ความแปรปรวนในอัตราการเต้นของหัวใจและระดับนอร์อิปิเนฟรินในพลาสมาของสุนัขที่เป็นโรคเบาหวานในขณะพัก

นางสาวประภาวดี ไพรินทร์

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HEART RATE VARIABILITY AND PLASMA NOREPINEPHRINE CONCENTRATION IN DIABETIC DOGS AT REST

Miss Prapawadee Pirintr

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ประภาวดี ไพรินทร์: ความแปรปรวนในอัตราการเต้นของหัวใจและระดับนอร์อิปิเนฟรินในพลาสมาของสุนัขที่เป็นโรคเบาหวานในขณะพัก (HEART RATE VARIABILITY AND PLASMA NOREPINEPHRINE CONCENTRATION IN DIABETIC DOGS AT REST) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.สพ.ญ.ดร. ชลลดา บูรณกาล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.สพ.ญ.ดร. สฤณี กลันทกานนท์ ทองทรง 62 หน้า

ความแปรปรวนในอัตราการเต้นของหัวใจเป็นการวัดการทำงานของระบบประสาทอัตโนวัติทางอ้อมซึ่งมีความน่าเชื่อถือสูง สามารถใช้ตรวจความผิดปกติจากภาวะเส้นประสาทอัตโนวัติที่ความคุมการทำงานของหัวใจเสื่อมในระยะที่ยังไม่แสดงอาการทางคลินิก การศึกษานี้มีวัตถุประสงค์เพื่อประเมินความผิดปกติจากภาวะเส้นประสาทอัตโนวัติที่ควบคุมการทำงานของหัวใจเสื่อมจากโรคเบาหวานโดยการวิเคราะห์การเปลี่ยนแปลงค่าความแปรปรวนในอัตราการเต้นของหัวใจแบบการวิเคราะห์ ช่วงเวลาและการวิเคราะห์ช่วงความถี่ และหาความสัมพันธ์ระหว่างค่าความแปรปรวนในอัตราการเต้นของหัวใจกับความดันโลหิตและระดับนอร์อิปิเนฟรินในพลาสมาของสุนัขที่เป็นโรคเบาหวานเทียบกับสุนัขปกติ แบ่งสุนัขเป็น 2 กลุ่มตามความถี่ในการปรับขนาดอินซูลินระดับกลูโคสในพลาสมาหลังการอดอาหารอย่างน้อย 8 ชั่วโมงและค่าระดับฟรุคโตซามีนในพลาสมา โดยแบ่งเป็น 1) สุนัขสุขภาพดี จำนวน 13 ตัว 2) สุนัขที่เป็นโรคเบาหวาน โดยแบ่งเป็น 2 กลุ่มย่อยคือ กลุ่มสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ดีจำนวน 11 ตัว และ สุนัขเบาหวานที่ควบคุมระดับได้ไม่ดีจำนวน 11 ตัว ซึ่งสุนัขทุกตัวไม่มีประวัติเป็นโรคหัวใจและไม่ได้รับยากลุ่ม α หรือ β blockers ที่จะส่งผลต่อค่าความแปรปรวนในอัตราการเต้นของหัวใจ ทำการวัด ความดันโลหิตวัดทางอ้อมโดยวิธีออสซิโลเมตริก เก็บตัวอย่างเลือดไปวิเคราะห์ค่าทางโลหิตวิทยา ค่าก๊าซในเลือด ค่าเคมีในเลือด ระดับความเข้มข้นของน้ำตาล ระดับฟรุคโตซามีน และระดับนอร์อิปิเนฟรินในพลาสมา ข้อมูลในการวิเคราะห์ความแปรปรวนในอัตราการเต้นของหัวใจได้จากการบันทึกคลื่นไฟฟ้าหัวใจต่อเนื่อง 30 นาทีและนำมาวิเคราะห์ช่วงเวลาและช่วงความถี่ด้วยโปรแกรมสำเร็จรูป ผลการศึกษาพบว่า สุนั ขเบาหวานทั้ง 2 กลุ่มมีระดับความเข้มข้นของน้ำตาลในเลือดหลังการอดอาหาร และระดับฟรุคโตซามีน สูงกว่ากลุ่มสุนัขสุขภาพดี อย่างมีนัยสำคัญทางสถิติ ($p< 0.001$) ค่าความแปรปรวนในอัตราการเต้นของหัวใจแบบการวิเคราะห์ช่วงเวลา ในสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ไม่ดีมีค่า NNA และ SDANN ต่ำกว่าสุนัขสุขภาพดีอย่างมีนัยสำคัญทางสถิติ (601.9$\pm$28.5 กับ 697.4$\pm$31.9 ms และ 22.40$\pm$5.53 กับ 50.96$\pm$10.74 ms ตามลำดับ) ($p< 0.05$)และมีค่าอัตราการเต้นของหัวใจสูงกว่า สุนัขสุขภาพดีและกลุ่มสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ดี อย่างมีนัยสำคัญทางสถิติ (102.7$\pm$6.6 กับ 88.1$\pm$3.9 และ 85.7$\pm$3.7 bpm ตามลำดับ) ($p< 0.05$) พบว่าสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ไม่ดีมีค่า NNA, SDNN, SDNN index และ pNN50% ต่ำกว่าสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ดีอย่างมีนัยสำคัญทางสถิติ (601.9$\pm$28.5 กับ 711.5$\pm$27.1 ms, 118.9 $\pm$12.3 กับ 178.1$\pm$19.0 ms, 115.4$\pm$12.2 กับ 174.5$\pm$18.7 ms และ 46.73$\pm$6.96 กับ 68.23$\pm$5.25 ms ตามลำดับ) ($p < 0.05$) ค่าความแปรปรวนในอัตราการเต้นของหัวใจแบบการวิเคราะห์ช่วงความถี่ในสุนัขกลุ่มเบาหวานที่ควบคุมระดับน้ำตาลได้ไม่ดีมีค่า HF และ Total power ต่ำและมีค่าอัตราส่วนระหว่าง LF ต่อ HF สูงกว่าสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ดี อย่างมีนัยสำคัญทางสถิติ (7,670$\pm$1,829 กับ 20,503$\pm$6,030 ms^2, 14,694 $\pm$2,591 กับ 32,097$\pm$7,148 ms^2 และ 0.54$\pm$0.15 กับ 0.21$\pm$0.04 ตามลำดับ) ($p < 0.05$) ในสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ไม่ดีมีค่าความดันซิสโตลิก ไดแอสโตลิก และค่าความดันเลือดแดงเฉลี่ยสูงกว่าสุนัขสุขภาพดีอย่างมีนัยสำคัญทางสถิติ ($p< 0.05$) และยังพบว่าระดับนอร์อิปิเนฟรินในพลาสมาในสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ไม่ดีมีค่าต่ำกว่ากลุ่มสุขภาพดีอย่างมีนัยสำคัญทางสถิติ ($p< 0.05$) (210.4+36.7 กับ 478.9+74.3 pg/ml) ไม่พบความแตกต่างระหว่างความดันโลหิต ค่าความแปรปรวนในอัตราการเต้นของหัวใจ และระดับนอร์อิปิเนฟรินในพลาสมาในสุนัขเบาหวานที่ควบคุมระดับน้ำตาลได้ดีกับกลุ่มสุนัขสุขภาพดี เมื่อนำข้อมูลมาวิเคราะห์ทางสถิติเพื่อศึกษาความสัมพันธ์พบว่าระดับนอร์อิปิเนฟรินในพลาสมามีค่าสหสัมพันธ์เชิงลบกับระดับฟรุคโตซามีนในพลาสมา ($p< 0.05$, r = -0.359) จากการศึกษานี้สรุปได้ว่าสุนัขโรคเบาหวานกลุ่มที่ควบคุมระดับน้ำตาลได้ไม่ดีมีแนวโน้มในการเกิดภาวะเส้นประสาทอัตโนวัติที่ควบคุมการทำงานของหัวใจเสื่อม

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต.....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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PRAPAWADEE PIRINTR: HEART RATE VARIABILITY AND PLASMA NOREPINEPHRINE CONCENTRATION IN DIABETIC DOGS AT REST. ADVISOR: PROF. CHOLLADA BURANAKARL, D.V.M., Ph.D., CO-ADVISOR: ASST. PROF. SARINEE KALANDAKANOND-THONGSONG, D.V.M., Ph.D. 62 pp.

Cardiac autonomic neuropathy in dogs with diabetes mellitus (DM) were evaluated using time-and frequency-domain parameters of heart rate variability (HRV) as well as the plasma norepinephrine (NE) concentration compared with control healthy non-diabetic dogs. Dogs were divided into 2 groups according to frequency of insulin adjustment, their fasting plasma glucose and plasma fructosamine concentrations: group 1, the control healthy non-DM group (n = 13), group 2, diabetic group (n = 22). The diabetic group was further divided into 2 subgroups, the well-controlled DM (n = 11) and the poorly-controlled DM (n = 11). None of these dogs had heart failure or received any drug that affected HRV or autonomic nervous system. The blood pressures were recorded along with the measurements of complete blood count, blood gas analysis, biochemical profiles and plasma NE concentrations. For HRV, the electrocardiogram (ECG) was recorded continuously for at least 30 minutes at rest. The results showed that the concentrations of fasting blood glucose and serum fructosamine were significantly higher in both DM subgroups compared with control group ($p < 0.001$). The time-domain parameters, NNA and SDANN in poorly-controlled DM were significantly lower than control group (601.9$\pm$28.5 vs 697.4$\pm$31.9 ms and 22.40$\pm$5.53 vs 50.96$\pm$10.74 ms, respectively) ($p < 0.05$) while heart rate was significantly higher than control and well-controlled DM (102.7$\pm$6.6 vs 88.1$\pm$3.9 and 85.7$\pm$3.7 bpm, respectively) ($p < 0.05$). In poorly-controlled DM, the NNA, SDNN, SDNN index and pNN50% were also significantly lower when compared with well-controlled DM (601.9$\pm$28.5 vs 711.5$\pm$27.1 ms, 118.9 $\pm$12.3 vs 178.1$\pm$19.0 ms, 115.4$\pm$12.2 vs 174.5$\pm$18.7 ms and 46.73$\pm$6.96 vs 68.23$\pm$5.25 ms, respectively) ($p < 0.05$). The frequency-domain parameters, HF and total power in poorly-controlled DM were significantly lower (7,670$\pm$1,829 vs 20,503$\pm$6,030 ms^2 and 14,694$\pm$2591 vs 32,097$\pm$7,148 ms^2,respectively) ($p < 0.05$) while the LF/HF was higher (0.54$\pm$0.15 vs 0.21$\pm$0.04) ($p < 0.05$) when compared with well-controlled DM. In poorly-controlled DM, systolic, diastolic and mean arterial blood pressures were significantly higher while plasma NE concentrations were significantly lower than control group (210.4$\pm$36.7 vs 478.9$\pm$74.3 pg/ml) ($p <0.05$). However, in well-controlled DM group, no significant change was found among all parameters when compared with control group. The plasma NE concentrations had negative correlation with plasma fructosamine concentrations ($p < 0.05$, r = -0.359). It is concluded that the cardiac autonomic neuropathy occurred only in poorly-controlled DM dogs. The sympathetic activity in this group was suppressed as shown by decrease in both plasma NE concentration and LF component resulting in higher arterial blood pressure and heart rate.

Department : Veterinary Physiology Student's Signature……..
Field of Study : Animal Physiology Advisor's Signature……
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%	percent
α	alpha
β	beta
β-OHB	beta-hydroxybutyrate
µl	microliter
µmol	micromole
ALP	alkaline phosphatase
ANS	autonomic nervous system
BCS	body condition score
BP	blood pressure
bpm	beat per minute
BUN	blood urea nitrogen
CAN	cardiac autonomic neuropathy
CBC	complete blood count
CHF	congestive heart failure
CIPA	cardiac index of the parasympathetic activity
DAN	diabetic autonomic neuropathy
DBP	diastolic blood pressure
DCAN	diabetic cardiac autonomic neuropathy
DHBA	3,4-dihydroxy-benzyl-amine hydrobromide
DKA	diabetic ketoacidosis
dl	deciliter
DM	diabetes mellitus
E	epinephrine
E:I ratio	the ratio of the duration of expiration to inspiration
ECG	electrocardiogram
EDTA	ethylene diamine tetra-acetic acid
EGTA	ethylene glycol bis (β-aminoethyl ether)-N-N,N',N'-tetracetic acid

F	female
FFT	fast Fourier transformation
Fs	spayed female
HCO_3^-	bicarbonate
HDL	high density lipoprotein
HF	high frequency component
HPLC-EC	high pressure lipid chromatography-electrochemical detection
HRV	heart rate variability
Hz	hertz
IDDM	insulin dependent diabetes mellitus
K^+	potassium ion
Kg	kilogram
L	liter
LDL	low density lipoprotein
LF	low frequency component
LF/HF ratio	the ratio of LF to HF components
M	male
MABP	mean arterial blood pressure
Mc	castrated male
mg	milligram
MI	myocardial infarction
ml	milliliter
mM	millimolar
mmHg	millimeter of mercury
ms	millisecond
ms^2	millisecond square
n	number
nA	nano ampere

Na^+	sodium ion
NaCl	sodium chloride
NE	norepinephrine
NN interval	normal to normal RR interval
NNA	average of normal-to-normal intervals where normal-to-normal intervals patterns appear in all the division
^OC	degree celsius
pCO_2	the partial pressure of carbon dioxide
PD	polydipsia
Pg	picogram
pH	the potential of hydrogen
pNN_{50}	percentage of differences between adjacent normal RR intervals that are >50 ms computed in the entire recording
PSD	power spectral density
PU	polyuria
p-value	the probability of obtaining a test static at least as extreme as the one that was actually observed
QT interval	the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTc	corrected QT interval
r	the correlation coefficient
r^2	the squared correlation coefficient
RMSSD	square root of the mean of the squared differences between adjacent RR intervals in the entire recording
RR intervals	the interval from the peak of one QRS complex to the peak of the next as shown on an electrocardiogram
SA node	sinoatrial node
SBP	systolic blood pressure
SD	standard deviation

SDANN	standard deviation of all 5 minutes segments of normal RR intervals in the entire recording
SDANN index	mean of the standard deviations of all normal RR intervals for all 5 minutes segment in the entire recording
SEM	the standard error of the mean
SGPT	serum glutamic pyruvic transaminase
TP	total power
ULF	ultra low frequency component
V	voltage
VLDL	very low density lipoprotein
VLF	very low frequency component
VVTI	the vasovagal tonus index
x g	times gravity

CHAPTER I

INTRODUCTION

Diabetic retinopathy, nephropathy and neuropathy are late complications of diabetes mellitus (DM) which are significant causes of morbidity and mortality in diabetic patients (Ziegler, 1994). A risk factor of these complications in human is the duration of diabetes (Nathan, 1993; Muñana, 1995). In small animals, especially dogs and cats, late complications of DM are uncommon (Muñana, 1995). The shorter life span of dogs after the diagnosis of diabetes (2-5 years) is a factor which put animals at a lower risk (Feldman and Nelson, 1987). However, a report of complications in dogs and cats with both spontaneous and experimentally induced DM are presented (Muñana, 1995).

Diabetes mellitus is considered causes of autonomic neuropathy and cardiac autonomic neuropathy (CAN) (SchÖnauer et al., 2008). CAN produce abnormalities in heart rate control (Maser and Lenhard, 2005). Diabetic patients at rest may have prolong QTc (Flugelman et al., 1980; Mathur and Gupta, 2006), tachycardia, subnormal plasma norepinephrine levels (Eckberg et al., 1986) and blood pressure changes (Wieling et al., 1983), and reduced heart rate variability (HRV) (Freeman et al., 1991; Ziegler et al., 2001; Kudat et al., 2006; SchÖnauer et al., 2008).

HRV has been used as a standard screening method in the diagnosis of autonomic dysfunction (Seung-Hyun et al., 2008). Decreased HRV in diabetic patients depend on severity and complications of DM. Diabetic patients with complications had the lowest HRV parameters compared with diabetic patients without complications (Kudat et al., 2006). An earliest sign of diabetic cardiac autonomic neuropathy (DCAN) is a reduction in HRV, which is detectable in the subclinical stage (Mésangeau et al., 2000; Ziegler et al., 2001; SchÖnauer et al., 2008). Up to date, there are many researches on human DCAN, but there are only two studies of the autonomic function in diabetic dogs. Atkins et al. (1989) found the absence of electrocardiographic indicators of autonomic neuropathy in their study which suggested that the dog was less

susceptible to the development of diabetic autonomic neuropathy than diabetic humans. On the other hand, the study by Kenefick et al. (2007) reported different results in which the diabetic dogs had the mean, median and modal cardiac indices of parasympathetic activity (CIPA) values significantly lower than healthy dogs. Therefore, the diabetic dog may develop autonomic neuropathy similar to the diabetic humans.

In long term, diabetic humans with severe autonomic neuropathy, the basal plasma NE concentration at rest is lower than in the non-neuropathic diabetics or healthy control subjects (Caviezel et al., 1982; Eckberg et al., 1986; Hilsted, 1995). In the dog, the relationship between HRV, blood pressure and plasma NE concentration in DM with or without autonomic neuropathy has not yet been reported.

The research questions of the present study were therefore: firstly, does HRV in diabetic dogs decrease similar to diabetic humans? Secondly, are there any differences in HRV values between a normal and well-controlled or poor-controlled DM dogs? Lastly, is there a relationship between HRV, blood pressure and plasma NE in diabetic or normal dogs?

The research objectives of the present study were then to evaluate cardiac autonomic neuropathy in diabetic dogs using HRV and to investigate the relationship between HRV, blood pressure and the plasma NE levels in diabetic dogs compared with control healthy dogs.

The present study hypothesized that HRV, an indicator of cardiac autonomic neuropathy, blood pressure and plasma NE concentrations may be altered in diabetic dogs and may depend on severity of diabetes mellitus.

Keywords (English):

autonomic neuropathy, diabetes mellitus, dog, heart rate variability, norepinephrine

Advantages of study

1. HRV could be used as a clinically effective noninvasive method to investigate on autonomic status in diabetic dogs.

2. Early detection of diabetic cardiac autonomic neuropathy in diabetic dogs by using HRV would be clinically meaningful for the prevention of adverse cardiovascular outcome in diabetic dogs.

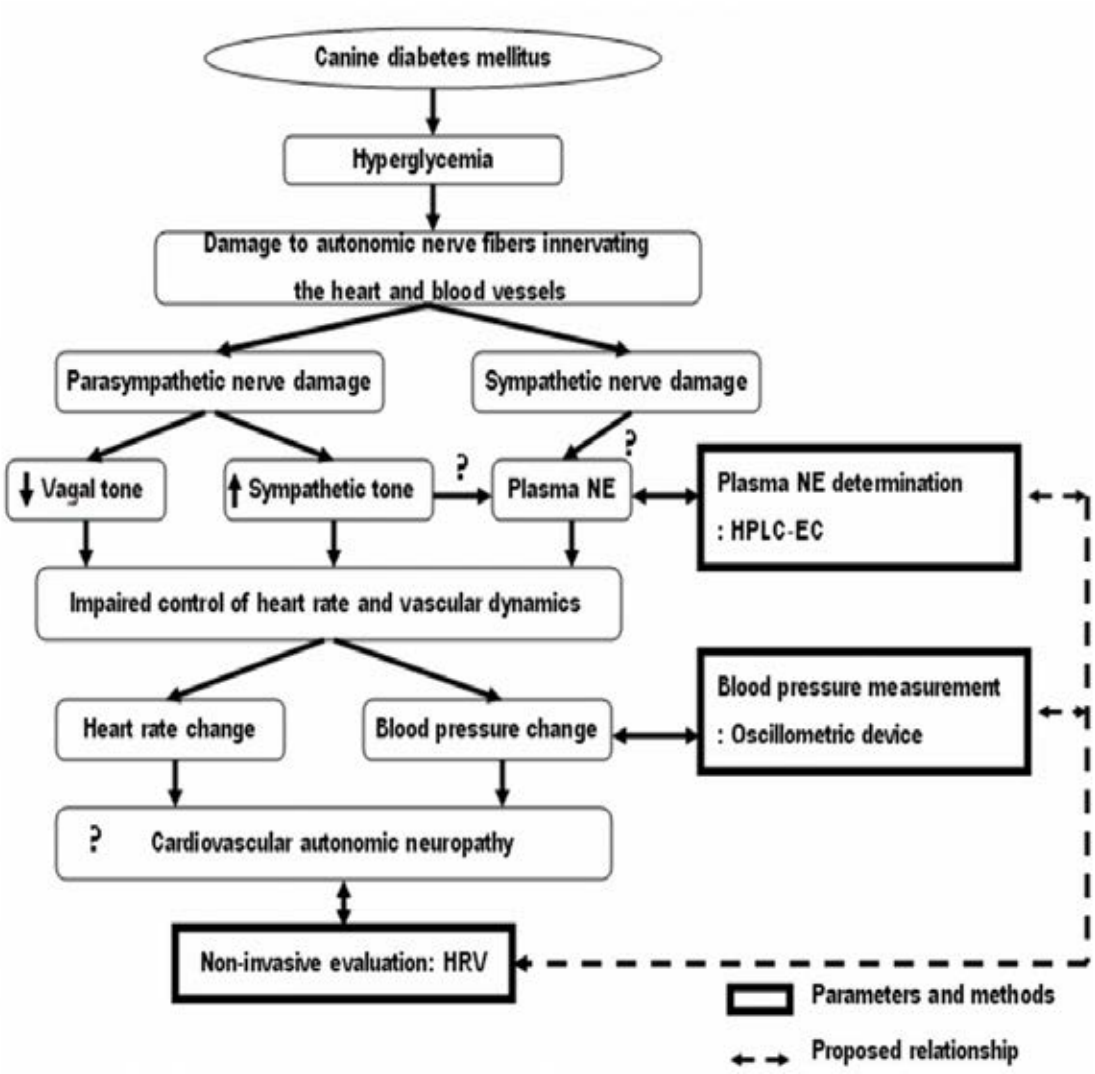


Figure 1-1 Conceptual framework

CHAPTER II

LITERATURE REVIEWS

A. Canine diabetes mellitus

Diabetes mellitus (DM) is a disorder of the endocrine pancreas (Islet cells) that commonly occurs in dogs as well as in cats (Feldman and Nelson, 1987; Hess, 2008). DM is often caused by pancreatic disease such as β-cell damage following pancreatitis or immune-mediated destruction of pancreatic β-cells. Secondary DM can be caused by other endocrine diseases such as glucagonomas (excess glucagons), hyperadrenocorticism (excess cortisol), pheochromocytomas (excess catecholamines) and acromegaly (excess growth hormone) (Hess, 2008). In addition, DM is categorized as metabolic disease that characterized by fasted hyperglycemia (>120 mg/dl in dogs and > 130 mg/dl in cats) and glucosuria resulting from either impairment of insulin secretion (type 1 DM) or defects in insulin action (type 2 DM; insulin resistance) (ADA, 2003). The clinical signs of DM are including, polyuria and polydipsia (PU/PD), weight loss, sometimes with polyphagia, blurred vision from cataract in dogs and plantigrade stance in cats (ADA, 2003; Hess, 2008).

Almost all DM in dogs are type 1 or insulin dependent diabetes mellitus (IDDM) that generally required exogenous insulin adjustment throughout their uptake blood glucose, lead to hyperglycemia, catabolism of glycogen, protein and storage fat, ketogenesis and dyslipidemia (Hess, 2008).

B. Diabetes cardiac autonomic neuropathy

Canine diabetes mellitus can cause many complications such as diabetic ketoacidosis (DKA), cataracts, lowered resistance to infection, retinopathy, nephropathy and neuropathy (Hess, 2008), the same as in humans (Ziegler, 1994). Long-term complications of diabetes mellitus are significant cause of morbidity and mortality in human diabetic patients, Diabetic autonomic neuropathy (DAN), and its incidence in

human diabetic patient has been reported to be 20-40% (Ewing and Clark, 1982). DAN is a condition that develops at some point after an onset and then progresses slowly over the course of the diabetic disease. DAN can affect all organs, including the gastrointestinal tract, the urogenital tract and the cardiovascular system (ADA, 1988).

Diabetic cardiac autonomic neuropathy (DCAN) is the primary disease of DAN and is one of the most frequent complication (Maser et al., 2003; Vinik and Mitchell, 2003; SchÖnauer et al., 2008). DCAN is characterized by widespread neurological degeneration of the small nerve fiber of the parasympathetic and sympathetic branches (Freeman et al., 1991). Damage to autonomic nerve fibers innervating the heart and blood vessels caused by diabetes can result in the impaired control of heart rate and vascular dynamics (Schumer et al., 1998). An imbalance of the sympathetic/ parasympathetic control of cardiac functions can lead to the development of fatal cardiac arrhythmias (Sztajzel, 2004). DCAN can cause prolongation of the corrected QT interval (Flugelman et al., 1980; Mathur and Gupta, 2006), abnormalities in heart rate control causing excessive rapid heart rates, reduced heart rate variability, subnormal plasma catecholamine (norepinephrine) levels (Eckberg et al., 1986; Porojan et al., 2010) and blood pressure change after standing (Wieling et al., 1983).

Late complications of diabetes mellitus are uncommon in diabetic dogs and cats because it is only detected in human patients with diabetes of long duration (5 years or more) (Rollins et al., 1992). Dogs after the diagnosis of diabetes have shorter life span (2-5 years) (Feldman and Nelson, 1987). However, late complications have been infrequently reported in diabetic dogs and cats (Muñana, 1995). Clinical signs in dogs typically involve progressive symmetric paraparesis with postural deficits, depressed spinal reflexes and muscle atrophy.

There are only two studies of the autonomic function of diabetic dogs. Atkins et al. (1989) found no evidence of any difference in cardiovascular autonomic tone between diabetic and non-diabetic dogs. On the other hand, Kenefick et al. (2007) reported that the diabetic dogs had the cardiac index of parasympathetic activity (CIPA) values significantly lower than control healthy dogs. It is suggested that the diabetic dog may develop autonomic neuropathy like in humans.

C. Heart rate variability

The beat to beat (RR intervals) of the healthy heart is not absolutely regular. Under resting conditions, the ECG of healthy individuals exhibits the variation in RR intervals (Figure 2-1). This fluctuation in heart beats is known as heart rate variability (HRV) (Stein et al., 1999; Subbalakshmi et al., 2009). HRV is the result of various influences of the autonomic nervous system on the heart rate (Stein et al., 1999).

HRV is a noninvasive and highly reproducible Electrocardiogram (ECG) reflecting the activity of the sympathetic and vagal components of the autonomic nervous system (ANS) on the Sinoatrial node (SA node) of the heart. Variability of the sinus rate is largely a result of variations in autonomic input to the sinus node, and alterations in variability can be used as reliable markers of autonomic input to the heart (Van Ravenswaaij-Arts et al., 1993; Stein et al., 1994). The vagal tone has a protective role by reducing the incidence of ventricular arrhythmia, whereas sympathetic tone can increase arrhythmia (Calvert, 1998). The parasympathetic function is reduced early in the development of CAN and HRV induced by deep breathing is almost exclusively mediated by parasympathetic fibers. HRV expresses the total amount of variations of both instantaneous heart rate and RR intervals of normal sinus depolarizations (Van Ravenswaaij-Arts et al., 1993; Stein et al., 1994). Recently, HRV analysis has been investigated in a variety of clinical situations including diabetic neuropathy, myocardial infarction (MI), congestive heart failure (CHF) and sudden death (Sztajzel, 2004). Abnormalities of autonomic inputs to the heart resulting in the decreased indices of HRV (Stein et al., 1994). Apart from reduced HRV, the clinical manifestations of CAN include fixed heart rate, increased resting heart rate, sinus tachycardia, orthostatic hypotension, reduced circadian rhythm of heart rate and blood pressure, abnormal hormonal regulation to standing and exercise, antibodies to autonomic tissue (vagal nerve, sympathetic ganglia), denervation hypersensitivity to α- and β-adrenergic agonists, exercise intolerance, reduced left ventricular diastolic filling/ejection fraction, intraoperative cardiovascular instability, QTc interval prolongation and increased susceptibility to silent MI (Vinik and Ziegler, 2007). Decreased HRV can be used as a predictor of risk after MI and as an early warning sign of diabetic neuropathy

(Freeman et al., 1991; Task Force, 1996; Ziegler et al., 2001; Kudat et al., 2006; SchÖnauer et al., 2008). Different physiological factors may influence HRV such as gender, age, circadian rhythm, respiration and body position (Bonnemeier et al., 2003).

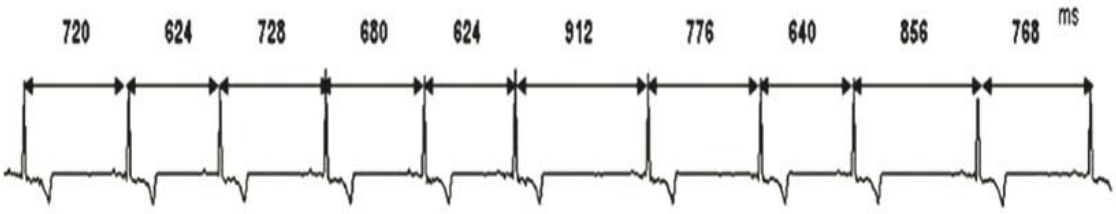


Figure 2-1 The variation of RR intervals in the normal canine heart.

1. HRV measurements and analysis

Measurements of HRV may generally be performed on the basis of 24 hour Holter recordings or on shorter periods ranging from 0.5 to 5 minutes ECG recordings (Van Ravenswaaij-Arts et al., 1993). Most holter apparatus manufacturers nowadays propose HRV analysis programs which are incorporated into their instrument systems (Jung et al., 1996). The standard clinical measurements in the analysis of HRV are time and frequency domain analysis. The first step in HRV analysis is measuring consecutive R waves (RR interval) on the surface ECG. Premature beats of both atrial and ventricular origins are deleted and the RR interval signal is corrected using statistical calculation. A tachogram (an oscillatory curve) is plotted from that varies in amplitude and frequency that is based on the ECG RR interval (Figure 2-2). Time domain analysis includes calculations of RR interval averaged (mean, variances, and root mean square values), whereas frequency domain analysis includes calculations of RR interval frequency transformations such as power spectral analysis (Stein et al., 1994; Calvert, 1998).

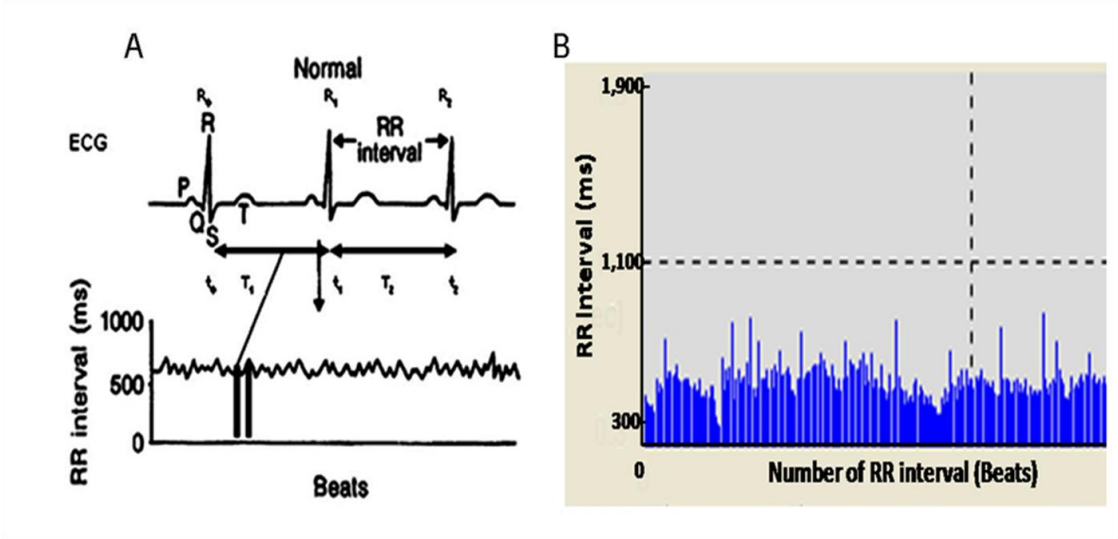


Figure 2-2 A tachogram of canine HRV: **A.** A computer-derived R-wave process is calculated after a surface ECG is recorded, which represented variability in SA node outputs. Derived R-waves are numbered t_0, t_1, t_2. The time intervals (cardiac cycle lengths) between consecutive R waves are numbered T_1,T_2. **B.** These time intervals are used to construct a wave form that varied in amplitude and frequency and are based on the ECG RR interval.

1.1 Time domain analysis

Time domain measurements are commonly used. Many parameters can be extracted from the original RR interval signals to show the changes in the ANS (Calvert, 1998). Time domain analysis measures the changes in heart rate over time or the intervals between successive normal cardiac cycles (Kleiger et al., 1992; Malik, 1995; Task Force, 1996; Sztajzel, 2004). From a continuous ECG recording (Holter), each QRS complex is detected and the normal RR intervals (NN intervals), due to sinus depolarizations, or the instantaneous heart rate are then determined.

Table 2-1 summarizes the most frequently used parameters of the time domain. Parameters of the first category are night-day, SDNN, SDNN index, SDANN, and SD; and those of the second category are RMSSD and pNN50. SDNN is a global index of HRV and reflects all the long-term components and circadian rhythms

responsible for variability in the recording period. SDANN is an index of the variability of the average of 5-minute intervals over 24 hours. Thus, it provides long-term information. It is a sensitive index of low frequencies like physical activity, changes in position, circadian rhythm. SD is generally considered to reflect the day/night changes of HRV. RMSSD and pNN50 are the most common parameters based on interval differences. These measurements correspond to short-term HRV changes and are not dependent on day/night variations (Van Ravenswaaij-Arts et al., 1993; Malik 1995; Task Force ,1996; Tsuji et al., 1996; Calvert, 1998; Sztajzel, 2004). RMSSD and pNN50 reflect alterations in autonomic tone that are predominantly vagally mediated.

Table 2-1 Description for time domain parameters

Variables	Units	Description
NNA	ms	Mean of the normal RR interval in the entire recording
SDNN	ms	SD of all normal RR intervals in the entire recording
SDNN index	ms	Mean of the SDs of all normal RR intervals for all 5-min segment in the entire recording
SDANN	ms	SD of the mean of all 5-min segments of normal RR intervals in the entire recording
RMSSD	ms	Square root of the mean of the squared differences between adjacent RR intervals in the entire recording
pNN_{50}	%	Percentage of differences between adjacent normal RR intervals that are >50 ms computed in the entire recording

Abbreviations: SD, Standard deviation; ms, millisecond

Source: (Stein et al., 1994; Calvert, 1998; Sztajzel, 2004).

1.2 Frequency domain analysis

Frequency domain (power spectral density; PSD) analysis describes the periodic oscillations of the heart rate signal decomposed at different frequencies and amplitudes and provides information on the amount of their relative intensity (termed variance or power) in the heart's sinus rhythm (Task Force, 1996; Calvert, 1998; Sztajzel, 2004). Analysis in the frequency domain is mathematically complex and requires a Holter system with an accurate timing track (Stein et al., 1994; Calvert, 1998; Stein et al., 1999). PSD analysis can be performed in two ways: 1) by a nonparametric method, the fast Fourier transformation (FFT), which is characterized by discrete peaks for the several frequency components, and 2) by a parametric method, the autoregressive model estimation resulting in a continuous smooth spectrum of activity (Malliani et al., 1991; Öri et al., 1992; Malliani et al., 1994). While the FFT is a simple and rapid method; the two components of ANS, sympathetic and parasympathetic influence different bands in the spectrum of RR intervals. Therefore, frequency domain analysis can be used to monitor the state of the ANS (Calvert, 1998).

The power spectrum consists of frequency bands ranging from 0 to 0.5 Hz and can be classified into four bands: the ultra low frequency band (ULF), the very low frequency band (VLF), the low frequency band (LF) and the high frequency band (HF). The total power (TP) is the total variance which corresponds to the sum of the four spectral bands (Sztajzel, 2004). Short-term spectral recordings (5 to 10 minutes) are characterized by the VLF, HF and LF components, while long-term recordings include a ULF component in addition to the three others. High-frequency component (HF; 0.15 to 0.4 Hz): representing quicker changes in the heart rate, is primarily due to the parasympathetic nervous system (vagal activity) during respiration. Low-frequency component (LF; 0.04 to 0.15 Hz): representing slow changes in the heart rate, is related to baroreflexes and represents sympathetic and parasympathetic activity. An increase in LF component during activity (tilt, physical or mental stress, received sympathomimetic agents etc.) seems to reflect primary sympathetic activity. The very Low Frequency band (VLF; 0.0033 to 0.04 Hz): representing slower changes in the heart rate, is an index of thermal regulation of the body's internal systems. The LF/HF ratio is the index

of sympathetic to parasympathetic balance (Stein et al., 1994; Calvert, 1998; Sztajzel, 2004; Litscher et al., 2009).

2. HRV in dogs

HRV in small animals was used to determine the clinical usefulness of cardiac event and many medical research fields.

To date, many articles have been published about HRV in dogs. These articles observe about relationships between HRV and heart diseases such as the mitral valves regurgitation (Häggström,1996; Fujii and Wakao, 2003; Spiljak Pakkanen et. al., 2012), dilated cardiomyopathy (Minors and O'Grady, 1997; Calvert and Jacobs, 2000; Calvert and Wall, 2000; Pereira et al., 2008) and myocardial infarction (Hull Jr et al.,1990), medications that affect the autonomic nervous system such as chronically morphine used (Napier et al.,1998), topical application of lavender oil (Komiya et al., 2009), hemodynamic changes such as hemorrhage (Kawase et al., 2000; Kawase et al., 2002), exercise (Billman and Kukielka, 2006), pre-operative stress (Väisänen et al., 2005) and etc.

Doxey and Boswood (2004) reported that there was a significant difference in HRV (the vasovagal tonus index; VVTI) between brachycephalic breeds and non-brachycephalic breeds in which the brachycephalic breeds tended to have higher VVTI than non-brachycephalic breeds. The result was supported by study of Rasmussen et al. (2011) that HRV in dogs were not significantly associated with age, sex and body weight, but significantly associated only with breed.

3. Comparative study of HRV between human and animals

In 2009, Manzo et al. reported that dogs and calves showed similar LF (991.1 $\pm$ 646.1 ms^2/Hz and 547.0 $\pm$ 256.9 ms^2/Hz, respectively), HF (702.1 $\pm$ 394.1 ms^2/Hz and 601.0 $\pm$ 666.6 ms^2/Hz, respectively) and LF/HF (2.0 $\pm$ 1.3 and 2.5 $\pm$ 1.9, respectively) when compared with the human data, human LF (1,216 $\pm$ 1,221 ms^2/Hz) and HF (570.9 $\pm$ 581.3 ms^2/Hz). Then dogs and calves might be used to reflect human clinical situations.

D. Blood pressure changes in diabetes mellitus patients

Hypertension is a common problem in human diabetic patients (Epstein and Sowers, 1992). Hypertension in the diabetic individual markedly increases the risk and accelerates the course of cardiac disease, peripheral vascular disease, stroke, retinopathy and nephropathy (Parving et al., 1988). Diabetes mellitus and hypertension frequently occur concurrently and probably have synergistic detrimental effects on the cardiovascular system (The Hypertension in Diabetes Study Group, 1993[a,b]). The mortality in diabetic patients with hypertension is several folds higher than in normotensive diabetic patients (Dupree and Meyer, 1980). Many previous studies have demonstrated that both diabetes and hypertension impair cardiac autonomic function (Head, 1995; Ewing, 1996; Liao et al., 1996; Takahashi et al., 2001), suggesting that the association of these two diseases promotes cardiac autonomic dysfunction. In diabetic dogs, systemic hypertension and proteinuria are common (Struble et al., 1998). Data from their study have shown that hypertension was detected in 23 (from 50 dogs) on the basis of a systolic pressure > 160 mm Hg (12 dogs), a diastolic pressure > 100 mm Hg (21 dogs) or a mean pressure > 120 mm Hg (23 dogs). All dogs with systolic hypertension had concurrent diastolic and mean hypertension, and 19 of 21 dogs with diastolic hypertension had concurrent high mean pressure. Albumin concentration and albumin-to-creatinine ratio were significantly higher in urine from diabetic dogs, compared with healthy, non-diabetic dogs. However, the combined effects of diabetes and hypertension on cardiac autonomic control in diabetic dogs have not yet been reported.

E. Plasma norepinephrine concentrations in diabetes mellitus patients

Venous plasma norepinephrine (NE) concentrations are related to sympathetic neural activity in human and animals, plasma NE increases during sympathetic stimulation and decreases during sympathetic inhibition (Goldstein et al., 1983). Patients undergone sympathectomy for Raymond's phenomenon have low plasma NE in the venous effluent of the sympathectomized arm (Nielsen et al., 1980). Plasma NE levels are diminished in idiopathic orthostatic hypotension apparently due to peripheral

sympathetic failure (Ziegler et al., 1977; Polinsky et al., 1981). Norepinephrine basal levels and cold responses are diminished in patients with definite and severe autonomic neuropathy (Granados et al., 2000). In human with long duration of diabetes with clinical severe autonomic neuropathy, plasma NE concentration is low (Hilsted, 1995). Caviezel et al. (1982) reported that the basal plasma NE levels at rest were significantly lower in diabetic patients with autonomic neuropathy than in the non-neuropathic diabetics and healthy control subjects. After standing, plasma NE rose to significantly higher levels in both control and diabetic subjects without neuropathy than in the patients with autonomic neuropathy. During exercise, plasma NE rose to similar levels in healthy controls and patients with diabetic neuropathy. These indicated that in DAN neuropathy there was a reduction in peripheral neurosympathetic tone at rest but a normal response to moderate exercise.

In dog, the relationship between plasma NE concentration and diabetic with or without autonomic neuropathy has not yet been reported.

F. Baroreflex change in diabetes mellitus patients

The baroreflex or baroreceptor reflex is one of the body's homeostatic mechanisms for maintaining blood pressure. The baroreflex pathway consists of the autonomic nervous system (sympathetic and parasympathetic nerves). The sympathetic and parasympathetic nerves have opposing effect on blood pressure. Sympathetic activation leads to an elevation of total peripheral resistance and cardiac output via increased cardiac contractility, heart rate and arterial vasoconstriction, which tends to increase blood pressure. On the other hand, parasympathetic activation leads to decreased cardiac output via decrease in heart rate, resulting in a tendency to lower blood pressure. The baroreflex may be responsible for a part of the low-frequency component of heart rate variability, the so-called Mayer waves, at 0.1 Hz (Sleight, 1995).

In 1986, Eckberg et al. reported that the diabetic patients with no symtomatic cardiac autonomic neuropathy may have the resting excessive rapid heart rate, reduced heart rate variability, subnormal baseline plasma norepinephrine concentrations during change of arterial blood pressure and supranormal blood pressure response to

phynylephrine. All of these relate to baroreflex impairment. Furthermore, It has been shown that the baroreflex control of heart rate is impaired in diabetic patients and experimental diabetic animals (Dall's Ago et al., 2007).

From these data, it is therefore interesting to determine whether diabetic dogs has impared autonomic control of the heart, this reflecting changes in HRV parameters and NE levels.

CHAPTER III

MATERIALS AND METHODS

A. Animals and grouping

The healthy non-diabetic and diabetic dogs were recruited from The Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University with official allowances from the owners. The physical examination, biochemical profiles, continuous electrocardiograms (ECG) and blood pressure were determined. Diabetes mellitus was diagnosed on the basis of laboratory data composed of fasting blood glucose and plasma fructosamine concentrations. In this study, diabetic dogs were received veterinary porcine lente insulin (Caninsulin®, Intervet) or insulin human recombinant (Humulin N, Eli Lilly and Company) treatments. Diabetic dogs with diabetic ketoacidosis or DKA (plasma β-OHB concentration is higher than 3.8 mmol/l) (Duarte et al., 2002) were not included in this study. None of the control and DM dogs had heart failure or received any drugs that affected heart rate, such as α- and β-blockers.

The study consisted of 35 dogs, which were divided into 2 groups according to their fasting plasma glucose and plasma fructosamine concentrations which were modified from criterion described by Feldman and Nelson (2004).

Group 1 (control group) (n = 13): the healthy non-diabetic dogs had fasting plasma glucose concentrations less than 120 mg/dl and plasma fructosamine concentrations between 225-364 µmol/l. These individuals were age and gender matched with the diabetes groups.

Group 2 (Diabetic group) (n = 22): dogs had fasting plasma glucose concentrations higher than 120 mg/dl. The dogs in group 2 were further divided into 2 subgroups;

The well-controlled DM subgroup (n = 11) in which their plasma fructosamine concentrations between 365-500 µmol/l. this group had no clinical sign of DM such as polyuria, polydipsia, lethargy or depression and received veterinary porcine

lente insulin (Caninsulin®, Intervet) or insulin human recombinant (Humulin N, Eli Lilly and Company) treatments without changing the dose of insulin for periods of time (12.64 ± 6.09 months). The fasting blood glucose concentrations especially at the nadir could be controlled to the reference normal range (90-145 mg/dl) (Fleeman and Rand, 2001).

The poorly-controlled DM subgroup (n = 11) in which their plasma fructosamine concentrations higher than 500 µmol/l. The dogs in this group had clinical signs of DM such as polyuria, polydipsia, weight loss despite constant hunger, lethargy or depression. Insulin dose adjustment was required at the time of investigation.

B. Experimental protocol

The experimental protocol was approved by the Institutinal Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University. All dogs were allowed by the owners to be recruited in this study. The age, gender, body weight, body condition score (BCS) and diabetic duration were documented at the beginning of the study. The body condition score was defined as 1 to 5 scoring system (1 = emaciated; 2 = thin; 3 = ideal; 4 = overweight and 5 = obese) (Ellictt, 2010). Physical examinations such as gaiting behavior, cataracts, uveitis, etc were recorded. The systolic, diastolic and mean arterial blood pressure were measured by an oscillometric device (PetiTelemo, FUKUDA DENSHI CO., LTD., JAPAN) at rest and systemic arterial hypertension was defined as a systolic BP > 160 mmHg, a diastolic BP > 100 mmHg and a mean arterial BP > 120 mmHg (Struble et al., 1998). An indwelling intravenous catheter was inserted into the cephalic vein for blood collection. Blood samples were collected for a routine blood check including complete blood count (CBC), fasting glucose concentrations, fructosamine concentrations, blood gas analysis and plasma norepinephrine concentrations. Single-voided urine sample was collected for routine urinalysis and was determined for the presence of urinary glucose and ketone. The continuous ECG recording was performed 15 minutes after the dog was fed and insulin was administered. ECG was recorded by Holter (FUKUDA DENSHI CO., LTD.,

JAPAN) for 30 minutes in a quiet room. The same procedures were also done in the control healthy dogs except no insulin administration.

C. Experimental procedures

1. Procedure for blood pressure measurement

Indirect blood pressure measurement was performed according to the previous studies (Kallet et al., 1997; Haberman et al., 2006; McMurphy et al., 2006). The systolic, diastolic and mean blood pressures were measured by an oscillometric device (PetiTelemo, FUKUDA DENSHI CO., LTD., JAPAN), when the dog was calm and relaxed. A pressure cuff of appropriate width (approximately 40% the leg's circumference) was placed upon the median artery between the elbow and the carpal pad (Figure 3-1) or the proximal tail. Three consecutive measurements of blood pressure were recorded and averaged.

Figure 3-1 The procedure to measure indirect blood pressure, the photo depicted from a control healthy dog.

2. Blood collection and analytical procedures

Five milliliters of blood were collected from an indwelling intravenous catheter which was inserted into the left cephalic vein of each dog. A drop of whole blood was used to measure glucose concentration by Biamperometry (Accu-check®, Advantage II, Roche Diagnostics, USA). Another one drop of whole blood was used to measure plasma ketone (beta-hydroxybutyrate; β-OHB) concentration by a portable ketone meter and Optium™ β-Ketone test strips (MediSense Optium™, Abbott Laboratories, USA). The 1.5 ml of blood was placed into sodium heparinized tube for determination of fructosamine concentration using the colorimetric test (COBAS Integra, Roche Diagnostics) by reaction with nitroblue tetrazolium. A half of ml of blood was collected in ethylene diamine tetra-acetic acid (EDTA) for analysis of a complete blood count. Another half of ml of blood was put into sodium heparinized tube for analysis of blood gas analysis using a blood gas analyzer (RapidlabTM348, Bayer Diagnostics). The rest of blood was collected in a tube containing ethylene glycol bis (β-aminoethyl ether)-N-N, N', N'-tetracetic acid (EGTA) with reduced glutathione for analysis of plasma norepinephrine.

3. Procedure for plasma catecholamines determination

Approximately 2-3 ml of blood was put into a prechilled tube, containing a 60 µl mixture of ethylene glycol bis (β-aminoethyl ether)-N-N,N',N'-tetracetic acid (EGTA; 90 mg ml^{-1}) and reduced glutathione acid (60 mg ml^{-1}) as an antioxidation for catecholamine preservation. The collected blood was centrifuged at 1500-x g, at 4°C for 5 minutes. The plasma supernatant was then transferred into a test tube and kept frozen at -70°C until analyze. The analysis for plasma catecholamines was done within 2 months after collection.

Extraction procedure for plasma catecholamines composed of epinephrine (E) and norepinephrine (NE) with 3,4-dihydroxy-benzyl-amine hydrobromide (DHBA), as an internal standard, was done according to previous published procedures by Anton and Sayre (1962) with some modifications. One ml of the plasma was placed in a 3 ml column containing frit (Alltech Associates Inc., Deerfied, II, U.S.A.) along with 20 mg of

acid-activated alumina (Sigma, St. Louis, Mo), 1 ml of 1.5 Tris, pH 8.8 and 50 µl of DHBA (500 pg), as an internal standard. NE, E and DHBA were allowed to adsorb to an acid-activated alumina by gentle mixed on a vertical shaker for 20 minutes. The adsorbed alumina were then washed three times with ice-cold ultrapure water and centrifuged at 3,000 g, at 4°C for 3 min to remove excessed water. NE, E and DHBA were eluted from the alumina, following the addition of 100 µL 0.1 M PCA (Sigma), suspended by a horizontal shaker for 20 minutes and centrifuged at 3000-x g, at 4°C for 3 minutes. The extracts were collected and saved for injection into the HPLC system. All samples from each dog were extracted twice to provide the data of E and NE in duplicate.

The HPLC system with an electrochemical detector (HPLC-EC), a glassy carbon working electrode and amperometric control (Bioanalytical systems, West Lafayette, IN, USA.) was used to measure the concentrations of E, NE and DHBA. A Shimadzu Model LC-10 AD pump (Kyoto, Japan) was connected to a Rheodyne (Cotati, CA, USA.) injector, equipped with a 20 µl fixed loop and a 15 cm spherisorb® column, packed with 5 µm particles. The mobile phase solution was composed of 1.5 mM heptane sulfonate, 100 mM NaH_2PO_4, 1 mM Na_2EDTA and 4% methanol, adjusted to pH 4.1 with saturated citric acid. The mobile phase was filtered through a 0.22 µm filter, degassed by ultrasonic agitation and pumped at a flow-rate of 0.8 ml min^{-1}. The amperometer was set at a positive potential of 0.700 V with respect to the Ag/AgCl reference electrode, with a sensitivity of 0.2 nA. The extract (40 µl) from the plasma sample was injected into the HPLC-EC system to separate NE, E and DHBA. Data was collected and analyzed by delta 5.0 software (Digital Solutions, Margate, QLD, Australia).

Standard solutions at different concentration were injected into the HPLC-EC system. The retention time was evaluated by injecting both standard catecholamine individually and by the injection of a standard mixture (Figure 3-2). Standard solutions of the same concentration were injected repeatedly everyday for several days to verify the repeatability of the assay.

To obtain plasma calibration curves, plasma samples were pooled. Different concentrations of NE and E with a fixed amount of DHBA (as an internal standard) were added to 1 ml pooled plasma. The mixtures with different concentrations of standard were extracted similarly to the plasma samples. The absolute level of catecholamine was calculated as the percentual ratio between the peak areas of catecholamines and the corresponding internal standard, after alumina extraction. The plasma calibration curve was plotted after subtraction from the baseline endogenous NE and E. The levels of NE and E were presented as a mean $\pm$ S.E.M.

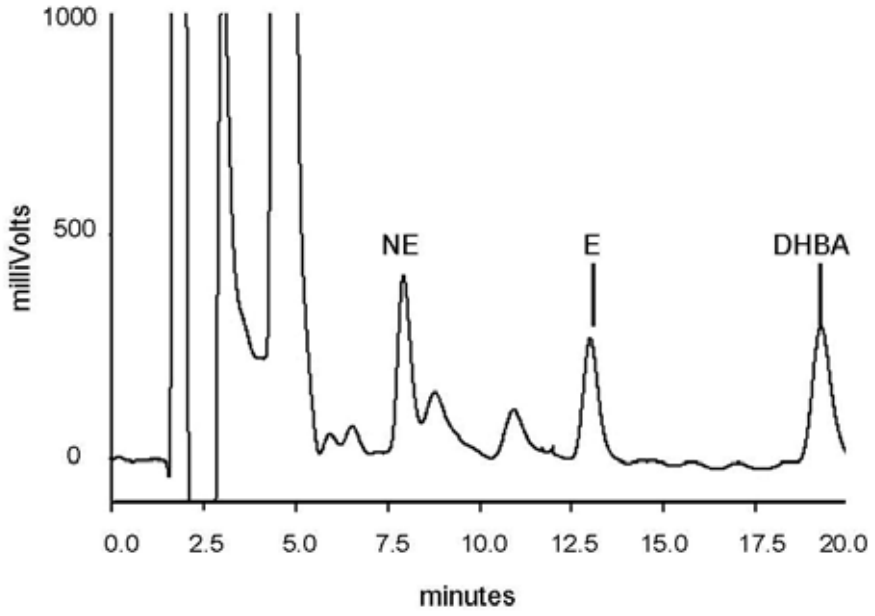


Figure 3-2 The chromatogram represents peak of standard NE, E and DHBA (concentration 500 pg/ml) measured by HPLC-EC. The retention time of NE, E and DHBA were approximately 7.75, 12.75 and 19.50 minutes, respectively. Abbreviations: NE, norepinephrine; E, epinephrine; DHBA, 3,4-dihydroxy-benzyl-amine hydrobromide.

4. Procedure for measurements and analysis of heart rate variability

The continuous ECG recording was performed for 15 minutes after the DM dog was fed and insulin was administered. The ECG recording was also recorded in control healthy dog but without insulin treatment. Standard 3-channel, 7-ECG electrodes were

attached to the anterior chest wall of dogs with standard bipolar leads (Figure 3-3) and were connected to the monitor system (FUKUDA DENSHI CO., LTD., JAPAN). The continuous ECG was done using by Holter recording for at least 30 minutes in all dogs during resting period in a recumbency position, either sternal or lateral. The subjects were instructed to fully relax with minimal restraint, stay awake and breath regularly (Figure 3-4).

After a surface ECG was recorded, a SCM-510 Holter software (FUKUDA DENSHI CO., LTD., JAPAN) was used to obtain a derived R-wave process. All QRS complexes from ECG were first edited automatically and then manually by careful inspection of the RR intervals. The extracting normal RR intervals were done by deleted both supraventricular and ventricular premature beats. The recording was acceptable if 85% or more of R waves were normal beats. Signals were filtered through a Hamming window. The filtered signals were transformed into a spectrum by Fast Fourier Transformation. Heart rate variability parameters were measured from 512 samples of consecutive R-R intervals. Separate frequency components of the HRV were obtained including ULF, VLF, LF, HF, TP and LF/HF ratio. The power of spectral bands were calculated, the ultra low frequency component at 0 to 0.004 Hz (ULF), very low frequency component at 0.004 to 0.041 Hz (VLF), low frequency component at 0.041 to 0.150 Hz (LF) and the high frequency component at 0.150 to 0.500 Hz (HF) (Stevens, 2007) (Figure 3-5). The time domain parameters were analyzed as following; Mean NN (NNA), SDNN, SDNN index (SSDA), SDANN (SASD), and pNN50 (RR (+/-) 50%).

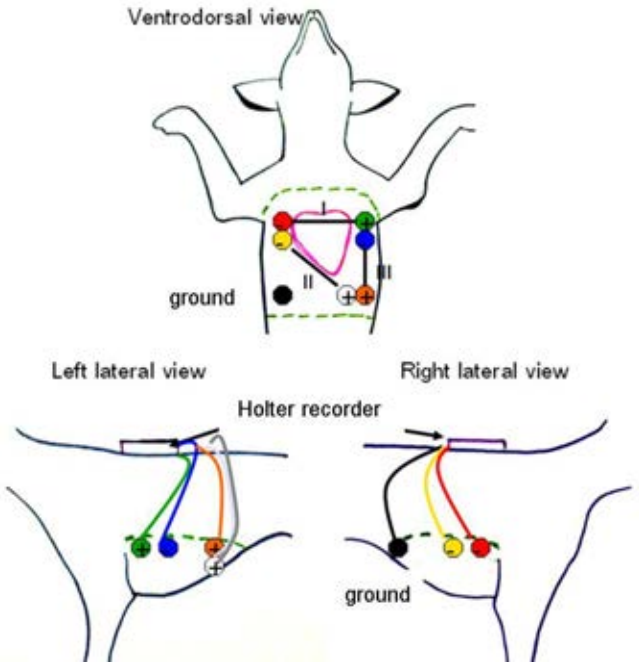


Figure 3-3 Standard three-channel ECG electrodes were attached to the anterior chest wall of dogs and were connected to the monitor system (modified from bipolar leads placement).

Figure 3-4 Continuous ECG recording in DM dog at rest, the subjects were fully relaxed with minimal restraint, stay awake and breath regularly.

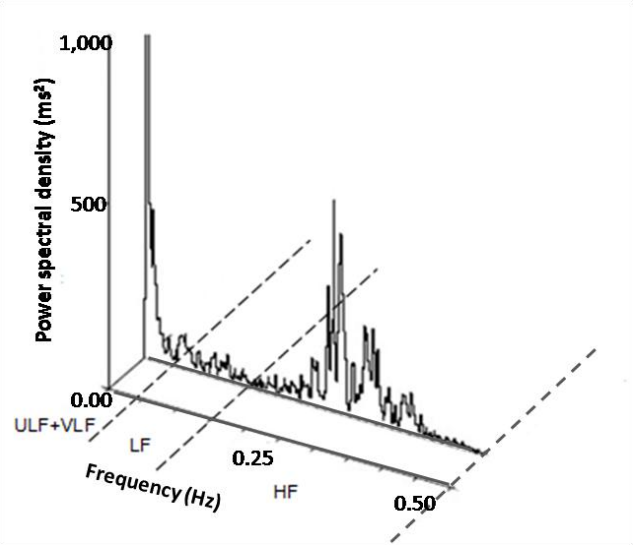


Figure 3-5 The canine power spectral components corresponding to different pieces of frequency bands: HF = high frequency power (0.15 to 0.50 Hz) ; LF = low frequency powe (0.04 to 0.15 Hz) r; VLF = very low frequency power (0.004-0.04 Hz); ULF = ultra low frequency (< 0.004 Hz); PSD= power spectral density.

D. Statistical Analysis

All numerical data are presented as mean $\pm$ SEM. Statistical analyses were performed using SAS program. The differences of general characteristics, blood parameters, HRV parameters, blood pressures and plasma norepinephrine concentration between DM subgroups (well-controlled or poorly-controlled DM) and control group using orthogonal contrast. Pearson and linear regression analysis were used to determine the relationship among each parameter. A *p*-value < 0.05 was considered a significant difference.

CHAPTER IV

RESULTS

A. General characteristics of dogs in control and study groups

The characteristics of the subjects are shown in Table 4-1. The mean age, weight and body condition score in DM group was not different from control healthy group. The mean age of the control healthy group was matched to both well-controlled DM and poorly-controlled DM subgroups. In control group, well-controlled DM and poorly-controlled DM subgroups, the ranges of age were 4-14, 7-14 and 4-13 years, respectively. The means of body weights were higher in both DM subgroups compared with the control group but a statistical significance was found only in poorly-controlled DM subgroup ($p < 0.05$).

For the body condition score, the mean values were more than 3 (from the score of 1-5) in all groups. There was no significant difference in body condition score between the control, well-controlled DM and poorly-controlled DM subgroups. Furthermore, the duration of DM was longer in well-controlled DM subgroup than poorly-controlled DM subgroup.

Table 4-1 Characteristics of the subjects

Variables	Control group	Total DM	DM subgroups	
			Well-controlled	Poorly-controlled
	(n = 13)	(n = 22)	(n = 11)	(n = 11)
Age (years)	9.18 ± 0.79	9.92 ± 0.57	10.47 ± 0.72	9.36 ± 0.88
Body weight (kg)	5.78 ± 0.39	8.11 ± 1.09	6.84 ± 1.21	9.38 ± 1.79 *
Body Condition Score (1-5)	3.73 ± 0.20	3.45 ± 0.15	3.41 ± 0.23	3.50 ± 0.20
Diabetic duration (months)	0	15.03 ± 4.00	25.64 ± 6.56	4.42 ± 1.31

Data are presented as mean ± SEM.

* $p < 0.05$ compared with control group using orthogonal contrast.

Abbreviations: n, number; DM, diabetes mellitus; kg, kilogram.

The breeds of the dogs are shown in Figure 4-1. Most of the dogs in all groups were poodle. In the well-controlled DM subgroup, most dogs were small breed while in the poorly-controlled DM subgroup, the poodle was predominant breed leaving the mixed breed for only 18%.

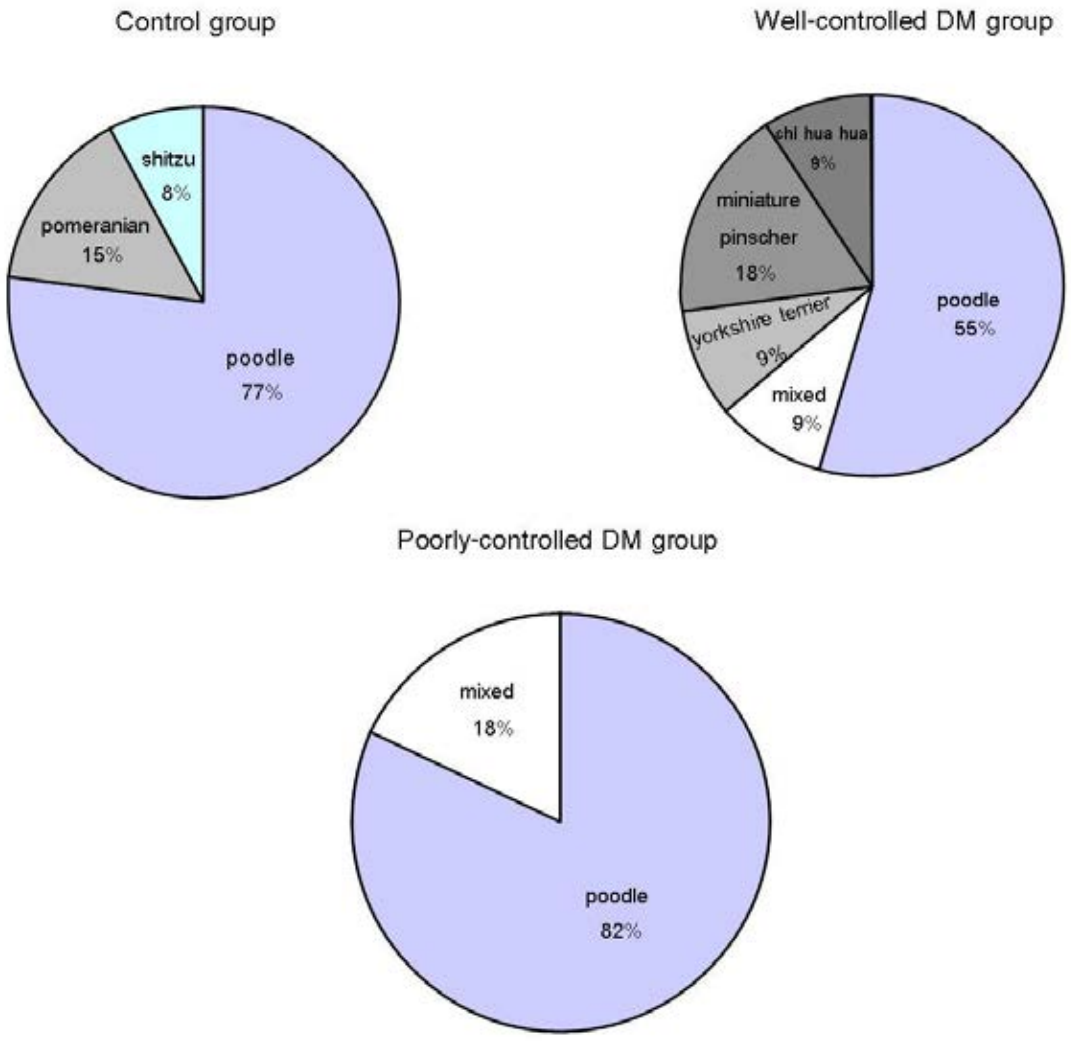


Figure 4-1 The percentages of breed in the subject groups.

The genders of the dogs are shown in Figure 4-2. In the well-controlled DM subgroup, the ratio of female to male was 0.85:1. In poorly-controlled DM subgroup, the ratio of female to male was 1.77:1. In summary, the ratio of female to male in all diabetic group was 1.22:1. For control group, the ratio of female to male was 0.61:1.

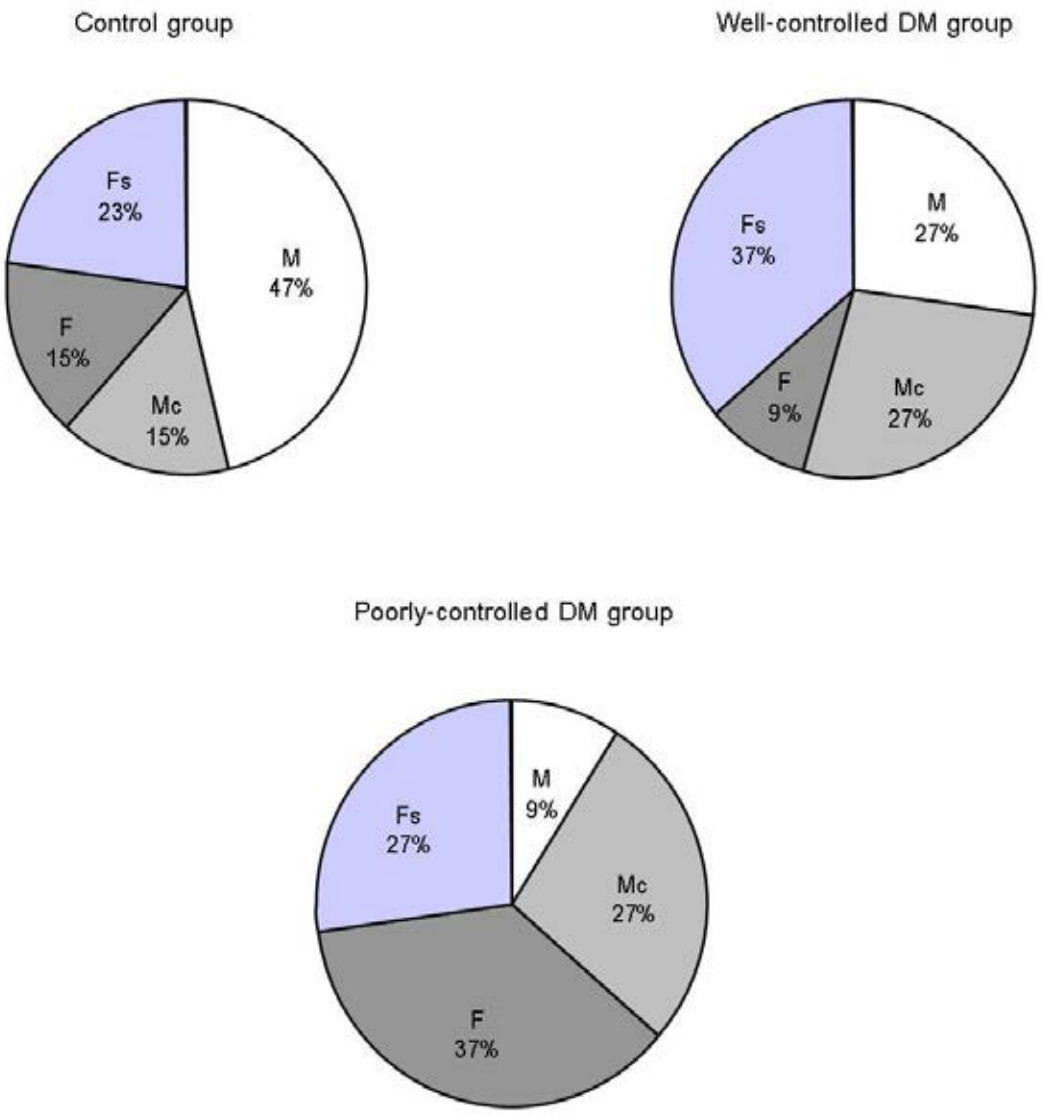


Figure 4-2 The percentages of gender status in the subject groups.

Abbreviations: M, Male; Mc, castrated male; F, Female; Fs, spayed female.

B. Hematological and serum blood chemistry profiles in control and study groups

The hematocrit and serum blood chemistry profiles are presented in Table 4-2. The hematocrit values were significantly lower in both DM subgroups compared with control group ($p < 0.05$). There was no difference in hematocrit between both DM subgroups. However, the hematocrit values of all study groups were in the normal range. Fasting blood glucose concentrations were significantly higher in both DM subgroups compared with the control group ($p < 0.001$). There was no difference in fasting blood glucose concentration between both DM subgroups. Serum fructosamine concentrations were significantly higher in the well-controlled DM subgroup ($p < 0.001$) and significantly highest in the poorly-controlled DM subgroup ($p < 0.001$) compared with control group and statistical significance was found between well-controlled DM and poorly-controlled DM subgroups ($p < 0.001$). The plasma ketone concentrations were highest in the poorly-controlled DM subgroup and were significance compared with the control group ($p < 0.01$) and the well-controlled DM subgroup ($p < 0.05$). There were no statistically significant difference among study groups for serum creatinine and BUN which were in the normal range. The plasma SGPT concentrations were higher in the DM group compared with the control group ($p < 0.01$) with a statistical significance was found only in the well-controlled DM subgroup ($p < 0.01$). However, they tended to be differences of plasma SGPT concentrations in the poorly-controlled DM subgroup compared with the control group ($p = 0.077$). The plasma ALP concentrations in both DM subgroups were significantly higher than the control group ($p < 0.05$). The cholesterol concentration was higher in the well-controlled DM subgroup ($p < 0.01$) and in the poorly-controlled DM subgroup ($p < 0.05$) than in the control group. For total protein and plasma albumin concentrations, in the DM group, total protein concentrations were lower compared with the control ($p < 0.01$) with a significant difference only in the well-controlled DM subgroup ($p < 0.05$). However, they tended to be a difference in the total protein concentration between the poorly-controlled DM and the control group ($p = 0.051$). There was no statistical significance for plasma albumin among study groups.

Table 4-2 Hematocrit and serum blood chemistry in control, total DM, well-controlled DM subgroup and poorly-controlled DM subgroup.

Variables	Control group	Total DM	DM subgroups	
			Well-controlled	Poorly-controlled
	(n = 13)	(n = 22)	(n = 11)	(n = 11)
Hematocrit (29.8-57.5 %)	50.69±1.37	43.32±1.99 *	43.00±3.87 *	43.64±1.27 *
FBG (79-126 mg/dl)	78.62±3.82	300.4±26.3 ***	273.6±34.9 ***	327.1±39.4 ***
Plasma fructosamine (225-364 μM)	265.9±14.3	514.1±25.2 ***	422.5±21.7 ***	605.7±22.9 ***,†††
Plasma ketone (DKA > 3.8 mM)	0.12±0.02	0.37±0.11 *	0.20±0.06	0.61±0.23 **,†
Creatinine (0.6-1.4 mg/dl)	0.86±0.07	0.81±0.03	0.81±0.05	0.81±0.04
BUN (7-26 mg/dl)	5.68±1.60	15.42±2.55	17.75±4.60	13.09±2.25
SGPT (4-91 U/L)	38.62±5.04	121.8±22.1 **	142.9±39.3 **	100.7±20.5
ALP (20-120 U/L)	95.39±22.42	432.3±80.0 **	426.3±112.3 *	438.3±119.3 *
Cholesterol (125-300 mg/dl)	203.6±15.9	322.2±28.4 **	344.3±29.2 **	297.0±51.8 *
Total protein (5.8-9.7 g/dl)	8.78±0.37	7.37±0.32 **	7.21±0.50 *	7.56±0.40
Plasma albumin (2.6-4 g/dl)	3.44±0.09	3.29±0.10	3.32±0.15	3.26±0.14

Data are presented as mean ± SEM.

* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ compared with control group using orthogonal contrast.

† $p < 0.05$ and ††† $p < 0.001$ compared with well-controlled DM subgroup using orthogonal contrast.

Abbreviations: n, number; DM, diabetes mellitus; FBG, Fasting blood glucose; BUN, blood urea nitrogen; ALP, alkaline phosphatase; SGPT, serum glutamic pyruvic transaminase.

C. Blood gas analysis and plasma electrolyte concentrations in control and study groups

Blood gas analysis and plasma electrolyte concentrations are shown in Table 4-3. There was no statistically significant difference between both DM subgroups compared with the control group for blood pH, pCO_2, HCO_3^- and anion gap although the anion gap in the poorly-controlled DM tended to be the highest and pCO_2 and HCO_3^- in the control group were slightly lower than the normal range. The phosphorus and sodium concentrations were not different between both DM subgroups and the control group and all were within the normal range. A potassium concentration was significantly higher ($p < 0.05$) while a chloride concentration was lower ($p < 0.05$) in the DM group compared with the control group. When compared potassium concentrations with the control group, the value of potassium in the well-controlled DM subgroup was significantly higher ($p < 0.05$) while those of the poorly-controlled DM subgroup was high without a significant difference. For the chloride concentration, the significant difference was found only in the poorly controlled DM subgroup ($p < 0.05$) compared with the control group.

Table 4-3 Blood gas analysis and plasma electrolytes in the control, total DM, well-controlled DM subgroup and poorly-controlled DM subgroup.

Variables	Control group	Total DM	DM subgroups	
			Well-controlled	Poorly-controlled
	(n = 13)	(n = 22)	(n = 11)	(n = 11)
Blood pH (7.35-7.45)	7.46±0.01	7.42±0.01	7.41±0.02	7.43±0.02
pCO_2 (32.0-45.0 mmHg)	30.43±1.77	35.64±2.24	36.91±3.91	34.37±2.34
HCO_3^- (22.3±0.43 mEq/L)	20.68±0.72	22.20±0.80	22.33±1.25	22.07±1.06
Anion gap (12-24 mEq/L)	13.73±1.93	16.94±2.17	14.68±2.74	19.20±3.34
Phosphorus (2.5-6.2 mEq/L)	3.85±0.34	4.14±0.24	4.29±0.31	3.96±0.40
Sodium (134-146 mEq/L)	142.8±1.3	141.1±1.0	140.2±1.5	142.0±1.5
Potassium (3.4-4.5 mEq/L)	3.47±0.11	3.91±0.13 *	3.98±0.16 *	3.84±0.20
Chloride (96-108 mEq/L)	111.9±2.3	106.0±1.3*	107.2±2.1	104.9±1.5 *

Data are presented as mean ± SEM.

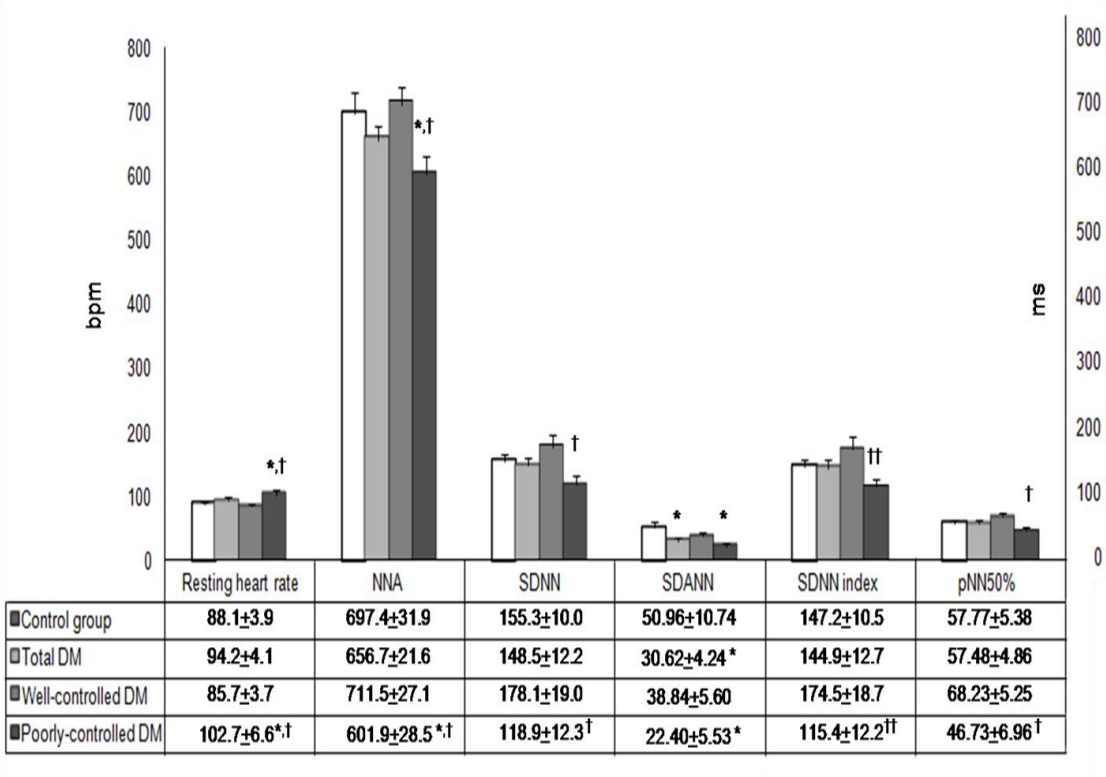
* $p < 0.05$ compared with control group using orthogonal contrast.

Abbreviations: n, number; DM, diabetes mellitus; pCO_2, the partial pressure of carbon dioxide; HCO_3^-, bicarbonate.

D. Time-and frequency domain analysis of heart rate variability in control and study groups

1. The resting heart rate and time-domain parameters of HRV

The resting heart rate and time-domain parameters of HRV in all study groups are shown in Figure 4-3. The resting heart rate was highest in the poorly-controlled DM subgroup and was significant differences compared with the control group (p <0.05) and the well-controlled DM subgroup (p <0.05). There was no significant difference in resting heart rate between the control group and the well-controlled DM subgroup. The NNA value was significantly lower in the poorly-controlled DM subgroup than boths of the control group and the well-controlled DM subgroup (p < 0.05). There was no significant difference in the NNA value between the control group and the well-controlled DM subgroup. The SDNN values were lowest in the poorly-controlled DM subgroup and were significant differences compared with the well-controlled DM subgroup (p < 0.05) and tended to be lower than in the control group (p = 0.07). The SDANN values in the DM group were lower than the control group significantly (p <0.05) with a significance was found only in poorly-controlled DM subgroup (p <0.05). The SDNN index and pNN50% were not different in the DM group compared with the control. The SDNN index was lower in the poorly-controlled DM subgroup than the control group and the well-controlled DM subgroup but a statistical significance was found only in the poorly-controlled DM subgroup compared with the well-controlled DM subgroup (p < 0.01). There was no significant difference in the SDNN index between the control group and the well-controlled DM subgroup. The pNN50% were lower in the poorly-controlled DM subgroup than the control group and the well-controlled DM subgroup but a statistical significance was found only in the poorly-controlled DM subgroup compared with the well-controlled DM subgroup (p < 0.05). There was no significant difference in the pNN50% between the control group and the well-controlled DM subgroup.



	Resting heart rate	NNA	SDNN	SDANN	SDNN index	pNN50%
Control group	88.1±3.9	697.4±31.9	155.3±10.0	50.96±10.74	147.2±10.5	57.77±5.38
Total DM	94.2±4.1	656.7±21.6	148.5±12.2	30.62±4.24 *	144.9±12.7	57.48±4.86
Well-controlled DM	85.7±3.7	711.5±27.1	178.1±19.0	38.84±5.60	174.5±18.7	68.23±5.25
Poorly-controlled DM	102.7±6.6*,†	601.9±28.5 *,†	118.9±12.3†	22.40±5.53 *	115.4±12.2††	46.73±6.96†

Figure 4-3 Histogram illustrates mean values of heart rate and time-domain parameters of HRV; NNA, SDNN, SDANN, SDNN index and pNN50% of all study groups.

Data are presented as mean ± SEM.

* $p < 0.05$ compared with the control group using orthogonal contrast.

† $p < 0.05$ and †† $p < 0.01$ compared with the well-controlled DM subgroup using orthogonal contrast.

Abbreviations: HRV, heart rate variability; bpm, beat per minute; ms, millisecond; NNA, mean of the normal RR intervals in the entire recording; SDNN, standard deviation of all normal RR intervals in the entire recording; SDANN, standard deviation of the average of all 5 min segments of normal RR intervals in the entire recording; SDNN index, mean of the standard deviations of all normal RR intervals for all 5 min segment in the entire recording; pNN50 %, percentage of differences between adjacent normal RR intervals that are >50 ms computed in the entire recording.

2. The frequency-domain parameters of HRV

The frequency domain (PSD, power spectral density) analysis of a dog from each study groups are shown in Figure 4-4 (A-C). The decrease in power spectrum in both low and high frequencies were seen in the poorly-controlled DM dog.

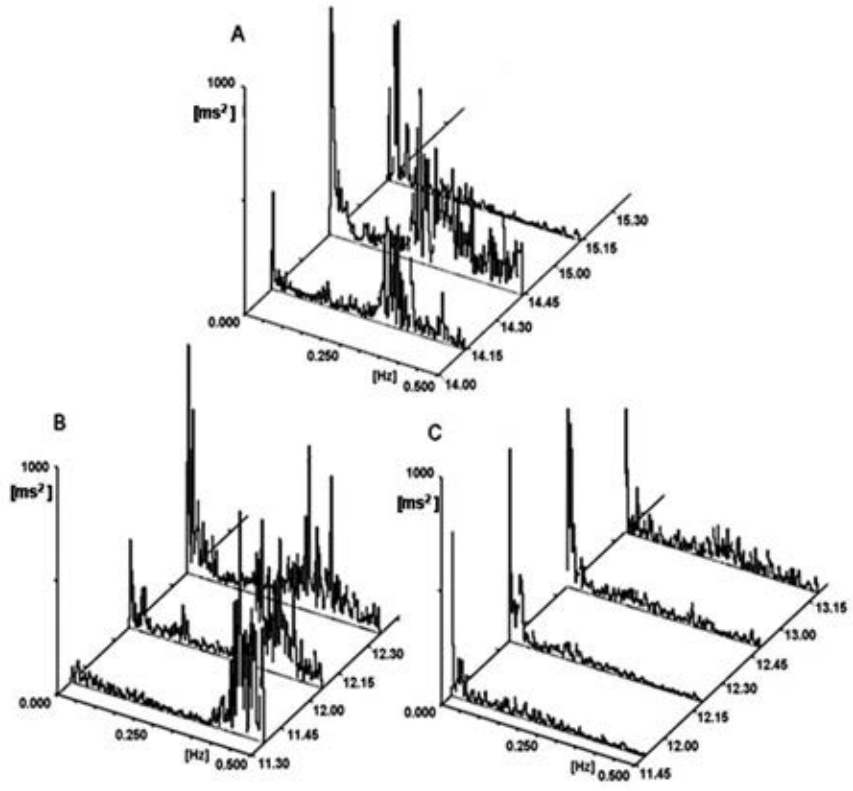
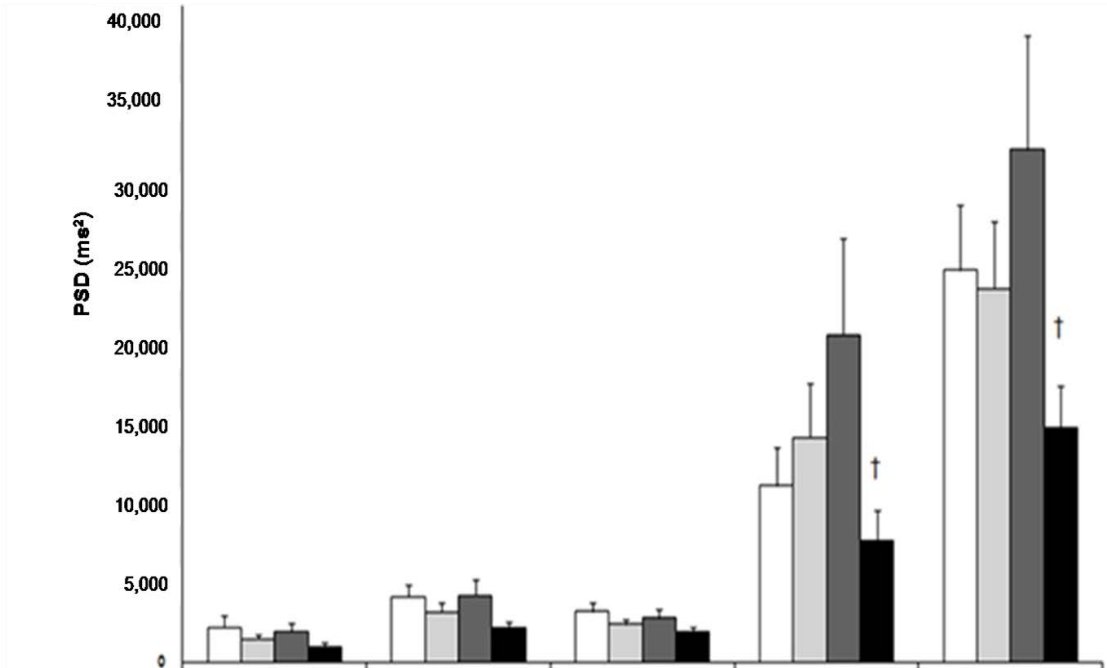


Figure 4-4 The examples of power spectrum density of one dog from (A) control group, (B) well-controlled DM and (C) poorly-controlled DM subgroups.

In the Figure 4-5, the values of ULF were slightly lower in both DM subgroups than in the control group, although there was no significant difference. The VLF value tended to be lower in the poorly-controlled DM subgroup than in the control group ($p < 0.07$) and the well-controlled DM subgroup ($p < 0.07$). There was no significant difference in the VLF between the control group and the well-controlled DM subgroup. The values of LF were slightly lower in both DM subgroups than in the control group and the lowest value was found in the poorly-controlled DM subgroup. The LF value tended to be lower in the poorly-controlled DM subgroup than in the control group ($p < 0.06$). There was no significant difference in the LF between the control group and

the well-controlled DM subgroup. HF and total power values were lower in the poorly-controlled DM subgroup than the control group and the well-controlled DM subgroup but statistical significance was found only in the poorly-controlled DM subgroup compared with the well-controlled DM subgroup ($p < 0.05$). There was no significant difference in the HF and total power values between the control group and the well-controlled DM subgroup. The highest LF/HF ratio was found in the poorly-controlled DM subgroup (0.54 $\pm$ 0.15) and the lowest ratio was found in the well-controlled DM subgroup (0.21 $\pm$ 0.04), therefore, the statistical significance was found only in the poorly-controlled DM subgroup compared with the well-controlled DM subgroup ($p < 0.05$).



	ULF	VLF	LF	HF	Total power
Control group	2,199±708	4,120±770	3,220±495	11,095±2,381	24,562±4,116
Total DM	1,474±295	3,182±554.7	2,386±312	14,086±3,378	23,395±4,168
Well-controlled DM	1,9567±475	4,206±976	2,824±526	20,503±6,030	32,097±7,148
Poorly-controlled DM	992.4±307.5	2,158±361	1,948±311	7,670±1,829 †	14,694±2,591 †

Figure 4-5 Histogram illustrates mean values of heart rate and frequency-domain parameters of HRV including ULF, VLF, LF and total power of all study groups.

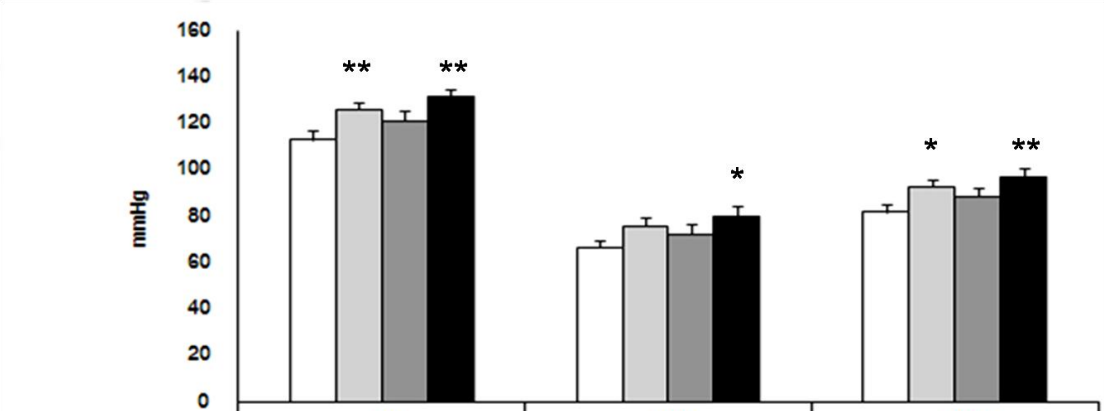
Data are presented as mean $\pm$ SEM.

† $p < 0.05$ compared with the well-controlled DM using orthogonal contrast.

Abbreviations: HRV, heart rate variability; ULF, Ultra low frequency; VLF, Very low frequency; LF, low frequency; HF, high frequency; Hz, hertz; ms^2, millisecond square.

E. The systolic, diastolic and mean arterial blood pressures in control and study groups

The blood pressures are presented in Figure 4-6. The systolic and mean pressures in the DM group were significantly higher than the control group (p <0.01 and p <0.05, respectively). The systolic, diastolic and mean pressures in the poorly-controlled DM subgroup were significantly higher than the control group (p <0.01, p <0.05 and p <0.01, respectively). However, in the well-controlled DM subgroup, no change in systolic, diastolic and mean blood pressures were found compared with both control group and poorly-controlled DM subgroup.



	SBP	DBP	MABP
Control group	113.1±4.1	66.6±3.2	82.1±3.1
Total DM	126.2±2.9**	76.0±3.4	92.8±2.9*
Well-controlled DM	121.2±4.4	72.2±4.8	88.6±4.1
Poorly-controlled DM	131.3±3.2**	79.8±4.8*	97.0±4.0**

Figure 4-6 Histogram illustrates mean values of systolic blood pressure, diastolic blood pressure and mean arterial blood pressure of all study groups

Data are presented as mean ± SEM.

* p < 0.05 and ** p < 0.01 compared with control group using orthogonal contrast.

Abbreviations: n, number; DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP, mean arterial blood pressure; mmHg, millimeter of mercury.

F. Plasma catecholamines concentrations in control and study groups

The chromatograms of plasma catecholamines depicted in a dog from the control group, the well-controlled DM and the poorly-controlled DM subgroups were shown in Figure 4-7. The plasma norepinephrine in the DM group was lower than the control group although there was no significant difference (p = 0.12). However, the poorly-controlled DM subgroup had the lowest NE concentration. The plasma norepinephrine concentration in the poorly-controlled DM subgroup was significantly lower than both the control group and the well-controlled DM subgroup ($p < 0.05$). There was no difference in plasma norepinephrine concentrations between the well-controlled DM subgroup and the control group. No significance of epinephrine concentrations was found among the control, well-controlled DM and poorly-controlled DM subgroups (Figure 4-8).

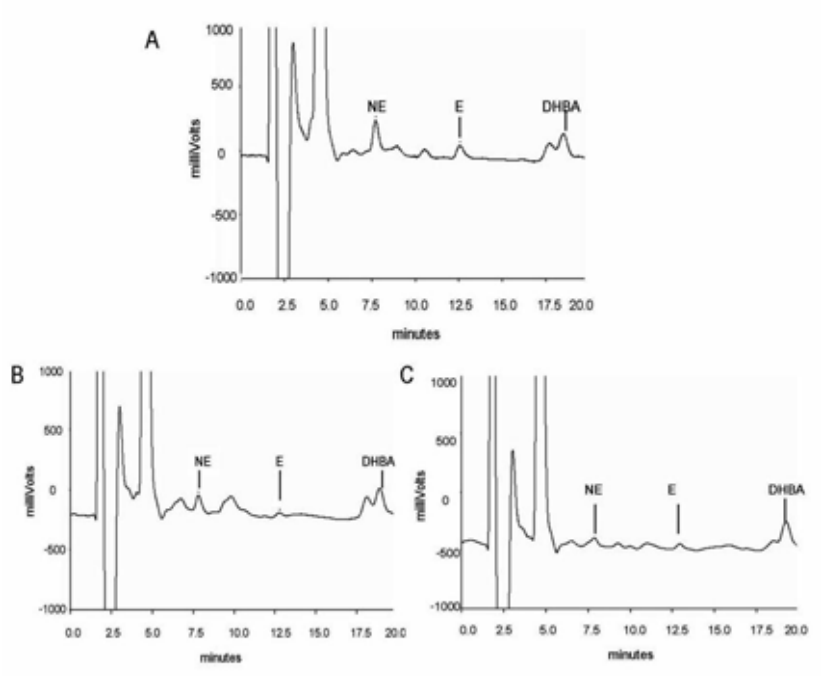
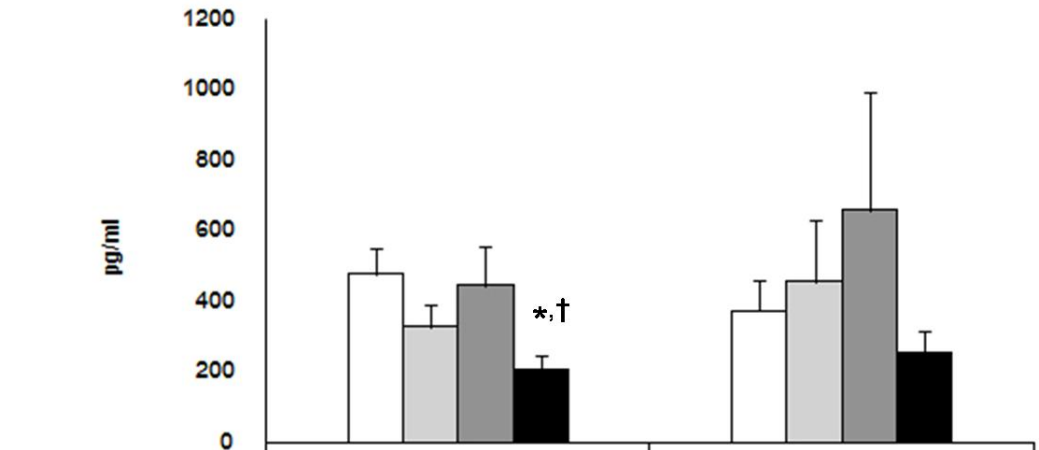


Figure 4-7 The examples of chromatograms represent plasma catecholamines (NE, E) and internal standard (DHBA) concentrations of one dog from each study groups, (A) control dog, (B) well-controlled DM dog and (C) poorly-controlled DM dog measured by HPLC-EC. The retention times of NE, E and DHBA were approximately 7.75, 12.75 and 19.5 minutes, respectively.



	Norepinephrine	Epinephrine
Control group	478.9±74.3	374.3±85.3
Total DM	329.1±62.0	458.0±172.8
Well-controlled DM	447.7±109.4	658.5±337.1
Poorly-controlled DM	210.4±36.7*,†	257.4±60.7

Figure 4-8 Histogram illustrates mean values of plasma norepinephrine and epinephrine concentrations of all study groups.

Data are presented as mean ± SEM.

* $p < 0.05$ compared with control group using orthogonal contrast.

† $p < 0.05$ compared with well-controlled DM group using orthogonal contrast.

Abbreviations: n, number; DM, diabetes mellitus; pg, picogram; ml, milliliter.

G. The correlations between parameters

1. The correlations between plasma NE concentrations and diabetic durations

In Figure 4-9, there was no correlation between plasma NE concentrations and diabetic durations in all subjects (r = 0.070).

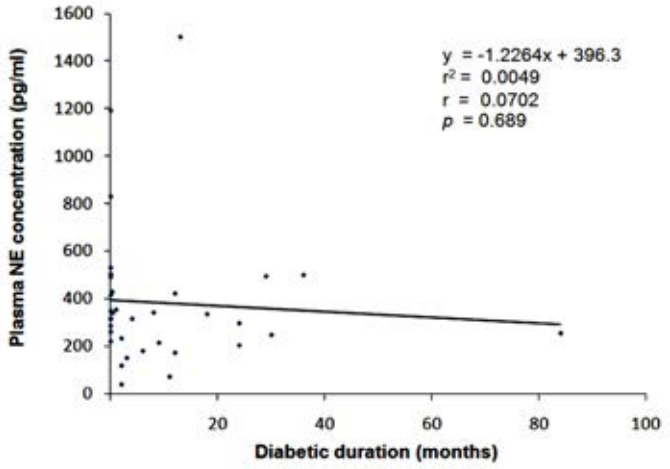


Figure 4-9 Relationship between plasma NE and diabetic durations in all subjects.

2. The correlations between plasma NE concentrations and plasma fructosamine concentrations in all subjects

The negative correlation was found between plasma NE concentrations and plasma fructosamine concentrations in all subjects (p <0.05, r= 0.359) (Figure 4-10). However, no correlation was found between plasma fructosamine concentrations and HRV paramaters, blood pressure or heart rate.

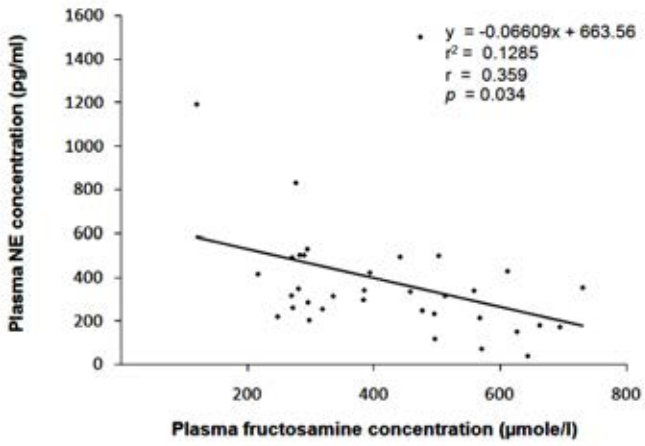


Figure 4-10 Relationship between plasma NE and fructosamine concentrations in all subjects.

3. The correlations between time-and frequency-domain parameter in all subjects

When plotting between time-domain parameters and frequency-domain parameters, the significant positive correlations were found between LF and SDANN ($p < 0.01$, r = 0.524) (Figure 4-11; A) and SDNN index ($p < 0.001$, r = 0.623) (Figure 4-11; B). The HF also had positive correlation with pNN50% ($p < 0.001$, r = 0.575) (Figure 4-12).

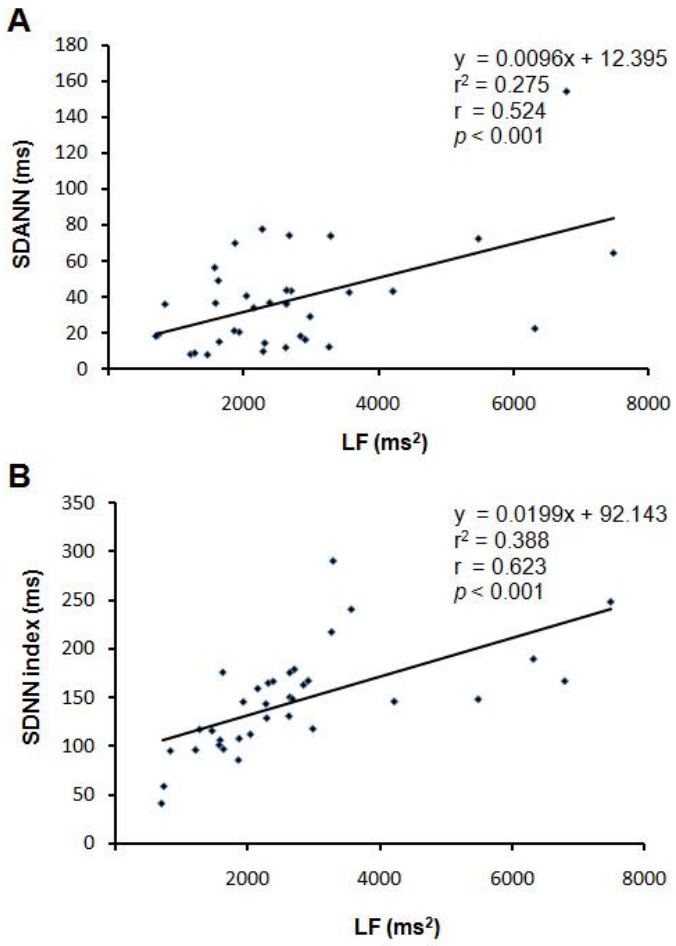


Figure 4-11 Relationship between low frequency domain parameter (LF) and SDANN (A) and SDNN index (B) of time-domain parameters in all subjects.

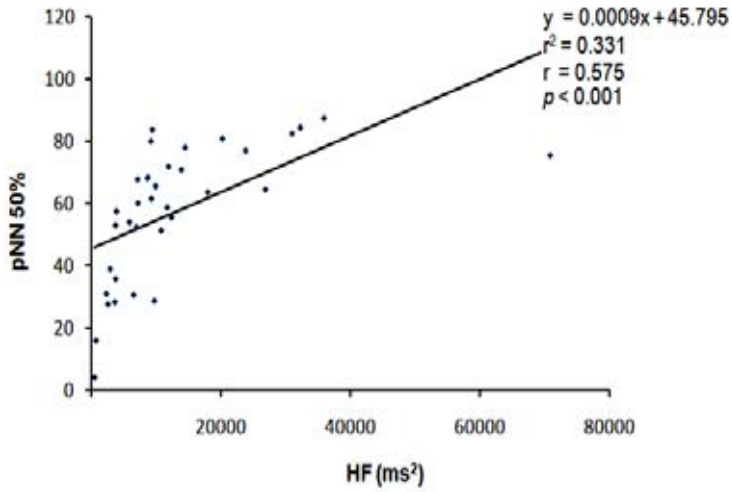


Figure 4-12 Relationship between high frequency domain parameter (HF) and pNN50% of time-domain parameter in all subjects.

4. The correlations between HRV parameters, blood pressures and the plasma norepinephrine concentrations in all subjects

There was no correlation between HRV parameters, blood pressures and the plasma norepinephrine concentrations in all subjects in this study.

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CHAPTER V

DISCUSSION

A. General characteristics of control and study groups

The diabetic dogs in this study had body condition scores higher than 3/5 similar to the previous study that DM was found in obese dogs (Lund et al., 2006). Most of dogs were middle to older aged as previously reported (Guptill et al., 2003; Davison et al., 2005; Fall et al., 2007; Hess, 2008; Chansaisakorn et al., 2009). Since most of the DM dogs had inactive lifestyle, the diabetic dogs were gained body weight. The means diabetic duration of the well-controlled DM subgroup was longer than the poorly-controlled DM subgroup (25.64$\pm$6.56 and 4.42$\pm$1.31 months, respectively) since the well-controlled DM subgroup was presented to the veterinarian for a longer period and got a better adjustment of insulin therapy compared with the poorly-controlled DM subgroup.

Although a variety of breeds were found in DM subgroups included poodle, miniature pinscher, chi hua hua, yorkshire terrier, shih tzu and mixed breed dogs, the main breed in all of study groups was poodle. Similarly, the most frequent reported breed were samoyeds, miniature schnauzers, miniature poodles, toy poodles and pugs (Hess, 2008). The prevalence of DM in the study was found to be female more than male with the ratio of 2:1; similarly, others have shown that DM occurs mainly in female (Ricketts et al., 1953; Guptill et al., 2003; Fall et al., 2007) but with a slightly different ratio ranging from the ratio of female to male of 2-4 to 1. These different may due to the limited number of the dog in the present study. However, these suggested that female dogs were had risks of DM than male dogs.

B. Hematological profiles and serum blood chemistry in control and study groups

The hematocrit in the poorly-controlled DM and the well-controlled DM subgroups were slightly lower than the control; however, there was within reference ranges. These results may be caused by overhydration as a result of polydipsia. The fasting blood glucose concentrations in the well-controlled DM and the poorly-controlled DM subgroups were 3.5 and 4 times higher than the control group. Although many factors can increase blood glucose such as stress (McCowen et al., 2001; Hess, 2008), medications such as formamidine insecticide (Amitraz), thaizide diuretic, β-blockers, sympathomimetic drugs, corticosteroids, sex hormones (progesterone, megestrol acetate), pentamidine, protease inhibitor, atypical antipsychotic, and etc. (Hsu and Schaffer, 1988; Chan et al., 1996; Luna and Feinglos, 2001; ADA, 2004; Hess, 2008) and other diseases such as hyperadrenocorticism, pheochromocytoma, acromegaly, acute pancreatitis, and etc. (Hess, 2008); the increased blood glucose concentration in this study was mainly due to the lacking of insulin. DM in dogs is well known to be type 1 DM which islet cells cannot produce and secrete insulin. Thus, exogenous insulin administration is needed. The types of insulin used in this study were veterinary porcine lente insulin (Caninsulin®, Intervet) or human recombinant insulin (Humulin N, Eli Lilly and Company). Some animals in this study may switch between these two types of insulins for controlled their blood glucose concentrations to the reference range. The frequency of injection was once to twice daily depended on the glucose response curve. Dogs those glucose can be controlled well would be maintained on insulin for a period of time, and they were categorized as the well-controlled DM subgroup. However, dog who presented at the hospital for the first episode or glucose could not be adjusted to the reference range was categorized as the poorly-controlled DM subgroup. Therefore, dogs in the well-controlled DM subgroup did not show clinical signs of DM like polyuria, polydipsia, weight loss despite constant hunger, lethargy or depression. In contrast, the polyuria, polydipsia, weight loss in addition to constant hunger, lethargy or depression were presented in the poorly-controlled DM subgroup.

A plasma fructosamine which is the glycated proteins in the DM groups was higher than the control group. Plasma fructosamine represents hyperglycemia for at least 2-3 weeks of duration. It is not significantly affected by acute or transient hyperglycemia (Marca et al., 2000) such as epinephrine-induced hyperglycemia (Gerich et al., 1976). However, the plasma fructosamine concentration could be affected by plasma protein and albumin, since plasma fructosamine in dog can be lowered by hypoproteinemia and hypoalbuminemia (Loste and Marca, 1999). Total protein and plasma albumin in this study were within normal ranges. Then this parameter was used for diagnosis and monitoring glucose concentrations while insulin therapy in diabetic dogs and cats (Feldman and Nelson, 2004). Plasma fructosamine was widely used in Veterinary medicine although the hemoglobin A1C which is commonly used in human DM has been reported in small animals (Marca et al., 2000; Davison et al., 2002; Catchpole et al., 2008; Chansaisakorn et al., 2009).

Although both DM subgroups had higher blood glucose and fructosamine concentration, none of these dogs had diabetic ketoacidosis. The ketone body (beta-hydroxybutyrate; β-OHB) was higher in both DM subgroups and was highest in the poorly-controlled DM subgroup. However, the blood pH was in a normal range in all groups. The diabetic ketoacidosis (DKA) was not included in this study since the dog did not had diabetic ketoacidosis and the β-OHB was in the normal range (0 to 0.6 mmol/l). If plasma β-OHB concentration is higher than 2.0 mmol/l, dogs should receive ambulatory monitoring and treatment. If the plasma beta-hydroxybutyrate concentration is higher than 3.8 mmol/l, the diagnosis of DKA is confirmed and an intensive care is warranted (Duarte et al., 2002).

For the biochemical profiles, serum creatinine and BUN were not different among the study groups and they were in a normal range, it was then concluded that dogs had no evidence of diabetic nephropathy in this study. Serum SGPT and ALP were increased, the hepatic impairment was thus suspected in DM dogs although an increase of serum SGPT concentration could indicate an impairment of insulin signaling rather than purely hepatocytes injury in type 2 DM (Harris, 2005).

Increase cholesterol concentrations in both DM groups compared with the control group may be due to an abnormality in the level of lipoproteins, the particles that carry cholesterol into the bloodstream (Goldberg, 2001). Type 1 DM patients with poor glucose control have diabetic dyslipidemia that tends to lower HDL cholesterol levels and moderately raised triglyceride and VLDL and LDL cholesterol levels (Shen, 2007). Therefore, DM patients have an increase in the risk for atherosclerosis which lead to coronary heart disease and stroke (Messier et al., 2004). However, the prevalence in dogs was unknown.

C. Blood gas analysis and plasma electrolyte concentrations in control and study groups

The plasma HCO_3^- content was in the normal range while the anion gap was elevated in the poorly-controlled DM. An increased anion gap may be due to a rise of the ketone body. The presence of ketone bodies such as acetone, acetoacetate, and β-OHB could be found in uncontrolled DM patients (Fulop et al., 1999). The serum D-lactate may increase anion gap in diabetic ketoacidosis patients (Christopher et al., 1995; Lu et al., 2011). However, D-lactate was not measured in this study. The phosphate was not contributed to increased anion gap since the plasma phosphorus was unchanged. The electrolytes Na^+, K^+, were in the normal range and not significantly different from the control suggested that electrolyte hemostasis was still able to be regulated in order to get the normal blood pH and water regulation. The significant decreased chloride in DM dogs may be due to the NaCl restriction in the diet or may be partly due to an exchange with HCO_3^- during pH regulation.

D. Time-and frequency-domain analysis of heart rate variability in control and study groups

The present results showed that heart rate in the poorly-controlled DM subgroup was the highest. An Increased in heart rate suggested that this DM subgroup may have autonomic dysfunction (Murray et al., 1975; Eckberg et al., 1986). The time domain parameters included NNA, SDNN, SDANN, SDNN index and PNN50% were widely used by clinicians to demonstrate cardiac autonomic function (Task Force, 1996; Calvert,

1998; Sztajzel, 2004). The NNA is an inverse of heart rate which will be changed if autonomic function alters. The SDNN was correlated with the low-frequency power spectrum which reflected from change in sympathetic and parasympathetic tones (Calvert, 1998; Sztajzel, 2004). The SDANN and SDNN index were correlated with the low-frequency power spectrum which reflected from sympathetic and parasympathetic tones attributable to baroreceptors (Calvert, 1998; Sztajzel, 2004). They will be decreased when the baroreceptor is impaired. The pNN50% correlated with the high-frequency power spectrum which represents the parasympathetic change during respiratory changes (Calvert, 1998; Sztajzel, 2004). Reduction in pNN50% indicates parasympathetic impairment. In this study, the poorly-controlled DM subgroup showed a significant reduction in NNA and SDANN while SDNN, SDNN index and pNN50% also reduced but not significant compared with the control group. The reductions in all components suggest the impairment of baroreceptors and some part of parasympathetic nerve occurred in the poorly-controlled DM subgroup.

For frequency domain parameters, the origin of ULF component of heart rate variability is unknown (Task Force, 1996) but physical activity may be a major contributor to the ULF component (Serrador et al., 1999). The VLF component indicates the part of thermal regulation of the body's internal systems (Task Force, 1996; Calvert, 1998; Sztajzel, 2004). Both ULF and VLF in this study tended to reduce which may in part due to the reduction in physical activity and decrease the ability to regulate thermal adjustment in animals who suffered from DM. However, the body temperature was not recorded. In elderly DM patients, diabetic ketoacidosis or hypoglycemia usually had hypothermia (Strauch et al., 1969; Gale and Tattersall, 1978; Neil et al., 1986). The LF component is mainly due to both sympathetic and parasympathetic components that controlled cardiac function. However, the HF component is represented only the parasympathetic control. The total power is the sum of all frequency components and represents all autonomic neural control of the heart. The LF/HF ratio is then indicates the balance between sympathetic and parasympathetic activity (Task Force, 1996; Calvert, 1998; Sztajzel, 2004).

In the present study, the LF, HF and total power components were significantly lower and LF/HF ratio was significantly higher in the poorly-controlled DM subgroup than in the well-controlled DM subgroup. The poorly-controlled DM subgroup tended to have lower VLF and LF levels than the control group. Reduction in both sympathetic and parasympathetic cardiac control was suspected.

E. The relationship between HRV parameters, blood pressures and the plasma norepinephrine concentrations in control and study groups

Blood pressure was significantly higher in the DM group and the highest value was found in the poorly-controlled DM subgroup. This increased BP was similar to those reported in human patients (Epstein and Sowers, 1992). In humans, increased blood pressure was due to old age with reduced elasticity of the arterial system resulting in increased total peripheral resistance, vessel constriction or heart disease. Moreover, DM patients may have abnormal cholesterol and lipid metabolism that leading to atherosclerosis (Goldberg, 2001). In dog, atherosclerosis was rarely presented due to short life expectancy in dogs. Moreover, the HDL/LDL ratio (High-density lipoprotein cholesterol to Low-density lipoprotein cholesterol) in dog is higher than in humans since HDL in dog is the major cholesterol-carrying lipoproteins, in contrast to human (Watson, 1996; Kagawa et al., 1998; Maldonado et al., 2001; Bailhache et al., 2003). Increased blood pressure in this study may be due to increased heart rate. This heart rate is mainly controlled by vagal activity (Heymans and Neil, 1958; Mendelowitz, 1999). Our results showed the defect in parasympathetic activity by decreased HF. Therefore, suppressed vagal activity may be responsible for increased blood pressure.

The impaired baroreceptor reflex can lead to increase blood pressure. However, study by Cowley et al. (1973) showed that buffer nerve denervation could cause only temporary increase in mean arterial blood pressure. However, blood pressure would come back to a normal range thereafter but the variability of blood pressure would be higher. This result suggested that baroreceptor may not be solely responsible for blood pressure control. Our study showed that baroreceptors may be reset since the high blood pressure did not respond by suppression of heart rate.

In the present study, the relationships were found between either SDANN or SDNN index and LF component. These relationships indicate that the 2 parameters from time domains were related to the LF component in frequency domain. Thus, the sympathetic and parasympathetic were related to the baroreceptor reflex. The similar relationship was also supported by Calvert (1998). In the well-controlled DM subgroup which had DM for a longer period and was controlled well by insulin treatment, the LF component was lower which was corresponded to the reduction in norepinephrine concentrations in the plasma. Moreover, in the poorly-controlled DM subgroup, LF tended to decrease compared with the control group and it was decreased along with the decrease in plasma norepinephrine concentrations. Thus, suppression of sympathetic activity occurs in DM dogs.

The present study also showed the relationship between plasma fructosamine concentrations and plasma norepinerhine concentrations. This result suggested that sympathetic activity was suppressed especially when the DM was uncontrollable. The plasma fructosamine did not represent the duration of diabetic but showed the goodness of glucose control by the treatment. Thus, sympathetic nerve activity could be altered by hyperglycemia rather than the duration of DM. Animals which can control DM may not have autonomic insufficiency but the neuropathy should be detected in a very poorly-controlled DM. We proposed that the neuropathy may occur at the beginning of DM in dogs without treatment but may be resolved after the hyperglycemia was controllable. The similar result was found in type I DM in humans which showed that in the early stage of diabetic autonomic neuropathy, the striking of glycemic control can slow the onset of diabetic autonomic neuropathy (DAN) and sometimes can reverse to a normal (Hussein et al., 2011). Moreover, in this study, plasma epinephrine concentrations were unchanged among groups which suggested that no adrenal gland responsive to DM.

In poorly-controlled DM subgroup, the HF was also decreased but not significant. The relationship of pNN50% and HF component was found in this study suggested that the vagal tone had been altered during respiration. This result was supported by Calvert (1998). These results suggested that in order to evaluate the

parasympathetic activity in DM, the variability of heart rate during inspiration and expiration should be determined using E:I ratio as previous reported (Watkins and Mackay, 1980; Subbalakshmi, et al., 2009). A study by Kenefick et al. (2007) reported that the cardiac index of parasympathetic activity (CIPA) values, which represented parasympathetic activity, was decreased in DM dogs compared with control healthy dogs. The results support that cardiac autonomic neuropathy can occur in diabetic dogs. The controversy was demonstrated in dogs with alloxan induced DM for a duration of 3 years, the QT intervals of electrocardiogram were unchanged. There is no evidence of parasympathetic autonomic neuropathy (Atkins et al., 1989). However, QT interval may be affected by many factors such as fever, heart rate, metabolic diseases especially K^+. Thus, the measurement of QT interval may not be a good index for parasympathetic control of the heart.

In this study, the reduction in total power in the poorly-controlled DM subgroup along with decreases in both LF and HF suggests the impairment of both sympathetic and parasympathetic components. The LF/HF ratio in the poorly-controlled DM was higher than the well-controlled DM subgroup suggests that the parasympathetic component may be affected more than the sympathetic component. The malfunction in autonomic nerve and baroreceptor reflex can be demonstrated by increase in both systolic and mean arterial pressures along with increased heart rates in the poorly-controlled DM compared with control healthy dogs.

Decreased in time- and frequency-domain parameters in this study was similar to the study of Porojan et al. (2010). They conducted a study in patients with type 2 DM and found the decrease in all parameters of time- and frequency-domains. These patients did not show any clinical signs of cardiac autonomic neuropathy. The NE was also lower than control healthy humans. The epinephrine concentration, similar to this study, was not different from the control DM since the dog was type I DM. Therefore, the changes in autonomic dysfunction can occur in both type 1 DM dogs and type 2 DM patients. Moreover, in DM humans and animals, the tachycardia was found along with an increased LF component and a decreased HF component in the early stage of DM (Eckberg et al., 1986; Freeman et al., 1991; Rollins et al., 1992; Task Force, 1996;

Mésangeau et al., 2000; SchÖnauer et al., 2008). The LF component seems to be declined when DM was progressed which corresponded to the poorly-controlled DM dogs in this study. These results suggested that the parasympathetic nerve may be impaired before the sympathetic component.

CHAPTER VI

CONCLUSION

This study showed the decreases in both time- and frequency-domains in poorly-controlled DM dogs. The decreased sympathetic activity was corresponded to a decrease in plasma norepinephrine concentration and the LF component resulting in higher arterial blood pressure in diabetic groups than in the control healthy non-diabetic group. The reduction in parasympathetic also presented as shown by decrease in pNN50% and HF component in the poorly-controlled DM subgroup compared with the control healthy non-diabetic group. The baroreflex parameters demonstrated reflex impairment. These changes were supported by increased in blood pressure without decreased in heart rate.

In conclusion, HRV could be a clinically and effectively noninvasive method to investigate the autonomic status in diabetic dogs. Poorly glycemic control or chronic elevation of blood glucose in diabetic dogs lead to cardiac autonomic neuropathy which is a complication of diabetes mellitus. Early detection of diabetic cardiac autonomic neuropathy in diabetic dogs using HRV and strict glycemic control could be clinically meaningful for the prevention of adverse cardiovascular outcome, slowing the onset of in cardiac autonomic neuropathy and sometimes reversing the neural impairment.

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