# NO (Nitric Oxide) to Type 2 Diabetes Induced Endothelial Dysfunction: Crosstalk with ET-1 (Endothelin-1)

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**Abstract**

Vascular endothelium is a fundamental part of the blood vessel and sustains homeostasis of the circulatory system and normal arterial function. Experimental and clinical studies suggest that insulin resistance in diabetes is associated with functional disorder of endothelium and higher risk of cardiovascular disease (CVD). Insulin resistance causes reduction in nitric oxide (NO) bioavailability and increase in endothelin-1 (ET-1) secretion which plays a vital part in the development of endothelial dysfunction. ET-1 causes a decrease in adiponectin secretion (hypo adiponectinaemia which is correlated with diabetes/impaired insulin signaling) which has also been identified as an independent risk factor for CVD. This mini review investigates and appraises the impact of insulin resistant Type 2 diabetes (T2DM) on endothelium derived vasodilator, NO, and vasoconstrictor ET-1 highlighting crosstalk between them.

**Keywords:** Diabetes; Cardiovascular disease; Endothelium; Insulin; NO; ET-1; Oxidative stress; Adiponectin

## Mini Review

Cardiovascular diseases (CVD) are one of the major causes for the most non communicable disease (NCD) related deaths worldwide (17.5 million deaths annually). Diabetes is the third highest contributor to NCD deaths (1.5 million) trailing behind cancers and respiratory diseases [1].

A key feature of CVDs is endothelial dysfunction characterized by a reduction in bioavailability of the signaling radical NO. T2DM is associated with systemic insulin resistance, which promotes hyperglycemia and dyslipidemia [2] and leads to reduced bioavailability of NO, indicative of a critical role for insulin in regulating NO bioavailability [3-5].

Insulin is the most effective anabolic peptide hormone which helps in the synthesis and storage of carbohydrates, lipids and proteins and is made by the β cells, of the islets of Langerhans in the pancreas [6]. As a major regulator of vascular homeostasis, insulin helps the endothelium in maintaining the balance between vasodilation and vasoconstriction, by stimulating production of both NO and ET-1. Impairment in the functioning of insulin (T2DM) or production of insulin (type I diabetes) or both disturbs this tightly regulated equilibrium between NO and ET-1 leading to endothelial dysfunction, decreased blood flow and ultimately to CVDs [7]. Decreased blood flow due to endothelial dysfunction further aggravates diabetes as it causes reduced delivery of blood to pancreas, resulting in further decrease in insulin secretion. A vicious cycle between insulin resistance and endothelial dysfunction develops which is amplified synergistically. A recent study has demonstrated that ET-1 also has the ability to regulate adiponectin secretion [8]. Adiponectin is an insulin sensitizing hormone which is a potent vasodilator and antioxidant and, its vasodilatory effect is also mediated via a NO-dependent mechanism [9-11]. This mechanism is disrupted in T2DM, a phenomenon potentiated by excessive accumulation of reactive oxygen species (ROS). Though multiple independent and interdependent factors such as hyperglycemia, dyslipidemia, inflammatory cytokines hypoadiponectinemia, oxidative stress contribute in the fierce synergy linking insulin resistance and endothelial dysfunction, this mini review focuses only on the vasoactive role of insulin in T2DM on endothelial function and vice versa due to the interplay of NO and ET-1.

### NO and ET-1 cross talk in T2DM

The vascular endothelium has fundamental control on the maintenance and modulation of vascular tone. In the

healthy state, endothelium continually produces NO and other vasodilator molecules (prostacyclin, endothelium–derived hyperpolarizing factor (EDHF), bradykinin, adrenomedullin, C-natriuretic peptide), thereby promoting a relaxed vascular state however, they also concurrently form and release ET-1 and other constricting factors, (angiotensin-II, thromboxane A2, prostaglandins, hydrogen peroxide $(H_2O_2)$ and free radicals) which help to modulate the tone of the vascular bed [12]. The L-arginine-NO pathway is thought to be the most important enzymatic vasodilator source and ET-1 the most potent vasoconstrictor source. The basal and stimulated production of NO inhibits the synthesis of ET-1 by endothelial cells. Thus, the direct vasoconstrictor action of ET-1 is modulated by the stimulation of the release of NO. It appears that a delicate balance exists between ET-1 and NO released. When there is an increase in NO release, enhanced endothelial function is observed. In pathological conditions like T2DM the balance between NO and ET-1 is disturbed by generation of free radicals (due to dyslipedemia, hyperglycemia, hypoadeponectinemia ) which causes a decrease in bioavailability NO, increased ET-1 production directly or indirectly by modulation of insulin signaling. Several groups have proposed that the hemodynamic effects of insulin are regulated by the balance between the vasodilator and vasoconstrictor effects of NO and ET-1, respectively [13-15]. In addition, as the actions of ET-1 and NO are mutually antagonistic, any imbalance, is amplified leading to endothelial dysfunction and subsequently CVDs. NO and ET-1 have emerged as central pathophysiological players in endothelial dysfunction [16,17].

Insulin exerts its metabolic and vasoactive actions via the phosphatidylinositol 3-kinase (PI-3 kinase) and mitogen-activated protein kinase (MAPK) signaling pathways. Insulin binds and activates the insulin receptor tyrosine kinase (IR) resulting in phosphorylation of insulin receptor substrates (IRS) and activation of PI-3 kinase and protein kinase B (PKB or Akt) [18]. This causes the translocation of glucose transporter 4 (GLUT4) and subsequently glucose uptake in myocytes and the phosphorylation of eNOS [19-21]. On the other hand, activation of MAPK pathway results in secretion of ET-1 by the endothelial cells [22,23] and up-regulation of ET-1 gene expression through PI3K-dependent inactivation [24-26]. High levels of insulin in healthy individuals, result in increase in circulating NO and ET-1 levels, a phenomenon observed also in T2DM patients, in respect to only ET-1 and not with NO production [27]. In T2DM, insulin resistance is classically accompanied by compensatory hyperinsulinemia so as to maintain euglycemia which have important pathophysiological implications. At the cellular level, insulin resistance causes selective impairment in PI3K-dependent signaling pathways with no influence on MAPK-dependent pathways. In the vasculature, hyperinsulinemia over stimulates the unaffected MAPK-dependent pathways causing an imbalance between PI3K-and MAPK-dependent functions of insulin and consequently resulting in an imbalance of NO and ET-1. The imbalance between PI3K/Akt/eNOS/NO and MAPK/ET-1 vascular actions of insulin attributes to endothelial dysfunction and subsequently to CVD [28,29]. Apart from hyperinsulinenia, T2DM is accompanied by dyslipedemia, hyperglycemia, hypoadiponectemia and inflammatory cytokines which contribute in the generation of oxidative stress. One of the other mechanism which links ET-1 to NO in T2DM may be via formation of ROS, which results in decreased bioactivity of NO by virtue of formation of peroxynitrite. Recent data demonstrate that ET-1 mediates superoxide production along with vasoconstriction [30]. Further, increase in ET-1 in T2DM causes downregulation in the expression of eNOS [31] and adiponectin deficiency [8]. Adiponectin, is down-regulated in obesity and its related pathologies [32]. There is some evidence suggesting involvement of PI3K in adiponectin-induced production of endothelial NO, possibly via activation of AMP kinase [33-35]. Adiponectin improves the redox state in human vessels by restoring eNOS coupling [36]. In an *ex vivo* study on aorta of T2DM mice an increase NO bioavailability was observed after treatment with adiponectin indicating that adiponectin decreases superoxide production [37]. The adiponectin has the ability to oppose ET-1-mediated vasoconstriction which may be an important aspect of its insulin-sensitizing actions [38].

Mostly endothelial dysfunction often refers to a situation of reduced bioavailability and consequently impaired vasodilator effect of endothelium-derived relaxing factors of NO, along with an additional important alteration in endothelial dysfunction is an increased production and biological activity ET-1. Insulin produces NO through PI3/Akt pathway and ET-1 by MAPK pathway. In T2DM, compensatory hyperinsulinemia results in endothelium dysfunction due to decreased NO bioavailability (disruptive PI3/Akt pathway, oxidative stress and decrease in adiponectin) and increase in ET-1 (activated MAPK pathway, oxidative stress). Progressive deregulation of the relationship between ET-1 and NO due to insulin resistance, adiponectin deficiency and oxidative stress, may be one of the major contributory factor for endothelial dysfunction observed in T2DM.

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