1.00
3	คุณนาย	238	475	709	Humulin N®	0.88
4	ซูชิ	239	706	420	Caninsulin®	0.50
5	ลูกหมี	221	463	828	Caninsulin®	0.62
6	ปุ๊กกี้	203	358	1722	Humulin N®	0.88
7	รถถัง	219	413	635	Caninsulin®	0.63
8	คาเมล	467	330	1375	Humulin N®	0.67
9	สโนว์	259	350	494	Caninsulin®	0.50
10	ดุ๊กดิ๊ก	393	576	1797	Caninsulin®	0.72
11	ป๊อปคอร์น	438	403	1313	Caninsulin®	0.55
12	ขนมครก	268	408	888	Humulin N®	1.00
13	โคล่า	232	373	7	Caninsulin®	0.67
14	อิ๋ง อิ๋ง	430	474	12	Caninsulin®	0.47
15	มอมแมม	559	528	44	Caninsulin®	0.33
16	บอสซี่	250	637	11	Caninsulin®	0.43
17	แมทธิว	375	811	1029	Caninsulin®	0.47
18	ถุงทอง	600	732	12	Caninsulin®	0.40
19	เก็บตก	281	474	9	Caninsulin®	0.36

Appendix D: Cardiac biomarker concentrations of the diabetic group

No.	Name	cTnI (ng/ml)	Gal-3 (ng/ml)	NT-proBNP (pg/ml)
1	ปั๊กกี้	0.019	5.94	73.89
2	เจ้าหญิง	0.100	1.22	39.84
3	คุณนาย	0.021	1.11	100.20
4	ซูชิ	0.004	0.32	46.42
5	ลูกหมี	0.016	0.68	48.36
6	ปุ๊กกี้	0.008	1.10	64.22
7	รถถัง	0.021	1.36	36.75
8	คาเมล	0.014	0.46	65.76
9	สโนว์	0.015	0.43	151.65
10	ดุ๊กดิ๊ก	0.019	0.89	24.76
11	ป๊อปคอร์น	0.016	0.24	95.94
12	ขนมครก	0.054	0.08	21.27
13	โคล่า	0.005	1.24	67.70
14	อิ๋ง อิ๋ง	0.047	1.14	100.58
15	มอมแมม	0.021	0.47	149.39
16	บอสซี่	0.020	0.09	126.89
17	แมทธิว	0.007	0.37	174.09
18	ถุงทอง	0.032	0.61	163.26
19	เก็บตก	0.016	0.89	131.92

Appendix E: Cardiac biomarker concentrations of the control group

No.	Name	cTnI (ng/ml)	Gal-3 (ng/ml)	NT-proBNP (pg/ml)
1	ชิโร่	0.013	0.53	45.98
2	ปุ๊กกี้	0.006	3.57	160.93
3	ดุ๊กดิ๊ก	0.023	0.54	252.23
4	วิงกี้	0.030	0.43	107.16
5	โบนัส	0.073	0.49	250.00
6	บราวนี่	0.021	0.35	130.75
7	ตัวเล็ก	0.066	0.40	157.06
8	ฮาชิ	0.012	0.33	277.08
9	ออสการ์	0.013	0.37	78.91
10	โมจิ	0.054	0.15	159.38
11	น้ำหวาน	0.014	0.37	131.52
12	ขนม	0.018	0.53	37.90
13	ชารอน	0.018	0.74	152.08
14	เต็งหนึ่ง	0.022	1.35	101.05
15	มาเฟีย	0.025	0.81	66.53
16	ซีโตส	0.021	0.53	124.91
17	อาร์ตี้	0.008	0.59	92.24
18	ซูชิ	0.021	0.58	94.071
19	ข้าวปุ้น	0.027	0.39	66.54
20	โอเลี้ยง	0.017	0.28	83.42

Appendix F: Echocardiographic parameters of the diabetic group

No.	Name	Peak E	Peak A	E/A	Dec time	IVRT	Peak pul S
1	ปั๊กกี้	0.88	0.55	1.60	128	67	0.62
2	เจ้าหญิง	0.79	0.66	1.21	117	79	0.49
3	คุณนาย	0.53	0.59	0.90	138	75	0.58
4	ซูชิ	0.43	0.51	0.85	108	75	0.49
5	ลูกหมี	0.45	0.35	1.29	167	83	0.35
6	ปุ๊กกี้	1.07	0.63	1.72	61	78	0.29
7	รถถัง	0.41	0.6	0.69	117	72	0.42
8	คาเมล	0.68	0.86	0.79	172	78	0.51
9	สโนว์	0.78	0.67	1.16	83	44	0.49
10	ดุ๊กดิ๊ก	0.54	0.89	0.61	117	72	0.38
11	ป๊อปคอร์น	0.55	0.53	1.04	158	83	0.43
12	ขนมครก	0.70	0.60	1.14	117	72	0.41
13	โคล่า	0.98	0.50	1.97	211	47	0.76
14	อิ๋ง อิ๋ง	0.81	0.61	1.21	128	67	0.73
15	มอมแมม	0.75	0.63	1.19	111	61	0.66
16	บอสซี่	0.73	0.67	1.09	44	56	0.81
17	แมทธิว	0.73	0.50	1.44	133	61	0.56
18	ถุงทอง	0.65	0.45	1.44	117	61	0.69
19	เก็บตก	0.79	0.52	1.52	161	56	0.38

Appendix F: Echocardiographic parameters of the diabetic group

No.	Name	Peak pul D	Peak pul A	S/D	PV ar dur	AV max	AV PG
1	ปั๊กกี้	0.56	0.23	1.11	78	1.60	10.22
2	เจ้าหญิง	0.41	0.17	1.20	65	1.01	4.10
3	คุณนาย	0.46	0.29	1.25	92	0.77	2.36
4	ซูชิ	0.34	0.28	1.45	75	1.14	5.15
5	ลูกหมี	0.38	0.18	0.92	71	0.95	3.60
6	ปุ๊กกี้	0.38	0.14	0.77	72	1.14	5.15
7	รถถัง	0.52	0.20	0.81	67	1.30	6.76
8	คาเมล	0.48	0.17	1.08	56	1.43	8.14
9	สโนว์	0.49	0.18	1.01	78	1.42	8.09
10	ดุ๊กดิ๊ก	0.48	0.21	0.86	72	1.06	4.52
11	ป๊อปคอร์น	0.39	0.15	1.08	72	1.38	7.61
12	ขนมครก	0.29	0.22	1.40	72	1.36	7.44
13	โคล่า	0.67	0.33	1.12	83	1.40	7.80
14	อิ๋ง อิ๋ง	0.58	0.35	1.25	72	1.40	7.80
15	มอมแมม	0.63	0.26	1.05	56	1.46	8.50
16	บอสซี่	0.46	0.14	1.75	67	1.36	7.37
17	แมทธิว	0.60	0.25	0.92	56	1.33	7.03
18	ถุงทอง	0.58	0.24	1.20	72	1.63	10.63
19	เก็บตก	0.47	0.15	0.82	78	1.57	9.91

Appendix F: Echocardiographic parameters of the diabetic group

No.	Name	Peak E'	Peak A'	E'/A'	Peak S'	IVSd	LVIDd	LVPWd
1	ปั๊กกี้	0.08	0.09	0.89	0.13	0.69	1.48	0.49
2	เจ้าหญิง	0.06	0.08	0.75	0.06	0.79	1.46	0.55
3	คุณนาย	0.07	0.10	0.70	0.08	0.69	1.35	0.47
4	ซูชิ	0.11	0.15	0.73	0.10	0.65	1.61	0.57
5	ลูกหมี	0.10	0.10	1.00	0.10	0.61	1.55	0.59
6	ปุ๊กกี้	0.10	0.08	1.25	0.11	0.53	1.54	0.47
7	รถถัง	0.09	0.11	0.82	0.10	0.67	1.80	0.44
8	คาเมล	0.11	0.12	0.92	0.12	0.49	1.07	0.51
9	สโนว์	0.07	0.09	0.78	0.13	0.55	1.58	0.41
10	ดุ๊กดิ๊ก	0.07	0.09	0.78	0.08	0.75	1.31	0.63
11	ป๊อปคอร์น	0.11	0.11	1.00	0.14	0.51	1.60	0.49
12	ขนมครก	0.14	0.15	0.93	0.14	0.51	1.47	0.39
13	โคล่า	0.14	0.12	1.17	0.17	0.60	1.81	0.43
14	อิ๋ง อิ๋ง	0.14	0.14	1.00	0.14	0.44	1.43	0.38
15	มอมแมม	0.14	0.13	1.08	0.11	0.52	1.58	0.40
16	บอสซี่	0.11	0.08	1.38	0.09	0.59	1.80	0.46
17	แมทธิว	0.15	0.13	1.15	0.17	0.56	1.66	0.54
18	ถุงทอง	0.13	0.15	0.87	0.14	0.40	1.53	0.38
19	เก็บตก	0.08	0.12	0.67	0.17	0.48	1.75	0.40

Appendix F: Echocardiographic parameters of the diabetic group

No.	Name	IVSs	LVIDs	LVPWs	LA	LA/AO	%FS	%EF
1	ปั๊กกี้	0.72	0.95	0.67	0.86	0.84	36.13	68.52
2	เจ้าหญิง	0.92	0.88	0.79	1.20	1.22	39.33	73.01
3	คุณนาย	0.66	1.10	0.65	1.33	1.2	18.75	40.80
4	ซูชิ	0.72	1.08	0.74	1.47	1.51	32.35	63.32
5	ลูกหมี	0.79	1.00	0.87	1.30	1.11	35.59	67.77
6	ปุ๊กกี้	0.73	0.97	0.68	1.20	1.37	37.32	69.88
7	รถถัง	0.80	1.03	0.69	1.66	1.48	43.01	76.39
8	คาเมล	0.57	0.66	0.76	1.15	1.35	38.26	71.84
9	สโนว์	0.62	1.14	0.58	1.14	1.38	28.00	56.92
10	ดุ๊กดิ๊ก	0.89	0.77	0.80	1.23	1.07	41.67	75.77
11	ป๊อปคอร์น	0.62	1.05	0.72	1.27	1.29	34.62	66.13
12	ขนมครก	0.54	0.89	0.54	1.16	1.38	39.28	72.19
13	โคล่า	0.79	1.20	0.57	1.43	1.28	33.76	64.94
14	อิ๋ง อิ๋ง	0.79	0.80	0.61	1.17	1.42	44.30	78.21
15	มอมแมม	0.66	1.06	0.64	1.11	1.33	32.92	64.47
16	บอสซี่	0.66	1.25	0.66	1.35	1.37	30.77	60.96
17	แมทธิว	0.76	1.05	0.72	1.19	1.32	37.00	69.17
18	ถุงทอง	0.59	1.04	0.55	1.09	1.18	32.05	63.35
19	เก็บตก	0.55	1.16	0.63	1.12	1.13	33.73	64.36

Appendix G: Echocardiographic parameters of the control group

No.	Name	Peak E	Peak A	E/A	Dec time	IVRT	Peak pul S
1	ชิโร่	0.64	0.38	1.71	79	63	0.53
2	ปุ๊กกี้	0.84	0.52	1.62	83	67	0.61
3	ดุ๊กดิ๊ก	0.58	0.46	1.26	78	72	0.28
4	วิงกี้	0.55	0.34	1.60	83	61	0.24
5	โบนัส	0.88	0.88	1.00	28	50	0.58
6	บราวนี่	0.53	0.69	0.76	94	67	0.56
7	ตัวเล็ก	0.67	0.62	1.09	122	67	0.52
8	ฮาชิ	0.64	0.47	1.34	94	44	0.37
9	ออสการ์	0.74	0.59	1.27	72	50	0.49
10	โมจิ	0.84	0.82	1.03	100	61	0.45
11	น้ำหวาน	0.80	0.78	1.03	89	56	0.46
12	ขนม	0.73	0.61	1.20	106	61	0.37
13	ชารอน	0.58	0.59	1.00	83	61	0.44
14	เต็งหนึ่ง	0.73	0.72	1.02	100	72	0.50
15	มาเฟีย	0.58	0.55	1.07	67	72	0.51
16	ชีโตส	0.59	0.34	1.72	78	89	0.55
17	อาร์ตี้	0.46	0.39	1.16	122	83	0.63
18	ซูชิ	0.58	0.39	1.50	94	56	0.52
19	ข้าวปุ้น	0.98	0.58	1.71	111	56	0.67
20	โอเลี้ยง	0.68	0.62	1.10	78	61	0.60

Appendix G: Echocardiographic parameters of the control group

No.	Name	Peak pul D	Peak pul A	S/D	PV ar dur	AV max	AV PG
1	ชิโร่	0.41	0.15	1.29	58	1.14	5.15
2	ปุ๊กกี้	0.47	0.24	1.30	61	1.54	9.44
3	ดุ๊กดิ๊ก	0.26	0.17	1.06	67	0.78	2.45
4	วิงกี้	0.24	0.15	1.00	61	1.27	6.45
5	โบนัส	0.53	0.20	1.09	61	1.29	6.69
6	บราวนี่	0.50	0.22	1.11	67	1.17	5.48
7	ตัวเล็ก	0.43	0.21	1.22	56	1.19	5.68
8	ฮาชิ	0.44	0.18	0.84	67	1.33	7.07
9	ออสการ์	0.32	0.20	1.56	61	1.19	5.64
10	โมจิ	0.48	0.15	0.94	61	1.29	6.61
11	น้ำหวาน	0.49	0.15	0.95	56	1.32	6.99
12	ขนม	0.37	0.17	1.00	39	1.06	4.46
13	ชารอน	0.43	0.15	1.02	44	1.17	5.49
14	เต็งหนึ่ง	0.38	0.21	1.30	72	1.28	6.58
15	มาเฟีย	0.56	0.18	0.92	61	1.39	7.78
16	ซีโตส	0.56	0.18	0.97	67	1.27	6.45
17	อาร์ตี้	0.53	0.20	1.20	72	1.29	6.61
18	ซูชิ	0.71	0.25	0.74	67	1.27	6.45
19	ข้าวปุ้น	0.43	0.20	1.58	67	1.65	10.83
20	โอเลี้ยง	0.44	0.19	1.36	61	1.32	6.93

Appendix G: Echocardiographic parameters of the control group

No.	Name	Peak E'	Peak A'	E'/A'	Peak S'	IVSd	LVIDd	LVPWd
1	ชิโร่	0.11	0.08	1.38	0.09	0.51	1.70	0.48
2	ปุ๊กกี้	0.15	0.11	1.36	0.13	0.52	1.60	0.34
3	ดุ๊กดิ๊ก	0.17	0.05	3.40	0.07	0.72	1.34	0.64
4	วิงกี้	0.07	0.05	1.40	0.06	0.88	1.30	0.78
5	โบนัส	0.08	0.06	1.33	0.07	0.54	1.60	0.48
6	บราวนี่	0.06	0.07	0.86	0.06	0.66	1.31	0.62
7	ตัวเล็ก	0.09	0.07	1.29	0.10	0.48	1.22	0.48
8	ฮาชิ	0.16	0.11	1.45	0.09	0.59	1.51	0.45
9	ออสการ์	0.08	0.07	1.14	0.08	0.55	1.37	0.55
10	โมจิ	0.10	0.09	1.11	0.08	0.60	1.29	0.42
11	น้ำหวาน	0.10	0.10	1.00	0.09	0.55	1.44	0.38
12	ขนม	0.07	0.06	1.17	0.09	0.62	1.35	0.47
13	ชารอน	0.12	0.09	1.33	0.11	1.01	1.51	0.55
14	เต็งหนึ่ง	0.15	0.14	1.07	0.15	0.81	1.66	0.59
15	มาเฟีย	0.16	0.12	1.33	0.11	0.66	1.29	0.55
16	ซีโตส	0.12	0.10	1.20	0.11	0.68	1.19	0.53
17	อาร์ตี้	0.11	0.11	1.00	0.10	0.45	1.37	0.40
18	ซูชิ	0.12	0.11	1.09	0.10	0.51	1.36	0.40
19	ข้าวปุ้น	0.18	0.11	1.64	0.16	0.41	1.90	0.35
20	โอเลี้ยง	0.13	0.14	1.03	0.14	0.70	1.88	0.40

Appendix G: Echocardiographic parameters of the control group

No.	Name	IVSs	LVIDs	LVPWs	LA	LA/AO	%FS	%EF
1	ชิโร่	0.65	1.22	0.62	1.12	1.20	28.35	57.10
2	ปุ๊กกี้	0.76	0.96	0.61	1.20	1.19	39.76	72.70
3	ดุ๊กดิ๊ก	1.01	0.78	0.87	1.22	1.37	41.22	75.36
4	วิงกี้	1.14	0.69	0.97	1.41	1.46	35.94	70.12
5	โบนัส	0.74	0.92	0.72	1.06	1.15	42.85	77.29
6	บราวนี่	0.82	0.71	1.02	1.25	1.44	45.83	80.57
7	ตัวเล็ก	0.59	0.85	0.66	1.07	1.21	30.30	61.37
8	ฮาชิ	0.74	0.47	0.77	1.02	1.04	45.46	80.02
9	ออสการ์	0.84	0.67	0.80	1.08	1.15	50.75	85.20
10	โมจิ	0.70	0.81	0.65	1.07	1.59	36.92	70.89
11	น้ำหวาน	0.78	0.91	0.65	1.17	1.38	36.84	70.32
12	ขนม	0.62	0.94	0.69	1.32	1.34	30.00	61.62
13	ชารอน	1.05	0.63	0.86	1.25	1.22	58.10	90.39
14	เต็งหนึ่ง	0.90	1.18	0.75	1.38	1.58	28.95	58.08
15	มาเฟีย	0.87	0.74	0.79	1.21	1.10	42.50	76.83
16	ชีโตส	0.75	0.73	0.78	1.09	1.00	38.89	73.01
17	อาร์ตี้	0.59	1.02	0.57	1.11	1.24	25.86	54.14
18	ซูชิ	0.71	0.89	0.51	1.11	1.32	35.37	67.74
19	ข้าวปุ้น	0.71	0.47	0.56	1.35	1.48	33.97	65.26
20	โอเลี้ยง	0.90	1.00	0.63	1.13	1.15	36.48	68.52

VITA

Miss Pleansaung Vichit was born on August 13, 1988 in Kanchanaburi, Thailand. She finished her high school education from Srinagarindra The Princess Mother School, Kanchanaburi, and graduated from Faculty of Veterinary Science, Chulalongkorn University, 2007-2013. She received her Bachelor degree of Veterinary Science with 1st class Honors in 2013. After that, she decided to continue her education in Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University in 2013 under Chulalongkorn University Graduate Commemorate the 72nd Anniversary of His Majesty King Bhumibol Adulyadej Scholarship.