ผลของความผิดปกติของระบบประสาทออโตโนมิก ต่อการไหลเวียนเลือดบริเวณแขนใน ผู้ป่วยเบาหวานชนิดที่ 2

นายพิริยะ สุวรรณดิษฐ์

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EFFECT OF AUTONOMIC NEUROPATHY ON FOREARM BLOOD FLOW IN TYPE 2 DIABETES

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A Thesis Submitted in Partial Fulfillment of the Requirements

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ภาวะความผิดปกติทางระบบประสาทออโตโนมิกของระบบหัวใจและหลอดเลือด (cardiovascular autonomic neuropathy, CAN) เป็นภาวะแทรกซ้อนที่พบได้บ่อยในผู้ป่วยเบาหวาน โดยอาจส่งผลให้เกิดความผิดปกติในการควบคุมความตึงตัว และการตอบสนองของหลอดเลือดทั่วร่างกาย **วัตถุประสงค์** : เพื่อศึกษาว่าการเปลี่ยนแปลงของระบบประสาทออโตโนมิกในการควบคุมความตึงตัวของหลอดเลือดนั้นจะมีผลต่อการไหลเวียนเลือด บริเวณปลายแขนในขณะ reactive hyperemia (maxFBF) และภายหลังการออกกำลังกายหรือไม่ในผู้ป่วยเบาหวาน **ระเบียบวิธีวิจัย** : ในงานวิจัยนี้ ผู้ป่วยเบาหวานจะถูกแบ่งออกเป็น กลุ่มที่มี และไม่มีความผิดปกติของ CAN โดยแบ่งได้จากการตอบสนองของระบบหัวใจและหลอดเลือดต่อการทดสอบของ Ewing et al. 5 ขั้นตอน และได้มีการวัดอัตราการไหลเวียนเลือดบริเวณปลายแขนโดยใช้ venous occlusion plethysmograph ในภาวะ reactive hyperemia และ เมื่อออกกำลังกายเป็นจังหวะโดยใช้ handgrip exercise ที่ความหนัก 45% ของความสามารถสูงสุดที่ทำได้ รวมทั้งได้มีการวัดการไหลเวียนเลือดในกลุ่มควบคุมที่มีสุขภาพดีด้วยเช่นกัน **ผลการทดสอบ** : ในผู้ที่มีสุขภาพดีจำนวน 33 คน และในผู้ป่วยเบาหวาน จำนวน 57 คน (ชาย 24 คน, หญิง 33 คน อายุระหว่าง 40-75 ปี) แบ่งเป็น ผู้ป่วยที่มี CAN จำนวน 21 ราย และไม่มี CAN จำนวน 36 ราย พบว่า maxFBF ในผู้ป่วยที่มี CAN มีค่าสูงกว่าผู้ป่วยที่ไม่มี CAN อย่างมีนัยสำคัญทางสถิติ (27.71 ± 6.94 vs. 21.56 ± 4.17 ml/100ml/min, P=0.001) นอกจากนี้ผู้ป่วยที่มี CAN จะมีค่าความสามารถในการนำเลือดของหลอดเลือดบริเวณแขนมากกว่า (FVC, P=0.012) และความต้านทานการไหลเวียนเลือดน้อยกว่า (FVR, P=0.011) เมื่อเทียบกับผู้ป่วยที่ไม่มี CAN แต่ในผู้ที่มีสุขภาพดีจะมีค่า maxFBF, FVC มากที่สุด และ FVR น้อยที่สุด อย่างไรก็ตาม การกระตุ้นด้วย handgrip exercise นั้นยังไม่ทำให้เกิดความแตกต่างของการตอบสนองของหลอดเลือดอย่างมีนัยสำคัญ **สรุปผลการทดลอง** : ผู้ป่วยเบาหวานที่มี CAN มีการปรับเปลี่ยนการควบคุมความต้านของหลอดเลือดส่วนปลาย ซึ่งช่วยให้มีการขยายตัวมากขึ้น โดยมีค่า maxFBF เพิ่มสูงขึ้น เพราะเป็นไปได้ว่าในภาวะ CAN อาจมีกระแสประสาทซิมพาเทติกมาที่หลอดเลือดลดลง หรือมีการตอบสนองที่มากกว่าปกติต่อการกระตุ้นที่กล้ามเนื้อเรียบบริเวณหลอดเลือดให้เพิ่มการขยายตัวของหลอดเลือด

สาขาวิชา.....เวชศาสตร์การกีฬา.... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2554..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5174801030 : MAJOR SPORTS MEDICINE
KEYWORDS: EXERCISE HYPEREMIA / REACTIVE HYPEREMIA / FOREARM BLOOD FLOW

PIRIYA SUWONDIT : EFFECT OF AUTONOMIC NEUROPATHY ON FOREARM BLOOD FLOW IN TYPE 2 DIABETES. ADVISOR : ASSOC.PROF.ONANONG KULAPUTANA, M.D., Ph.D., CO-ADVISOR : PROF.NARISA FUTAKUL, M.D., Ph.D., 84 pp.

Cardiovascular autonomic neuropathy (CAN), is a common but devastating complication of diabetes, resulting in an impairment of autonomic control of vascular tone and reactivity. **Objective** : To determine whether alterations in neural control of the vascular tone contribute to changes in forearm blood flow response to exercise and transient ischemia in diabetics. **Methods** : Diabetic patients were categorized into CAN and without CAN groups. Autonomic function was assessed by five simple noninvasive cardiovascular reflex tests proposed by Ewing et al. Forearm blood flow was determined during reactive hyperemia (maximal flow) and immediately after a 5-minute rhythmic handgrip exercise at 45% of maximal voluntary contraction (submaximal flow) using venous occlusion plethysmography. A healthy control group was also studied in the same protocol. **Results** : Reactive and exercise hyperemias were measured in 33 healthy volunteers and 57 type 2 diabetes patients (24 men, 33 women, age range 40-75 years) with (n=21) and without CAN (n=36). Patients with CAN showed a significant higher maximal blood flow than patients without CAN (27.71 ± 6.94 vs. 21.56 ± 4.17 ml/100ml/min, P=0.001). Patients with CAN exhibited a higher forearm vascular conductance (FVC, P=0.012) and lower forearm vascular resistance (FVR, P=0.011) compared to patients without CAN. However, the healthy group had the greatest maximal blood flow and FVC and lowest FVR. In addition, submaximal blood flow, FVC and FVR in exercise hyperemic condition did not differ between the 3 groups. **Conclusion** : Increased maximal blood flow in patients with CAN suggests an adjustment in control of peripheral vascular resistance in favor of augmented vasodilation. Despite an impaired autonomic control, increased reactivity to reactive hyperemia may hypothetically be explained by mechanism such as decreased sympathetic vasoconstrictor tone and/or hypersensitivity to vasodilating stimulation at the level of vascular smooth muscle cells.

Field of Study..Sports Medicine...Student's Signature.....
Academic Year.....2011..... Advisor's Signature.....
Co-advisor's Signature.....

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ABBREVIATIONS	
Ach	acetylcholine
ANS	autonomic nervous system
ACE	angiotensin converting enzyme
BMI	body mass index
BSBF	basal skin blood flow
BP	blood pressure
CHF	chronic heart failure
CVD	cardiovascular disease
CAN	cardiovascular autonomic neuropathy
DAN	diabetic autonomic neuropathy
DM	diabetes mellitus
DM without CAN	type 2 diabetics without cardiovascular autonomic neuropathy
DM with CAN	type 2 diabetics with cardiovascular autonomic neuropathy
ExBF	exercise hyperemia blood flow
FBF	forearm blood flow
FVR	forearm vascular resistance
FVC	forearm vascular conductance
HRV	heart rate variability
HR	heart rate
IDDM	type 1 diabetes
L-NMMA	N^G-monomethyl-L-arginine
MVC	maximal voluntary contraction
MSNA	muscle sympathetic nerve activity
MAP	mean arterial pressure
NO	nitric oxide

LIST OF ABBREVIATIONS

ABBREVIATIONS	
PAD	peripheral arterial disease
RHBF	reactive hyperemia blood flow
SND	sympathetic nerve dysfunction
SNS	sympathetic nervous system
SNP	sodium nitroprusside

CHAPTER I

INTRODUCTION

Background and Rationale

Cardiovascular autonomic neuropathy (CAN) is a common form of autonomic dysfunction found in patients with diabetes mellitus and probably one of the most disregard of all serious complications of diabetes, causing abnormalities in heart rate control, as well as defects in central and peripheral vascular dynamics (1). CAN is found in one forth of type 1 while roughly one third of type 2 diabetic patients are reported with CAN (2). It is involved with increased mortality and silent myocardial ischemia and may even predict the progression of stroke (2). CAN also increases risk of mortality by an approximately five-fold in patients with diabetes just as in those with chronic liver diseases (3).

One major clinical manifestations of autonomic neuropathy in diabetes is neurovascular function (1). During hyperemia, the increase in myocardial blood flow (% change from baseline) was slightly greater in the diabetics with sympathetic nerve dysfunction (SND) than those without SND (4). Additionally, flow-mediated brachial artery dilation was greater in the diabetics with SND compared with those without SND although no statistical difference was detected(4). When compared with healthy volunteers, coronary hyperemia and brachial artery diameter were lower in diabetes (4). Thus, these data suggest that abnormal vasoactivity progresses early in the course of diabetic neuropathy (4). Additionally, its severity is related to the degree of CAN (4). These findings also demonstrate that cardiac sympathetic signals play a vital role in adjusting myocardial blood flow during periods of stimulation of the sympathetic nervous system, such as exercise (4). Myocardial ischemia and left ventricular dysfunction can be induced by the defective dilator response of resistance vessels during times of

increased oxygen demand such as exercise, despite a lack of overt coronary atherosclerosis (4).

Nowadays, the caliber of the resistance vessels response to arterial occlusion was a crucial clinical parameter to assess endothelial function. Forearm blood flow measured by venous occlusion plethysmography under resting conditions, consists of blood flow from skeletal muscle roughly ~70% of total forearm blood flow (FBF), with skin blood flow accounting for most of the remainder (5). There was a close relation between coronary arteries in patients with cardiac risk factors and flow-mediated vasodilation in the brachial artery or brachial artery diameter (6). Therefore, the detection of abnormal vascular reflex in brachial artery that might be caused by endothelial or neurovascular dysfunction, might become a useful surrogate in assessing the predisposition to coronary atherosclerosis and advanced stage of CAN in patients with cardiac risk factors.

Vasoconstrictor and vasodilator influences are known for their acting on arteries and veins, determining their state of vascular tone. Tonicity of a blood vessel reflects the balance between intrinsic factors such as myogenic mechanisms, endothelial factors (i.e., nitric oxide and endothelin) and metabolic by-products or hypoxia, and extrinsic factors such as sympathetic nerves and circulating angiotensin II (7, 8). This balance between intrinsic and extrinsic factors enable the body to direct blood flow to areas where the need is greater and to divert it away from areas where the need is less during exercise (8).

The increase in heart rate (HR), blood pressure (BP), and peripheral vasoconstriction during exercise is a consequence of the activation of sympathetic nervous system (SNS). As part of this process, renal vasoconstriction occurs and attends to sustain BP as well as to rearrange blood flow to the contracting skeletal muscle bed (9). During exercise, activation of SNS is mediated by two major control mechanisms (10-12): *1*) central command, which refers to a signal arising from within the central nervous system that is linked to the perceived effort of exercise; and *2*) the

exercise pressor reflex, mediated by sensory nerve endings within the skeletal muscle that, when stimulated during exercise, results in a reflex initiation of central sympathetic outflow. These sensory nerve endings include the metaboreceptors and mechanoreceptors. Mechanoreceptors are largely activated by mechanical stretch while the metaboreceptors are sensitized by ischemic metabolites generated during exercise but it is possible that largely negated by metabolic vasodilation, thereby optimizing muscle perfusion, initially termed “functional sympatholysis” (12, 13).

Tissue metabolic activity or metabolic mechanism is the main factor in acute control of local blood flow. The chemical effects acting directly on the muscle arterioles to cause dilation (exercise hyperemia) result in the tremendous increase in muscle blood flow during skeletal muscle activity. Rhythmic handgrip at 45% maximal voluntary contraction (MVC) is accompanied by increases in muscle sympathetic nerve activity (MSNA) due to activation of the muscle metaboreflex, mechanoreflex, and central command (11, 13). This exploratory test with exercise stimuated-hyperemia may disclose some effect of autonomic neuropathy in patients with type 2 diabetes on forearm blood flow.

Another phenomenon of vasodilation that may reveal some effect of autonomic neuropathy is blood flow immediately after the release of the occlusion exceeding the flow before the occlusion, called reactive hyperemia (8). The well accepted mechanism controlling blood flow in reactive hyperemia includes metabolic and myogenic autoregulation (7). Metabolic products of arterial occlusion may activate some metaboreceptor muscle afferents that reflexively increase efferent-sympathetic vasoconstrictor discharge (13) and effect on blood flow too. In healthy humans, the muscle metaboreceptors are principal in generating the reflex increases in SNS activity during static exercise (13, 14). However, patients with chronic heart failure, a disease state accompanied with a baseline SNS overactivity and exercise intolerance, reveal abnormalities of the exercise pressor reflex (15). Such abnormalities are characterized by a blunted metaboreceptor activation of SNS activity during exercise (15). Recently

study show that during posthandgrip circulatory arrest, MSNA remained elevated in controls but decreased rapidly to baseline levels in end-stage renal disease, indicative of markedly blunted metaboreceptor control of MSNA (11).

Vascular function has been assessed invasively by a number of studies. In a study that stimulated forearm blood flow with intra-arterial injection of acetylcholine and sodium nitroprusside (endothelium-dependent vasodilator and endothelium-independent vasodilator, respectively), it was found that the FBF inversely correlates with various indicators of autonomic dysfunction (16). As the increasing blood flow increased through the group with more severity of autonomic dysfunction in patients with type 1 diabetes, they were complicated with a leak of albumin in the urine (macroalbuminuria) (16). Besides, the study of basal skin blood flow (BSBF) and its dynamic components found a higher BSBF in diabetic patients with autonomic neuropathy than those without autonomic neuropathy and healthy control subjects (17).

Due to the fact that autonomic nervous system is an important part in controlling the systemic vascular resistance by affecting the sympathetic vasoconstrictor tone. It is hypothesized that in diabetes when cardiovascular autonomic neuropathy, a neurologic complications manifests, the mechanisms that control blood flow in the acute phase may be affected too. This condition may cause alterations in forearm blood flow (FBF) during hyperemia in response to certain stimuli including exercise as well as arterial occlusion. The main purpose of this study is to search for these changes using a noninvasive method of reactive hyperemia and exercise hyperemia. These techniques are relatively easier and safer than injecting chemicals into the blood vessels to assess their function.

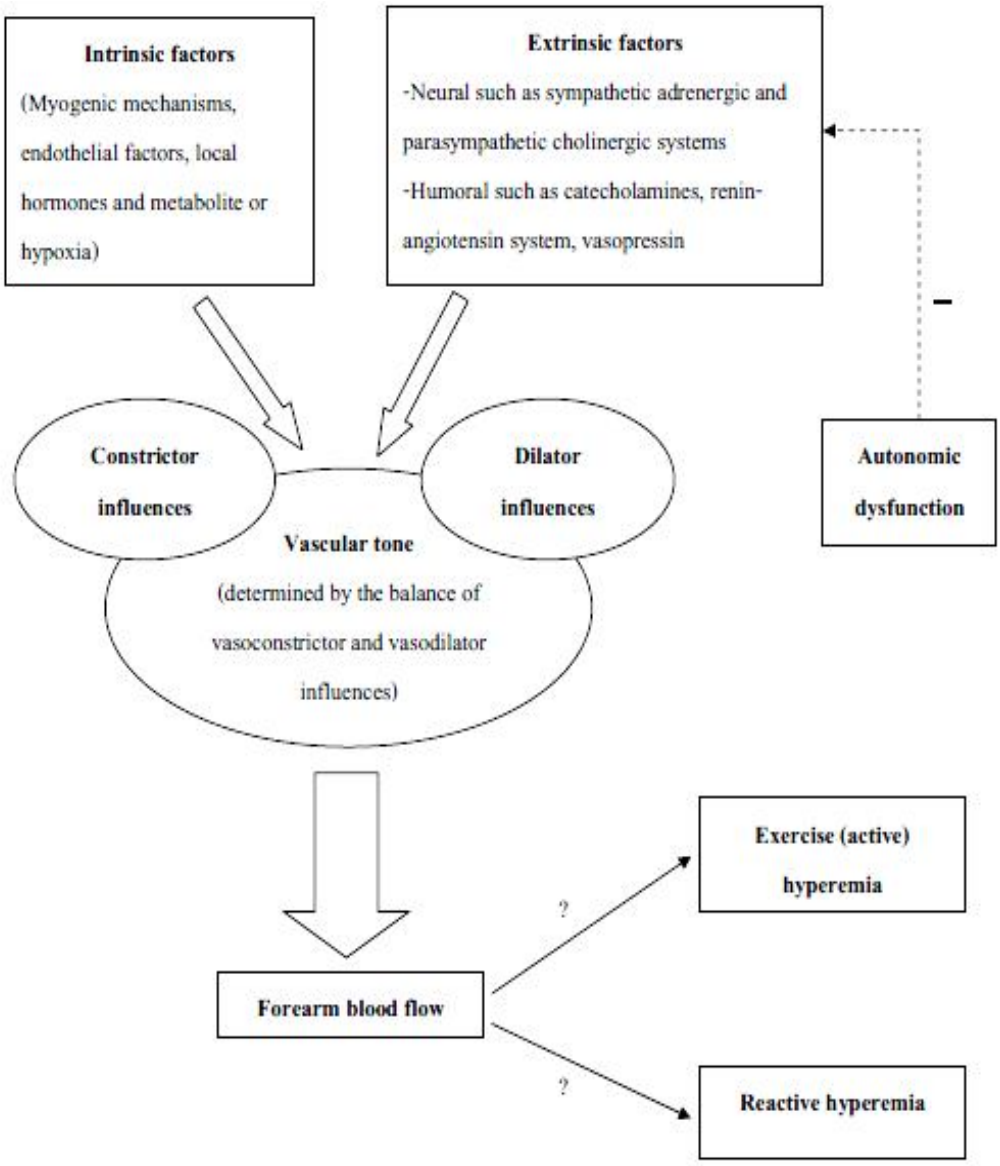
Research question

Does autonomic neuropathy in patients with type 2 diabetes affect forearm blood flow ?

Objectives

To study forearm blood flow in response to exercise and transient ischemia in type 2 diabetic patients with and without autonomic neuropathy.

Conceptual framework



Hypothesis

1. Forearm blood flow during exercise hyperemia in type 2 diabetic patients with autonomic neuropathy differ from those without autonomic neuropathy.

2. Forearm blood flow during reactive hyperemia in type 2 diabetic patients with autonomic neuropathy differ from those without autonomic neuropathy.

Scope of research

This is a cross sectional analytic design study in which the healthy individuals and diabetics with and without autonomic neuropathy participated as subjects.

The study approval was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University. Written inform consent was obtained from each subject before the experiment started. All subjects were given a briefing on the experimental procedure and risk involved, and reminded of their right to withdraw at any stage.

Assumptions

1. The equipment was calibrated to meet the standard of accuracy and reliability.

2. All volunteers participated as subjects in this study with voluntariness.

Limitations

1. The possible confounding factors included age, hypertension, severity of diabetes. These are minimized by appropriate inclusion and exclusion criteria.

2. Some patients with diabetes received medications for hypertension that may affect vascular function such as calcium channel blockers and ACE inhibitors for some period of time. However, all of which were withheld for 24 hours before the study.

3. Plethysmographic measurement of flow is not continuous, it takes a few seconds to obtain the first hyperemia flow, so that the first highest peak may not necessarily be the true maximum hyperemia flow, and minimum forearm vascular resistance (FVR).

Operational definition

1. Forearm blood flow is defined as the blood flow rate measured at the non-dominant forearm using venous occlusion strain gauge plethysmography.

2. Exercise hyperemia is the increase in organ (forearm) blood flow after 5 minutes of rhythmic handgrip exercise at 45 % of maximum voluntary contraction.

3. Reactive hyperemia is the transient increase in organ (forearm) blood flow that occurs following 5 minutes of arterial occlusion.

4. Cardiovascular autonomic neuropathy (CAN) results from damage to the fibers of the autonomic nervous system (ANS) with associated abnormalities of heart rate control and vascular dynamics (1) and defined by the standard battery of cardiovascular reflex tests (18-20) (score 0-0.5=normal, 1-2.5=mild CAN and $\geq$ 3= severe CAN)

Expected benefits and applications

1. Understanding vascular responses during reactive and exercise hyperemias in diabetic patients with and without autonomic neuropathy.

2. Providing the preliminary data for further research.

CHAPTER II

REVIEW LITERATURES

Diabetes is a common cause of neuropathy that involves motor, sensory, and autonomic nerve fibers. Additionally, the autonomic nervous system (ANS) regulates individual organ function and homeostasis not under voluntary control. An efferent and afferent system of the ANS transmit impulses between the central nervous system and peripheral organ system. This results in control of heart rate and force of contraction, constriction and dilatation of blood vessels, contraction and relaxation of smooth muscle in various organs, visual accommodation, papillary size, and secretions from exocrine and endocrine glands. The ANS is also responsible for conveying visceral sensation. The ANS is typically divided into two divisions: the parasympathetic and the sympathetic systems on the basis of anatomical and functional differences (1). When diabetes affects the ANS, it may alter function in the cardiovascular system, where autonomic regulation of both the heart and peripheral circulation may be affected, leading to a wide range of disorders.

Diabetic autonomic neuropathy (DAN) is a serious and common complication of diabetes and frequently happen together with other diabetic complications, but DAN may be isolated, frequently preceding the detection of other complications (1). One common form of DAN is cardiovascular autonomic neuropathy (CAN) resulting from damage to the fibers of the ANS with associated abnormalities of heart rate control and vascular dynamics. The sympathetic-vagal balance modulates the function of three of the main cardiovascular system textures: the sinus node (heart rate), the ventricles (end-systolic and end-diastolic volumes) and the blood vessels, including microcirculation (total peripheral resistance). Also, CAN is the most studied and clinically important form of DAN (1).

Pathogenesis of DAN

There are excellent pathogenesis reviews of DAN in the literature (1, 3). Briefly, the hypotheses concerning the multiple etiologies of diabetic neuropathy include a metabolic insult to nerve fibers, neurovascular insufficiency, autoimmune damage, and neurohormonal growth factor deficiency. The result of this multifactorial process may be stimulation of polyADP ribosylation depletion of ATP, resulting in cell necrosis and activation of genes involved in neuronal damage.

It should be noted that neuropathies accompanying type 1 and type 2 diabetes are different. Etiological factors other than hyperglycaemia seems to be more important in patients with type 2 diabetes (21). Comparing 11 patients with type 1 and 17 with type 2 diabetes, Sima et al. (22, 23) suggested that the pattern of nerve fiber loss seemed similar although in type 2 diabetes wallerian degeneration was more prominent with a more obviously patchy loss of fibers, perhaps supporting a microvascular caused neuropathy .

Prevalence and risk factors

CAN seems to be more prevalent and progress rapidly in type 2 than type 1 diabetic patients, appearing earlier and causing higher mortality rates. It appears to be due to the extended period of time being exposed to abnormal metabolism particularly hyperglycemia. This is likely happening during the conditions of prediabetic and metabolic syndrome which exists a long time prior to the diagnosis of type 2 DM (1, 24). However, subclinical autonomic dysfunction (in which functional and reversible alterations are predominant) can occur within a year of diagnosis in type 2 diabetes and within two years in type 1diabetes (25).

The increasing prevalence of CAN positively correlates with age, duration of DM and poor glycemic control. In type 2 diabetes, the following risk factors

are associated with a reduced heart rate variation: age, obesity, hyperinsulinemia, diabetic duration, hypertension, retinopathy, diabetic polyneuropathy and smoking (24), suggested that autonomic neuropathy was associated with an increased cardiovascular risk (3).

In patients with diabetes, it was shown that patients with CAN had five times higher the 5-year mortality rate than individuals without CAN. Moreover, the reduced cardiovascular autonomic function as measured by heart rate variability (HRV) is strongly (i.e., relative risk is doubled) associated with an increased risk of silent myocardial ischemia and mortality (1). Meta-analysis of eight studies showed that mortality after 5–8 years in diabetic patients with CAN was 29%, while it was 6% in those without CAN (3). Fifteen studies involving a total of 2,900 diabetic patients with and without CAN were reviewed in this meta-analysis (26). During the follow-up period, which ranged from 0.5 to 16 years, mortality rates were invariably higher in patients with CAN (30%) than in those without CAN (13%). The study of type 2 diabetic patients to ascertain risk factors associated with the incident of strokes in type 2 diabetes over a 5-year follow-up period manifested that DAN is a significant independent risk factor for the occurrence of stroke. The incidence may have been ascribed to enhanced damage rate of cerebral vessels and alterations in the cerebral blood flow control in diabetic patients with DAN (27).

Clinical Manifestations of CAN

There are common clinical manifestations of diabetic autonomic neuropathy. Those clinical signs include resting tachycardia, exercise intolerance, orthostatic hypotension, orthostatic tachycardia and bradycardia syndromes, intraoperative and perioperative cardiovascular instability, silent myocardial ischemia/cardiac denervation syndrome. Also with DAN which is typically assessed by focusing on symptoms or dysfunction attributable to a specific organ system such as constipation, gastroparesis, erectile dysfunction, sudomotor dysfunction, impaired neurovascular function, and potentially, autonomic failure in response to hypoglycemia

(28). Neurovascular dysfunction resulting from DAN contributes to a wide spectrum of clinical disorders including loss of skin integrity, erectile dysfunction and abnormal vascular reflexes. Generally, clinical symptoms of autonomic neuropathy do not occur until long after the onset of diabetes. Whereas symptoms suggestive of autonomic dysfunction may be common they may frequently be due to other causes rather than to true autonomic neuropathy.

When classified the autonomic neuropathy by clinical features, DAN can be identified as symmetrical polyneuropathy which was the most common form of diabetic neuropathies. DAN regularly occurs as a system-wide disorder affecting all parts of the ANS. In fact, because the vagus nerve (the longest of the ANS nerves) accounts for approximately 75% of all parasympathetic activity, and DAN manifests first in longer nerves (length-dependent pattern), even early effects of DAN are widespread (1). There is a close correlation between the changes in microcirculation and diabetic neuropathy. Moreover, sympathetic impairment may dilate microvasculars leading to arteriovenous shunting (29) and the rhythmic contraction of arterioles and small arteries is disordered (1). The study of basal skin blood flow (BSBF) and its dynamic components found that higher BSBF in diabetic patients with autonomic neuropathy than without it and healthy control subjects (17). On the other hand, microcirculatory changes can participate in the ischemic etiology of diabetic neuropathy. These also may affect forearm blood flow in type 2 diabetes with CAN.

Cardiovascular autonomic neuropathy tests

Practically, the clinical diagnosis and staging of CAN using simple and noninvasive autonomic tests which are reproducible and sensitive for both branches of the autonomic nervous system is indicated in the Table 2.1. The evaluation should be based on the results of a battery of autonomic tests rather than one single test. There are currently two standardized methods for this purpose.

Table 2.1 Diagnostic assessment of cardiovascular autonomic function (2)

Parasympathetic	Sympathetic
1. Resting heart rate	1. Resting heart rate
2. Beat-to-beat variation with deep breathing (E:I ratio)	2. Spectral analysis of heart rate variation, very- low-frequency power (VLFP; 0.003–0.04 Hz)
3. 30:15 Heart rate ratio with standing	3. Orthostasis blood pressure
4. Valsalva ratio	4. Hand grip blood pressure
5. Spectral analysis of heart rate variation, high-frequency power (HFP; 0.15–0.50 Hz)	5. Cold pressor response
	6. Sympathetic skin galvanic response (cholinergic)
	7. Sudorometry (cholinergic)
	8. Cutaneous blood flow (peptidergic)

Computerized heart rate variability (HRV) study

Heart rate variability, a noninvasive electrocardiographic marker refers to the variations in the beat intervals or correspondingly in the instantaneous HR, is a reliable reflection of the activity of the sympathetic and vagal components or the status of the autonomic nervous system on the sinus node of the heart (balanced sympthovagal state). HRV can be assessed either by calculation of beat-to-beat indices represent fast changes in HR based on statistical analysis of R-R intervals (time domain analysis, it reveals the total amount of variations of both instantaneous heart rate and RR intervals) or by spectral analysis (frequency domain analysis) of an array. The result of power spectrum can be displayed with the magnitude of variability as a function of frequency. In other words, the power spectrum reflects the amplitude of the heart rate fluctuations (Y axis) present at different oscillation frequencies (X axis). The power spectrum peaks of HRV that mediated primarily by the sympathetic and

parasympathetic system has been shown in table 2.1. However, spectral analysis is carried out under resting conditions, it has the advantage that active cooperation of the patient is not required. Many factors may influence the test results: age, HR, respiratory rate, BP, eating, drinking coffee, smoking, body position, volume status, mental stress, drugs, exercise, and time of day. Also, it requires computerized equipment and coupled mathematical software. (2, 24).

Recently, seven parameters and commercially available computer programs (eg, Neuro-Diag II, Ansar) are used for the early detection of CAN, but conventional ECG equipment can also be used (2):

1) Coefficient of variation of R-R intervals or spectral power in the HF band at rest;
2) Spectral power in the very-low-frequency band;
3) Spectral power in the LF band associated with the baroreceptor reflex;
4) HRV during deep breathing, including mean circular resultant of vector analysis or expiration/inspiration ratio (E:I ratio);
5) Orthostatic test (maximum/minimum 30:15 ratio);
6) Valsalva ratio; and
7) Postural change in systolic blood pressure.

The age-related normal ranges of the 7 indices included in this battery have been determined.

Ewing tests (The standard battery of cardiovascular reflex tests)

The most commonly used battery of non-invasive tests for assessment of cardiovascular reflexes (cardiovascular reflex tests) was proposed by Ewing and Clarke (18, 30, 31) which included heart rate variation in response to deep breathing, standing and Valsalva maneouvre to assess cardiac parasympathetic activity, as well as blood pressure responses to standing and sustained handgrip to evaluate sympathetic nervous activity. These five tests are validated, reliable and reproducible, correlate with

each other and with tests of peripheral somatic nerve function and are of prognostic value (3). These tests still form the core of diagnosis of CAN. Normal, borderline and abnormal values (18, 31) of the five standard cardiovascular reflex tests are summarised in table 3.1.

In keeping with the recommendations of the American Diabetes Association and American Academy of Neurology (1, 24), the five tests for outpatients classically described by Ewing, three (deep breathing test, Valsalva maneuver and orthostatic test) are currently recommended and used to assess cardiovascular autonomic function for type 2 diabetes at the time of diagnosis, and for type 1 diabetes five years after the diagnosis. After the first survey, these tests should be yearly repeated. These three tests have a good reproducibility, specificity higher than 91% and sensitivity from 93% (deep breathing and orthostatic test) to 98% (Valsalva).

The standard battery of cardiovascular reflex tests might be less influenced by the mention above confounders (such as age or the severity of the underlying cardiovascular disease) because they are combined with a stimulus increasing either the activity of the parasympathetic and sympathetic nervous system. Moreover, HRV in spectral analysis did not detect cardiac autonomic neuropathy in older diabetes patients (55±10 years) better than the standard battery of cardiovascular reflex tests (20). Though these methods are used widely, they may be associated with difficulties in patient compliance, particularly those who are elderly.

All cardiovascular tests should be performed in the morning, under fasting conditions, with a capillary blood glucose level lower than 180 mg/dl and all cardiovascular medication, anxiolytics, antidepressants, caffeine and decongestants discontinued for at least eight hours and optimally 24 hours before (because it will depend on the half-life of each drug in particular). Normal values always depend on the age range of the patient and are standardized.

Mechanical response of the standard battery of cardiovascular reflex tests (31)

Ewing et al. proposed 5 simple noninvasive cardiovascular reflex tests that have been applied successfully by many investigators. The clinical literature has consistently identified these 5 tests as they have been widely used in variety of studies (1, 4, 16, 17, 19, 32, 33).

Heart rate variation during deep breathing

Normally the heart rate varies continually but this depends on an intact parasympathetic nerve supply. The variation is abolished with atropine (pharmacological blockade of the vagus nerve) but uninfluenced by propranolol (sympathetic blockade) and is more pronounced at slow heart rates, during deep breathing, and in younger patients. Diabetics with autonomic neuropathy may have a noticeable reduction in, and sometimes complete absence of, heart-rate variation during deep breathing or deep inspiration.

Heart rate response to Valsalva maneuver

During the strain period of the Valsalva manoeuvre the blood pressure drops and the heart rate rises (tachycardia) and peripheral vasoconstriction. After release the blood pressure rises, overshooting its resting value, and the heart slows (bradycardia). Though these reflex changes are complex, Pharmacological blockade studies demonstrate the drugs' varied effects of attenuation or augmentation of the hemodynamic response to the maneuver at specific times during the response, suggesting that it is mediated by dual involvement of autonomic nerve branches. In patients with autonomic damage (the reflex pathways are damaged) the blood pressure slowly falls during strain and slowly returns to normal after release, with no overshoot rise in blood pressure and no change in heart rate.

Immediate heart rate response to standing

During the change from lying to standing a characteristic immediate rapid increase in heart rate occurs which is maximal at about the 15th beat after standing. This is followed by a relative overshoot bradycardia that is maximal at about the 30th beat. This response is mediated by the vagus nerve (blockade achieved with atropine). Diabetics with autonomic neuropathy show only a gradual or no increase in heart rate after standing.

Blood pressure response to standing

On standing pooling of blood in the legs causes a slightly fall in blood pressure, which is normally rapidly corrected by baroreflex-mediated peripheral vasoconstriction and tachycardia by sympathetic nerve fibers. In patients with autonomic damage the systolic blood pressure falls on standing and remains lower than in the lying position.

Blood pressure response to sustained handgrip

During sustained handgrip a sharp rise in blood pressure occurs, due to a reflex arc from the exercising muscle to central command and back along efferent fibers. The efferent fibers innervate the heart and muscle, resulting a heart-rate-dependent increase in cardiac output with unchanged peripheral vascular resistance. Should the normal reflex pathways be damaged, as in diabetics with extensive peripheral sympathetic abnormalities, the rise in diastolic blood pressure is abnormally small.

Control of blood flow

Muscle blood flow (34, 35)

The blood flow to skeletal muscle, like that to any other tissue or organ, depends primarily on the perfusion pressure and the caliber of the resistance vessels.

Changes in caliber are regulated by chemical and physical changes that originate within (local) and in the immediate environment of the resistance vessels, by alterations in activity of the nerves to the vessels, and by circulating vasoactive agents (humoral).

In resting muscle most of the flow circulates through exchanging blood vessels. The blood flow to the human forearm and calf muscles is approximately 3-5 ml/100ml/min. The control of blood flow based on metabolic mechanisms (vasodilator and oxygen lack theory) is a crucial regulation of local blood flow, likewise a forearm blood flow. For example, two spontaneous local regulation of blood flow was shown below.

1. **Reactive hyperemia** is the transient increase in organ blood flow (hyperemia) that occurs following a brief period of ischemia (e.g., arterial occlusion). It has also been studied widely using venous occlusion plethysmography. The traditional concept is that both metabolic (substances that accumulate during period of circulatory arrest) and myogenic autoregulation contribute to reactive hyperemia. Early studies showed that (36-38), as the period of ischemia increased up to 5 min, the peak forearm blood flow response after the restoration of flow increased and found that during reactive hyperemia, forearm blood flow can increase up to 10-15 times from baseline measurement. When the longer the period of occlusion (less than 5 minutes), the greater the metabolic stimulus for vasodilation leading to increases in peak reactive hyperemia and duration of hyperemia. Depending upon the organ, maximal vasodilation as predicted by peak blood flow, may occur following less than one minute (e.g., coronary circulation) of complete arterial occlusion, or may require several minutes of occlusion (gastrointestinal circulation). If the period of ischemia was longer than about 5 minutes, there was little further increase in peak flow, but the increase is mainly evident in the duration of the high flows (7). Moreover, an ischemia period longer than four and a half minutes does not lead to a further increase in forearm blood flow (39). This implicates a vasodilator substances whose concentration increases with time. The reactive hyperemia subsides exponentially, in keeping with a wash out or conversion of

vasodilator metabolites. In addition venous blood collected during reactive hyperemia has vasodilator properties too (40).

During arterial occlusion, tissue hypoxia and an accumulation of vasodilator metabolites (e.g., adenosine) dilate arterioles and decrease vascular resistance. Several studies suggest that endogenous nitric oxide (NO) plays only a minor role in vasodilation during reactive hyperemia, and that reactive hyperemia is mostly caused by endothelium related mechanisms other than NO, such as adenosine, prostaglandins, and endothelium derived hyperpolarizing factor (41). Nevertheless, there are reports that NO may provide significantly to the late stage of reactive hyperemia (42), as well as to the peak hyperaemic flow (43). It was recently shown that inhibition of NO production by N^{G}-monomethyl-L-arginine (L-NMMA) infusion significantly decreases peak hyperaemic flow as well as the total hyperaemic flow in flow-time curves by 30-50% (43). Furthermore, myogenic mechanisms may also contribute to reactive hyperemia in some tissues. By this mechanism, arterial occlusion results in a reduction of pressure downstream in arterioles, which can contribute to myogenic-mediated vasodilation (35). In additional, the mechanical forces originated by rapid cuff inflation to obstruct blood flow might cause an increase in BP through an increase in total peripheral resistance. The increase in BP could activate the baroreceptors and consequently inhibit muscle sympathetic nerve activity (MSNA) (44)

When perfusion pressure is restored (immediately after released the occlusion cuff), blood flow rapidly elevated (peak or maximum forearm blood flow) that lasts for several minutes because of the reduced vascular resistance (minimum forearm vascular resistance). During the hyperemia, the tissue becomes reoxygenated and vasodilator metabolites are washed out of the tissue. This causes the resistance vessels to recover their normal vascular tone, thereby returning flow to control.

Interestingly, it was also demonstrated that the total flow during the hyperemic period (total flow) was far in excess of that demanded to repay any metabolic debt (flow debt) occured during the ischemia (reactive hyperemia) (7).

2. Exercise hyperemia (7, 35) is the increase in organ blood flow (hyperemia) that is associated with increased metabolic activity of an organ or tissue. An example of active hyperemia is the increase in blood flow that accompanies muscle contraction, which is also called exercise or functional hyperemia in skeletal muscle. Blood flow increases because the increased oxygen consumption during muscle contraction (tissue hypoxia) activates the production of vasoactive substances or vasodilator metabolites that dilate the resistance vessels in the skeletal muscle such as adenosine, carbon dioxide, NO and potassium ion . The magnitude of exercise hyperemia responses vary among organs because of the relative changes in metabolic activity from rest and their vasodilatory capacity. Exercise hyperemia can result in up to a 30-50 fold increase in muscle blood flow with maximal exercise (depending on the type of exercise and which parts of the body to be measured or the difference in muscle mass), whereas cerebral blood flow may only increase 2-fold with increased neuronal activity.

The comparison between exercise and reactive hyperemia mechanisms (35)

When compared the forearm blood flow in the human after a period of circulatory occlusion and after exercise of the forearm muscles, with both conditions adjusted so that the initial peak blood flow are similar, the flow after the exercise remain above the resting level for a much longer period.

With prolonged circulatory arrest in the human there is a local release of histamine, but this does not occur with exercise, provided the muscle blood flow is permitted to increase normally. In additional, during circulatory arrest the resultant hypoxia may provide a greater opportunity for ATP to cross the skeletal muscle cell membranes and so participate in the relaxation of the muscle resistance vessels. The myogenic response and endogenously formed prostaglandins also seem to have a more assured role in reactive than exercise hyperemia. With intense exercise of the muscles, both dynamic and static, presumably the resultant mechanical hindrance to

flow will cause additional metabolic and other changes similar to those that occur during artificial occlusion of the blood supply.

The assessment of reactive hyperemic blood flow

Diabetes mellitus is a strong risk factor for endothelial dysfunction and likely to develop atherosclerotic vascular disease such as cardiovascular disease (CVD), coronary artery disease, myocardial infraction and cerebrovascular disease etc (45, 46). Therefore, to identify these patients before the onset of CVD, ankle-brachial index (ABI) is generally used as a predictive marker of CVD (47, 48). However, the study that evaluated baseline total flow volume at the popliteal artery using gated two-dimensional cine-mode phase-contrast magnetic resonance imaging (MRI) found that type 2 diabetic patients with low total flow volume (< 62.7 ml/min) increased probability of developing CVD events (hazard ratio=8.60) when compared with type 2 diabetic patients with high total flow volume (> 85.5 ml/min), while both groups had normal ABI (> 0.9) (47).

The arterial stress testing used to determine the presence and severity of lower extremity arterial disease. Also, postocclusion reactive hyperemia should probably replace treadmill exercise as the first method of stress testing lower extremities (49). Due to, the resting blood flow in patients with limb vascular dysfunction (moderate claudication) using venous occlusion plethysmography were normal or no significant difference. Thus, the assessment of reactive hyperemic blood flow which the period of occlusion causes tissue hypoxia and a build up of vasodilator metabolites would increase sensitivity to detect peripheral arterial disease (PAD) or other disorders.

There were not much difference in resting blood flow between the PAD patients with normal subjects. Although, when compared the first flow, peak flow, time of peak flow and duration of hyperemia that response to arterial occlusion (reactive hyperemia) in normal subjects and PAD patients revealed that the PAD patients were delay in peak flow or not the same value with the first flow. Besides, peak flow was a

greatly decreased when compared with normal subjects. The recovery time for basal flow was prolonged after exercise and reactive hyperemia (50).

Flow-mediated endothelium-dependent dilation (reactive hyperemia) was impaired in type 2 diabetes mellitus and it was further greatly decreased in patients with peripheral vascular disease. Percent flow change after responsing to reactive hyperemia in Diabetics with and without peripheral vascular disease were 364 ± 67 % ad 440 ± 64 % respectively and the normal subjects were 535 ± 70 %. In addition, nitroglyerin-induced endothelium independent dilatation showed a trend of impairment in patients with peripheral vascular disease but did not reach statistical significance (46). So, the maximal vasodilator and minimal vascular resistance in limb blood flow that response to transient ischemia reflecting the endothelial function were a useful prognostic marker in coronary artery disease patients (39, 42). This approach has proved substantial in assessing structural as opposed to vasomotor changes in the circulation in conditions such as hypertension and heart failure (7).

The maximal forearm blood flow responses during reactive hyperemia using strain-gauge plethysmography was used to study the effects of long-term (12 weeks) moderate aerobic exercise (brisk walking) on endothelial function in patients with essential hypertension found that improved reactive hyperemia, an index of endothelium-dependent vasorelaxation (not vascular smooth muscle), through an increase in release of nitric oxide (NO) . Basal forearm blood flow did not differ with exercise (36).

Ishibashi et al (38) revealed that The number of cardiovascular risk factors significantly correlated with the duration of reactive hyperemia in both men ($r = -0.56$, $p<0.001$). Reactive hyperemia was measured following 5-min occlusion of the upper arm in 449 healthy subjects. Maximum blood flow and minimum vascular resistance in reactive hyperemia did not differ between subjects with and without cardiovascular risk factors regardless of gender but duration of reactive hyperemia was significantly shorter in subjects with risk factors. For explain these, they showed that

reduction of endogenous adenosine, as well as prostaglandin, might decrease reactive hyperemia, and that this attenuation was caused by both a reduction of maximum flow and a shortening of the duration of hyperemia. Also, there was a constant trend toward a short duration of reactive hyperemia with an increasing number of risk factors clustering in a single individual of either gender ($r=0.56$ in men, $r=0.62$ in women, $p<0.001$ for both).

The assessment of exercise hyperemic blood flow

Arnold JM et al (51) studied local forearm blood flow (FBF) during exercise in patients with chronic heart failure (CHF) which was often a muscle fatigue symptom caused by decreased in nutritive blood flow, changed in muscle metabolism, and histological muscle fiber atrophy have been implicated. 13 patients with severe CHF and eight normal untrained subjects of similar age were performed intermittent forearm static exercise by squeezing a hand grip dynamometer for 5 seconds, three times per minute, for 5 minutes at 15%, 30%, and 45% of maximum voluntary contraction. Forearm blood flow was measured at baseline before exercise and during the last 3 minutes of each exercise stage. Exercise was repeated after 24 hours of intravenous administration of milrinone in the patients with CHF. Twenty-four hours of intravenous milrinone administration increased FBF at baseline and during exercise, also with forearm vascular resistance but did not significantly alteration (compared with before administration of milrinone and with normal subjects). However, baseline FBF before and after administration of milrinone in patients with CHF still significantly lower than the normal subjects (51).

Effect of autonomic nervous system on exercise blood flow

Exercise hyperemia can also be affected by competing vasoconstrictor mechanisms. For example, the maximal skeletal muscle hyperemia can be reduced by

sympathetic activation during exercise compared to what would happen in the absence of sympathetic activation.

During exercise, activation of skeletal muscle fibers by somatic nerves results in vasodilation and functional hyperemia. Sympathetic nerve activity is crucial to vasoconstriction and the preservation of systemic arterial pressure (52). The accumulation of chemical metabolic products of contraction in skeletal muscle such as H^+ activate chemically sensitive skeletal muscle afferents that reflexively increase efferent-sympathetic vasoconstrictor discharge. This reflex mechanism (termed the "muscle metaboreflex") has been shown to trigger parallel sympathetic activation in resting and exercising human skeletal muscle. Moreover, the reflex from mechanoreceptor that was stimulated immediately at the start of exercise might be responsible too (12, 13, 52), but the resultant effect of this reflex-sympathetic activation on muscle blood flow remains poorly understood.

Muscle blood flow increases in proportion to the intensity of activity despite accompanying increases in sympathetic neural discharge to the active muscles, indicating a reduced responsiveness to sympathetic activation (52). In other words, sympathetic vasoconstriction in contracting muscle might largely abolish by metabolic vasodilation, therefore optimizing muscle perfusion. This concept, initially termed "functional sympatholysis" recently has been extended by reductionist microcirculatory preparations describing that some local metabolic consequences of contraction (e.g., intramuscular acidosis, hypoxia) intervene with specific signal transduction pathways mediating alpha-adrenergic vasoconstriction (12, 13).

The study of vascular function in diabetic autonomic neuropathy

There was an altered neurotransmission in type 1 diabetes (IDDM) with autonomic dysfunction or decreased sympathetic vasoconstrictor tone that was the key role in the control of vascular reactivity, might contribute to blood flow responses to intra-brachial infusions of endothelial dependent vasodilation (acetylcholine) and endothelium

independent vasodilation (sodium nitroprusside). IDDM with macroalbuminuria exhibited hyperresponsiveness in forearm blood flow to both acetylcholine (Ach) and sodium nitroprusside (SNP) compared with the patients with normoalbuminuria or normal subjects. Also, Reflex sympathetic vasoconstriction to cold was severely impaired in the IDDM patients with macroalbuminuria compared with the patients with normoalbuminuria and normal subjects (16).

In the study that exercised in the supine position by pedalling a cycle ergometer at workloads of 25, 50 and then 75 W, each for 3 min. The patients with peripheral autonomic dysfunction shown that there was a difference in the response of hemodynamic and catecholamine compared with normal subjects and IDDM. With exercise, It was found that heart rate increased, blood pressure decreased, cardiac index slightly increased and systemic vascular resistance greatly decreased, whereas Plasma noradrenaline slightly increased (53).

It was possible that exercise-induced reflex sympathetic activation could limit skeletal muscle exercise performance. This could occur through a sympathetically induced vasoconstriction limiting oxygen and other substrate delivery to the working muscle or a direct effect on muscle bioenergetics. Kardos et al (54) mentioned sympathetic denervation of the upper limb significantly improves forearm skeletal muscle bioenergetics and exercise performance, exercise duration increased in 11 patients that performed rhythmic handgrip exercise at 30% of their maximal voluntary contraction at 40 pulls/min until exhaustion, in patients with idiopathic palmar hyperhidrosis after 4-7 weeks of thoracoscopic sympathetic trunkotomy (TST) on right forearm. In addition, right TST significantly increased resting forearm blood flow (FBF) and decreased forearm vascular resistance (FVR) in the right forearm but not in the left. In 5 patients, right FBF was also determined at the end of each minute of rhythmic handgrip and found that FBF at peak exercise was significantly higher after TST, and FVR was lower too (54).

CHAPTER III

RESEARCH METHODOLOGY

Research design

This study is a cross-sectional analytic research which examined the changes of forearm blood flow in response to a period of arterial occlusion and exercise the diabetics with and without autonomic neuropathy and healthy subjects using venous occlusion strain gauge plethysmography. The study protocol was approved by The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

Population

Target population

In this study, the 3 target population groups were patients with type 2 diabetes and healthy volunteers. All subjects met the inclusion and exclusion criteria of the study.

Study population

Most type 2 diabetes patients were recruited mainly from the diabetes clinic at King Chulalongkorn Memorial Hospital, Bangkok Thailand. Healthy subjects were recruited by flyers and personal contact. The study participants were recruited according to the following criteria and all subjects were initially contacted by telephone to determine their qualification before included in the study.

Inclusion criteria

Control group

1. Men or women aged 40 - 75 years old.
2. No history or symptoms of any disease below

2.1 Diabetes

2.2 Cardiovascular disease

2.3 High blood pressure (≥160/100 mmHg)

2.4 Peripheral vascular disease (PAD)

Diabetes group

1. Diabetic subjects of either sex, aged 40-75 years
2. No history, clinical sign or symtoms of myocardial infaction, angina pectoris, stroke and PAD.
3. Not receiving any antiarrhythmic drugs or beta blocker
4. Asymtomatic autonomic neuropathy (no clinical signs of CAN e.g. Orthostatic hypotension, resting heart rate > 100 beats/min.)

Exclusion criteria (for both healthy and diabetic groups)

1. History of heavy smoking or stop smoking less than 10 years.
2. Pregnancy
3. History of cardiovascular or peripheral vascular diseases.
4. Having neurological or muscular pathologies.
5. History of recent trauma or injury to the arm.
6. Presence of any soreness or open wounds on the arm.
7. Having abnormal sensory neuropathy employed by a Semmes-Weinstein nylon monofilament.
8. Taking any medications that may affect heart rate and blood flow such as β-adrenergic blocking drugs and vasodilators and cannot refrain from these medications at least 24 hrs before the testing day.
9. BMI ≥ 30 kg/m^2

Sample

Sampling technique

This study used purposive sampling technique and voluntariness for recruiting subjects.

Sample size determination

In this study, sample size determination was calculated from the previous pilot study in 5 healthy men and 5 diabetes with cardiovascular autonomic neuropathy (DM with CAN) and 5 diabetes without cardiovascular autonomic neuropathy (CAN) ranging in age between 43 – 72 years participated as subjects. The pilot study was examined forearm blood flow during reactive and exercise hyperemia by using venous occlusion strain gauge plethysmography. The sample size was calculated from Cohen statistical power analysis.

$$f = \frac{\sigma_m}{\sigma}$$

Effect size estimation

By $\sigma_m = \sqrt{\sum_{i=1}^{k} \frac{(m_i - m)^2}{k}}$

m_i = the average value of group i

m = the average value of all groups

k = number of groups - 1

$\sigma = \sqrt{MSE}$ (Root Mean Square Error)

m_1 is peak blood flow averaged during reactive hyperemia in the healthy group (40.13 ml/100 ml tissue/min)

m_2 is peak blood flow averaged during reactive hyperemia in the DM without CAN group (23.78 ml/100 ml tissue/min)

m_3 is peak blood flow averaged during reactive hyperemia in the DM with CAN group (31.67 ml/100 ml tissue/min)

So σ_m = 6.676 σ = 9.489

Thence f = 0.70, from the power tables for effect size Cohen (1988) and power =0.90 therefore, the number of subjects per group is 10

In case of, peak blood flow during exercise hyperemia

m_1 is peak blood flow averaged during exercise hyperemia in the healthy group (20.68 ml/100 ml tissue/min)

m_2 is peak blood flow averaged during exercise hyperemia in the DM without CAN group (16.05 ml/100 ml tissue/min)

m_3 is peak blood flow averaged during exercise hyperemia in the DM with CAN group (19.15 ml/100 ml tissue/min)

So σ_m = 1.924 σ = 4.11

Thence f = 0.468, from the power tables for effect size Cohen (1988) and power =0.90 therefore, the number of subjects per group is 27

As a result, from this pilot study we would have to recruit 27 subjects per group and the 5% allowance for error, so all subjects is 87 volunteers for the study.

Instruments

1. Case record form
2. Oscillometric blood pressure and heart rate monitor (ES-H55, Terumo Corporation, Tokyo, Japan)

3. Venous occlusion strain gauge plethysmography (EC6, DE Hokanson Inc, WA, USA) including arterial occlusion cuffs.
4. Handgrip dynamometer (T.K.K. 5401 Grip-D, Takei Scientific Instrument, Japan)
5. Arm and forearm support
6. Metronome
7. Cardiac electrical signal for heart rate variability was recorded by the physiograph system (Biopac MP 100 system with ECG100A transducer module with an acqknowledge® software version 3.7.3., Biopac System Inc., CA, USA)
8. Bioelectrical impedance analysis (Inbody230, Biospace co., ltd, Seoul, Korea)

Subject Preparation

Prior to each test session, subjects were asked to abstain from food, caffeine for at least 4 hours and alcohol for 48 hours. In addition, vigorous physical activity was not allowed 24 hours prior to testing. Comfortable clothing (short sleeves) for forearm blood flow testing should be worn. In diabetic patients, about 2 days before appointment, the patients were contacted by telephone and asked to refrain from medications as discussed in exclusion criteria by telephone.

Room temperature was controlled between 25 ± 1°C throughout the experiment. Upon arrival to the laboratory, the individual was allowed to relax for 5-10 minutes before weight, percent body fat, resting heart rate and resting blood pressure were recorded.

Standard measurements

Measurements of weight, percent body fat, resting heart rate, and blood pressure provide a baseline characteristics of the subjects. The following procedures were performed and baseline characteristics of the subjects were recorded.

Weight and body composition: Bioelectrical impedance technique (Inbody230, Biospace co., ltd, Seoul, Korea) provides quantitative analysis of current body compositon by using difference of electrical conductivity for biological character of body tissue. The impedance is measured by using handle sensor and foot sensor in the 8-point tactile sensor part (supposed that the body consists of five cylindrical conductors; trunk, arms and legs). This technology also uses Direct Segmental Multi-frequency Bioelectrical Impedance Analysis (DSM-BIA) too separately measure the impedance of the trunk, arms, and legs of the body. Prior to measurement, the subjects were asked to void within 30 minutes to ensure test reliability.

Resting blood pressure and heart rate: The individual was sitting upright in a straight backed chair. The left arm was resting on a table with the elbow flexed. The position was relaxed and comfortable. Conversation was discouraged. Blood pressures and heart rate were measured with an oscillometric monitor (ES-H55, Terumo Corporation, Tokyo, Japan) and recorded in millimeters of mercury (mmHg) and beats per minute, respectively.

Forearm blood flow measurement

Subject setup

Subject was in the supine position on the treatment bed and relaxed. The non-dominant arms were extend, slightly external rotated and supported approximately 10 cm. above the heart level. This position was set in order to lower the initial venous pressure, ensure adequate venous emptying and facilitate outflow during

the period of venous cuff deflation (5, 55) which is usually achieved by resting the elbows on foam pads and supporting the hands with pillows (Figure 3.1). Consequently, the appropriate size strain gaug which is placed around the largest part of the forearm, does not touch anything other than the forearm. Then the gauge was fixed to the skin by putting a piece of masking tape over the head of the gauge. Subjects were asked to hold the position without hand and forearm movement to prevent gauge disturbance.

Basic concept

Venous occlusion plethysmography measures arterial inflow by abruptly stopping venous outflow with a upper arm cuff (venous occlusion cuff) inflated to above venous pressure but below arterial pressure (about 50 mmHg (56)), arterial inflow is unaltered and blood can enter the forearm but cannot escape. This results in a linear increase in forearm volume over time, which is proportional to arterial blood inflow.

A mercury – in – rubber gauges around the forearm is used to measure the volume change in the forearm. The rate of change of the volume, in percent per minute, is the arterial flow rate (forearm blood flow) at the moment of venous occlusion. Often the flow rate is reported as being ml/100 ml/minute or %change/minute.

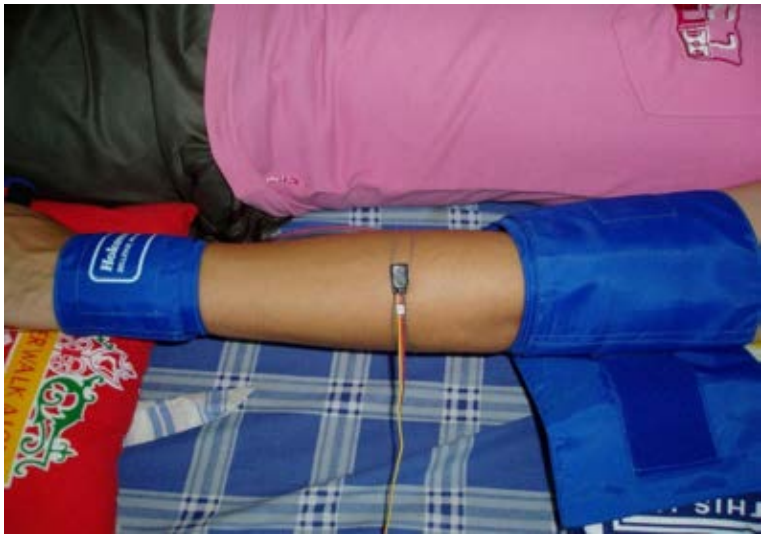


Fig 3.1 Subject starting position and instruments

Instrumentation

This arterial inflow or venous occlusion plethysmography (Fig 3.2) measurement system requires, an E20 rapid cuff inflator to abruptly occlude the upper arm cuff (venous cuff). The cuff pressure was maintained at 50 mmHg for 5 seconds (inflow time) in each 15 seconds cycle (reading interval) to occlude venous outflow from the arm (according to the manufacturer guidelines). The plethysmograph was automatically set to inflate and deflate the venous cuff at the specified interval.

The noninvasive Vascular Program (NIVP3) software that interfaces the laptop computer was compatible with the plethysmography. The NIVP3 software stores patient information and waveforms for arterial inflow measurements (Fig 3.3). The program very accurately set the slope according to the chosen intercept points and displays the flow rate as the slope is edited. The ideal sensitivity will give an inflow slope of approximately 45 degrees. So, strain gauge plethysmography measures the rate of change (slope) of the circumference of a forearm. This represents a change in the volume of the limb segment over a brief period of time rather than the change in blood flow. However, because the change in volume is assumed to be the result of arterial inflow of blood, the convention is to report the change as blood flow (57).

The DC (vein) mode was set on the EC6 plethysmography in accordance with the other settings. In this mode, the signal from the strain gauge is directly coupled to the recorder without any filters to distort the signal, which allows the instrument to record continuous changes in blood flow (every 15 seconds).

A second wrist cuff is placed distal to the strain gauge. Hand blood flow was predominantly through skin blood vessels rather than skeletal muscle and thus had different control mechanisms than FBF (58). Therefore, the hands were excluded from the circulation by inflating cuffs around the wrists to above systolic pressure (about 220 mmHg) at least 1 minute before each measurement and throughout measurement of FBF.

Blood pressure and heart rate were measured on the contra-lateral arm at 1-min intervals in every test of FBF using an oscillometric blood pressure and heart rate monitor (ES-H55, Terumo Corporation, Tokyo, Japan)

Fig 3.2 EC6 strain gauge plethysmography and E20 rapid cuff inflator

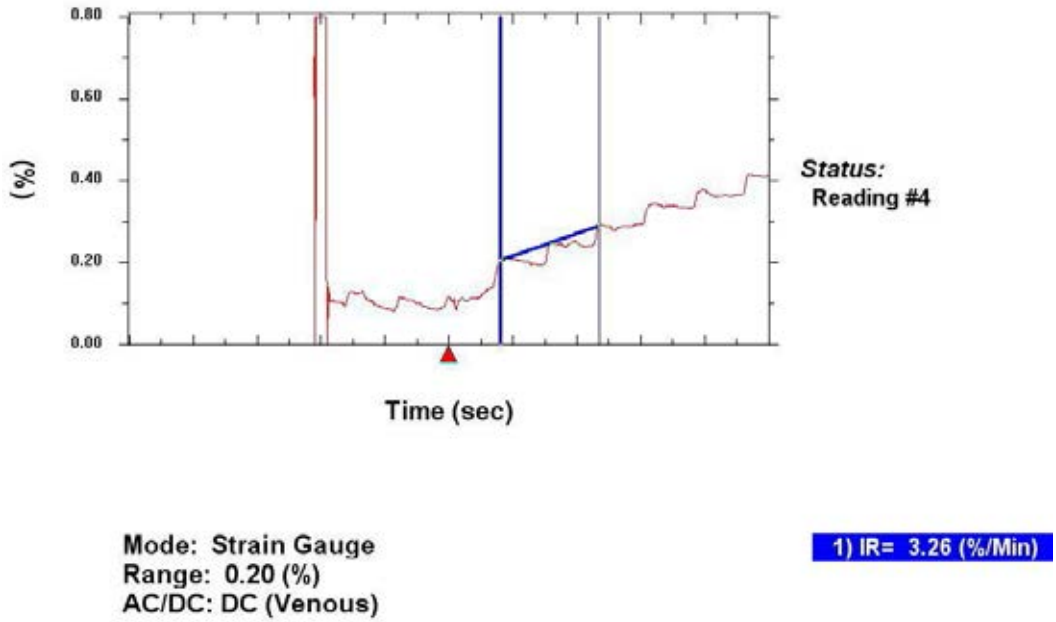


Fig 3.3 The waveforms for arterial inflow measurements

Resting forearm blood flow measurement

As mentioned above, a strain gauge was placed around the forearm at the point of greatest circumference and two cuffs were placed around the upper arm and the wrist. FBF was obtained from the mean of the all reading in every 15 sec cycle. Resting FBF was recorded for 3-5 minutes before the reactive (defined as baseline FBF) and exercise hyperemia measurement (to ensure the return of FBF that repose to reactive hyperemia to baseline).

Reactive hyperemia measurement (36-38, 58)

Following limb ischaemia there is a rapid increase in forearm blood flow, which slowly returns to baseline values and is termed reactive hyperemia. Reactive hyperemia was then induced by manually inflating the another occlusion cuff on the non-dominant upper arm (above venous cuff) to at least 60 mmHg above the systolic blood pressure (220 mmHg in most subjects) for a period of 5 min to occlude the FBF. After the cuff was released, FBF was measured for a 5 minute duration.

Exercise hyperemia measurement (13)

Handgrip exercise was performed with a handgrip dynamometer (T.K.K. 5401 Grip-D, Takei Scientific Instrument, Japan). Before the experiment, each subject's maximum voluntary contraction (MVC) was determined as the best of three trials with verbal encouragement to improve at each trial. Force output from the handgrip device was recorded on paper and communicated by the test administrator (observer) to provide the subject feedback (to maintain constant intensity at 45% MVC). The exercise protocol consisted of 5 min of intermittent isometric exercise where subjects matched force production to a notification of the observer, to the rhythm of a metronome (40 beats/min) with a 50% duty cycle (1.5 sec contraction and 1.5 sec relaxation).

Rhythmic handgrip at 45% MVC were used in this study which was accompanied by increases in muscle SNA due to activation of the muscle metaboreflex

(13). In contrast, rhythmic handgrip at 5, 10, 20, or 33% MVC does not engage the muscle metaboreflex and does not increase muscle SNA. Immediately after completion of rhythmic handgrip exercise, FBF was measured for 5 min.

Recovery time was given (15 min) between each intervention and following measurement, allowing sufficient time for blood pressure, HR, and FBF to return to baseline levels before next intervention.

Fig 3.4 Reactive hyperemia and handgrip exercise measurement

The standard battery of cardiovascular reflex test (1, 18, 20, 31)

To obtain the information of the cardiovascular autonomic function test, Cardiac electrical signal for heart rate variability was recorded by the physiograph system (Biopac MP 100 system with ECG100A transducer Inc., CA, USA) with data acquisition software (Acknowledge® version 3.7.3) for measurement of Lead I. Signals from this electrode montage can be used to calculated heart rate and beat to beat time

interval (RR interval). Blood pressure was observed by an oscillometric blood pressure monitor (ES-H55, Terumo Corporation, Tokyo, Japan).

When surface electrodes had been placed, cardiovascular autonomic neuropathy (CAN) was assessed by a standard battery cardiovascular autonomic reflex test. This battery composed heart rate variation during deep breathing, the Valsalva test, heart rate response to tilting, systolic blood pressure response to tilting and diastolic blood pressure response to sustained handgrip.

Heart rate variation during deep breathing

Prior to the test start, the subject seated quietly. As the subjects were asked to breath deeply and evenly at six deep breaths within 1 minute (5 seconds inspiration and 5 seconds expiration). The maximum-minimum heart rate during each 10 second breathing cycle was measured. To ensure correct technique, the subjects had several breathing practices before actual test and data record. The heart rate difference was calculated from the mean maximum and minimum heart rates. A difference of >14 beats as normal (score=0), a difference of 11-14 beats was regarded as borderline (score=0.5) and a difference of ≤ 10 beats as abnormal (score=1) (Fig 3.5).

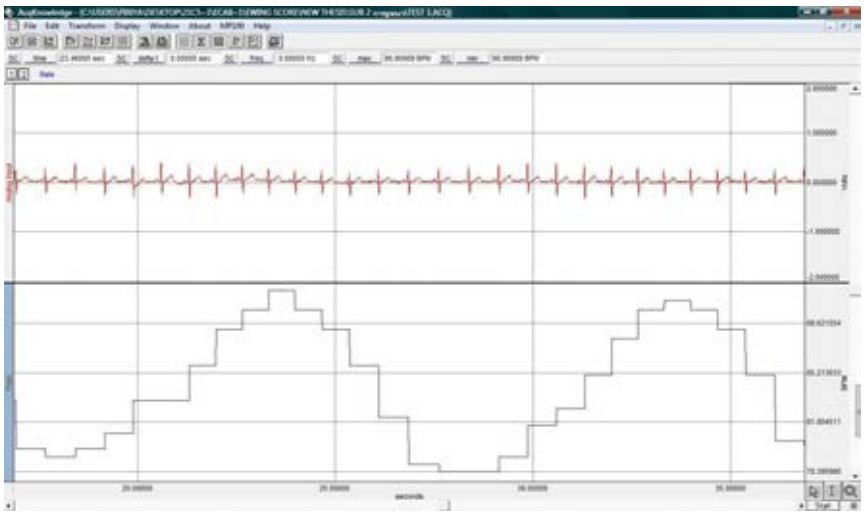


Fig 3.5 Heart rate variation during deep breathing test

Valsalva maneuver

The subject seated quietly and then blows into a mouthpiece connected to a modified sphygmomanometer and maintaining a pressure of 40 mmHg for 15 seconds while a continuous electrocardiogram was recorded. The result was expressed as the Valsalva ratio, which is the ratio of the longest RR interval (reflecting the overshoot bradycardia following release) after the maneuver (during 30s) to the shortest RR interval during the maneuver (reflecting the tachycardia during strain). We have defined a Valsalva ratio (as the mean ratio from two successive valsalva maneuvers) of $\geq$ 1.21 was defined as normal (score=0), a ratio of 1.11-1.20 as borderline (score=0.5) and a ratio of $\leq$ 1.10 as abnormal (score=1).

Heart rate response to standing

The subject was asked to lie down resting for 5-10 min in the supine position and then was asked to actively stand up unaided and remained so for 3 min while the heart rate was recorded continuously on an electrocardiograph. The shortest RR interval (usually $15^{th} \pm 5$ beats after starting to stand) and after that the longest RR interval (usually $30^{th} \pm 5$ beats) were selected and could be quantified as the 30/15 ratio, which was the ratio of the longest RR interval to the shortest RR interval. An normal 30/15 ratio was defined as $\geq$ 1.04 as normal (score=0), a ratio of 1.01-1.03 as borderline (score=0.5) and a ratio of $\leq$ 1.0 as abnormal (score=1)

Blood pressure response to standing

Similar to the heart rate response to standing, postural fall in blood pressure test was performed and the patient's blood pressure was determined with an oscillometric blood pressure monitor (ES-H55, Terumo Corporation, Tokyo, Japan). The patient was asked to lie down quietly and again when the patient actively stood up immediately, blood pressure was recorded at 1-min intervals and continuously for 3-5 min duration. Blood pressure was determined before standing and the minimum value of the all measurement during standing. A fall in systolic blood pressure of ≤ 10 mmHg was regarded as normal (score=0), a decrease of 11-29 mmHg as borderline (score= 0.5) and a decrease of ≥ 30 mmHg as abnormal (score=1).

Blood pressure response to sustained handgrip (30)

The subject was asked to squeeze as strongly as possible a handgrip dynamometer three times to get the maximum voluntary contraction (MVC). The mean of these attempts was calculated. The handgrip was then maintained steadily at 30% of MVC for as long as possible up to a maximum of 5 minutes while the subject's blood pressure was measured with an oscillometric blood pressure monitor on the non-exercising arm at 1 minute intervals during handgrip. An increase in diastolic blood pressure was measured as the difference between resting reading and the last reading before the release of handgrip. A rising in diastolic blood pressure of ≥ 16 mmHg was defined as normal (score=0), a rising of 11-15 mmHg as borderline (score = 0.5) and a rising of ≤10 mmHg as abnormal (score=1).

Cardiovascular autonomic neuropathy (CAN) has been classified by the results of tests of cardiovascular autonomic function score. The sum of 5 tests were used (18-20) as shown below (Table 3.1). This scoring system uses five tests and the corresponding values commonly accepted in many studies. In each test, a score from 0 to 1 was assigned. If the value in one test falls in normal range a score of 0 is assigned; if it falls within the borderline range a score 0.5 is assigned and if it falls within the borderline range a score 1 is assigned. The sum of the obtained scores for each test provides the definite classification of the patient's degree of autonomic involvement.

-The score value of < 1 (0-0.5) was regarded as normal.

-The score value of 1-2.5 was regarded as mild cardiovascular autonomic neuropathy.

-The score value ≥3 was regarded as severe cardiovascular autonomic neuropathy.

This method was used as a screening for diabetic patients with CAN complications and divided diabetic patients into 2 groups (the DM with and without CAN).

Table 3.1 Test of Cardiovascular Autonomic Function.

Test	Normal	Borderline	Abnormal
Test reflecting parasympathetic function 1. Heart rate (R-R interval) variation during Deep rate breathing (maxHR - minHR)	≥15	11-14	≤10
2. Heart rate response to Valsava manoeuvre (Valsalva ratio)	≥1.21	1.11-1.20	≤1.10
3. Immediate heart rate response to standing (30:15 ratio)	≥1.04	1.01-1.03	≤1.00
Test reflecting sympathetic function 1. Blood pressure (mmHg) response to standing (Fall in systolic blood pressure)	≥10	11-29	≥30
2. Blood pressure (mmHg) response to sustained handgrip (increase in diastolic blood pressure)	≥16	11-15	≤ 10
Score	0	0.5	1

Semmes weinstein monofilament test (28, 59, 60)

American Diabetes Association standard of care guidelines recommend screening all diabetic patients' neurological status using the 5.07 (10g force) monofilament annually and quarterly for high risk diabetics. Monofilament testing is a simple noninvasive independent predictor of risk for diabetic foot lesions. The 5.07 monofilament has been accepted as the medical standard for screening of the minimum level of protective sensation or sensory neuropathy in the foot. Monofilament test was performed as follows.

1. The subject was asked to lie down in the supine position with shoes and socks removed.

2. The patient's skin on arm or hand was lightly touched with the monofilament wire to demonstrate what the touch feels like.

3. The subject was instructed to respond "yes" each time he/she feel the pressure of the monofilament on his/her foot during the examination.

4. The subject was instructed to close his/her eyes with toes pointing straight up during the examination.

5. The monofilament perpendicular was hold to the patient's foot and it was pressed against the foot, with the pressure increasing until the monofilament bends into a C-shape. The area with ulcer, callus, scar, or necrotic tissue were avoided.

7. The filament was hold in place for 1 sec. and then was pressed to the skin such that it buckles at one of two times as you say "time one" or "time two." The patients were asked to identify at which time they were touched. The sequence of applying the filament was randomized throughout the examination.

8. Testing 10 sites: dorsal midfoot, plantar aspect of foot including pulp of the first, third, and fifth digits, the first, third and fifth metatarsal heads, the medial and lateral midfoot and the calcaneus (Fig 3.6)

9. Response on foot screening was recorded. A foot was classified to have loss of protective sensation or sensory neuropathy when less than seven sites are felt by the patient. A foot is classified as normal if seven or more sites are felt by the patient.

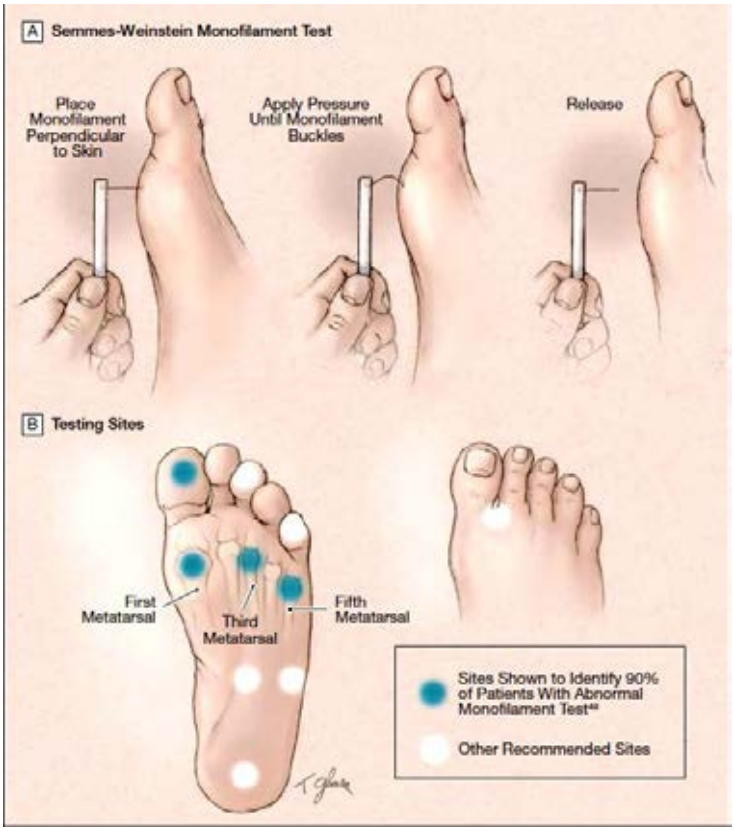


Fig 3.6 Monofilament test for light touch sensation (60)

Procedure (**Fig 3.7**)

1. The subjects were asked to refrain from consuming food and caffeine for at least 4 hours and alcohol for 48 hours. In addition, vigorous physical activity was not allowed 24 hours before the testing session to help stabilize extremity blood flow and maintain normal fluid.

2. Furthermore, upon arrival to the laboratory, each individual was allowed to relax for 5-10 minutes before screened for sensory neuropathy of the foot using the 5.07 (10g force) monofilament. After that, weight, percent body fat, resting heart rate and resting blood pressure were recorded. The largest non-dominant forearm circumference was measured when the FBF equipment has been set before the

measurement session started. Room temperature was controlled between 25 ± 1°C throughout the experiments.

3. The subjects then assumed a supine position on a treatment bed. And then the inflation cuffs were placed around the upper arm and wrist. The appropriate size of mercury-in-rubber strain gauge was fastened around the largest forearm area and secured with tape. To measure the forearm blood flow at rest and during reactive and exercise hyperemia for 5 minutes in each sessions. Moreover, during the experiment, the subject's blood pressure and heart rate were repetitively measured at 1 minute interval.

4. After finished FBF measurements, the subjects were allowed to relax for 15 minutes.

5. The standard battery of cardiovascular reflex tests were performed measuring mainly the parasympathetic limb included the heart rate variation during deep breathing, valsalva ratio and heart rate change with standing. In addition, the blood pressure responses to sustained handgrip and to standing were also determined. The latter test are thought to reflect the integrity of the sympathetic nervous system. Upon completion, with normal vital sign, the subjects were allowed to leave the lab.

Rest	Resting BF	Reactive Hyperemia	Rest	Resting BF	Exercise Hyperemia	Rest	Cardiovascular Reflex Tests
10 min	5 min	5 min arrest + 5 min bf	15 min	5 min	5 min ex + 5 min bf	15 min	30 min (5 tests)

Fig 3.7 Timeline of experimental protocol

Data processing and analysis

Flow measurements (FBF) were recorded for 5 sec every 15 sec. Therefore 4 readings were obtained and averaged for each mean value. It takes a few seconds to obtain the first hyperemic flow, almost the first measurement for each protocol assumed as the maximum hyperemia flow (peak blood flow), maximum forearm vascular conductance (FVC) and minimum forearm vascular resistance FVR. However, these maximum and minimum parameters may be the second or third waveform measurement in some subjects (a delay of peak blood flow). Reactive and exercise hyperemia blood flow were calculated as follows:

Mean arterial pressure (MAP) was calculated using the standard equation: MAP = diastolic blood pressure + [(systolic blood pressure - diastolic blood pressure)/3] expressed as mmHg.

Forearm vascular resistance (FVR) was calculated by dividing MAP by forearm blood inflow (FVR = MAP/forearm blood inflow) expressed as $mmHg \cdot ml^{-1} \cdot min \cdot 100\ ml$

Forearm vascular conductance (FVC) was calculated by dividing FBF by MAP and multiplied by 100 (FVC = FBF × 100/ MAP) expressed as $ml \cdot min^{-1} \cdot 100\ ml^{-1} \cdot mmHg^{-1}$.

Percent increased in FBF was calculated by: %increase FBF = [(peak FBF - resting FBF)/ resting FBF] × 100

Percent recovery in FBF after the first minute was calculated by: %recovery after 1 min = [(peak FBF – 4^{th} reading FBF)/ (peak FBF – resting FBF)] × 100

Statistical analysis

All data were presented as mean ± standard deviation (S.D). Statistical comparison within each measured group parameter were performed by one-way analysis of variance (ANOVA) for factor measurements. When the overall F-test was significant, the pairwise testing for differences between groups were determined by a Fisher's least significant difference test (LSD) post hoc comparisons. However, there were unequal variances in weight, resting blood flow, peak reactive blood flow and maximal forearm vascular conductance during reactive hyperemia, so the results of these tests had to be analysed using a log transformation.

An alpha level of 0.05 was used to determine statistical significant. All analyses were performed on the Statistical Package for the Social Sciences version 16.0 (SPSS,Chicago,IL,USA).

CHAPTER IV

RESULTS

In this study, 60 of type 2 diabetes patients and 33 healthy volunteers who met inclusion/exclusion criteria were recruited for this study. Three patients were excluded because they can not conform some cardiovascular autonomic function procedures. A total of 90 subjects (57 diabetes patients and 33 healthy volunteers) underwent forearm blood flow and cardiovascular autonomic function test. All subjects were initially contacted by telephone to determine their qualification and announce the research project before included in the study. The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University.

Characteristics of the subjects

The characteristics of study subjects in the diabetics without cardiovascular autonomic neuropathy (DM without CAN group), diabetics with cardiovascular autonomic neuropathy (DM with CAN group) and healthy non-diabetics (Healthy group) are summarized in Table 4.1 and 4.2. Age of the 36 DM without CAN subjects ranged from 45 – 75 years (mean 59.42 ± 9.12 years), the 21 DM with CAN subjects aged from 45 – 75 years (mean 61.52 ± 9.81 years) and the 33 healthy subjects ranged from 42 – 75 years (mean 58.61± 8.12 years). Both the DM with and without CAN groups were heavier and had more percent body fat and body mass index (BMI) than the healthy group ($P < 0.001$ for all three variables) but there were no significant differences between the DM with and without CAN. Resting heart rate was not significantly different in all subjects. The mean duration of diabetes in both groups of

diabetic patients was similar (P=0.359). The level of HbA1c and fasting blood sugar (FBS) of DM with (7.17 ± 0.95 % and 120.6 ± 23.1 mg/dl) and without CAN (7.13 ± 0.83 % and 125.3 ± 29.5 mg/dl) were not different but DM groups had a significantly higher fasting blood sugar than the healthy group (94.2 ± 9.3 mg/dl, P<0.001 for both DM groups vs. control). Also, systolic and mean arterial blood pressure were higher in the DM with and without CAN groups than the healthy group (P < 0.001 and P=0.032, respectively) but no significant difference in blood pressure between the DM with and without CAN was found. Interestingly, the DM with CAN group had significantly higher resting blood flow than the healthy group (6.03 ± 2.65 vs. 4.3 ± 1.39 ml/100ml/min, respectively). DM with and without CAN did not significantly differ in any baseline characteristics (t-test, 0.079< P <0.865) except cardiovascular autonomic function score as shown in Table 4.3 (P < 0.001) and no loss of protective sensation or sensory neuropathy were detected in all subjects.

Baseline resting forearm blood flow and cardiovascular autonomic function score

At rest, the DM with CAN group had the highest baseline forearm blood flow (resting blood flow) measured before undergoing reactive hyperemia (Figure 4.1). Interestingly, resting blood flow of DM with CAN was statistically higher than that of the healthy group (P=0.012) while there was no different between DM without CAN and healthy groups (P0.093). Regarding, forearm blood flow before exercise hyperemia the DM with CAN group also had the highest forearm blood flow (Figure 4.2). The cardiovascular autonomic function score of DM with and without CAN groups and healthy group were 1.6 ± 0.41, 0.31 ± 0.25 and 0.14 ± 0.26, respectively. Scores in each cardiovascular autonomic function test were shown in Table 4.3. It should be mentioned here that 76.2% of subjects in the DM with CAN were abnormal in testing of heart rate

variation during deep breathing and 47.6% of subjects in this particular group had borderline systolic blood pressure response to standing.

Table 4.1. Basic characteristics of the 90 subjects shown in mean ± SD

Variable	DM without CAN	DM with CAN	Healthy
Number of subjects	36	21	33
Age(years)	59 ± 9	62 ± 10	59 ± 8
Gender(Male:Female)	12 : 24	12 : 9	7 : 26
Number of subjects with sensory neuropathy	0	0	0
Height(cm)	158 ± 10	161 ± 8	157 ± 7
Body weight(kg)	63 ± 13	67 ± 10	52 ± 9*#
Body Mass Index(kg/m^2)	25.3 ± 4	25.6 ± 2.6	21.3 ± 3.7*#
Percent body fat(%)	33.1 ± 9	32.2 ± 8	27.4 ± 8.7*#
Nondominant arm muscle mass(kg)	2.12 ± 0.6	2.27 ± 0.56	1.73 ± 0.49*#
% Fat in nondominant arm(%)	38.89 ± 11.63	37.92 ± 10.88	34.2 ± 10.97
Fat mass in nondominant arm(kg)	1.59 ± 0.89	1.54 ± 0.59	0.99 ± 0.47*#
Resting heart rate(beats/min)	68 ± 12	72 ± 9	68 ± 11
Fasting Blood Sugar(mg/dl)	125 ± 29	121 ± 23	94 ± 9*#
HbA1c(%)	7.13 ± 0.8	7.17 ± 0.9	N/A
Duration of diabetes(years)	10 ± 8	12 ± 7	N/A
Systolic BP(mmHg)	127 ± 15	132 ± 17	114 ± 18*#
Diastolic BP(mmHg)	70 ± 10	68 ±10	67 ± 10
Mean Arterial Pressure(mmHg)	88.7 ± 10	89.5 ± 11.3	82.3 ± 12.3*#
Resting blood flow(ml/100ml/min)	5.36 ± 2.62	6.03 ± 2.65	4.3 ± 1.39#

* $P<0.05$ VS. DM without CAN and # $P<0.05$ VS. DM with CAN

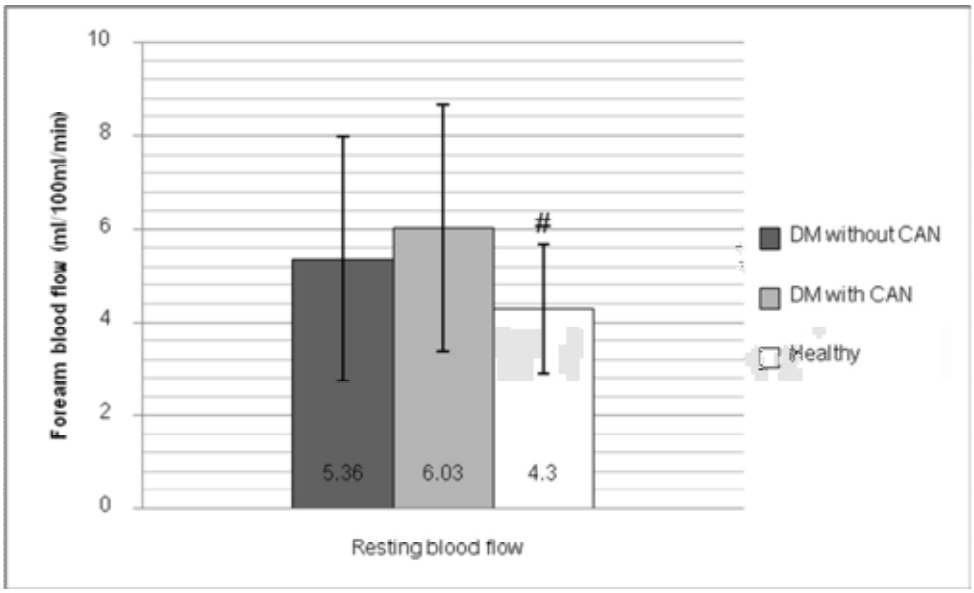


Fig 4.1 Baseline forearm blood flow (* Significant difference with a log transformation, P<0.05 as compared with the DM with CAN.)

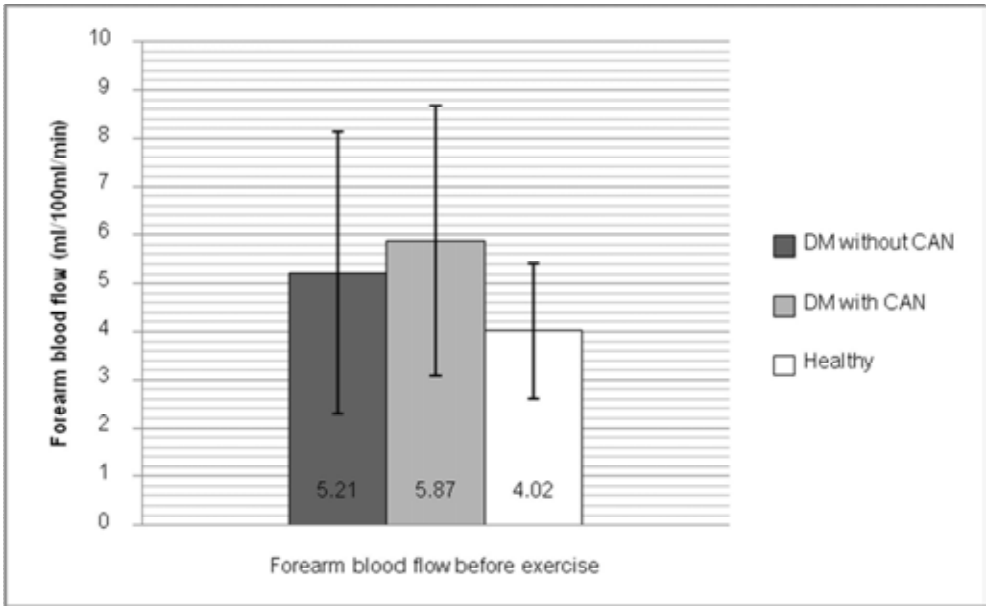


Fig 4.2 Forearm blood flow before exercise hyperemia

Type 2 diabetic patients, 36 DM without CAN and 16 (information missing from 5 out of 21 patients) DM with CAN provided their information on current medication including oral hypoglycemic agents, antihypertensive agents and hypolipidaemic agents. Those patients with missing medication information had lost their contact. List of medications was shown in Table 4.2.

Table 4.2 Medications in diabetic patients

Medications	DM without CAN (n=36)	DM with CAN (n=16)
Sulfonylureas	23 (63.8)	7 (43.7)
Biguanides	31 (86.1)	12 (75.0)
Thaiazolidinediones	3 (8.3)	2 (12.5)
α-Glucosidase inhibitor	8 (22.2)	2 (12.5)
Diuretics	1 (2.7)	2 (12.5)
β-Blockers	2 (5.5)	3 (18.7)
Calcium Channel Blockers	6 (16.6)	7 (43.8)
Angiotensin Converting Enzyme Inhibitors	9 (25.0)	7 (43.8)
Angiotensin Receptor Blockers	8 (22.2)	6 (37.5)
Hypolipidaemic agents	15 (41.6)	7 (43.8)

Data are number of patients using the medication (%)

Table 4.3. Characteristics of cardiovascular autonomic function score

Variable	DM without CAN	DM with CAN	Healthy
Cardiovascular autonomic function score (mean ± SD)	0.31 ± 0.25	1.6 ± 0.41	0.14 ± 0.26
No. subject with score=0 (%)	14 (38.9)	0	25 (75.75)
No. subject with score=0.5 (%)	22 (61.1)	0	8 (24.24)
No. subject with score=1 (%)	0	4 (19)	0
No. subject with score=1.5 (%)	0	10 (47.6)	0
No. subject with score=2 (%)	0	6 (28.6)	0
No. subject with score=2.5 (%)	0	1 (4.8)	0

All diabetic patients were evaluated for cardiovascular autonomic neuropathy, divided into two groups (the sum of cardiovascular autonomic function score $\leq$ 0.5 was considered as those without CAN and 1 – 2.5 as with early/mild CAN as shown in Table 4.2. None of subjects had score value $\geq$ 3

Table 4.4. The five tests for cardiovascular autonomic function.

Autonomic Function Tests	DM without CAN	DM with CAN	Healthy
Tests reflecting parasympathetic function	(n=36)	(n=21)	(n=33)
1.Heart rate(R-R interval) variation during deep breathing			
Abnormal (score=1)	0	16 (76.2%)	0
Borderline (score=0.5)	13 (36.1%)	4 (19%)	8 (24.24%)
Normal (score=0)	23 (63.9%)	1 (4.8%)	25 (75.75%)
2.Heart rate response to valsava maneuver			
Abnormal (score=1)	0	1 (4.8%)	0
Borderline (score=0.5)	1 (2.8%)	5 (23.8%)	0
Normal (score=0)	34 (94.4%)	14 (66.7%)	33 (100%)
3.Immediate heart rate response to standing			
Abnormal (score=1)	0	1 (4.8%)	0
Borderline (score=0.5)	1 (2.8%)	0	0
Normal (score=0)	35 (97.2%)	20 (95.2%)	33 (100%)
Tests reflecting sympathetic function			
1.Systolic blood pressure response to standing			
Abnormal (score=1)	0	1 (4.8%)	0
Borderline (score=0.5)	0	10 (47.6%)	0
Normal (score=0)	36 (100%)	10 (47.6%)	33 (100%)
2.Diastolic pressure response to sustained handgrip			
Abnormal (score=1)	0	3 (14.3%)	0
Borderline (score=0.5)	7 (19.4%)	4 (19.0%)	1 (3.03%)
Normal (score=0)	29 (80.6)	12 (57.1%)	32 (96.96%)

Forearm blood flow responses during reactive hyperemia (RHBF)

The average RHBF measures following 5 min. of upper arm occlusion were summarized in Table 4.4. ANOVA showed a significant difference in peak blood flow, % increase reactive blood flow, forearm vascular conductance (FVC) and forearm vascular resistance (FVR) during reactive hyperemia between the three groups ($P<0.02$). However, there was no significant difference in % recovery blood flow after 1 min. between the three groups ($P=0.094$). Post hoc analysis on a log transformation data showed a higher peak reactive blood flow (Figure 4.3) and forearm vascular conductance (Figure 4.4) in the DM with CAN and healthy groups than the DM without CAN group (peak RHBF $P=0.001$, $P<0.001$ and FVC $P=0.012$, $P<0.001$, respectively). Also, Post hoc analysis with LSD showed a lower forearm vascular resistance in the DM with CAN and healthy groups (Figure 4.5) than the DM without CAN group ($P=0.011$ and $P<0.001$, respectively) and a higher % increase reactive blood flow in the healthy group (Figure 4.6) than the DM with and without CAN groups ($P=0.003$ and $P<0.001$, respectively). Interestingly, there were no significant difference in peak blood flow, FVC and FVR between the Healthy and DM with CAN groups, also there was no significant difference in % increase reactive blood flow between the DM with and without CAN groups. However, it seemed that the DM with CAN group had lowest %recovery blood flow after 1 min. (69.77 ± 24.21 %) but this was not significantly different (Figure 4.7). Additionally, the healthy group had highest % increase in reactive blood flow. Concerning with the standard deviation (SD) of % increase in reactive blood flow, SD > 50% of % increase in reactive blood flow averaged. Thus, data distribution (scatter plot) of peak reactive blood flow and resting forearm blood flow in all subjects is shown in Figure 4.8.

Table 4.5 Reactive hyperemia study results in the healthy and the diabetics with and without CAN shown in mean ± SD

Variable	DM without CAN (n=36)	DM with CAN (n=21)	Healthy (n=33)
Resting blood flow (ml/100ml/min)	5.36 ± 2.62	6.03 ± 2.65	4.3 ± 1.39#
Peak blood flow (ml/100ml/min)	21.56 ± 4.17	27.71 ± 6.94*	30.73 ± 8.98*
% Increase reactive blood flow	384.08 ± 213.29	438.69 ± 249.23	665.96 ± 328.32* #
Forearm vascular conductance (ml/100ml/min/100mmHg)	24.79 ± 5.26	31.39 ± 8.37*	37.94 ± 12.32* #
Forearm vascular resistance (mmHg.min.100 $ml.ml^{-1}$)	4.21 ± 0.92	3.46 ± 1.2*	2.94 ± 1.04*
% Recovery blood flow after 1 min.	81.79 ± 20.76	69.77 ± 24.21	76.1 ± 15.85

* $P<0.05$ VS. DM without CAN and # $P<0.05$ VS. DM with CAN

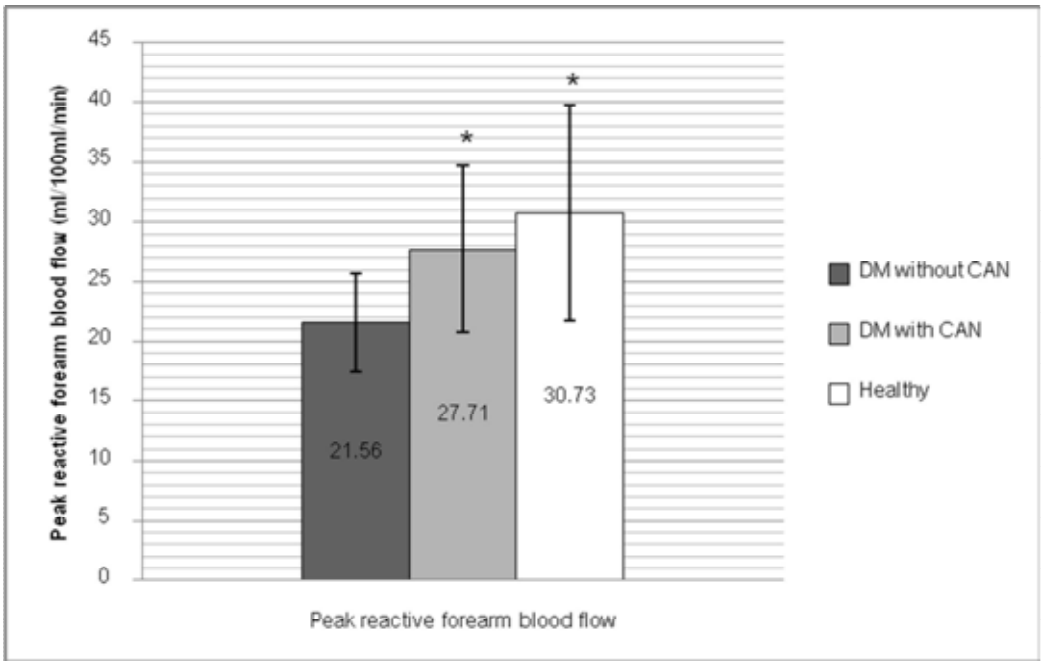


Fig 4.3 Peak RHBF (* Significant difference with a log transformation, P<0.05 as compared with the DM without CAN group.)

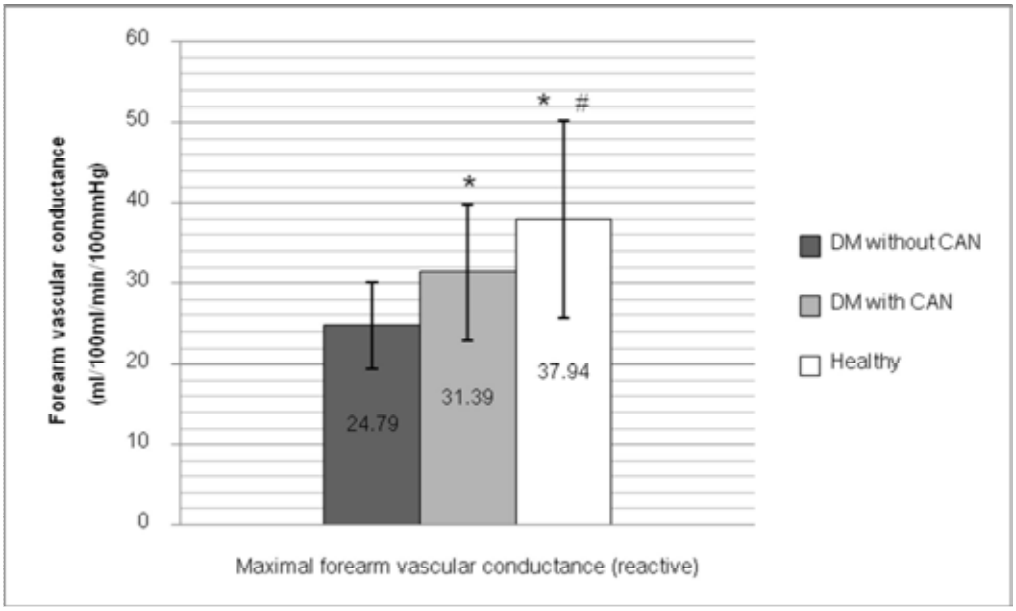


Fig 4.4 FVC during reactive hyperemia (Significant difference with a log transformation, * P<0.05 as compared with the DM without CAN and # P<0.05 as compared with the DM with CAN).

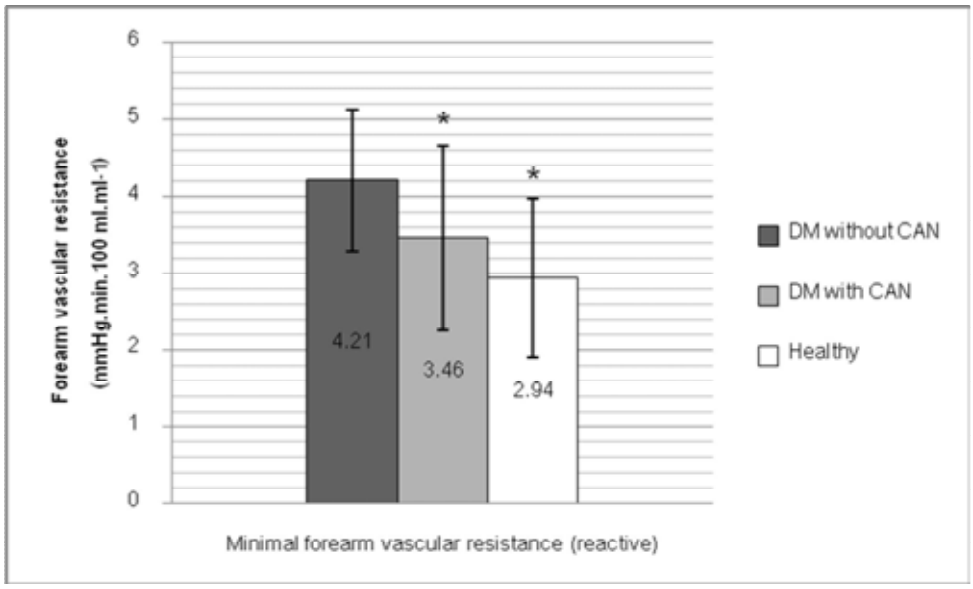


Fig 4.5 FVR during reactive hyperemia (* Significant difference with LSD, P<0.05 as compared with the DM without CAN group.)

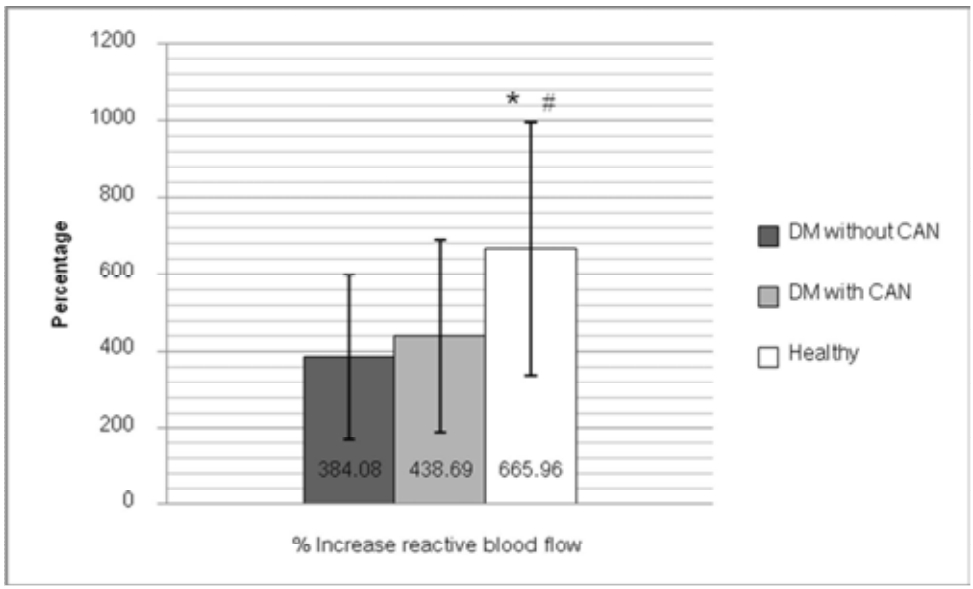


Fig 4.6 Percentage of increase reactive blood flow (Significant difference with LSD, * P<0.05 as compared with the DM without CAN and # P<0.05 as compared with the DM with CAN)

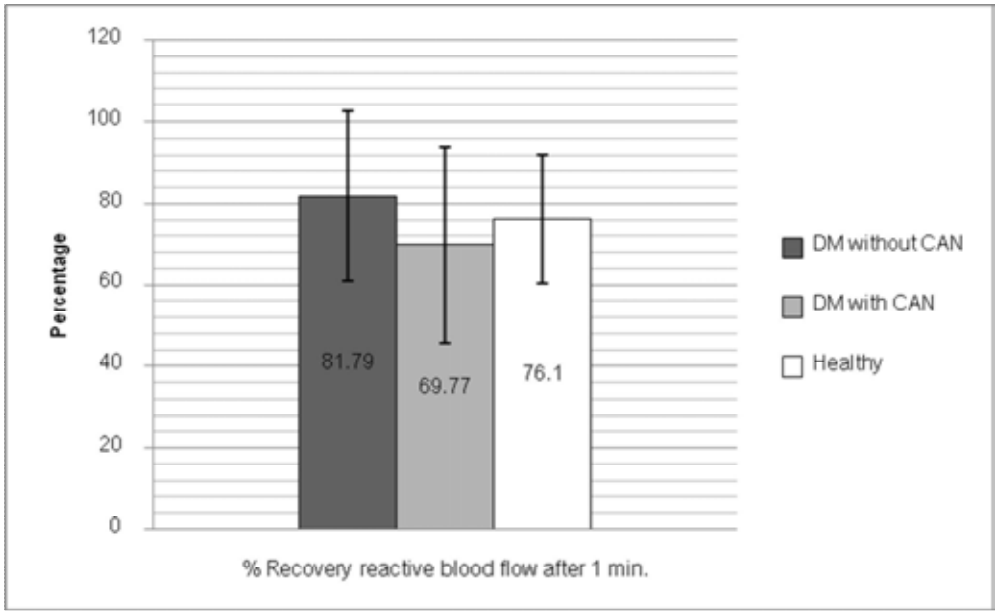


Fig 4.7 Percentage of recovery reactive blood flow after 1 min.

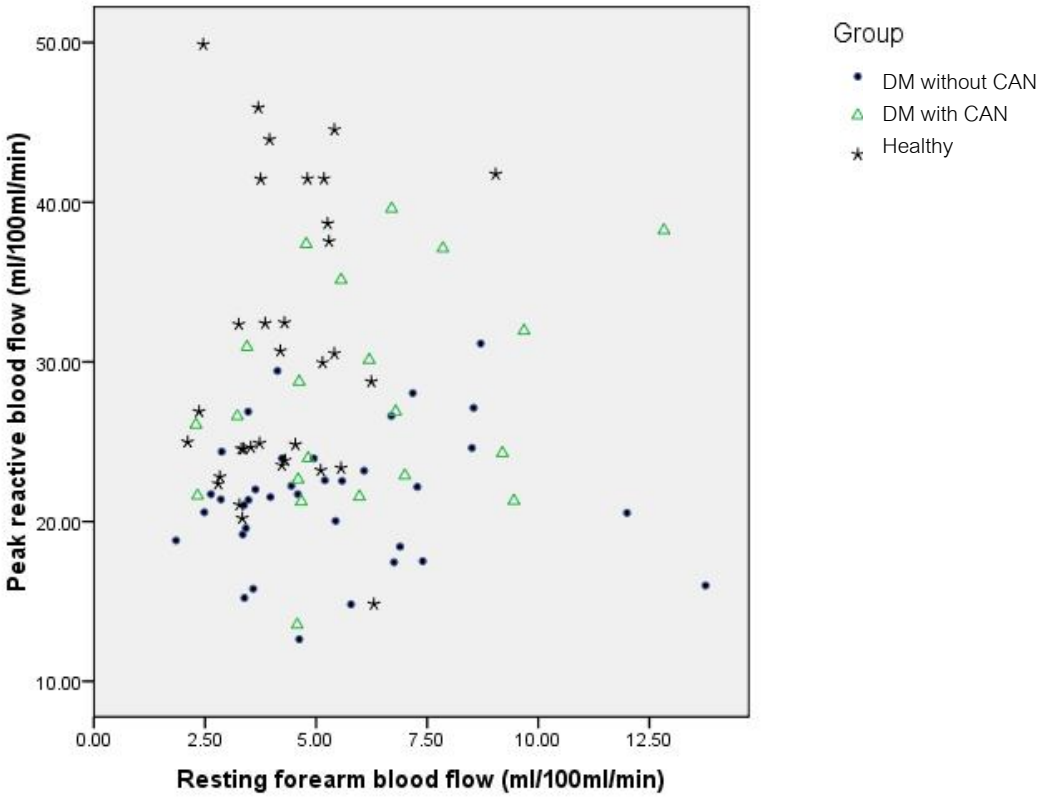


Fig 4.8 Data distribution of peak reactive blood flow and resting forearm blood flow in all subjects (n=90).

Forearm blood flow during exercise hyperemia (ExBF)

The new resting blood flow in the 3 groups prior to handgrip exercise test were similar to the values measured at baseline of reactive experiment (5.21 ± 2.92 for DM without CAN, 5.87 ± 2.79 for DM with CAN and 4.02 ± 1.4 for healthy). The results for ExBF which was measured immediately following 5 min. of rhythmic handgrip exercise were depicted in Table 4.6. ANOVA showed that no significant difference in peak blood flow, % increase exercise blood flow, forearm vascular conductance (FVC) and forearm vascular resistance (FVR) during exercise hyperemia between the three groups ($P>0.1$). However, it seemed that the DM with CAN group had the lowest % recovery blood flow after 1 min. (48.79 ± 39.67 %) and the healthy group had greater % recovery blood flow after 1 min (55.58 ± 20.23 %) as shown in Figure 4.9 but there was no significant difference. Furthermore, the DM with CAN group had the highest peak exercise blood flow followed by the healthy and DM without CAN groups respectively (Figure 4.10) but there was no significant difference. Concerning with the standard deviation (SD) of % increase in exercise blood flow and % recovery blood flow after 1 min, some of these values were highly spread out. Thus, data distribution (scatter plot) of peak exercise blood flow and resting forearm blood flow in all subjects is shown in Figure 4.11.

Table 4.6 Exercise hyperemia study results in the healthy and the diabetics with and without CAN shown in mean ± SD

Variable	DM without CAN (n=36)	DM with CAN (n=21)	Healthy (n=33)	*P* Value ANOVA
Peak exercise blood flow (ml/100ml/min)	17.58 ± 5.15	20 ± 5.78	18.23 ± 6.36	0.309
% Increase exercise blood flow	294.02 ± 161.41	319.89 ± 243.76	379.34 ± 175.74	0.169
Forearm vascular conductance (ml/min/100mmHg)	19.36 ± 6.78	21.13 ± 6.52	20.98 ± 8.43	0.587
Forearm vascular resistance (mmHg.min.100 $ml.ml^{-1}$)	5.8 ± 1.98	5.13 ± 1.43	5.68 ± 3.19	0.589
% Recovery blood flow after 1 min.	49.35 ± 21.17	48.79 ± 39.67	55.58 ± 20.23	0.536

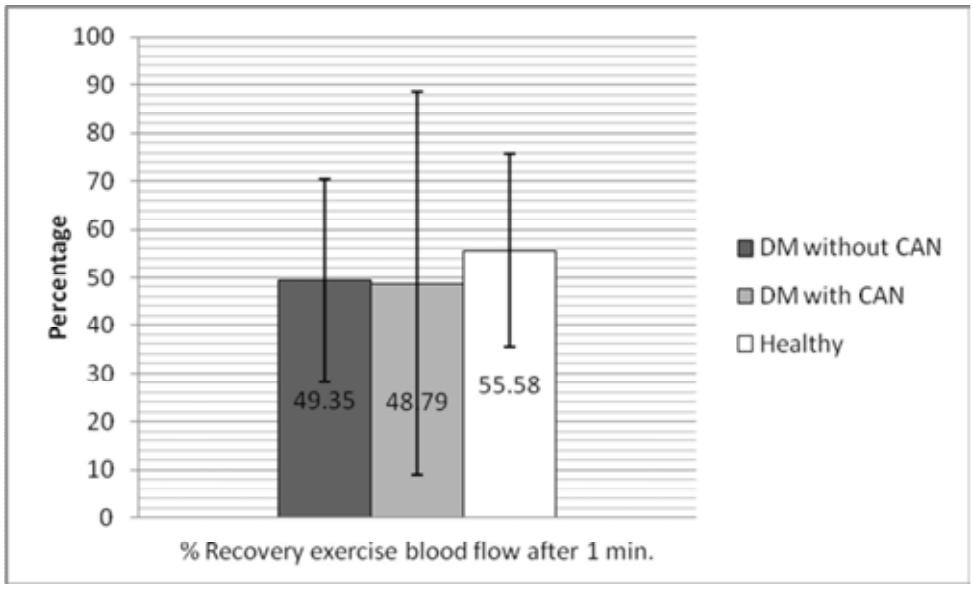


Fig 4.9 Percentage of recovery exercise blood flow after 1 min

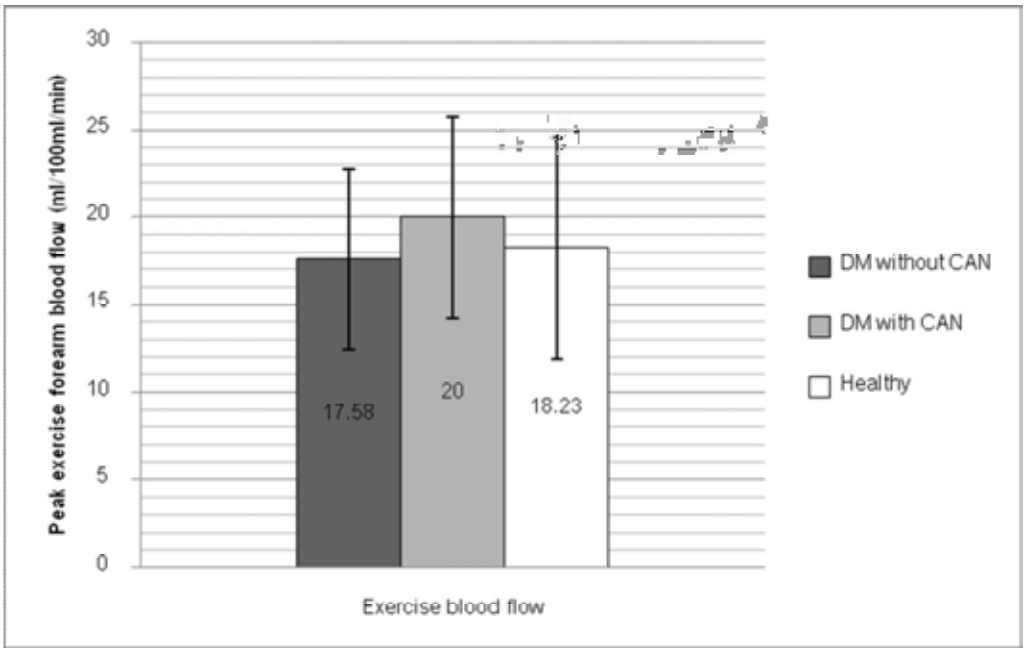


Fig 4.10 Peak exercise blood flow

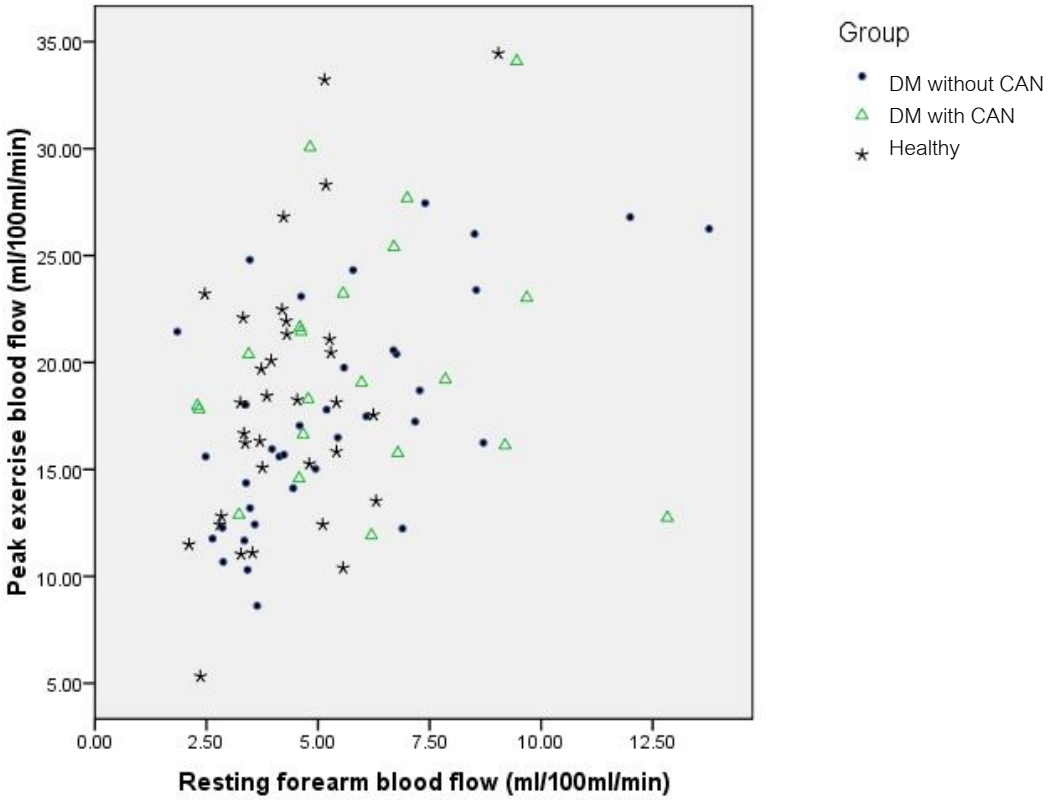


Fig 4.11 Data distribution of peak exercise blood flow and resting forearm blood flow in all subjects (n=90).

CHAPTER V

DISCUSSION AND CONCLUSION

In this study, we determined whether alterations in autonomic nervous system specifically cardiovascular autonomic neuropathy in diabetes affect vascular reactivity. The contribution of autonomic control to forearm blood flow that responds to reactive hyperemia (maximal flow) and to submaximal rhythmic handgrip exercise was determined using venous occlusion plethysmography. There was a significant greater forearm blood flow during reactive hyperemia in diabetes with autonomic neuropathy. However, there was no significant difference in forearm blood flow in response to rhythmic handgrip exercise between the 2 diabetic groups. This suggests that evidence of autonomic dysfunction in the control of vascular reactivity can be seen during a large flow stage as in reactive hyperemia.

Characteristics of the study population

A survey of baseline characteristics showed that all subjects in this study have normal protective sensation and the DM with CAN group has been classified as mild CAN (none of diabetic patients has been classified as severe CAN with a score $\geq$3). Moreover, baseline characteristics such as fasting blood sugar and HbA1c etc. were similar between DM with and without CAN. Both DM groups had a good glycemic control suggesting that diabetic condition of the patients in this study were not sever. Gender differences (male : female) are similar between DM with and without CAN but the majority in the healthy group were female. However, most female subjects were

post menopausal and not taking hormone replacement therapy. Thus, there should not have any differences in female hormone effects between groups.

Cardiovascualr autonomic function score

By the fundamental of this scoring system, for example the score 1 may be due to one test turning as completely abnormal or two tests of all five tests showing borderline involvement, indicating dysfunction of both parasympathetic and sympathetic pathways. In other words, the diabetic patients with CAN may indicate both parasympathetic and sympathetic dysfunction or definite only parasympathetic damage.

Results in the standard battery of cardiovascular reflex test in Table 4.3 indicated that the parasympathetic impairment was more prevalent than sympathetic impairment. This was evident by 76.2% of subjects in the DM with CAN showing abnormal in testing of heart rate variation during deep breathing. This finding is consistent with natural history of cardiovascular autonomic neuropathy which parasympathetic impairment preceded sympathetic dysfunction in diabetic patients (1, 61, 62). However, 47.6% of subjects in the DM with CAN were a borderline in systolic blood pressure response to standing (orthostatic hypotension) reflecting sympathetic impairment. Thus, an autonomic impairment in DM with CAN in this study appeared to affect both cardiac and vascular functions.

Baseline forearm blood flow

Notably, resting forearm blood flow in the DM with CAN (before reactive hyperemia procedures) was significantly greater than that in the healthy group. Similar to the recent study, resting skin blood flow at the feet of diabetic patients with neuropathy showed a higher value when compared with diabetic patients without neuropathy and in control subjects (63). Another study reported resting skeletal muscle

blood flow was greater in diabetic type I compared to normal controls(64). The mechanism responsible for this was unclear. However, DM with CAN having a reduction in vascular tone may be contributed to decreased sympathetic tone to forearm blood vessels. Moreover, it has been reported that arterial plasma norepinephrine concentrations were decreased in diabetic patients(64). This suggested that reduced circulating catecholamines may be partly responsible for the reduced peripheral vascular resistance, hence, increased blood flow.

Effect of cardiovascular autonomic neuropathy on reactive hyperemia

The main finding in this study is that peak reactive hyperemia of forearm blood flow (confirmed with % increased reactive blood flow) is highest in the healthy group and FVR is also lowest in the healthy group. However, peak reactive hyperemic blood flow and FVR of normal controls were not significantly different from the diabetic patients with CAN. Additionally, the DM with CAN had greater forearm blood flow during reactive hyperemia than the DM without CAN. It has been shown that autonomic control, sympathetic in particular, may play a significant role in blood flow control during reactive hyperemia. In support of this notion, the endothelium-dependent vasodilation response to insulin has been described recently in patients who have undergone sympathectomy is enhanced (65). Also, local alpha-adrenergic blockade by phentolamine showed increase forearm blood flow in response to ACh (65). Thus, increased vasodilatory responses in DM with CAN may be due to decreased sympathetic vasoconstrictor tone.

Furthermore, in healthy humans, the muscle metaboreceptors play a key role in generating the reflex increases in sympathetic nervous system (SNS) activity during static exercise as well as dynamic handgrip exercise protocol (13). However, patients with chronic heart failure (CHF), another disease state distinguished by resting SNS excess and exercise intolerance, show abnormalities of the exercise pressor reflex identified by a blunted metaboreceptor activation of SNS activity during exercise and an overstated reflex-sympathetic activation mediated by the mechanoreceptors (11). The

mechanisms underlying blunted metaboreceptor activation of MSNA are unclear. Potential mechanisms might include the decline of metabolites generated during ischemia that stimulate the metaboreceptors. ATP is one commonly accepted trigger of metaboreceptor activation, because of impaired oxidative energy metabolism from limited muscle conductance; therefore, reduced ATP availability may result in diminished metaboreceptor activation (11). According to the metabolic hypothesis, the metabolic activity during arterial occlusion may activate muscle metaboreflex too. However, on possibility is that DM with CAN may have less metaboreceptor activation than DM without CAN. Furthermore, arterial occlusion maintained for 5 min. before the forearm blood flow measurement may slightly reduced MSNA. To explain this, the increase in total peripheral resistance from mechanical forces created by rapid thigh cuff inflation caused an increase in blood pressure (44). Thus, the increase in blood pressure could stimulate the baroreceptors and subsequently restrain MSNA.

Moreover, in a study that augmented forearm blood flow with acetylcholine (endothelium-dependent vasodilator) and sodium nitroprusside (endothelium-independent vasodilator) reflecting maximal forearm blood flow, it was found that the forearm blood flow inversely correlated with various indicators of autonomic dysfunction. As the increased blood flow increases through the group with more severity of autonomic dysfunction in patients with type 1 diabetes that found a leak of albumin in the urine (macroalbuminuria) (16). However, the strong relationship between CAN and the nitrosovasodilators response might reflect the gradually withdrawal of sympathetic pressor tone with long-standing diabetic neuropathy. This would change the balance between the endothelial vasodilator factors and sympathetic pressor tone in preference of raised vasodilation. Thus, taken together, increased reactivity to nitrovasodilators in IDDM patients with macroalbuminuria could be described by hypersensitivity at the level of the vascular smooth muscle and/or decreased sympathetic vasoconstrictor tone (16). It is of interest whether the diabetic

patients with CAN in the present study would also have such hypersensitivity of vascular smooth muscle or simply have a decreased sympathetic vasoconstrictor tone.

Effect of cardiovascular autonomic neuropathy on exercise hyperemia

In this study, we did not find any significant difference on blood flow in response to handgrip exercise between the three groups. However, it appeared that the DM with CAN group had the highest peak exercise forearm blood flow although there was no statistical significance. A previous study reported that a 5-minute rhythmic handgrip exercise at 45% of maximum voluntary contraction, similar to the present study, was accompanied by increases in muscle sympathetic nerve activity (MSNA) (13). This was due to accumulation of metabolites and reflex from mechanoreceptor within the skeletal muscle, stimulating somatosensory fiber group III and IV afferent neurons which evoke a reflex increase in MSNA, known as the muscle metaboreflex and mechanoreflex (66). The result of the present study showed that DM with CAN group tended to have a greater peak exercise forearm blood flow, which means there was possibility in decreasing sympathetic vasoconstriction. In addition, research has found that forearm exercise performance (duration of exercise and forearm blood flow during exercise) was higher in patients with idiopathic palmar hyperhydrosis with depressed sympathetic denervation of the upper limb as a result of the treatment by thoracoscopic sympathetic trunkotomy (TST) (54). Therefore a tendency of elevated peak forearm blood flow in response to exercise may possibly explained in part by decreased sympathetic control in diabetic patients with CAN.

Percentage of recovery forearm blood flow after 1 minute

There were unexpected results of the present study. In the healthy group, in stead of having lowest percent recovery forearm blood flow after 1 min, the values were not different among the 3 groups. It was also expected that the DM with

CAN complications would have short duration of reactive hyperemia, but ANOVA revealed no significant difference on reactive hyperemia duration among the groups. In this study, percent recovery forearm blood flow after 1 min. of the DM with CAN tended to be lowest in both reactive and exercise hyperemia. Our results were in conflict with Yutaka et al (38) that duration of reactive hyperemia, however, was significantly shorter in subjects with risk factors. Age-adjusted mean value of duration of reactive hyperemia was significantly smaller in men with a smoking habit, diabetes mellitus, hypercholesterolemia or obesity, and in women with smoking habit, hypertension, diabetes mellitus or obesity. The number of risk factors significantly correlated with the duration of reactive hyperemia in both men ($r=-0.56$, $p<0.001$) and women ($r=-0.62$, $p<0.001$), suggesting that duration of reactive hyperemia reflects cardiovascular risk factors and decreases with the number of risk conditions. To explain this controversy, the duration of reactive hyperemia, defined as the interval (seconds) between release of occlusion and a return of blood flow to the level of within 5% of baseline forearm blood flow, may be used as alternative outcome. In contrast, in the present study, percent recovery forearm blood flow after 1 min was used instead because many subjects took several times more than 5 minutes for a recovery to baseline.

Conclusion

The present study shows that there was a significant greater in forearm blood flow (peak blood flow, %increase reactive blood flow, FVC and FVR) during reactive hyperemia in diabetes with CAN. Increase reactivity to reactive hyperemia may hypothetically be explained by mechanism such as during exercise (mechanoreceptor and metaboreceptor activation) and reactive hyperemia (metaboreceptor activation), accumulation of metabolites and reflex from mechanoreceptor within the skeletal muscle. The mechanism stimulates somatosensory fiber group III and IV afferent

neurons which evoke a reflex increase in MSNA, known as the muscle metaboreflex, possibly be reduced in DM with CAN. In addition, there may be certain changes in the balance between the endothelial vasodilator system and sympathetic pressor tone in favor of augmented vasodilation. These findings suggest that introducing reactive hyperemia (but not found in exercise hyperemia) which is a noninvasive measure in assessing vascular function which can be done more frequently yields effects similar to stimulation with invasively administering agents causing vasodilatation.

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APPENDICES

APPENDIX A

แบบสอบถามข้อมูลเพื่อการคัดกรองขั้นต้น

หากท่านอยู่ในเกณฑ์ของแต่ละข้อ ให้ขีดเครื่องหมาย ✓ ลงในช่อง ❑ ใช่ และหากท่านไม่ได้อยู่ในเกณฑ์ ให้ขีดเครื่องหมาย ✓ ลงในช่อง ❑ ไม่ใช่

เกณฑ์ในการคัดเข้า

<u>กลุ่มควบคุม</u>

	ใช่	ไม่ใช่
1. ท่านมีอายุระหว่าง 40-75 ปี	❑	❑
2. เป็นผู้มีสุขภาพดีที่ไม่มีประวัติอาการของโรค หรือภาวะ ต่อไปนี้		
2.1 โรคเบาหวาน	❑	❑
2.2 โรคหัวใจและระบบไหลเวียนเลือด	❑	❑
2.3 โรคความดันโลหิตสูง (≥160/100 mmHg)	❑	❑
2.4 โรคหลอดเลือดส่วนปลาย	❑	❑
2.5 โรคติดเชื้อทางเดินหายใจ (respiratory tract infection) ภายใน 4 สัปดาห์ ก่อนวันทดสอบ	❑	❑
3. ท่านสมัครใจเข้าร่วมโครงการศึกษาวิจัย	❑	❑

<u>กลุ่มผู้ป่วยเบาหวาน</u>

	ใช่	ไม่ใช่
1. ท่านเป็นโรคเบาหวานชนิดที่ 2	❑	❑
2. ท่านมีอายุระหว่าง 40-75 ปี	❑	❑
4. ไม่เคยได้รับการวินิจฉัยว่าเป็นโรคระบบการไหลเวียนของเลือดส่วนปลาย (peripheral vascular disease)	❑	❑
5. ไม่เคยได้รับการวินิจฉัยว่าเป็นโรคหลอดเลือดหัวใจ (coronary heart disease)	❑	❑
6. ไม่มีประวัติโรคความดันโลหิตสูง (hypertension) ที่ความรุนแรงระดับ ≥ 160/100 mmHg	❑	❑
7. ไม่เป็นโรคติดเชื้อทางเดินหายใจ (respiratory tract infection) ภายใน 4 สัปดาห์ ก่อนวันทดสอบ	❑	❑
8. ท่านสมัครใจเข้าร่วมโครงการศึกษาวิจัย	❑	❑

เกณฑ์ในการคัดออก

	ใช่	ไม่ใช่
1.ท่านมีพยาธิสภาพของกล้ามเนื้อ (muscle) หรือเส้นประสาท (nerve) ที่ยังคงแสดงอาการอยู่	❑	❑
2. มีแผลเปิด บริเวณแขนข้างที่ไม่ถนัด	❑	❑
3. BMI $\geq$ 30 kg/m2	❑	❑
4. ตั้งครรภ์	❑	❑
5. มีโรคอื่นๆ ที่อาจเป็นอุปสรรคหรือรบกวนผลการทดสอบได้	❑	❑
6. มีประวัติการสูบบุหรี่ หรือหยุดสูบบุหรี่มาอย่างน้อย 10 ปี	❑	❑

APPENDIX B

แบบบันทึกข้อมูลส่วนบุคคล

การวิจัยเรื่อง ผลของความผิดปกติของระบบประสาทออโตโนมิก ต่อการไหลเวียนเลือดบริเวณแขนในผู้ป่วยเบาหวานชนิดที่ 2

ส่วนที่ 1 ข้อมูลพื้นฐาน

เลขที่ .....

น้ำหนัก ..... กิโลกรัม ส่วนสูง .....เซนติเมตร

วัน/เดือน/ปีเกิด ..... อายุ ..... ปี

ระยะเวลาในการเป็นโรคเบาหวาน.....ปี ถนัดแขนข้าง ซ้าย / ขวา

โรคประจำตัวอื่นๆ.....

เพศ ❑ ชาย ❑ หญิง ระยะเวลาที่ประจำเดือนหมดอย่างถาวร (Menopause)……ปี

ท่านได้รับ ยาฮอร์โมนทดแทน ❑ ใช่ ❑ ไม่ใช่

ชื่อยา.....ปริมาณ.....

Oral hypoglycaemic drugs

ชื่อยา.....ปริมาณ.....

ชื่อยา.....ปริมาณ.....

ชื่อยา.....ปริมาณ.....

Insulin injection ❑ ไม่ใช้

❑ ใช้ .....

Cardiovascular/ antihypertensive drugs ❑ ใช้

❑ ไม่ใช้

ชื่อยา.....ปริมาณ.....

ชื่อยา.....ปริมาณ.....

ชื่อยา.....ปริมาณ.....

ชนิดและปริมาณของยาอื่นๆที่ใช้……

……

ลักษณะกิจกรรมทางกาย.....

จำนวน.....ครั้ง ต่อ สัปดาห์ ระยะเวลาในแต่ละครั้ง..... นาที

ลักษณะกิจกรรมทางกาย.....

จำนวน.....ครั้ง ต่อ สัปดาห์ ระยะเวลาในแต่ละครั้ง..... นาที

ส่วนที่ 2 แบบรายงานผลการวิจัย

วัน/เดือน/ปี ที่ทำการทดสอบ ..... เวลาที่ทำการทดสอบ.....น.

หมายเหตุ .....

ข้อมูลการทดสอบ

อัตราการเต้นของหัวใจขณะพัก ท่านั่ง ..... ครั้งต่อนาที ท่านอน ..... ครั้งต่อนาที

ความดันโลหิตขณะพัก ท่านั่ง..... มิลลิเมตรปรอท ท่านอน ..... มิลลิเมตรปรอท

แรงบีบมือสูงสุด ครั้งที่ 1).....ครั้งที่ 2)……ครั้งที่ 3)…….. ค่าเฉลี่ย..... 30% MVC =……..45% MVC=……..

แขนข้างที่วัด…….

ความกว้างรอบแขน ..... เซนติเมตร

ความยาวของ strain gauge ที่ใช้ ..... เซนติเมตร

การวัด autonomic function test

-การเปลี่ยนแปลงของอัตราการเต้นของหัวใจขณะหายใจเข้าขออก อย่างช้าๆ 6 ครั้ง

(Heart rate response to deep breathing) score =…….

HRmax …… – HRmin…….. =……...

- การเปลี่ยนแปลงของอัตราการเต้นของหัวใจขณะหายใจต้านแรงดันเมื่อหายใจออก

(Heart rate response to Valsalva maneuver) score =…….

HRmax……÷ HRmin……. = ……..

- การเปลี่ยนแปลงของอัตราการเต้นของหัวใจ ต่อการเปลี่ยนแปลงท่าในทันที (Heart rate response to standing up) score =…….

HRmax(10-20)…….. ÷ HRmin(25-35)……. = ……

- การเปลี่ยนแปลงของความดันโลหิตขณะเปลี่ยนจากท่านอนเป็นยืน (Blood pressure response to standing up) score =…….

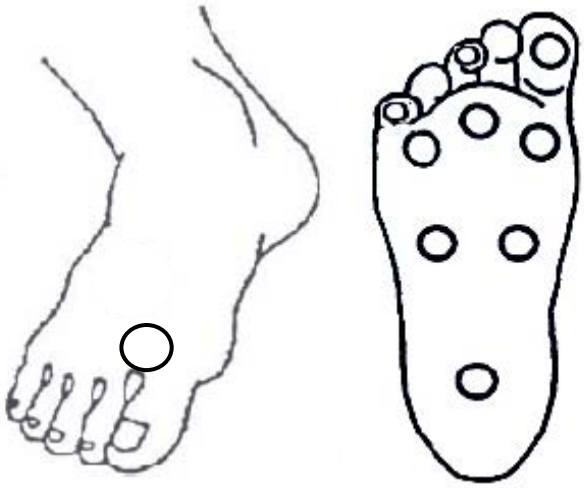
Systolic BP (supine)……. – Systolic BP (stand)……. = ……..

- การเปลี่ยนแปลงของความดันโลหิตขณะเกร็งกล้ามเนื้อมือ (Blood Pressure response to handgrip exercise) score =…….

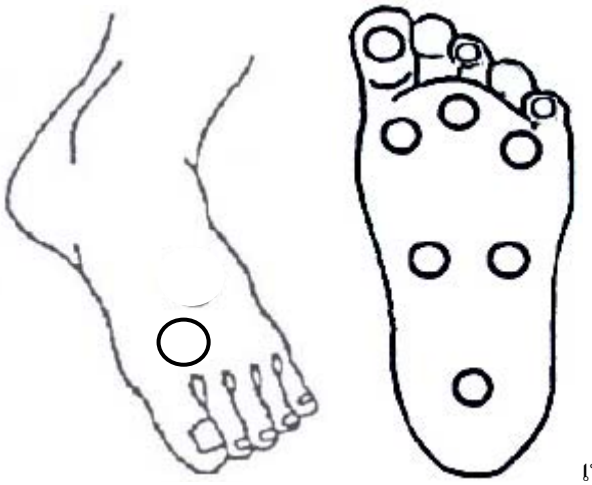
Diastolic BP after…… - Diastolic BP before…….= ……

Total score =…….

การตรวจวัดการรับรู้ความรู้สึก : Semmes-Weinstein Monofilament



เท้าขวา



เท้าซ้าย

จำนวนตำแหน่งที่ไม่ตอบสนอง.....ตำแหน่ง

- ❑ Impairment of Protective sensation
- ❑ Loss of Protective sensation
- ❑ Normal

ข้อมูล	ผลการทดสอบ																			
	วิธีการวัด	นาทีที่ 1				นาทีที่ 2				นาทีที่ 3				นาทีที่ 4				นาทีที่ 5			
การไหลเวียนเลือดและความดัน	Blood pressure																			
	Resting blood flow																			
	Blood pressure																			
	Exercise hyperemia																			
	Blood pressure																			
	Resting blood flow																			
	Blood pressure																			
	Reactive hyperemia																			

ปริมาณโลหิตบริเวณแขนส่วนปลาย (mL/100 mL/min)

BIOGRAPHY

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