Journal of Cellular Biochemistry 96:1149–1156 (2005)

# Mechanisms of Endothelial Dysfunction With Development of Type 1 Diabetes Mellitus: Role of Insulin and C-Peptide

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**Abstract** Complications associated with insulin-dependent diabetes mellitus (type-1diabetes) primarily represent vascular dysfunction that has its origin in the endothelium. While many of the vascular changes are more accountable in the late stages of type-1diabetes, changes that occur in the early or initial functional stages of this disease may precipitate these later complications. The early stages of type-1diabetes are characterized by a diminished production of both insulin and C-peptide with a significant hyperglycemia. During the last decade numerous speculations and theories have been developed to try to explain the mechanisms responsible for the selective changes in vascular reactivity and/or tone and the vascular permeability changes that characterize the development of type-1diabetes. Much of this research has suggested that hyperglycemia and/or the lack of insulin may mediate the observed functional changes in both endothelial cells and vascular smooth muscle. Recent studies suggest several possible mechanisms that might be involved in the observed decreases in vascular nitric oxide (NO) availability with the development of type-1 diabetes. In addition more recent studies have indicated a direct role for both endogenous insulin and C-peptide in the amelioration of the observed endothelial dysfunction. These results suggest a synergistic action between insulin and C-peptide that facilitates increase NO availability and may suggest new clinical treatment modalities for type-1 diabetes mellitus. J. Cell. Biochem. 96: 1149–1156, 2005. 

**Key words:** type-1 diabetes mellitus; endothelium; insulin and C-peptide; nitric oxide; oxidative stress

## VASCULAR ENDOTHELIUM AND NITRIC OXIDE SYNTHESIS

The importance of the vascular endothelium in all aspects of cardiovascular physiology has been realized during the past 15 years. Normal endothelial cells maintain a delicate balance in the vascular between vasoconstriction and vasodilation [Kuo et al., 1988; Unthank et al., 1996; Furchgott and Vanhoutte, 1998]. Endothelium-derived NO (EDNO) is now recognized as a potent vasodilating substance, which modulates endothelium-dependent vasodilator responses. NO also appears to regulate blood flow distribution among arteriolar branches, suggesting an important function in regulating the blood flow distribution in vascular networks [Falcone and Bohlen, 1990; Welch and Loscalzo, 1994].

Studies performed in isolated human blood vessels [Saenz de Tejada et al., 1989] and in rats [Bucala et al., 1991], show the existence of an endothelial cell dysfunction or a reduced response to endothelial NO in diabetes [Cohen, 1995]. These results have been confirmed by in vivo studies of diabetic human forearm blood flow [Halkin et al., 1991]. Recent observations using cultured endothelial cells and high glucose concentrations show an increased apoptosis [Baumgartner-Parzer et al., 1995], suggesting the potential for endothelial damage by hyperglycemia in diabetic patients.

At the onset of diabetes, there is a selective dysfunction of receptor- and endothelium-

Grant sponsor: American Heart Association (grant-in-aid); Grant sponsor: American Physiological Society (Fellowship award).

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Received 14 June 2005; Accepted 1 July 2005

DOI 10.1002/jcb.20620

dependent agents, such as acetylcholine and ADP that lead to a reduced release or response to NO [Hattori et al., 1991]. In advanced states of diabetes, the cGMP levels are decreased, suggesting that NO release or its action on guanylate cyclase is markedly reduced [Kamata et al., 1992]. The reduced response to NO in diabetic vessels may be due to an increased destruction of endothelial NO by oxygen-derived free radicals.

## MICROVASCULAR DYSFUNCTION IN TYPE-1 DIABETES MELLITUS

Vascular diseases, including atherosclerosis and microangiopathy, are the principal causes of death and disability in patients with diabetes mellitus [Ruderman and Haudenschild, 1984]. Diabetic microvascular disease, particularly that affecting the eye and kidney, contributes importantly to morbidity [Zatz and Brenner, 1986]. "Diabetic microangiopathy" is very important in understanding the basis of the late complications of diabetes. Retinopathy, nephropathy, and diabetic cardiopathy [Yarom et al., 1992] undeniably involve microvessels.

It has been postulated that abnormalities in endothelial function are present in patients with diabetes and may contribute to the pathogenesis of vascular disease in these individuals. The normal endothelium plays an important role in maintaining vessel wall homeostasis, the synthesizing of biologically active substances that modulate vascular tone, prevention of thrombosis, and influencing smooth muscle growth [Vane et al., 1990]. Important among these vasoactive substances is endothelium-derived relaxing factor (EDRF), identified as nitric oxide (NO) by Furchgott and Zawadzki [1980]. NO causes vasodilation by stimulating the activity of soluble guanylate cyclase within the vascular smooth muscle, thereby elevating tissue levels of cyclic GMP. Reduced levels of NO could contribute to vascular injury and disease by facilitating platelet-vascular wall interaction, adhesion of circulating monocytes to the endothelial surface, and vascular smooth muscle proliferation [Johnstone et al., 1993]. There is now substantial evidence that endothelium-dependent vasodilation is abnormal in both conduit and resistance arteries of chemically induced experimental diabetic animals [Mayhan, 1989; Pieper and Gross, 1990; Mayhan et al., 1991; Diederich et al., 1994; Rosen et al., 1995; Taylor et al., 1995]. In two genetic models of IDDM, a similar impaired relaxation has been documented in the aorta [Durante et al., 1988; Pieper et al., 1996] and mesenteric arteries [Heygate et al., 1995; Lindsay et al., 1997] of diabetes-prone BB/W or BB/E rats. Similar results are reported for aorta [Miyata et al., 1992] and mesenteric arteries [Miyata et al., 1993] of the diabetes-prone WBN/Kob rat. Also, cGMP levels are low in aortas of diabetic rats, suggesting that basal concentrations of NO are reduced in these vessels.

Almost without exception, studies that have shown impaired endothelium-dependent relaxation have found normal relaxation to nitrovasodilators. These agents relax vascular smooth muscle by activating guanylate cyclase. But, unlike NO, they do not require the presence of the endothelium. Thus, the intrinsic property to activate vascular smooth muscle guanylate cyclase appears not to be altered by experimental diabetes.

Several mechanisms have been proposed to explain abnormal endothelium-dependent relaxation in diabetes. These include abnormalities in signal transduction, reduced synthesis of NO, accelerated inactivation of nitric oxide, and generation and release of competing vasoconstrictor substances. Faulty signal transduction has been variably attributed to decreased expression of inhibitory G proteins, reduced phosphoinositol metabolism, and increased activation of protein kinase C [Gawler et al., 1987; Lee et al., 1989]. Abnormalities in the endothelial milieu might hasten the inactivation of nitric oxide. These include high levels of oxygen-derived free radicals, advanced glycosylation end-products, and transport barrier such as thickened basement membranes [Johnstone et al., 1993].

## OXIDATIVE STRESS AND DIABETES

Evidence associating reactive oxygen species (ROS) with the etiology of diabetic complications in the vasculature is founded in published studies showing increased generation of ROS and/or decreased endogenous antioxidant defense mechanisms in diabetic endothelial dysfunction. ROS may impair endothelial function through inactivation of nitric oxide or by serving as an endothelium-derived contracting factor [Rubanyi and Vanhoutte, 1986; Katusic and Vanhoutte, 1989]. A major hallmark of diabetes is an abnormally high blood glucose

level (i.e., hyperglycemia), Thus, endothelial cell damage during diabetes appears linked to hyperglycemia. There is also widespread understanding that hyperglycemia causes or exacerbates ROS production the important question has been how?

Hyperglycemia-induced ROS production (in the mitochondria) occurs in endothelial cells and in platelets through auto-oxidation of glucose [Wolff and Dean, 1987], advanced glycation end (AGE) product formation and the binding of AGEs to their receptors [Yan et al., 1994; Ceriello, 1999], increased substrate flux through the polyol pathway [Giugliano et al., 1996], and/or through stimulation of the eicosanoid metabolic pathways [Tesfamariam and Cohen, 1992a,b; Quilley and Chen, 2003]. The major sources of $O_2^-$ in cardiovascular cells are: NADH/NADPH oxidase [Griendling et al., 2000], which transfers electrons from NADH or NADPH to molecular oxygen, producing $O_2^-$; xanthine oxidase [Wolin, 1996]; endothelial nitric oxide synthase [Ignarro et al., 1999]; cyclooxygenase-2 [Adeagbo et al., 2003]; myeloperoxidase [Berliner and Heinecke, 1996]; and lipoxygenases [Kunsch and Medford, 1999]. The $O_2^-$ that is generated via any of these pathways is converted by superoxide dismutase to $H_2O_2$, which is scavenged by catalase or by peroxidases.

## INSULIN-INDUCED VASODILATION

It is now generally acknowledged that insulin administration can have pronounced effects on the cardiovascular system of either animals or humans. The reported actions of insulin include increases in heart rate, cardiac output, forearm and animal hindlimb blood flow, and decreases in systemic vascular resistance [Baron, 1994]. Increases in human forearm and animal hindlimb blood flows, as well as decreases in total peripheral resistance, have been mainly attributable to a decrease in skeletal muscle vascular resistance and an increase in skeletal muscle blood flow [Baron, 1994]. When insulin activates its receptors, two major pathways become active. The first is the phosphatidylinositol 3-kinase pathway, which is important for glucose transport in skeletal muscle. More recently, this pathway has been shown to be important for endothelial nitric oxide production and leads to insulin-induced vasodilation. The second insulin-activated pathway is the MAPK pathway, which not only promotes insulin-mediated growth of the VSMC, but also stimulates VSMC migration.

Observation by Scherrer et al. [1994] and Steinberg et al. [1994] showed that the insulin-induced increases in blood flow could be abolished by inhibiting NO synthesis-dependent vasodilatation using L-*N*-monomethylarginine (L-NMMA) but not by other vasoconstrictors such as norepinephrine. These in vivo observations are supported by recent studies performed in skeletal muscle arterioles [Chen and Messina, 1996; Porter et al., 1997] and vascular endothelial cells [Zeng and Quon, 1996]. In arterioles from rat cremaster muscle, insulin-induced vasodilation can be completely abolished by removal of the endothelium and by $N^G$-nitro-L-arginine (L-NNA, another inhibitor of eNOS). Insulin also increases NO production in cultured human umbilical vein endothelial cells [Zeng and Quon, 1996]. Inhibition of eNOS, and inhibition of tyrosine kinases by genestein, both block insulin-induced NO release, while wortmannin, an inhibitor of phosphatidylinositol 3-kinase, partly, blocks NO-release, suggesting that insulin-stimulated NO release and glucose transport may share common signaling elements in endothelial cells.

## C-PEPTIDE AND ITS BIOLOGICAL EFFECT

Since the discovery in 1967 of the mode of insulin biosynthesis, it has generally been held that C-peptide, the connecting segment of pro-insulin, does not possess biological activity of its own (Fig. 1). However, recently, several studies

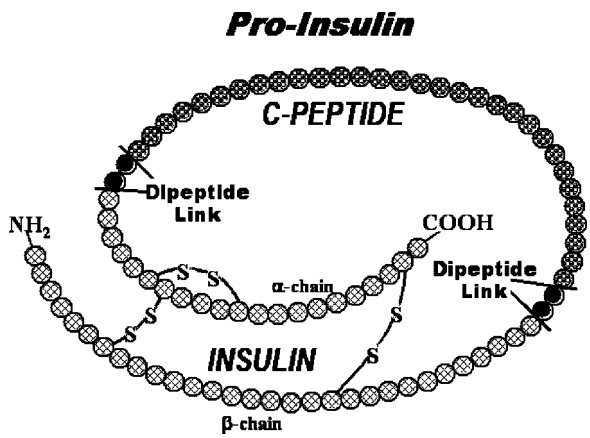


**Fig. 1.** Diagram of pro-insulin, illustrating the dipeptide insulin portion and the linking peptide portion (C-peptide). Modified from a figure by Chance RE, Ellis RM, Broomer WW. Porcine proinsulin: Characterization and amino acid sequence. Science 161, 1968.

have raised doubts concerning this view. Short-term C-peptide replacement in animals with experimental diabetes and patients with type-1 diabetes is accompanied by improved renal function, augmented glucose utilization, increased blood flow in muscle and skin, and improved autonomic nerve function. Prolonged C-peptide administration in type-I diabetic patients results in improvement of renal function and amelioration of autonomic and sensory nerve dysfunction [Johansson et al., 2000]. In vitro studies have confirmed that C-peptide stimulates glucose transport in skeletal muscle and that this effect is mediated via pathways other than the insulin receptor. C-peptide also improves RBC deformability in type I diabetes patients [Forst et al., 2000]. C-peptide signal transduction, appear to follow the normal rules of ligand and receptor biochemistry. However, the cellular mechanisms that form the basis of the biological effect of C-peptide are not fully understood.

C-peptide has been shown to modify several cellular mechanisms that have been shown to be altered with development of clinical or experimental IDDM and could relate to modulation of vascular function. As stated earlier, changes in $Na^+$–$K^+$ ATPase and nitric oxide synthase activity are both suggested as mechanisms that might mediate the vascular action of C-peptide.

$Na^+$–$K^+$ ATPase maintains the electrochemical gradient of sodium and potassium in a number of cells including those in the vasculature. $Na^+$–$K^+$ ATPase activity has been shown to be reduced in a variety of cell types in human and experimental IDDM [Wald et al., 1993; Gerbi et al., 1997]. C-peptide has been shown to stimulate $Na^+$–$K^+$ ATPase activity in a number of tissues including erythrocytes and renal tubules in diabetic patients and animal models [Ohtomo et al., 1996; Dufayet et al., 1998; Forst et al., 2000]. Speculations are that decreased $Na^+$–$K^+$ ATPase activity could directly cause an increase in vascular tone by increasing intracellular sodium and calcium in vascular smooth muscle [Forst et al., 2000]. Conversely, elevation in vascular nitric oxide and cGMP has been shown to stimulate $Na^+$–$K^+$ ATPase activity [Gupta et al., 1994]. Several recent studies suggest that blocking nitric oxide production or the $Na^+$–$K^+$ pump could attenuate the vascular effects of C-peptide [Wahren et al., 2000]. Since impaired $Na^+$–$K^+$ ATPase activity is a characteristic of a number of cells in the type 1 diabetic human subjects and animal models [Forst and Kunt, 2004] a role for C-peptide in mediating a portion of the observed endothelial dysfunction.

## ROLE OF INSULIN AND C-PEPTIDE SYNERGISM

A number of studies have suggested that decreased circulating levels of C-peptide may play a role in vascular dysfunction in peripheral and renal vascular beds associated with IDDM. Patients with IDDM have been reported to develop glomerular hyperfiltration that is not reverse with insulin therapy alone [Sandahl-Christiansen et al., 1981]. Interestingly, in patients suffering from non-insulin dependent diabetes (NIDDM), where plasma C-peptide levels are normal or above normal, no significant glomerular hyperfiltration is observed [Friedman et al., 1981; Schmitz et al., 1988]. Infusion of C-peptide into IDDM patients to produce physiological levels of this hormone has been shown to produce a significant reduction in glomerular filtration rate and an elevation in renal plasma flow [Johansson et al., 1992]. In subsequent studies, these investigators confirmed the ability of this hormone to improve diabetic nephropathy by decreasing urinary albumin excretion in diabetic patients in both early and established stages of the disease [Johansson et al., 1993, 2000]. Similarly, administration of C-peptide to streptozotocin diabetic rats significantly reduced glomerular hyperfiltration and markedly reduced protein leakage [Sjöquist et al., 1998].

Administration of C-peptide has been show to increase blood flow to skeletal muscle of IDDM patients. Johansson et al. [1992] first demonstrated that acute administration of C-peptide could increase blood flow in the exercising forearm of IDDM patients but not in normal individuals. Later these investigators confirmed the vasodilator ability of C-peptide in the forearm at rest [Johansson and Pernow, 1999]. Jensen and Messina [1999] found that C-peptide when topically applied to isolated skeletal muscle arterioles produced a marked vasodilation, but only in the presence of insulin. While there is no direct evidence of a role for C-peptide in modulating vascular permeability in the skeletal muscle vasculature, an increase capillary recruitment in the isolated rat hindlimb has been observed following infusion of C-peptide [Lindström et al., 1996]. The mechanism by which C-peptide produces dilation in

skeletal muscle is also thought to involve stimulation of nitric oxide.

Preliminary studies in our laboratory show that insulin and C-peptide both produce significant dose-dependent arteriolar dilation [Zhang and Joshua, 2001]. The dilation to insulin or C-peptide was abolished by pretreatment with L-NAME, a non-specific inhibitor of nitric oxide synthase. In addition the pretreatment with the Na–K pump antagonist, ouabain also antagonized the c-peptide-induced dilation in cremaster arterioles. Responses to a non-endothelium dependent dilator (sodium nitroprusside) were not affected by treatment with L-NAME or ouabain.

In the STZ-induced diabetic rat the in vivo arteriolar dilation to insulin was greatly depressed [Zhang and Joshua, 2000]. However, pretreatment with a non-vasodilator dose of C-peptide restored the arteriolar vasodilation to insulin in the STZ-induced diabetic rats, suggesting a synergistic interaction between these two factors. Thus these data support attenuation in an endothelium-mediated dilation that involves NO in the STZ-diabetic rat. In addition, we demonstrate the ability of both insulin and C-peptide to restore dilator capacity of these vessels and thus suggest some synergy in the action of these two hormones.

## SUMMARY

Collectively the research literature along with preliminary findings from our laboratories suggest some interesting possibilities related to mechanisms underlying the endothelial dysfunction related to development of type-1 diabetes mellitus (Fig. 2). Clearly both the lack of insulin and C-peptide mediate the attenuated dilator response to stimulation of endogenous NO. The synergism between these two hormones suggests that two separate and facilitating mechanisms for stimulating the production and or release of NO exist. The current evidence suggest that a lack of insulin facilitates an oxidative stress environment in the cell that could lead to a decrease in NO availability via a decrease in available NO due to it utilization in generation of reactive nitrogen species (ROS) or

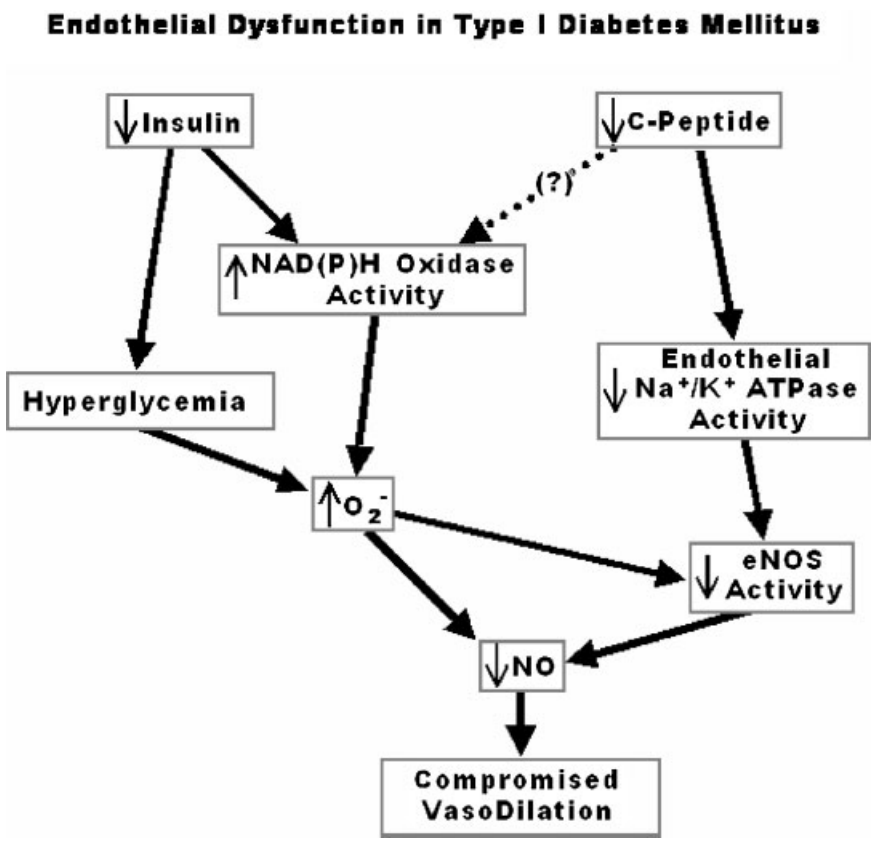


**Fig. 2.** Diagram to represent the possible mechanisms to explain the attenuated endothelial mediated dilator responses observed with the development of type 1 diabetes mellitus.

due to a reactive oxygen species generated uncoupling of endothelial nitric oxide synthase (eNOS). C-peptide on the other hand appears to be necessary for normal cellular Na–K+ ATPase activity and its loss with development of type-1 diabetes may in some way impair the activation of eNOS. Thus a synergism between these two biological agents may be necessary for normal endothelial cell dilator function.

In closing, treatment of type-1 diabetes mellitus with insulin replacement is an effective tool for addressing perturbations in glucose metabolisms however many of the vascular abnormalities associated with this disease persist. A review of recent cardiovascular related studies suggest that chronic administration of the bridging peptide fragment from pro-insulin, C-peptide along with insulin can attenuate or prevent the vascular complications associated with this disease. These recent findings appear to suggest that supplemental treatment with both insulin and C-peptide may be a more effect treatment for type-1 diabetes mellitus. Currently little is known about the mechanism by which these peptides alone or in concert exerts their effects on arteriolar resistance vessels. Initiation of studies to elucidate the mechanism(s) of this fascinating synergy between these two pancreatic hormones could provide valuable data that may have potential value in developing new treatments for human subjects suffering from type-1 diabetes mellitus.

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