

Endothelial Nitric Oxide Synthase (T-786C) Gene Polymorphism and Coronary Artery Disease: A Review

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ABSTRACT

Coronary Artery Disease (CAD) is the most common type of heart disease. Globally, CAD is the leading cause of death and is predicted to remain so for the next 20 years. NO has been reported to exert various physiological roles due to its ability to induce vasodilatation. Other physiological roles of NO have been demonstrated that include its role in immune system, nervous system, inflammation and blood flow. NO has been noted to relax the smooth muscle and walls of arterioles. A common feature of many cardiovascular risk factors (including hypertension, diabetes, insulin resistance, obesity and hyperlipidemia) is endothelial dysfunction. Because the endothelium normally protects against the processes involved in atherogenesis – namely, smooth muscle cell proliferation, inflammation and thrombosis – endothelial dysfunction is an important final common pathway by which these risk factors increase atherosclerosis. This review article aims to understand the complex relationships between insulin resistance, visceral adiposity and endothelial dysfunction; the task before us is to translate this knowledge into effective treatments to reduce CVD, which is the leading cause of morbidity and mortality from diabetes and related metabolic diseases.

Key words: Endothelial nitric oxide synthase (T-786C), gene polymorphism, Coronary artery disease, CAD

INTRODUCTION

Coronary Artery Disease (CAD) is the most common type of heart disease. Globally, CAD is the leading cause of death and is predicted to remain so for the next 20 years¹.

Each year, approximately 3.8 million men and 3.4 million women die from CAD. In 2020, it is estimated that this disease will be responsible for a total of 11.1 million deaths globally⁹. Cardiovascular disease (CVD) currently accounts for nearly half of non-communicable diseases (NCDs). NCDs have overtaken communicable diseases as the world's major disease burden, with CVD remaining the leading global cause of death. Increasingly, the populations affected are those in low and middle-income countries, where 80% of these deaths occur, usually at younger ages than in higher income countries, and where the human and financial resources to address them are most limited.

Someone suffers a coronary event every 26 seconds, and someone dies from one every minute in the USA¹¹. In Europe, between 1 in 5 and 1 in 7 European women die from CAD, and the disease accounts for between 16% and 25% of all deaths in European men.

By 2005, the total number of CVD deaths (mainly coronary heart disease, stroke, and rheumatic heart disease) had increased globally to 17.5 million from 14.4 million in 1990. Of these, 7.6 million were attributed to coronary heart disease and 5.7 million to stroke. The World Health Organization (WHO) estimated there will be about 20 million CVD deaths in 2015, accounting for 30 percent of all deaths worldwide. By 2030, researchers project that non-communicable diseases will account for more than three-quarters of

deaths worldwide; CVD alone will be responsible for more deaths in low income countries than infectious diseases (including HIV/AIDS, tuberculosis, and malaria), maternal and perinatal conditions, and nutritional disorders combined¹⁴. Thus, CVD is today the single largest contributor to global mortality and will continue to dominate mortality trends in the future.

There is paucity of literature regarding association of eNOS gene (T786C) polymorphism and Insulin resistance in patients with CAD in Indian population. The studies associating eNOS gene (T786C) polymorphism and risk of insulin resistance have yielded variable results. Hence the present study is planned to review the association of eNOS gene (T786C) polymorphism and Insulin resistance in patients with Coronary Artery Disease.

An international epidemic

Due to this increasing incidence across the world CAD has been described as an epidemic.

Studies suggest that the average age-adjusted incidence rates of CAD per 1,000 person-years are 12.5 for white men, 10.6 for black men and 4.0 for white women.

According to American Heart Association (AHA) statistics, 770 000 Americans suffered a new coronary attack in 2008, and a further 430 000 experienced a recurrent attack. An additional 190 000 silent first heart attacks are estimated to occur each year⁴.

High Cost burden

In addition to its mortality burden, CAD is a leading cause of morbidity and loss of quality of life. This makes CAD a major public health problem which exerts heavy economic costs. Overall, CAD is estimated to have cost the EU €45 billion in 2003⁵.

Approximately one million working years were lost because of CAD mortality, with a total cost of €11.7 billion. An additional 90 million working days were lost because of CAD morbidity⁶.

The association between CAD and diabetes is strong despite the fact that there are wide ethnic and geographic variations in their prevalence. The protective female gender effect is lost in diabetic subjects, and indeed, women with diabetes are possibly more prone to develop CAD than men with diabetes⁷. It was established in the Organization to Assess Strategies for Ischaemic Syndromes study that diabetic subjects without prior CAD had a similar risk of CAD as nondiabetic subjects with prior CAD, with poorer prognosis seen in diabetic subjects than nondiabetic subjects after a clinical event. In addition to cardiovascular risk factors seen in nondiabetic subjects, diabetes-specific cardiovascular risk factors also contribute to CAD in diabetic subjects⁸.

Epidemiology of Coronary Artery Disease in Indians

India is predicted to bear the greatest CAD burden, according to the estimates from the Global Burden of Disease Study. Of the more than 9 million deaths due to CAD in 1990 in developing countries, 2.4 million (25%) had occurred in India. In the same year, mortality rates in India due to acute myocardial infarction (MI) were 141 per 100,000 in males and 136 per 100,000 in females, which was much higher than in China (66 per 100,000 in males and 69 per 100,000 in females) and Latin American countries (81 per 100,000 in males and 76 per 100,000 in females). The overall cardiovascular mortality in Indians is predicted to rise by 103% in men and 90% in women between 1985 and 2015. A matter of serious concern is that 52% of the CAD deaths in India occurred in people aged below 70 years, while the same was just 22% in developed countries.

A meta-analysis of the CAD prevalence based on the surveys conducted since 2010 suggested that the increase in prevalence of CAD in the urban and rural populations were nine-fold and two-fold, respectively. Thus, in the next 15 years, a phenomenal increase in the prevalence of

CAD is expected in India, adding to the health burden due to CAD among Indians⁹.

Epidemiology of Diabetes in Indians

There are currently 135 million people with diabetes in the world, and India leads the world with 40.9 million people in diabetes in 2007. Moreover, it is projected that, by the year 2025, 80.9 million will have diabetes in India. The prevalence of diabetes in urban Indians has steadily increased from 2.1% in the 1970s to 8.2% in the 1980s, later climbing to 12–16%. Thus the phenomenon of high prevalence of diabetes reported among migrant Asian Indians has now spread to urban India and is rapidly moving to rural areas as well. There is still inadequate population-based data on the prevalence of CAD in India, particularly comparing diabetic and non-diabetic subjects¹⁰.

Insulin resistance

Insulin resistance (IR) is a syndrome characterised by a diminished ability of insulin to perform its normal physiological functions. It has often been linked to endothelial dysfunction, defined as paradoxical or inadequate endothelial-mediated vasodilation.

Specifically, IR has been associated with the decreased synthesis and/or release of NO, as occurs in many clinical conditions including heart failure, or with its exaggerated consumption due to high tissue levels of reactive oxygen and nitrogen species in a condition of altered glucose and lipid metabolism. Alternatively, genetically determined deficiency of endothelial-derived NO could predispose to IR.

Signs and symptoms

Chest pain that occurs regularly with activity, after eating, or at other predictable times is termed stable angina and is associated with narrowings of the arteries of the heart. Angina that changes in intensity, character or frequency is termed unstable. Unstable angina may precede myocardial infarction. In adults who go to the emergency with an unclear cause of pain, about 30% have pain due to coronary artery disease.

Risk factors

Coronary artery disease has a number of well determined risk factors. The most common risk factors include smoking, family history, hypertension, obesity, diabetes, lack of exercise, stress, and high blood lipids. Smoking is associated with about 36% of cases and obesity 20%. Lack of exercise has been linked to 7–12% of cases.

Job stress appears to play a minor role accounting for about 3% of cases. In one study, women who were free of stress from work life saw an increase in the diameter of their blood vessels, leading to decreased progression of atherosclerosis. Contrastingly, women who had high levels of work-related stress experienced a decrease in the diameter of their blood vessels and significantly increased disease progression. Also, having a type A behavior pattern, a group of personality characteristics including time urgency, competitiveness, hostility, and impatience is linked to an increased risk of coronary disease.

Nitric Oxide

Nitric oxide, a colorless gas, has been considered as an important biological regulator which is a fundamental component in the fields of neuroscience, physiology and immunology. The term NO was first termed in 1772 by Joseph Priestley when he named it as 'nitrous air'. It was known as a toxic gas and an air pollutant till 1987, when it was shown that it is actually produced naturally in the body and thus its role has been described in the regulation of blood pressure and protection from various cardiovascular diseases¹². Moreover, the cardioprotective roles of NO regulation include blood pressure and vascular tone²⁸, inhibition of platelet aggregation and leukocyte adhesion along with prevention of smooth muscle proliferation⁶. In addition, it has been comprehensively demonstrated that reduced bioavailability of NO leads to development and progression of various cardiovascular disease¹⁸. Further, the reactivity of NO depends on various

physical parameters like small size, high diffusion rate and lipophilicity. NO has been shown to possess various direct and indirect effects leading to its effect on various biological systems. NO has been reported to exert various physiological roles due to its ability to induce vasodilatation. Other physiological roles of NO have been demonstrated that include its role in immune system, nervous system, inflammation and blood flow. NO has been noted to relax the smooth muscle and walls of arterioles. The complex endothelial cells lining the blood vessels release a puff of NO at each systole, which gets diffused into the underlying smooth muscle cell, and thus permits the surge of blood to pass through easily¹³.

NOS3 GENE

Endothelial nitric oxide synthase (eNOS), along with inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS), catalyze the generation of nitric oxide and L-citrulline from L-arginine and molecular oxygen. It is activated at concentrations of calcium greater than 100 nM and requires tetrahydrobiopterin, flavin adenine dinucleotide, flavin mononucleotide and NADPH for catalytic activity. eNOS is tightly regulated by co- and post-translational lipid modifications, phosphorylation by Akt/PKB, PKA and AMPK and protein-protein interactions. Negative protein-protein interactions are mediated by Hsp90 and Cav-1 in the Golgi

and by NOSIP and NOSTRIN at the plasma membrane, whilst dynamin, porin and soluble guanylylcyclase are positive regulators of eNOS activity. eNOS is a critical mediator of cardiovascular homeostasis through regulation of the diameter of blood vessels and maintenance of an antiproliferative and antiapoptotic environment in the vasculature. The human gene encoding eNOS is localized to chromosome 7q36^{6,18,26}.

NOS3 Polymorphisms

In the human genome, the NOS3 gene spans about 24 Kb on chromosome 7. It contains 26 exons and 25 introns. A number of polymorphic sites, including a variable number of tandem repeats (VNTRs), dinucleotides repeats (CA)_n and SNPs in the NOS3 gene have been identified. Three common and clinically relevant polymorphisms in the eNOS gene have been widely studied because they may affect NO formation in healthy subjects and in patients: the T-786C (rs2070744) in the promoter region; the 4b/a VNTR in intron 4; and the Glu298Asp (rs1799983) in exon 7. The organization of NOS3 gene and localization of the three common polymorphisms. The top line and the numbers represent the scale of the DNA in kilobases. The bottom line and the solid bars on it represent the exons and their relative locations¹⁴.

GENE	NOS3	NOS1	NOS2
Protein	eNOS (Endothelial NOS)	nNOS (Neuronal NOS)	iNOS (Inducible NOS)
Tissue Distribution	endothelium, cardiac myocytes, blood platelets, mast cells, erythrocytes, leukocytes	neurons, skeletal muscle cells, endothelial cells	immune system, cardiovascular system, hepatocytes, chondrocytes, keratinocytes, respiratory epithelia, macrophages
Physiological Function	Vasodilation	Neurotransmitter	Inflammatory response
Chromosomal location	7q35-36	12q24.2	17q12
Expression	Constitutive	Constitutive	Inducible
Enzyme Activity	Ca ²⁺ dependent	Ca ²⁺ dependent	Ca ²⁺ independent

The T-786C polymorphism, due to a thymine nucleotide replacement by a cytosine in 786 nucleotides upstream of the coding region, reduces the expression of eNOS gene by about 50%¹⁵. The reduced expression might be the result of the altered transcription factor binding caused by the

minor allele. Although eNOS is normally constitutively expressed at basal level, it is up-regulated by the cytokine IL-10 through the transcription factor STAT-3 upon shear stress stimulation. A cell based study showed that in the -786 CC genotype cells, the IL-10 response is abolished. It was

suggested that this insensitivity to IL-10 stimulation played a role in the increased risk for rheumatoid arthritis in CC genotype carriers¹⁵.

In the 4b/a VNTR polymorphism, the wild-type allele (4b) has five 27-bp repeats while the minor allele (4a) has four. It has been proposed that the 27-bp repeats may act as small RNA in controlling the eNOS expression through a negative feedback since endothelial cells with the wild type allele (five repeats) had lower levels of eNOS expression than the cells with the minor allele (four repeats)¹⁶. However, the NO level in the 4a carriers was found to be lower¹⁷. Therefore the molecular mechanism of this polymorphism is inconclusive so far. In addition to 4b/a polymorphism, two other VNTR polymorphisms in intron 4, 4c that correspond to six 27-bp repeats and 4y that has only two 27-bp repeats were also reported in rare cases, confined to the group of African-Americans with severe cardiovascular diseases¹⁸.

The Glu298Asp polymorphism, also known as G894T, is characterized by a guanine conversion to thymine at position 894 of the gene. Consequently the amino acid residue at 298 of the protein eNOS is converted from glutamine to aspartate. This amino acid change reduces the binding of eNOS to caveolin-1, the major protein located in the caveolae of epithelial cells, resulting in less eNOS protein storage there and subsequently a reduced NO production upon activation¹⁹.

Haplotype analysis shows that these three polymorphisms are not randomly distributed. The Asp298 allele is negatively associated with the 4a allele, meaning that an Asp298 minor allele carrier is very unlikely to carry the VNTR 4a minor allele. The Asp298 allele is also positively associated with the -786C allele in Caucasians, with the Asp298-786C-4b haplotype found in 24% frequency in the population²⁰.

Endothelial dysfunction and eNOS

The endothelium comprises the inner lining of blood vessels. In contrast to the earlier viewpoint that the function of the endothelial lining of vessels is to serve as a mechanical barrier, we now know that the endothelium also senses and responds to physiologic and pathologic stimuli. It produces vasoactive substances, including nitric oxide (NO), prostacyclin and endothelins. The expression of surface cell adhesion molecules governs interactions with circulating cells; these are leukocytes and monocytes, affecting inflammation, and platelets, ultimately affecting thrombosis. The endothelium also modulates the proliferation and injury response of the vascular smooth muscle layer, which contributes to neointima formation during the development of atherosclerotic plaques. These roles of the endothelium parallel current concepts about the pathogenesis of atherogenesis²¹⁻²², which involve abnormalities in vascular signaling, oxidative stress, inflammatory cells and thrombosis. Normal endothelial function protects against these processes, and endothelial dysfunction is central to the pathogenesis of atherosclerotic lesion development.

NO is a gas produced by nitric oxide synthase (NOS) enzymes. There are three major isoforms of NOS, encoded by separate genes on separate chromosomes: neuronal NOS (nNOS), or type 1 NOS; inducible NOS (iNOS), or type 2 NOS; and eNOS, or type 3 NOS. The essential role of NO as the elusive endothelium-derived relaxing factor (EDRF) was the topic of the 1998 Nobel Prize in Physiology or Medicine. eNOS-derived NO serves important functions, including regulation of vascular tone and regional blood flow, suppression of vascular smooth muscle cell proliferation, modulation of leukocyte-endothelial interactions and modulation of thrombosis. These functions are confirmed by the phenotypes of eNOS-knockout mice. eNOS gene deficiency results in hypertension, increased vascular smooth muscle cell proliferation in response to

vessel injury, increased leukocyte–endothelial interactions, hypercoagulability and increased diet-induced atherosclerosis.

A common feature of many cardiovascular risk factors (including hypertension, diabetes, insulin resistance, obesity and hyperlipidemia) is endothelial dysfunction. Because the endothelium normally protects against the processes involved in atherogenesis – namely, smooth muscle cell proliferation, inflammation and thrombosis – endothelial dysfunction is an important final common pathway by which these risk factors increase atherosclerosis.

Insulin signaling in endothelial cells occurs after insulin binding to the insulin receptor. This causes activation of two separate and parallel pathways: (i) PI3K–Akt and (ii) Ras/Raf/MAP kinase. (i) Akt kinase phosphorylates eNOS at S1177, resulting in increased NO production and vasodilation. (ii) The MAP kinase pathway results in endothelin-1 production and vasoconstriction. In skeletal muscle the PI3K–Akt pathway results in translocation of GLUT4 and glucose uptake. In vascular smooth muscle the MAP kinase pathway results in growth and mitogenesis.

Insulin resistance alters the balance between the two pathways to downstream insulin signaling. Specifically, the PI3K–Akt pathway is altered in insulin resistance²³. This results in diminished eNOS activity, less NO generation and diminished insulin-mediated vasodilation. In the peripheral tissues, insulin resistance leads to decreased glucose uptake in skeletal muscle and adipose tissues because of downregulation of GLUT4 translocation. By contrast, the Ras/Raf/MAP kinase pathway is generally preserved in insulin resistance. Thus, unopposed ET-1 production and mitogenic effects persist and contribute to the vascular effects of insulin resistance.

The relationship between endothelial dysfunction and insulin resistance is complex. Metabolic abnormalities seen in obesity, diabetes and metabolic syndrome include insulin resistance and visceral adiposity. Both might cause endothelial

dysfunction and, as a result, lead to development of atherosclerosis²⁴. Conversely, endothelial dysfunction, specifically deficiency of endothelial NO, might lead to metabolic abnormalities, including insulin resistance²⁵. In addition, insulin resistance can cause atherogenic dyslipidemia and contribute to visceral adiposity²⁶.

Under conditions of oxidative stress, O_2^- production increases. O_2^- reacts with NO to form OONO– in an extremely rapid reaction that is essentially diffusion limited²⁷. Thus, O_2^- scavenges NO and renders it unavailable to mediate its physiologic functions, including binding to soluble guanylatecyclase to stimulate vasodilation. In addition, OONO– can cause direct oxidative damage, as well as tyrosine phosphorylation of endogenous proteins, affecting their function.

Because uncoupled eNOS not only generates NO but also generates O_2^- , increasing eNOS transcription, translation or activity does not guarantee improved endothelial function. In fact, as a potential source of O_2^- , uncoupled eNOS might actually worsen the situation as compared with enzymatically inactive or absent eNOS. This is an important limitation to transgenic or gene therapy approaches to increase vascular eNOS expression.

Endothelial dysfunction causes Insulin resistance

In addition to the effects of obesity, insulin resistance and metabolic abnormalities on eNOS and endothelial function, eNOS itself is required for intact insulin signaling²⁸. Insulin-mediated increases in eNOS activity and NO production lead to increased blood flow and functional capillary recruitment to peripheral tissues. This results in increased delivery of insulin and glucose to skeletal muscle and fat, which contributes to insulin-mediated glucose uptake. Thus, when endothelial dysfunction occurs and vascular redistribution effects of insulin are blunted, there is a vicious cycle that results in further reduction in the metabolic effects of insulin

in peripheral tissues owing to decreased delivery of glucose and insulin to the tissues.

Insulin-Stimulated Production of NO

Insulin-signaling pathways in vascular endothelium leading to the activation of endothelial NO synthase (eNOS) and increased production of NO are completely distinct, separable, and independent from classical calcium-dependent mechanisms used by G-protein-coupled receptors, such as the acetylcholine receptor. Recently, a complete biochemical insulin-signaling pathway in endothelium regulating the production of NO has been elucidated. This involves insulin receptor phosphorylation of IRS-1, which then binds and activates PI 3-kinase, leading to phosphorylation and activation of PDK-1, which in turn phosphorylates and activates Akt. Akt directly phosphorylates eNOS at Ser1177, resulting in increased Enos activity and NO production. Activation of PI 3-kinase is necessary for insulin-stimulated production of NO in endothelium. However, it is not sufficient because stimulation of endothelial cells with growth factors such as platelet-derived growth factor activates PI 3-kinase and Akt without leading to phosphorylation or activation of eNOS. In addition to phosphorylation, other posttranslational modifications, including palmitoylation, nitrosylation, and O-GlcNacylation, are important regulatory mechanisms for subcellular targeting and regulation of eNOS activity. All of these regulatory mechanisms may contribute to basal and insulin-stimulated production of NO. The Ras/MAP-kinase branch of insulin-signaling pathways does not contribute to activation of eNOS in response to insulin inasmuch as insulin-stimulated production of NO is not substantially affected by inhibition of these pathways.

Shared Mechanisms Underlying Insulin Resistance and Endothelial Dysfunction

Genetic and environmental factors contribute to insulin resistance and endothelial dysfunction. Interestingly, some of the same mechanisms underlying

acquired insulin resistance also contribute to endothelial dysfunction. In particular, the hyperglycemia of diabetes leads to glucotoxicity, which causes insulin resistance and endothelial dysfunction. Similarly, elevated free fatty acid (FFA) levels in diabetes, obesity, and dyslipidemias lead to lipotoxicity, which underlies other shared mechanisms of insulin resistance and endothelial dysfunction. Pro-inflammatory states associated with metabolic and cardiovascular diseases represent a third category of shared mechanisms between insulin resistance and endothelial dysfunction²⁹.

Glucotoxicity and Insulin Resistance

Hyperglycemia associated with diabetes causes insulin resistance by increasing oxidative stress, formation of advanced glycation end products (AGEs), and flux through the hexosamine biosynthetic pathway.

Shared Stressors Causing Simultaneous Insulin Resistance and Endothelial Dysfunction

One reason that metabolic and cardiovascular diseases are often associated is that multiple stressors independently cause insulin resistance and endothelial dysfunction. Hyperglycemia leads to increased oxidative stress, increased AGE, inflammation, and increased flux through the HSP, and elevated levels of FFA promote oxidative stress and inflammation. Thus, in diabetes, obesity, metabolic syndrome, dyslipidemias, and cardiovascular diseases, multiple pathogenic stressors simultaneously cause insulin resistance in metabolic tissues and endothelial dysfunction in vascular tissues³⁰.

Cardiovascular disease and the dyslipidemia of insulin resistance

The available in vitro and in vivo data suggest that, as long as adequate insulin is available to prevent excessive lipid oxidation, FFA-driven lipid synthesis is the major determinant of VLDL TG secretion. Hyperinsulinemia probably is a marker of insulin resistance, rather than a major, direct

contributor to the process. The basis for the rise in FFA flux in insulin resistance is probably oligogenic in nature, but whatever its ultimate cause, the rest of the dyslipidemic phenotype associated with insulin resistance follows once VLDL secretion increases. Hypertriglyceridemia, leading to low HDL cholesterol and increased small dense LDL particles, is due mainly to the actions of cholesteryl ester transfer protein (CETP). In plasma, collisions between VLDL and HDL, in the presence of CETP, stimulate the transfer of VLDL TG to HDL in exchange for HDL cholesteryl esters. The resulting TG-enriched HDL becomes a good substrate for hepatic lipase (and possibly LPL), and the TG is hydrolyzed. This, in turn, generates a smaller HDL that must shed some of its surface, including apoA-I. ApoA-I is a small protein that can be filtered by the kidney and then degraded by renal tubular cells. In a similar fashion, intravascular collisions between VLDL and LDL allow for CETP-mediated exchange of VLDL TG for LDL cholesteryl esters. The succeeding hydrolysis of LDL TG generates small dense LDL particles.

Since both normoglycemic insulin-resistant individuals and patients with type 2 diabetes mellitus do not have higher LDL cholesterol levels than the general population, how does the resulting dyslipidemia increase the risk of insulin-resistant individuals to cardiovascular disease? It is suggested that multiple aspects of their lipid profiles are atherogenic. First, not only are there increased levels of VLDL particles, which can enter the vessel wall and accumulate in atherosclerotic plaques³¹, but these VLDL are, by virtue of receiving CETP-transferred cholesteryl esters, able to deliver more cholesterol per particle to the vessel wall. Additionally, increased VLDL secretion can contribute to postprandial hyperlipidemia by providing competition for chylomicron clearance pathways; postprandial hyperlipidemia is independently associated with CAD. Second, reduced HDL cholesterol and

apoA-I levels mean that there are fewer HDL particles engaged in cholesterol efflux from peripheral tissues, which is the first step in reverse cholesterol transport. Fewer HDL particles also mean that HDL cannot fulfill several proposed direct antiatherogenic actions at the vessel wall, including the role of HDL as an antioxidant. Krieger and colleagues recently identified scavenger receptor B1 (SRB1), which appears to mediate the selective delivery of HDL cholesteryl esters to the liver (delivery of core lipid by the HDL particle without endocytosis and degradation of the whole particle), targeting that cholesterol for excretion via the biliary pathway. CETP-mediated transfer of HDL cholesteryl esters to VLDL not only may enrich an atherogenic lipoprotein with cholesterol but also can divert that cholesterol from the specific reverse cholesterol transport pathway. Theoretically, the reduced HDL cholesterol characteristic of insulin resistance could be the result of increased SRB1 expression; this is unlikely, however, because such an increase would likely reduce the risk of atherosclerosis³². Finally, small dense LDL, first identified by Sniderman and colleagues and then studied in depth by Krauss and Austin and their colleagues, may be more atherogenic than an equal number of larger more cholesteryl ester-rich LDL, because small dense LDL may be more liable to oxidation or may more readily penetrate and stick to the ECM of the artery wall.

CONCLUSIONS

With pharmacologic or transgenic interventions designed to increase eNOS activity, it will be important to assess possible effects on eNOS uncoupling because eNOS can serve not only as a source for NO but also as a source for O₂⁻. For future studies, it will be crucial to use experimental models of human disease in intact animals that reflect these complexities of NO signalling. Better yet, to the extent possible, examination of responses in patients will yield the most clinically

relevant and dependable information. Now that we understand the complex relationships between insulin resistance, visceral adiposity and endothelial dysfunction, the task before us is to translate this knowledge into effective treatments to reduce CVD, which is the leading cause of morbidity and mortality from diabetes and related metabolic diseases.

REFERENCES

1. WHO | Cardiovascular diseases (CVDs). World Health Organization; [cited 2015 Apr 19]; Available from: <http://www.who.int/mediacentre/factsheets/fs317/en/>
2. What Are Coronary Heart Disease Risk Factors? - NHLBI, NIH [Internet]. [cited 2015 Apr 20]. Available from: <https://www.nhlbi.nih.gov/health/health-topics/topics/hd>
3. Goyal A, Yusuf S. The burden of cardiovascular disease in the Indian subcontinent. *Indian J Med Res.* 2006; 124(3): 235–44.
4. Hadi HA, Suwaidi J Al. Endothelial dysfunction in diabetes mellitus. *Vasc Health Risk Manag.* 2007;3(6):853–76.
5. Ray S, Bairagi AK, Guha S, Ganguly S, Ray D, Basu AK, et al. A simple way to identify insulin resistance in non-diabetic acute coronary syndrome patients with impaired fasting glucose. *Indian J Endocrinol Metab.* 2012;16:S460–4.
6. Vecoli C, Andreassi MG, Liga R, Colombo MG, Carpeggiani C, L'Abbate A, et al. T-786→C polymorphism of the endothelial nitric oxide synthase gene is associated with insulin resistance in patients with ischemic or non ischemic cardiomyopathy. *BMC Med Genet.* 2012;13(1):92.
7. Samant P, Chavan P, Rai S. Association of eNOS gene variant with insulin resistance in Metabolic Syndrome Material & Methods. 2014;2(6):784–91.
8. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, et al. Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation.* 2001;104(3):342–5.
9. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 2006;3(11):2011–30.
10. WHO | The global burden of disease: 2004 update. World Health Organization; [cited 2015 Apr 28]; Available from: http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/
11. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, et al. Heart disease and stroke statistics--2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation.* 2009; 119(3):e21–181.
12. Coronary artery disease management: to relieve symptoms and reduce the risk of cardiovascular events | CLARIFY registry official web site [Internet]. [cited 2015 Apr 28]. Available from: <http://www.clarify-registry.com/journalists/about-coronary-artery-disease/4-coronary-artery-disease-management/>
13. World Health Organization. a Vital Investment. World Health. 2005;202.
14. Beaglehole R, Bonita R. Global public health: a scorecard. *Lancet.* 2008; 372(9654):1988–96.
15. Who. Burden of disease: DALYs. *Glob Burd Dis 2004 Updat.* 2008;40–51.
16. Jones DW, Chambless LE, Folsom AR, Heiss G, Hutchinson RG, Sharrett AR, et al. Risk Factors for Coronary Heart Disease in African Americans. *Arch Intern Med.* 2002;162(22):2565.
17. Tendera M. How much does Europe invest in the treatment of cardiovascular diseases? *Eur Heart J.* 2006;27(13):1521–2.
18. Yoshimura T, Hisatomi A, Kajihara S, Yasutake T, Ogawa Y, Mizuta T, et al. The relationship between insulin resistance and polymorphisms of the endothelial nitric oxide synthase gene in patients with coronary artery disease. *J Atheroscler Thromb.* 2003;10(1):43–7.
19. Fox KA. Implications of the Organization to Assess Strategies for Ischemic Syndromes-2 (OASIS-2) study and the results in the context of other trials. *Am J Cardio.* 1999; 84(5A):26M – 31M.
20. Mohan V, Venkatraman JV, Pradeepa R. Epidemiology of cardiovascular disease in type 2 diabetes: the Indian scenario. *J Diabetes Sci Technol.* 2010;4(1):158–70.
21. Okita K, Iwahashi H, Kozawa J, Okauchi Y, Funahashi T, Imagawa A, et al. Homeostasis model assessment of insulin resistance for evaluating insulin sensitivity

- in patients with type 2 diabetes on insulin therapy. *Endocr J.* 2013;60(3):283–90.
22. Kontos MC, Diercks DB, Kirk JD. Emergency department and office-based evaluation of patients with chest pain. *Mayo Clin Proc.* 2010;85(3):284–99.
 23. Kivimäki M, Nyberg ST, Batty GD, Fransson EI, Heikkilä K, Alfredsson L, et al. Job strain as a risk factor for coronary heart disease: a collaborative meta-analysis of individual participant data. *Lancet.* 2012;380(9852):1491–7.
 24. Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet.* 2012;380(9838):219–29.
 25. Wang HX, Leineweber C, Kirkeeide R, Svane B, Schenck-Gustafsson K, Theorell T, et al. Psychosocial stress and atherosclerosis: family and work stress accelerate progression of coronary disease in women. The Stockholm Female Coronary Angiography Study. *J Intern Med.* 2007;261(3):245–54.
 26. McCann SJH. The Precocity-Longevity Hypothesis: Earlier Peaks in Career Achievement Predict Shorter Lives. *Personal Soc Psychol Bull.* 2001;27(11): 1429–39.
 27. AliReza Y, Fatemeh KK, Mohammad RN. T-786C Polymorphism of Endothelial Nitric Oxide Synthase Gene and Serum Level of Nitric Oxide in Nonsmoker and Nondiabetic Patients Suffering from Coronary Artery Disease. *J Biotechnol Biomater.* 2012; 02:10–3.
 28. Arun Kumar AS, Umamaheswaran G, Padmapriya R, Balachandar J, Adithan C. Endothelial nitric oxide synthase gene polymorphisms and the risk of acute myocardial infarction in a South Indian population. *Mol Biol Rep.* 2013;40(2):1275–81.
 29. Casas JP, Cavalleri GL, Bautista LE, Smeeth L, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: A HuGE review. *Am J Epidemiol.* 2006; 164(10):921–35.
 30. Shah VN. Endothelial Nitric Oxide Synthase Gene Polymorphism and the Risk of Diabetic Neuropathy in Asian Indian Patients with Type 2 Diabetes. *J Diabetes Metab.* 2013;04(02).
 31. NOS3 Gene - GeneCards | NOS3 Protein | NOS3 Antibody [Internet]. [cited 2015 Apr 29]. Available from: <http://www.genecards.org/cgi-bin/carddisp.pl?gene=NOS3>
 32. Tanus-Santos JE, Desai M, Flockhart DA. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. *Pharmacogenetics.* 2001;11(8): 719–25.

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