CHAPTER V
DISCUSSION

Studies of cardiac functions

The results of this study demonstrated that the hearts of streptozotocin-induced diabetic rats at 8, 12, and 16 weeks exhibited the decreased cardiovascular function which were characterized by increased systolic pressure, diastolic pressure and mean arterial pressure. The pathologic basis for the abnormal systolic and diastolic function remain uncertain. Major histologic abnormalities are found in the diabetic heart, this encompasses virtually all levels of the myocardium, extending from the basement membrane to major intramural arteries. Marked myocardial fibrosis is a frequent finding in diabetic hearts. The arterial vasculature also exhibit changes, consisting of medial hypertrophy, endothelial thickening and a thickenced extracellular matrix of the intramural arterioles. It appears that the extensive fibrous tissue, which forms the interstitium as a result of abnormalities in the interactions between the vascular cell walls and hematogenous elements, or perhaps because of alterations of interstitial protein, may be one of the major factor responsible for the abnormality of both systolic and diastolic functions (Becker, 1990).

In the present experiment also found that cardiac output and aortic flow rate were decreased in heart of streptozotocin-induced diabetic rats at 8,12,and 16 weeks. Previous study showed that the decreased cardiac output and aortic flow rate in diabetes are due to the relative inability of the heart to utilize physiologic concentration of glucose as substrate for energy production. And, the decreased cardiac output and aortic flow rate in diabetes were reversed by insulin or by increasing substrate-related defects in energy metabolism rather than a general muscle weakness caused by acute insulin deficiency. Thus, the ventricular dysfunctions and decreased ATP levels are most likely due to well-known defect in cardiac glucose utilization associated with diabetes (Miller,

1979). Numerous studies examining the myocardium of experimental animals and also of diabetic patients have demonstrated that altered glucose metabolism results in a derangement of myofibrillar performance. Thus, changes in carbohydrate metabolism are responsible for cardiac dysfunction of the diabetic heart. Besides abnormal glucose metabolism calcium transport between the sarcolemma and the sarcoplasmic reticulum also have been reported. Because Ca^{2+} fluxes are important in cardiac contractility, the decrease in the Ca^{2+} pool may be responsible for the reduction of contractility found in cardiac muscle from diabetic animals and humans (Becker, 1990). The studies in diabetic patients found that diabetes may be responsible for both structural and functional alterations of the coronary circulation. These alterations may contribute to the progressive deterioration of the myocardium and to the pathogenesis of diabetic cardiomyopathy (Penpargkul et al., 1980). From the ultrastructural studies in ventricular myocardium of streptozotocin-induced diabetic rats found that myocardium revealed increased lipid deposition and progressive deterioration of the myocardial cell intergrity. This deterioration was characterized by loss of contractile protein, vascuolization (swollen sarcoplasmic reticulum), myelin formations, myocytolysis and contracture bands. These alterations paralled the depression of cardiac function (Jackson et al., 1985). Therefore, light and electron microscopy studies showed intimal proliferation of small myocardial arterioles, focal myocardial fibrosis and perivascular loss of contractile myocardial element in diabetes. These demonstrated that in diabetes has linked diabetic cardiomyopathy to changes in small vessels of the myocardium(Nitenberg,1993). In the normal heart, a balance between increased oxygen demand and supply is obtained by dilation of both epicardial coronary arteries and arterioles. Dilation of epicardial coronary circulation partly is caused by EDRF release. The impairment of EDRF production in diabetes may participate in the decreased conductance of the diabetic coronary arterial bed and limit blood supply when myocardial oxygen demand is increased. These alterations of the coronary circulation in diabetes may participate in the progressive deterioration of the myocardium and the

pathogenesis of diabetic cardiomyopathy (Penpargkul et al., 1980). Overall, the cardiac dysfunction in diabetes can be due to atherosclerotic changes in the coronary vessels or to metabolic alterations of the myocardium resulting from exposure to the abnormal metabolism in diabetes (Becker, 1990).

Studies of dose responses to vasoactive agents

The studies of coronary arteriolar responses to different concentrations of Ach, SNP, and NE found that coronary arterioles didn't response to 10^{-7}M of these vasoactive agents. However, at 10^{-4}M of Ach, SNP, and NE cause maximum responses of these coronary arterioles. And, this dose was also reported by several investigators (Mayhan et al., 1987; Tesfamariam et al.,1989; Abebe and Macleod, 1990; Leech and Faber, 1996). These results indicated that coronary arterioles in isolated arrested hearts have physiologic and pharmacologic properties. Indeed, McDonagh et al. (1984) have demonstrated that the isolated arrested heart model is appropiate for both physiologic and pharmacologic studies. It is particularly well suited for determining the direct effects of an intervention on coronary tone and the coronary microcirculation.

Studies of coronary arteriolar responses to vasoactive agents

1. Endothelium-dependent relaxation

In the present experiment found that acetylcholine-induced vasodilation of coronary arterioles in controls at all three different groups. From the studies by using light/dye treatment, resulting in selective impairment of the endothelium without affecting vascular smooth muscle reactivity, demonstrated that arteriolar dilation to acetycholine is endothelium-dependent and is mediated exclusively via endothelial factor (Koller et al., 1989 a ; 1989 b). Indeed, several investigators found that acetylcholine cause vasodilation through a mechanism that appear to be mediated by endothelium-derived relaxing factor (EDRF) or nitric oxide (Furgchott and Zawadski, 1980; Kaley et al.,

1989; Kaley et al., 1992). The action of EDRF on blood vessels has been demonstrated to be mediated by stimulation guanylate cyclase, leading to elevated levels of cyclic GMP in vascular smooth muscle which initiates the process of relaxation (Rapoport and Murad, 1983).

The results of this study demonstrated that the endothelium-dependent relaxation in response to acetylcholine was attenuated in diabetic rats compared to controls. In contrast, the relaxant effect of sodium nitroprusside, endothelium-independent agents (Koller et al., 1989 a), showed no depression of relaxant response. It is likely that the decrease in the relaxant response to acetylcholine may be due to an impairment of endothelial cells (Oyama et al., 1986; Kamata et al., 1989). These observations are consistent with the findings in isolated aortae of alloxan-induced diabetic rabbit which showed impaired endothelium-dependent relaxation induced by acetylcholine (Tesfamariam et al., 1989). Other have reported that endothelium-dependent relaxations to acetylcholine are impaired in aortae and cerebral arterioles of streptozotocin-induced diabetic rats (Oyama et al., 1986, Mayhan, Simmons, and Sharpe, 1991; Tesfamariam et al., 1991) and aortae of spontaneously diabetic BB Wister rats (Meraji et al., 1987). In additionally, decreased endothelium-dependent relaxations to acetylcholine have been reported in isolated penile corpus cavenosum tissue of impotent diabetic men (Tejada et al., 1989). A possible explanation for the impairment of endothelium-dependent relaxation by acetylcholine in diabetes may be a reduced production and release of EDRF(Durante et al., 1988). Moreover, the production of cyclic GMP induced by acetylcholine was lower in vessels from diabetic rats than in those from age-matched control rats. It is most likely, therefore, that decrease in acelylcholine-induced levels of cyclic GMP may be due to an impairment of the endothelium in diabetic rats. This decreased production of cyclic GMP may be responsible for the decreased relaxation induced by acetylcholine in diabetes (Kamata et al., 1989). As EDRF leads to vasodilation through elevation of cyclic GMP, this is indicative of abnormal EDRF production (Taylor et al., 1992). Several investigators have

suggest that nitric oxide synthase activity is reduced in diabetes. It is possible that hyperglycemia could affect nitric oxide synthase activity through a common metabolic pathway. Moreover, it has been proposed that hyperglycemia may impair nitric oxide production in vascular endothelium though the stimulation of the polyol pathway (Williamson et al., 1990; Cameron and Colter, 1992; Taylor and Poston, 1994). This pathway associated with increased utilization of reduced nicotinamide-adenine dinucleotide phosphate (NADPH), which may lead to reduced availability of cellular NADPH , an essential cofactor of nitric oxide synthase (Cameron and Colter, 1992). And, increased polyol pathway activation also lead to reduction in glutathione which in turn may reduce superoxide dimutase activity (Taylor et al., 1994). Several studies have shown that treatment with an aldose reductase inhibitor could prevent the impairment of endothelium-dependent relaxation to acetylcholine in streptozotocin-induced diabetic rats (Cameron and Coltex, 1992). Under hyperglycemic condition, several investigators have demonstrated that there is selective impairment in receptor-mediated endothelium-dependent relaxation and enhanced generation of vasoconstrictor prostanoid (Tesfamariam et al., 1990; 1991). The studies in diabetic vessels and normal ressels exposed to elevated glucose found that prostaglandin H_2/thromboxane A_2 receptor blockade could prevent the impaired endothelium-dependent relaxation to acetylcholine, suggesting that prostaglandin H_2, the precursor prostaglandin, or other vasoconstrictor prostaglandins could account for this impairment. Therefore, experiments by using exogenous prostaglandin H_2 point to its involvement as the endothelium-derived vasoconstrictor prostanoid (Tesfamariam and Cohen, 1992a; Tesfamariam, 1994). Moreover, some observations also indicated that oxygen-derived free radicals, most likely superoxide anions, participate in the contractile response to prostaglandin H_2 in diabetes (Tesfamariam and Cohen, 1992a; 1992b). Superoxide anions could attenuate vasodilation to acetylcholine by inactivates EDRF during transfer from the endothelium to smooth muscle (Kaley et al., 1989).

2. Endothelium-independent relaxation

From the study by using light/dye treatment, resulting in selective impairment of the endothelium without affecting vascular smooth muscle reactivity, demonstrated that arteriolar dilation to sodium nitroprusside was not altered by this treatment (Koller et al., 1989a). This result indicated that arteriolar dilation to sodium nitroprusside is endothelium-independent. In this study found that coronary arteriolar dilation caused by sodium nitroprusside was not impaired in streptozotocin-induced diabetic rats. Because sodium nitroprusside relax vascular smooth muscle in a direct endothelium-independent way (Durante et al., 1988; Koller et al., 1989a). And, this result also indicated that the relaxing capacity of smooth muscle is not altered in diabetes (Durante et al., 1988). Because, sodium nitroprusside cause vasodilation by activating guanylate cyclase within the smooth muscle cell, probably through the generation of nitric oxide free radical and direct activation of guanylate cyclase. This activation lead to increasing rates of formation of cyclic GMP in vascular smooth muscle cells which initiates the process of relaxation (Rapoport and Murad, 1983). The relaxant effect of sodium nitroprusside in this study are consistent with the findings of conduit arteries (Durante et al., 1988; Kamata et al., 1989; Tesfamariam et al., 1989) and resistance vessels (Mayhan et al., 1991; Taylor et al., 1992) in diabetic animal models.

3. Contractile response to norepinephrine

The present findings demonstrated that norepinephrine caused vasoconstriction in controls at all three different groups. Previous study indicated that norepinephrine-induced contraction is mediated by alpha-adenoceptor, which resembles the $alpha_1$-adenoceptor subtype (White and Carrier, 1988). Therefore, the results of this present study also found that coronary arterioles from rats with streptozotocin-induced diabetes are more response to the contractile effects of norepinephrine than are the corresponding arterioles from age-matched control rats. This enhanced pressor reactivity is agreement with most studies of conduit arteries and resistance arteries from streptozotocin-induced diabetic rats (White and Carrier,

1988; Abebe and MacLeod, 1990; Taylor et al., 1992; Taylor et al., 1994). Previous findings indicated that enhanced contractile response of vessels from diabetic rats to norepinephrine were inhibited by the protein kinase C (PKC) inhibitor suggest that at least part of the norepinephrine response of these vessels is mediated via activation of PKC (Abebe and MacLeod, 1990). This activation leads to opening of Ca^{2+} channels allowing the increased influx of extracellular Ca^{2+} into smooth muscle cells, thereby resulting in increased tension development (White and Carrier, 1988; Abebe and MacLeod, 1990). However, they do not demonstrate whether this results from increased activation of PKC by increased production of diacylglycerol, and/or from increased responsiveness of contractile process of activation of PKC (Abebe and MacLead, 1991). Some studies in resistance arteries of streptozotocin-induced diabetic rats also indicated that enhanced vascular contraction to norepinephrine predominantly due to reduced EDRF release (Taylor et al., 1994). This results from norepinephrine-induced contraction are normally attenuated by nitric oxide release (Kaley et al., 1992; Taylor et al., 1994). Indeed, the vasoconstriction to norepinephrine was enhanced after blocking the L-arginine pathway (Kaley et al., 1992).

4. Effect of cyclooxygenase inhibifor

Prostacyclin is the main product of arachidonic acid in all vascular tissues and strongly vasodilates all vascular bed studied (Van and Botting, 1995). In the present study found that indomethacin caused vasoconstriction in controls at all three different groups. These results indicated that the cyclooxygenase inhibitor, indomethacin induced vasoconstriction resulting from reduced production of vasodilator prostanoid, most likely prostacyclin. Moreover, the changes in basal arteriolar diameter after indomethacin administration suggest that arteriolar endothelium participates in the maintenance of basal arteriolar tone and prostaglandins have influence on the resting tone of resistance vessels (Koller et al., 1989b). In this experiment also found that the effects of indomethacin in normal vessels unlikely in diabetic vessels. Indomethacin administration that caused

vasodilation in streptozotocin-induced diabetic rats at all three different groups suggested that this results might be due to the from decreased production of vasoconstrictor prostanoids and superoxide anions. Several studies in diabetes found that hyperglycemia results in increased production of vasoconstrictor prostanoids including thromboxane A_2, prostaglandin $F_{2\ alpha}$ and it precursor, prostaglandin H_2 (Tesfamariam et al., 1989; Tesfamariam et al., 1990; Shimizu et al., 1993). An increased production of thromboxane A_2 associated with decreased prostacyclin production (Tesfamariam et al., 1989). This abnormal prostanoid production by diabetic vessels may have effect other than vasoconstriction. These included the increase of superoxide anions which could inactivate EDRF during transfer from the endothelium to smooth muscle resulting in enhanced vasoconstriction in diabetic vessels (Kaley et al., 1989).

Studies of garlic extract of cardiovascular functions

The present finding demonstrated that daily feeding of garlic extract could decrease systolic pressure, diastolic pressure, and mean arterial pressure in STZ-rats. The hypotensive activity of garlic has been observed in a variety of experimental studied. The mechanisms of the hypotensive effect of garlic were earlier thought to be due to endogenous release of various hypotensive substances in the body. Because this effect was blocked by dimetane (an anti-histamine), indicating that garlic act by releasing histamine (Sail and Ahmad, 1982). Later, it was postulated that hypotensive effect of garlic may either be due to its prostaglandin-like content by release of prostaglandins in the body (Rashid and Khan, 1985). The prostaglandin-like activity of garlic extract was supported by the study using isolated rat colon. This showed that effects of garlic extract were similar to prostaglandin E_2 (Rashid et al., 1986). The study in anesthelized dogs found that garlic could induce natriuretic and diuretic responses resulting in decreased in blood pressure which seem to resemble the effects of atrial natriuretic peptide (ANP). Since natural inhibitors of ANP-degrading proteinase, as well as of the metalloproteinase involved in the renin-angiotensin II

system have been identified in several plants, it would seem reasonable to expect that garlic may also contain one or more inhibitor of this nature, which could decrease in blood pressure (Pentoja et al., 1991). Recent study showed that garlic caused relaxation in isolated rat aorta which were attenuated by the removal of endothelium. However, the relaxant response to garlic was not completely abolished by the endothelial denudation. These strongly suggested that the vasorelaxant effect of garlic is important in its hypotensive activity and mediated by the production of endothelium-derived relaxing factors and/or muscle-derived relaxing factors (Ozturk et al., 1994).

The hypoglycemic effect of garlic was demonstrated by many investigators and most of them attributed such effects to allicin type compounds. The study by using alloxan-induced diabetes found that after allicin administration significantly increased the liver glycogen levels. This effect is consistent with the proposed role of oral antidiabetic agents, tolbutamide, in increasing serum insulin-like activity. A probable action of allicin may be to spare insulin from sulphydryl group inactivation as it can effectively combine with compounds like cysteine. Insulin activation by endogenous sources of cysteine, glutathione and serum albumin fracting rich in -SH groups may be blocked by allicin and thus enhance endogenous insulin effect to bring about a reduction in blood glucose (Mathew and Augusti, 1973). Later study suggested that garlic may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the beta-cells of the islets or it release from bound insulin (Jain and Vyas, 1975), or by an enhanced conversion of blood glucose into glycogen in liver (Chang and Johnson, 1980). From the many metabolic dysturbances of diabetes,hyperglycemia is the most likely cause of abnormal endothelial functions and cardiac dysfunctions (Becker, 1990; Tesfamariam and Cohen, 1992). Overall studies indicated that garlic act as antidiabetic agent. Thus, the hypoglycemic actions of garlic may improve cardiovascular dysfunctions in diabetes.

In the response to injury hypothesis of the pathogenesis of atherosclerosis, interaction of the blood platelets with damaged endothelium appears to play a pivotal role (Ross, 1986).

Many studies have demonstrated an increased sensitivity of platelets from diabetic humans and animals to platelet aggregating agents. And, these studies also showed enhanced arachidonic acid metabolism, leading to increased production of thromboxane A_2, a potent agonist for platelet aggregation, and release of platelet granule contents. Fibrinogen has recently been identified as a risk factor for coronary thrombosis. In addition, plasma concentrations are frequently elevated in people with diabetes (Rifkin, 1991). Defect in fibrinolytic activity is main factor for the development of thrombus (Gadkari and Loshi, 1991), leading to risk factor for arterial occlusion in diabetes (Kiesewetter et al., 1991). Both platelet and low-density lipoproteins (LDL) are known to play an important role in the development of atherosclerosis. LDL may contribute to the hyperaggregability of platelets in diabetes not only by enhancing the reactivity of platelets to proaggregatory agents but also by increasing the production of thromboxane A_2 (Rifkin, 1991). Garlic extracts contains a compound termed ajoene which, among other compound from garlic, is a potent inhibitor of platelet aggregation. Ajoene could inhibit the fibrinogen-induced aggregation and inhibits binding of fibrinogen to ADP-stimulated platelets. The antiaggregatory effect of ajoene is causally related to it inhibition of fibrinogen binding by direct interation with fibrinogen receptor (Apitz-Castro et al., 1986). Further study found that garlic was effective in inhibiting aggregation induced by calcium ionophore A23187, therefore, its effect may be related to mobilization of Ca^{2+}. Moreover, garlic also inhibit the formation of thromboxane A_2 and lipoxygenase products formed in platelets and inhibit the phospholipase activity (Srivastava, 1986), and increase fibrinolytic activity (Gadkari and Joshi, 1991; Mirhadi, Singh, and Gupta, 1991). From histopathological studies of aorta and heart from hypercholesterolemic animal indicated that garlic could prevent the development of atherosclerosis (Mirhadi et al., 1991). Overall, the vascular protection of garlic act as atherosclerosis prevention by influencing the mentioned risk parameters for the development of atherosclerosis. And, the incidence of cardiovascular disorder could be reduced by anti-athrosclerosis effect of garlic (Kiesewetter et al., 1991).

Among the most factors identified for coronary artery disease, lipid is the almost importance. Risk of coronary artery disease is proportional to the cholesterol levels (Gadkari and Joshi, 1991). Several labolatories demonstrated that endothelial functions might be altered by hypercholesterolemia. Pharmacologic and physiologic studies have been shown excessive contraction and markedly impair endothelium-dependent relaxation of coronary microvessels in animals and humans with hypercholesterolemia. In diabetic rats found that garlic extract could decrease total cholesterol, triglyceride and LDL, and increase high-density lipoprotein (HDL) (Anchalee Jatapai, 1994). Garlic may act by decreasing hepatic cholesterogenesis, whereas the triacylglycerol-lowering effect appears to be due to inhibition of fatty acid synthesis (Yeh and Yeh, 1994). And, it also increase liver and intestinal HMG CoA reductase activity (Sheela and Augusti, 1992), leading to increase the excretion of cholesterol end products. There is evidence that excretion of bile acids increases after garlic ingestion and excretion of sterol is seen in feces (Gadkari and Joshi, 1991). Moreover, garlic could oxidize NADPH, which is nesscessary for lipid synthesis, leading to decrease lipid synthesis (Sheela and Augusti, 1992). These indicated that hypocholesterolemic effect of garlic could improve impairment of endothelial functions and cardiac dysfunctions in diabetes.

There is evidence that hypercholesterolemia could impair endothelium-dependent relaxation via oxidative inactivation of the EDRF (Harrison and Ohara, 1995). Moreover, selective impairment of endothelium-dependent relaxation has been also demonstrated in vessels from diabetes. The oxidative injury is increased in diabetes because of a weakened defense due to reduced endogenous antioxidants (Vitamin E, reduced glutathion) (Barnett, 1991). Nitric oxide produced by endothelium is inactivated shortly before or after release from the endothelial cells and is thus incapable of producing vascular relaxation (Harrison and Ohara, 1995). An imbalance due to reduced production of nitric oxide or increased production of free radicals, mainly superoxide anion, may facilitate the development of an arterial functional spasm (Barnett, 1991). Previous study showed that vitamin E supplement could reduce the

elevated lipid peroxides. And, the study in streptozotocin-induced diabetic rats found that vitamin E could increase aortic prostacyclin and decrease platelet thromboxane A_2 production (Karpen et al., 1982). Recent study in this model demonstrated that vitamin E prevent the activation of protein kinase C by hyperglycemia, leading to normalizing the elevated levels of diacylglycerol (Kunisaki et al., 1996). Several studies demonstrated that garlic could inhibit lipid peroxidation, and also suggested that its action like the effect of vitamin E (Phelps and Harris, 1993). These indicated that antioxidant effect of garlic could decrease production of free radicals, leading to attenuate impairment of endothelial function resulting from hyperglycemia and hypercholesterolemia in diabetes.

The results of this finding demonstrated the impairment of endothelium-dependent relaxation to acetylcholine and enhancement of norepinephrine-induced contraction in streptozotocin-induced diabetic rats. These abnormalities of endothelial functions result from reduction of EDRF release and production (Durante et al., 1988). Recent study showed that garlic extract could increase nitric oxide synthase activity (Das, Khan and Sooranna, 1995), indicating that garlic extract could attenuate impairment of endothelium-dependent relaxation to acetylcholine and decrease enhancement of contraction to norepinephrine in diabetes. Moreover, the study by using garlic juice also inhibit the contractions of rabbit and guinea pig aortic rings induced by norepinephrine (Aquel et al., 1991). The extract of garlic is known to contain essential oils (Block, 1985), and such oil should have the capacity to penetrate cell membrane. It seem that garlic extract interferes with the contracton process beyond the cell membrane. This content is supported by the fact that garlic could inhibit contraction of aorta induced by norepinephrine in Ca^{2+}-free, indicating that inhibitory effect of garlic depend on intracellular Ca^{2+}-containing Krebs-Henseleit solution, indicating that its effect depend on extracellular Ca^{2+} release. Therefore, garlic also inhibit the contraction of aorta to norepinephrine in Ca^{2+}-containing Krebs-Henseleit solution, indicating that its effect depend on extracellular Ca^{2+} influx (Aquel et al., 1991). Later study found that garlic could improve

microcirculatory disorder by decreased plasma viscosity, and increased erythrocyte velocity resulting from vasodilation of arterioles. This dilation leads to enhance peripheral blood flow (Kiesewetter et al., 1991). Tesfamariam et al. (1989) demonstrated an increased production of thromboxane A_2 associated with decreased prostacyclin production, leading to enhanced vasoconstriction in diabetic vessels. In this study found that indomethacin cause vasoconstriction in garlic-treated STZ-rats, indicating that garlic-treated may decrease vasoconstrictor prostanoid production in STZ-rats. Indeed, the study by using rabbit aorta found that garlic could increase prostacyclin synthesis and decrease thromboxane A_2 production (Ali and Mohammed, 1986).

According to these major actions of garlic, I believed that they might be the reason why garlic could delay or prevent those diabetic cardiovascular functional changes as oberved in this present study. As garlic can normalize dyslipidemia, hyperglycemia, antihypertension, therefore it could offset the risks of cardiovascular abnormalities. The endothelial cells are then able to perform their normal physiological functions. Thus, the effects of garlic extract as a preservation for endothelium can improve the endothelial dysfunction in diabetes.