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The effect of sex on cardiovascular dynamics in normal and diabetic rates

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THE EFFECT OF SEX ON CARDIOVASCULAR DYNAMICS IN NORMAL AND DIABETIC RATS

by

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DEDICATION

To my husband,

Juan A. Morales Torres

my parents,

Agustín Martínez Nieves

Aida E. Nieves Baladejo

my sister,

Belinda E. Martínez Nieves

my niece and nephew,

Aidamar and Abner N. Torres Martínez

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CHAPTER I

OVERVIEW

GENERAL INTRODUCTION

Diabetes mellitus is one of the most prevalent endocrine disorders. It is the fourth leading cause of death by disease in the United States (CDC, 1997). It accounts for approximately 15% ($92 billion) of the annual health care expenditures and afflicts more that 5% of the total population in the United States alone (Sacks, et al, 1996). In 1997, 143 million adults were diagnosed with diabetes and it is estimated that by the year 2025 the number of cases of diabetes will have more than doubled to 300 million world wide (WHO, 1998). The trademark of this metabolic disorder is insulin insufficiency/deficiency (Ganong, 1993). It is also characterized by hyperglycemia, glucose underutilization, glycosuria, osmotic diuresis, polydipsia, polyphagia (with weight loss), increased protein and fat catabolism, ketosis, acidosis, and coma (Ganong, 1993; Sacks et al, 1996; National Diabetes Data Group, 1995). Four main types of diabetes have been described: Type I also known as insulin-dependent diabetes mellitus (IDDM), Type II or non-insulin-dependent diabetes mellitus (NIDDM), gestational diabetes mellitus (GDM), and diabetes secondary to other conditions (National Diabetes Data Group, 1995). But, the focus of this discussion will be on IDDM and NIDDM.

The incidence of this disorder increases with age (~50 % in individuals > 55 years old) and there seen to be racial as well as gender predisposition among individuals (Sacks et al, 1996; National Diabetes Data Group, 1995). In 1991, by the age of 65, 33% Hispanics, 25% Blacks, and 17% Whites were afflicted by this disorder

(Harris, 1991). In addition, according to Wenger (1995) and Morgan, et al, (1996) women are at a greater risk than men to develop diabetes mellitus (Barrett-Connor, et al, 1991).

Type I diabetes (IDDM) is the result of autoimmune destruction of the pancreatic β-cells leading to insulinopenia and is usually due to a genetic predisposition (Sacks et al, 1996; Dotta, et al, 1996; Roep, 1996). IDDM is also known as early onset diabetes (< 30 years, although it can set in at any age) and it accounts for approximately 5-10% of all diabetic cases in the U.S. (David, et al, 1994; National Diabetes Data Group, 1995).

On the other hand, Type II diabetes (NIDDM) accounts for 90-95% of the individuals diagnosed with diabetes (National Diabetes Data Group, 1995). According to current reports, approximately 15 million Americans, one in every 17 individuals, are afflicted with diabetes (Makrilakis, et al, 1998). Type II diabetes features are: defective insulin secretion which is insufficient to compensate for the coexisting insulin resistance and defects on insulin's actions (insulin resistance) thus, inability of insulin to act on its target tissues (Sacks, et al, 1996; Kruszynska, et al; 1996). NIDDM is also referred to as late onset or mature-onset diabetes (> 40 years) (Ganong, 1993).

A. DIABETES IN FEMALES

According to the National Institute of Health, individuals afflicted by diabetes in U.S. are "more likely to be older, female, member of a race or ethnic minority, less educated," and of low income when compared with non-diabetic individuals (National Diabetes Data Group, 1995). Moreover, after age 45 the likelihood of women to develop diabetes doubles when compared to men. This predisposition is influenced by a woman's genetic background and lifestyle. Therefore, diabetes mellitus imposes a greater risk for

women than for men. Researchers have demonstrated that women are at a greater disadvantage than men once they develop diabetes (Wenger, 1995; Morgan, et al., 1996; Barrett-Connor, et al., 1991; Howard, et al., 1996). Several lines of evidence provide reasoning for this disadvantage. According to the Rancho Bernardo Study (1991), the association between women disadvantage and diabetes is due to the greater survival rate among non-diabetic women. Moreover, this study also demonstrated that diabetes is a significant predictor of death due to ischemic heart disease among women. As a result, they established that diabetes places women at a greater disadvantage than men, and that this gender discrepancy is independent of other factors (Barrett-Connor, et al 1991).

The major disadvantage brought about by diabetes to women is that it eradicates the female's premenopausal protection against cardiovascular diseases (Wenger, 1995; Morgan, et al., 1996; Legato, 1996). This is confirmed by the fact that diabetes has been related to a three to seven-fold increase risk of a cardiovascular episode and this risk is amplified by related risk factors (lipid abnormalities, hypertension, fibrinogen abnormalities, and obesity) which are all prevalent among diabetics (Morgan, et al., 1996). Furthermore, a similar statistical result is also seen in women and their risk for coronary artery disease (CAD). But, this outcome is more striking since contrary to public belief men have a better survival rate than women (Legato, 1996). This is also true among women who suffer myocardial infarction (MI) and diabetes. Additionally, there is a higher prevalence of diabetes among women than men who undergo coronary artery bypass and coronary angioplasty and as a result is it likely to be a contributing factor to increased mortality and poor outcomes for women following myocardial revascularization surgeries (Wenger, 1995). Therefore, it is concluded that controlling and understanding

diabetes in women should be a key factor for ameliorating the gender-gap and women survival rate.

B. DIABETIC COMPLICATIONS

A major complication of diabetes mellitus is vascular disease (Kamata, et al., 1989; King, et al., 1996) that leads to altered peripheral blood flow at the micro- and macrovascular level, artherosclerosis, hypertension, retinopathy and chronic ulceration (Cipolla, et al., 1996; Leese, et al. 1996; Sowers, et al, 1995). Since, circulation abnormalities have been implicated in diabetic complications (Veves, 1998), investigations have focused in the role of the endothelium in the regulation of vascular tone. However, the exact mechanism involving these abnormalities remains unclear.

NITRIC OXIDE

The endothelial production of nitric oxide (NO), a potent vasodilator, plays a regulatory role in the maintenance of blood pressure and the regulation of resting vascular tone in different vascular beds (Abiru, et al., 1993; Costa e Forti, et al., 1998; Koltai, et al., 1997; Dunbar, et al., 1996). NO agonists and antagonists have been useful to characterize the functional role of NO in the regulation of mean arterial pressure (MAP), the control of peripheral vascular tone, the regulation of sympathetic nerve discharge, and endothelial dysfunction in diabetes (Häbler, et al., 1997; Hirai, et al., 1995).

In addition to the decreased NO production associated with diabetes, endothelial NO-mediated vasodilation may also be impaired in diabetes (Calver, et al., 1992). It has been suggested that this endothelial dysfunction or reduced response to endothelial NO in diabetes contributes to the development of diabetic vascular diseases (Abiru, et al., 1993; Calver, et al., 1992).

Furthermore, researchers infer that vascular dysfunction is a consequence of the loss of insulin-dependent vascular homeostasis and endothelial dysfunction as a whole (King, et al, 1996; Baron, 1996; Tooke, 1995). Investigators also refer to an interconnection between microvascular dysfunction, impaired NO-dilation, hyperglycemia, insulin resistance, and the incidence of IDDM and NIDDM (Klein, 1995; Giugliano, et al., 1994; Baron, 1994).

In addition to endothelial functional changes and injury to endothelium, we should also include platelet function abnormalities, and lipoprotein abnormalities (Sank, et al, 1994; Sowers, et al, 1995; Colwell, 1981). Platelet function abnormalities in diabetic individuals have been documented for many years. Among these abnormalities are increased adhesion, aggregation, and generation as well as many other characteristics (Sowers, et al, 1995; Colwell, 1981). In turn these abnormalities disrupt the equilibrium between coagulation and fibrinolysis which makes them key factors for diabetic vascular diseases. Another aberration among diabetic individuals is metabolic anomalies which result in increased lipoproteins in general and increased glycosylation. Both of these factors assist in the development of atherosclerotic plaque and therefore, upregulation of atherogenesis (Sowers, et al, 1995; Colwell, 1981). Therefore, one can conclude that the pathogenesis of diabetic vascular complications is not the result of a single contributing factor but instead the activation of a pathogenic cascade.

SYMPATHETIC NERVOUS SYSTEM

It is acknowledged that the prevalence of diabetic complications increase with the duration of this condition and, as a consequence, diabetic individuals are also afflicted with autonomic dysfunction (Bergström, et al., 1987; Low, 1993). This

phenomenon/condition has been documented well before the discovery of insulin in diabetic patients (Low, 1993). Investigations have suggested alterations in sympathetic-mediated vascular tone as a cause of diabetic vascular disease. The enhanced vessel reactivity, especially of the resistance vessels to specific agonist has been demonstrated (Cipolla, et al., 1996). Both, in vitro and in vivo studies, have demonstrated an increase sensitivity especially to adrenergic agonist in animals with experimental-diabetes (Jackson, et al, 1981), and to normal levels of circulating catecholamines (Beretta-Picolli, et al, 1981). Diabetes exhibits a progressive deterioration of peripheral somatic and autonomic nerve function also known as "diabetic neuropathy" (Bannister, et al, 1992; Taylor, 1994). Diabetic neuropathy is characterized by diffuse or focal damage to peripheral somatic and autonomic nerve fibers (Greene et al., 1992; Vinik et al., 1992). Particularly, deterioration of postganglionic sympathetic neurons and impairment of parasympathetic function (Taylor, 1994). Diabetic neuropathy occurs at a similar rate in IDDM and NIDDM (Greene, et al., 1992).

According to Taylor (1994), vascular α-adrenergic receptor upregulation in individuals with autonomic failure occurs because of deteriorated function of postganglionic sympathetic neurons and a subsequent decrease in norepinephrine (NE) release. Moreover, studies have also documented the coexistence of hyperactivity of the sympathetic nervous system (SNS), hypertension, reduced insulin sensitivity, hyperinsulinemia and diabetes (Tack, et al., 1996; Moan, et al., 1995; Chan, et al., 1995; Ralevic, et al., 1995; Moreau, et al., 1995). To further, support the link between diabetes and the SNS, Tack, et al., (1996), have stated that sympathetic hyperactivity could be a route by which hyperinsulinemia induces atherosclerosis and cardiovascular damage.

They also declare that increased sympathetic activity is not only cause of cardiovascular damage but is also implicated in increased cardiovascular morbidity, characteristics which are also prevalent in diabetes (Julius, 1993).

The administration of insulin in healthy individuals leads to a distinctive increase in sympathetic noradrenergic activity to skeletal muscle. In turn this sympathetic activity may modify muscle blood flow in two ways. Initially, sympathetic activity leads to vasoconstriction because of NE release. Secondly, sympathetic activation results in activation of neural sympathetic vasodilator mechanisms and as a consequence increase skeletal muscle blood flow (Anderson, et al, 1993; Vollenweinder, et al., 1994; Schultz-Klarr, et al., 1994; Pete, et al, 1998). Furthermore, chronic hyperinsulinemia or insulin resistance have been associated with modifications to sympathetic activity and these are implicated in hypertension (Moreau, et al., 1995; Esler, 1995).

The SNS is directly linked to the control of peripheral vascular tone. This association is because NO is synthesized in both systems and has been demonstrated to have a functional role in the regulation of sympathetic nerve discharge and vascular tone (Hirai, et al., 1995; Modin, et al., 1994). The interaction between NO, SNS, and vascular tone is demonstrated when the increased blood pressure mediated by a nitric oxide synthase (NOS) inhibitor is neutralized by an adrenergic blockade (Pohl, et al, 1996).

The presence of NO allows further integration between the incidence of diabetes vascular disease and the involvement of the SNS. It should be mentioned that, according to investigators, altered microvascular function is a contributing factor to nerve fiber dysfunction (nerve atrophy, nerve injury and nerve loss) and these in turn are linked to hyperglycemia and/or insulin deficiency (Greene, et al., 1992; Vinik, et al., 1992).

The central purpose of these studies were to evaluate comparatively the effects of sex and/or diabetes on (a) the inhibition of NO production, (b) the relative contribution of altered NO sensitivity, and (c) adrenergic tone on the selected regional blood flows (iliac, renal and superior mesenteric), mean arterial pressure and heart rate in normal and diabetic animals.

MATERIALS AND METHODS

A. GENERAL ANIMAL CARE

Female and male wistar rats (BW: 250-344 g) from Harlan Laboratory were utilized in our experimental procedures. These animals were kept in a control environment with a 12 hour light cycle and a 23°C room temperature. Prior to the induction of diabetes animals were housed two per cage. All experimental protocols were pre-approved by the Institutional Animal Care and Use Committee.

B. INDUCTION OF EXPERIMENTAL DIABETES

Diabetes was induced in Wistar rats by a single IV injection of streptozotocin (STZ) of 50 mg/kg dissolved in sodium citrate (0.1 mM, pH 4.5). STZ was administered via the tail vein. Approximately, two days after the STZ injection a blood sample was collected from the orbital sinus to determine if diabetes had developed. Animals labeled as "diabetic" displayed a non-fasting blood glucose greater than 275 mg/dl. Animals were utilized 4-6 weeks post-STZ injection.

C. MONITORING OF CARDIOVASCULAR PARAMETERS

1. Surgical Procedures

On the day of the study and following a 24 hr fast, normal and diabetic rats were anesthetized with urethane/α-chloralose 500 mg/kg and 80 mg/kg, respectively. Animals

were placed on a heating pad to maintained their body temperature at 37 ± 1.0°C. After placing and securing animals in position a rectal thermometer was inserted to monitor body temperature. To diminish the occurrence of respiratory obstruction during surgery, a tracheotomy was performed. Once the tracheotomy was completed a 4 cm polyethylene 200 (PE 200) tube was inserted and secured to maintain an open airway.

2. Catherization

Catheters for the blood vessels were made of polyethylene 50 (PE 50) and filled with heparinized saline (2000 U/ml). Bilateral incisions were made in the inguinal area above the femoral artery and vein. Both vessels were isolated, securely tied at the distal end and a loose suture and a clamp were placed at the proximal end. Subsequently, a small incision was made in the vessel and the catheter was inserted and secure with the loose suture placed at the proximal end. The right femoral vein catheter was used to deliver the anesthetic (constant infusion: urethane/α-chloralose: 25 and 4 mg/ml, respectively, at a rate of 0.49 ml/hr). The left femoral vein catheter was utilized for blood sample collection and treatments. The same procedure mentioned above was followed for the catherization of the right femoral artery. The femoral artery cannula was utilized for cardiovascular recording via a Spectra-Med pressure transducer.

Two blood samples (0.2 mls) were collected during all experimental procedures. An initial blood sample was collected 5 or 10 minutes before treatment (t = -5 or 10 min) and a second one at 30, 60, or 80 minutes after treatment or at the end of the experimental procedure. The blood volume was immediately replaced with heparinized saline. Promptly after blood collection, samples were transfer into microtubes containing 0.2 mls of heparinized saline, centrifuged and stored at -30 °C for subsequent blood

glucose analysis.

3. Blood Flow Monitoring

A laparotomy was performed for the placement of pulsed-Doppler blood flow probes on three preselected vessels: left common iliac, left renal, and superior mesenteric arteries. All three vessels were isolated and a velocity transducer (flow probe, Baylor electronics) was placed around each vessel. We utilized a 1.0 velocity transducer for the iliac and renal artery, and for the superior mesenteric artery we utilized a 2.0 velocity transducer. Following all of the instrumentation attachments, laparatomy and inguinal incisions were secured with 9 mm stainless steel autoclips (Becton Dickinson and company). The arterial catheter was connected to a pressure transducer and the flow probes connected to a pulsed-Doppler flowmeter (Baylor electronics). A micro 5000 signal processing system was used to measure the cardiovascular responses.

D. EXPERIMENTAL PROCEDURES

The experimental procedures were performed in three different parts: 1) A single dose response to L-NAME (10 mg/kg) compared to saline controls 2) A dose response curve to sodium nitroprusside (SNP) concentrations (1-20 μg/kg) compared to saline controls 3) A single dose response to Prazosin (4 mg/kg) compared to vehicle. All of these procedures were performed in normal and diabetic female and male rats and compared to their respective controls. The dose response curve consisted of four subsequent infusions of 0.2 ml of saline or the experimental agent administered in twenty minutes intervals as a bolus via the femoral vein.

Blood samples were collected prior to drug infusion, 60 minutes after treatment or at the end of the experimental protocol. Mean arterial pressure, systolic and diastolic

pressure, pulse pressure, heart rate, and blood flows (iliac, renal, and superior mesenteric) were monitored continuously.

E. DATA COLLECTION

The Biowindows Software program and a Micro 5000 signal processing system were utilized to monitor cardiovascular responses. The Biowindows Program records all cardiovascular parameters: mean arterial pressure (mm Hg), systolic and diastolic pressure (mm Hg), pulse pressure (mm Hg), heart rate (beats/min), and blood flow (KHz doppler shift).

The cardiovascular responses were monitored continuously for approximately 80 minutes. The data for the first protocol are averages of 30 second intervals for the reported period following L-NAME treatment. The data points for the second protocol are peak responses of 15 seconds intervals from the control period up to 4 minutes post-SNP treatments. The data for the third experimental procedure are averages of one minute intervals for the reported periods following Prazosin administration.

F. GLUCOSE ANALYSIS

Plasma glucose levels were determined by a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH).

G. EUTHANASIA

Once the experiment was concluded, animals were terminated by pneumothorax.

H. STATISTICAL ANALYSIS

All the data is reported as mean ± S.E.M. A one-way analysis of variance (ANOVA) was used to determine if there was a significant response difference within a group as function of concentration and to determined whether mean values had changed

as a function of time within a group. Two-way ANOVA was utilized to compare different groups. The student t-test was utilized to determine if there was a significant difference between pairs of means (normal vs diabetic) for each parameter.

CHAPTER II

ENDOTHELIAL-DEPENDENT VASODILATION AND THE INFLUENCE OF SEX IN DIABETES

INTRODUCTION

Vascular disease is the major complication of diabetes mellitus (King, et al, 1996). It can result in modifications in peripheral blood flow at the micro- and macrovasculature level. Modifications at the microvasculature level are mainly associated with retinal and renal disease whereas, changes in the macrovasculature are more commonly seen in lower limbs, cerebral vessels and coronary arteries (King, et al, 1996; Chakir, et al, 1996; Pete, et al, 1998). However, the exact mechanisms involving these complications remain unclear.

Nitric oxide (NO) is a potent vasodilator and its production by the endothelium plays an important role in the maintenance of blood pressure and the control of vascular resting tone of different vascular beds (Abiru, et al., 1993; Koltai, et al., 1997). The synthesis of NO from L-arginine can be antagonized by nitric oxide synthetase (NOS) antagonist such as N^G-mono-methyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME). These inhibitors have been utilized to characterized the functional role of NO in the regulation of blood pressure, in the control of peripheral vascular tone, and in the regulation of sympathetic nerve discharge (Habler, et al., 1997; Hirai, et al., 1995).

Systemic infusion of L-NAME or L-NMMA results in a dose-dependent pressor effect associated with regional vascular constriction. This pressor effect is consistently associated with bradycardia. These observations reiterate the involvement of NO in the

control of regional vascular conductance and blood pressure (Calver, et al., 1992; Gardiner, et al., 1990; Kiff, et al., 1991). Reports in the literature indicate that there is a decrease in NO formation in diabetic vessels leading to an impairment or dysfunction of the vascular endothelium (Abiru, et al., 1993; Calver, et al., 1992).

The emphasis of this study, was to evaluate and compare the effects of the inhibition of NO production on regional blood flow (iliac, renal and superior mesenteric), mean arterial pressure and heart rate in female and male normal and diabetic rats.

MATERIALS AND METHODS

Normal and diabetic female and male, Wistar rats (BW: 250-275 g) were used in our experimental procedures. They were kept in a control environment with a 12 hour light cycle and a 23°C room temperature with free access to water and food. Diabetes was induced in normal rats by a single IV injection in the tail vein of streptozotocin (STZ) of 50 mg/kg dissolved in sodium citrate (0.1 mM, pH 4.5). Five days after the STZ injection a blood sample was collected to determine hyperglycemia and maintained 4-6 weeks post STZ injection.

On the day of the study and following a 24 hr fast, normal or diabetic rats were anesthetized with urethane (0.5 mg/kg) and α-chloralose (70 mg/kg) and placed on a heating pad to maintained their body temperature. A tracheotomy was performed to diminish respiratory obstructions and catheters with heparinized saline were placed into the femoral artery and veins. The venous catheter was utilized for blood sample collection and infusions. The femoral artery cannula was utilized for cardiovascular recording.

Pulsed-Doppler blood flow transducers (flow probe, Baylor electronics) were

placed around the iliac, renal, and the superior mesenteric arteries. The arterial catheter was connected to a pressure transducer and the flow probes connected to a pulsed-Doppler flowmeter (Baylor electronics). A micro 5000 signal processing system was utilized to measure the cardiovascular responses.

Normal and diabetic female and male rats were given a single bolus injection of N-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg) five minutes after a stable baseline. Mean arterial pressure (MAP), heart rate (HR), and blood flows (iliac, renal, and superior mesenteric) were monitored continuously.

The Biowindows Software Program and a Micro 5000 signal processing system were used to monitor cardiovascular responses. The Biowindows Program records all cardiovascular parameters: mean arterial pressure (MAP), heart rate (HR), and blood flow (Hz Ds units).

Blood samples, 0.2 mls with saline replacement were collected prior to the study. The blood sample was centrifuged and used for glucose analysis (glucose analyzer; Yellow Springs Instruments Co., Yellow Springs, OH.

The cardiovascular responses were monitor for approximately 60 minutes. The data presented are averages of 30 seconds intervals for the reported periods following L-NAME treatment. The data was analyzed using student t-test or ANOVA with repeated measures and Post-hoc analysis where appropriate.

RESULTS

A. THE EFFECT OF A SINGLE SALINE INFUSION ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC RATS

The intent of this protocol was to established baseline responses for

cardiovascular responses and blood flow in normal and diabetic rats. Normal saline was administered in a single bolus injection of 0.2 ml via the femoral vein. Instrumentation of all animals was as described above (normal female, N = 6; diabetic female, N = 5; normal male, N = 6; diabetic male, N = 4).

The body weight and heart rate were decreased, however, not significantly except for the body weight of diabetic male rats when compared to normals (Table 1). Blood glucose was significantly increased in diabetic animals when compared with its normal counterpart (Table 1). Basal mean arterial pressure was not different between the groups (Table 1).

The administration of saline resulted in a similar pattern in normal and diabetic animals with respect to MAP and HR (Fig. 1 and 2). MAP displayed an initial increase in percent change following the treatment and in less than five minutes returned to baseline (Fig. 1A and 2A). Saline elicited an increase in HR which also returned to baseline in less than five minutes (Fig. 1B and 2B). Blood flow in the three vascular beds of normal and diabetic animals were not significantly different (Fig. 3 and Fig. 4). However, diabetic males exhibited a significant increased in blood flow in the iliac bed following saline treatment (Fig. 4). Diabetic females displayed a significant two-way ANOVA group effect with respect to the superior mesenteric artery blood flow ($p < 0.01$) (Fig. 3C). On the other hand, diabetic males exhibited a significant two-way ANOVA group effect in the iliac ($p < 0.001$), renal ($p < 0.01$) and superior mesenteric ($p < 0.01$) arteries blood flow (Fig. 4).

Conductance (g) was calculated as blood flow divided by mean arterial pressure (g = blood flow/MAP). This parameter is an index of vasodilation and vasoconstriction and

it is also defined as the reciprocal of resistance. In normal female rats, saline administration initially decreased conductance in all three vascular beds (Fig. 5). On the other hand, diabetic female rats exhibited a minor increase in conductance in all three vascular beds with no significant differences when compared to normals with the exception of the iliac bed (Fig. 5). When a two-way ANOVA group effect test was performed normal females were significantly different from diabetic females superior mesenteric conductance ($p < 0.001$) (Fig. 5C). In male rats, no significant differences were observed in the renal and superior mesenteric bed (Fig. 6B and 6C). However, diabetic males displayed a significant increased in iliac conductance when compared to normals (Fig. 6A). In addition, diabetic males also exhibited a significant Two-way ANOVA group effect with respect to iliac conductance (Fig. 6A).

TABLE 1 Basal Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP, mm Hg), and Heart Rate (HR) In Saline Treated Normal And Diabetic Rats.

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	252 ± 7	97 ± 4	72 ± 4	360 ± 14
Female	(6)	(6)	(6)	(6)
Diabetic	198 ± 9	354 ± 29*	70 ± 2	325 ± 14
Female	(5)	(5)	(5)	(5)
Normal	282 ± 14	74 ± 4	82 ± 5	387 ± 25
Male	(6)	(6)	(6)	(6)
Diabetic	216 ± 4*	409 ± 45*	79 ± 8	309 ± 12
Male	(4)	(4)	(4)	(4)

The values represent the mean ± S.E.M. * = $p < 0.01$ vs. normal counterpart, ANOVA. Number in parenthesis = N

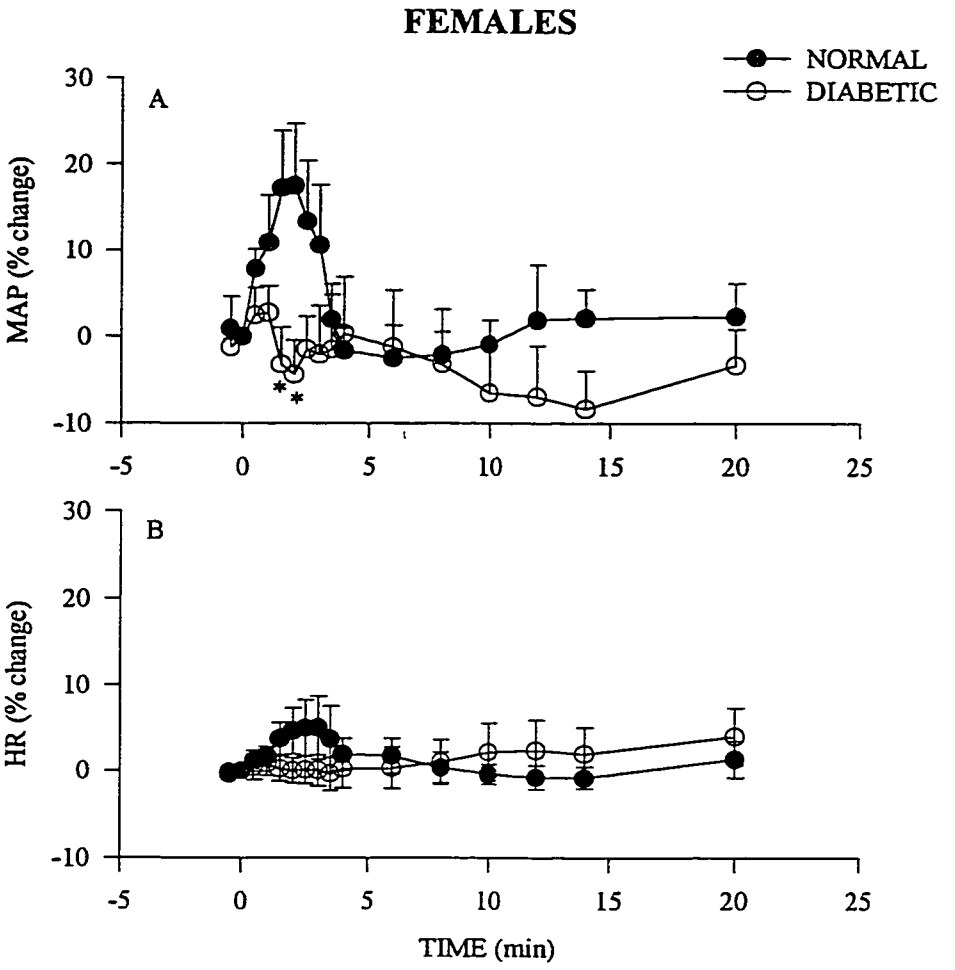

Figure 1. The effect of a single saline treatment (0.2 mls) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) in normal (N = 6) and diabetic (N = 5) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female MAP, $p < 0.001$.

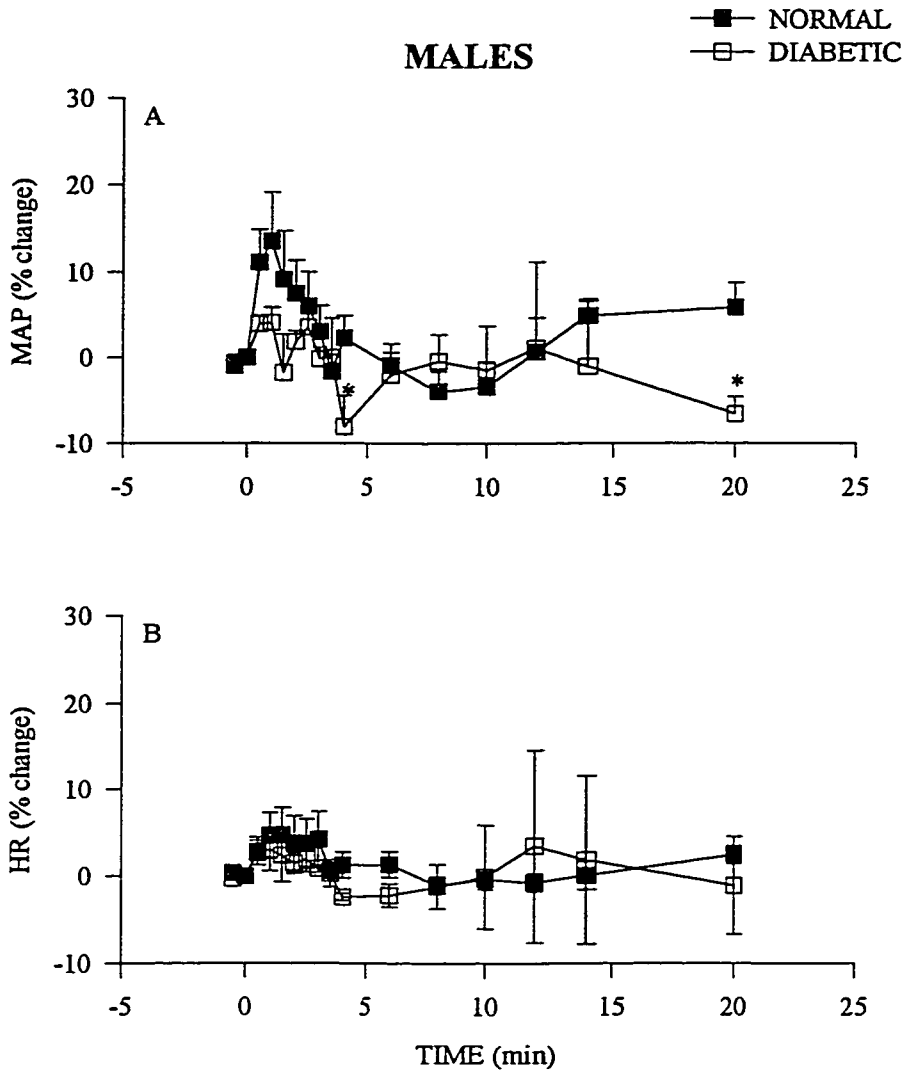

Figure 2. The effect of a single saline treatment (0.2 mls) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) in normal (N = 6) and diabetic (N = 4) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male MAP, $p < 0.05$.

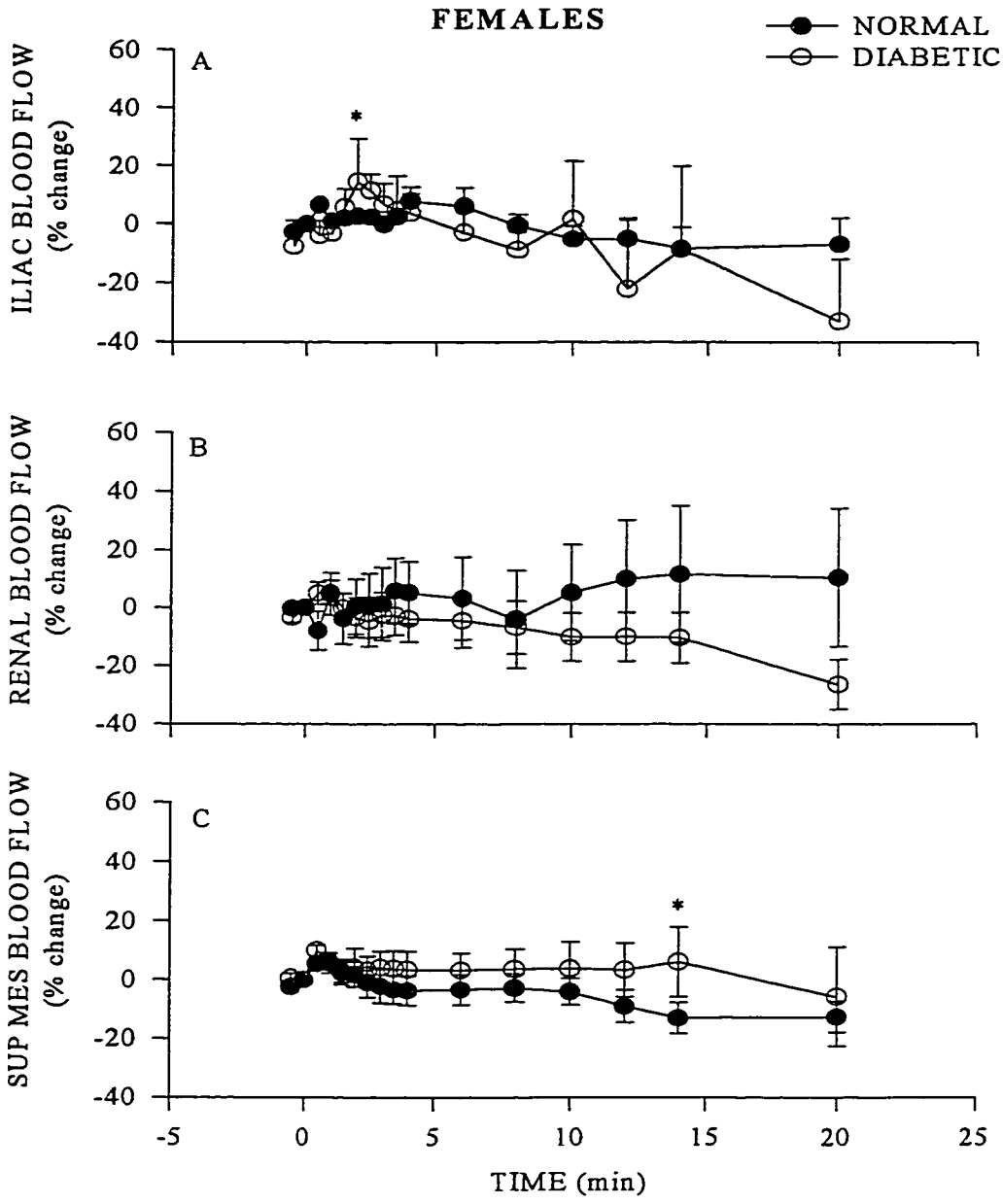

Figure 3. The effect of a single saline treatment (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 6) and diabetic (N = 5) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female Sup Mes blood flow, $p < 0.01$. The close circles represent normal females and the close circles represent the diabetic female rats.

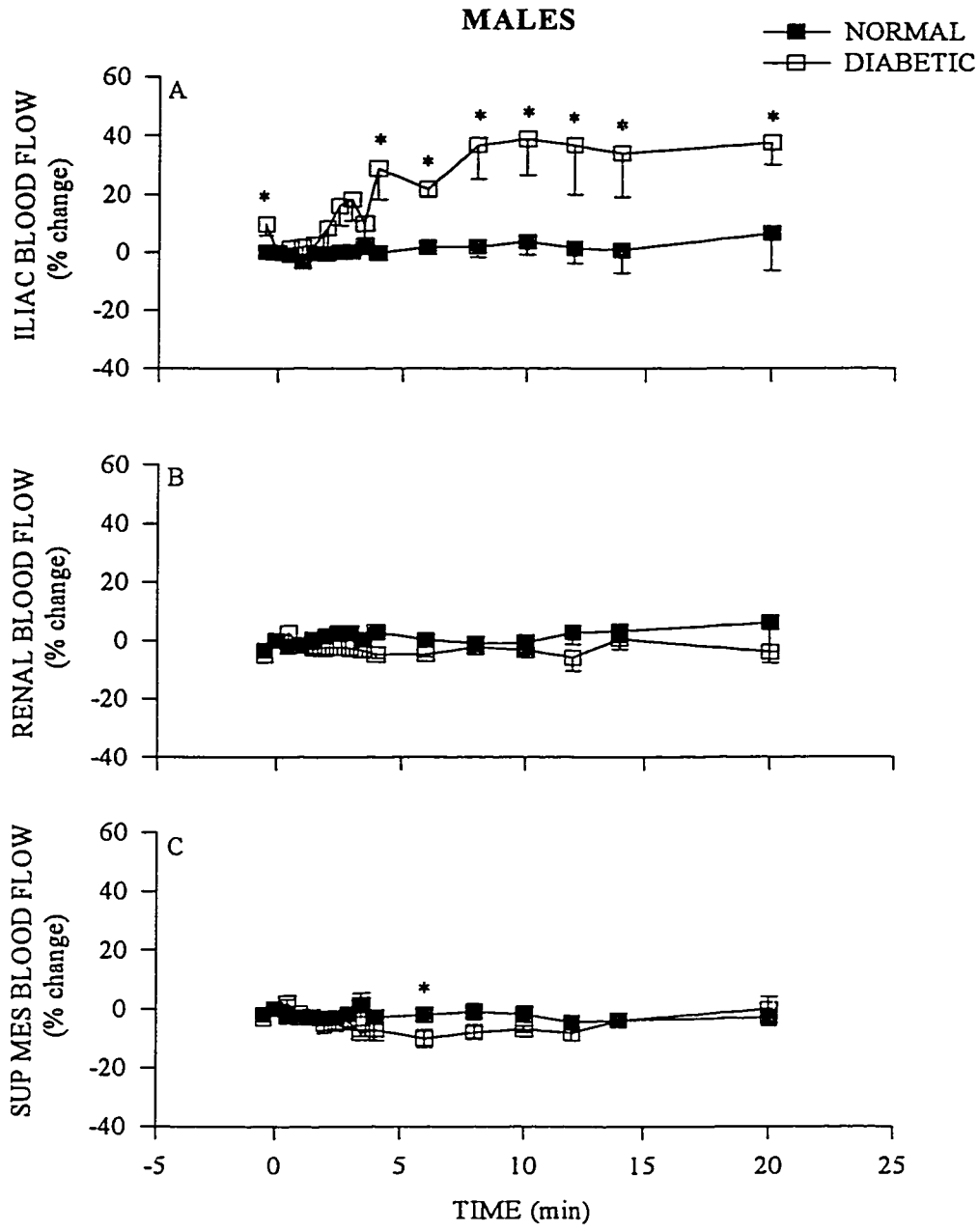

Figure 4. The effect of a single saline treatment (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 6) and diabetic (N = 4) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male Iliac blood flow, $p < 0.001$, Renal blood flow, $p < 0.01$ and Sup Mes blood flow, $p < 0.01$.

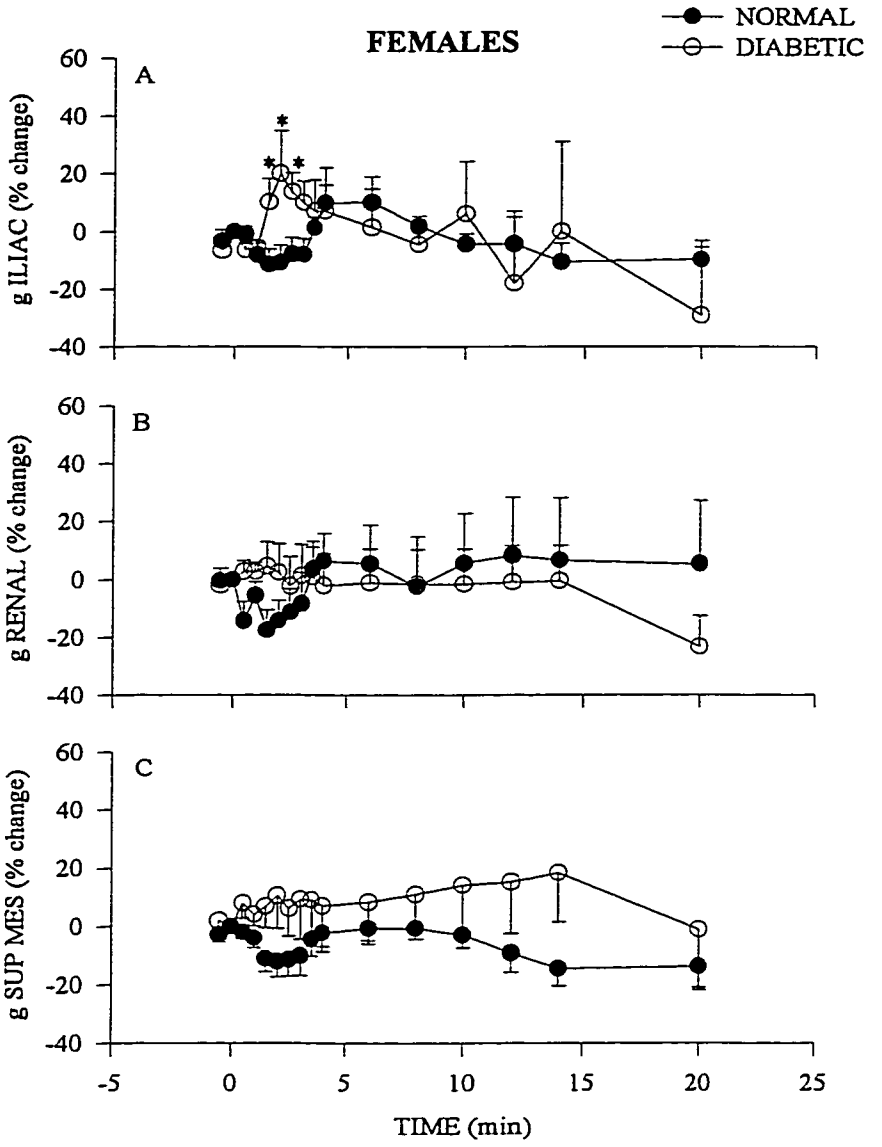

Figure 5. The effect of a single saline treatment (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 6) and diabetic (N = 5) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female g Sup Mes, $p < 0.001$.

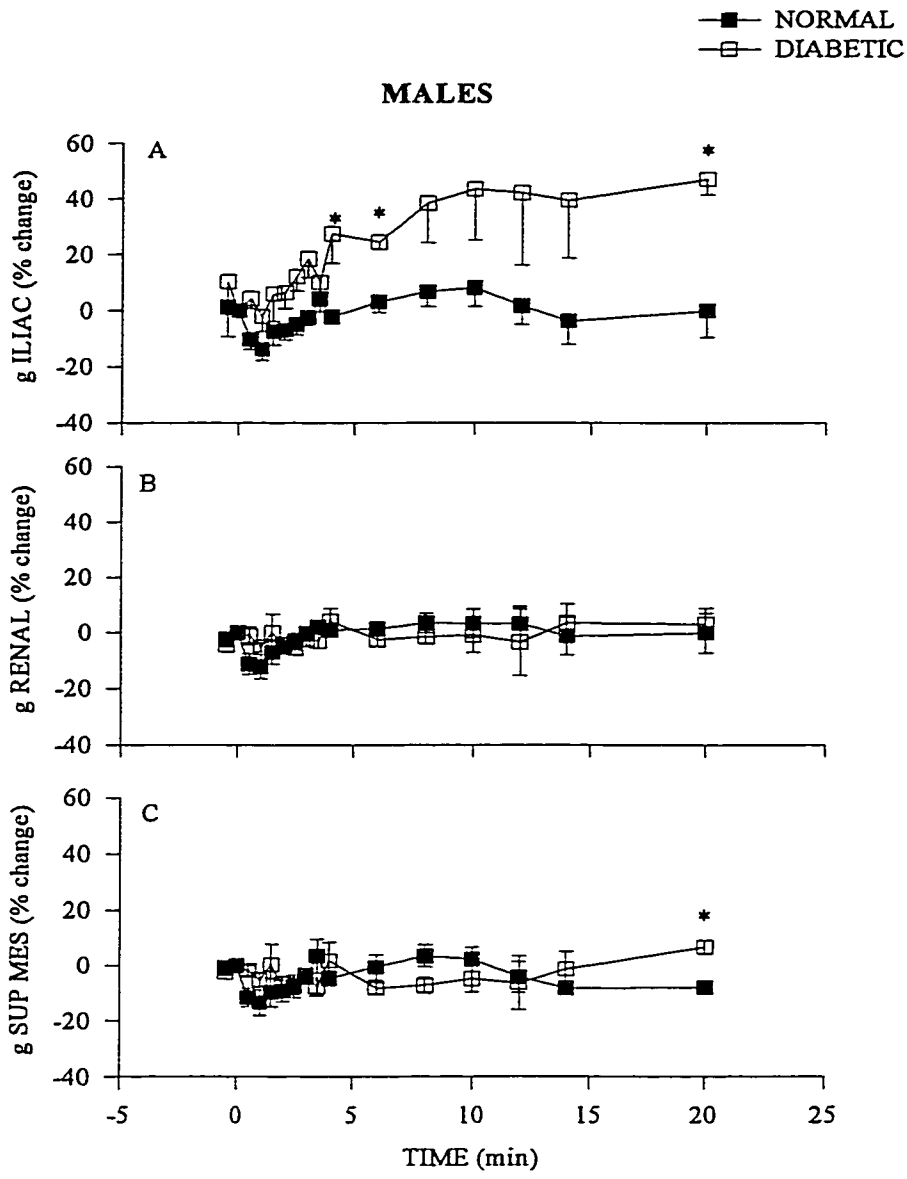

Figure 6. The effect of a single saline treatment (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 6) and diabetic (N = 4) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male g Iliac, $p < 0.001$.

B. THE EFFECT OF SYSTEMIC INFUSION OF L-NAME (10 mg/kg) ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC RATS

The purpose of this protocol was to evaluate the influence of a NO synthase (NOS) inhibitor, L-NAME (10 mg/kg), on cardiovascular responses and blood flow in normal and diabetic female vs. male rats. The systemic administration of the NOS inhibitor, consisted of a single bolus injection of L-NAME (10 mg/kg) (0.2 ml) via the femoral vein (normal female, N = 9; diabetic female, N = 8; normal male, N = 8; diabetic male, N = 6).

Animals were instrumented as described above. Blood samples (0.2 mls) were collected five minutes prior to the treatment infusions and 30 minutes after L-NAME treatment. Monitoring was recorded continuously for 40 minutes. The blood volume was immediately replaced with heparinized saline. Control animals runs were carried out similarly however, they were infused with normal saline (0.2 mls) instead of L-NAME.

The body weight and HR were decreased and the blood glucose increased significantly in the diabetic rats when compared to normals (Table 2). Basal mean arterial pressure was not different between the groups (Table 2).

The administration of L-NAME increased the MAP in normal and diabetic female rats (Fig. 7A). Normal females had a higher peak MAP and greater average pressure response to L-NAME when compared to diabetic females (Table 3 and Fig. 7A). When a two-way ANOVA group effect was performed, diabetic females were significantly different to normal females ($p < 0.001$). The HR in response to the pressor effect of NO antagonist was decreased to a greater degree in the normals compared to diabetics ($p < 0.001$; Two-way ANOVA group effect) (Table 3 and Fig. 7B).

Normal and diabetic males basal and peak MAP response to the NO antagonist was increased. A two-way ANOVA demonstrated that diabetic males are significantly different to normal males with respect to HR ($p < 0.001$). Again, the pressor response to NO suppression was significantly less in diabetic males (Fig. 8A and Table 3). The HR in response to NO antagonist-induced pressor response was decreased to a greater degree in diabetic males (Fig. 8B).

As indicated in Fig. 9 and 10, the administration of L-NAME resulted in a decrease in blood flow in all three vascular beds in normal and diabetic females. However, diabetic females exhibited an increase in renal blood flow when compared to normal females (Fig. 9B). Like females, normal and diabetic males displayed a decrease in blood flow in all three vascular beds with no change in the iliac bed of normal males upon the administration of L-NAME. However, this decrease in blood flow was significantly greater in the iliac bed in diabetic males (Fig. 10A). When a two-way ANOVA group effect was performed diabetic females were significantly different compared to its normal counterpart in the renal bed ($p < 0.001$), whereas, diabetic males were significantly different compared to its normal counterpart in the iliac bed ($p < 0.001$).

In female rats, L-NAME decreased the conductance to comparable degrees in the iliac but was attenuated in the superior mesenteric vascular beds in normal versus diabetic animals. However, L-NAME increased conductance significantly in the renal vessels of diabetic females (Fig. 11B). When a two-way ANOVA group effect was performed diabetic females were significantly different than normal females in the renal ($p < 0.001$) and superior mesenteric ($p < 0.01$) conductance.

In male rats, L-NAME decreased the conductance in all three vascular beds in normal and diabetic animals (Fig. 12). However, this decrease was significantly greater in the iliac vascular bed of the diabetic animals (Two-way ANOVA group effect; $p < 0.01$) (Fig. 12A). It should also be noted that upon a two-way ANOVA group effect test diabetic males were significantly different when compare to normal males in the superior mesenteric bed in terms of conductance.

In this study, we evaluated the average changes in MAP and the reflex changes in HR as an index of baroreflex sensitivity 10 minutes post-L-NAME administration (Fig. 13). The increased MAP following L-NAME resulted in a greater bradycardia in normal females when compared to diabetic females, normal and diabetic males. However, no significant differences were found when all four groups were evaluated (normal vs. diabetic animals for both sexes). On the other hand, when animals were evaluated by sex group, diabetic males displayed a significantly different relationship between MAP and HR when compared to normal males. No significant differences were observed among females.

TABLE 2 Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP) And Heart Rate (HR) In L-NAME (10 mg/kg) Treated Normal And Diabetic Rats

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	254 ± 5	90 ± 6	79 ± 4	469 ± 23
Female	(9)	(9)	(9)	(9)
Diabetic	213 ± 11*	382 ± 48*	72 ± 4	341 ± 9*
Female	(8)	(8)	(8)	(8)
Normal	286 ± 3†	78 ± 9	72 ± 2	419 ± 27
Male	(8)	(8)	(8)	(8)
Diabetic	230 ± 13*	412 ± 32*	77 ± 6	393 ± 20
Male	(6)	(6)	(6)	(6)

The values represent the mean ± S.E.M. * = $p < 0.05$ diabetic vs. normals, † = $p < 0.05$ males vs. females ANOVA. Number in parenthesis = N.

TABLE 3 Mean Arterial Pressure (MAP), Heart Rate (HR), Basal, Absolute, Percent Change Plateau, and Peak Responses to L-NAME (10 mg/kg) In Normal And Diabetic Rats

Group	Basal MAP (mm Hg)	Peak MAP (mm Hg)	MAP Plateau (mm Hg)	MAP Plateau (% change)	Basal HR (beats/min)	Post L-NAME HR (beats/min)
Normal	79 ± 4	120 ± 3	117 ± 1	49 ± 1	469 ± 23	405 ± 18
Female	(9)	(9)	(9)	(9)	(9)	(9)
Diabetic	72 ± 4	104 ± 3*	81 ± 1*	15 ± 2*	341 ± 9**	321 ± 20*
Female	(8)	(8)	(8)	(8)	(8)	(8)
Normal	72 ± 2	121 ± 4	112 ± 1**	58 ± 2**	419 ± 27	429 ± 24
Male	(8)	(8)	(8)	(8)	(8)	(8)
Diabetic	77 ± 6	112 ± 1*	99 ± 1*†	30 ± 1*	393 ± 20	357 ± 19*
Male	(6)	(6)	(6)	(6)	(6)	(6)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normals, ** = $p < 0.05$ vs. normal female, † = $p < 0.0001$ vs. diabetic female, student t-test. Number in parenthesis = N.

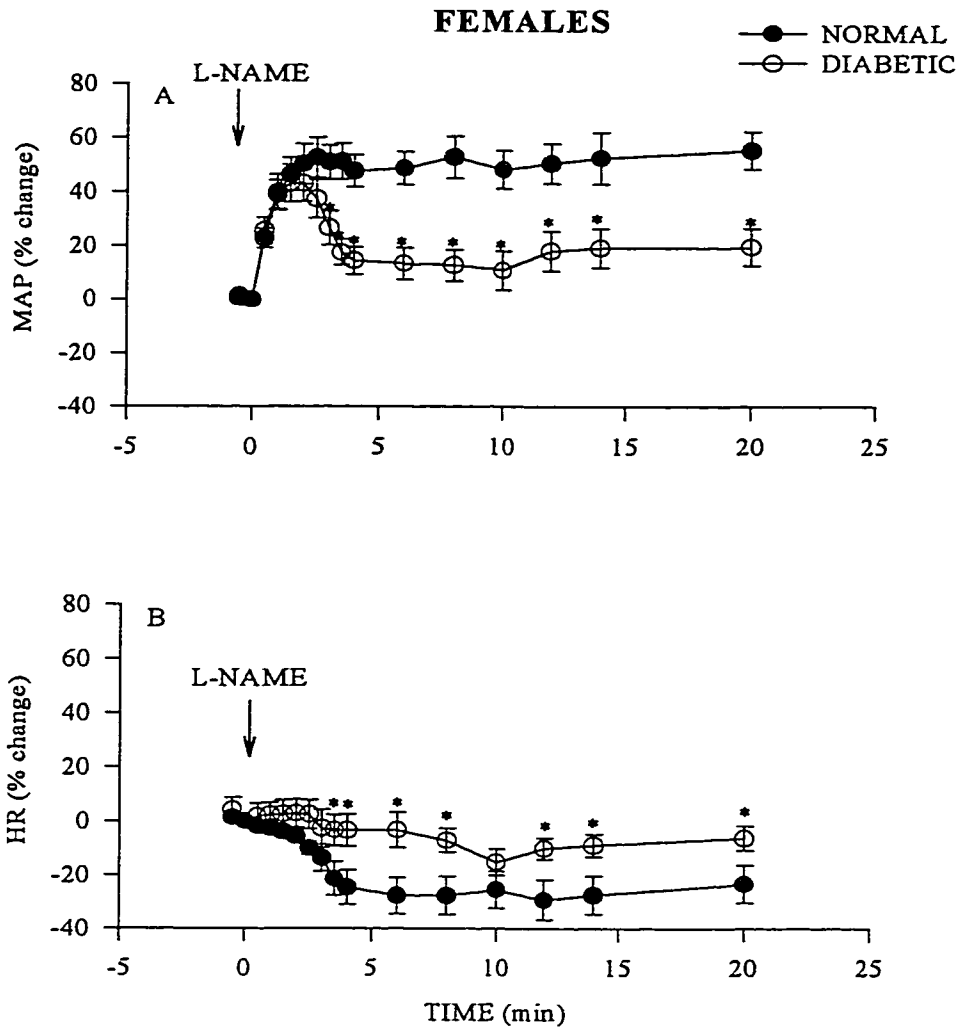

Figure 7. The effect of L-NAME (10 mg/kg) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) in normal (N = 9) and diabetic (N = 8) female rats expressed as percent change. * = $p < 0.05$ vs. normal female rats. Two-way ANOVA group effect normal vs. diabetic female MAP, $p < 0.001$ and normal vs. diabetic female HR, $p < 0.001$.

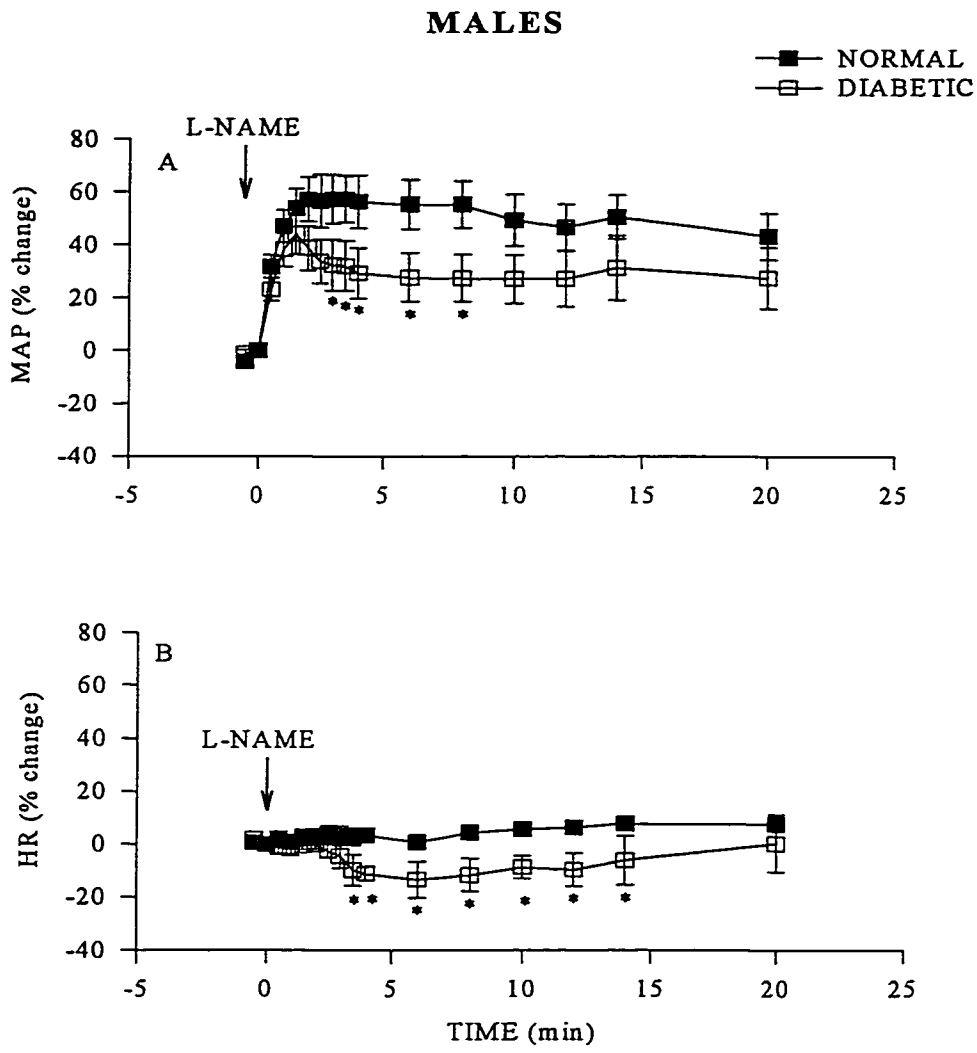

Figure 8. The effect of L-NAME (10 mg/kg) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 8) and diabetic (N = 6) male rats. * = $p < 0.05$ vs. normal male rats. Two-way ANOVA group effect normal vs. diabetic male MAP, $p < 0.001$; normal vs. diabetic male HR, $p < 0.001$.

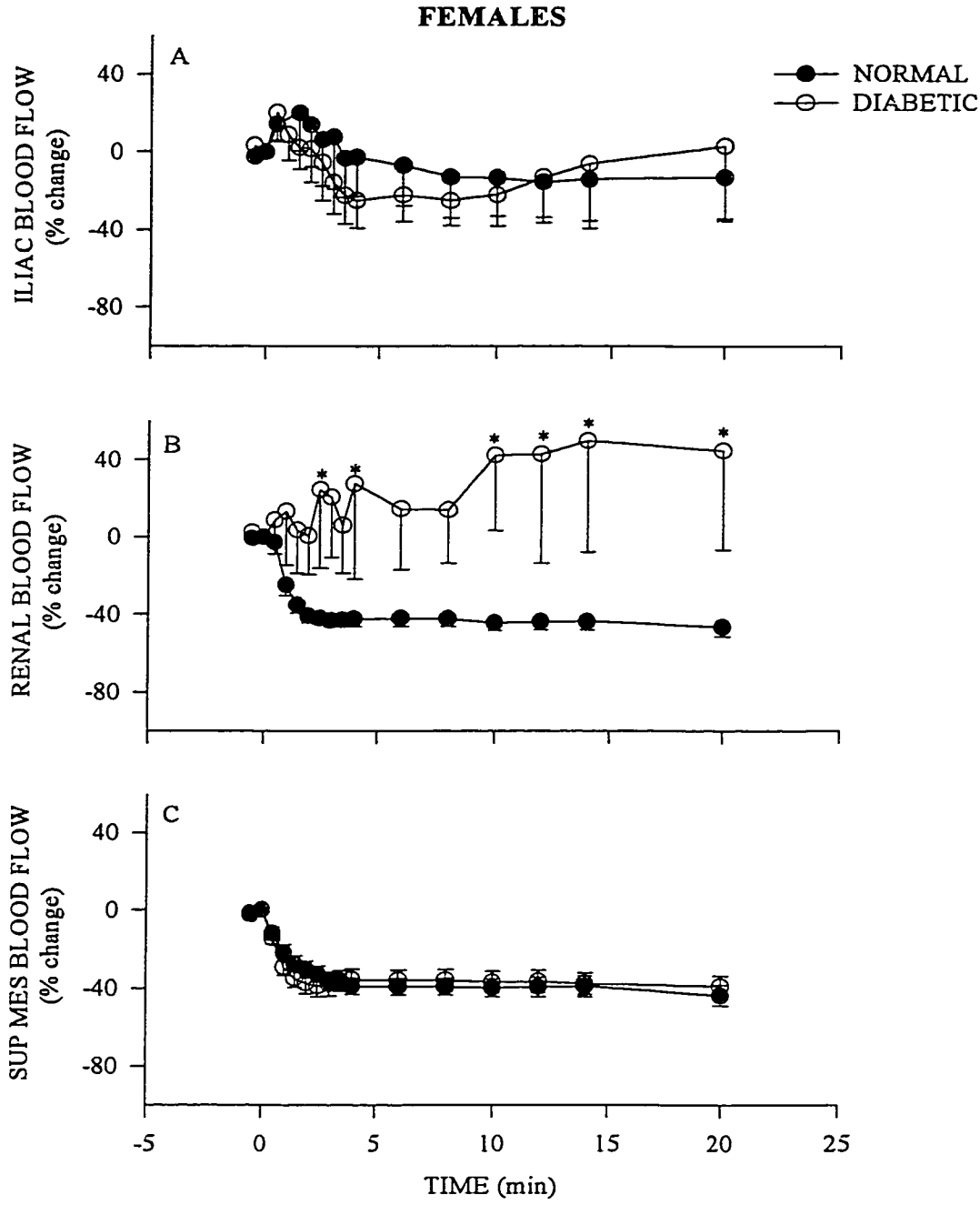

Figure 9. The effect of L-NAME (10 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 9) and diabetic (N = 8) female rats. * $p < 0.05$ vs. normal female rats. Two-way ANOVA group effect renal blood flow, $p < 0.001$.

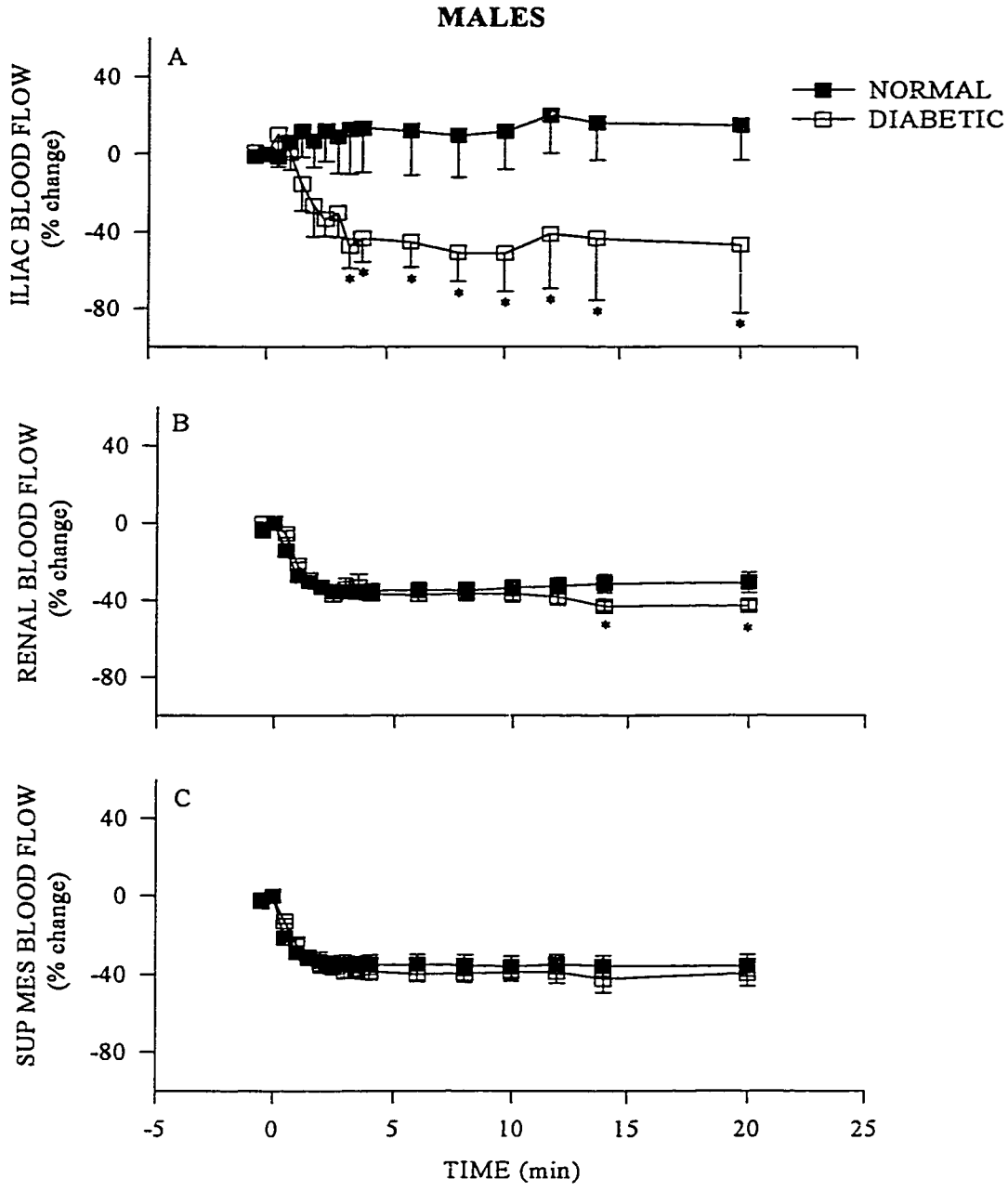

Figure 10. The effect of L-NAME (10 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries expressed as percent change in normal (N = 8) and diabetic (N = 6) male rats. * = $p < 0.05$ vs. normal male rats. Two-way ANOVA group effect normal vs. diabetic male iliac blood flow, $p < 0.001$.

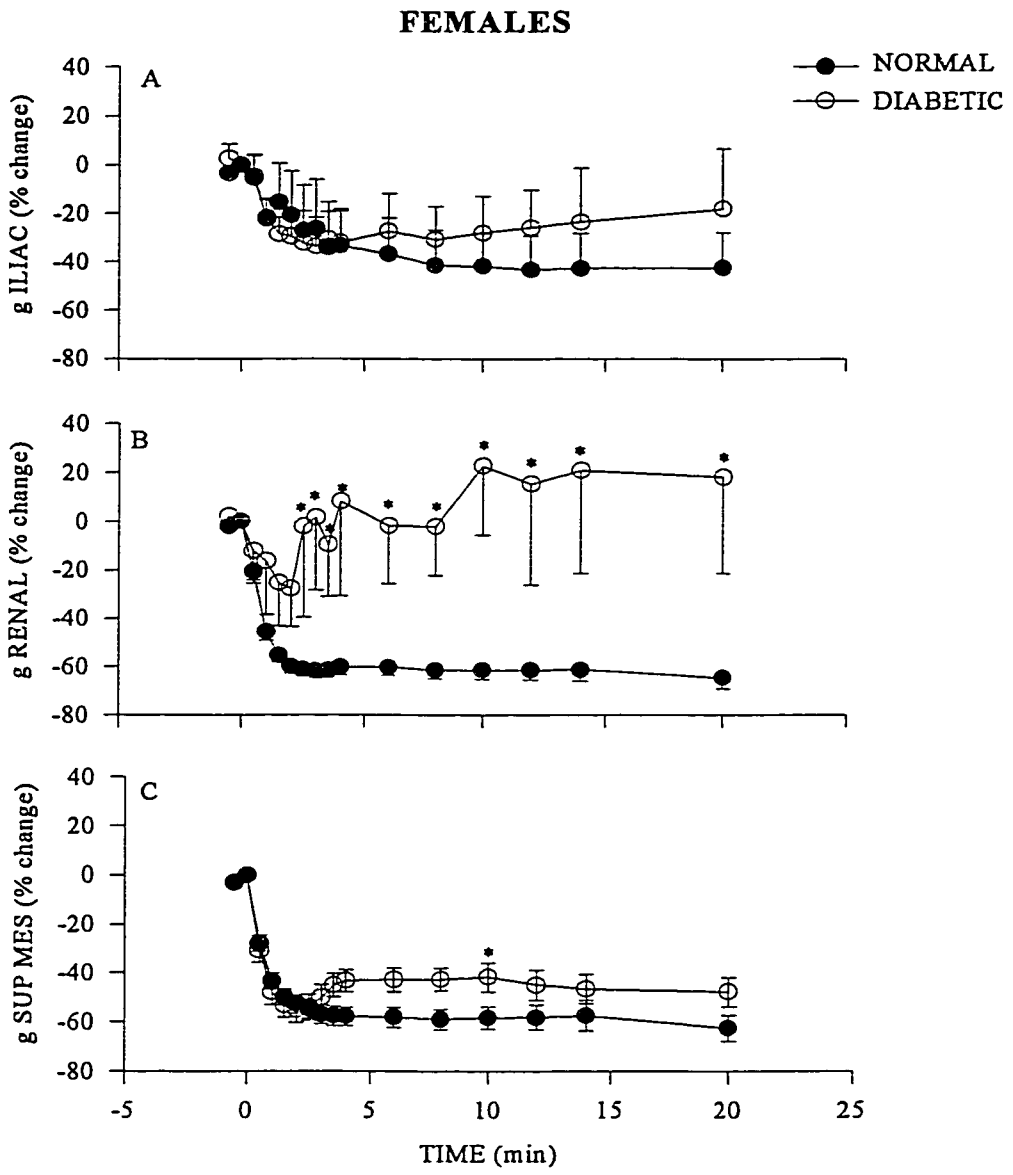

Figure 11. The effect of L-NAME (10 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 9) and diabetic female (N = 8) rats. * = $p < 0.05$ vs. normal female. Two-way ANOVA group effect normal vs. diabetic female g Renal, $p < 0.001$ and normal vs. diabetic female g Sup Mes, $p < 0.01$.

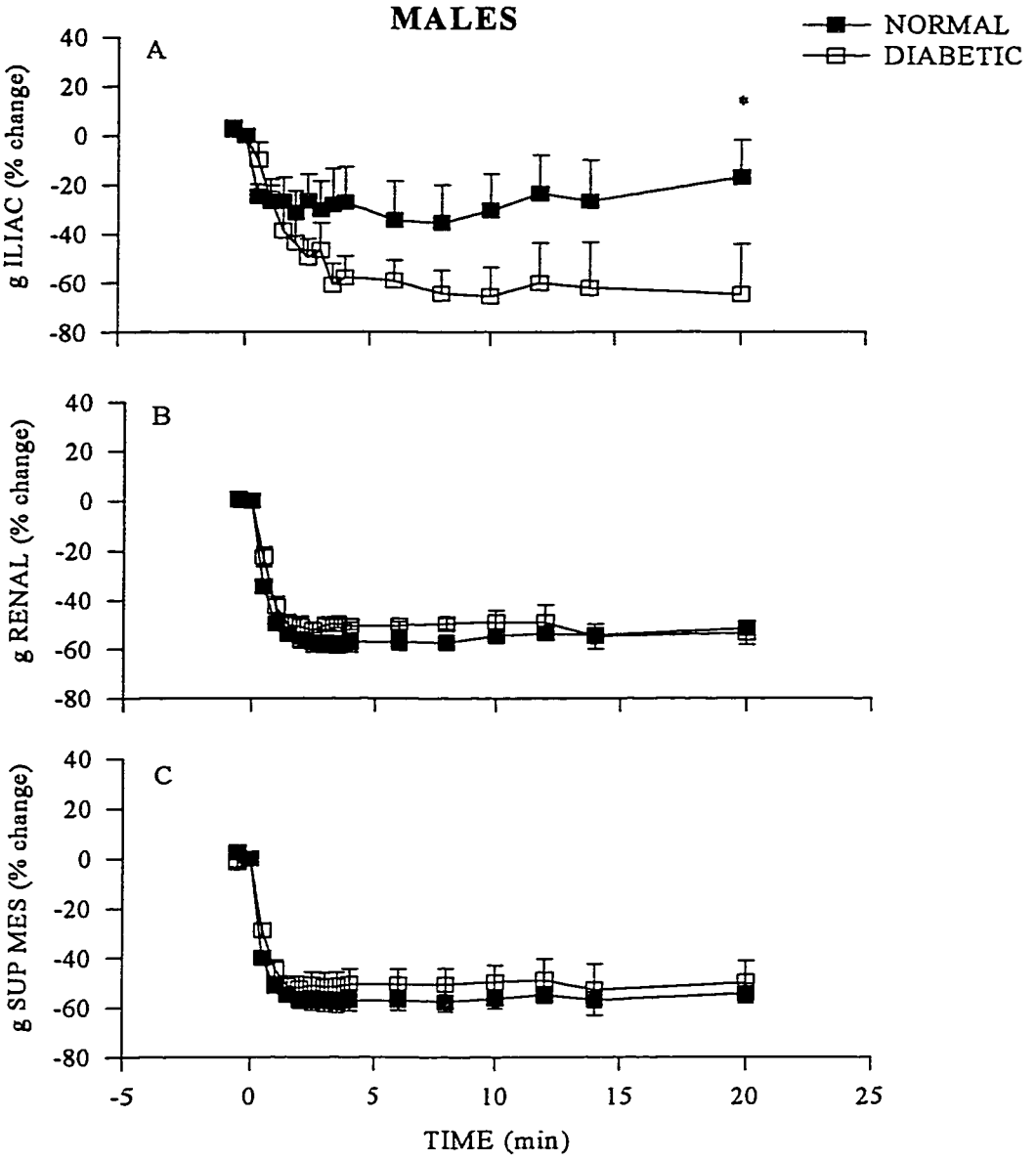

Figure 12. The effect of L-NAME (10 mg/kg) on iliac (A), renal (B), superior mesenteric (C) arteries conductance (g) in normal (N = 8) and diabetic (N = 6) male rats expressed as percent change. * = $p < 0.05$ vs. normal male rats. Two-way ANOVA group effect normal vs. diabetic male g Iliac, $p < 0.01$ and normal vs. diabetic male g Sup Mes, $p < 0.01$.

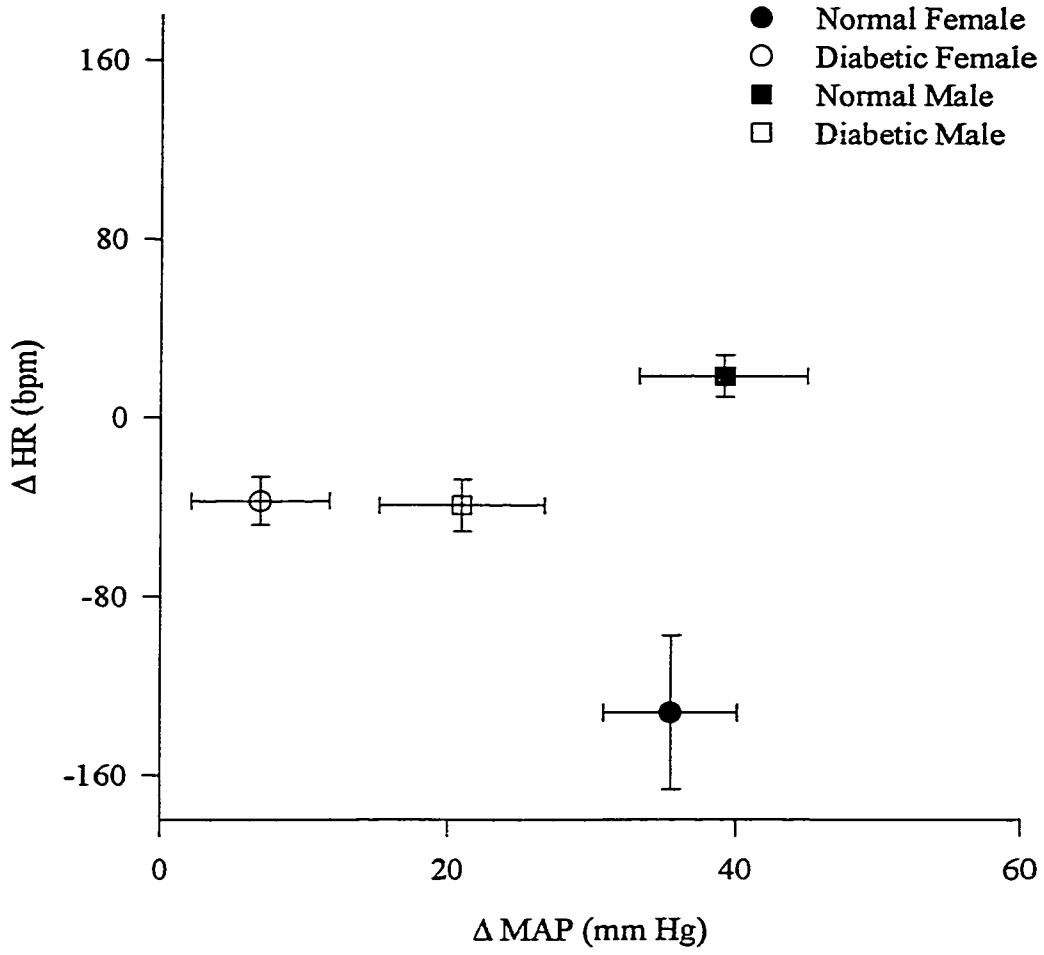

Figure 13. Females (normal, N = 9; diabetic, N = 8) and Males (normal, N = 8; diabetic, N = 6) Baroreflex Sensitivity Index 10 minutes Post-L-NAME (10 mg/kg) Treatment.

DISCUSSION

The present study indicates that a NOS antagonist, L-NAME acts to significantly increase the blood pressure in normal and diabetic female and male rats. These findings are consistent with previous observations of NO's action in the regulation of mean arterial pressure (Abiru, et al., 1993; Gardiner, et al., 1990; Mattar, et al., 1996). We observed that the pressor response to L-NAME was significantly greater in normals compared to diabetics. These results are comparable with the study by Abiru, et al., (1993) and Kiff, et al. (1991) in which the pressor response induced by L-NAME was significantly attenuated in STZ-diabetic rats compared to control. It is suggested that the failure of the NOS inhibitor to have a comparable pressor effect in diabetics may be associated with a decreased NO synthesis or release from endothelial cells in diabetic animals leading to a decreased dependency on NO for cardiovascular control (Calver, et al., 1992).

The administration of a NOS antagonist resulted in a comparable regional vasoconstriction in all three vascular beds (iliac, renal and superior mesenteric) in normal female and male rats. These results are also in agreement with previous reports in normal and diabetic male rats (Gardiner, et al., 1990; Reckelhoff, et al., 1998; Tolins, et al., 1993; Zappellini, et al., 1996). However, it can be noted that in our study diabetic male rats exhibit an enhanced constrictor response in the iliac bed in response to NOS inhibition suggesting that there is an increase in the overall balance in the constrictor tone versus the dilatory tone in skeletal muscle in diabetic males. On the other hand, the renal bed of diabetic female rats failed to constrict or had an increase conductance following a NOS antagonist.

There is substantial evidence of the contribution of endothelial nitric oxide in the

control of regional vascular differential conductances in vivo (Gardiner, et al., 1990; Lacolley, et al, 1991). The different responses to L-NAME in the iliac, renal and superior mesenteric bed could be due to different degrees of regulatory influence of NO versus neural control on other mechanisms in the control of conductances in the different vascular beds. In support of this notion Häbler, et al. (1997), demonstrated that the increase in skeletal muscle vascular resistance induced by L-NAME was more dramatic in intact lumbar sympathetic trunk (LST) animals than in the LST-denervated (sectioned LST) preparation. Additionally, the different responses could be the result of different interactions between L-NAME and the L-arg/nitric oxide pathway in the different vascular beds (Gardiner, et al., 1990; Lacolley, et al., 1991). It is conceivable that in diabetic males the iliac bed increased vasoconstrictive response to L-NAME is a consequence of a more pronounced endothelial dysfunction (lack or inability to produce NO) and greater dependence in NO, leading to an increase sensitivity to L-NAME. Investigators have also demonstrated that the inhibition of NO leads to an enhanced sympathoexcitatory response which results in an exaggerated vasoconstriction (Lacolley, et al., 1991; Owlya, et al., 1997; Sakuma, et al., 1992; Vo, et al., 1992; Zanzinger, et al., 1994). We suggest that this sympathoexcitatory response might be exacerbated in male diabetics and may account for the increase vasoconstrictive response in the iliac bed to L-NAME.

Diabetes is also characterized by abnormal renal hemodynamics, vasodilation, pronounced glomerular hyperfusion and hyperfiltration (Tolins, et al., 1993; Ballermann, et al., 1984; Mattar, et al., 1996). Reports have demonstrated that such abnormalities are completely abolished by the administration of L-NAME to diabetic rats (Tolins, et al.,

1993; Mattar, et al., 1996). Our results in normal and diabetic males are consistent with the above findings. However, we also observed an increase in renal blood flow in diabetic female rats. A possible explanation to the lack of renal constrictal response or vasodilatation seen in diabetic females might be an enhanced predisposition to vasodilatation due to estrogen enhanced NO production (Darkow, et al., 1997; Rahimian, et al., 1997; Tagawa, et al., 1997). Chronic estrogen administration has been demonstrated to enhance NO production from endothelium. We believe that the renal vasodilatory response to L-NAME can be attributed to the combination of enhanced tendency for vasodilation in females and the dramatic increase in mean arterial pressure. Another possibility in the renal vasodilatory response in diabetic females is provided by the greater levels of mRNA and endothelial nitric oxide synthase (eNOS) protein in females than in male rats kidneys (Reckelhoff, et al., 1998). However, the lessor response to L-NAME in females when compared to males led those investigators to suggest that there is a greater production of NO in males kidneys and the renal bed was more responsive to chronic inhibition of NOS with L-NAME than females or that the males kidneys are more NO dependent than females (Reckelhoff, et al., 1998). Therefore, these studies might provide further evidence that endothelial cell (EC) dysfunction alone is not responsible for the discrepancies seen between normal and diabetic male and female rats renal conductance response to L-NAME (Reckelhoff, et al., 1998). Instead, there is likely a combine dysfunction between sympathetic discharge and the synthesis/release of NO (Lacolley et al., 1991) which can lead to the discrepancies observed among diabetic male and female rats.

Our observations of the blood flow dynamics in response to NOS inhibition in the

superior mesenteric vascular bed of normal females and males are consistent with previous studies (Chakir, et al., 1996). Since, the ability of L-NAME to decrease diabetic females superior mesenteric conductance is compromised when compared to normal females, upon the administration of a NOS inhibitor, EC dysfunction might be greater among diabetic female rats (Nase, et al., 1996).

L-NAME pressor responses were associated with bradycardia for all four groups of animals and is consistent with previous studies in female and male dogs, and male rats (Gardiner, et al., 1990; Zappellini, et al., 1996). In addition, it should also be noted that L-NAME has been reported as a muscarinic receptor antagonist and consequently it will damper the reflex bradycardia elicited by L-NAME's pressor response (Hoover, et al., 1997; Buxton, et al., 1993). This antagonistic response seems more prevalent in diabetic animals and normal males. Contrarily, studies have also reported that L-NAME is not a generalized muscarinic receptor antagonist and therefore is an effective selective NOS antagonist and useful tool for experiments evaluating NOS inhibition (Koss, 1997). Normal females have a more sensitive baroreflex function compared to diabetic females and normal and diabetic males (Piha, 1993; McDowell, et al., 1994).

In summary, bolus administration of L-NAME results in a significant pressor effect and bradycardia in all four groups of animals and the pressor response was significantly attenuated in diabetic animals and to a greater degree in diabetic females. Blockade of NOS by L-NAME decreased conductance in all three vascular beds in normal animals. This decreased conductance was greater in the iliac bed of diabetic males and contrastly, in diabetic female rats NOS inhibition resulted in an increase in renal conductance.

CHAPTER III

THE EFFECT OF SEX AND DIABETES ON SMOOTH MUSCLE SENSITIVITY TO NITRIC OXIDE

INTRODUCTION

Vascular smooth muscle and endothelial reactivity to vasoactive agents have been extensively addressed in diabetes. As a result, several in vitro (Abiru et al., 1990; Taylor et al., 1994) and in vivo studies (Mayhan, 1992; McNally, et al., 1994; Kauser, et al., 1994) have reported contradicting results with respect to nitric oxide-independent vasodilation and diabetes (Veves, 1998).

Sodium nitroprusside (SNP, an NO donor) is a potent vasodilator frequently used to control hypertension, congestive heart failure and for the improvement of systemic blood flow (Maseda, et al., 1981; Lovell, et al., 1995). This NO donor generates NO nonenzymatically in a reaction with cysteine and acts directly at the level of the vascular smooth muscle (Lovell, et al., 1995; Baron, 1996). Therefore, it is a feasible agent to address endothelium-independent vasodilation. Systemic administration of SNP is associated with a dose-dependent hypotensive response, decrease total peripheral resistance and tachycardia (Chen, et al., 1982; Musialek, et al., 1997; Palmer, et al., 1975). The spontaneously generated NO from SNP activates guanylate cyclase, increases intracellular cGMP which leads to a protein phosphorylation resulting in vascular smooth muscle relaxation (Lovell, et al., 1995).

Consequently, the present study was performed to determine the relationship between sex, diabetes and endothelium- independent vasodilation as modulated by the

administration of SNP in normal and diabetic female and male rats.

MATERIALS AND METHODS

Normal and diabetic female and male, wistar rats (BW: 250-275 g) were used in our experimental procedures. They were kept in a control environment with a 12 hour light cycle and a 23°C room temperature with free access to water and food. Diabetes was induced in normal rats by a single IV injection in the tail vein of streptozotocin (STZ) of 50 mg/kg dissolved in sodium citrate (0.1 mM, pH 4.5). Five days after the STZ injection, a blood sample was collected to determine hyperglycemia and maintained 4-6 weeks post STZ injection.

On the day of the study and following a 24 hr fast, normal or diabetic rats were anesthetized with urethane (0.5 mg/kg) and α-chloralose (70 mg/kg) and placed on a heating pad to maintained their body temperature. A tracheotomy was performed to diminish respiratory obstructions and catheters with heparinized saline were placed into the femoral artery and veins. The venous catheter was utilized for blood sample collection and infusions. The femoral artery cannula was utilized for cardiovascular recording.

Pulsed-Doppler blood flow transducers (flow probe, Baylor electronics) were placed around the iliac, renal, and the superior mesenteric arteries. The arterial catheter was connected to a pressure transducer and the flow probes connected to a pulsed-Doppler flowmeter (Baylor electronics). A micro 5000 signal processing system was utilized to measure the cardiovascular responses.

Female and male normal and diabetic rats were given subsequent bolus injections of increasing concentrations of sodium nitroprusside (SNP; 1, 5, 10, and 20 μg/kg) in

twenty minutes intervals following the establishment of a baseline. Mean arterial pressure (MAP), heart rate (HR), and blood flows (iliac, renal, and superior mesenteric) were monitored continuously.

The Biowindows Software Program and a Micro 5000 signal processing system were used to monitor cardiovascular responses. The Biowindows Program records all cardiovascular parameters: mean arterial pressure (MAP), heart rate (HR), and blood flow (Hz Ds units).

Blood samples, 0.2 mls with saline replacement were collected prior to the study and used for glucose analysis (glucose analyzer; Yellow Springs Instruments Co., Yellow Springs, OH.

The SNP data presented are peak responses following treatments. The data was analyzed using two-way ANOVA, Post-hoc analysis where appropriate, and student t-test.

RESULTS

A. THE EFFECT OF SUBSEQUENT SYSTEMIC INFUSIONS OF SALINE ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC FEMALE VS. MALE RATS

The aim of this set of experiments was to establish baseline values for cardiovascular responses and blood flow in normal and diabetic female and male rats. Animals were instrumented as described above. Normal saline was administered in three/four subsequent systemic injections of 0.2 mls at twenty minutes intervals as a bolus via the femoral vein (normal female, N = 5; diabetic female, N = 5; normal male, N = 5, and diabetic male, N = 6).

The body weight and heart rate (HR) were decreased and blood glucose increased in the diabetic rats when compared to normals (Table 4). Mean arterial pressure (MAP) was not significantly different between the groups (Table 4).

The subsequent systemic administration of saline in female rats resulted in no significant difference in MAP, except for the initial treatment to diabetic female rats which elicited a lesser increase in MAP when compared to normals (Fig. 14A). In terms of group effect diabetic female's MAP was significantly different to its normal counterpart MAP. On the other hand, male rats displayed similar responses in terms of MAP between normal and diabetics animals, with the exception of the second and third administration which resulted in significantly lower responses when compared to its normal counterpart (Fig. 15A). When a Two-way ANOVA group effect was performed normal vs. diabetic group were significantly different in terms of MAP, $p < 0.01$. Normal and diabetic female and male animals displayed similar responses in terms of HR (Fig. 14B and Fig. 15B). Blood flow in the three vascular beds of normal and diabetic females were not significantly different (Fig. 16). On the other hand, subsequent administrations of saline resulted in a significant increase in blood flow in all three vascular beds of diabetic males (Fig. 17). The iliac and superior mesenteric arteries of diabetic males display a significant response after the third and the first saline treatment, respectively (Fig. 17A and 17C). Whereas, the renal bed responses were significant for all four saline treatments (Fig. 17B). A two-way ANOVA group effect was performed to evaluate normal vs. diabetic females blood flow. It was evident that diabetic female's superior mesenteric ($p < 0.05$) artery blood flow was significantly different when compared to normals. Contrastly, diabetic males were significantly different in the renal bed (Two-way ANOVA group effect; $p <$

0.001).

When blood flow was expressed as conductance (conductance = blood flow/MAP), no significant differences were observed between normal and diabetic females, except for the initial treatment in the iliac bed (Fig. 18A). Normal and diabetic males displayed similar responses to the subsequent saline treatments for all three vascular beds (Fig. 19). However, it should be noted that diabetic males exhibited less of a decrease in the iliac and renal conductance in response to the third saline treatment when compared to normals (Fig. 19A and 19B).

Normal and diabetic males conductance response were evaluated in terms of group effect by a two-way ANOVA. This evaluation implied as significant difference between normal and diabetic males with respect to the renal ($p < 0.05$) and superior mesenteric ($p < 0.01$) conductance.

TABLE 4 Basal Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP, mm Hg), and Heart Rate (HR) In Subsequently Saline Treated Normal And Diabetic Rats.

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	262 ± 12	89 ± 10	66 ± 6	354 ± 13
Female	(5)	(5)	(5)	(5)
Diabetic	203 ± 12*	443 ± 21*	71 ± 6	338 ± 25
Female	(5)	(5)	(5)	(5)
Normal	272 ± 10	70 ± 7	82 ± 7	383 ± 23
Male	(5)	(5)	(5)	(5)
Diabetic	245 ± 18†	394 ± 36*	70 ± 2	316 ± 10
Male	(6)	(6)	(6)	(6)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normal counterpart, ANOVA. † = $p < 0.05$ vs. diabetic female, ANOVA. Number in parenthesis = N.

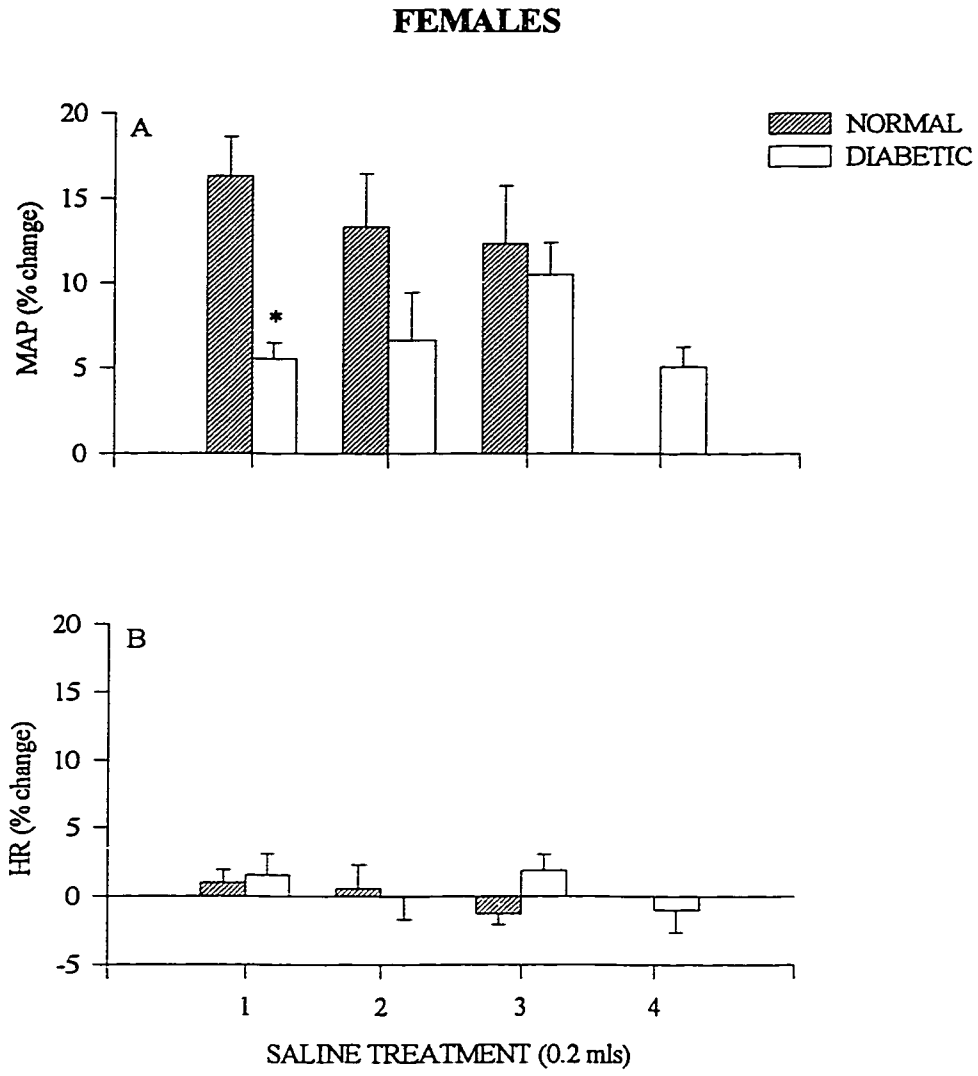

Figure 14. The effect of subsequent saline treatments on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 5) and diabetic (N = 5) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female MAP, $p < 0.01$.

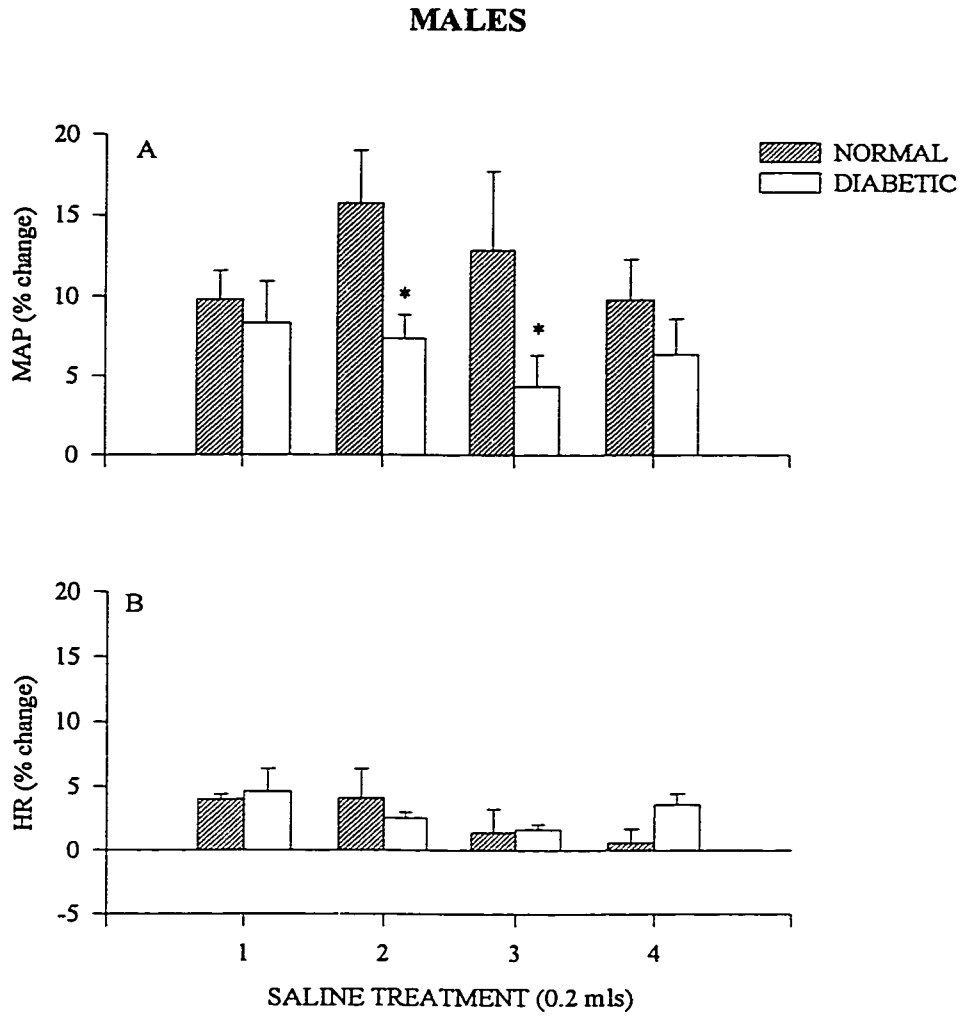

Figure 15. The effect of subsequent saline treatments (0.2 mls) on mean arterial pressure (MAP) (A), and heart rate (HR) (B) expressed as percent change in normal (N = 5) and diabetic (N = 6) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male MAP, $p < 0.01$.

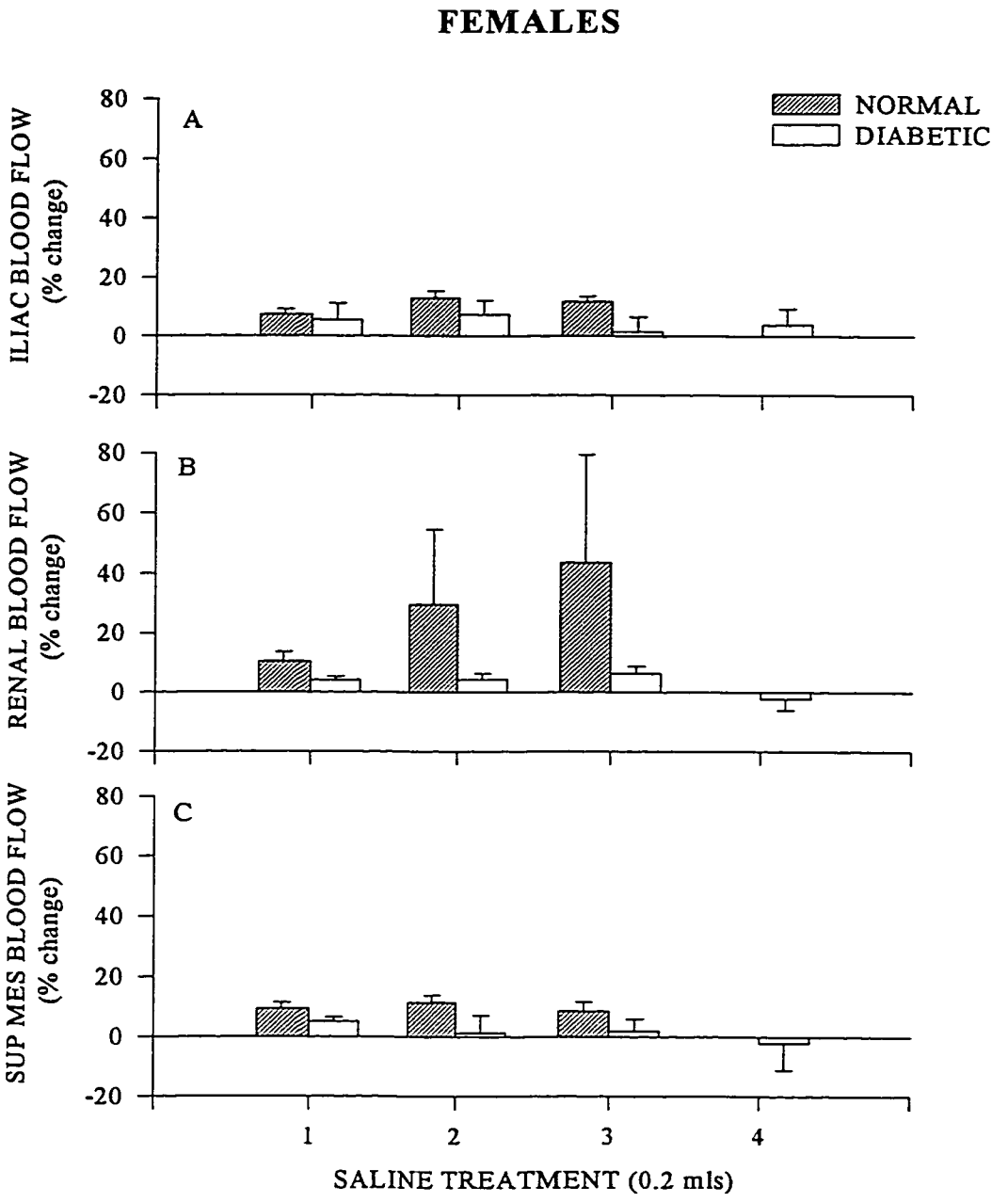

Figure 16. The effect of subsequent saline treatments (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow in normal (N = 5) and diabetic (N = 5) female rats expressed as percent change. Two-way ANOVA group effect normal vs. diabetic female Sup Mes blood flow, $p < 0.05$.

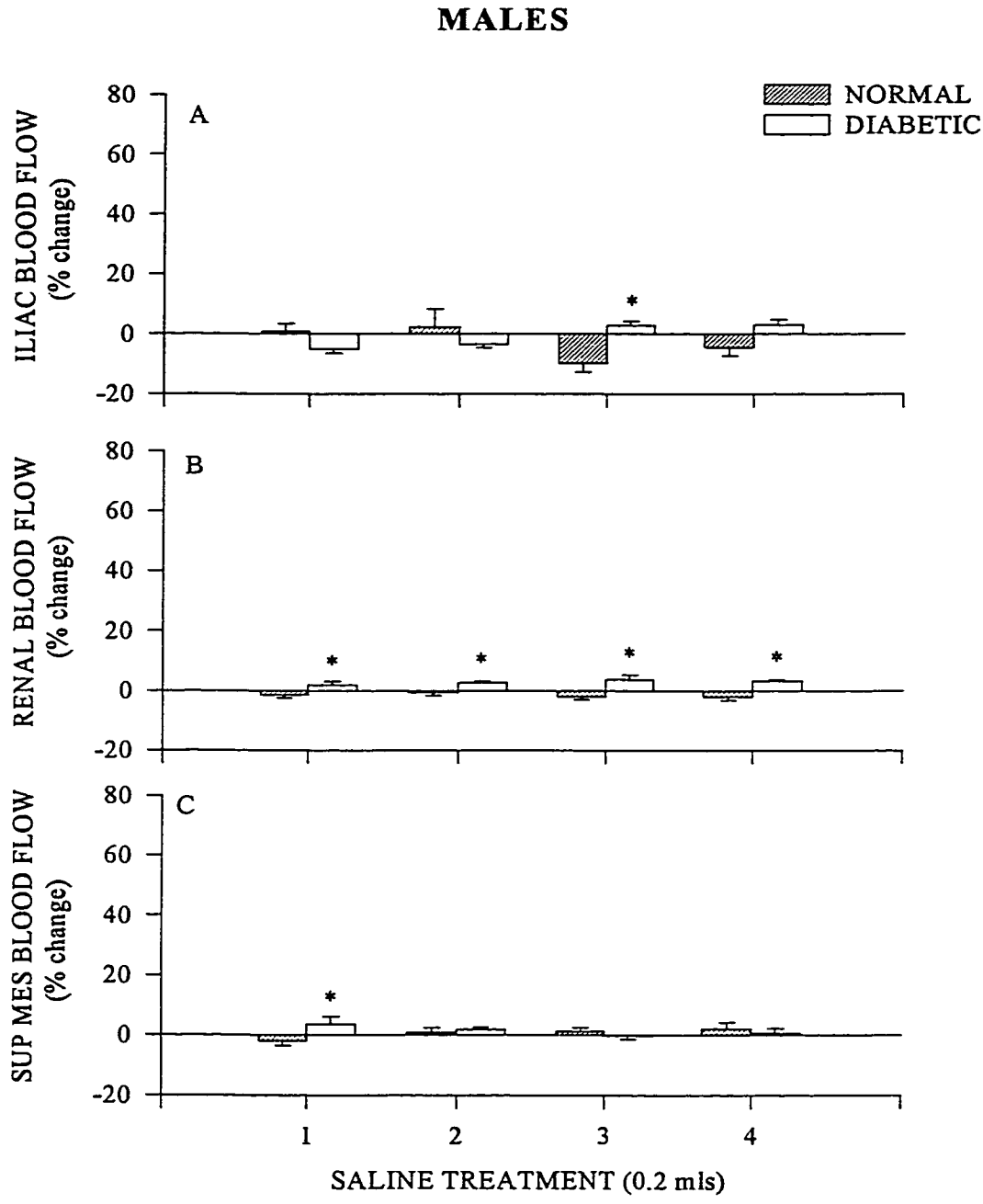

Figure 17. The effect of subsequent saline treatments (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries expressed as percent change in normal (N = 5) and diabetic (N = 6) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male renal blood flow, $p < 0.001$.

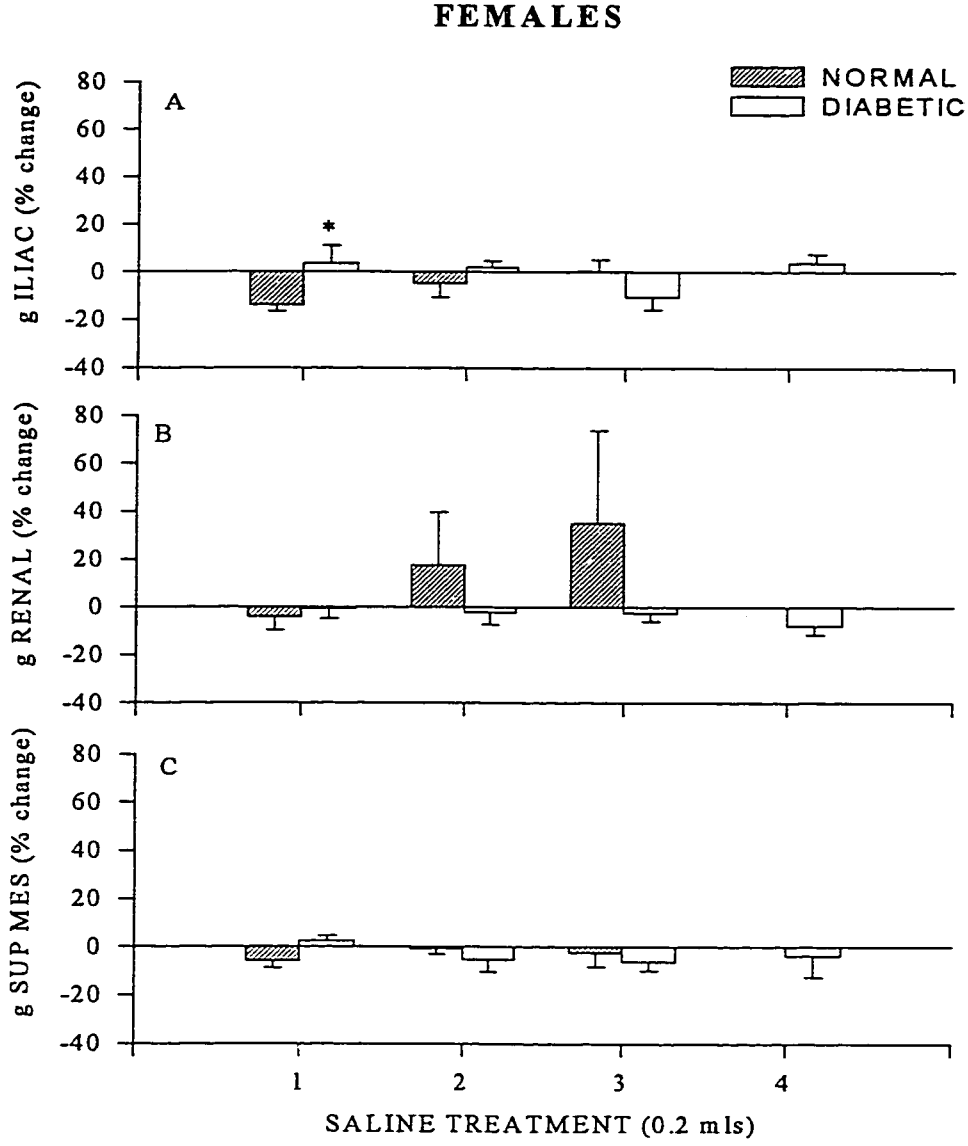

Figure 18. The effect of subsequent saline treatments (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 5) and diabetic (N = 5) female rats. * = $p < 0.05$, student t-test.

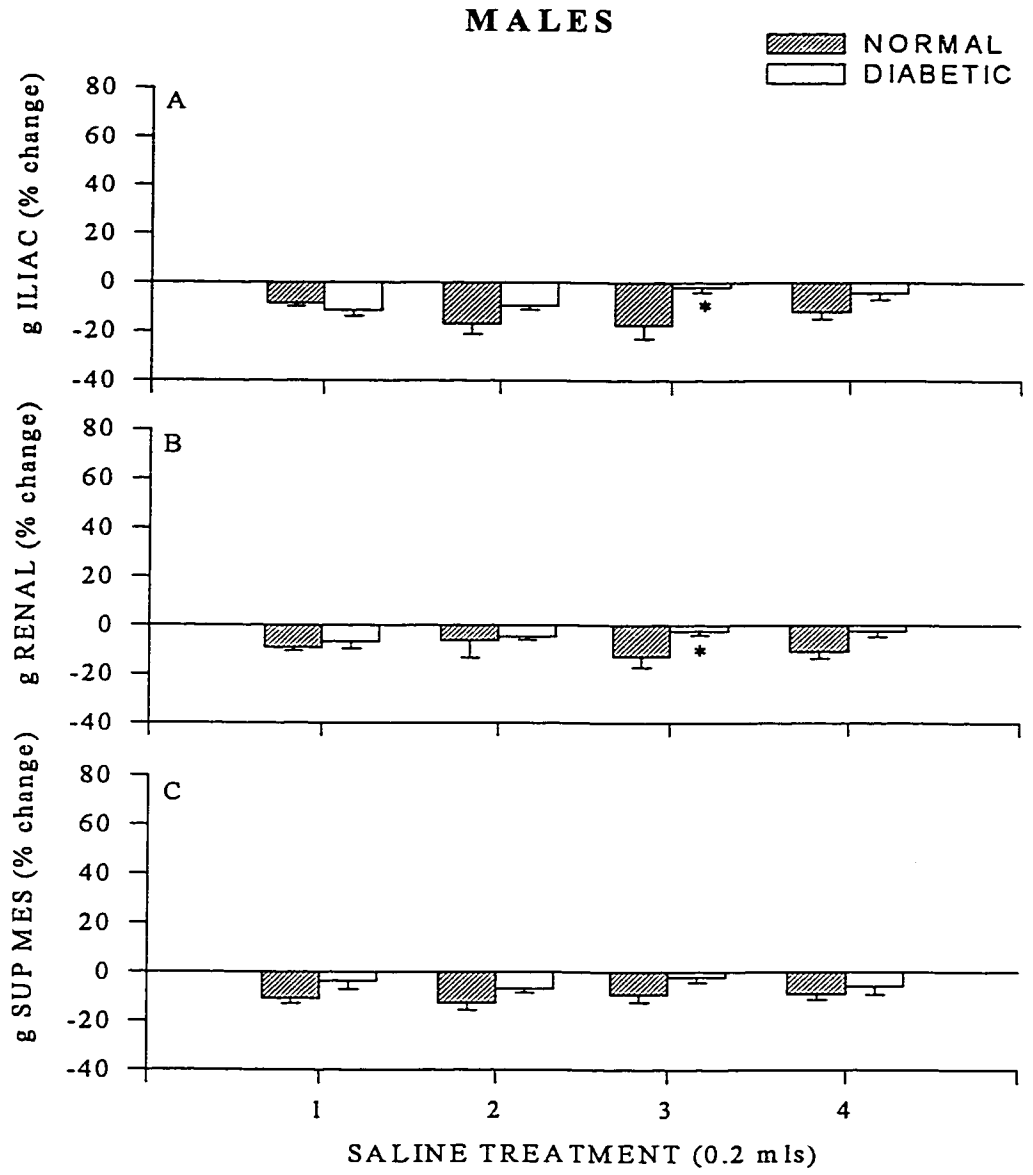

Figure 19. The effect of subsequent saline treatments (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 5) and diabetic (N = 6) male rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male g ILIAC, $p < 0.01$; normal vs. diabetic male g RENAL, $p < 0.05$; and normal vs. diabetic male g SUP MES, $p < 0.01$.

B. THE EFFECT OF INCREASING CONCENTRATIONS OF SYSTEMIC INFUSIONS OF SODIUM NITROPRUSSIDE (SNP) ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC RATS

In this study we evaluated the effect of a NO donor, sodium nitroprusside (SNP) on cardiovascular responses and blood flow in normal female (N = 10), diabetic female (N = 7), normal male (N = 6) and diabetic male (N = 7) rats. SNP was infused in four different concentrations (1, 5, 10, and 20 μg/kg) via the femoral vein.

Animals were instrumented as described before. Blood samples (0.2 mls) were collected five minutes prior to treatment and 80 minutes after the initial treatment. The blood volume was immediately replaced with heparinized saline. Responses were recorded continuously for 80 minutes. Control animals runs were performed similarly however, they were infused with normal saline (0.2 mls) instead of SNP (1, 5, 10, and 20 μg/kg).

The body weight was decreased in females diabetics and the blood glucose was increased in female and male diabetics when compared to normals. No significant differences were seen in basal MAP between groups. However, diabetic males had a significantly lower basal HR when compared with its corresponding counterpart (Table 5).

The administration of SNP resulted in a rapid decreased in MAP in normal and diabetic animals in a dose-dependent fashion (Fig. 20A and 21A). Normal males tended to have less of a decrease in MAP with increasing concentrations of SNP when compared to normal females (Fig. 20A and 21 A) (Table 6). However, this response was significant only at the 20 μg/kg concentration of SNP (Table 6). The HR was increased in normal

and diabetic animals (Table 6) (Fig. 20B and 21B). All four groups of animals demonstrated a reflexe increase in HR (20B and 21B) following SNP treatments. Normal animals tended to have greater increases in HR when compared to diabetic animals and diabetic males had a significantly smaller increase in HR when compared to diabetic females at the lower concentration of SNP, 1 μg/kg (Table 6).

Female's iliac, renal and superior mesenteric blood flow responses are displayed in Fig. 22. Normal females displayed a decrease in iliac blood flow upon the administration of two of the SNP treatments (1 and 5 μg/kg) and an increase in flow due to the other two concentrations (10 and 20 μg/kg) (Fig. 22A). Diabetic animals exhibited a reduction in blood flow for all four SNP treatments (Fig. 22A). Normal and diabetic females displayed a decrease in renal blood flow except for diabetic animals at its lowest concentration which had a minor increase in flow (Fig. 22B). On the other hand, the superior mesenteric artery exhibited a slightly different response pattern than the iliac and renal vascular bed (Fig. 22C). SNP augmented the blood flow for normal and diabetic females at all concentrations except for the diabetic animals at the 20 μg/kg concentration which resulted in a decrease in blood flow. Females blood flows were evaluated in terms of group effect with a two-way ANOVA test and it resulted in a significant difference between normal and diabetic rats for all three vascular beds (iliac: $p < 0.05$; renal: $p < 0.05$ and superior mesenteric: $p < 0.05$).

Normal and diabetic males blood flow responses to increasing concentrations of SNP can be seen in Fig. 23. Males displayed an increase in iliac blood flow in response to SNP for all concentrations except for the initial SNP concentration (1 μg/kg) (Fig. 23A). Like females, normal and diabetic males exhibited a decrease in renal blood flow except

for diabetic animals at its lowest concentration (1 μg/kg) which had a minor increase in flow (Fig. 23B). SNP administration augmented superior mesenteric artery blood flow in normal males at all concentrations (Fig. 23C). On the other hand, diabetic males exhibited a decrease in flow for three of the SNP treatments (5, 10, and 20 μg/kg) and an increase in flow to the 1 μg/kg concentration (Fig. 23C). Males blood flow group effect was evaluated by a two-way ANOVA and it demonstrated a significant difference in normal versus diabetic males in the renal ($p < 0.001$) and superior mesenteric ($P < 0.05$) bed.

SNP administration resulted in a dose-dependent increase in conductance (g) in all three vascular beds (Fig. 24 and Fig. 25). The dose-dependent response to SNP in the iliac artery (Fig. 24A and Fig. 25A) was significantly decreased in the male diabetics but not the female diabetics. This is particularly true for the 10 μg/kg concentration of SNP when all experimental groups are compared (Table 7). The increased iliac conductance in response to SNP was significantly greater in normal males when compared to normal females (Table 7). The increased conductance in response to SNP in the superior mesenteric artery was less in both diabetic females and males (Fig. 24C and Fig. 25C). When compared to normals, diabetic males had a greater increase in conductance in response to SNP in superior mesenteric conductance than diabetic females (Table 7). On the other hand, the responsiveness to SNP was greater in the renal artery in both diabetic females and males when compared to normals (Fig. 24B and Fig. 25B). In addition, when all four experimental groups were considered, diabetic females at the highest concentration of SNP (20 μg/kg) were significantly less responsive than normal females (Table 7). The renal conductance had an even greater increase in diabetic males when

compared to normals (Table 7) (Fig. 25B). However, diabetic females had a greater increase in conductance in response to the highest concentrations of SNP (10 and 20 μg/kg) in the renal artery when compared to normals (Table 7).

SNP's two-way ANOVA group effect across concentration with respect to conductance in females resulted in a significant difference between normal and diabetics in the renal ($p < 0.05$) and superior mesenteric ($p < 0.01$) vascular bed. On the other hand, evaluation of males demonstrated a significant difference between normal and diabetic males for all three vascular beds (iliac: $p < 0.01$; renal: $p < 0.01$ and superior mesenteric: $p < 0.01$).

TABLE 5 Body Weight (g), Basal Blood Glucose (mg/dl), Mean Arterial Pressure (MAP, mm Hg), And Heart rate (HR, beats/min) In Sodium Nitroprusside (SNP) Treated Normal And Diabetic Rats

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	271 ± 11	77 ± 5	70 ± 4	354 ± 11
Female	(10)	(10)	(10)	(10)
Diabetic	228 ± 11*	421 ± 63*	69 ± 5	340 ± 24
Female	(7)	(7)	(7)	(7)
Normal	246 ± 5	59 ± 3	78 ± 4	380 ± 13
Male	(6)	(6)	(6)	(6)
Diabetic	279 ± 23	377 ± 23*	71 ± 3	326 ± 6*
Male	(7)	(7)	(7)	(7)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normals, ANOVA.
Number in parenthesis = N.

TABLE 6 Mean Arterial Pressure (MAP) and Heart Rate (HR) responses to increasing concentrations of Sodium Nitroprusside (SNP)

MAP (% change)				
[SNP]	**NORMAL**		**DIABETIC**	
(μg/kg)	**Female**	**Male**	**Female**	**Male**
1	-10.42 ± 4.1	-5.04 ± 1.4	-13.67 ± 3.2	-13.23 ± 2.8
	(5)	(6)	(5)	(7)
5	-38.87 ± 2.2	-35.10 ± 2.4	-31.27 ± 4.9	-28.95 ± 2.1
	(5)	(6)	(4)	(7)
10	-48.36 ± 2.1	-46.80 ± 2.7	-42.74 ± 3.6	-37.55 ± 2.1
	(10)	(6)	(5)	(7)
20	-53.11 ± 2.8	-33.10 ± 17†	-58.57 ± 2.1	-43.11 ± 2
	(5)	(6)	(2)	(7)
HR (% change)				
1	2.07 ± 0.6	6.24 ± 1.7	8.40 ± 4.2	0.84 ± 0.46*
	(5)	(6)	(5)	(7)
5	5.85 ± 1.3	3.44 ± 1.2	3.42 ± 1.1	2.36 ± 0.5
	(5)	(6)	(4)	(7)
10	5.92 ± 1.3	4.72 ± 4.6	1.87 ± 1.3	2.52 ± 1.6
	(10)	(6)	(5)	(7)
20	5.76 ± 3.2	4.33 ± 1.6	4.36 ± 4.84	3.67 ± 1.9
	(5)	(6)	(2)	(7)

The values represent the mean ± S.E.M. † = $p < 0.01$ vs. normal female.
* = $p < 0.05$ vs. diabetic female, ANOVA. Number in parenthesis = N.

TABLE 7 Vascular Conductance(g) Response to Increasing Concentrations of Sodium Nitroprusside (SNP) In The Iliac, Renal, and Superior Mesenteric Arteries

[SNP] (μg/kg)	NORMAL Female	NORMAL Male	DIABETIC Female	DIABETIC Male
ILIAC g (% change)				
1	-2.90 ± 2.3 (5)	-0.80 ± 4 (6)	-24.45 ± 16.9 (5)	5.14 ± 6.6 (7)
5	4.41 ± 27.8 (5)	81.23 ± 18* (6)	27.22 ± 17.5 (4)	47.9 ± 5.6 (7)
10	94.21 ± 28 (10)	152.48 ± 28* (6)	69.58 ± 11.7 (5)	87.63 ± 20§ (7)
20	136.39 ± 27.3 (5)	142.0 ± 27 (6)	127.45 ± 1.2 (2)	88.23 ± 11.9 (7)
RENAL g (% change)				
1	8.62 ± 9.5 (5)	1.63 ± 1.0 (6)	21.22 ± 7.1 (4)	14.30 ± 3.4 (7)
5	14.79 ± 17.1 (4)	20.88 ± 6.7 (6)	33.5 ± 10.0 (4)	28.58 ± 6.8 (7)
10	20.39 ± 19.3 (10)	23.17 ± 9.2 (6)	54.82 ± 13.5* (5)	53.59 ± 11.2 (7)
20	44.75 ± 15.3 (5)	25.3 ± 5.3 (6)	101.79 ± 39.6* (2)	54.49 ± 11.6 (7)
SUP MES g (% change)				
1	32.39 ± 8.0 (5)	8.11 ± 3.05 (6)	19.28 ± 9.7 (5)	21.37 ± 4.3 (7)
5	71.01 ± 10.7 (5)	65.90 ± 8.8 (6)	32.82 ± 11.4 (4)	38.48 ± 5.9 (7)
10	90.34 ± 9.6 (10)	94.90 ± 13.6 (6)	52.46 ± 18.8 (5)	56.00 ± 10.7 (7)
20	131.55 ± 40.8 (5)	102.14 ± 13.1 (6)	0.288 ± 97* (2)	73.48 ± 8.3† (7)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normal female. § = $p < 0.05$ vs. normal male. † = $p < 0.05$ vs. diabetic female, ANOVA. Number in parenthesis = N.

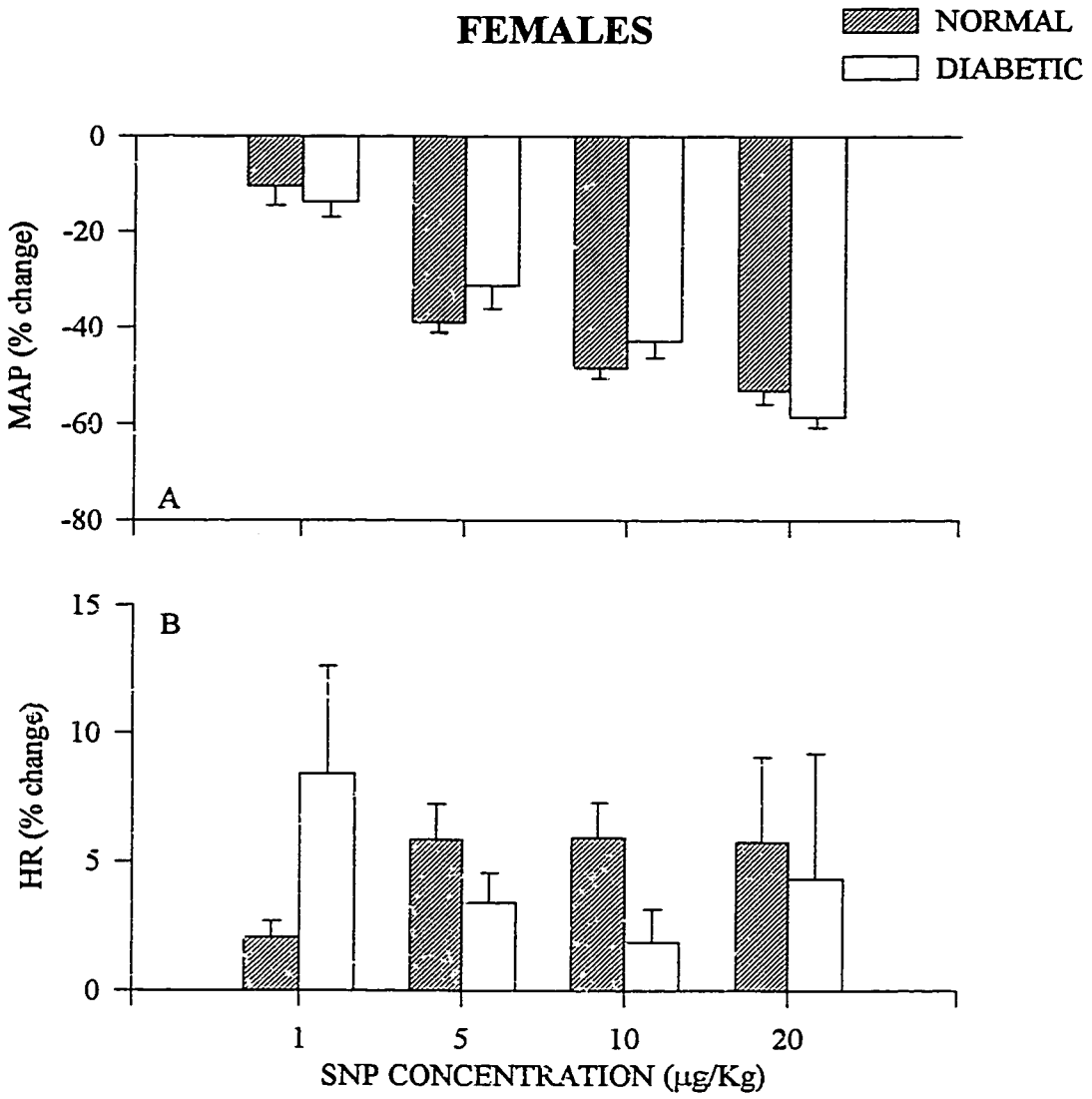

Figure 20. The effect of increasing concentrations of sodium nitroprusside (SNP) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 5, 5, 10, and 5) and diabetic (N = 5, 4, 6, and 2) female rats.

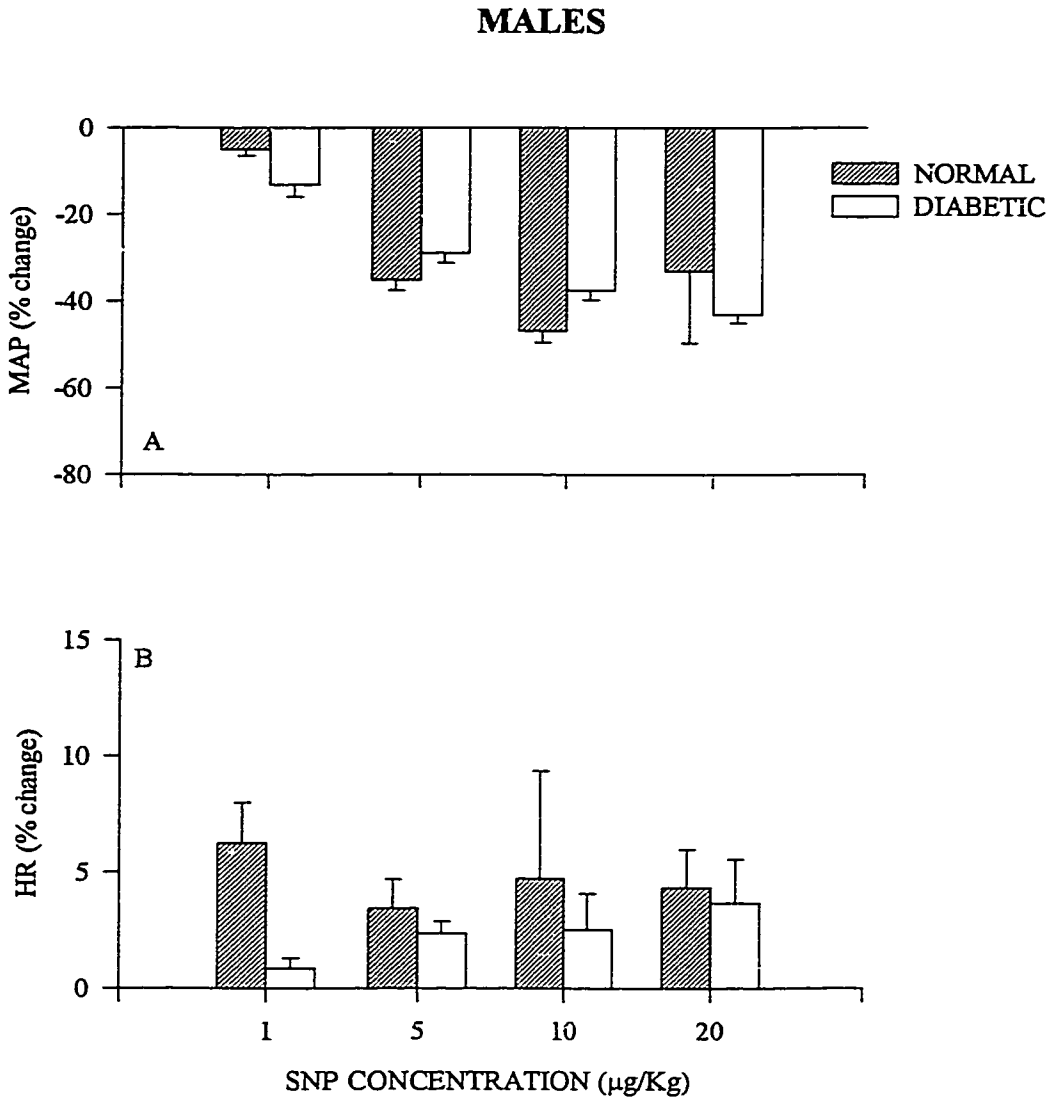

Figure 21. The effect of increasing concentrations of sodium nitroprusside (SNP) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 6) and diabetic (N = 7) male rats.

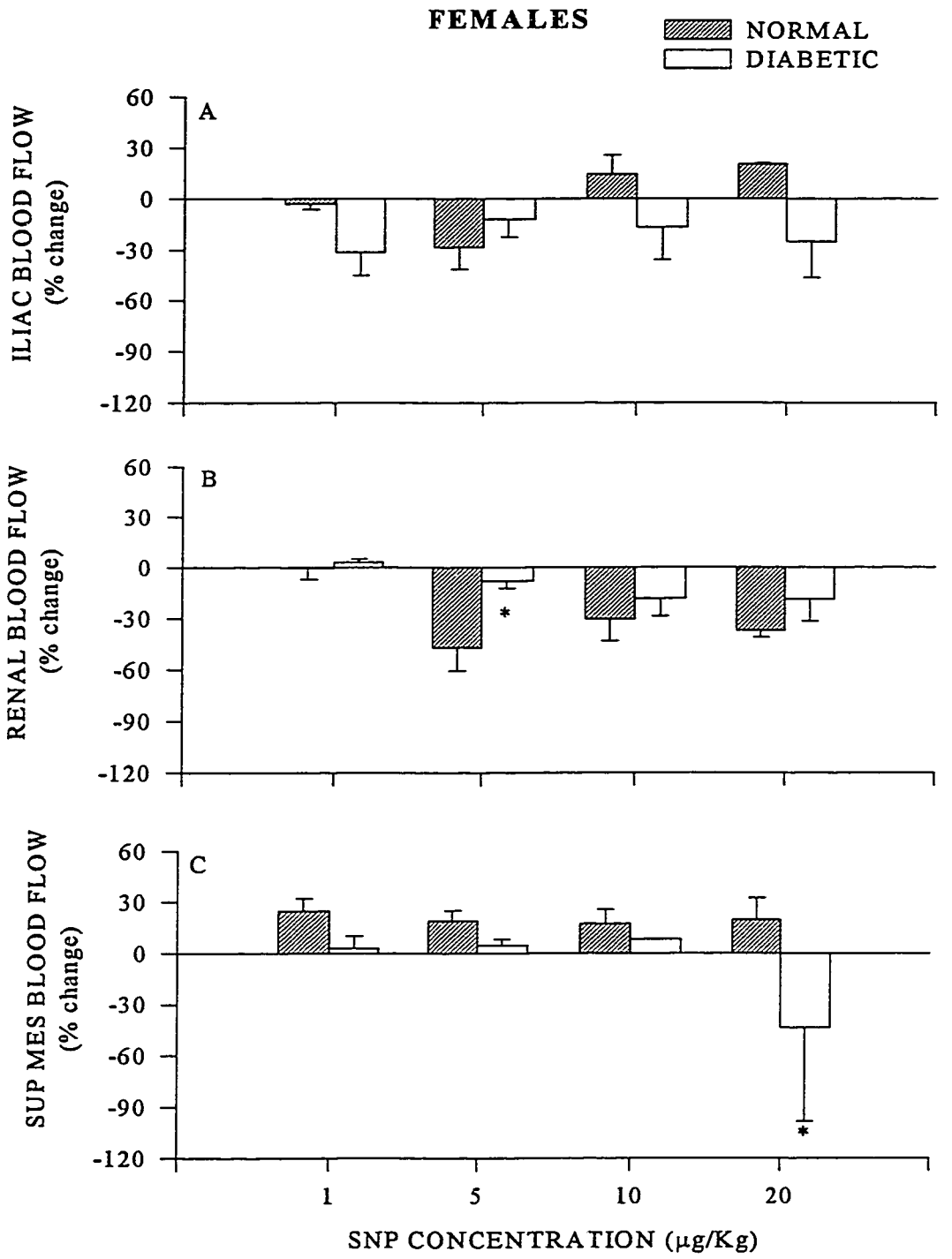

Figure 22. The effect of increasing concentrations of sodium nitroprusside (SNP) on iliac (A) renal (B), and superior mesenteric (C) blood flow expressed as percent change in normal (N = 5, 5, 10, and 5) and diabetic (N = 5, 4, 6, and 2) female rats. * = $p < 0.05$ vs. normal female. Two-way ANOVA across concentration group effect normal vs. diabetic female iliac blood flow, $p < 0.05$; renal blood flow, $p < 0.05$; and superior mesenteric blood flow, $p < 0.05$.

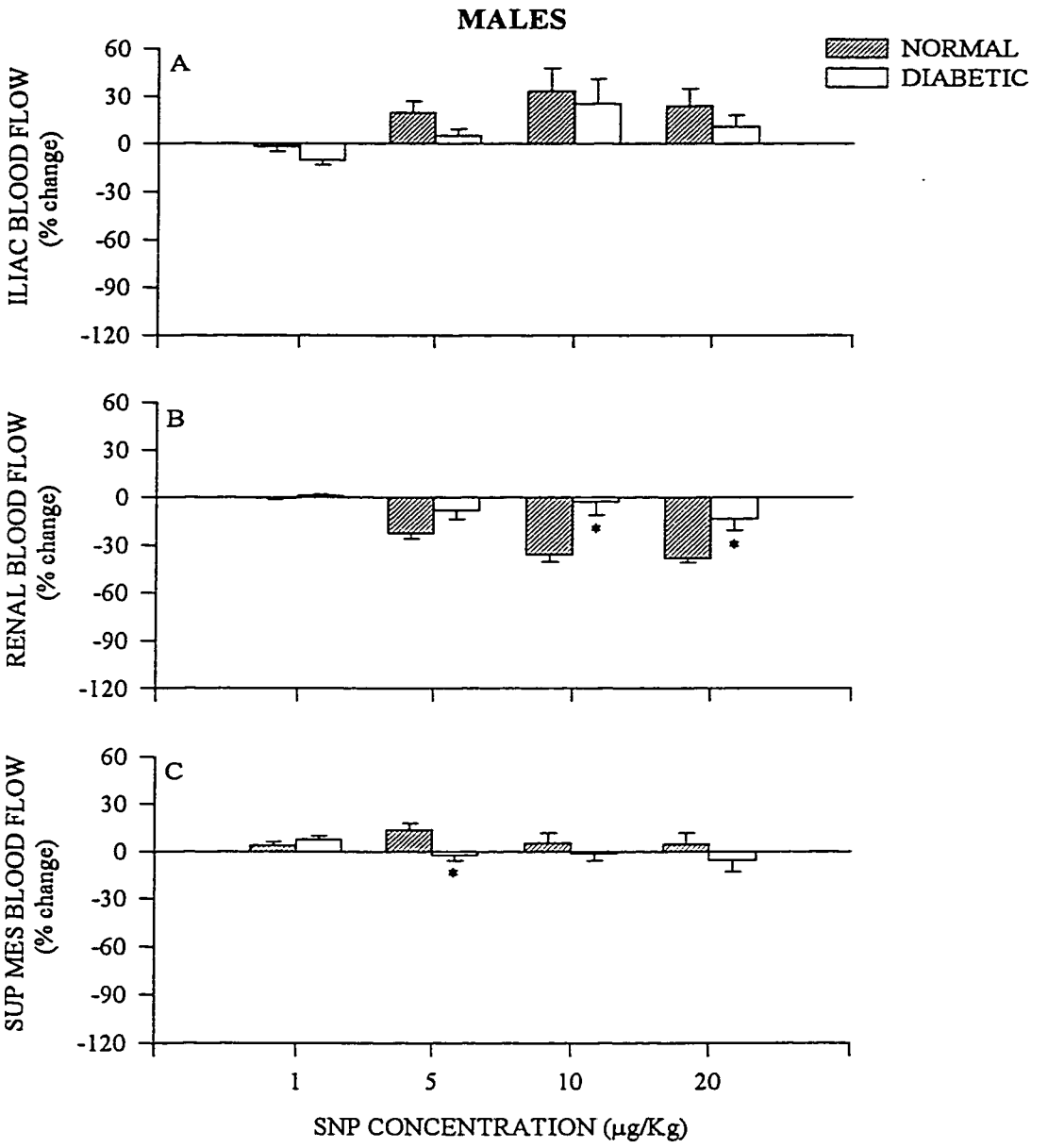

Figure 23. The effect of increasing concentrations of sodium nitroprusside (SNP) on iliac (A) renal (B), and superior mesenteric (C) blood flow expressed as percent change in normal (N = 6) and diabetic (N = 7) male rats. * = $p < 0.05$ vs. normal male. Two-way ANOVA across concentration group effect normal vs. diabetic male renal blood flow, $p < 0.001$; and superior mesenteric blood flow, $p < 0.05$.

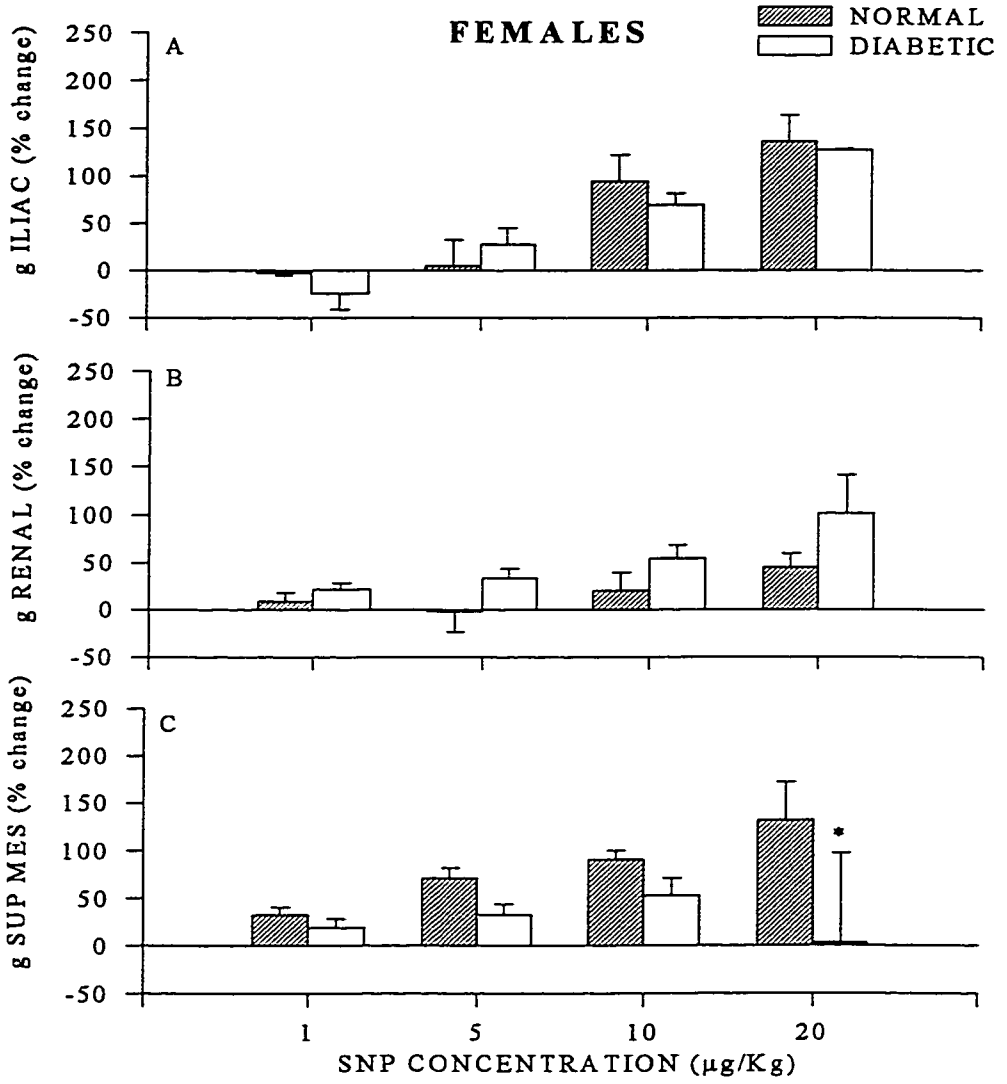

Figure 24. The effect of increasing concentrations of sodium nitroprusside (SNP) on iliac (A), renal (B), and superior mesenteric (C) conductance (g) expressed as percent change in normal (N = 5, 5, 10, and 5) and diabetic (N = 5, 4, 6, and 2) female rats. * $p < 0.05$ vs. normal female. Two-way ANOVA across concentration group effect normal vs. diabetic female g Renal, $p < 0.05$; normal vs. diabetic female g SUP MES, $p < 0.01$.

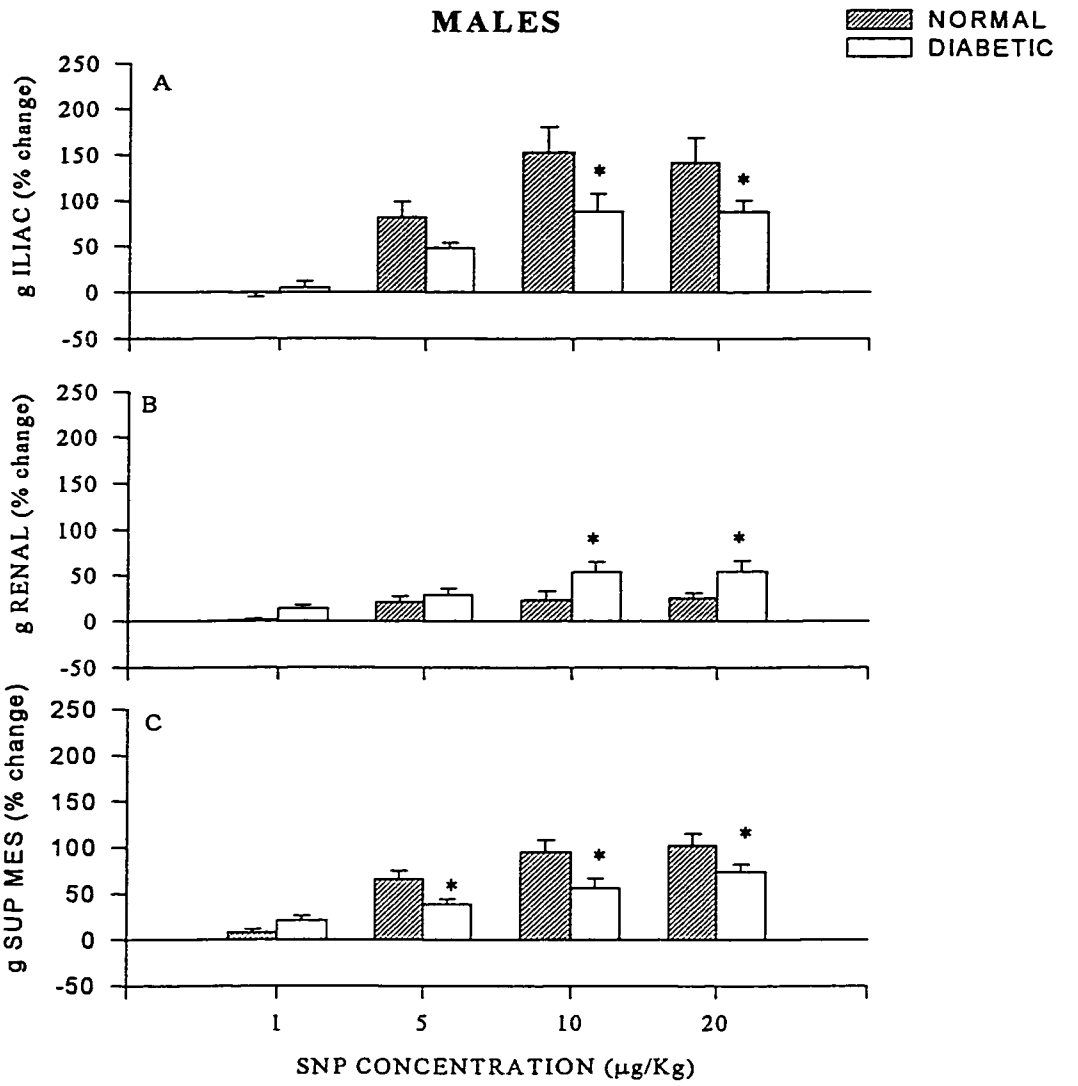

Figure 25. The effect of increasing concentrations of sodium nitroprusside (SNP) on iliac (A), renal (B), and superior mesenteric (C) conductance (g) expressed as percent change in normal (N = 6) and diabetic (N = 7) male rats. * = $p < 0.05$ vs. normal male. Two-way ANOVA across concentration group effect normal vs. diabetic male g iliac, $p < 0.01$; normal vs. diabetic male g renal, $p < 0.01$; and normal vs. diabetic male g SUP MES, $p < 0.01$.

DISCUSSION

The results presented in this study demonstrates that SNP administration results in a dose-dependent decrease in MAP and decreased peripheral resistance associated with an increased HR in normal and diabetic rats. These findings are consistent with previous observations of systemic administration of SNP (Veves, et al., 1998; Chen, et al., 1982 and Musialek, et al., 1997). Since, a primary focus of our investigation was to conduct a comparative study, we observed that the sensitivity to SNP-mediated vasodilation was less in diabetic iliac and superior mesenteric arteries when compared to normals (Pete and Dunbar, 1998). This reduced response to SNP is consistent with observations in humans forearm arterial bed and dorsal foot of diabetic patients (Veves, et al., 1998; Calver, et al., 1992; Cipolla, et al., 1996). When a comparison was made based on sex, diabetic females dilatory response to SNP was significantly less when compared to diabetic males in the iliac and superior mesenteric bed. These results are also consistent with previous observations of sex difference in diabetic patients (Ewald, 1997). Two possibilities to the reduced response to SNP in diabetes has been suggested: (1) that there is an abnormality/dysfunction in vascular smooth muscle sensitivity to NO in diabetic patients (Calver, et al., 1992) and (2) that endothelial dysfunction is responsible for the decreased responsiveness to SNP (Veves, et al., 1998). Since, SNP administration acts at sites distal to endothelial-mediated NO release, our results suggest a decreased sensitivity to NO in our diabetic animals and this is manifested to a greater extent in skeletal muscle and the splanchnic circulation.

Contrary to what was observed in the blood vessels of the skeletal muscle and the splanchnic vessels, diabetic females and males displayed a significant increased sensitivity

in the renal bed in response to SNP. These results are also supported by several investigators who have demonstrated that local and systemic infusions of SNP lead to increase renal blood flow, renal vasodilation and decreased renal vascular resistance (Costa e Forti, et al., 1998; Mesada, et al., 1981; and Lovell, et al., 1995). Diabetes is independently characterized by abnormal renal hemodynamics, vasodilation, pronounced glomerular hyperfusion and hyperfiltration (Ballermann, et al., 1984; Mattar, et al., 1996 and Tolins, et al., 1993). Therefore, the combination of the two observations suggest that the increase responsiveness to SNP in diabetic animals may add to the abnormal renal hemodynamics, and increase responsiveness to SNP in the renal bed.

In summary, systemic infusions of SNP results in a dose-dependent decreased blood pressure and increased HR in normal and diabetic rats. SNP increased the vascular conductance in normal and diabetic rats in all three vascular beds in a dose-dependent fashion. Diabetes decreased the responsiveness to SNP in the iliac and superior mesenteric vessels and increased in renal vessels. The sex difference is associated with an enhanced responsiveness to NO in the renal vessels of females.

We concluded that diabetes leads to a decrease sensitivity to NO in arteries of skeletal muscle and the splachnic circulation and increases it in the renal arteries. In addition, males appear to have a generally lower vascular sensitivity to NO when compared to females.

CHAPTER IV

THE EFFECT OF SEX AND/OR DIABETES ON BASAL ADRENERGIC TONE

INTRODUCTION

Investigations by our laboratory and others have suggested alterations in sympathetic-mediated vascular tone as a cause of diabetic vascular disease (Pete, et al., 1998; Hu, et al., 1998; Schultz-Klarr, et al., 1994; Dunbar, et al., 1991). The enhanced vessel reactivity, especially of the resistance vessels to specific agonist has been demonstrated (Cipolla, et al., 1996; Pete, et al., 1998). Both, in vitro and in vivo studies, have demonstrated an increase sensitivity especially to adrenergic agonist in animals with experimental-diabetes (Dunbar, et al., 1991; Jackson, et al., 1981), and to control levels of circulating catecholamines (Beretta-Picolli, et al., 1981).

Systemic administration of prazosin, an α-adrenergic antagonist, is associated with a hypotensive response and decreased peripheral resistance due to its action on vascular smooth muscle (Cavero, et al., 1980). This antagonist can be used to determine the relative contribution of alpha adrenergic tone to cardiovascular function (Hu, et al., 1997).

The aim of this study was to evaluate comparatively the effect of sex and/or diabetes on adrenergic tone. We examined the effects of an α_1-adrenergic antagonist on mean arterial pressure, heart rate and selected regional blood flows (iliac, renal and superior mesenteric).

MATERIALS AND METHODS

Normal and diabetic female and male, wistar rats (BW: 250-275 g) were used in

our experimental procedures. They were kept in a control environment with a 12 hour light cycle and a 23°C room temperature with free access to water and food. Diabetes was induced in normal rats by a single I injection in the tail vein of streptozotocin (STZ) of 50 mg/kg dissolved in sodium citrate (0.1 mM, pH 4.5). Five days after the STZ injection, a blood sample was collected to determine hyperglycemia and maintained 4-6 weeks post STZ injection.

On the day of the study and following a 24 hr fast, normal or diabetic rats were anesthetized with urethane (0.5 mg/kg) and α-chloralose (70 mg/kg) and placed on a heating pad to maintained their body temperature. A tracheotomy was performed to diminish respiratory obstructions and catheters with heparinized saline were placed into the femoral artery and veins. The venous catheter was utilized for blood sample collection and infusions. The femoral artery cannula was utilized for cardiovascular recording.

Pulsed-Doppler blood flow transducers (flow probe, Baylor electronics) were placed around the iliac, renal, and the superior mesenteric arteries. The arterial catheter was connected to a pressure transducer and the flow probes connected to a pulsed-Doppler flowmeter (Baylor electronics). A micro 5000 signal processing system was utilized to measure the cardiovascular responses.

Female and male normal and diabetic rats were administered a single bolus injection of prazosin (4 mg/kg) ten minutes after the establishment of a baseline. Mean arterial pressure (MAP), heart rate (HR), and blood flows (iliac, renal, and superior mesenteric) were monitored continuously.

The Biowindows Software Program and a Micro 5000 signal processing system

were used to monitor cardiovascular responses. The Biowindows Program records all cardiovascular parameters: mean arterial pressure (MAP); heart rate (HR); and blood flow (Hz Ds units).

Blood samples, 0.2 mls with saline replacement were collected prior to the study and used for glucose analysis (glucose analyzer; Yellow Springs Instruments Co., Yellow Springs, OH.

Prazosin data are averages of one minute intervals for the reported periods post-treatment. The data was analyzed using two-way ANOVA, Post-hoc analysis where appropriate, and student t-test.

RESULTS

A. THE EFFECT OF VEHICLE INFUSION ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC FEMALE VS. MALE RATS

The intent of this protocol was to established the cardiovascular responses and blood flow in normal and diabetic animals. Prazosin's vehicle was administered in a single bolus injection of 0.2 mls via the femoral vein. Instrumentation of animals was as described above (normal female, N = 6; diabetic female, N = 6; normal male, N = 4; diabetic male, N = 4).

The body weight, mean arterial pressure and heart rate was significantly lower in diabetic females when compared to its normal counterpart (Table 8). Blood glucose was significantly increased in diabetic animals when compared with their respective counterpart (Table 8).

The systemic administration of prazosin's vehicle resulted in a decrease in MAP and no significant differences between normal and diabetic female rats (Fig. 26A).

However, thirty minutes after its administration MAP was significantly lower than that of normal female rats (Fig. 26A). HR responses were similar between normal and diabetic female rats (Fig. 26B).

Males MAP and HR responses to prazosin's vehicle can be seen in Fig. 27. The administration of the vehicle resulted in a significant decrease in MAP in diabetic males (Fig. 27A). No significant differences were observed in HR (Fig. 27B).

The administration of vehicle resulted in similar blood flow patterns, an increase in flow, in the renal and superior mesenteric arteries in normal and diabetic female rats (Fig. 28B and 28C). However, diabetic females exhibited a significant decrease in iliac blood flow when compared to normals ($p < 0.01$) (Fig. 28A). Similar responses were depicted by males (iliac blood flow: $P < 0.0001$) (Fig. 29).

In terms of conductance, normal and diabetic females behaved alike for all three vascular beds (Fig. 30). However, it should be noted that after 20 minutes diabetic female rats exhibited a significant decrease in iliac conductance when compare to normal (Fig. 30A). In addition, when a Two-way ANOVA group effect was conducted and diabetic female rats conductance was significantly different from its normal counterpart ($p < 0.01$) (Fig. 30A). Males conductance responses are depicted in Fig. 31. The administration of prazosin's vehicle resulted in a significant increase in conductance in the renal and superior mesenteric bed with no significant differences in the iliac bed.

TABLE 8 Basal Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP, mm Hg), and Heart Rate (HR) In Vehicle (Saline-50 % ETOH + NH_4OH) Treated Normal And Diabetic Rats.

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	286 ± 6	80 ± 7	98 ± 5	408 ± 19
Female	(6)	(6)	(6)	(6)
Diabetic	188 ± 10*	388 ± 35*	73 ± 2*	307 ± 8*
Female	(6)	(6)	(6)	(6)
Normal	293 ± 7	75 ± 6	81 ± 3	418 ± 25
Male	(4)	(4)	(4)	(4)
Diabetic	238 ± 40	356 ± 23*	83 ± 13	359 ± 19
Male	(4)	(4)	(4)	(4)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normal counterpart, ANOVA. Number in parenthesis = N.

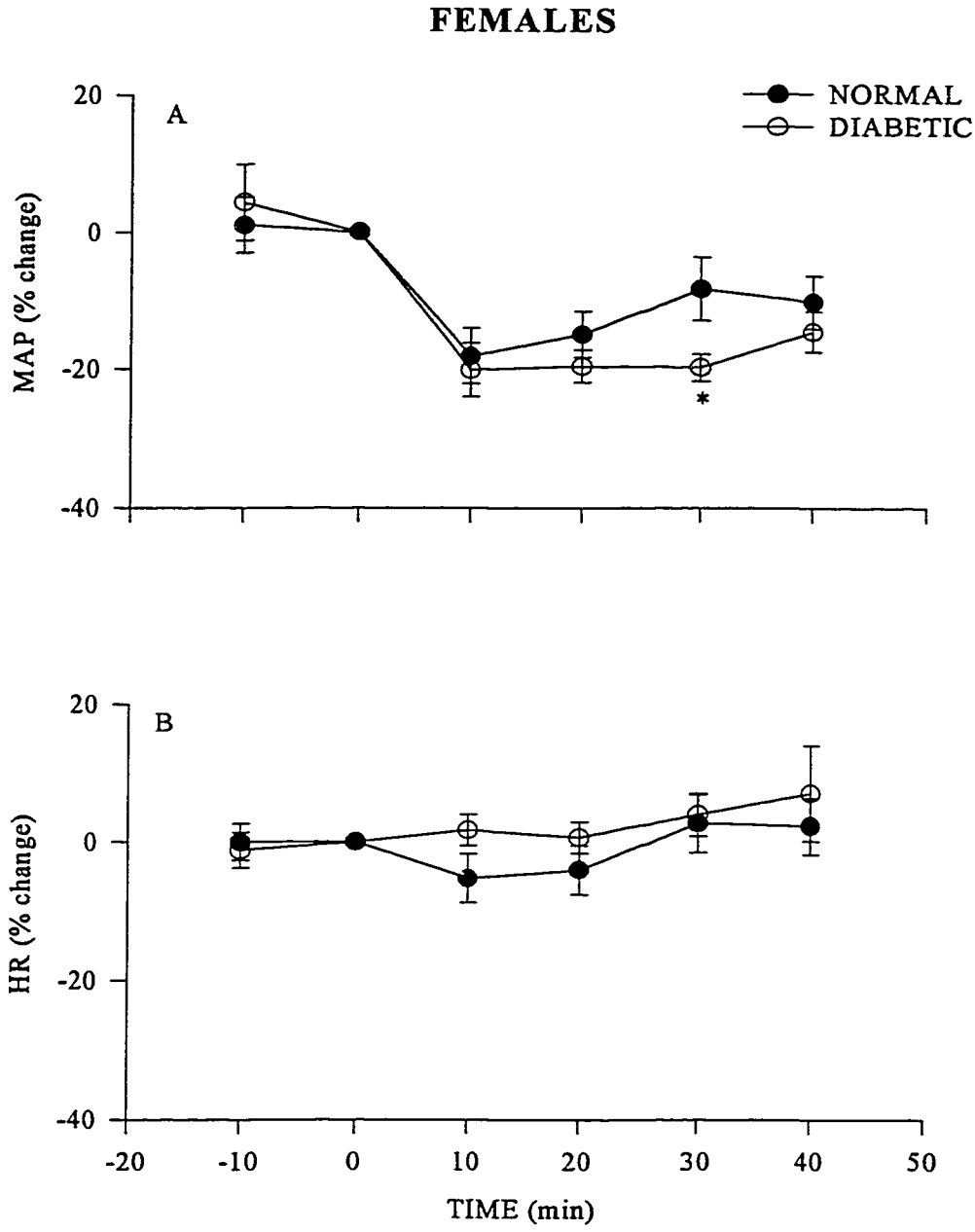

Figure 26. The effect of vehicle (50 % Sal-ETOH+NH_4OH) (0.2 mls) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 6) and diabetic (N = 6) female rats. * = $p < 0.05$, student t-test.

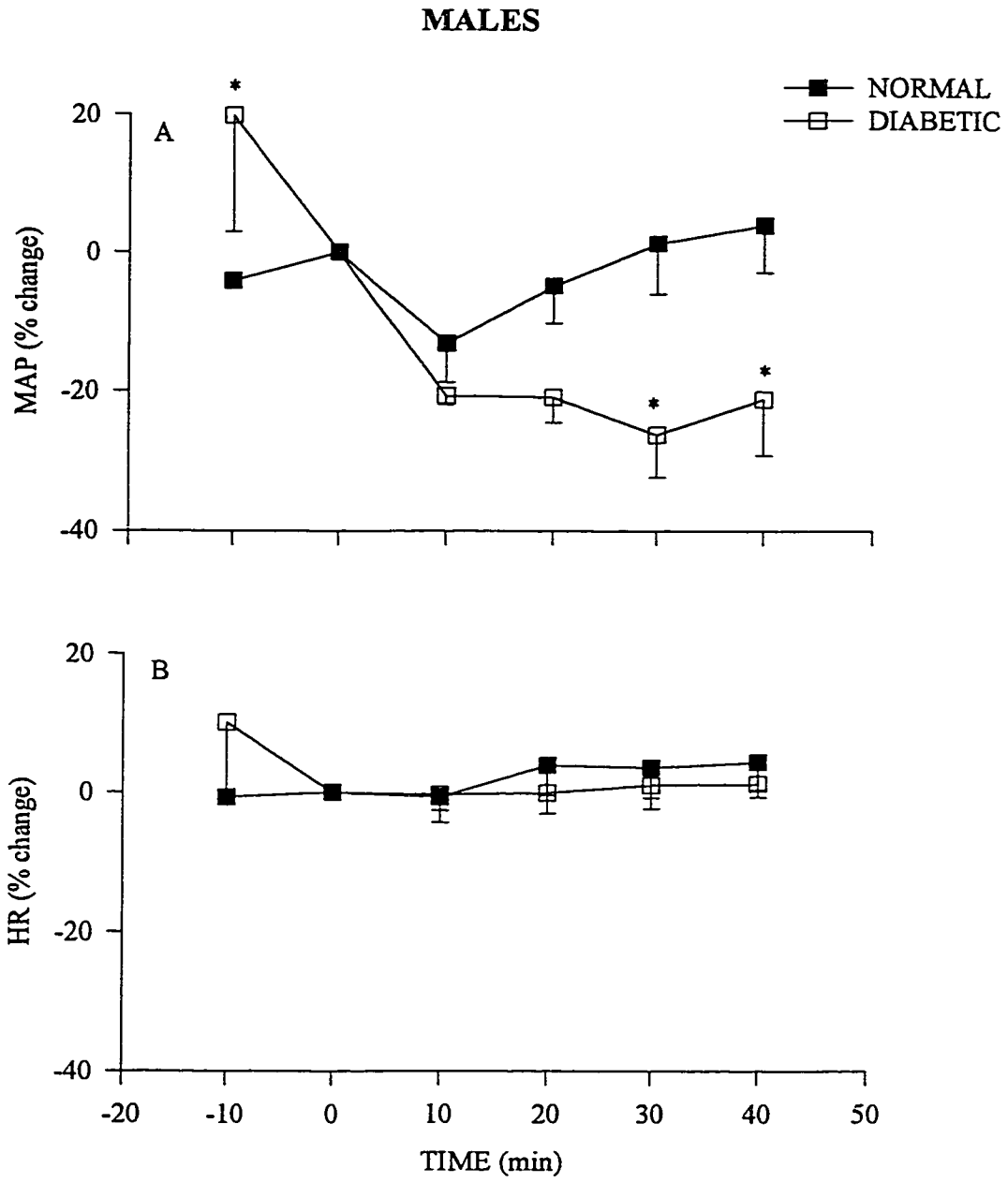

Figure 27. The effect of vehicle (0.2 mls) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) in normal (N = 4) and diabetic (N = 4) male rats. * = $p < 0.05$, student t-test.

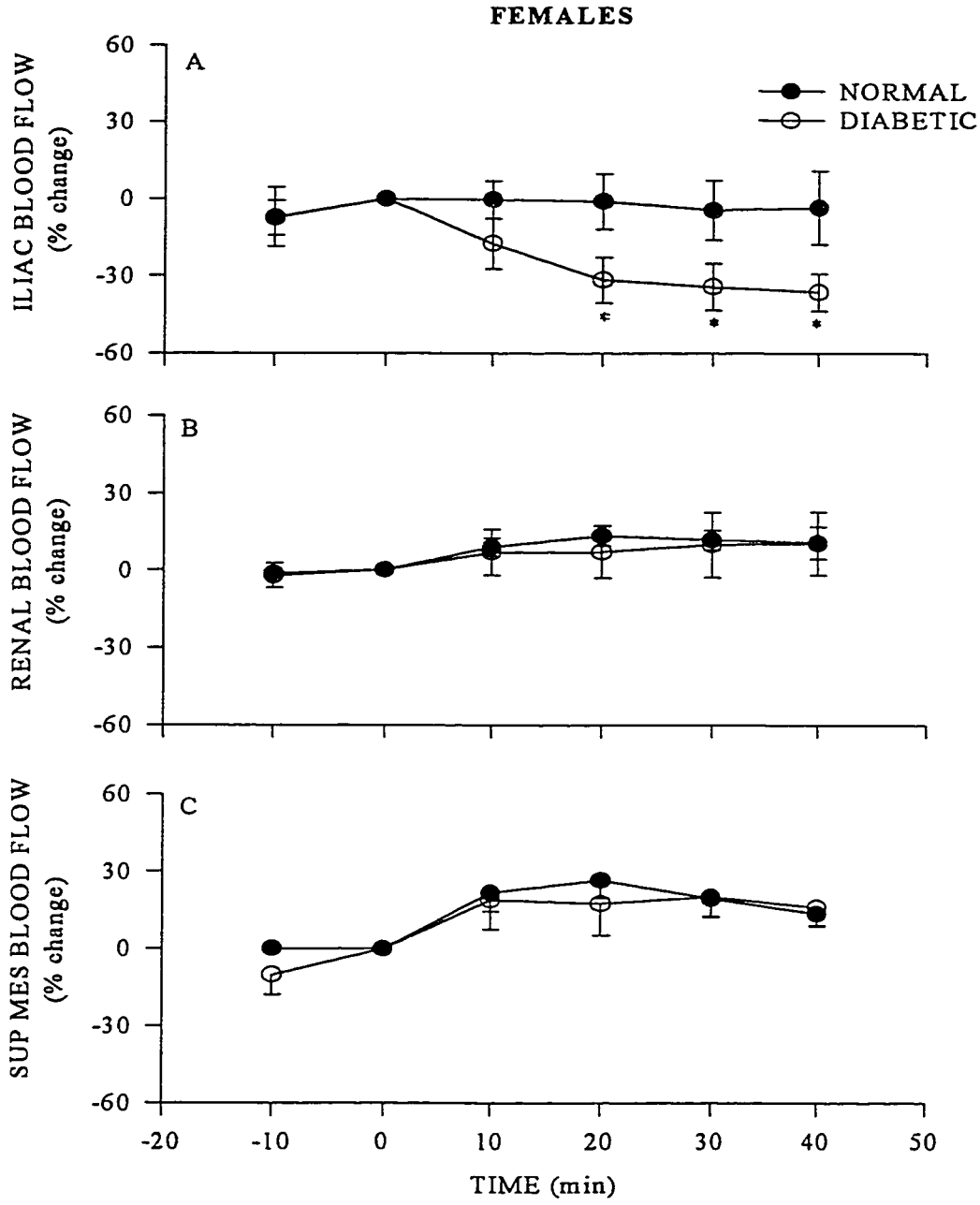

Figure 28. The effect of vehicle (50% Sal-ETOH+NH_4OH) (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 6) and diabetic (N = 6) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female iliac blood flow, $p < 0.01$.

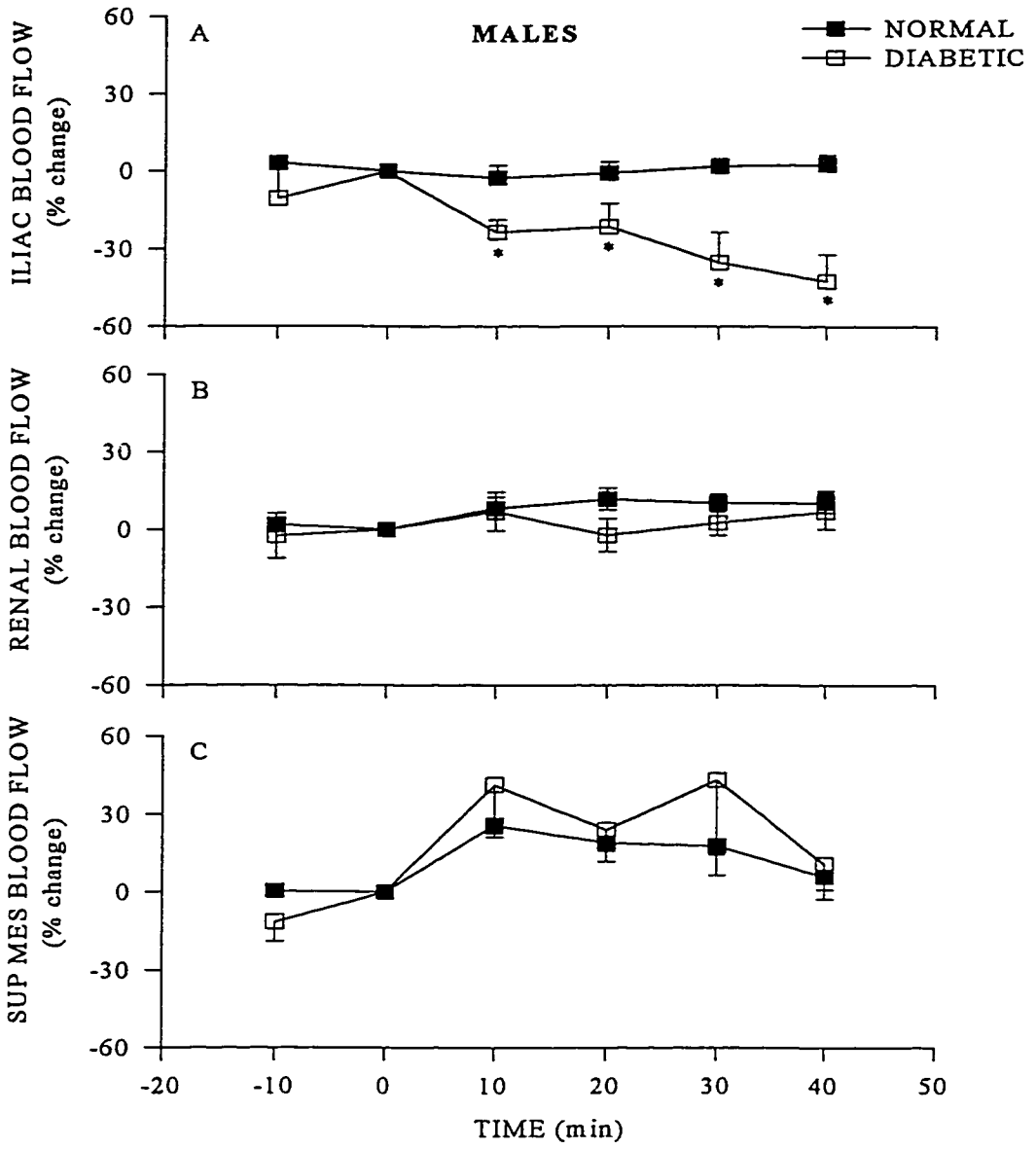

Figure 29. The effect of vehicle (50% Sal-ETOH+NH_4OH) (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 4) and diabetic (N = 4) male rats. Normal males are represented by the closed squares and diabetic males are represented by the open squares. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male ILIAC blood flow, $p < 0.0001$.

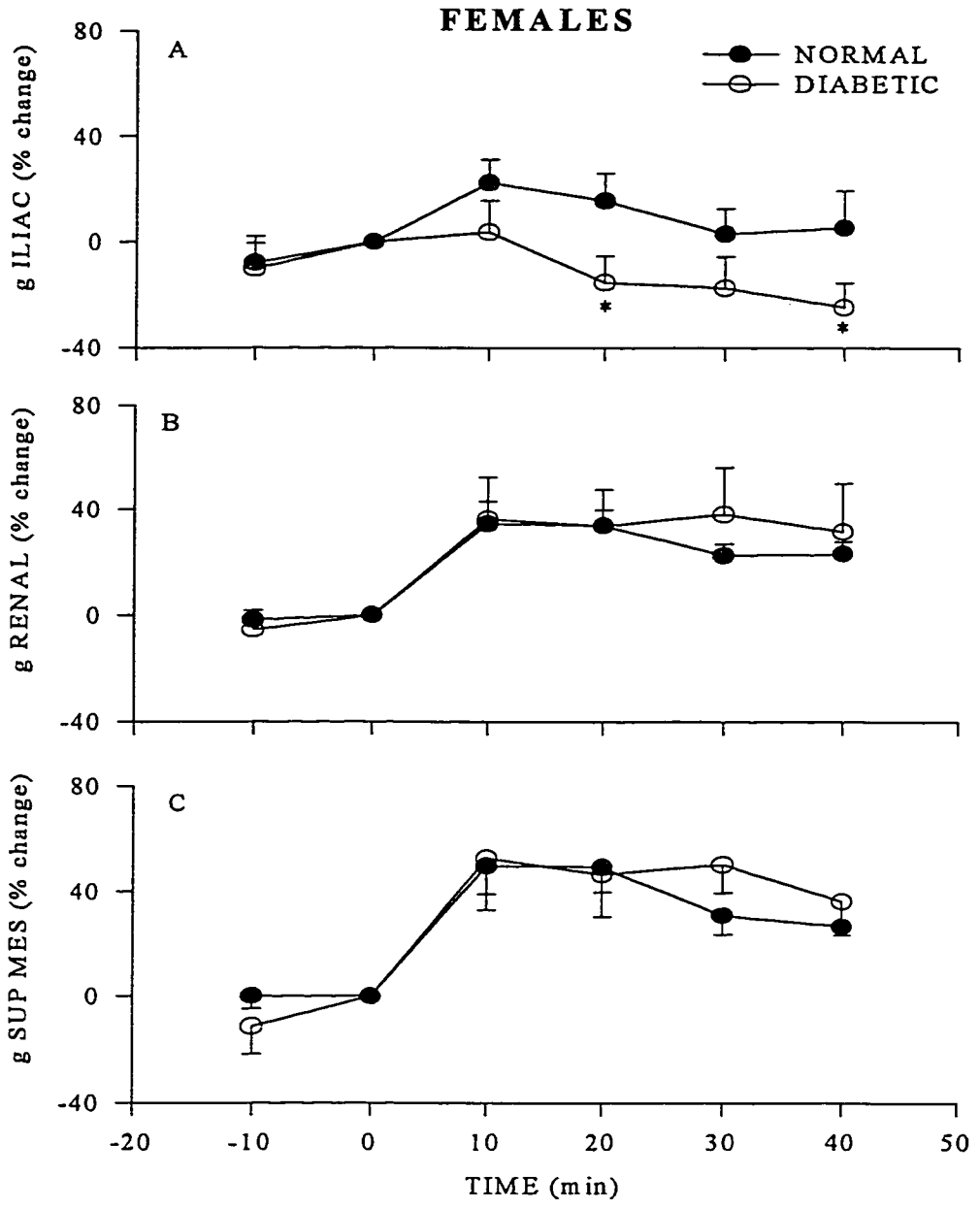

Figure 30. The effect of vehicle (50 % Sal-ETOH+NH_4OH) (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 6) and diabetic (N = 6) female rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic female g ILIAC, $p < 0.01$.

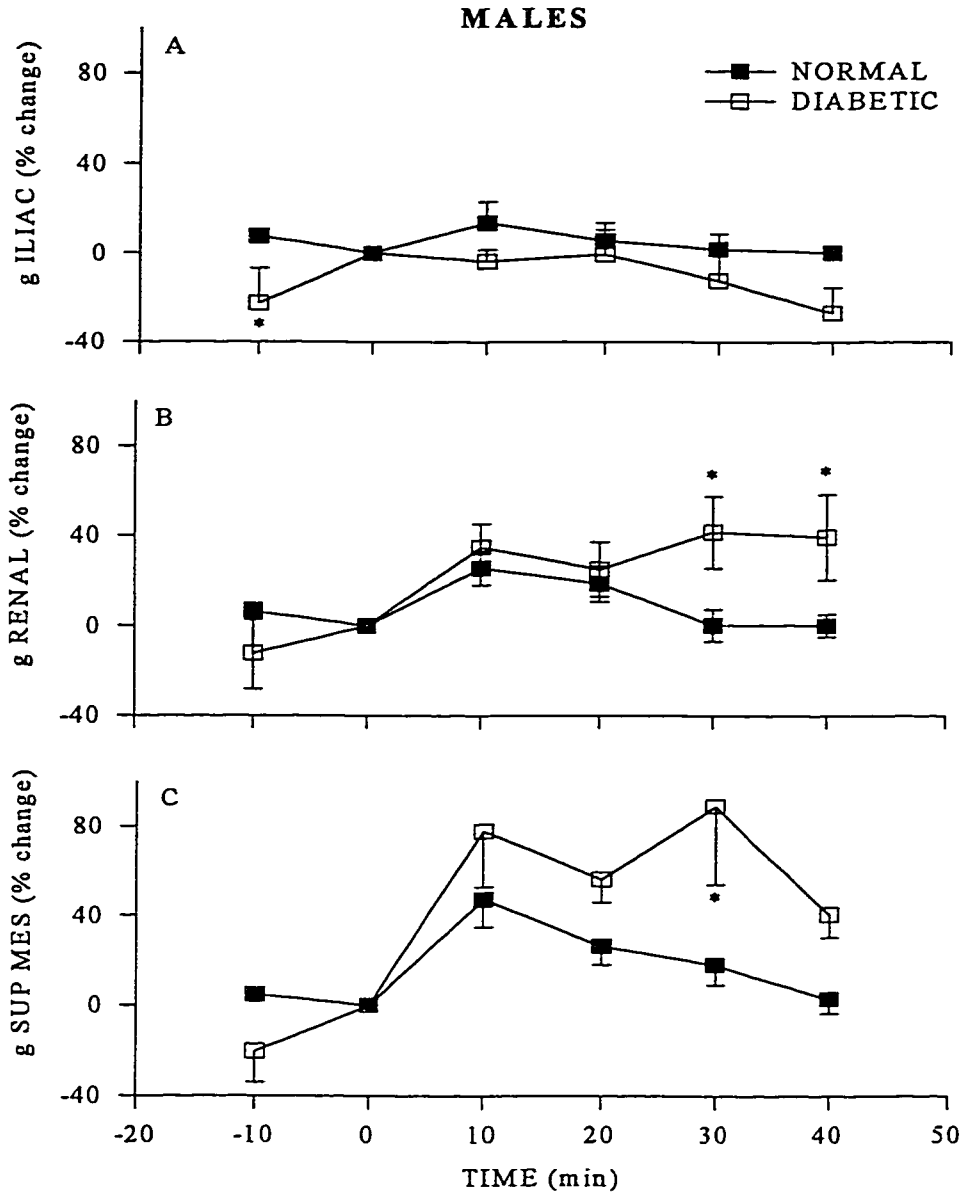

Figure 31. The effect of vehicle (50% Sal-ETOH+NH_4OH) (0.2 mls) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 4) and diabetic (N = 4) males rats. * = $p < 0.05$, student t-test. Two-way ANOVA group effect normal vs. diabetic male g ILIAC, $p < 0.01$; g Sup Mes, $P < 0.01$.

B. THE EFFECT OF PRAZOSIN (4 mg/kg) ON CARDIOVASCULAR RESPONSES IN NORMAL AND DIABETIC FEMALE VS. MALE RATS

The intent of this final protocol was to evaluate the effect of an α_1-adrenoceptor antagonist, prazosin (4 mg/kg), on cardiovascular responses and blood flow in normal and diabetic female vs. male rats. This study consisted of a single bolus administration of prazosin (0.2 ml) via the femoral vein (normal female, N = 6; diabetic female, N = 7; normal male, N = 5; diabetic male, N = 6).

Animals were instrumented as described before. Blood samples (0.2 mls) were collected 10 minutes prior to prazosin (4 mg/kg) treatment and 50 minutes after treatment. Blood volume was immediately replaced with heparinized saline. Cardiovascular responses and blood flow were continuously recorded for 50 minutes. Prazosin administration consisted of a single bolus administration of 0.2 mls slowly over a 1.5-2.0 minute period via the femoral vein approximately 10 minutes after a stable baseline was achieved.

No significant differences were observed between normal and diabetic female rats in terms of body weight. However, males body weight was significantly lower in diabetics when compared to normals. Diabetic animals had a significantly higher blood glucose levels when compared to normals. No significant differences were seen in terms of basal MAP or HR (Table 9).

Alpha adrenergic antagonism using prazosin resulted in a decrease in MAP in normal and diabetic animals (Fig. 32A and 33A). However, in normal males this decrease in MAP was less when compared to normal females (Table 10). In contrast to female (Fig. 32A), diabetic male had a greater decreased in MAP when compared to their normal

counterpart (Table 10) (Fig. 33A). The heart rates were initially decreased after the administration of prazosin, but, after approximately 10 minutes the HR returned toward baseline in all four groups of animals (Fig. 32B and 33B). Nonetheless, the initial decrease in HR was greater in diabetic females when compared to diabetic males (Table 10). However, prazosin had no significant effect in HR. When MAP and HR responses were evaluated in terms of group effect by a Two-way ANOVA, it reported no differences between normal and diabetic females in MAP and a significant difference in HR ($p < 0.05$). However, the two-way ANOVA evaluation resulted in a significant difference in MAP ($p < 0.001$) and no difference in HR among males.

The administration of the alpha adrenergic blocker resulted in an increase in blood flow in the iliac and superior and mesenteric arteries (Fig. 34A and 34C) (Fig. 35A and 35C) and in a decrease in the renal blood flow (Fig. 34B) (Fig. 35B) in female and male rats, respectively. Diabetic females seem more sensitive to the adrenergic blockade in the iliac bed vascular than normal female rats (Fig. 34). In addition, diabetic females were significantly less responsive/sensitive to the alpha adrenergic blockade in the renal and superior mesenteric vascular beds (Fig. 34B and 34C). On the other hand, no significant differences were observed between normal and diabetic male rats iliac and renal blood flow (Fig. 35A and 35B). However, the superior mesenteric artery of diabetic males seem slightly less responsive to prazosin than normal males (Fig. 35C). Females group effect responses in blood flow were compared with a two-way ANOVA and it resulted in a significant difference between normal and diabetic animals in all three vascular beds (iliac: $p < 0.05$; renal: $p < 0.01$ and superior mesenteric: $p < 0.01$). On the contrary, males group effect responses were only significant in the superior mesenteric bed ($p <$

0.01).

The conductance was increased in the three vascular beds in response to prazosin (Fig. 36 and 37). Prazosin increased blood flow in the iliac (Fig. 36A and 37A), renal (Fig. 36B and 37B), and superior mesenteric bed (Fig. 36C and 37C), to a greater extent in diabetic animals, except for diabetic females in the superior mesenteric artery (Table 11). Diabetic females superior mesenteric conductance significantly decrease after approximately 20 minutes of prazosin administration (Fig. 36C). In addition, the response to prazosin was less in the renal bed of normals and diabetics (Table 11) (Fig. 36B and 37B). Renal conductance in response to prazosin (Table 11) was increased to a greater extent in diabetic females when compared to diabetic males and also when compared to normal females (Table 11). In addition, renal conductance in diabetic male was significantly increased when compared to normals (Table 11). Superior mesenteric conductance was significantly lower in normal males when compared to normal females (Table 11). Diabetic females superior mesenteric conductance was significantly less when compared to normal females (Table 11).

A two-way ANOVA group effect demonstrated that normal and diabetic females were significantly different in terms of conductance in the renal ($p < 0.05$) and superior mesenteric ($p < 0.001$) bed. Males conductance group effect were significant in the iliac ($p < 0.05$) and the renal ($p < 0.01$) vascular bed.

TABLE 9 Basal Body Weight (g), Blood Glucose (mg/dl), Mean Arterial Pressure (MAP, mm Hg), and Heart Rate (HR) In Prazosin Treated Normal And Diabetic Rats

Group	Body Weight (g)	Glucose (mg/dl)	MAP (mm Hg)	HR (beats/min)
Normal	264 ± 4	90 ± 8	69 ± 3	349 ± 15
Female	(6)	(6)	(6)	(6)
Diabetic	228 ± 13	401 ± 42*	62 ± 4	331 ± 11
Female	(7)	(7)	(7)	(7)
Normal	344 ± 29	79 ± 4	68 ± 4	362 ± 28
Male	(5)	(5)	(5)	(5)
Diabetic	235 ± 17*	436 ± 18*	65 ± 2	322 ± 17
Male	(6)	(6)	(6)	(6)

The values represent the mean ± S.E.M. * $p < 0.05$ vs. normal counterpart, ANOVA. Number in parenthesis = N.

TABLE 10 Average Mean Arterial Pressure (MAP) and Heart Rate (HR) Responses (Between 10-30 minutes) to Prazosin (4 mg/kg)

MAP (% change)			
NORMAL		**DIABETIC**	
Female	**Male**	**Female**	**Male**
-47.16 ± 1.4	-30.09 ± 3.0*	-43.93 ± 2.2	-43.90 ± 2.2‡
(6)	(5)	(7)	(6)
HR (% change)			
-6.57 ± 3.1	-2.52 ± 4.4	-14.33 ± 2.6	-1.47 ± 3.4†
(6)	(5)	(7)	(6)

The values represent the mean ± S.E.M. * = $p < 0.001$ vs. normal female. ‡ = $p < 0.001$ vs. normal male. † = $p < 0.01$ vs. diabetic female, ANOVA. Number in parenthesis = N

TABLE 11 Average Iliac, Renal And Superior Mesenteric Arteries Vascular Conductance (g) Responses (Between 10-30 minutes) To Prazosin (4 mg/kg)

ILIAC g (% change)			
NORMAL		**DIABETIC**	
Female	**Male**	**Female**	**Male**
111.61 ± 14.3	85.75 ± 11.6	147.22 ± 26.3	126.60 ± 15.2
(6)	(5)	(7)	(6)
RENAL g (% change)			
38.42 ± 4.3	6.98 ± 3.2	73.26 ± 15.3*	42.00 ± 12.2†§
(6)	(5)	(7)	(6)
SUP MES g (% change)			
151.78 ± 9.7	88.21 ± 13.8‡	103.21 ± 16.2‡	95.68 ± 15.2
(6)	(5)	(7)	(6)

The values represent the mean ± S.E.M. * = $p < 0.05$ vs. normal female. † = $p < 0.05$ vs. normal male. § = $p < 0.05$ vs. diabetic female. ‡ = $p < 0.05$ vs. normal female, ANOVA. Number in parenthesis = N.

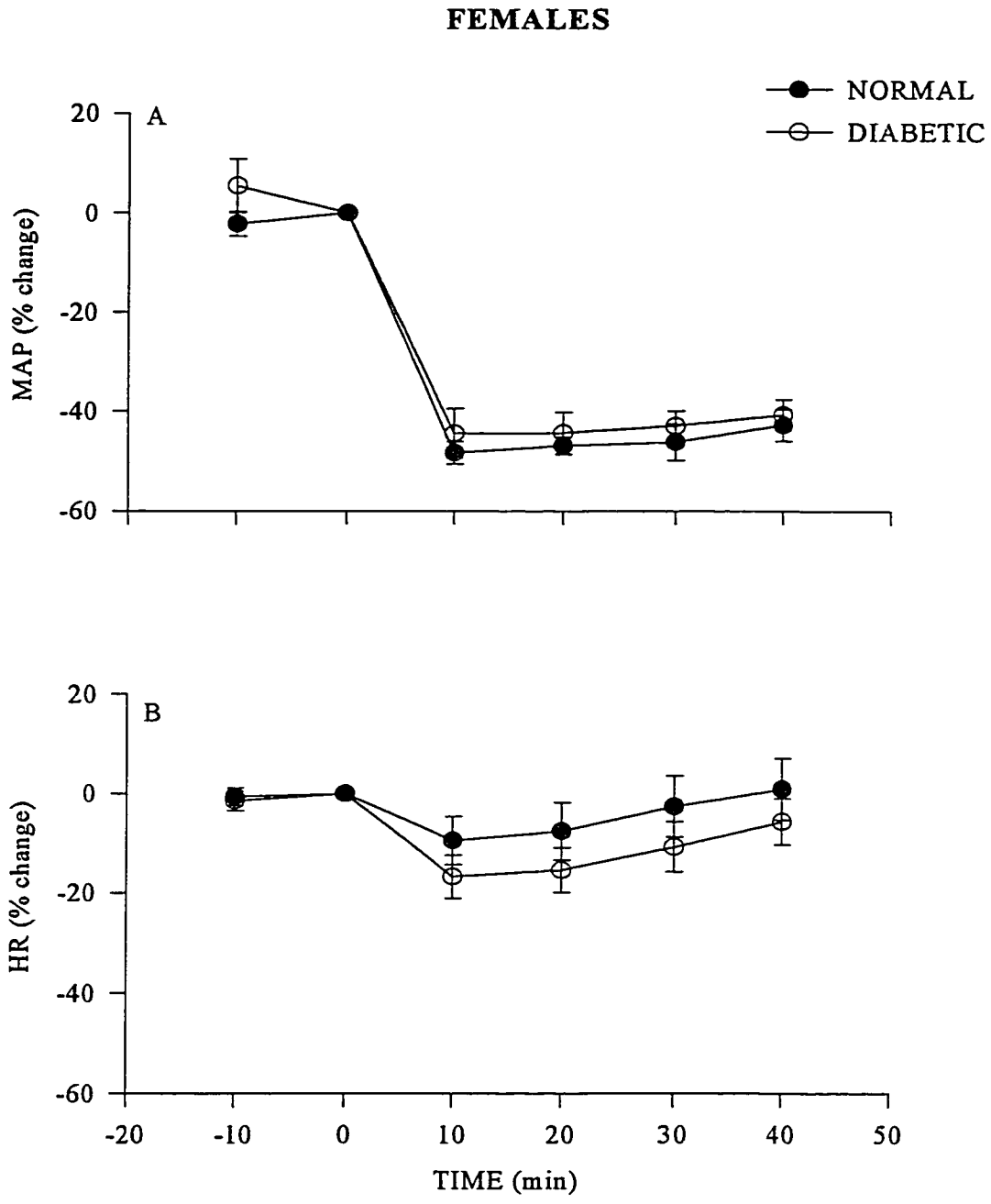

Figure 32. The effect of prazosin (4 mg/kg) on mean arterial pressure (MAP) (A) and heart rate (HR) (B) expressed as percent change in normal (N = 6) and diabetic (N = 7) females rats. Two-way ANOVA group effect normal vs. diabetic females HR, $p < 0.05$.

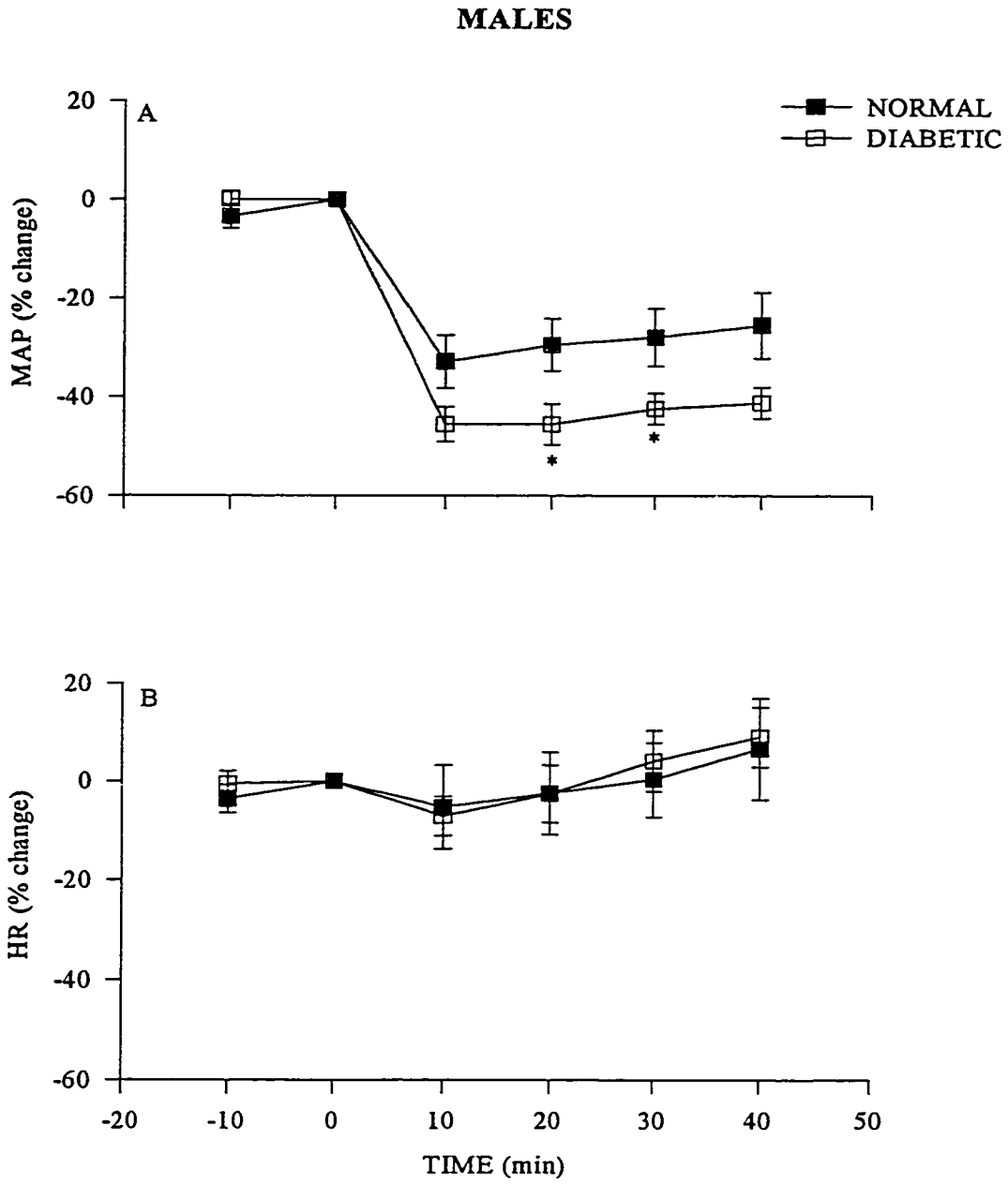

Figure 33. The effect of prazosin (4 mg/kg) on mean arterial pressure (A) (MAP) and heart rate (HR) (B) expressed as percent change in normal (N = 5) and diabetic (N = 6) males rats. * = $p < 0.05$ vs. normal male, student t-test. Two-way ANOVA group effect normal vs. diabetic males MAP, $p < 0.001$.

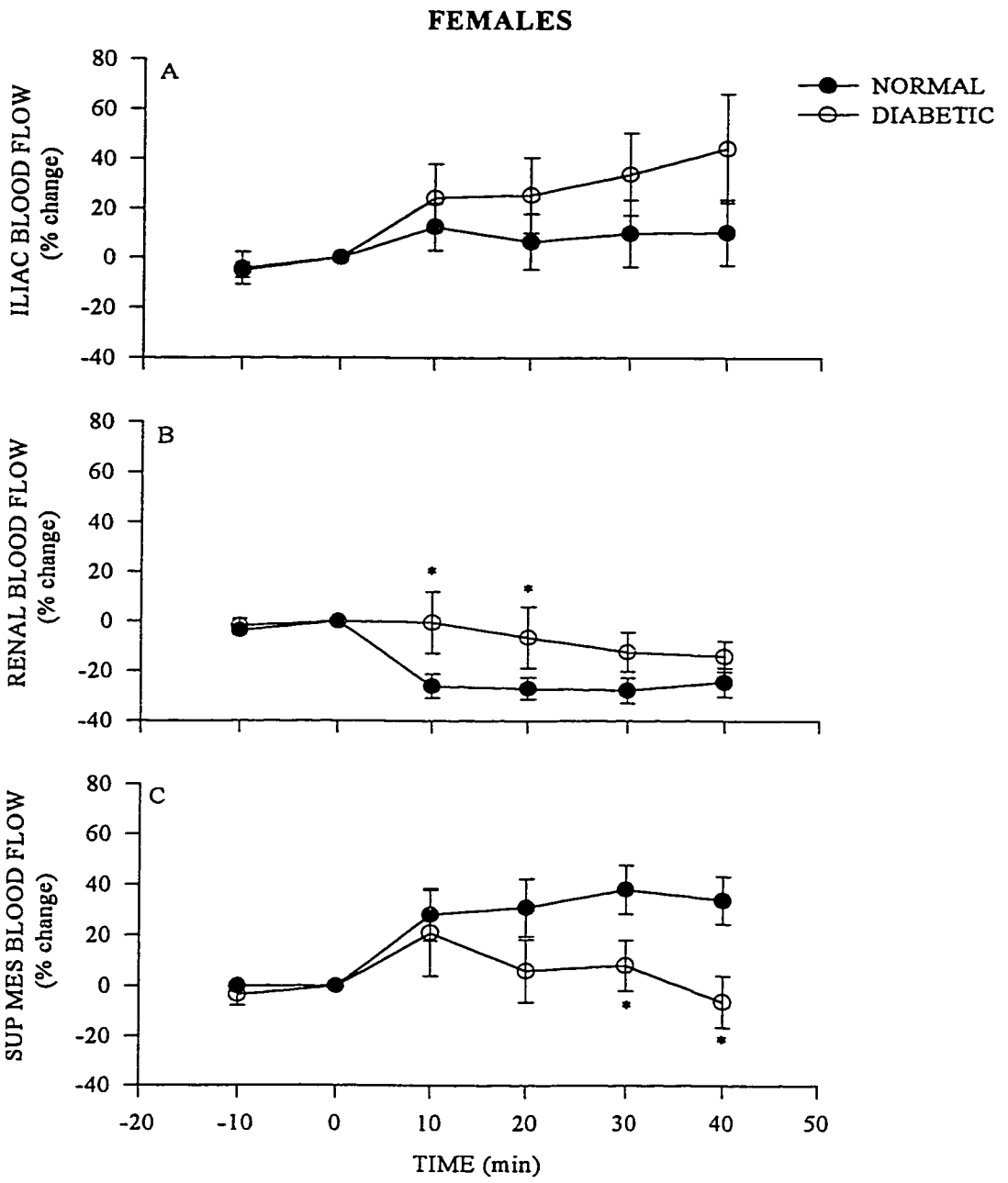

Figure 34. The effect of prazosin (4 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 6) and diabetic (N = 7) female rats. * = $p < 0.05$ vs. normal female. Two-way ANOVA group effect normal vs. diabetic females iliac blood flow, $p < 0.05$; renal blood flow, $p < 0.01$; and superior mesenteric blood flow, $p < 0.01$.

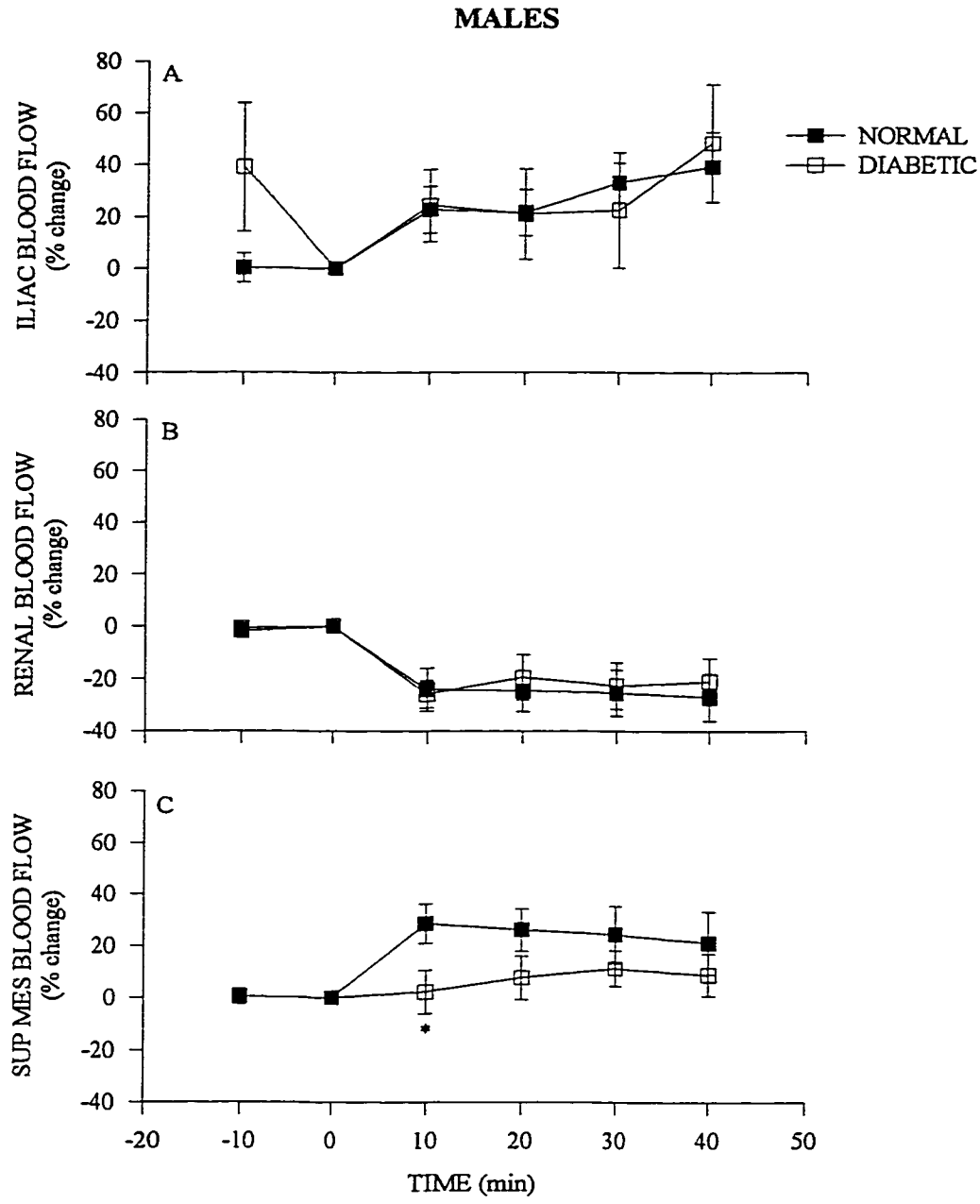

Figure 35. The effect of prazosin (4 mg/kg) on iliac (A), renal (B) and superior mesenteric (C) arteries blood flow expressed as percent change in normal (N = 5) and diabetic (N = 6) male rats. * = $p < 0.05$ vs. normal male, ANOVA. Two- way ANOVA group effect normal vs. diabetic male superior mesenteric blood flow, $p < 0.01$.

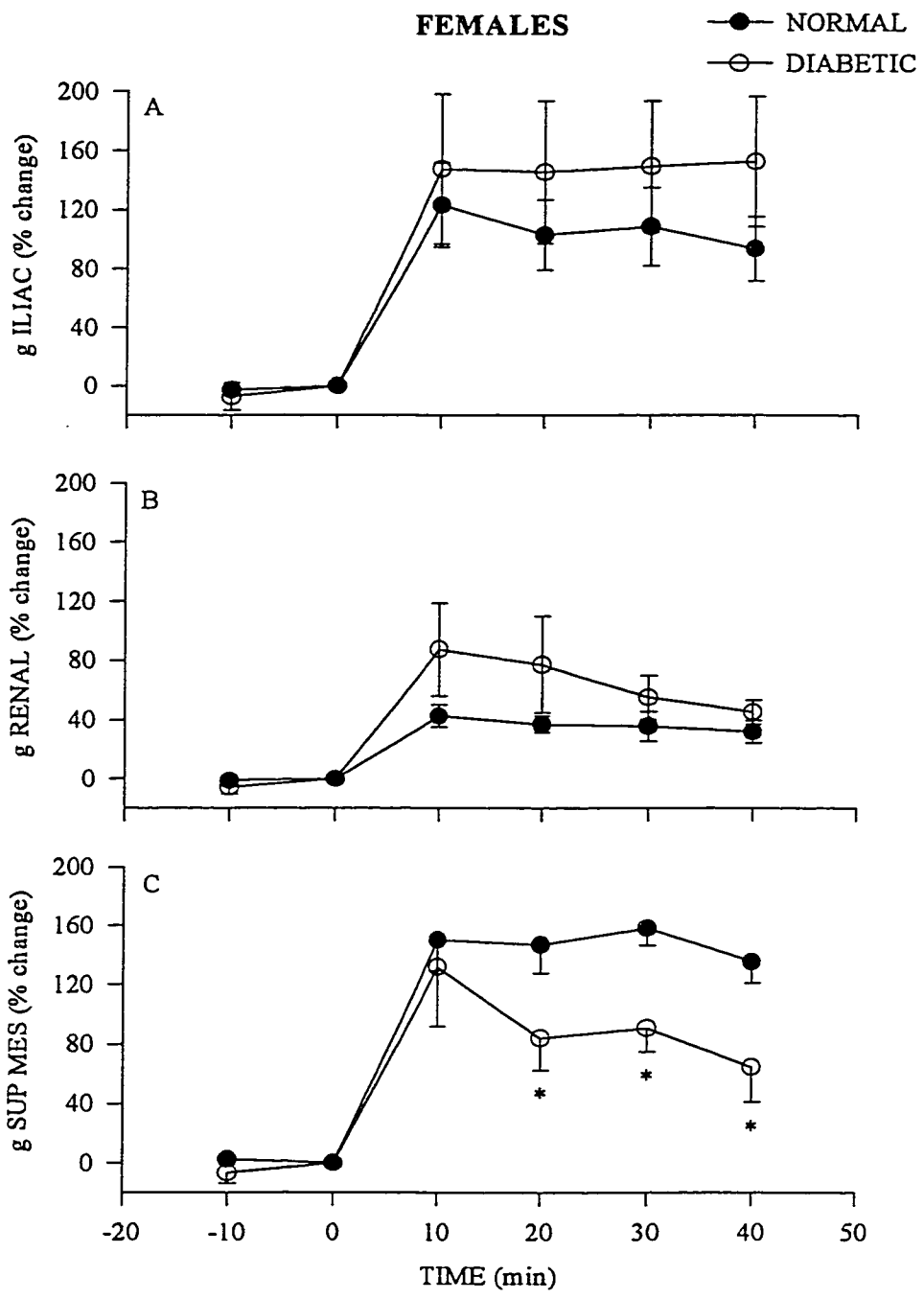

Figure 36. The effect of prazosin (4 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 6) and diabetic (N = 7) female rats. * = $p < 0.05$ vs. normal female, student t-test. Two-way ANOVA group effect normal vs. diabetic females g Renal, $p < 0.05$; g SUP MES, $p < 0.001$.

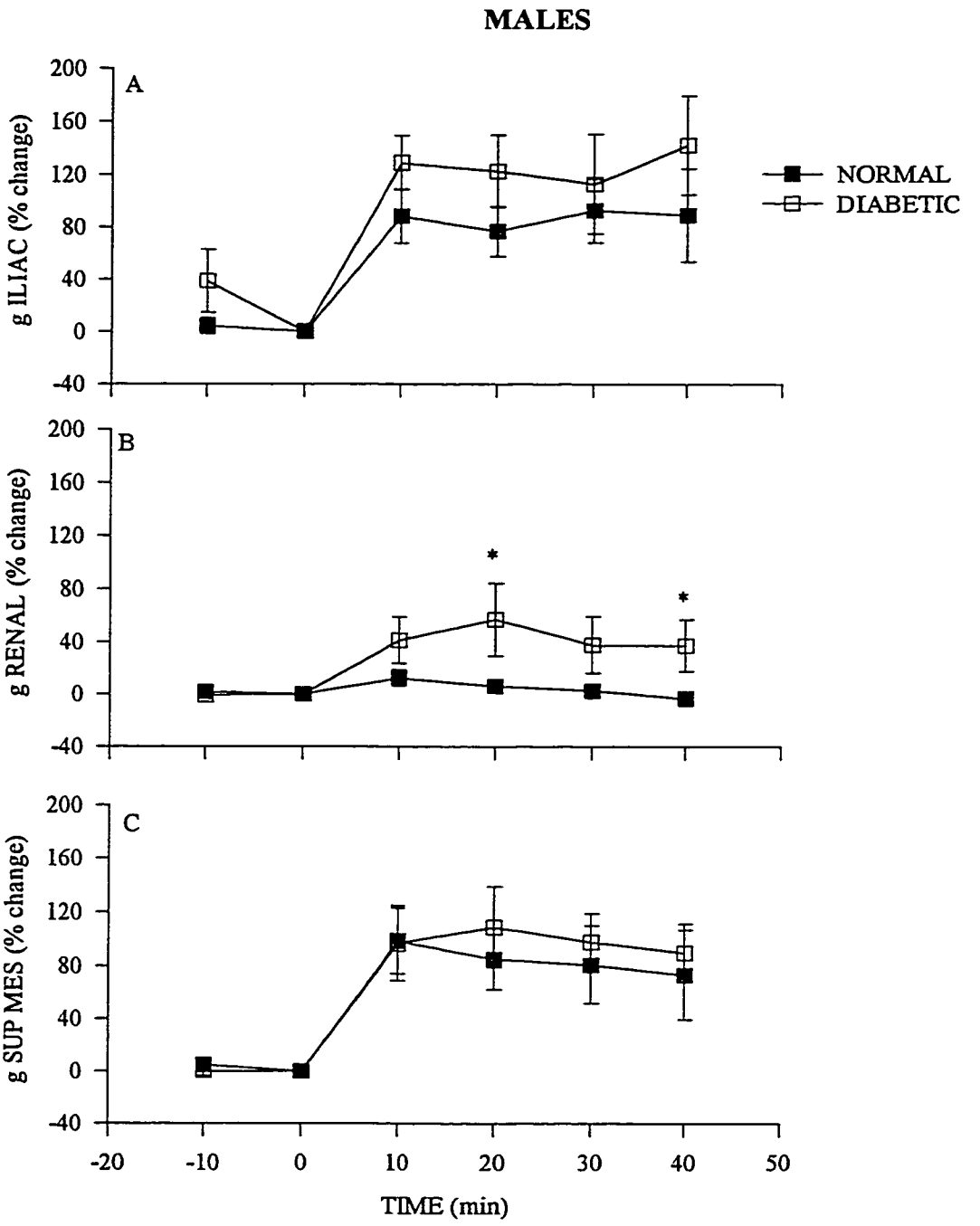

Figure 37. The effect of prazosin (4 mg/kg) on iliac (A), renal (B), and superior mesenteric (C) arteries conductance (g) expressed as percent change in normal (N = 5) and diabetic (N = 6) male rats. * = $p < 0.05$ vs. normal male, student t-test. Two-way ANOVA group effect normal vs. diabetic male g iliac, $p < 0.05$; and g Renal, $p < 0.01$.

DISCUSSION

The administration of the α_1-adrenoceptor antagonist, prazosin, resulted in a decrease in MAP and increase regional conductance findings which are consistent with previous observations (Hu, et al., 1997_a; Albillos, et al., 1995; and Hui, et al., 1996). According to previous studies, the hypotensive response to prazosin is a consequence of the decrease in α-adrenergic-mediated peripheral resistance at the level of vascular smooth muscle (Cauffield, et al., 1996 and Docherty, 1989). The initial bradycardia followed by an increase or returned to basal HR observed in all four groups of animals after the administration of prazosin has also been previously documented (Davidow, et al., 1996) as well as the diminished reflex tachycardia observed in our female rats (Bolli, et al., 1976). Conversely, our normal and diabetic male rats displayed a reflex tachycardia that was consistent with previous studies (Koltai, et al., 1997 and Davidow, et al., 1996).

Prazosin leads to an increase in vascular conductance/blood flow in the different vascular beds and our results are in agreement with these observations (Hui, et al., 1996; Holmes, et al., 1986; and Buckwalter, et al., 1997). This response is a reflection of blockade of the α_1-adrenoceptors vasoconstrictor tone and the consequent decreased resistance and increased in blood flow (Hui, et al., 1996; Holmes, et al., 1986; and Buckwalter, et al., 1997). The differential responses in different vascular beds is strongly influenced by the distribution of α-receptors, where increased α-receptors population is associated with an enhanced peripheral blood vessels response, specially in the kidney and the splanchnic circulation (Holmes, et al., 1986). In our study, the administration of prazosin resulted in a differential response in blood flow. This was especially so in the iliac and superior mesenteric bed where there was a greater response to the adrenergic

blockade than the renal bed in both normal and diabetic animals. A possible explanation for the increased sensitivity to prasozin in the iliac bed may be that the blood flow in skeletal muscle has a greater dependence on adrenergic regulation. Other investigators also support observations that skeletal muscle blood flow is indeed under tonic sympathetic constrictor tone especially at rest (Buckwalter, et al., 1997; and Leech, et al., 1996). Like the skeletal muscle bed, the splanchnic bed also exhibited an increase in conductance following α-adrenergic blockade. This observation is also supported by studies using the in situ autoprefused superior mesenteric arterial bed of rats which suggest that α_1-adrenoceptors are strongly represented in this vascular bed (Nichols, et al., 1985; and Piascik, et al., 1997). On the other hand, the mild response to prazosin in the renal bed when compared to the skeletal muscle and the splanchnic bed is not as easy to consolidate. Since, reports in the literature state that renal vasoconstriction is mainly mediated by α_1-adrenoceptors (Piascik, et al., 1997; Hesse, et al., 1984; and Elhawary, et al., 1992). However, investigators have also been able to demonstrate that α_2-adrenoceptors-mediate renal vasoconstriction in conscious rats when boluses of norepinephrine were administered into the kidney (Wolff, et al., 1989). Nevertheless, the basal vascular tone in the kidney may not be mediated by α-adrenergic mechanism and mostly by an autoregulatory mechanism by other mediators (Hu, et al., 1997_b; Cupples, 1993; and Takenaka, 1994). Therefore, the mild response observed in the renal bed to prazosin when compared to the iliac and superior mesenteric bed may be consistent with the autoregulatory mechanism in the kidney.

In summary, prazosin infusion decreased the MAP in normal and diabetic rats. The α_1-adrenoceptor antagonist, increased vascular conductance in all three vascular

beds, however, the relative responsiveness to prazosin was greater in the iliac and superior mesenteric arteries when compared to the renal bed. Again, females tended to be more responsive than males in their dilatory response.

Therefore, we concluded that skeletal muscle and the splachnic circulation is more dependent on alpha adrenergic tone when compared to the renal vessels and this tone is greater in both male and female diabetics.

CHAPTER V

SUMMARY

GENERAL DISCUSSION

According to our results the possible mechanism of diabetic vascular disease is likely a combination of endothelial dysfunction, abnormal vascular smooth muscle sensitivity to nitric oxide and increased dependence on alpha adrenergic tone. See Figures 38 and 39.

In our studies, blockade of NO synthesis by L-NAME resulted in an attenuated pressor response in diabetic animals and to a greater degree in diabetic females suggesting that a decreased NO synthesis or release from endothelial cells in diabetic animals leads to a decreased dependency on NO for cardiovascular control. This observation is supported by Calver, et al., 1992. The antagonism of NOS was also associated with a decreased conductance in all three vascular beds in normal animals. This decreased conductance was greater in the iliac bed of diabetic males and contrastly, in diabetic female rats NOS inhibition resulted in an increase in renal conductance.

The comparable regional vasoconstriction in all three vascular beds (iliac, renal and superior mesenteric) in normal animals has been reported previously in normal and diabetic male rats (Gardiner, et al., 1990; Reckelhoff, et al., 1998; Tolins, et al., 1993; Zappellini, et al., 1996). Diabetic male rats enhanced constrictor response in the iliac bed in response to NOS inhibition suggests an increase in the overall balance in the constrictor tone versus the dilatory tone in blood vessels of skeletal muscle in diabetic males. It is conceivable that in diabetic males the iliac bed increased vasoconstrictive response to L-

NAME is a consequence of a more pronounced endothelial dysfunction (lack or inability to produce NO) and greater dependence in NO, leading to an increase sensitivity to NO withdrawal with L-NAME. Other investigators have also demonstrated that the inhibition of NO leads to an enhanced sympathoexcitatory response which results in an exaggerated vasoconstriction (Lacolley, et al., 1991; Owlya, et al., 1997; Sakuma, et al., 1992; Vo, et al., 1992; Zanzinger, et al., 1994). We suggest that this sympathoexcitatory response might be exacerbated in male diabetics and may account for the increase vasoconstrictive response in the iliac bed to L-NAME.

Contrastingly to the observations above, the renal bed of diabetic females failed to constrict following the administration of L-NAME. A possible explanation for the lack of renal constrictal response or vasodilatation seen in diabetic females might be an enhanced vasodilatatory sensitivity due to estrogen enhanced NO production (Darkow, et al., 1997; Rahimian, et al., 1997; Tagawa, et al., 1997). Chronic estrogen administration has been demonstrated to enhance NO production from endothelium. We believe that the renal vasodilatory response to L-NAME can be attributed to the combination of enhanced vasodilatory responsiveness in females and the dramatic increase in mean arterial pressure. Another possibility in the renal vasodilatory response in diabetic females is provided by the greater levels of mRNA and eNOS protein in females than in male rats kidneys (Reckelhoff, et al., 1998). However, the reduced response to L-NAME in females when compared to males led those investigators to suggest that there is a greater production of NO in males kidneys and the renal bed was more responsive to chronic inhibition of NOS with L-NAME than females or that the males kidneys are more NO dependent than females (Reckelhoff, et al., 1998). Nevertheless, these studies provide further evidence

that EC dysfunction alone is not responsible for the discrepancies seen between normal and diabetic male and female rats renal conductance to L-NAME (Reckelhoff, et al., 1998). Instead, there is likely a combine dysfunction between sympathetic discharge and the synthesis/release of NO (Lacolley, et al., 1991) which can lead to the discrepancies observed among diabetic male and female rats.

Our observations of the blood flow dynamics in response to NOS inhibition in the superior mesenteric vascular bed of normal females and males are consistent with previous studies (Chakir, et al., 1996). Since, the ability of L-NAME to decrease diabetic females superior mesenteric conductance is compromised when compared to normal females, upon the administration of a NOS inhibitor, EC dysfunction might be greater among diabetic female rats (Nase, et al., 1996).

The results of this study are also consistent with a decreased vascular smooth muscle sensitivity to NO in diabetes which lead to a decrease in cGMP and consequently less vasodilation as depicted in Figure 39 (Wang, et al., 1993; Kamata, et al., 1989; Koltai, et al., 1997). The smooth muscle decreased sensitivity to NO was particularly true in the splachnic and skeletal muscle bed of diabetic animals when compared to normals. Two possibilities to the reduced response to NO in diabetes has been suggested: (1) that there is an abnormality/dysfunction in vascular smooth muscle sensitivity to NO in diabetic patients (Calver, et al., 1992) and/or (2) that endothelial dysfunction is responsible for the decreased responsiveness to SNP (Veves, et al., 1998).

Contrastingly, diabetic animals exhibited an increased sensitivity to NO in the renal bed. This observation is supported by several investigators who have demonstrated that local and systemic infusions of SNP lead to increase renal blood flow, renal

vasodilation and decreased renal vascular resistance (Costa e Forti, et al., 1998; Mesada, et al., 1981; and Lovell, et al., 1995). Diabetes is independently characterized by abnormal renal hemodynamics, vasodilation, pronounced glomerular hyperfusion and hyperfiltration (Ballermann, et al., 1984; Mattar, et al., 1996 and Tolins, et al., 1993). Therefore, the combination of the two observations suggest that the increase responsiveness to SNP in diabetic animals may add to the abnormal renal hemodynamics, and increase responsiveness to SNP in the renal bed.

The last factor that we believe contribute to diabetic vascular disease is alterations in sympathetic-mediated vascular tone (see Figure 39; Pete, et al., 1998; Hu, et al., 1997; Schultz-Klarr, et al., 1994; Dunbar, et al., 1991). Our studies clearly demonstrate an increased dependence on alpha adreneregic tone on the splachnic and skeletal muscle bed and a decrease dependence in the renal bed. A possible explanation for the increased sensitivity to prasozin in the iliac bed may be that the blood flow in skeletal muscle has a greater dependence on adrenergic regulation. Other investigators also support observations that skeletal muscle blood flow is indeed under tonic sympathetic constrictor tone especially at rest (Buckwalter, et al., 1997; Leech, et al., 1996). Like the skeletal muscle bed, the splanchnic bed also exhibited an increase in conductance following α_1-adrenergic blockade. This observation is also supported by studies using the *in situ* autoprefused superior mesenteric arterial bed of rats which suggest that α_1-adrenoceptors are strongly represented in this vascular bed (Nichols, et al., 1985; Piascik, et al., 1997). Since conflicting reports in the literature state that renal vasoconstriction is mediated by α_1-adrenoceptors (Piascik. et al., 1997; Hesse, et al., 1984; Elhawary, et al., 1992) or by α_2-adrenoceptors in conscious rats when boluses of norepinephrine were administered

into the kidney (Wolff, et al., 1989), the mild response to prazosin in the renal bed when compared to the skeletal muscle and the splanchnic bed is not as easy to consolidate. Nevertheless, the basal vascular tone in the kidney may not be mediated by α-adrenergic mechanism and mostly by an autoregulatory mechanism by other mediators (Hu, et al., 1997_b; Cupples, 1993; and Takenaka, 1994). Therefore, the mild response observed in the renal bed to prazosin when compared to the iliac and superior mesenteric bed may be consistent with the autoregulatory mechanism in the kidney.

In summary, it is clear that diabetic vascular disease have altered endothelial function and nitric oxide sensitivity in smooth muscle which may contribute to an imbalance that leads to enhanced adrenergic activity or dependence. This would promote altered vascular responses of the skeletal muscle, renal and splachnic vascular bed in diabetic individuals.

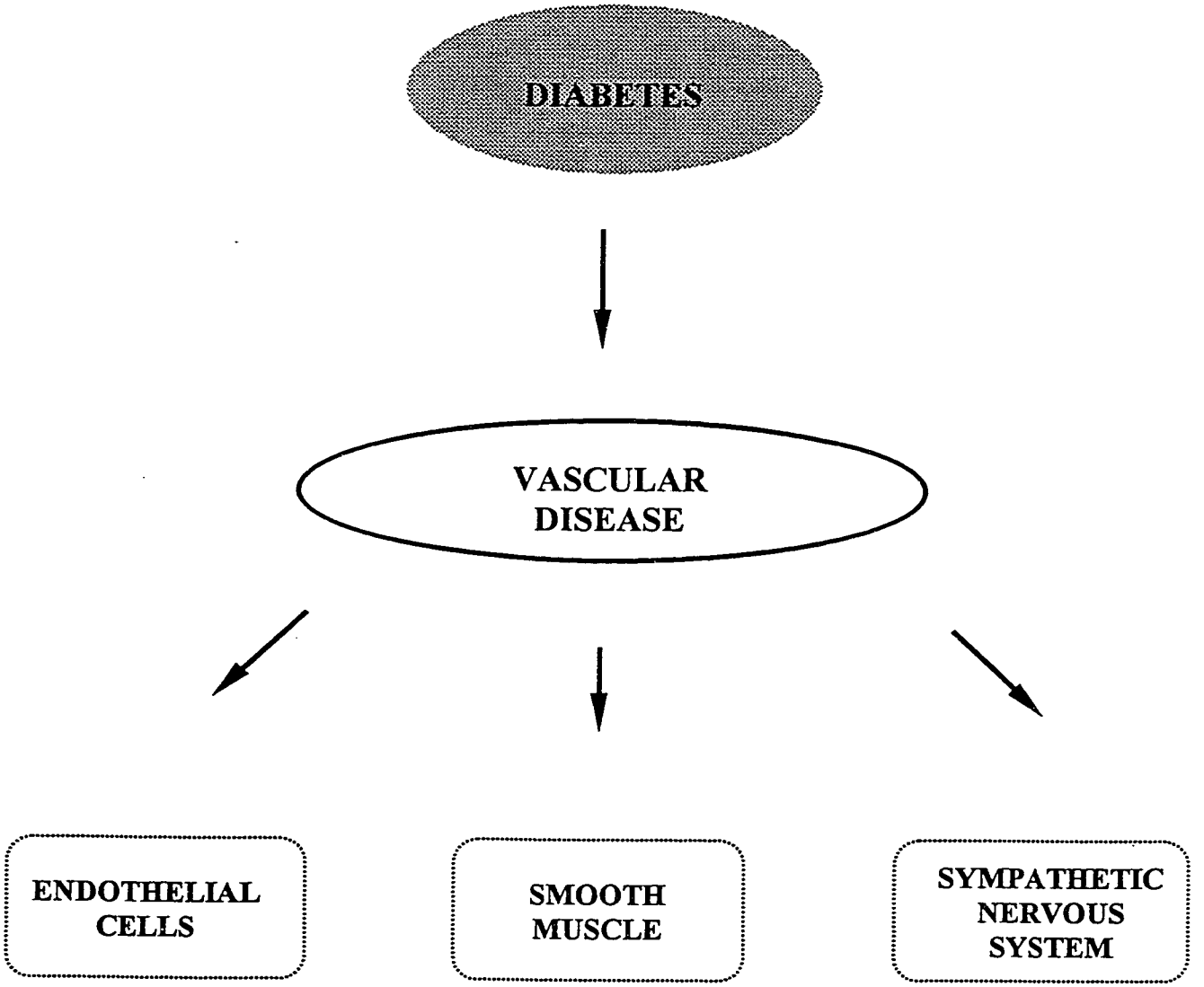

Figure 38. Diabetes Pathogenic Cascade

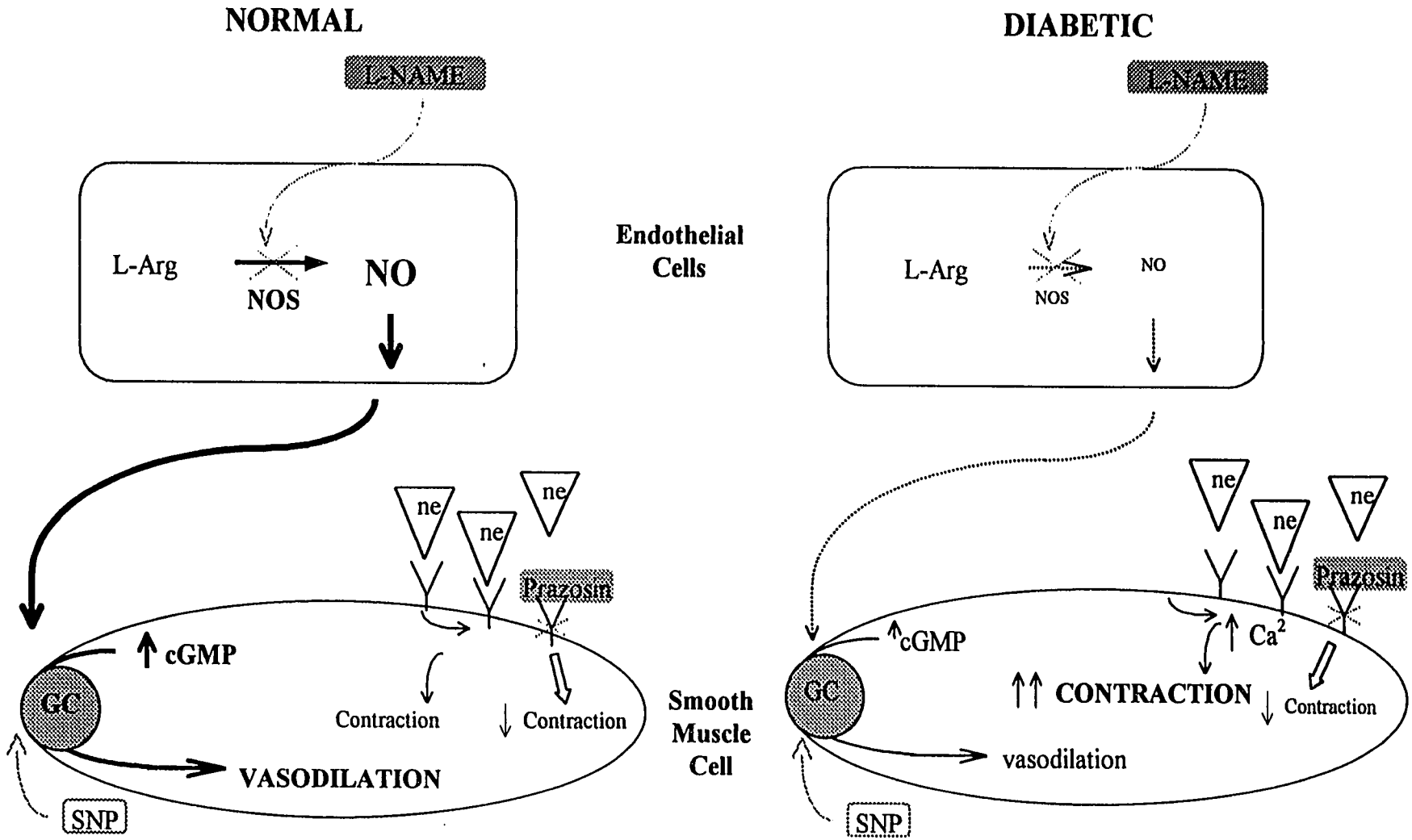

Figure 39. Schematic diagram of the pathogenesis of diabetic vascular disease. The normal nitric oxide cascade is depicted on the left.

GENERAL CONCLUSION

Systemic administration of L-NAME resulted in a significant increase in MAP in normal females and males. However, this response was significantly attenuated in diabetic animals and to a greater extent in diabetic females. HR was decreased in all groups. Blood flow and conductances were decreased in the iliac, renal and superior mesenteric vascular beds in normal rats. In diabetic animals, L-NAME decreased iliac bed conductance to a greater extent in diabetic males while the renal conductance was significantly increased in diabetic females. These observations suggest a decreased in NO synthesis or release from endothelial cells in diabetic animals leading to a decreased dependency on NO for cardiovascular control.

Intravenous infusion of SNP resulted in a dose-dependent decrease in blood pressure in normal and diabetic rats while HR was not significantly changed. Blood flow and vascular conductance were increased by SNP in normal and diabetic rats in a dose-dependent fashion. However, the responsiveness of diabetic animals was attenuated in the iliac and superior mesenteric and increased in the renal artery when compared to normals. Diabetic females exhibited an enhanced responsiveness to NO in the renal vascular bed. Thus, we propose that diabetes is associated with an abnormal vascular smooth muscle sensitivity to NO which leads to a decrease in cGMP and consequently less vasodilation in the splachnic and skeletal muscle bed in conjunction with abnormal renal hemodynamics.

Systemic administration of prazosin decreased MAP in normal and diabetic rats with no significant differences in HR. Conductance was increased in all three vascular beds in normal and diabetic rats with the greatest responsiveness in the iliac and superior

mesenteric arteries. Females seem to be more responsive than males in their dilatory response. Alpha-adrenergic tone was greatest in diabetic animals. These observations suggest that diabetes is linked to an increased adrenergic tone in the splachnic and skeletal muscle bed in both diabetic female and male rats. It is possible that the exacerbated adrenergic tone in these beds is a consequence of the altered endothelial and smooth muscle function in diabetes.

These studies provide insight into the role of the endothelium, the smooth muscle and the sympathetic nervous system in modulating cardiovascular and regulatory changes associated with diabetes and/or sex in whole body dynamics, specifically changes in heart and vascular reactivity. It also has significant implications in the understanding of local and overall cardiovascular responses in diabetes. Furthermore, it sheds some light in the rapid deterioration of females physiological/cardiovascular responses to diabetes.

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ABSTRACT

THE EFFECT OF SEX ON CARDIOVASCULAR DYNAMICS IN NORMAL AND DIABETIC RATS

by

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May, 1999

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CHAPTER II

In this study, we evaluated the effect of L-NAME, a NOS antagonist, on MAP, HR, and selective vascular flows in male and female normal and diabetic rats. Rats were made diabetic using streptozotocin (50 mg/kg, IV) and maintained for 5-6 weeks. Following anesthesia with urethane/α-chloralose, the femoral artery and vein were cannulated for recording and sampling and flow probes were placed on the iliac, renal and superior mesenteric arteries. A bolus infusion of L-NAME resulted in a rapid increase in MAP in normal females and males. However, in diabetic females and males this response was significantly lower and especially so in diabetic females. L-NAME decreased the conductance in all three vascular beds in normal rats. In diabetic animals, the response to L-NAME was decreased to a greater extent in the iliac bed of diabetic males while the renal conductance was actually increased in females. We concluded that diabetics decreased pressor response to NOS inhibition and the lack of response of the renal vessels in female diabetics may play a role in the increased renal pathology in diabetic females.

CHAPTER III

SNP (1-20 μg/kg) infusions resulted in a dose-dependent decrease in MAP in normal and diabetic rats. Reflex tachycardia was more prominent in diabetic males. The vascular conductance was increase in normal and diabetic rats in a dose-dependent fashion, however, the responsiveness was less in the iliac and superior mesenteric and increased in the renal arteries in diabetics when compared to normals. We concluded that diabetes is associated with an increased response to NO in the renal vessels and a decreased response in the iliac and superior mesenteric vessels in both females and males.

CHAPTER IV

Prazosin (4 mg/kg) administration decreased the MAP in normal and diabetic rats to a comparable degree. It increased the vascular conductance in all three vascular beds in normal and diabetic rats with the greater increase occurring in the iliac and superior mesenteric arteries. α-adrenergic tone was greatest in diabetic female and male rats. We concluded that decreased vascular flow in diabetes is associated with an increased adrenergic tone in the splachnic and skeletal muscle bed in both diabetic female and male rats.

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Abstracts:

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