ALTERATIONS IN SKELETAL MUSCLE ARTERIOLAR VASOREACTIVITY DURING THE PROGRESSION OF TYPE 2 DIABETES IN THE ZUCKER DIABETIC FATTY RAT

A Dissertation

by

LISA ANNMARIE LESNIEWSKI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2004

Major Subject: Kinesiology

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ABSTRACT

Alterations in Skeletal Muscle Arteriolar Vasoreactivity During the Progression of Type 2 Diabetes in the Zucker Diabetic Fatty Rat. (May 2004)

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Altered vasoreactivity and mechanical properties of skeletal muscle arterioles could impact peripheral insulin resistance and hypertension observed in type 2 diabetes. The purpose was to determine if increased vasoconstrictor reactivity, decreased vasodilator reactivity and alterations in the structural properties of 1A arterioles from both high-oxidative and low-oxidative glycolytic skeletal muscles is present during prediabetes as well as acute and chronic diabetes, and to determine if this dysfunction precedes the development of elevated arterial pressure in type 2 diabetes. Zucker Diabetic Fatty (ZDF) rats and lean age-matched controls were studied at 7 (prediabetes), 13 (acute diabetes) and 20 (chronic diabetes) weeks of age. Following measurement of arterial pressure, vasoconstrictor responsiveness to norepinephrine (NE), potassium chloride (KCl), and increasing intraluminal pressure (MYO), vasodilator responsiveness to acetylcholine (ACh), sodium nitroprusside (SNP) and intraluminal flow and passive mechanical properties were examined in arterioles from soleus and gastrocnemius muscles. Vasoconstriction to NE was enhanced in gastrocnemius muscle arterioles during prediabetes and preceded elevated arterial pressure. Alterations in the passive mechanical properties of arterioles from both muscles

were observed throughout the progression of diabetes. Flow-induced vasodilation was decreased in the high-oxidative muscle arterioles during acute diabetes, and was coincident with the emergence of elevated arterial pressure. During chronic diabetes, vasodilation to ACh and flow were reduced in soleus muscle arterioles. The reduced vasodilation to ACh was the result of a loss of NO. Although the vasodilator capacity of low-oxidative glycolytic skeletal muscle arterioles was not diminished throughout the progression of diabetes, the contribution of NO to ACh-induced dilation was lost in the prediabetic and acute diabetic rats. The data demonstrate that alterations in both the vasoconstrictor and passive properties of low-oxidative glycolytic skeletal muscle arterioles are present during prediabetes, and precede the development of type 2 diabetes, and that although endothelial dysfunction does not become manifest in these skeletal muscle arterioles, alterations in the signaling mechanisms to achieve that vasodilation are present in prediabetes. Moreover, overt type 2 diabetes results in endothelial dysfunction and altered mechanical properties in high-oxidative skeletal muscle arterioles.

DEDICATION

To the memory of my father

Jerome M. Lesniewski.

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CHAPTER I

INTRODUCTION

Diabetes Mellitus, the sixth leading cause of death in the US, accounted for more than 193,000 deaths in 1997. With a worldwide prevalence that is steadily increasing, a reported 151 million cases worldwide in the year 2000 is projected to increase in the next decade by 46%. Of these individuals, approximately 90% are type 2 diabetics. In the US alone, 16 million people are already affected, with an estimated 800,000 new cases each year. This number is expected to increase to 23 million within 10 years. Alarmingly, another 200 million people worldwide suffer from some degree of glucose intolerance, and 40 % of these individuals are expected to progress to frank type 2 diabetes within 5-10 years. Moreover, although once considered a disease of the middle aged and aged, its prevalence is increasing among our nation's youth, mirroring the increase in obesity in that population. This will undoubtedly place an enormous burden on our health care systems. At present, the cost of diabetes-related health care has reached $105 billion annually, which accounts for one tenth of all US health care dollars and one quarter of all Medicare dollars spent.

With the advent of insulin therapy, diabetes is no longer a fatal ketotic disease, but rather leads to a number of serious morbidities that often develop slowly and insidiously over the duration of the disease. The cardiovascular complications of diabetes are now the leading cause of morbidity and mortality in patients, and it is

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This dissertation follows the style of the *American Journal of Physiology.*

becoming evident that these complications have an onset much earlier than previously assumed. The macrovascular complications of diabetes have been associated with hypertension, atherosclerotic disease, myocardial infarction and stroke. Microvascular complications are associated with retinopathy, neuropathy, and nephropathy, making diabetes the leading cause of blindness in adults aged 20-74, lower limb amputations, and end-stage renal failure. The recognition that at least 40% of type 2 diabetics present with hypertension at the time of diagnosis led investigators to postulate that the cardiovascular complications of the disease, normally thought to be the consequence of prolonged disease duration, are actually beginning prior to the onset of overt diabetes during the preceding period of subclinical hyperglycemia and insulin resistance, termed prediabetes. Moreover, vascular dysfunction may be both the cause and the consequence of insulin resistance as blood flow to insulin sensitive tissues, and subsequent insulin delivery, limits glucose uptake. Thus, it is necessary to examine vascular function not only after the development of overt diabetes but also during this prediabetic period.

The purpose of this study was to examine alterations in microvascular function during the progression of type 2 diabetes from prediabetes to acute and chronic type 2 diabetes. As the skeletal muscle is both an insulin-sensitive tissue and the largest sink for plasma glucose, dysfunction in this circulation can have profound implications on glucose uptake. In addition, the skeletal muscle microvasculature is a large contributor to whole body vascular resistance, and alterations in its function could contribute to the hypertension often seen in prediabetic and type 2 diabetic individuals. The skeletal muscle is not

homogeneous and can be characterized into several types, including high-oxidative and low-oxidative glycolytic. These muscle fiber types differ in regards to their contractile characteristics, as well as blood flow, glucose uptake and insulin sensitivity profiles. Differential alterations in the function of arterioles that supply blood to low-oxidative glycolytic and high-oxidative muscles have been found in other circumstances, e.g. normal physiological aging, and if present in type 2 diabetes, could profoundly effect delivery of glucose not only to the skeletal muscle as a whole, but also its distribution within the muscle mass. To examine this possibility, we studied the structure and function of resistance arteries that supply both predominately high-oxidative and predominately low-oxidative glycolytic locomotor skeletal muscles.

The specific aims of this study were to test the hypotheses that, 1) type 2 diabetes will enhance myogenic responsiveness of resistance arterioles isolated from muscles composed of different fiber types, 2) type 2 diabetes will increase the passive stiffness of these skeletal muscle resistance arterioles, 3) type 2 diabetes increases responsiveness of skeletal muscle resistance arterioles to vasoconstrictor agonists, 4) type 2 diabetes will attenuate skeletal muscle arteriolar responsiveness to either endothelium-dependent or endothelium-independent vasodilator stimuli, and 5) that these changes will be present during the prediabetic state as well as during acute and chronic type 2 diabetes.

CHAPTER II

REVIEW OF LITERATURE

2.1 Discovery and Classification of Diabetes

Diabetes mellitus has been recognized for centuries; however until 1776 it was diagnosed solely on the clinical manifestations of thirst, excessive urination, hunger, weight loss, and muscle weakness (66). An ancient physician, Arateaus of Cappadocia, first coined the term 'diabetes' from the Greek for 'syphon' owing to the excessive urine output of afflicted patients. The sweet nature of the urine of diabetic patients was described in ancient times as well, specifically, diabetes was termed 'sweet pissing disease' in ancient Hindu texts, although the substance in the urine that led to the sweet taste was not elucidated until much later. Moreover, in the 17th century, Thomas Willis again made this same observation (i.e. sweet tasting urine), and coined the term 'diabetes mellitus', from the Greek for honey (41). However, sugar was not revealed to be the source of the sweetness until 1776 when Dobson evaporated the urine of diabetic patients and found a residue that tasted like brown sugar. Along with this discovery, Dobson also identified the presence of excess blood sugar in patients (41, 66). Although the sugar was not identified as glucose until 1815, the discovery of sugar in the urine allowed for a means of clinically diagnosing diabetes. However, even with a means of biochemically diagnosing the disease, the mechanisms and/or organs responsible for the disease remained unknown.

The clinical manifestation of polyuria first led researchers to believe that the kidney was the seat of the disorder. However, at the end of the 18th century Rollo demonstrated that the content of the diet influenced the amount of urinary sugar

excreted, then focusing attention to the gastrointestinal system (66). However, the liver emerged as the most likely candidate, as Claude Bernard had recently described the liver's glycogen stores and role in glucose excretion, leading investigators to surmise that diabetes was the result of disordered liver glucose excretion. This contention was further supported by the work of Bernard and Schiff, who failed to induce diabetes following degeneration of the pancreas following occlusion of its secretory duct.

In 1889, Minkowski and Von Mering finally determined the pancreas to be the site of the disorder, when they were able to induce diabetes in a pancreatectomized dog. A decade later, a secretion from the Islets of Langerhans was determined to be the anti-diabetogenic agent (67). This secretion was not described as insulin until 1921 by Fredrick Banting, Charles Best, J.J.R. Macleod and J. Bertram Collip, for which Banting and Macleod were awarded the Nobel Prize less than three years later (67). In the following year, the first successful insulin treatment of a diabetic human began on a 14 year old patient, Leonard Thompson. Before insulin therapy, the life expectancy of a type I diabetic patient was less than 12 months, with insulin therapy, Thompson lived to age 59 when he died of cancer (67). However, it became apparent that insulin therapy was not effective in all diabetic patients, suggesting possible subgroups of the disease.

During the time of the elucidation of the pancreas as the diseased organ, some physicians began to note differences in the etiology of the disease among their patients. In 1875, Bouchardat compiled findings published over 35 years which described at least two forms of diabetes, one in the young, presenting acutely with

dramatic weight loss, and one in older adults who tended to be overweight. These findings were later confirmed by Lancereauex in 1877 and 1880 (66). However, after the discovery of insulin and the resultant introduction of insulin therapy, these observations were largely ignored and diabetes was treated as a single disorder.

Attention finally returned to the classifications first introduced by Bouchardat and Lancereauex in the twentieth century. Himsworth, in 1935, proposed an arbitrary classification, made by age at the onset of diabetes, could distinguish between different underlying pathophysiologies. Specifically, younger and thinner patients were more sensitive to insulin treatment then were their older, more obese counterparts. In the 1950's type 1 and type 2 diabetes were finally recognized as the distinct disorders that Himsworth had described by demonstrating that serum from type 1 diabetics behaved as though it contained no insulin, in contrast to serum from type 2 diabetic subjects which behaved as if it did. Thus, the current view of diabetes is as a syndrome of chronic nontransient hyperglycemia with or without glycosuria, most commonly of three origins termed type 1, type 2, and gestational diabetes (66).

Classification of the subtypes of diabetes is an ongoing process. The World Health Organization released a report in 1999 on the diagnosis and classification of diabetes, in which it defines not only the aforementioned diabetes subtypes, but also defines impaired glucose regulation, an intermediate state of glucose regulation between normal and pathological (1). Type 1 diabetes includes cases in which the primary insult is pancreatic beta-cell failure, with affected individuals prone to ketoacidosis. Type 2 diabetes is the result of systemic insulin resistance and defects in insulin secretion leading to a relative insulin deficiency. The latter form of diabetes

may go undiagnosed for many years since the level of hyperglycemia is not enough to produce the noticeable symptoms of diabetes. Moreover, ketoacidosis is rare in this type of diabetes and insulin treatment is generally not required. Along with gestational diabetes, these distinct forms represent the three major classifications of diabetes. However, a stage in the development of type 2 diabetes has also been defined, that of impaired glucose regulation.

Impaired glucose regulation, although not a separate class of diabetes *per se*, represents a stage in the natural progression of type 2 diabetes, including both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), two distinct forms of disordered glucose metabolism, the former during fasting conditions and the latter post-prandially. In addition, IGT is often associated with the metabolic syndrome which is a cluster of disorders including hypertension, central obesity and dyslipidemia with or without hyperglycemia. Insulin resistance appears to be a common factor in the syndrome, although the mechanisms for this resistance remain to be elucidated.

The metabolic syndrome includes compensatory hyperinsulinemia concurrent with transient postprandial hyperglycemia or subclinical fasting hyperglycemia. This hyperinsulinemia is the result of the body's attempt to overcome this peripheral insulin resistance. Furthermore, features of the metabolic syndrome can be present for up to a decade prior to the development of glycemic disorders, and are associated with cardiovascular disease risk. Individuals with the metabolic syndrome are at high risk of future diabetes (1), leading to the term prediabetes (19). Following the development of overt diabetes plasma insulin will decline to near normal values

despite the maintenance of fasting hyperglycemia (28). In severely diabetic individuals, those with plasma glucose exceeding 10-11 mM, insolinopenia will develop. Therefore, the prediabetic state itself has been shown to be associated with increased cardiovascular disease risk, and has become a point of interest in the chronology of vascular dysfunction associated with type 2 diabetes.

2.2 Epidemiology of Type 2 Diabetes

Diabetes mellitus accounted for more than 193,000 deaths in the US in 1997, and this may be an underestimation of diabetes-related mortality, as many deaths related to diabetic complications are attributed to other causes, e.g. cardiovascular disease (80). In the year 2000, diabetes affected approximately 151 million worldwide, with a projected increase of 46% by the year 2010. Of these individuals, 90% are type 2 diabetics. In addition, at least 200 million people worldwide have some degree of impaired glucose tolerance, and an estimated 40% of these individuals will progress to overt diabetes within 5-10 years (127). In the US alone, 15 million suffer from type 2 diabetes, with an estimated 20 million more suffering from some degree of glucose intolerance (81). Moreover, the prevalence of type 2 diabetes among the youth of the US is increasing. For example, Pinhas-Hamiel and colleagues (85) reported a 12% increase in the diagnosis of type 2 diabetes among new diabetes diagnoses in patients aged 10-19 between 1992-1994. This increase in type 2 diabetes among our nation's younth appears to parallel the national increase in obesity in children (107).

The presence of diabetes increases cardiovascular disease risk 2 to 6-fold over that in normal healthy adults (8, 10, 43, 54), with 40% of type 2 diabetics presenting with concurrent hypertension (114). Moreover, patients with the metabolic syndrome, individuals with at least three of the following conditions, abdominal obesity, hypertension, atherogenic dyslipidemia, and high fasting glucose (58) are at a significantly greater risk of cardiovascular disease than normal healthy adults, and an estimated 40% of these patients will progress to overt type 2 diabetes within 5-10 years (127).

Cardiovascular complications are now the leading cause of the morbidity and mortality associated with diabetes (4). The macrovascular complications of diabetes result in atherosclerotic disease, hypertension, increased risk of myocardial infarction and stroke (4, 43). Dysfunction of the microvasculature resulting from diabetes is responsible for a number of co-morbidities associated with prolonged diabetes, e.g. retinopathy, neuropathy, and nephropathy. As a result, diabetes has become the leading cause of blindness in adults aged 20-74 (31), lower limb amputations, and accounts for 40% of all new cases of end stage renal failure (91). All of these complications pose a significant burden on the health care system with the cost of diabetes-related health care reaching $105 billion dollars annually (80).

It is becoming clear that the cardiovascular complications normally associated with advanced diabetes are beginning prior to the diagnosis of overt type 2 diabetes and are related to long term impaired glucose tolerance and hyperinsulinemia related to a prediabetic dysfunction in glucose metabolism (45). Insulin resistance in the absence of frank diabetes is itself a significant risk factor

for cardiovascular disease. For example, the metabolic syndrome is associated with more than a 2-fold increase in the risk of microvascular disease and myocardial infarction (8, 10, 43, 54). Hyperinsulinemia itself could be the cause of hypertension seen prior to frank diabetes. Elevated plasma insulin concentrations can alter both the renal sodium reabsorption leading to an increase in vascular volume, and can also increase sympathetic nerve activity (81). In addition, hyperinsulinemia has been implicated in the development of hypertension by activition of ion channels, increasing cellular calcium concentrations and stimulating production of growth factors (23). As a result, attention is now being given to vascular function not only after the development of diabetes but also during prediabetes.

2.3 Insulin Action

Insulin acts as an anabolic hormone, stimulating protein synthesis and the uptake of glucose, triglycerides, and amino acids into tissues, but also acts on vascular tissues as a vasodilator via nitric oxide and has anticoagulent effects on the vascular endothelium. The vascular effects of euglycemic insulin infusion are time and dose dependent. Insulin infusion first induces an increase in glucose extraction that lasts approximately 60 minutes. However, if insulin infusion is continued, glucose extraction plateaus, whereas blood flow increases continuously in response to insulin infusion. The flow response to insulin infusion is dependent on the particular vascular bed examined. Muscles composed primarily of highly-oxidative type I muscle fibers display a greater increase in blood flow to insulin infusion than

do muscles composed primarily of type IIa or IIb fibers, and are therefore considered insulin sensitive (123).

Insulin resistant non-diabetic subjects are at a higher risk of cardiovascular disease than are subjects with impaired beta cell function but normal insulin sensitivity (44). This relationship between insulin resistance and macroangiopathy could be responsible for the significant cardiovascular disease often present at the time of diagnosis in type 2 diabetic patients. A reduction in insulin signaling through particular transduction pathways will result in an impaired peripheral action, whereas the elevated circulating insulin concentrations may also result in an enhanced hormonal effect (25). The net effect of these alterations on the vasculature may be a decrease in nitric oxide synthase activity in insulin resistant states and a subsequent loss of the anti-artherogenic effects of NO (2), as well as an enhanced production of the procoagulent, plasminogen activator inhibitor 1 (PAI-1) (16).

Insulin also elevates muscle sympathetic nerve activity that induces vasoconstriction via stimulation of the α-adrenergic receptors. In addition, insulin will stimulate the secretion of endothelin-1 from the vascular endothelium, a potent vasoconstrictor (104). Thus, insulin has a dual effect on the vasculature, vasodilation via increases in NO and the Na^+-K^+ ATPase and vasoconstriction via sympathetic nerve activity and endothelin-1 production. At normal physiological insulin concentrations the net effect of insulin is vasodilation in skeletal muscle (50). Clearly, alterations in tissue sensitivity to insulin or insulin availability in the prediabetic state could have dramatic effects on vascular structure and function

even before true diabetes is manifest. The two major defects in type 2 diabetes are impaired insulin sensitivity and secretion. Impaired secretion may be biphasic, such that IGT induces an increased insulin secretion and subsequent hyperinsulinemia and increased circulating preproinsulin and c-peptide, all of which may affect vascular function independent of the hyperglycemia that will later accompany frank diabetes.

Insulin's vasodilator action is blunted in diabetic and insulin resistant states independent of frank endothelial dysfunction (60). The diabetic state appears to predispose an individual to endothelial dysfunction. The insulin resistance that precedes frank diabetes in type 2 individuals is associated with impaired insulin-induced dilation. However, kinetic studies of insulin action point to a rate-limiting step in the pathway that precedes insulin binding to the receptor, and may reflect a defect in transendothelial transport (104). Impaired endothelium-dependent dilation in metabolically important vascular beds such as skeletal muscle and adipose tissue may be both cause and consequence of insulin-resistance (60). However, at present, endothelium-dependent vasodilation has not been studied adequately in Type 2 diabetes and prediabetes (104) and as a result, a better description of the progression of cardiovascular complications prior to frank diabetes is warranted.

2.4. Blood Flow and Glucose Uptake

The skeletal muscle is the largest sink for plasma glucose with approximately 50-60% of an oral glucose load being cleared by the skeletal muscle.

Predominately high-oxidative skeletal muscle has a higher rate of glucose uptake than low-oxidative glycolytic skeletal muscle, and substrate delivery to the tissue, e.g. blood flow to the muscle, is a primary determinant of the quantity of glucose cleared from the blood. Although non-insulin mediated skeletal muscle glucose uptake accounts for approximately 75-85% of the total rate of glucose utilization, under conditions of elevated plasma glucose, as occurs post-prandially, greater than 80% of this additional glucose is cleared from the plasma via an insulin-dependent mechanism (121). Within a given skeletal muscle, rates of glucose uptake differ in a fiber type specific manner, with high rates of 2-deoxyglucose uptake being found in the deep portion of the gastrocnemius muscle, consisting of high-oxidative muscle fibers and a low rate of glucose uptake in both the middle and superficial portions which consist primarily of low-oxidative glycolytic muscle fibers (47). This difference in glucose utilization may be influenced by the distribution of blood within the muscle.

The microvasculature is the network of arteries in the vascular tree that are responsible for the distribution of blood flow between and within tissues, as well as for the majority of systemic vascular resistance. During resting conditions, approximately half of the skeletal muscle is blood-perfused, with the other half of the capillaries plasma filled (96). Capillary perfusion is determined by the pressure gradient across the capillary bed, which is itself a function of blood flow and precapillary resistance determined at the level of the first, second, and third-order arterioles (39, 68, 108). Vascular tone in the resistance arterioles will determine which vascular beds are perfused with blood. *In vivo*, insulin stimulated glucose

uptake is accompanied by an increase in blood flow to the splanchnic and other peripheral circulations (121), with insulin playing an active role in this increase in glucose utilization by inducing peripheral vasodilation (8, 86, 121).

Baron (8) reported an attenuated whole leg blood flow response to systemic insulin infusion in insulin resistant and type 2 diabetic individuals resulting in a 30% reduction in glucose utilization. In addition to reductions in total blood flow, alterations in the distribution of flow between areas of primarily high-oxidative or low-oxidative glycolytic skeletal muscle may affect glucose utilization. Such differential changes in microvascular reactivity from predominately high-oxidative or low-oxidative glycolytic locomotor skeletal muscles in aged Fisher rats (77) have previously been described. It is possible that similar changes in reactivity between high-oxidative or low-oxidative glycolytic muscles may also exist in type 2 diabetic animals. However, no such comparisons have been made in insulin resistance and diabetes. If the resistance arterioles of high-oxidative or low-oxidative glycolytic skeletal muscles are also differentially affected by hyperinsulinemia, insulin resistance and hyperglycemia, as occurs with aging, then these alterations in vascular function may alter the normal distribution of the blood flow between glycolytic or oxidative fibers within or between skeletal muscles and result in diminished glucose clearance.

Total peripheral resistance may also be affected by altered reactivity of specific skeletal muscle vascular beds. Peripheral resistance is determined, at least in part, by endothelial NO production via its modulation of vasoconstrictor tone at the level of the resistance arterioles (42). Even at rest, the rat hindlimb skeletal

muscle receives approximate 15-20% of the total cardiac output and that fraction increases to approximately 90% with exercise (42). Alterations in the balance of vasoconstrictor and vasodilator function in skeletal muscle arterioles in the insulin resistant state or in diabetes could increase total peripheral vascular resistance and contribute to the hypertension often associated with prediabetes and type 2 diabetes.

2.5 Proposed Cellular Mechanisms of Vascular Complications

Intracellular hyperglycemia leads to increases in blood flow and vascular permeability due to changes in the balance between the activities of vasoconstrictors and vasodilators and alterations in matrix components (13). Insulin resistance leads to pathway specific alterations such that the vasodilator actions of insulin are lost through a specific dysfunction of the phosphyatidylinositol-3-OH pathway. However, a concurrent increase in the insulin-induced activation of the mitogen-activated protein kinase pathway results in an increase in vascular smooth muscle proliferation and a pro-coagulent state (13). Four major hypotheses have emerged to explain vascular dysfunction in diabetes, including increased flux through the polyol pathway, increased advanced glycated endproduct (AGE) formation, increased protein kinase C activation (PKC), and increased hexosamine pathway flux (13).

Hyperglycemia itself can have injurious effects on tissues, particularly on those that are not dependent on insulin for glucose uptake. Tissues lacking sensitivity to insulin action are unable to downregulate glucose entry in response to

increases in extracellular concentrations. Transient postprandial hyperglycemic episodes in prediabetic individuals may contribute to the early onset of diabetic complications in type 2 diabetic individuals. These short term elevations in glycemia have been shown to increase flux through alternate biochemical pathways of glucose disposal such as the polyol, hexosamine, and diacylglycerol pathways resulting in an increase in oxidative stress. Further, a sustained increase in blood glucose concentrations will additionally have a cumulative effect on long-lived macromolecules, e.g. the non-enzymatic glycosylation of extracellular matrix proteins (100).

The polyol pathway converts glucose to sorbitol via the enzyme aldose reductase (AR) when glucose is in excess of glycolytic needs. A consequence of an increased flux through the polyol pathway is the generation of an excess of NADH which occurs when sorbitol is converted to fructose via sorbitol dehydrogenase. The resultant increase in the NADH to NAD^+ ratio mimics ischemic changes and has been termed 'hyperglycemic pseudohypoxia' or 'reductive stress'(103). Moreover, the increase in NADH drives the generation of glyceraldehydes-3-phosphate (GA-3-P), a precursor to the AGE forming methylglyoxal (MGO) and diacylglycerol (DAG), an endogenous activator of PKC via glyceraldehydes-3-phosphate dehydrogenase (GAPDH). This increase in glucose flux through the polyol pathway is accompanied by a depletion of myoinositol, altered phosphoinositide turnover, a decrease in intracellular calcium concentrations, and a decrease in the activity of the Na^+-K^+ ATPase, all of which may contribute to cardiovascular complications (103). Alterations in the redox potential may be

responsible for an increase in free radical generation, AGE accumulation, PKC activation, and hexosamine formation.

Hexosamine formation occurs when fructose-6-phosphate is converted to glucosamine-6-phosphate via glucosamine-fructose-amidotransferase (GFAT). Increased concentrations of hexosamines may mediate the vascular damage by hyperglycemia as well as induce insulin resistance, termed glucose toxicity. Increased hexosamine concentrations enhance PKC translocation, induce glycosylation, and can activate growth factors like TGF-β1 and PAI-1. Moreover, they may inhibit PKC activated GLUT4 translocation, thereby reducing glucose transport and utilization.

The formation of advanced glycated endproducts (AGEs), which is correlated with the level of glycemic control, can also alter the structure and function of membrane proteins. AGEs are formed when reducing sugars react non-enzymatically with amino groups of proteins. Through a series of reactions, Schiff bases and Amadori products form AGEs. This process, discovered in the 1900's, is termed the Maillard reaction. AGE formation is catalyzed by transitional metals and is inhibited by reducing compounds (100). Reactive intermediates form during the Maillard reaction and are implicated as a mechanism of diabetes induced damage. The formation of these intermediates is accomplished by non-oxidative rearrangement and hydrolysis of Amadori products, examples of which are 3-deoxyglucosone (3-DG) and methylglyoxal (MGO). These products can be further modified into oxidative AGEs leading to carbonyl stress, which may be involved in the accelerated vascular damage seen in diabetes (100). AGE-related damage to

vascular tissues may be more dependent on the rate of accumulation than the concentration of AGE, as suggested by a lower concentration of AGE in insulin-dependent diabetic subjects despite microvascular complications when compared to healthy older non-diabetic patients (100).

AGEs were once believed to simply tag senescent proteins for degradation, but recently it has been discovered that AGEs activate signaling pathways that lead to the synthesis and release of growth factors and cytokines that may both initiate tissue turnover and repair, but may also contribute to diabetic vascular complications (111, Bierhaus, 1998 #185). For example, glycated membrane proteins are more highly cross-linked resulting in a decrease in solubility and proteolytic turnover (30).

AGE modified proteins on the ECM will decrease membrane elasticity, increase wall thickness, and enhance rigidity, and can result in a reduction in luminal diameter (9). AGEs on ECM proteins can interfere with endothelial function in a number of ways. AGEs inhibit normal network formation in type IV collagen, decrease proteoglycan binding by vitronectin and laminin, quench NO during passage from endothelium to smooth muscle layer, bind and activate cell surface receptors, increase membrane permeability, and induce tumor necrosis factor-alpha (TNF-α). TNF-α may itself induce insulin resistance in muscle and adipose tissues by impairing autophosphorylation of insulin-receptor and insulin receptor substrate. In addition, AGEs also induce the expression of endothelin-1, a potent endothelium-derived vasoconstrictor (9).

Diabetic vascular disease also appears to result in an unregulated process of

tissue remodeling sustained by an abnormal expression of factors regulating tissue homeostasis of both resident and non-resident vascular cells (25). During normal vascular remodeling, adhesion molecules provide communication between the lumen and vessel wall in both directions. The endothelial barrier protects against passage of macromolecules from the lumen to the interstitium and comes into contact with circulating elements such as leuckocytes. Contractile elements respond to perfusion pressure and oxygen demand to establish vessel tone, and endothelial cell interactions with adhesion molecules and non-resident cells establish balance between pro- and anticoagulent states to prevent thrombus formation. With diabetes, vascular remodeling is perturbed. The cell-matrix composition of the vascular wall is altered such that there is an enhanced deposition of the vascular basement membrane resulting from increased synthesis(38, 88) and decreased degradation of matrix proteins (79, 90). However, changes in the cell compartment are more complex.

Vascular cellularity may either increase due to a proliferation of vascular smooth muscle cells or the inflow of non-resident cells, or decrease as the result of a decrease in proliferation or an increase in apoptosis. Moreover, cell-to-cell, and cell-to-matrix communication is disturbed. An abnormal pattern of contact molecule expression has been observed in diabetic vascular tissues. Increases in integrin expression have been reported leading to an upregulation of fibronectin and enhanced endothelial cell-matrix attachments(92). Similarly, an abnormal pattern of adhesion molecules leads to increased leukocyte rolling and adhesion, which allows for enhanced deposition of non-resident macromolecules into the

vessel wall (74).

Contributing to this increase in macromolecule deposition is a loss of the barrier function of the vascular endothelium. Barrier function loss is the result of basement membrane thickening, hemodynamic changes, endothelial dysfunction, and alterations in the plasma. Functional alterations can result from these changes in vessel structure. Basement membrane thickening is a hallmark of both type 1 and type 2 diabetes and is positively correlated with disease duration (30). Hyperglycemia, hyperinsulinemia and cytokines have been shown to result in an increase in the mRNA expression for laminin, fibronectin and type IV collagen. Yu et al. (124) reported an increase in type IV collagen, fibronectin and laminin following 2 weeks of streptozotocin-induced Type 1 diabetes in cremaster and femoral arteries. Increased shear stress and pressure have also been shown to increase production of these membrane components and may be present concomitantly in some diabetic patients. Increases in these extracellular matrix proteins could reduce vascular elasticity, alter membrane permeability, interfere with normal cell-matrix interactions, and cause the endothelium to lose its antithrombotic, profibrinolytic effect (30, Stehouwer, 1997 #191), all of which may contribute to a stiffer, less compliant vessel.

2.6 Complications of Diabetes

Diabetic vascular complications affect a number of tissues beds and result in characteristic features unique to the tissues involved. Moreover, the incidence of these complications varies among tissue beds and the commonly affected tissues

appear to vary by the type of diabetes. For example, among type I diabetics approximately 30% will develop nephropathy, whereas 80-90% will develop some degree of retinopathy (25). Of the 90% of diabetics who will develop background retinopathy, 8-26% will develop proliferative retinopathy leading to vessel hemorrhages, ischemia, and infarctions. Both retinopathy and nephropathy are believed to be the result of dysfunction of the microvasculature leading to these organs. However, among type 2 diabetics, macrovascular disease is more common than among type 1 diabetic patients, and is manifest as a high incidence of hypertension in this population (25). Complications may develop independent of one another, such that 17% and 25% of diabetics will present with cardiovascular disease or retinopathy alone, respectively. However, in those patients with diabetic nephropathy, some degree of cardiovascular disease and retinopathy is usually present as well (25). There also appears to be a time dependence related to the development of these complications. For example, in type 2 diabetics, macroangiopathy is often present at the time of diagnosis whereas microangiopathy is believed to develop as a late consequence of the disease. Moreover, the risk of retinopathy increases linearly with disease duration while the risk of nephropathy peaks at 15 years of disease and then declines (25). Cardiovascular complications of overt diabetes have been extensively studied in type 1 diabetic patients and animal models of the disease. Although late complications of type 1 and type 2 diabetes vary, similar alterations in vascular structure and function to those seen in type 1 diabetes may contribute to vascular dysfunction in type 2 diabetes and may become manifest prior to the onset of frank diabetes.

Following development of overt diabetes, structural and functional changes in the vasculature occur in a tissue-dependent manner. The majority of investigations examined the effects of type I diabetes on the morphology and vasoreactivity of both macro- and microvessels, and the results may not be consistent with changes found with type 2 diabetes. The hemodynamic hypothesis put forth by Parving et al. (83) attempts to define the sequelae of changes leading to diabetic vascular complications. This hypothesis implicates hyperglycemia-mediated increases in capillary perfusion pressure in the overproduction of basement membrane components as well as shear stress induced damage to the endothelium, leading to a stiffening of the vessels and altered endothelial cell function that results in a reduction in the maximal hyperemic response in diabetic vessels. This is an attractive hypothesis that has been supported in the type 1 diabetes literature, however increased capillary pressure is not a consequence of the type 2 diabetic state, and thus, does not appear to describe the progression of the disease in type 2 diabetes (115). However, there is evidence that the ultimate result of type 2 diabetes on the vasculature does indeed appear to be a reduction in the maximal hyperemic response, consistent with the findings in type 1 diabetes.

Less is known about possible vascular alterations induced by type 2 diabetes and the time course of these putative changes. Type 2 diabetes related complications result from a variety of hormonal and metabolic derangements. Hyperinsulinemia, hyperglycemia and insulin resistance may coexist and contribute to vascular dysfunction. In addition to the multiple abnormalities that exist in type 2 diabetes, the progression of the disease is slow and often silent, resulting in long

periods of impaired glucose tolerance caused by a history of insulin resistance. Structural and functional alterations in the microvasculature may already be present during prediabetes. Microalbuminuria seen during prediabetes is suggestive of structural alterations in the basement membrane and endothelium, leading to increased permeability (25, 120). However, little information is available regarding structural alterations during this phase in the development of diabetes. An increase in muscle capillarization has been reported in rats exposed to 1 week of hyperinsulinemia and is most pronounced in low-oxidative glycolytic muscles of IGT patients (121). This increase in capillary density may be a transient adaptation to inadequate substrate availability, as capillary rarefaction has been reported to be the ultimate consequence of the diabetic state (120). Similarly, little functional data is available from prediabetic animals and patients. Due to methodological limitations, the information available is often obtained from large vessels and vascular beds that may not be representative of other areas, such as the cutaneous circulation (120). In normotensive type 2 diabetics, capillary pressures are normal despite an early profound decrease in cutaneous vasodilator capacity present at diagnosis (115). Few estimates of microvascular dysfunction are available in type 2 diabetic patients. In contrast to studies in type 1 diabetes, capillary pressure and filtration are normal in type 2 diabetics despite a profound decrease in maximal microvascular blood flow. This decrease in microvascular blood flow has been shown at the time of diagnosis and is equivalent to that seen in type 1 patients after 18 years of disease (113). It is presently unknown whether there is an enhanced vasoconstrictor or diminished vasodilator response in resistance vessels of type 2

diabetics. Such dysfunction could contribute to the chronic hypertension seen in type 2 diabetic patients.

2.7 Vascular Dysfunction in Type 2 Diabetes

The most profound abnormality in type 2 diabetes appears to be a decrease in the maximal hyperemic response, measured in the cutaneous circulation, which is present at diagnosis. A decreased hyperemic response to local heating has been reported in the cutaneous circulation of the forearm in patients with impaired fasting glucose (IFG), and this response was correlated to insulin concentrations but not to glycemia (55). Moreover, reduced flow-induced vasodilation has been found in first-degree relatives of type 2 diabetics (7), indicating that the alterations in vascular function are present not only as a precursor to diabetes but the result of an inherited vascular dysfunction. Hyperglycemic non-diabetic patients display a decreased vasodilator capacity that is correlated with insulin resistance. Moreover, Caballero et al. (15) and Brett et al. (2000)(12) found decreased acetylcholine (ACh) and sodium nitroprusside (SNP) induced vasodilations in the cutaneous circulation of type 2 diabetic patients. Vischer et al. (118) also found a diminished response in forearm blood flow to ACh in insulin resistant and type 2 diabetic individuals, but found no change in the SNP response.

The use of animal models allows for the examination of changes in vascular responsiveness in highly specific vascular beds. Although endothelial dysfunction and impaired vasodilator capacity have been documented in the skeletal muscle vasculature of diabetic rats and insulin resistant humans (8, 33, 37, 110, 112), this

finding has been inconsistent, as others have reported increased responsiveness to endothelium-dependent vasodilators in obese Zucker rats (3) and skeletal muscle arterioles of normoglycemic rats exposed to acute hyperglycemia (61).

In addition to the specificity of the vasculature studied, the use of animal models has allowed the examination of changes in vascular responsiveness as a function of duration of insulin resistance. Cox and Kikta (1992) (22) examined changes in the function of the thoracic aorta in obese Zucker rats across the lifespan and found increases in vasoconstrictor responsiveness that diminished with advancing age. Futhermore, ACh-induced vasodilation was enhanced in the youngest obese rats with no change in SNP-induced dilation at any age. Similar results were reported by Sexl et al. (99), who found relaxation to ACh and a calcium ionophore to be increased in the aorta of obese rats compared to their age-matched controls in young (12 week) obese Zucker rats, but not at any other age (24, 36, and 52 weeks). By examining the reactivity of the aorta from obese rats these investigators have been able to document changes in vessel function as the insulin resistant state worsens. However, no such examination has been made in the microcirculation.

In conjunction with altered vasodilator function, enhanced vasoconstrictor responsiveness could also play a role in diabetic microangiopathy. Previous investigations into the vasoconstrictor responsiveness of arterioles from diabetic and insulin resistant humans and animals have produced inconsistent results. Constriction to norepinephrine (NE) has been shown to be either unchanged (97) in human subcutaneous fat arterioles and type 1 diabetic arterioles (70) or increased in

gracilis muscle arterioles from 13-15 week old obese Zucker rats (OZR) (105). Constriction to other pharmacological agonists have been similarly variant, with either increased (70), unchanged (105), or decreased (122) constriction to angiotensin II and endothelin-1 being reported. Moreover, myogenic constriction has been shown to be both increased in gracilis muscle arterioles from streptozotocin-induced diabetic rats (116) as well as OZR rats (34) and decreased in subcutaneous adipose tissue arterioles from type 2 diabetic patients (97) and streptozotocin induced diabetic rats (48).

In addition to altered reactivity, the structure of the microvasculature may also be deranged by insulin resistance and diabetes. Altered permeability and basement membrane thickening have been well documented in both the renal and retinal circulations. Hyperinsulinemia has been implicated as stimuli for vascular remodeling (103). Previous investigators have found changes in vessel wall structural characteristics in type 2 diabetic human adipose arterioles (87, 97), type 2 diabetic coronary arterioles (125), and type 1 diabetic rat mesenteric arterioles (93). In these studies, increases in medial wall thickness, cross-sectional area, and wall-to-lumen ratio have been reported and are consistent with hypertrophic remodeling. However, others have reported decreases in medial wall thickness, wall-to-lumen ratio, and decreased distensibility in gracilis muscle arterioles from obese Zucker rats (34). Lacking however is an examination of the structural adaptations of skeletal muscle arterioles during the progression of type 2 diabetes from insulin resistance to prolonged overt diabetes.

2.8 Hypotheses

The specific aims of this study were therefore to test the hypotheses that 1) type 2 diabetes will enhance myogenic responsiveness of resistance arterioles isolated from muscles composed of different fiber types, 2) type 2 diabetes will increase the passive stiffness of these skeletal muscle resistance arterioles, 3) type 2 diabetes will increase responsiveness of skeletal muscle resistance arterioles to vasoconstrictor agonists, 4) type 2 diabetes will attenuate skeletal muscle arteriolar responsiveness to either endothelium-dependent or endothelium-independent vasodilator stimuli, and 5) that these changes will be present during the prediabetic state as well as during acute and chronic type 2 diabetes.

CHAPTER III

VASODILATOR DYSFUNCTION IN LOCOMOTOR SKELETAL MUSCLE ARTERIOLES FROM ZUCKER DIABETIC FATTY RATS IS DEPENDENT ON DISEASE PROGRESSION AND FIBER TYPE

3.1 Overview

Endothelial dysfunction in skeletal muscle arterioles could impact both peripheral insulin resistance and hypertension observed in type 2 diabetes. The purpose was to determine if endothelial dysfunction is present in arterioles isolated from high-oxidative and low-oxidative glycolytic skeletal muscles during prediabetes as well as acute and chronic diabetes, and to determine if this dysfunction precedes elevations in arterial pressure. Zucker Diabetic Fatty (ZDF) rats and lean age-matched controls were studied at 7 (prediabetes), 13 (acute diabetes) and 20 (chronic diabetes) weeks of age. Following measurement of arterial pressure, vasodilator responsiveness to acetylcholine (ACh), and intraluminal flow were examined in isolated arterioles from the soleus and white gastrocnemius muscles. Moreover, the contribution of nitric oxide (NO) in ACh-induced vasodilation was assessed. Flow-induced vasodilation was decreased in the high-oxidative muscle arterioles during acute diabetes, and this was coincident with the emergence of elevated arterial pressure. During chronic diabetes, vasodilation to ACh and flow were reduced in soleus muscle arterioles. The reduced vasodilation to ACh was the result of a loss of NO. Although the vasodilatory capacity of low-oxidative glycolytic skeletal muscle arterioles was not

diminished at any timepoint, the contribution of NO to ACh-induced dilation was lost in the prediabetic and acute diabetic rats. The data demonstrate that although endothelial dysfunction does not become manifest in low-oxidative glycolytic skeletal muscle arterioles, alterations in the signaling mechanisms to achieve that vasodilation occur in the prediabetic state. Moreover, endothelial dysfunction is a consequence of the diabetic state in high-oxidative skeletal muscle arterioles.

3.2 Introduction

Cardiovascular complications are now the leading cause of the morbidity and mortality associated with diabetes (4), and it appears that these complications begin years before the onset of overt diabetes and are related to long term impaired glucose tolerance and hyperinsulinemia (45). The skeletal muscle and its vasculature may have profound implications for the development and progression of insulin resistance and hypertension associated with prediabetes and frank type 2 diabetes. A diminished vasodilator capacity of the skeletal muscle resistance vasculature could affect the magnitude and distribution of cardiac output, glucose uptake, as well as total peripheral resistance. Furthermore, if resistance arterioles from high-oxidative or low-oxidative glycolytic skeletal muscles are differentially affected by hyperinsulinemia, insulin resistance, or hyperglycemia, then these alterations could function to change the normal distribution of the blood flow between glycolytic and oxidative fibers within or between skeletal muscles, and consequently result in diminished glucose clearance.

Inconsistencies have been reported in the literature regarding vasodilator function in insulin resistance, hyperglycemia, and type 2 diabetes. Although endothelial dysfunction and impaired vasodilator capacity have been shown in the vasculature of diabetic rats and insulin resistant humans by some investigators (8, 33, 37, 110, 112), others have reported enhanced responsiveness to endothelium-dependent and independent vasodilators in obese Zucker rats (3), (6) and skeletal muscle arterioles of normoglycemic rats exposed to acute hyperglycemia (61). Lacking, however, is an examination of the changes in microvascular vasodilator function during the progression of type 2 diabetes from prediabetes through the chronic overt diabetic state.

Therefore, the purpose of the present study was 1) to determine whether alterations in the vasodilator capacity of skeletal muscle arterioles exist during the natural progression of type 2 diabetes in a rat model, and 2) to determine whether the putative effect differentially occurs in arterioles from skeletal muscles composed of different fiber types and oxidative capacities. The Zucker diabetic fatty (ZDF) rat was chosen because it has been shown to initially develop peripheral insulin resistance and then, frank type 2 diabetes over a predictable age range (27, 56). Moreover, these rats develop many of the same conditions as prediabetes and type 2 diabetes in humans, such as obesity (11), hypertension (56, 126), and abnormal blood lipid profiles (11, 27, 101, 102), making it a clinically relevant model.

3.3 Materials and Methods

Male ZDF rats were obtained from Charles Rivers Laboratories / Genetic Models Inc. Diabetic (ZDF:fa/fa) and lean (ZDF: +/?) age-matched controls were studied at 7, 13, and 20 weeks of age to represent prediabetes and acute (1 week) and chronic (8 weeks) diabetes. Animals were housed separately and allowed free access to Purina 5008 diet and water. Animals were housed in a temperature controlled (23 ± 2 ºC) room with a 12:12 light-dark cycle. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied by the guidelines of the National Research Council *Guide for the Care and Use of Laboratory Animals* (Washington DC: National Academy Press, Revised 1996).

3.3.1 Animal Procedures

Rats were anesthetized with sodium pentobarbital (65 mg/kg) via i.p. injection. A polyurethane (Braintree Scientific, Micro-renathane; ID 0.36 mm, OD 0.84 mm) catheter filled with heparinized saline was inserted into the caudal artery via a 1 cm incision in the ventral surface of the tail. Electronically averaged mean arterial pressure was recorded while the rat was maintained under anesthesia. Following blood pressure measurement, blood was collected via cardiac puncture for assessment of plasma glucose and insulin content, and the heart removed. Whole heart and left and right ventricular masses were recorded. The gastrocnemius-plantaris-soleus muscle complex was then carefully excised from each leg.

3.3.2 Microvessel Preparation

Following excision, the gastrocnemius-plantaris-soleus muscle complex was placed in cold (4º C) physiological saline solution (PSS) that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer and 1 g/100 ml BSA, pH 7.4. Gastrocnemius and soleus muscle first-order (1A) arterioles were isolated with the aid of a dissecting microscope (Olympus SVH10) as previously described (71, 76). In the soleus muscle, 1A arterioles were defined as the first arterial branch after the feed artery entered the muscle. In the gastrocnemius muscle, 1A arterioles were defined as the first branch off the feed artery that runs over the superficial portion of the muscle. The arterioles (length, 0.5 - 1.0 mm; inner diameter, soleus: 31 – 163 μm, gastrocnemius: 53 -168 μm) were cleared of surrounding muscle fibers, removed from the muscle and placed in lucite chambers containing MOPS buffered PSS equilibrated to room air. The arterioles were cannulated on both ends to micropipettes and secured with nylon suture. After cannulation, the chambers were transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (Microcirculation Research Institute), and data acquisition system (MacLab/Macintosh) for recording of luminal diameter. Arterioles were initially pressurized to 44 mmHg with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the reservoirs verifying that diameter remained constant. Arterioles that exhibited leaks were

discarded. Arterioles free of leaks were warmed to 37º C and allowed to develop initial spontaneous tone during a 30-60 min equilibration period.

3.3.3 Experimental Design

To determine whether vasodilator function of the endothelium and vascular smooth muscle is altered at different periods in the natural progression of type 2 diabetes, vasodilator responses of 1A arterioles isolated from the superficial portion of the gastrocnemius and soleus muscles to increasing intraluminal flow, acetylcholine (ACh), and sodium nitroprusside (SNP) were measured. Sensitivity of the arterioles to pharmacological agonists was assessed by calculating the IC_{50}. In a second set of experiments, the contribution of nitric oxide synthase (NOS) and cyclooxygenase (COX) signaling pathways in ACh-induced vasodilation was assessed. The ACh dose responses were repeated in the presence of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) or the combined NOS and COX inhibitors L-NAME and indomethacin (INDO).

3.3.4 Vasodilation to Intraluminal Flow

After a steady-state spontaneous tone developed, arterioles were exposed to graded increases in intraluminal flow in the absence of changes in intraluminal pressure. This was accomplished by altering the heights of the independent fluid reservoirs in equal and opposite directions. Diameter measurements were made in response to incremental increases in intraluminal flow of 3-50 nl/sec (77).

3.3.5 Vasodilation to Pharmacological Agonists

Concentration-response relations to the cumulative addition of ACh (10^{-9} to 10^{-4}) and SNP (10^{-10} to 10^{-4}) were determined. These vasodilators were chosen because they cause vasodilation by activation of endothelial pathways and direct activation of smooth muscle guanylate cyclase (donation of NO), respectively. The vessels were allowed to equilibrate between successive dose responses and were discarded unless at least 20% baseline tone was achieved prior to addition of vasodilator agents. Maximal intraluminal diameter was determined by allowing the vessels to equilibrate in calcium free PSS following active dose responses.

3.3.6 Inhibitory Effects of N^G-nitro-L-arginine Methyl Ester and Indomethacin

After establishing spontaneous tone of at least 20%, vasodilator responses to ACh were evaluated after an 30 min incubation with 10^{-5} M L-NAME or the combination of 10^{-5} M L-NAME and 10^{-5} M INDO.

3.3.7 Solutions and Stocks

Stock solutions of drugs were prepared in distilled water and frozen. Fresh dilutions of these stocks were prepared daily. All drugs were purchased from Sigma Chemical (St. Louis, MO).

3.3.8 Data Presentation and Statistical Analysis

Vasodilator responses were recorded as actual diameters and expressed as a percentage of maximal relaxation according to the following formula:

$$\text{relaxation (\%)} = (D_s - D_b)/(D_m - D_b) * 100$$

Where D_m is maximal inner diameter at 44 mmHg, D_s is the steady-state inner diameter recorded after addition of drug or increase in intraluminal flow, and D_b is the initial baseline inner diameter before the first addition of drug or intraluminal flow. The IC_{50} was calculated for ACh and SNP, and differences were assessed by one-way analysis of variance (ANOVA). Repeated measures ANOVA was used to determine differences between age-matched diabetic and control groups for all dose responses. Fisher protected least significant difference post hoc was used where appropriate. For animal data, one-way ANOVA was used to determine significant differences between diabetic and age-matched controls. All data are presented as mean ± SE. Significance was set at $P \leq 0.05$.

3.4 Results

Body mass was greater in the 7 week old prediabetic fatty rats (F7) than the age-matched lean (L7) rats. There were no differences in body mass between groups at either 13 or 20 weeks of age. Heart mass was higher (10.4%) in the prediabetic fatty (F7) group, however, the heart-to-body mass ratio was lower in the F7 rats compared to age-matched controls (Table 3.1). With overt diabetes, (13 and 20 weeks), heart mass was lower in the fatty rats (6.5% and 17%, respectively) and this difference persisted when expressed relative to body mass (Table 3.1). Soleus muscle mass and soleus-to-body mass ratio did not differ between the prediabetic rats and the lean age-matched controls (Table 3.1). The absolute soleus muscle mass did not differ between lean and fatty rats during acute diabetes,

Table 3.1. Descriptive Characteristics of Prediabetic (7), Acute (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats

Weeks of Age	7		13		20	
Group	**L**	**F**	**L**	**F**	**L**	**F**
(n)	(15)	(15)	(13)	(14)	(13)	(11)
Body mass (g)	201 ± 4	256 ± 5*	358 ± 4	377 ± 14	435 ± 5	410 ± 14
Soleus muscle mass (mg)	109 ± 6	121 ± 6	194 ± 6	175 ± 7	227 ± 5	165 ± 9*
Soleus-to-body mass ratio (%)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01*	0.05 ± 0.01	0.04 ± 0.01*
Gastrocnemius muscle mass (g)	0.99 ± 0.07	1.03 ± 0.03	1.88 ± 0.02	1.45 ± 0.05*	2.07 ± 0.16	1.35 ± 0.10*
Gastrocnemius-to-body mass ratio (%)	0.49 ± 0.13	0.40 ± 0.04*	0.53 ± 0.03	0.39 ± 0.02*	0.47 ± 0.13	0.33 ± 0.08*
Whole heart mass (g)	0.70 ± 0.01	0.77 ± 0.02*	1.07 ± 0.02	1.00 ± 0.03*	1.29 ± 0.03	1.07 ± 0.02*
Heart-to-body mass ratio (%)	0.35 ± 0.01	0.30 ± 0.01*	0.30 ± 0.01	0.27 ± 0.01*	0.30 ± 0.01	0.26 ± 0.01*
Left ventricle mass (mg)	540 ± 13	693 ± 15*	810 ± 12	761 ± 22	993 ± 24	835 ± 17*
Right ventricle mass (mg)	129 ± 10	148 ± 7	218 ± 12	197 ± 12	231 ± 7	196 ± 7*

* Denotes significant difference from age-matched control, $P \leq 0.05$, (n) is the number of animals

however, the soleus-to-body mass ratio was lower in the fatty rats compared to age-matched controls. Both absolute and relative soleus muscle mass were lower in the fatty rats compared to lean age-matched controls during chronic diabetes.

The absolute mass of the gastrocnemius muscle did not differ between the fatty and lean rats during prediabetes (Table 3.1), but the gastrocnemius muscle-to-body mass ratio was lower in the prediabetic fatty rats compared to age-matched controls. During acute diabetes, gastrocnemius muscle mass was reduced in the fatty (F13) rats when compared to the lean controls (L13) and these differences persisted when expressed relative to body mass. Absolute and relative gastrocnemius muscle mass was reduced in the fatty (F20) rats compared to age-matched lean (L20) rats (Table 3.1).

Plasma glucose was higher in all fatty groups relative to age matched controls (Figure 3.1A). Plasma insulin was higher in the prediabetic rats, but was lower than that in control animals with both acute and chronic diabetes (Figure 3.1B). MAP did not differ between the fatty and lean rats during prediabetes, but was significantly elevated with acute and chronic diabetes (Figure 3.1C).

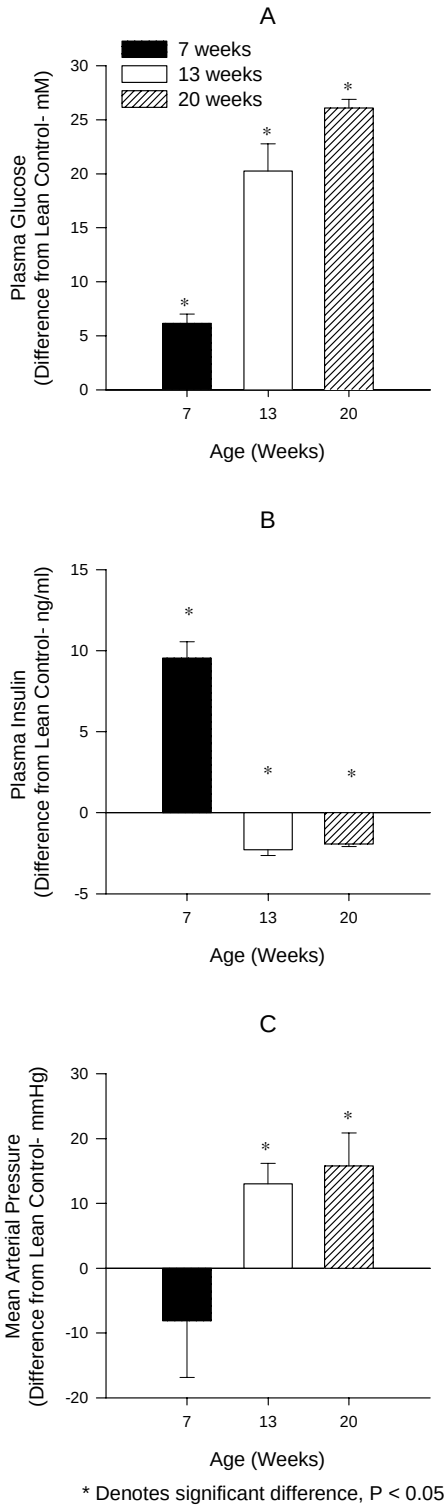


Figure 3.1. Difference from Mean Control Values for Plasma Glucose (A), Plasma Insulin (B) and Mean Arterial Pressure (C) of Prediabetic (7), Acutely (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats

Maximal diameter of soleus muscle arterioles did not differ between the fatty and lean rats at any stage of diabetes. Likewise, maximal diameter of the gastrocnemius muscle arterioles were similar between the fatty and lean rats at both 7 and 13 weeks of age (Table 3.2). However, with chronic diabetes, maximal diameter of gastrocnemius muscle arterioles from the fatty rats was lower compared to those from the age-matched controls.

Spontaneous tone did not differ between the fatty and lean rats in either muscle at any point in the progression of the disease (Table 3.2). Incubation with L-NAME increased tone in gastrocnemius muscle arterioles from both the lean and fatty rats at all three timepoints (Table 3.2). The combination of L-NAME and INDO increased tone in all gastrocnemius arterioles except in gastrocnemius muscle arterioles from the chronically diabetic rats (Table 3.2). Incubation of soleus muscle arterioles with L-NAME increased tone in both the lean and fatty rats at 7 weeks of age, as well as from arterioles isolated from the lean rats at 13 weeks of age (Table 3.2). No differences in soleus muscle arteriolar tone was seen between the chronically diabetic rats and their age-matched controls. The combined L-NAME and INDO blockade did not alter tone in soleus muscle arterioles.

Table 3.2. Characteristics of First Order Arterioles from Soleus and White Gastrocnemius Muscles

	Soleus Muscle					
Weeks of age	7		13		20	
Group	L	F	L	F	L	F
Maximal diameter (μm)	90 ± 10	100 ± 5	117 ± 8	113 ± 10	126 ± 6	122 ± 2
	Spontaneous tone (%)					
Flow	40 ± 5	46 ± 10	42 ± 4	44 ± 4	41 ± 6	40 ± 7
ACh	55 ± 7	54 ± 5	51 ± 5	51 ± 5	49 ± 5	46 ± 7
Pre-L-NAME	39 ± 5	49 ± 6	38 ± 3	41 ± 7	47 ± 6	41 ± 5
L-NAME	66 ± 8†	73 ± 5†	59 ± 6†	68 ± 7	60 ± 6	50 ± 8
Pre-L-NAME + INDO	62 ± 9	58 ± 6	53 ± 6	56 ± 5	52 ± 7	44 ± 7
L-NAME + INDO	68 ± 8	72 ± 5	65 ± 8	72 ± 5	67 ± 6	45 ± 10
	Gastrocnemius muscle					
Weeks of age	7		13		20	
Group	L	F	L	F	L	F
Maximal diameter (μm)	96 ± 7	101 ± 3	126 ± 10	116 ± 4	130 ± 4	110 ± 7*
	Spontaneous tone (%)					
Flow	36 ± 4	37 ± 4	36 ± 3	33 ± 7	32 ± 4	33 ± 5
ACh	37 ± 3	41 ± 4	47 ± 3	43 ± 5	34 ± 3	42 ± 5
Pre-L-NAME	40 ± 6	29 ± 4	28 ±3	24 ± 2	29 ± 3	31 ± 3
L-NAME	68 ± 7†	58 ± 7†	42 ± 7†	55 ± 5†	47 ± 5†	48 ± 5†
Pre-L-NAME + INDO	56 ± 4	36 ± 7*	37 ± 6	36 ± 3	39 ± 5	38 ± 3
L-NAME + INDO	71 ± 4†	63 ± 6†	55 ± 5‡	61 ± 5†	54 ± 5†	49 ± 6

* denotes significant difference from age-matched control,† denotes significant difference in % tone after addition of blocker compared to pre-, ‡ denotes significant difference in % tone between L-NAME alone and L-NAME and indomethacin, $p \leq 0.05$

3.4.1 Soleus Muscle Arteriolar Reactivity

Cumulative addition of ACh produced a dose dependent vasodilation in all groups (Figure 3.2). No differences were observed between the vasodilator responses from the prediabetic or acutely diabetic rats when compared to their age-matched lean controls. However, with chronic diabetes, the F20 group displayed a diminished maximal vasodilation to ACh compared to lean control rats (Figure 3.2C). No differences in sensitivity (IC_{50}) to ACh were observed in any group. Incubation with L-NAME reduced the maximal vasodilation to ACh in both the fatty and lean rats during both pre- and acute diabetes. The combined NOS and COX blockade produced no further decrement in vasodilation in either the fatty or lean rats at 7 and 13 weeks of age (Figure 3.2A&B). During chronic diabetes, L-NAME incubation reduced the vasodilation to ACh in the lean rats but had no effect in the arterioles from the fatty rats (Figure 3.2C). The combined L-NAME and INDO blockade produced no further decrement in ACh-induced vasodilation in either the lean or fatty rats at 20 weeks of age (Figure 3.2C).

Flow-induced vasodilation was diminished in the arterioles from the fatty rats during both overtly diabetic conditions (13 and 20 weeks of age) compared to lean age-matched controls (Figure 3.3B&C), but was similar between fatty and lean rats during prediabetes (Figure 3.3A).

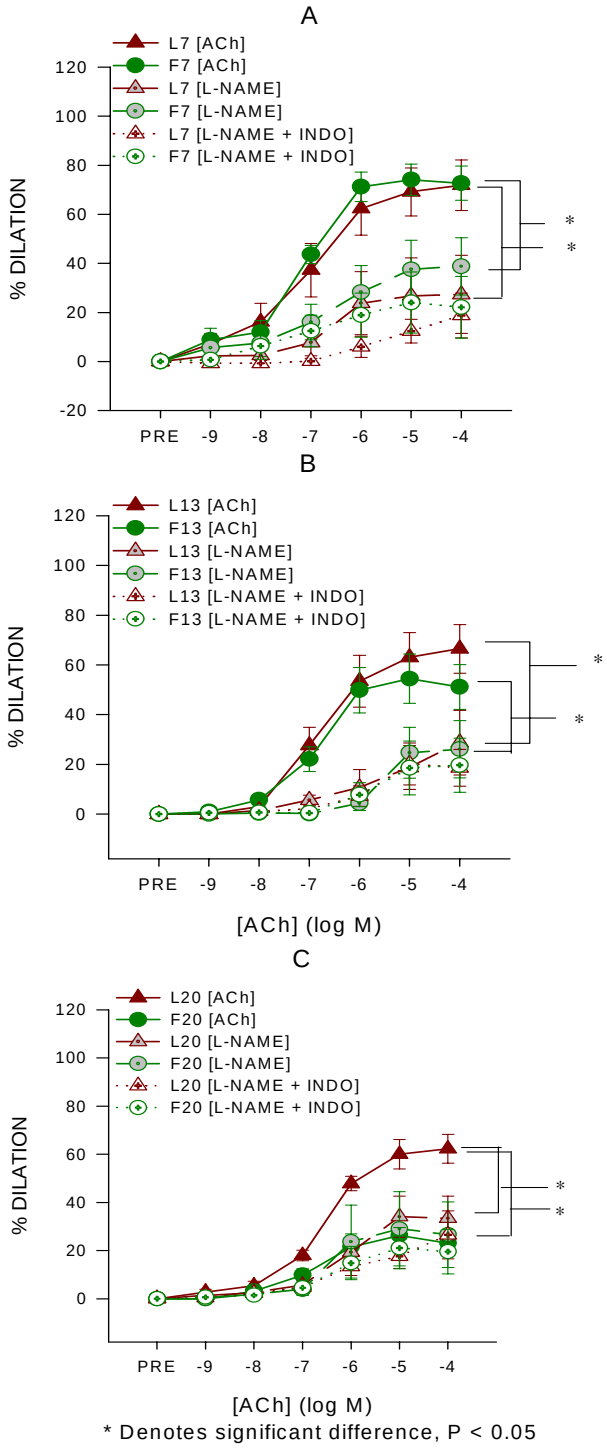


Figure 3.2. Vasodilator Responses to ACh in the Presences of L-NAME and the Combination of L-NAME and INDO in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

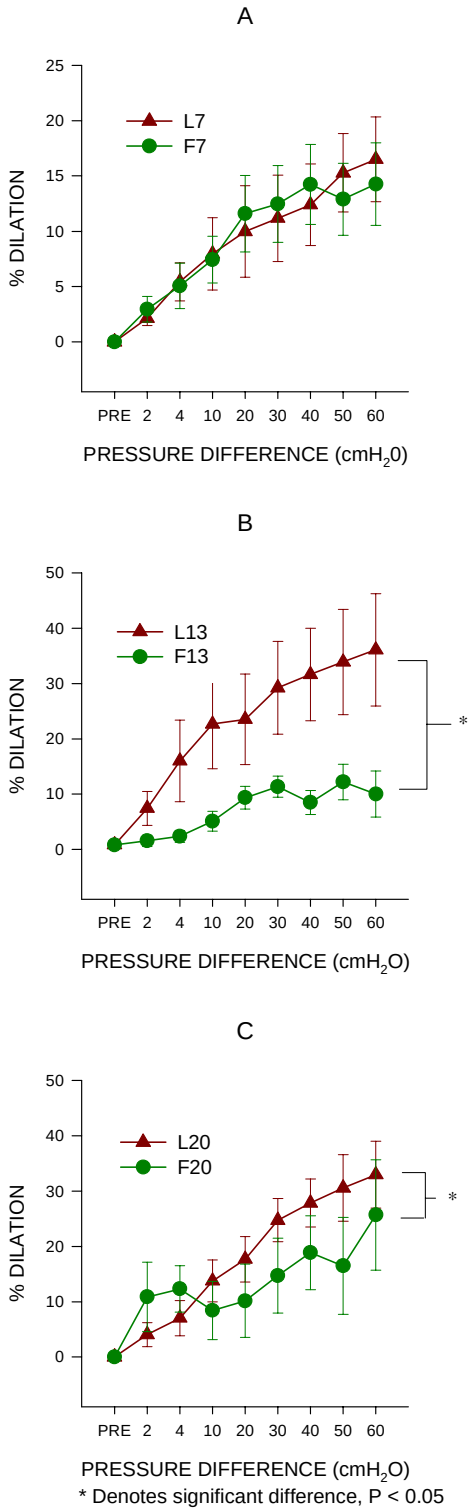


Figure 3.3 Vasodilator Responses to Intraluminal Flow in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

No differences in SNP-induced vasodilation were observed at any age between fatty and lean age-matched controls (Figure 3.4).

3.4.2 Gastrocnemius Muscle Arteriolar Reactivity

The cumulative addition of ACh produced a dose dependent vasodilation in all arterioles studied (Figure 3.5). No differences were observed between the vasodilator responses to ACh from the fatty groups and their age-matched lean controls at any point in the progression of type 2 diabetes (Figure 3.5). No differences in sensitivity to ACh were observed between the fatty and lean rats at any timepoint. Incubation with L-NAME reduced the maximal vasodilation to ACh in the lean rats at 7 and 13 weeks of age but not in the fatty rats at the same ages (Figure 3.5A&B). The combination of L-NAME and INDO produced no further decrement in vasodilation to ACh in either lean control group at 7 and 13 weeks of age (Figure 3.5A&B). However, the combination of L-NAME and INDO diminished the vasodilation to ACh in the fatty rats during both pre-and acute diabetes (Figure 3.5A&B). With chronic diabetes, L-NAME incubation did not reduce the vasodilation to ACh in the lean rats, however a reduction in vasodilation tended to be seen in the fatty rats during chronic diabetes ($p = 0.065$) (Figure 3.5C). However, INDO in combination with L-NAME produced a significant reduction in the dilation to ACh in both the fatty and lean rats during chronic diabetes (Figure 3.5C).

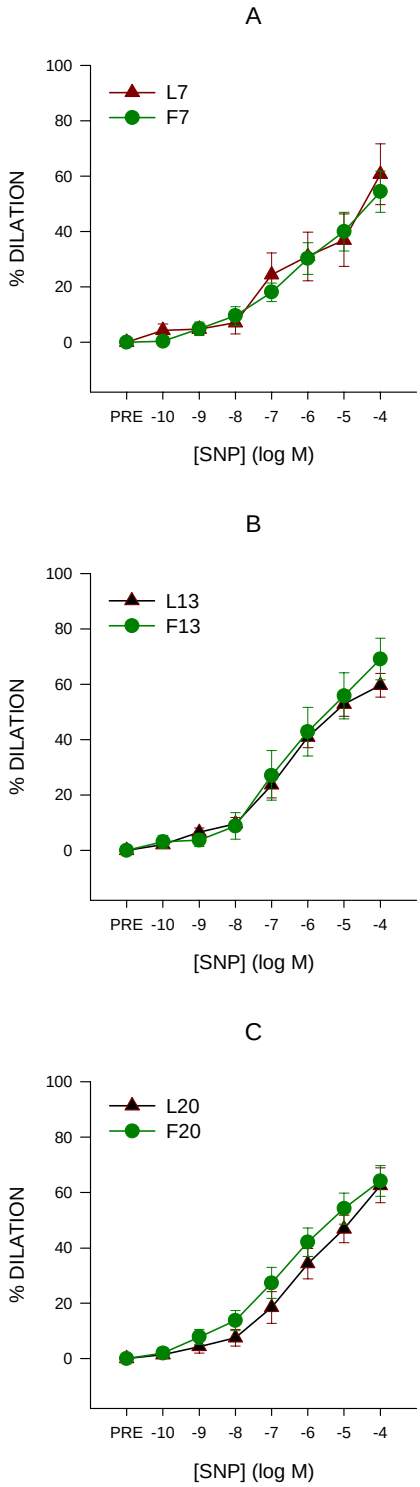


Figure 3.4 Vasodilator Responses to SNP in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

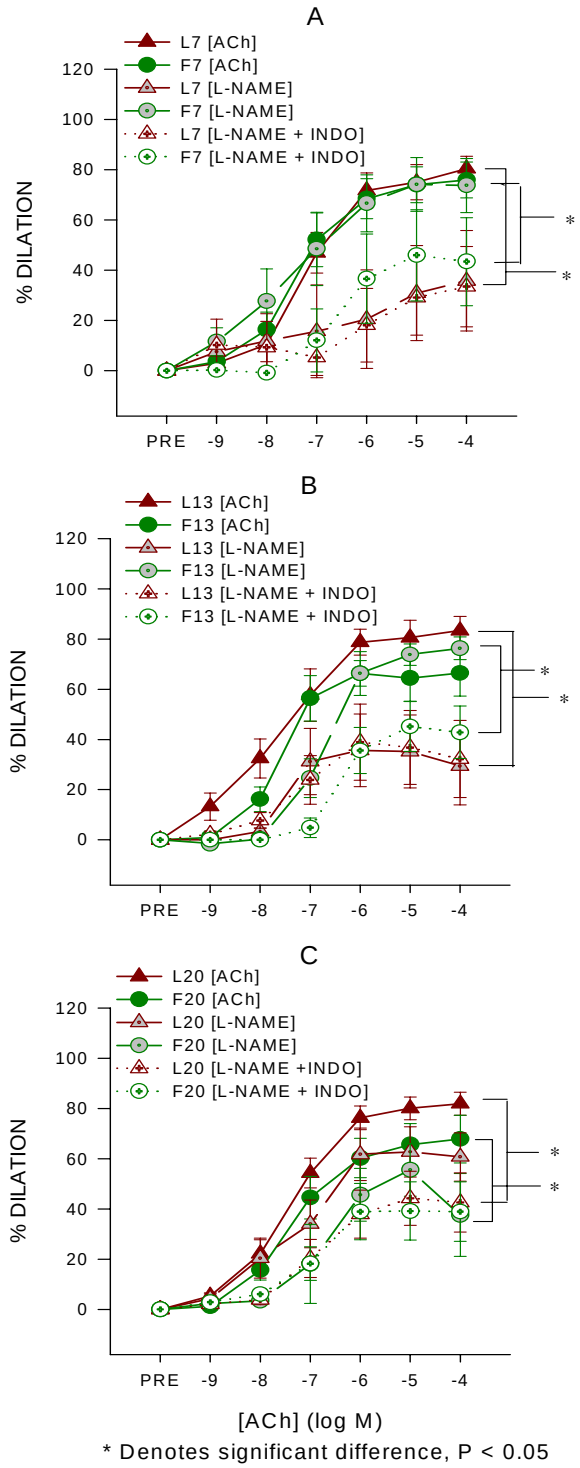


Figure 3.5 Vasodilator Responses to ACh in the Presences of L-NAME and the Combination of L-NAME and INDO in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

No differences occurred between fatty groups and their age-matched controls in flow-induced vasodilation (Figure 3.6).

During prediabetes, vasodilation to SNP was increased in the fatty rats when compared to the lean controls of the same age (Figure 3.7A). Vasodilation to SNP did not differ between the fatty and age-matched lean controls during either acute or chronic diabetes (Figure 3.7B&C).

3.5 Discussion

The purpose of the present study was 1) to determine if vasodilator dysfunction is present in prediabetes, 2) to examine the temporal relation of putative vasodilator dysfunction with the emergence of elevated arterial pressure and 3) to determine if arterioles from high-oxidative and low-oxidative glycolytic skeletal muscle are differentially affected by the progression of type 2 diabetes in the ZDF rat. In the present study, the results demonstrate that endothelium-dependent vasodilation is differentially affected by type 2 diabetes in muscles composed of different fiber types and having different oxidative capacities. The maximal flow-induced and ACh-mediated vasodilator response of low-oxidative glycolytic skeletal muscle is preserved in prediabetes and acute diabetes, but the mechanisms by which ACh-induced vasodilation is achieved is modified in the prediabetic state. Specifically, the NOS-dependent portion of ACh-stimulated vasodilation is absent in the gastrocnemius muscle arterioles of the prediabetic rats. In contrast, arterioles from the high-oxidative soleus muscle demonstrate endothelial dysfunction that becomes manifest after the development of type 2

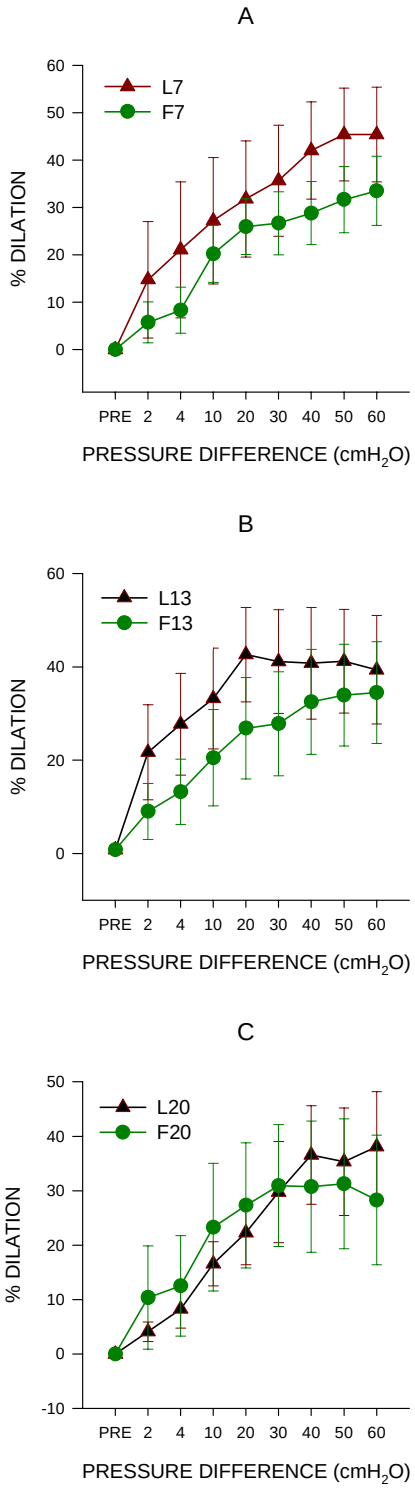


Figure 3.6 Vasodilator Responses to Intraluminal Flow in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

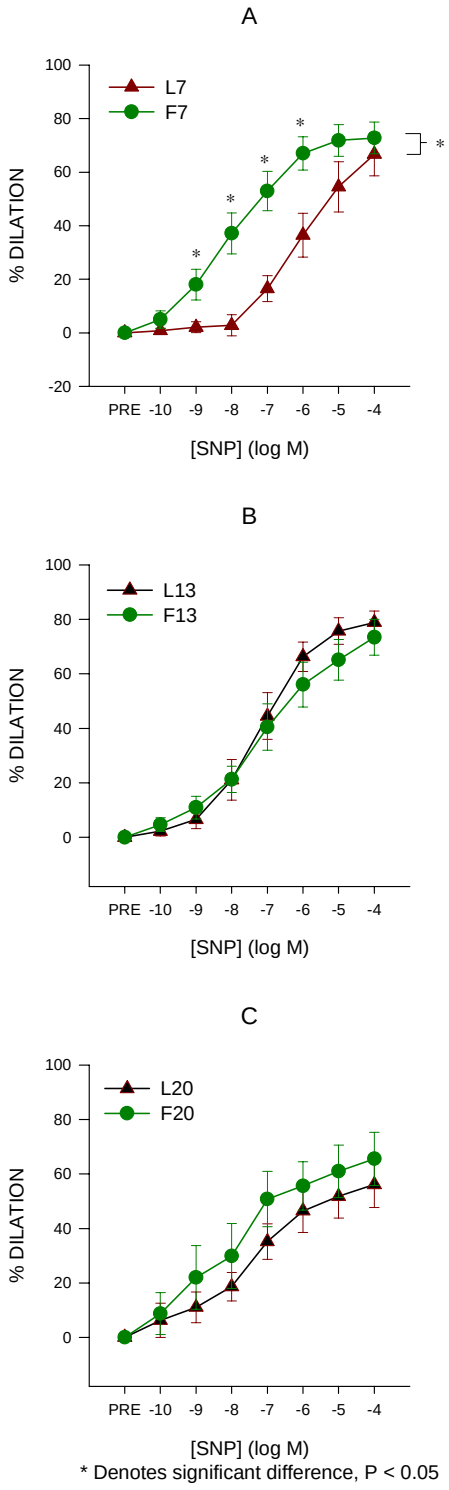


Figure 3.7 Vasodilator Responses to SNP in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

diabetes. Additionally, the results show that flow-induced vasodilation is diminished by type 2 diabetes earlier in the progression of the disease than is ACh-mediated vasodilation. This decrement in flow-induced vasodilation is coincident with the emergence of elevated arterial pressure in the ZDF rat. The data also demonstrate that the decrement in ACh-stimulated vasodilator capacity with chronic diabetes is the consequence of a loss of the nitroxidergic signaling mechanism.

Despite a preserved maximal vasodilator response in the gastrocnemius arterioles of the fatty rats, examination of the mechanisms of ACh-induced vasodilation reveals an alteration in endothelial function in both prediabetes and the diabetic conditions, resulting in a shift from predominately nitroxidergic to cyclooxygenase signaling mechanisms in the fatty rats. In addition to its role in vasodilation, NO has a number of vasoprotective actions, such as the inhibition of vascular smooth muscle growth and migration, platelet aggregation and thrombosis, oxidation, monocyte adhesion, and inflammation (51). Although the loss of NO production in these vessels does not adversely affect vasodilator function, it may however compromise these other vasoprotective effects, making the vessels susceptible to inflammatory damage, abnormal vascular remodeling or oxidative stress, and these in turn may influence the development of hypertension. Interestingly, a clear difference in the susceptibility to diabetes-associated endothelial dysfunction can be seen between high-oxidative and low-oxidative glycolytic muscles.

Skeletal muscle blood flow at rest and during exercise throughout the progression of type 2 diabetes has not been examined, but the data of the present study suggest that impaired vasodilator function of the arterioles of high-oxidative muscle could have profound implications for blood flow regulation during locomotion in diabetic conditions since these muscles are the most highly recruited during activity (5, 63). Moreover, the vasodilation in response to intraluminal flow is more sensitive to the diabetic condition than is ACh-mediated vasodilation, and is more likely to have an impact on exercise hyperemia. This speculation warrants further examination.

Previous investigators have provided evidence for impaired vasodilator function of skeletal muscle arterioles in obese Zucker rats (OZR), but an evaluation of the development of this dysfunction has not previously been elucidated. These previous studies report a loss of endothelium-dependent vasodilation in the gracilis and cremaster muscles (33, 36, 37). Although these muscles are made up of predominately low-oxidative glycolytic muscle fibers the effects of diabetes on vasodilator function differ greatly from those of arterioles in the present study from muscle of a similar fiber type composition. The disparity in findings between these investigations and the present study may not be a function of the fiber type of the surrounding muscle *per se* but of the peculiarities in function of the cremaster and gracilis muscles themselves. For example, the cremaster muscle, although a striated muscle, is not skeletal and not involved in gross skeletal movement. Unlike the cremaster muscle, the gracilis muscle is an adductor of the hindlimb made up of primarily fast-twitch muscle fibers (20% type I, 3% type IIA, 6% type

IIX, and 71 % type IIB) similar to the superficial gastrocnemius muscle (0% type I, 0% type IIA, 8% type IIX, and 92% type IIB)(24). However, although similarities in fiber populations between the gracilis and gastrocnemius muscle exist, differences in the blood flow response and consequently muscle recruitment during exercise are profound (5, 64) and may account for differences seen in the arteriolar function between investigations.

The differences in findings between the present study and those of Frisbee et al. (33, 36, 37) may also be a function of the animal model examined. The OZR rat is not necessarily an appropriate model of the prediabetic condition, as it may maintain subclinical hyperglycemia throughout its lifespan, and thus never progress to the overt diabetic condition. For example, the progression of the OZR rat to overt diabetes has not been consistent in the literature, with some investigator reporting severe hyperglycemia ((33-37, 105), and others reporting little to no elevation in plasma glucose (14, 53, 75, 106), (128). Moreover, even in cases where the plasma glucose is dramatically elevated, plasma insulin concentrations are often not reported (33-37, 105), making it difficult to determine whether the external environment of these vessels more closely resembles that of the prediabetic or overt diabetic condition.

Using the of the ZDF model of type 2 diabetes, progressive changes in microvascular vasodilator capacity of the skeletal muscle vasculature during the development of type 2 diabetes were determined. The data demonstrate that 1) although endothelium-dependent vasodilator responses are preserved in the low-oxidative glycolytic gastrocnemius muscle arterioles, the loss of the endothelial

nitroxidergic signaling mechanism occurs concurrently with hyperglycemia and hyperinsulinemia in the prediabetic state, 2) an increase in smooth muscle cell sensitivity to exogenous NO occurs coincidently with the diminished endothelial NO signaling in the low-oxidative glycolytic gastrocnemius muscle arterioles, 3) there is a loss of endothelium-dependent, NO-mediated vasodilator response in arterioles from the high-oxidative soleus muscle, and 4) this dysfunction occurs coincidently with elevations in mean arterial pressure. If applicable to the human condition, these data indicate that alterations in endothelial signaling and vasodilator capacity may contribute to the hypertension that occurs with type 2 diabetes.

CHAPTER IV

VASOCONSTRICTOR DYSFUNCTION AND STRUCTURAL ADAPTATIONS IN LOCOMOTOR SKELETAL MUSCLE ARTERIOLES FROM ZUCKER DIABETIC FATTY RATS IS DEPENDENT ON DISEASE PROGRESSION AND FIBER TYPE

4.1 Overview

Altered vasoconstrictor reactivity or mechanical properties of skeletal muscle arterioles could impact both peripheral insulin resistance and hypertension observed in type 2 diabetes. The purpose was to determine if increased vasoconstrictor reactivity and/or alterations in the structural properties of 1A arterioles isolated from both high-oxidative and low-oxidative glycolytic skeletal muscles is present during prediabetes as well as acute and chronic diabetes, and to determine if this putative dysfunction precedes the development of elevated arterial pressure in a rat model of type 2 diabetes. Zucker Diabetic Fatty (ZDF) rats and lean age-matched controls were studied at 7 (prediabetes), 13 (acute diabetes) and 20 (chronic diabetes) weeks of age. Following measurement of arterial pressure, vasoconstrictor responsiveness to norepinephrine (NE), potassium chloride (KCl), and increasing intraluminal pressure (MYO) and passive mechanical properties were examined in isolated arterioles from the soleus and superficial gastrocnemius muscle. Vasoconstriction to NE was enhanced in the gastrocnemius muscle arterioles during prediabetes and preceded the observed elevations in mean arterial pressure. Vasoconstrictor responses to both NE and KCl were increased in the

soleus muscle arterioles during acute diabetes. Vasoconstrictor responsiveness to both NE and MYO were increased in chronic diabetes, despite a diminished reactivity to KCl. Alterations in the passive mechanical properties of arterioles from both muscles were observed throughout the progression of diabetes. The data demonstrate that alterations in both the active and passive properties of low-oxidative glycolytic skeletal muscle arterioles are present during prediabetes, and precede the development of type 2 diabetes.

4.2 Introduction

Type 2 diabetes affects approximately 15 million Americans, with an estimated 20 million more suffering from some degree of glucose intolerance (81). The presence of diabetes increases cardiovascular disease risk 2 to 6-fold over that in normal healthy adults (8, 10, 43, 54). Hypertension, a common comorbidity of overt type 2 diabetes, often accompanies the diagnosis of type 2 diabetes with at least 40% of patients presenting with hypertension at the time of diabetes diagnosis (114).

Overt type 2 diabetes is preceded by a prolonged period of impaired glucose regulation, termed prediabetes. Prediabetes is characterized by peripheral insulin resistance, primarily in the skeletal muscle and adipose tissue, hyperinsulinemia, transient post-prandial and subclinical fasting hyperglycemia, hypertension, obesity, and dyslipidemia. With chronic type II diabetes, abnormal microvascular function has been implicated in the development of a number of vascular complications, such as peripheral neuropathy, renal failure and blindness (17, 31,

91). However, dysfunction of the skeletal muscle microvasculature may be manifest well before the onset of overt diabetes and contribute to both insulin resistance and hypertension seen during the prediabetic state. Increases in vasoconstrictor responsiveness in the skeletal muscle could result in diminished insulin delivery, thus limiting plasma glucose disposal, and increase total peripheral resistance, contributing to hypertension. Moreover, structural adaptations in the microvasculature related to hyperinsulinemia, hyperglycemia, or altered transmural pressure may result in vascular remodeling that could contribute to increases in peripheral vascular resistance as well as alter vasoreactivity. However, neither the vasoconstrictor reactivity nor structural properties of arterioles have been adequately studied during the development of type 2 diabetes.

The purpose of the present study was to examine alterations in the vasoconstrictor responsiveness and structural adaptations of skeletal muscle arterioles during the development of type 2 diabetes in a rat model. The Zucker diabetic fatty (ZDF) rat was chosen because it has been shown to develop peripheral insulin resistance and subsequently, frank type 2 diabetes at predictable ages (27, 56). Moreover, these rats develop many of the same conditions as prediabetes and type 2 diabetes in humans, such as obesity (11), hypertension (56, 126), and abnormal blood lipid profiles (11, 27, 101, 102), making it a clinically relevant model.

4.3 Materials and Methods

Male ZDF rats were obtained from Charles Rivers Laboratories / Genetic Models Inc. Diabetic (ZDF:fa/fa) and lean (ZDF: +/?) age-matched controls were studied at 7, 13, and 20 weeks of age to represent prediabetes, acute (1 week), and chronic (8 weeks) diabetes. Animals were housed separately and allowed free access to Purina 5008 diet and water. Animals were housed in a temperature controlled (23 ± 2 ºC) room with a 12:12 light-dark cycle. All animal procedures were approved by the Texas A&M University Laboratory Animal Care Committee and complied by the guidelines of the National Research Council *Guide for the Care and Use of Laboratory Animals* (Washington DC: National Academy Press, Revised 1996).

4.3.1 Animal Procedures

Rats were anesthetized with sodium pentobarbital (65 mg/kg) via i.p. injection. A polyurethane (Braintree Scientific, Micro-renathane; ID 0.36 mm, OD 0.84 mm) catheter filled with heparinized saline was inserted into the caudal artery via a 1 cm incision in the ventral surface of the tail. Electronically averaged mean arterial pressure was recorded while the rat was maintained under anesthesia. Following blood pressure measurement, blood was collected via cardiac puncture for assessment of plasma glucose and insulin content, and the heart removed. Whole heart and left and right ventricular masses were recorded. The gastrocnemius-plantaris-soleus muscle complex was then carefully excised from each leg.

4.3.2 Microvessel Preparation

Following excision, the gastrocnemius-plantaris-soleus muscle complex was placed in cold (4º C) physiological saline solution (PSS) that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM$MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer and 1 g/100 ml BSA, pH 7.4. Gastrocnemius and soleus muscle first-order (1A) arterioles were isolated with the aid of a dissecting microscope (Olympus SVH10) as previously described (71, 76). In the soleus muscle, 1A arterioles were defined as the first arterial branch after the feed artery entered the muscle. In the gastrocnemius muscle, 1A arterioles were defined as the first branch off the feed artery that runs over the superficial portion of the muscle. The arterioles (length, 0.5 - 1.0 mm; inner diameter, soleus muscle: 60-159 μm, gastrocnemius muscle: 84-160 μm) were cleared of surrounding muscle fibers, removed from the muscle and placed in lucite chambers containing MOPS buffered PSS equilibrated to room air. The arterioles were cannulated on both ends to micropipettes and secured with nylon suture. After cannulation, the chambers were transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (Microcirculation Research Institute), and data acquisition system (MacLab) for recording of luminal diameter. Arterioles were initially pressurized to 44 mmHg with two independent hydrostatic pressure reservoirs. Leaks were detected by pressurizing the vessel and then closing the reservoirs verifying that diameter remained constant. Arterioles that exhibited leaks were

discarded. Arterioles free of leaks were warmed to 37º C and allowed to develop initial spontaneous tone during a 30-60 min equilibration period.

4.3.3 Experimental Design

To determine whether vasoconstrictor function of the skeletal muscle arterioles is altered at different periods in the natural progression of type 2 diabetes, and if these alterations occur differentially in arterioles from predominately low-oxidative glycolytic and high-oxidative skeletal muscles, constriction of 1A arterioles isolated from the superficial portion of the gastrocnemius and soleus muscle arterioles was measured in response to increasing intraluminal pressure (myogenic), norepinephrine (NE), and isotonic potassium chloride (KCl). Sensitivity of the arterioles to pharmacological agonists was assessed by calculating the EC_{50}.

4.3.4 Vasoconstriction to Intraluminal Pressure

After a steady-state spontaneous tone was achieved, arterioles were exposed to graded increases in intraluminal pressure. This was accomplished by altering the height of the independent fluid reservoirs. Diameter measurements were made in response to incremental increases and decreases in pressure from 0-99 in 11 mmHg increments.

4.3.5 Vasoconstriction to Pharmacological Agonists

Concentration-response relations to the cumulative addition of NE (10^{-9} to 10^{-4}) and KCl (10-100 mM) were determined. These vasoconstrictors were chosen because they mediate constriction through receptor dependent (NE) and independent (KCl) mechanisms. The vessels were allowed to equilibrate between successive dose responses and were discarded unless at least 20% baseline tone was achieved prior to addition of vasoconstrictor agents.

4.3.6 Passive Mechanical Properties

To determine whether alterations in the mechanical or structural properties of arteries occurs with the development of type 2 diabetes, a second set of arterioles was used to measure passive pressure-diameter relations, circumferential stress and strain and incremental stiffness. Previous investigations of arteries from normal and hypertensive rats indicate that the structural behavior of a vessel segment may remain unchanged despite an alteration in the material properties of the vascular wall (89). Therefore, the passive pressure-diameter relation and measures of stiffness were used to assess the structural behavior and material properties of the arterial wall, respectively. These vessels were dissected and cannulated as described above, but placed in calcium-free PSS. Arterioles were allowed to equilibrate at 37ºC while pressurized at 44 mmHg to induce complete vasorelaxation. The vessels were then exposed to incremental increases in intraluminal pressure from 0-99 mmHg in 11 mmHg increments. Maximal diameter at each pressure step was recorded. Following the passive pressure-

diameter assessment, vessels were allowed to equilibrate at 44 mmHg for 5 minutes then fixed in 20% Bouin's fixative for 10 min. Following fixation, vessels were removed from the cannulae and frozen in OCT for histological analysis. Frozen samples were sectioned in a cryostat at 7 μm, and stained with Weigart's hematoxylin (Sigma #HT1079) and Maisson's trichrome stain (Sigma #HT15), which stains the smooth muscle red and connective tissue blue. Medial wall cross-sectional area was measured from the stained sections. Medial wall thickness at each pressure step was then calculated according to the following equation

$$V = \pi\,[(ID + 2WT)2 - ID^2]L = \pi[(ID_o + 2WT_o)^2 - (ID_o)^2]L$$

where V is wall volume (a constant), ID is inner diameter at any given pressure other than zero, WT is wall thickness, L is vessel segment length, ID_o is original diameter measured at 0 cmH_2O, and WT_o is wall thicknessmeasured at 0 cmH_2O. The vadidity of estimating WT at intraluminal pressures other than 60 cmH_2O is based on the assumption that wall volume remains constant with changes in diameter. Both circumferential Cauchy (σ) and circumferential second Piola-Kirchoff stresses (S) were calculated from intraluminal pressure (IP), inner diameter, and wall thickness in the following manner

$$\sigma_\theta = (IP \times ID)/(2WT)$$

and

$$S_\theta = \sigma_\theta \times [1/(\lambda_\theta)^2]$$

where λ_θ is the principal stretch (extension) ratio in the circumferential direction, which equals current inner radius(r) divided by initial inner radius (R). In these arterioles, λ_θ can be accurately represented by measurements of the inner radius

because the wall thickness is minimal, i.e., on average, wall thickness is approximately 4% of outer diameter. Because the deformations of the arterioles are large, principal stretch ratios were appropriate measures. The Cauchy stress, or true stress, is defined as the actual force acting on an oriented differential area in the current (deformed) configuration and is related to the principal stretch ratio (λ). The second Piola-Kirchoff stress, on the other hand, is defined as the theoretical force acting on an oriented differential area in the reference (undeformed) configuration, and is conjugate to the Green's strain (E), where conjugate indicates that the stress can be determined directly from an energy function by differentiating with respect to the conjugate measure of deformation. The circumferential Greesn's strain is calculated in the following manner

$$E_\theta = ½ [(\lambda_\theta)^2 - 1]$$

Stiffness can be determined from either the stress/strain or stress/stretch relations. In the present study, incremental stiffness was calculated as change in stress/ change in strain for each datum point. Incremental stiffness points were plotted as a function of their corresponding stress points, and linear regression analysis was used to calculate the slope of that relation (76).

4.3.7 Solutions and Stocks

Stock solutions of drugs were prepared in distilled water and frozen. Fresh dilutions of these stocks were prepared daily. All drugs were purchased from Sigma Chemical (St. Louis, MO).

4.3.8 Data Presentation and Statistical Analysis

Vasoconstrictor responses to NE and KCl were recorded as actual diameters and expressed as a percentage of possible constriction according to the following formula for analysis:

$$\text{Diameter (\% tone)} = 100\text{-}[(D_s - D_b)/(D_b) * 100]$$

Where D_s is the steady-state inner diameter recorded after each dose or change in intraluminal pressure, and D_b is the initial baseline inner diameter before the first addition of drug or change in intraluminal pressure. Myogenic vasoconstriction and passive pressure-diameter relations are presented as a percentage of the maximum diameter obtained at 44 mmHg. Spontaneous tone is presented at a percentage of maximal intraluminal diameter. Repeated measures analysis of variance (ANOVA) was used to determine differences between age-matched diabetic and control groups for all dose responses. Fisher protected least significant difference post hoc was used where appropriate. For animal characteristics data, a one-way ANOVA was used to determine significant differences between diabetic and age-matched controls. All data are presented as mean ± SEM. Significance was set at $P \leq 0.05$.

4.4 Results

Body mass was 27% greater in the 7 week old prediabetic fatty rats (F7) than the age-matched lean (L7) rats. There were no differences in body mass between the diabetic or lean groups at either 13 or 20 weeks of age (Table 4.1).

Table 4.1. Characteristics of Prediabetic (7), Acute (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats

Weeks of Age	**7**		**13**		**20**	
Group	**L**	**F**	**L**	**F**	**L**	**F**
(n)	(15)	(15)	(13)	(14)	(13)	(11)
Body mass (g)	201 ± 4	256 ± 5*	358 ± 4	377 ± 14	435 ± 5	410 ± 14
Soleus muscle mass (mg)	109 ± 6	121 ± 6	194 ± 6	175 ± 7	227 ± 5	165 ± 9*
Soleus-to-body mass ratio (%)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01*	0.05 ± 0.01	0.04 ± 0.01*
Gastrocnemius muscle mass (g)	0.99 ± 0.07	1.03 ± 0.03	1.88 ± 0.02	1.45 ± 0.05*	2.07 ± 0.16	1.35 ± 0.10*
Gastrocnemius-to-body mass ratio (%)	0.49 ± 0.13	0.40 ± 0.04*	0.53 ± 0.03	0.39 ± 0.02*	0.47 ± 0.13	0.33 ± 0.08*
Whole heart mass (g)	0.70 ± 0.01	0.77 ± 0.02*	1.07 ± 0.02	1.00 ± 0.03*	1.29 ± 0.03	1.07 ± 0.02*
Heart-to-body mass ratio (%)	0.35 ± 0.01	0.30 ± 0.01*	0.30 ± 0.01	0.27 ± 0.01*	0.30 ± 0.01	0.26 ± 0.01*
Left ventricle mass (mg)	540 ± 13	693 ± 15*	810 ± 12	761 ± 22	993 ± 24	835 ± 17*
Right ventricle mass (mg)	129 ± 10	148 ± 7	218 ± 12	197 ± 12	231 ± 7	196 ± 7*

* Denotes significant difference from age-matched control, $P \leq 0.05$, (n) is the number of animals

Although the absolute mass of the gastrocnemius muscle did not differ between the fatty and lean rats during prediabetes, the gastrocnemius muscle-to-body mass ratio was lower in the fatty rats. With acute diabetes, gastrocnemius muscle mass was reduced in the fatty (F13) rats when compared to the lean controls (L13), and these differences persisted when expressed relative to body mass. Although no differences were found in the absolute mass of the soleus muscle in acute diabetes, the muscle-to-body mass ratio was lower in the fatty rats (Table 4.1). Similar differences were seen in chronic diabetes with soleus and gastrocnemius muscle masses being lower in the fatty (F20) rats compared to age-matched lean (L20) rats, and these differences persisted when expressed relative to body mass (Table 4.1). Heart mass was higher (10.4%) in the prediabetic rats, however the heart-to-body mass ratio was lower in the F7 rats compared to age-matched controls (Table 4.1). Acute and chronic diabetes resulted in decreased heart mass (6.5% and 17%, respectively) and this difference persisted when expressed relative to body mass (Table 4.1).

Soleus and gastrocnemius muscle arteriolar maximal diameters did not differ between groups at any stage in the progression of type 2 diabetes (Table 4.2). Spontaneous tone in the soleus muscle arterioles did not differ between groups during any stage in the progression of type 2 diabetes (Table 4.2). Spontaneous tone in gastrocnemius muscle arterioles was larger in the fatty rats during chronic diabetes compared to age-matched controls prior to the KCl dose relation, but at no other point in the progression of the disease, and at no time point prior to the NE dose relation (Table 4.2).

Table 4.2. Soleus and Gastrocnemius Muscle Maximal Diameters and Spontaneous Tone of 1A Arterioles from Prediabetic (7), Acute (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats Prior to Active Responses

		Muscle					
		Soleus			**Gastrocnemius**		
Age (wks)	Grp	Max Diam (μm)	Pre-KCl Tone (%)	Pre-NE Tone (%)	Max Diam (μm)	Pre-KCl Tone (%)	Pre-NE Tone (%)
7	L	97 ± 4	36.4 ± 4.8	33.5 ± 4.2	101 ± 5	30.5 ± 3.8	32.4 ± 5.2
	F	93 ± 4	37.9 ± 5.7	33.7 ± 5.5	99 ± 3	28.7 ± 6.0	22.6 ± 3.6
13	L	117 ± 5	37.9 ± 4.1	47.6 ± 4.5	126 ± 5	26.5 ± 4.0	33.6 ± 4.1
	F	115 ± 7	37.3 ± 4.4	55.1 ± 6.3	124 ± 4	24.7 ± 4.0	30.7 ± 3.2
20	L	128 ± 4	43.5 ± 7.2	40.8 ± 5.2	124 ± 4	22.9 ± 2.3	32.5 ± 5.7
	F	122 ± 5	32.6 ± 5.8	42.2 ± 5.5	119 ± 6	31.6 ± 3.7*	36.1 ± 5.3

* Denotes significant difference from age-matched control, $P \leq 0.05$

Mean arterial pressure (MAP) did not differ between fatty and lean rats during prediabetes, but was significantly elevated after the onset of overt diabetes (Figure 4.1). Plasma glucose was elevated in all fatty groups compared to age-matched controls (Figure 4.1). Plasma insulin was elevated in the prediabetic rats relative to age-matched controls, but below control values in both the acute and chronic diabetic conditions (Figure 4.1).

4.4.1 Reactivity to Norepinephrine

Vasoreactivity to NE was greater in the soleus muscle arterioles from acutely diabetic rats compared to lean age-matched controls, but not at any other stage in the progression of type 2 diabetes (Figure 4.2). Conversely, vasoreactivity to NE was greater during both pre- and chronic diabetes in gastrocnemius muscle arterioles of the fatty rats compared to lean age-matched controls, but not during acute diabetes (Figure 4.3).

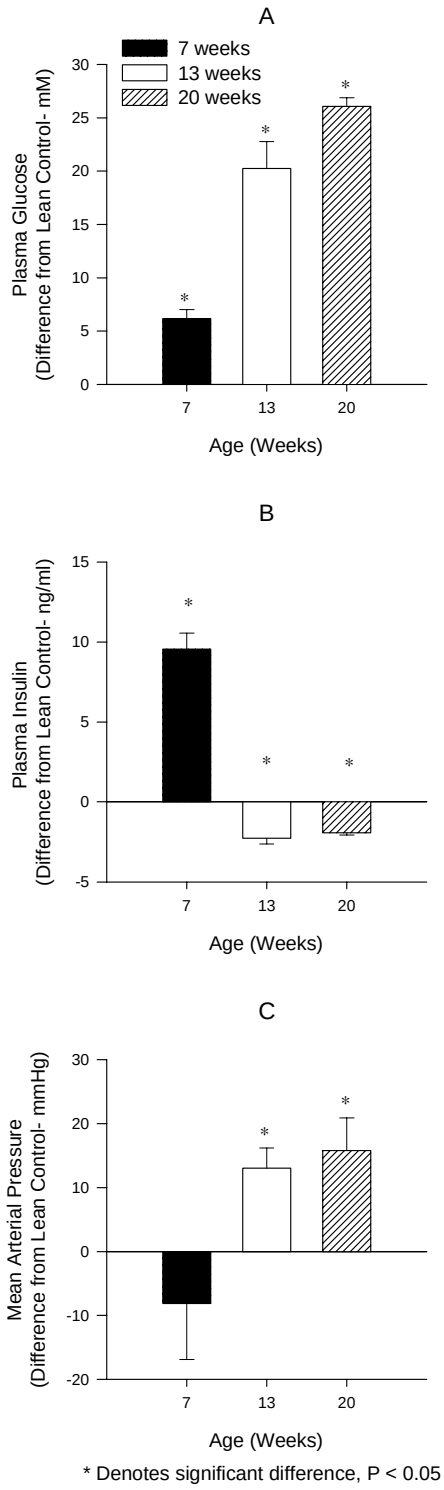


Figure 4.1. Values of Plasma Glucose Displayed as Difference from Control (A), Plasma Insulin (B) and Mean Arterial Pressure (C) of Prediabetic (7), Acutely (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats

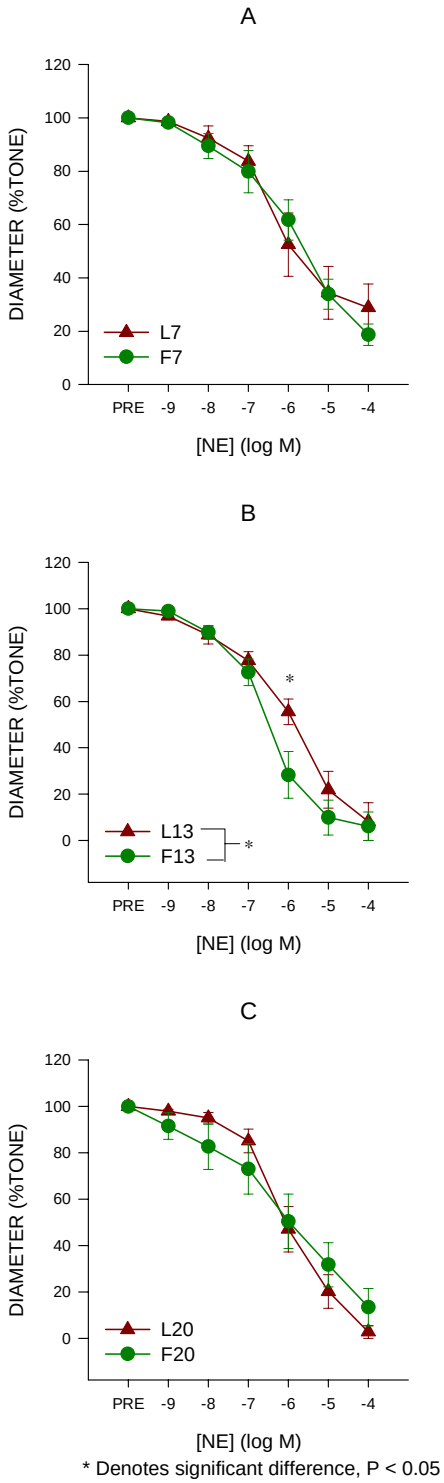


Figure 4.2. Vasoconstrictor Responses to Norepinephrine in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

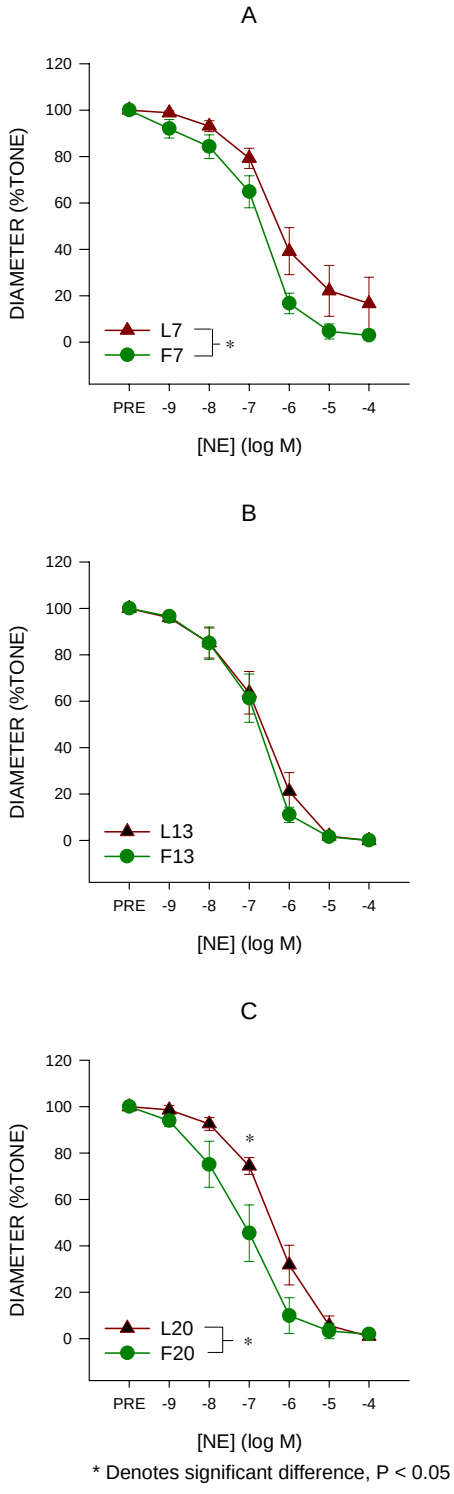


Figure 4.3. Vasoconstrictor Responses to Norepinephrine in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

4.4.2 Myogenic Response

No differences were found in the myogenic response at any stage in the progression of type 2 diabetes in arterioles from the soleus muscle (Figure 4.4). Myogenic reactivity was greater in gastrocnemius muscle arterioles of the chronically diabetic rats versus age-matched controls (Figure 4.5). No other differences were found.

4.4.3 Reactivity to Isotonic Potassium Chloride

Vasoreactivity to isotonic KCl was greater in acutely diabetic soleus muscle arterioles relative to age-matched controls (Fig 4.6). No other differences were found in reactivity to KCl in the soleus muscle arterioles. A reduced reactivity to KCl was found in gastrocnemius muscle arterioles from the acutely and chronically diabetic rats, whereas no differences occurred during prediabetes (Figure 4.7).

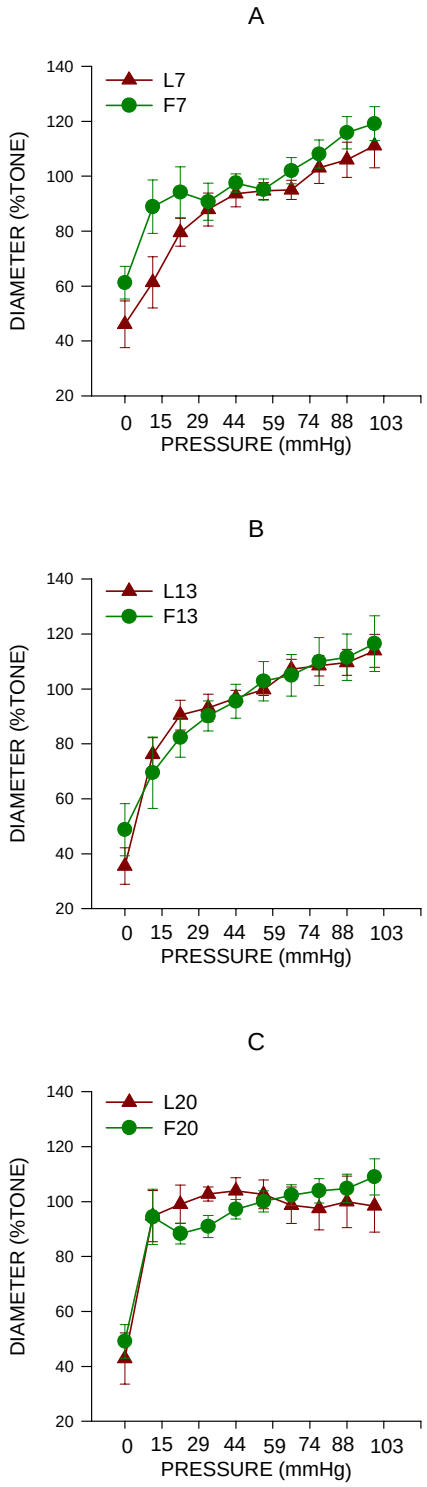


Figure 4.4. Vasoconstrictor Responses to Myogenic Stimuli in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

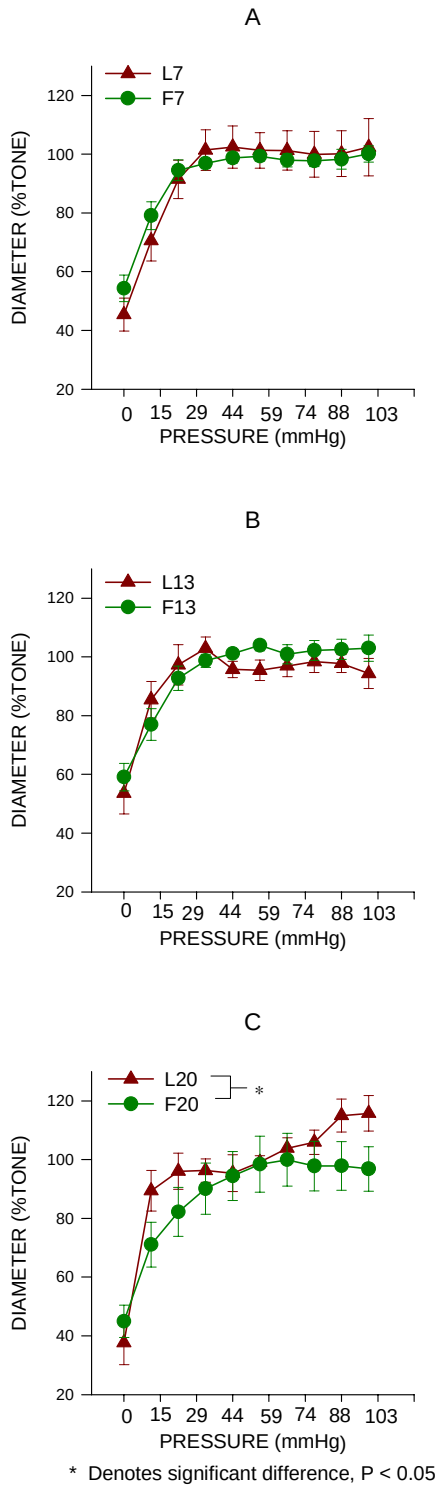


Figure 4.5. Vasoconstrictor Responses to Myogenic Stimuli in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

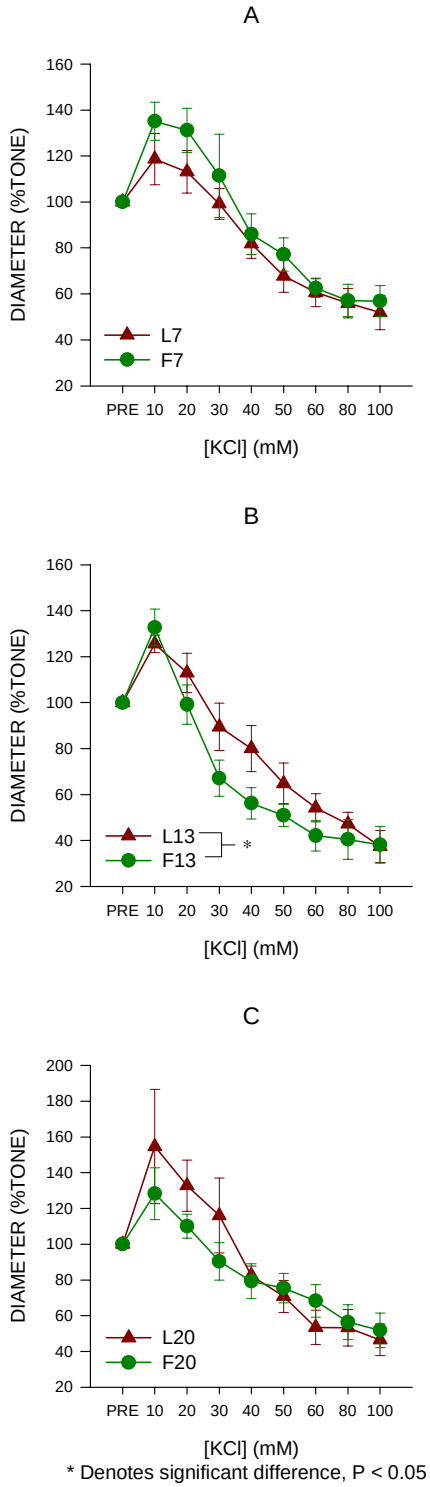


Figure 4.6. Vasoconstrictor Responses to Isotonic Potassium Chloride in Soleus Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

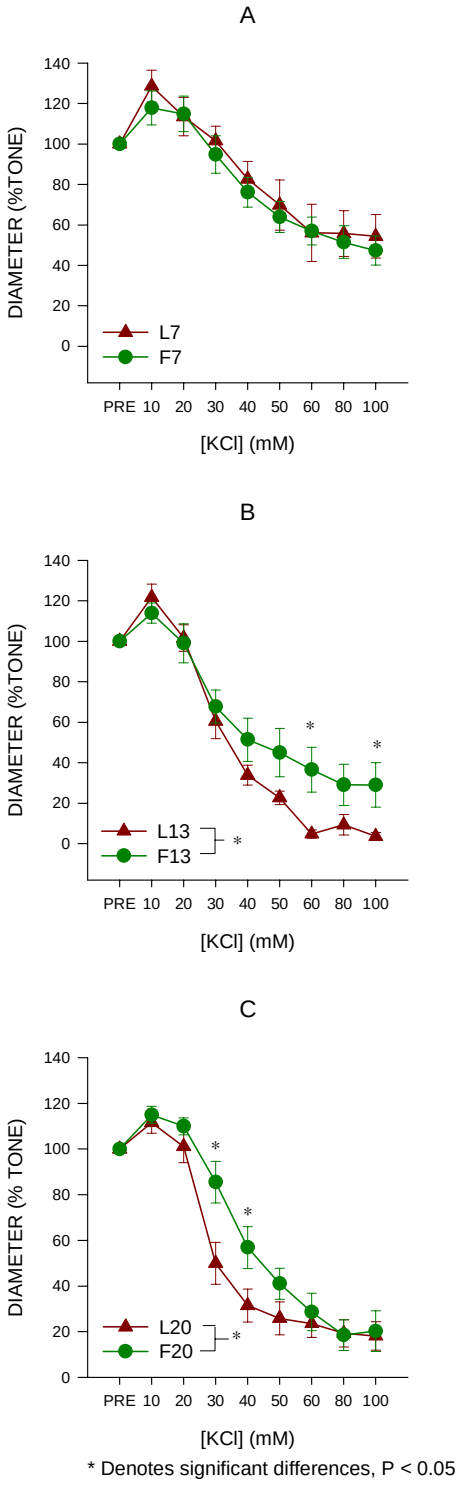


Figure 4.7. Vasoconstrictor Responses to Isotonic Potassium Chloride in Gastrocnemius Muscle Arterioles from Prediabetic (A), Acutely Diabetic (B) and Chronically Diabetic (C) Zucker Diabetic Fatty Rats and Age-Matched Control Rats

4.4.4 Vessel Morphology

No differences in maximal diameter were found in either the soleus or gastrocnemius muscle arterioles at any stage of diabetes (Table 4.3). Medial wall cross-sectional area and wall-to-lumen ratio were larger in the prediabetic gastrocnemius muscle arterioles compared to lean controls. No other differences in wall thickness or wall-to-lumen ratio were observed in arterioles from either muscle at any stage of diabetes.

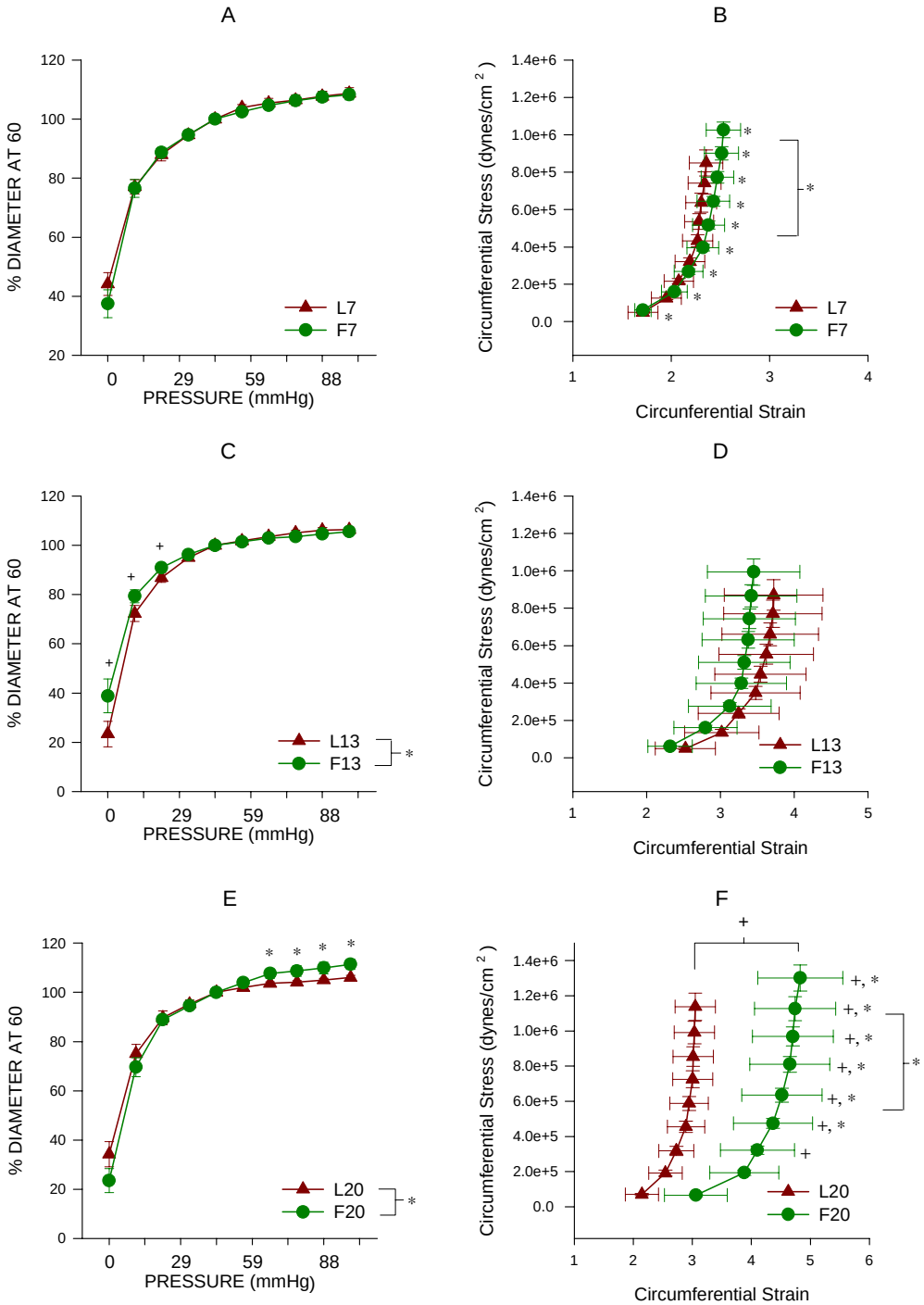
4.4.5 Passive Pressure-Diameter, Stress/Strain and Arteriolar Stiffness

During prediabetes, the passive pressure-diameter relation did not differ between the soleus muscle arterioles from the fatty or lean age-matched control rats (Figure 4.8A). However, stress/strain analysis revealed greater circumferential stress in the soleus muscle arterioles from the prediabetic rats (Figure 4.8B).

Table 4.3. Morphological Characteristics of 1A Arterioles from the Soleus and Gastrocnemius Muscles of Prediabetic (7), Acute (13) and Chronically (20) Diabetic Fatty (F) and Lean (L) Age-Matched Control Zucker Diabetic Fatty Rats

Soleus						
Weeks of Age	Group (n/n)	Maximal Diameter (μm)	Wall Thickness (μm)	Wall Cross Sectional Area (μm^2)	Wall to Lumen Ratio (%)	Stiffness Parameter
7	L (15/9)	94 ± 6	7.9 ±0.9	1218 ± 193	9.6 ± 1.1	5.8 ± 0.8
	F (16/11)	108 ± 4	7.5 ± 1.0	1615 ± 110	9.0 ± 1.9	5.5 ± 0.7
13	L (11/9)	117 ± 10	9.2 ± 1.4	2115 ± 390	7.8 ± 0.5	3.4 ± 0.8
	F (12/8)	117 ± 7	8.2 ± 1.0	2275 ± 621	7.0 ± 0.4	3.7 ± 0.5
20	L (12/11)	126 ± 8	7.7 ± 1.0	1863 ± 214	6.0 ± 0.8	5.9 ± 1.0
	F (11/9)	128 ± 7	7.0 ± 0.5	1807 ± 301	5.6 ± 0.3	3.3 ± 0.5*
Gastrocnemius						
Weeks of Age	Group (n/n)	Maximal Diameter (μm)	Wall Thickness (μm)	Wall Cross Sectional Area (μm^2)	Wall to Lumen Ratio (%)	Stiffness Parameter
7	L (14/9)	105 ± 6	10.0 ± 0.9	2142 ± 358	8.8 ± 0.8	7.4 ± 1.3
	F (14/8)	112 ± 7	12.2 ± 0.4	3780 ± 749*	11.8 ± 0.8*	6.1 ± 1.1
13	L (12/8)	126 ± 7	13.0 ± 1.8	3772 ±848	10.4 ± 1.2	4.8 ± 0.7
	F (11/8)	131 ± 11	11.4 ± 1.1	3013 ± 453	9.6 ± 1.2	4.7 ± 0.8
20	L (12/9)	138 ± 9	10.6 ± 0.9	2528 ± 313	8.1 ± 0.8	6.8 ± 1.6
	F (11/9)	127 ± 11	10.7 ± 1.1	2651 ± 410	10.3 ± 1.1	5.5 ± 1.0

* Denotes significant difference from age-matched control, $P \leq 0.05$, (n/n) number of vessels measured for diameter / number of vessels measured with histology



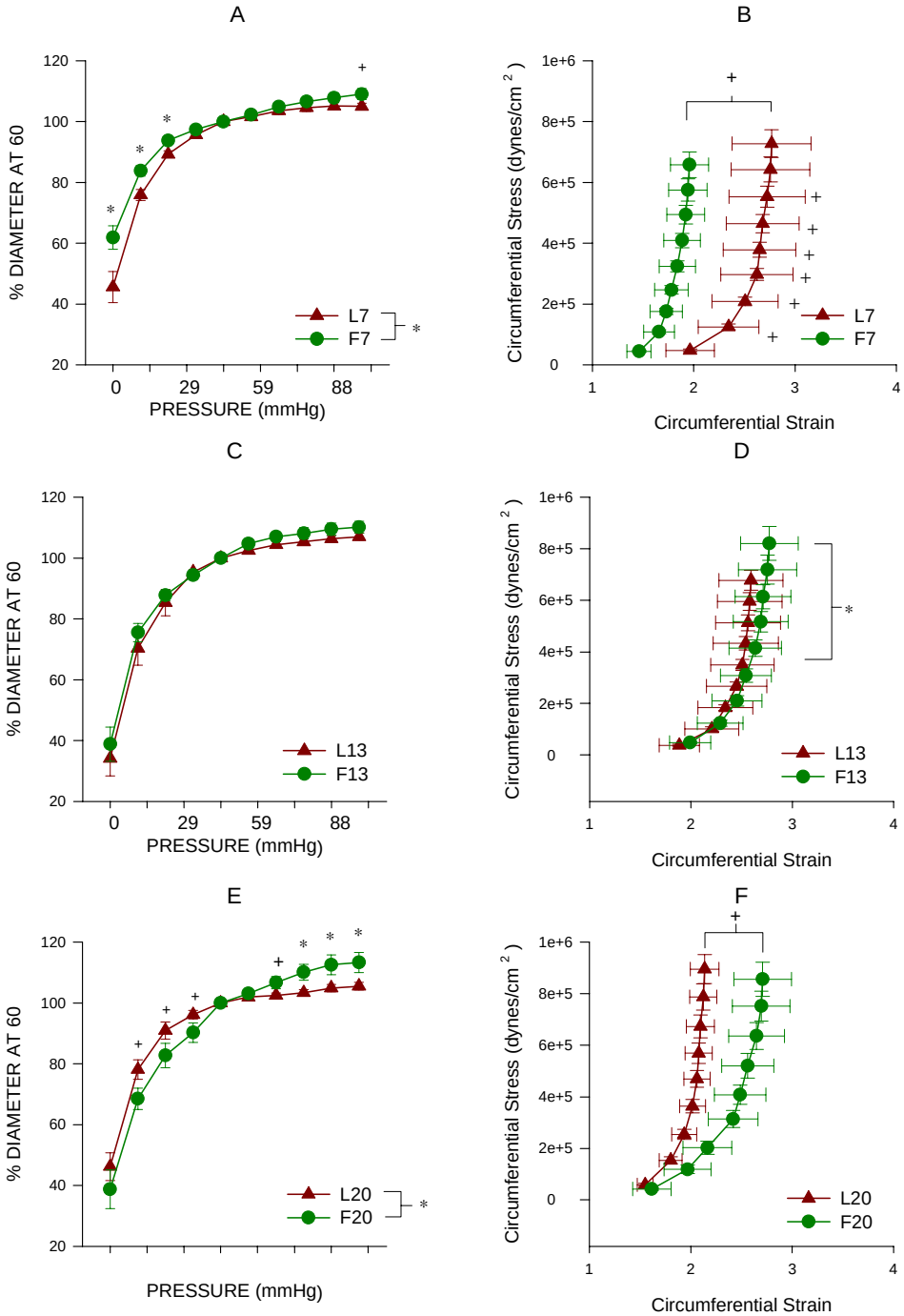
* Denotes difference in passive diameter (panels A,C,E) or circumferential stress (panels B,D,F) P < 0.05,
+ Denotes near significant difference in passive distension (panels A,C,E, P < 0.10)
or significant difference in circumferential strain (panels B.D,F) P< 0.05

Figure 4.8. Passive Pressure-Diameter Relationships (A,C,E) and Circumferential Stress and Strain Relationships (B,D,F) of Soleus Muscle Arterioles from Prediabetic, Acutely and Chronically Diabetic and Lean Age-Matched Control Zucker Diabetic Fatty Rats

Arterioles from the gastrocnemius muscle of prediabetic rats displayed larger relative diameters at low intraluminal pressures when compared to age matched control vessels (Figure 4.9A), and this was reflected in a lower circumferential strain in these vessels (Figure 4.9B). No differences in the incremental stiffness were observed during prediabetes in arterioles from either the soleus or gastrocnemius muscle (Table 4.3).

Larger relative diameters were observed in soleus muscle arterioles from the acutely diabetic rats compared to age-matched controls during assessment of the passive pressure-diameter relation (Figure 4.8C). However, no differences in the circumferential stress or strain were observed (Figure 4.8D). The passive-pressure diameter relation of gastrocnemius muscle arterioles from acutely diabetic rats was unaltered (Figure 4.9C). However, circumferential stress was greater in these arterioles (Figure 4.9D). No differences in incremental stiffness were observed during acute diabetes in arterioles from either muscle examined (Table 4.3).

Larger relative diameters were observed in response to high intraluminal pressure in soleus muscle arterioles from chronically diabetic rats (Figure 4.8E). Concurrently, circumferential strains were greater (Figure 4.8F). In addition, circumferential stress was also higher in the soleus muscle arterioles of the chronically diabetic rats (Figure 4.8F), resulting in a lower incremental stiffness in these arterioles (Table 4.3). Larger relative diameters were also observed in the gastrocnemius muscle arterioles of the chronically diabetic rats pressures (Figure 4.9E), and despite a normalization in circumferential stress in these arterioles,



* Denotes difference in passive diameter (panels A,C,E) or circumferential stress (panels B,D,F) P < 0.05,
\+ Denotes near significant difference in passive distension (panels A,C,E, P < 0.10)
or significant difference in circumferential strain (panels B.D,F) P< 0.05

Figure 4.9. Passive Pressure-Diameter Relationships (A,C,E) and Circumferential Stress and Strain Relationships (B,D,F) of Gastrocnemius Muscle Arterioles from Prediabetic, Acutely and Chronically Diabetic and Lean Age-Matched Control Zucker Diabetic Fatty Rats

circumferential strain was higher (Figure 4.9F). No differences in incremental stiffness were observed in the chronically diabetic gastrocnemius muscle arterioles (Table 4.3).

4.5 Discussion

The purpose of the present study was to determine 1) whether type 2 diabetes alters vasoconstrictor reactivity of skeletal muscle arterioles, 2) whether these putative changes precede the development of elevated arterial pressure, 3) whether structural vascular remodeling and alterations in the passive mechanical properties of skeletal muscle arterioles occur during the progression of type 2 diabetes, and 4) whether vasoconstrictor reactivity and passive mechanical properties from arterioles of high-oxidative and low-oxidative glycolytic skeletal muscles are differentially affected by the progression of type 2 diabetes. The data demonstrates that increases in vasoconstrictor reactivity in the low-oxidative glycolytic gastrocnemius muscle arterioles occurs during prediabetes, and therefore, precedes both the development of overt diabetes and elevated arterial pressures in the ZDF rat. The results also indicate that this prediabetic increase in vascoconstrictor reactivity to NE and KCl is unique to the low-oxidative glycolytic skeletal muscle arterioles, and that these enhanced responses are variable throughout the progression of the disease. In contrast, arterioles from the high-oxidative soleus muscle only have higher vascoconstrictor reactivity during the acute diabetic condition, and this increase in vasoconstrictor responsiveness coincides with the emergence of elevated arterial pressure. Finally, the data

demonstrates that arteriolar morphological and mechanical properties are altered during the prediabetic condition.

Chronic type 2 diabetes is often accompanied by hypertension. In fact, hypertension is frequently present at the time of diabetes diagnosis, and may be the result of enhanced arteriolar vasoconstrictor responsiveness present during the prediabetic state. During the progression of type 2 diabetes the external environment of the arterioles is in flux as a result of the patient's changing metabolic status, resulting in disparate stimuli for alterations in vascular structure and vasoreactivity throughout the progression of the disease. During prediabetes, when hyperinsulinemia and insulin resistance are coexistant, it is unknown whether increased vascular resistance will result from an insulin resistance-mediated diminution of insulin as a vasodilator (50) or if hyperinsulinemia leads to an increase in vasoconstriction via activation of the sympathetic nervous system (18, 73, 82, 104). Despite an established relation between insulin resistance and hypertension (29, 94), the role of altered vasoreactivity in the development of insulin resistance-related hypertension has not been established.

Enhanced vasoconstrictor responsiveness in the prediabetic state could contribute to the development of hypertension prior to overt diabetes. Result from the present study demonstrate elevated vasoconstrictor reactivity in low-oxidative glycolytic skeletal muscle in prediabetic ZDF rats. Previous examinations of the vasoconstrictor responsiveness of arterioles from insulin resistant humans and animals have produced inconsistent results. For example, vasoconstriction to norepinephrine (NE) has been shown to be either unchanged in insulin resistant

human subcutaneous fat arterioles (97) or increased in gracilis muscle arterioles from obese Zucker rats (OZR) (105), a low-oxidative glycolytic skeletal muscle. Neither the OZR rat nor insulin resistant humans are necessarily appropriate models of the prediabetic condition, since both may maintain subclinical hyperglycemia throughout their lifespan. For example, the progression of the OZR rat to overt diabetes has not been consistent in the literature, with some investigator reporting severe hyperglycemia (33-37, 105) while others report little to no elevation in plasma glucose (14, 53, 75, 106, 128). Moreover, even in cases where the plasma glucose is elevated, plasma insulin concentrations are often unknown (33-37, 105), making it difficult to assess whether the external environment of these vessels more closely resembles that of the prediabetic or overt diabetic condition.

Data in the present study are similar to those of previous investigators (105) in that vasoconstrictor responses to NE were enhanced in the low-oxidative glycolytic skeletal muscle during both pre- and chronic diabetes. This enhanced adrenergic vasoreactivity in the prediabetic state may indeed contribute to the hypertension which occurs with overt diabetes. Interestingly, the adrenergic vasoreactivity of the high-oxidative soleus muscle arterioles is unaffected during prediabetes, but is elevated during the acutely diabetic condition, which coincides with a large reduction in plasma insulin, increase in the level of hyperglycemia, and the development of elevated mean arterial pressure. Thus it appears that arterioles from high-oxidative and low-oxidative glycolytic skeletal muscles respond differently to these changing conditions.

Increases in adrenergic vasoconstrictor responsiveness in the low-oxidative glycolytic muscle arterioles precede and may be causally related to the development of elevations in arterial pressure, as this skeletal muscle is the predominant type in muscles, making up approximately 70% of the total skeletal muscle mass in rats (24). Increase s in adrenergic reactivity in the high-oxidative soleus muscle arterioles may be the consequence of either this elevated arterial pressure, the reduction in plasma insulin, or the pronounced elevation in plasma glucose.

Despite either unchanged or elevated adrenergic and myogenic vasoreactivity, KCl-induced vasoconstriction was found to be both increased and decreased at different time points during the progression of type 2 diabetes, even within the same muscle. The decreases in KCl-induced vasoconstriction present in the low-oxidative glycolytic gastrocnemius muscle occurred concurrently with the increases in both the adrenergic vasoreactivity and myogenic responsiveness, indicating that a generalized loss in smooth muscle contractility does not occur, but rather specific signaling mechanisms are adversely altered during acute and chronic diabetes. Similar variability in vasoconstrictor responses have been reported in the literature, for example, vasoconstriction to angiotensin II and endothelin-1 have been shown to be either increased (70), unchanged (105) or decreased (122) with overt disease. Moreover, myogenic constriction has been shown to be both increased in gracilis muscle arterioles from OZR rats (35) and decreased in subcutaneous adipose tissue arterioles from type 2 diabetic patients (97). Nonetheless, the present study demonstrates that an increase in the vasoconstrictor

reactivity of low-oxidative glycolytic skeletal muscles exists during the prediabetic state and may contribute to the later development of elevated mean arterial pressure.

In addition to changes in the vasoreactivity of the skeletal muscle arterioles, alterations in the vascular structure and mechanical properties may also contribute to the development of hypertension in type 2 diabetes. Stiffer arteries independent of increases in wall thickness have been reported in patients with impaired fasting glucose (95). Moreover, changes in vessel wall structural characteristics have been observed in type 2 diabetic human adipose arterioles (87, 97) and type 2 diabetic coronary arterioles (125) and have been proposed to contribute to peripheral insulin resistance and hypertension seen with type 2 diabetes. In these studies, increases in medial wall thickness, medial cross-sectional area, and wall-to-lumen ratio have been reported. However, these structural adaptations have not been consistently observed, as others have reported decreases in medial wall thickness, wall-to-lumen ratio, and decreased distensibility in gracilis muscle arterioles from OZR rats (34). Unlike the previous study (34), medial wall cross-sectional area and wall-to-lumen ratio were increased in the low-oxidative glycolytic gastrocnemius muscle arterioles during prediabetes in the present study. Moreover, an increase in the distensibility of these arterioles to low intraluminal pressures was observed. In contrast, no changes in vessel wall morphology were observed in the soleus muscle arterioles at any timepoint or in the gastrocnemius muscle arterioles at any other stage in the progression of type 2 diabetes, which is suggestive of a specific sensitivity of the gastrocnemius

arterioles to systemic hyperinsulinemia that was not present in the high-oxidative soleus muscle arterioles. This increase in medial wall cross-sectional area may have placed a physical limitation on changes in arteriolar diameter and resulted in the diminished circumferential strains observed in these vessels.

Examination of the circumferential stresses and strains of the gastrocnemius muscle arterioles throughout the progression type 2 diabetes indicate that the mechanical properties of the vessel are differentially altered by their changing external environment. Although smooth muscle hypertrophy is a consequence of only the prediabetic state, the circumferential strain in the gastrocnemius arterioles is progressively increasing relative to the age-matched controls as overt diabetes develops and is maintained. Moreover, the increased wall stress experienced during acute diabetes in these arterioles may be contributing to a rearrangement of vascular components (52) and, subsequently the large increase in distensibility and circumferential strain observed in the chronic diabetic state.

The soleus muscle arteriolar mechanical properties appear to be most effected by chronic diabetes in that the most dramatic alteration in vessel wall mechanics was observed in the chronically diabetic soleus muscle arterioles. The data demonstrate increases in both circumferential stress and strain that result in a decreased arteriolar stiffness. This is consistent with vascular changes observed in other hypertensive rat models, for example, an early decrease in stiffness of mesenteric small arteries has been reported from spontaneously hypertensive rats (SHR) (65). Moreover, the stiffness characteristics of mesenteric arterioles differ as a result of branch order in the SHR rat, such that in stroke prone SHR rats

second-order mesenteric arterioles exhibit increased stiffness while third-order arterioles of the same vascular bed display decreased stiffness (46). Thus, although the gastrocnemius muscle arteriolar morphology appears to be sensitive to the prediabetic state, the passive mechanical properties of the arterioles of the soleus muscle are most affected by chronic diabetes at which time elevations in mean arterial pressure may also be contributing to altered vessel wall mechanics.

In summary, increased adrenergic vasoreactivity is present in arterioles from both high-oxidative and low-oxidative glycolytic skeletal muscles, and this enhanced adrenergic reactivity in low-oxidative glycolytic muscle arterioles precedes the development of elevated arterial pressure. Enhanced vasoconstrictor responsiveness is not a generalized phenomenon, since increases in myogenic responsiveness are not present until chronic diabetes and then only in the gastrocnemius muscle arterioles, and KCl-induced vasoconstriction is both enhanced and diminished depending on the stage in the progression of diabetes and skeletal muscle being examined. Moreover, prediabetes results in a hypertrophic response in arterioles of the low-oxidative glycolytic gastrocnemius muscle arterioles that is not present following the development of overt diabetes. Lastly, although no changes in vessel morphology were observed with overt diabetes, the chronic diabetic condition led to increased distensibility in arterioles from both high-oxidative and low-oxidative glycolytic skeletal muscles, and a decreased stiffness in the high-oxidative muscle arterioles. If these findings are relevant to the development of type 2 diabetes in humans, they indicate that enhanced adrenergic vasoconstrictor reactivity in the prediabetic state concurrent with altered

vascular mechanical properties may be contributing to the development of both overt type 2 diabetes and hypertension.

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1 Overview

Using the ZDF model, we were able to examine differences in the vascoconstrictor and vasodilator responsiveness, as well as passive mechanical properties of resistance arterioles from high-oxidative and low-oxidative glycolytic skeletal muscles throughout the development of type 2 diabetes. Ultimately, it is not an alteration to any specific agonist of vasoconstriction or vasodilation that appears important singly, but rather how these influences along with structural adaptations might interact to influence total peripheral resistance and glucose uptake.

To that end, comparisons were made between the fatty genotype of the ZDF rat and its lean age-matched control in order to account for changes in vessel function that may be occurring as a result of maturation independent of the development of type 2 diabetes. Differences in vasomotor responses over time were selected to represent three distinct conditions in the progression to overt type 2 diabetes. The 7 week old ZDF animals represent the prediabetic condition, as both plasma insulin and glucose are significantly elevated. By 13 weeks of age, the fatty rats experience a significant loss of pancreatic function resulting in profound hyperglycemia with normal to below normal plasma insulin concentrations. The loss of pancreatic function is reported to occur at approximately 12 weeks in this animal, making this time point representative of an acutely diabetic condition.

Lastly, the 20 week old fatty rats remain hyperglycemic and hypoinsulinemic and are representative of uncontrolled chronic diabetes.

5.2 Prediabetes

Maximal vasodilator responsiveness to ACh and intraluminal flow was unaltered in prediabetes in both the soleus and gastrocnemius muscle 1A arterioles. However, the mechanisms by which the gastrocnemius muscle arterioles achieve ACh-induced vasodilation were altered in the fatty rats. Blockade of NOS by L-NAME failed to reduce the vasodilator response in the gastrocnemius muscle arterioles from the prediabetic and acute diabetic fatty rats, in contrast to the approximately 50% reduction in ACh-induced vasodilation in the control arterioles. In addition, blockade of both NOS and cyclooxygenase failed to further reduce the vasodilation to ACh in the control rats, but produced a reduction in vasodilation in the fatty rats similar to that seen in the controls with L-NAME alone. Thus, there was a shift in the endothelium signaling mechanisms in the prediabetic fatty rats from a primarily NO-mediated vasodilation to a prostaglandin-mediated vasodilation. Accompanying this decrease in the endothelial nitroxidergeric signaling was an increased responsiveness to SNP-induced vasodilation, indicating that smooth muscle reactivity to NO is enhanced in these arterioles. This enhanced sensitivity to NO may be the result of a diminished stimulated, but not basal bioavailability of NO in the gastrocnemius muscle arterioles. This assertion is supported by the fact the NOS blockade significantly increased spontaneous tone in all vessels, indicating that under non-stimulated conditions, NO still plays a role in

the modulation of basal vasoconstrictor tone in the fatty gastrocnemius muscle arterioles despite a lack of stimulated activity.

A number of possibilities exist to explain this lack of stimulated NO-mediated vasodilation. Acute hyperglycemia has been shown to decrease eNOS and result in diminished NO production by cultured endothelial cells (57, 59). In type 1 diabetic rats, it does not appear that a loss of NOS protein explains decrements in NO production, but rather a deficiency in a cofactor necessary for its production, tetrahydrobiopterin (72). AGEs in type 2 diabetes may induce oxidative stress production or directly quench NO and reduce its ability to relax the smooth muscle (17, 26, 119). At the present time, the precise mechanism inducing the shift from endothelial NO to prostaglandin vasodilation is unknown.

Vasoconstrictor responsiveness was unaltered in the soleus muscle arterioles from the prediabetic fatty rats. However, the gastrocnemius muscle arterioles displayed an enhancement in response to the receptor-mediated agonist NE with no change in the non-receptor-mediated vasoconstriction to KCl. A possible explanation for this difference may be the loss in stimulated NO production. Nitric oxide is released from the vascular endothelium in response to NE activation of the $alpha_2$-adrenoreceptors (40, 117) as well as in response to myogenic activation (117). A loss of this modulating influence to buffer vasoconstrictor reactivity may explain the enhancement of vasoconstriction seen with these vasoconstrictor stimuli, which is consistent with an unchanged smooth muscle response to KCl. Also possible is a change in the structural properties of

the vessel that may alter the mechanical distensibility of the vessels and alter active vasoconstrictor behavior.

The passive pressure-diameter relation, measures of circumferential stress and strain and incremental stiffness were assessed to determine the structural behavior of the vascular wall. The passive pressure-diameter relation assesses the distensibility of the vessel segment to increases in increasing intraluminal pressure, while the circumferential stress and strain and incremental stiffness are calculated parameters that assess the mechanical properties of the medial wall in response to increases in intraluminal pressure. In soleus muscle arterioles from prediabetic rats there were no changes in the passive pressure-diameter relation. And even though there was no change in medial wall morphology of the soleus muscle arterioles, there was an increase in circumferential wall stress in response to increasing intraluminal pressure.

The gastrocnemius muscle arterioles responded to increases in intraluminal pressure differently from that of the soleus muscle arterioles, such that at low intraluminal pressures the arterioles were more distensible in the fatty rats than were those from the lean age-matched controls. During this stage of diabetes, increases in medial wall cross-sectional area and wall-to-lumen ratio were observed in the gastrocnemius muscle arterioles of the fatty rats. Analysis of the circumferential stress, strain and incremental stiffness of the gastrocnemius arterioles revealed a decreased circumferential strain in response to changes in intraluminal pressure. However, no differences were observed in the slope of the

relation of these two characteristics, resulting in no change in vessel wall incremental stiffness in prediabetic gastrocnemius muscle arterioles.

Although elevated arterial pressure is not present in prediabetes, enhanced adrenergic vasoconstriction may be predisposing the rats to its development with overt diabetes. Previous investigators have shown sympathetic nerve activity to be elevated in response to insulin infusion in normal healthy humans, despite the maintenance of normal arterial pressures (32). Moreover, adrenergic vasoconstrictor responses have been shown to be elevated in the forearm of type 2 diabetic patients in response to norepinephrine administration (49). A similar enhancement in the NE-mediated vasoconstriction was observed in the present study. Interestingly, this enhancement is present only in arterioles from the low-oxidative glycolytic gastrocnemius muscle, which show a greater level of alpha-adrenergic than that seen in high-oxidative skeletal muscles (62). Thus, if sympathetic activity is similarly enhanced in the ZDF rat, the combined effect of enhanced sympathetic drive and enhanced adrenergic responsiveness would be a large increase in vasoconstrictor tone in the arterioles of low-oxidative skeletal muscles, which make up approximately 70% of the total skeletal muscle mass (24) and may contribute to increased regional vascular resistance and alterations in blood flow distribution. In addition to a role in arterial pressure, these putative changes in blood flow distribution could also contribute to an exacerbation of insulin resistance by diverting blood away from the skeletal muscle, thereby limiting glucose and insulin delivery and thus glucose disposal. The soleus muscle arterioles appear to be largely protected from alterations in function during

prediabetes, although increases in circumferential stress may be predisposing these arterioles to the altered mechanical properties observed in chronic type 2 diabetes.

5.3 Acute Diabetes

Maximal vasodilator response to ACh was unaffected by acute diabetes in both the soleus or the gastrocnemius muscle arterioles. However, as occurred during prediabetes, the gastrocnemius muscle arterioles lacked the NO component of ACh-induced vasodilation that was present in the lean control rats. Nitric oxide synthase inhibition through L-NAME produced approximately a 50% reduction in vasodilation to ACh in the lean gastrocnemius arterioles, but was without effect in arterioles from the fatty rats. Combination blockade with L-NAME and INDO reduced the ACh-induced vasodilation in the gastrocnemius muscle arterioles of the fatty rats, but failed to further diminish vasodilation in the lean age-matched controls beyond what was observed with NOS blockade alone. L-NAME blockade produced an increase in spontaneous tone in soleus and gastrocnemius muscles arterioles from both the lean and fatty rats at 13 weeks of age. Thus, the shift from NOS to cyclooxygenase appeared to affect stimulated endothelium-dependent vasodilation and not basal tone.

Flow-mediated vasodilation was significantly diminished in the soleus muscle arterioles, but unchanged in the gastrocnemius arterioles from diabetic rats compared to their lean age-matched controls. Muller-Delp et al. (2002) (77) and Schrage et al. (2002) (98) have previously demonstrated that flow-induced vasodilation is mediated through an NO-dependent mechanism in soleus muscle 1A

arterioles in Fisher 344 and Sprague-Dawley rats, respectively. If flow-induced vasodilation occurs similarly through a nitroxidergic mechanism in the ZDF rat, then a diminished flow-induced vasodilator response with a normal ACh-induced vasodilation is indicative of an alteration in the flow sensitive pathway in the vascular endothelium upstream of NO production. Thus, alterations in vessel morphology or shear/stretch responsive receptors may be responsible for this specific loss of endothelial function.

Increased vasoconstrictor responsiveness was observed to both KCl and NE in the soleus muscle arterioles from the acutely diabetic rats. However, no changes in myogenic vasoconstriction were found in the soleus muscle arterioles during this stage in the progression of diabetes. A different effect was found in the gastrocnemius muscle arterioles, with a diminished vasoconstriction to increasing concentrations of isotonic KCl in the acutely diabetic gastrocnemius arterioles. However, the responsiveness to NE and myogenic vasoconstriction was unaltered in the gastrocnemius muscle arterioles of the acutely diabetic rats relative to lean age-matched controls, and this lack of change may be the result of a reduced NO-mediated modulation of the vasoconstriction induced by increasing intraluminal pressure and NE.

No differences in medial wall thickness, cross-sectional area, wall-to-lumen ratio, or luminal diameter occurred in arterioles from either muscle in the acutely diabetic rats. A small but significant increase in distensibility to low intraluminal pressures occurred in the acutely diabetic soleus muscle arteriolar passive pressure-diameter relation. However, no changes in the mechanical properties of the vessel

wall were found. Conversely, no changes in the passive pressure-diameter relation were found in the gastrocnemius muscle arterioles of the acutely diabetic rats, although circumferential stress was increased in these vessels.

With the onset of overt diabetes, the external environment of the arterioles has been significantly altered such that, hyperinsulinemia is absent and marked hyperglycemia and elevated arterial pressure are both present. In response to these stimuli, the arterioles of the high-oxidative soleus muscle demonstrate a greater degree of dysfunction than do those of the low-oxidative glycolytic gastrocnemius muscle. For example, the altered adrenergic vasoconstriction that occurred in prediabetes in the gastrocnemius is absent and KCl-induced vasoconstriction is diminished. Whereas, in the soleus muscle arterioles, adrenergic and KCl-induced vasoconstrictor responsiveness are increased and flow-induced vasodilation is decreased. The combined effect of these alterations in soleus muscle vascular function may result in a net increase in vasoconstrictor tone, increased regional vascular resistance, and, subsequently, increased mean arterial pressure. In addition, increased passive distention of the soleus muscle arterioles manifests during acute diabetes, and appears to be the beginning of altered mechanical properties that worsen as the disease is prolonged. Thus, in acute diabetes increases in the vasoconstrictor responses and diminished vasodilator capacity in the arterioles of the high-oxidative soleus muscle may predominate, and in the presence of largely unaltered structure and function in the low-oxidative glycolytic skeletal muscle arterioles, may contribute to the elevations in arterial pressure that emerge concurrently with overt disease.

5.4 Chronic Diabetes

Chronic diabetes resulted in a reduced maximal vasodilator response to both ACh and intraluminal flow in the soleus muscle arterioles. The reduced ACh-induced vasodilator response is the consequence of a loss of the NOS-dependent signaling mechanism. Vasodilation in the gastrocnemius muscle arterioles remained unaffected by diabetes; however, at this age, L-NAME did not reduce the vasodilation to ACh in either the lean or fatty rats.

Vasoconstrictor responsiveness was unaffected by chronic diabetes in the soleus muscle arterioles compared to their age-matched counterparts. In the gastrocnemius muscle, however, despite a diminished responsiveness to KCl (30 and 40 mM KCl), vasoconstrictor responsiveness to NE and increasing intraluminal pressure were both higher.

No changes in vessel morphology occurred in either muscle with chronic diabetes. However, the passive pressure-diameter relation, circumferential stress and strain, and incremental stiffness revealed changes in the structural characteristics and mechanical properties of the vessels. In arterioles from both the soleus and gastrocnemius muscles, the passive pressure-diameter relation differed between the fatty and lean rats such that at high intraluminal pressures (77-99 mmHg) arterioles from both muscles display an increased relative diameter. In the soleus muscle arterioles, both circumferential stresses and strains were increased in the fatty rats compared to lean age-matched controls, and the slope of the relations indicated a reduction in vessel stiffness in the soleus muscle arterioles from the chronically diabetic rats. Gastrocnemius muscle arterioles from the chronically

diabetic rats also showed increased circumferential strains, but no differences were found for either circumferential stress or incremental stiffness.

With chronic diabetes, the external environment of the arterioles is similar to acute diabetes, but the arterioles have been exposed to the elevations in arterial pressure and marked hyperglycemia for a prolonged period. Under these conditions, arteriolar dysfunction occurs in both the soleus and gastrocnemius muscle arterioles, although altered mechanical properties of the arterioles is more pronounced in the soleus muscle arterioles. Chronic diabetes results in enhanced vasoconstrictor responses in the low-oxidative glycolytic gastrocnemius muscle arterioles and diminished vasodilator responses in the high-oxidative soleus muscle arterioles, the combined effect of which is likely to increase the vascular resistance of the skeletal muscle and contribute to the elevated arterial pressure. Moreover, the presence of type 2 diabetes for a prolonged period of time resulted in alterations in the passive mechanical properties of both soleus and gastrocnemius muscle arterioles, and may be indicative of alterations in the matrix components of the arterioles since wall morphology appears unaltered. Therefore, although chronic diabetes appears to present arterioles with a similar external environment to that which is experienced in acute diabetes, the prolongation of these stimuli results in different arteriolar adaptations than occurred in the acute phase of diabetes.

5.5 Endothelial Dysfunction in High-oxidative Skeletal Muscle Arterioles and the Emergence of Elevated Mean Arterial Pressure

The ZDF rat is a particularly useful model of type 2 diabetes, not only because of its predictable progression to overt disease, but also because the development of diabetes is accompanied by many of the same conditions that coexist in type 2 diabetic patients, including the coincidence of elevations of arterial pressure. Hypertension is often present at the time of diagnosis in type 2 diabetes patients (113), however, the slow, often silent progression of the disease may lead many patients to go undiagnosed for long periods of time even after frank diabetes has become manifest. With this model of type 2 diabetes it is possible to examine the temporal relation of alterations in arteriolar reactivity and elevations in arterial pressure.

Obesity and hyperinsulinemia have been associated with increases in sympathetic nerve activity (18, 73) and are both associated with the prediabetic state. This increase in sympathetic drive may be differentially affecting arterioles from high-oxidative and low-oxidative glycolytic skeletal muscles and contribute to endothelial dysfunction associated with overt diabetes. Responsiveness to NE is enhanced only in the gastrocnemius arterioles, setting the stage for a large increase in vasoconstriction to an increased NE availability under conditions of elevated sympathetic nerve activity. As previous investigators have shown, arteriolar and capillary recruitment in response to both muscular contraction and pharmacological agonists can vary between microvessels of similar architecture even within a single muscle (69, 109). It is reasonable to predict that, in the presence of a normal

responsiveness to NE and normal myogenic tone in the soleus muscle arterioles, a greater than normal percentage of the blood flow may be directed towards the high-oxidative skeletal muscles or towards other tissue that will not dispose of glucose during prediabetes, owing to this enhanced vasoconstrictor tone in the low-oxidative glycolytic skeletal muscle. Although a shunting toward the predominately high-oxidative soleus muscle could positively affect glucose disposal and therefore insulin resistance, it could also increase shear stress in these arterioles, increasing endothelial damage and leading to subsequent dysfunction. Such a sequence of events is not dissimilar from that proposed in the hemodynamic hypothesis of type 1 diabetic vascular complications, in which early increases in capillary pressure are proposed to enhance shear stress and lead to endothelial dysfunction and structural adaptations in the microcirculation (83). Endothelial dysfunction, as evidenced by reduced flow-induced dilation, is indeed present in type 2 diabetes, and is coincident with both the development of overt diabetes and elevated mean arterial pressure in this model. Moreover, this endothelial dysfunction persists throughout the progression of the disease, even after vasoconstrictor responses have normalized in the same skeletal muscle. However, this endothelial dysfunction appears to be limited to the arterioles of the high-oxidative soleus muscle.

5.6 Alterations in Arterioles from the Low-oxidative Glycolytic Gastrocnemius Muscle

Endothelial responses in the gastrocnemius muscle arterioles are protected from diabetes-induced dysfunction. In fact, although there appears to be a loss of

the NO-mediated portion of ACh-induced dilation in the prediabetic and diabetic arterioles, this defect is compensated for by an increase in the contribution of COX-1 products. Moreover, with maturation in the lean animals this NO component to ACh-induced vasodilation is normally lost by 20 weeks of age, and neither the maximal vasodilation nor mechanisms through which it is achieved differ between the diabetic or normal rats. Thus, abnormal vasodilator responses of the low-oxidative glycolytic gastrocnemius muscle arterioles do not occur as a result of the diabetic state, and therefore this mechanism may not contribute to the elevations in mean arterial pressure observed with the onset of overt disease in these rats.

Despite a normal vasodilator response, both the mechanical properties and vasoconstrictor responsiveness of the gastrocnemius muscle arterioles are affected by prediabetes, acute and chronic type 2 diabetes. Alterations in the mechanical properties of the gastrocnemius muscle arterioles may contribute to the changes in vasoconstrictor responses observed. Circumferential strain analysis demonstrated a progressive increase in wall distensibility during the development of chronic type 2 diabetes in the gastrocnemius muscle, despite the decreasing strain observed in the arterioles from the control rats across these same ages. This increase in the distensibility of the vessel may function to limit the contractility of the vascular smooth muscle and may be related to the diminished KCl-induced vasoconstriction in the acute and chronic diabetic arterioles.

However, despite lower contractile performance to KCl, these vessels are able to achieve normal or even enhanced vasoconstrictor responses to receptor-mediated agonists. It is possible that signaling cascades affected by the metabolic

derangement of the disease could be upregulated and increase the responsiveness of the arterioles to these agonists. One possible explanation may be protein kinase C (PKC) activation. Increased activation of PKC has been documented as a consequence of hyperglycemia and enhanced de novo synthesis of diacylglycerol (13, 21, 25). PKC activation decreases NOS activity leading to a reduced production of NO (78), which could result in enhanced constriction to both myogenic activation and NE. Moreover, the cellular activation of the myogenic response is itself PKC-dependent, and increased PKC activation may lead directly to the enhanced myogenic tone seen in the present study (20, 116).

The passive mechanical characteristics may also be influenced by alterations in this same signaling molecule. PKC activation can increase the accumulation of extracellular matrix proteins, such as type IV collagen and fibronectin, via the induction of transforming growth factor-beta (TGF-β) (13). Alterations in the extracellular matrix components of the vessels may result in the abnormal passive properties seen in these arterioles.

In conclusion, during the development of type 2 diabetes, the external environment of the skeletal muscle arterioles progresses from hyperinsulinemia and mild hyperglycemia to marked hyperglycemia in the absence of hyperinsulinemia with concurrent elevations in arterial pressure. The prediabetic condition results in increased adrenergic vasoconstriction in the low-oxidative glycolytic gastrocnemius arterioles. This may result in enhanced regional vascular resistance and contribute to the concurrent insulin resistance as well as to the elevations in arterial pressure that occurs with acute diabetes. Acute diabetes results in enhanced

vasoconstriction and diminished vasodilation in the high-oxidative skeletal muscle arterioles concurrent with the emergence of arterial pressure. Finally, chronic diabetes results in diminished vasodilation of soleus muscle arterioles and enhanced vasoconstriction in gastrocnemius arterioles, the combined affect of which would likely lead to enhanced regional vascular resistance and contribute to the concurrent elevations in arterial pressure. Moreover, the development of type 2 diabetes results in a progressive increase in passive distention and circumferential strain in arterioles from both skeletal muscle arterioles examined.

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