

# Vascular Calcification in Type 2 Diabetes: A Role for Allopurinol?

Dhruv K Singh\* and Ken Farrington

Renal Unit, Lister Hospital, Stevenage, SG1 4AB, UK

## Abstract

Vascular calcification contributes significantly to the vascular damage burden in type 2 diabetes. Chronic hyperglycaemia is the driver of initiation and progression of vascular damage in these subjects. Enhanced oxidative stress is widespread in these subjects due to complex metabolic, cytokine, inflammatory and ageing factors, on the background of chronic hyperglycaemia. All these factors together contribute to an increased risk of vascular damage leading to atherosclerosis and vascular calcification. The xanthine oxidase system is a major contributor to the development of enhanced oxidative stress through generation of excessive free radicals. Current standard therapy is focused on controlling hyperglycaemia and screening/treatment of known co-morbidities. However, these therapies do not target oxidative stress. Allopurinol, a potent xanthine oxidase inhibitor has demonstrated beneficial effects on several parameters of vascular health such as reduction in oxidative stress, proteinuria, reversal of vascular damage, regression of ventricular hypertrophy and arresting the rate of progression of chronic kidney disease. A randomised clinical trial of Allopurinol for amelioration of oxidative stress, endothelial cell dysfunction and vascular calcification in subjects with type 2 DM may be helpful to explore its potential in this area.

**Keywords:** Diabetes mellitus; Oxidative stress; Endothelial cell dysfunction; Atherosclerosis; Vascular calcification; Allopurinol

## Introduction

Vascular calcification (VC) is an important component of vasculopathy in type 2 diabetes mellitus (DM), leading to coronary artery disease (CAD) and peripheral vascular disease (PVD), the foremost causes of mortality and morbidity, respectively, in these subjects [1]. Although, chronic hyperglycaemia is the primary factor for initiation of vascular damage, the progression of vascular disease, may be due to several associated factors downstream such as enhanced oxidative stress (OS) [2]. The increased OS in DM may be due to impaired anti-oxidant response and/or excessive production of free radicals in close approximation to the vessel wall [3].

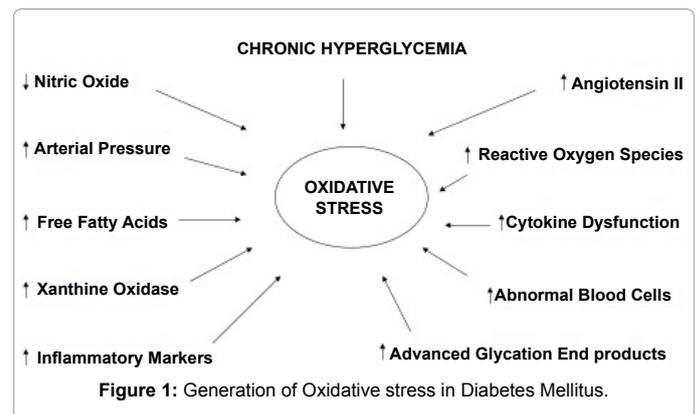
Persistent OS is detrimental to the endothelial lining of the vessel wall and has been demonstrated to promote endothelial cell dysfunction and apoptosis [4]. The various mechanisms by which the abnormal cellular and biochemical changes in endothelial cell dysfunction influence the vessel wall have been discussed previously [5,6]. Endothelial cell dysfunction is a major intermediate event in the vascular pathology between OS and progressive vascular disease such as atherosclerosis and VC [5] of the intimal layer (atherosclerotic calcification) and medial arterial calcification (MAC) [7].

Chronic OS manifests its effects by promotion of endothelial cell dysfunction [6], modulation of calcifying vascular smooth muscle cells (VSMC) [8] and influencing osteogenic transcription factors in the vessel wall [9]. The OS (Figure 1) seen in subjects with type 2 DM may be fuelled by excessive production of free radicals from major metabolic pathways/processes such as oxidative phosphorylation of glucose, excessive generation of advanced glycation end products, polyol pathway, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) pathway [3] and xanthine oxidase systems [10].

Subjects with type 2 DM have been found to have elevated levels of xanthine oxidase, which along with NADPH pathway plays a major role in promotion of OS [11]. The aims of this review are i) to explore the xanthine oxidase system in promoting OS ii) discuss the importance of OS in manifestation of VC and iii) postulate a role for Allopurinol in the amelioration of VC, through its inhibition of xanthine oxidase.

## Xanthine Oxidase - Metabolism

Xanthine oxidase is a major metabolic enzyme from the molybdenum iron-sulfur flavin hydroxylase group of enzymes. It is composed of two monomer subunits of approximately 150 kDa with an approximate total size of 300 kDa [12]. It has been located in several body tissues such as the liver, gastrointestinal tract, brain, kidneys and widely distributed in the cardiovascular system with high levels in the endothelial cells, cardiac and skeletal muscles [13]. Xanthine oxidase and xanthine dehydrogenase are two inter-changeable forms of the same enzyme, xanthine oxidoreductase [14]. While xanthine dehydrogenase can reduce oxygen to superoxide and hydrogen peroxide, and nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to NADH, xanthine oxidase can only reduce oxygen to superoxide [15].



\*Corresponding author: Dhruv K Singh, Renal Unit, Lister Hospital, Stevenage, SG1 4AB, UK, Tel: +44 1438781340; Fax: +44 1438781130; E-mail: dsingh4@nhs.net

Received April 30, 2015; Accepted May 27, 2015; Published May 30, 2015

Citation: Singh DK, Farrington K (2015) Vascular Calcification in Type 2 Diabetes: A Role for Allopurinol? J Diabetes Metab 6: 559. doi:10.4172/2155-6156.1000559

Copyright: © 2015 Singh DK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Xanthine oxidase and xanthine dehydrogenase play important roles in purine metabolism by catalyzing the conversion of hypoxanthine to xanthine and further to uric acid [14]. In mammals, the uric acid is degraded further by an enzyme uricase to allantoin, which is excreted in urine. Due to absence of uricase enzyme in humans, the uric acid cannot be further degraded and is directly handled by the kidneys for its excretion from the body [16]. In addition, both these enzymes can oxidize NADH with generation of reactive oxygen species such as superoxide and hydroxyl [17].

### Xanthine Oxidase and Oxidative Stress

Xanthine oxidase system is one of the major sources of reactive oxygen species, which includes free radicals such as superoxide (\*O<sub>2</sub>-), hydroxyl (\*OH) and non-radical molecules such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [18]. These oxidative molecules, with an unpaired electron in the outer orbit, are highly reactive and damaging when in contact with other cell structures and molecules [19]. Of the above radicals, hydroxyl radicals have an ultra-short half-life of about one nano-second and randomly oxidize the nearest molecule/s as seen in lipid peroxidation and amino acid oxidation leading to enzyme dysfunction [20]. In physiological states, these molecules carry out important roles in cell signalling and ageing, during metabolic processes.

The superoxide (\*O<sub>2</sub>-) generated by xanthine oxidase may react with nitric oxide (NO) in the vascular lumen to produce reactive nitrogen species - peroxynitrite (ONOO-), a highly reactive oxidant [18]. Peroxynitrite can potentially oxidize tetrahydrobiopterin, a major cofactor for nitric oxide synthase (NOS), and adversely reduce cellular transport of L-arginine, a substrate for the action of eNOS, to generate NO [21]. Chronic hyperglycaemia-induced excess formation of peroxynitrite plays a major role in the pathogenesis of endothelial cell dysfunction and vascular damage [22]. In addition, peroxynitrite is instrumental in oxidant-induced injury through activation of matrix metalloproteinase 2 (MMP-2) [23].

Xanthine oxidase has been reported to be an important source of free radicals in human cultured aortic endothelial cells [24]. Subjects with type 2 DM have been found to have significantly high levels of xanthine oxidase as compared to healthy controls [10]. The excessive free radical generation by xanthine oxidase in these subjects may be compounded by additional free radical generation by non-glucose

dependent pathways such as NADPH and glucose dependent pathways such as oxidative phosphorylation, polyol/sorbitol pathway and advanced glycation end product pathway. Aberrations in other minor metabolic pathways such as uncoupling of nitric oxide synthase, cytokine and growth factor signal transduction and amplification, glutathione pathway, mitochondrial uncoupling of the respiratory chain or as in primary inherited mitochondrial dysfunction, may add to the OS burden [25], by increased production of reactive oxygen species (ROS) and relatively subdued antioxidant activity in these subjects.

### Oxidative Stress and Vascular Calcification

Enhanced OS plays a key role in the initiation and progression of vascular damage from endothelial cell dysfunction to atherosclerosis and finally VC [26]. MAC, the characteristic calcification in subjects with type 2 DM leads to compromised vessel compliance, as a result of reduced elasticity [7]. Traditionally, MAC has been considered as a benign process, usually related to the ageing process, however recent studies have deciphered this as a slow but dynamic process of vascular mineralisation, with involvement and contribution from several cell types such as the endothelium, inflammatory cells and vascular smooth muscle cells (VSMC) amongst others, leading to significant cardiovascular functional compromise [27].

The manifestation and progression of MAC on the background of progressive OS is primarily an imbalance between inhibitors of VC in the vascular milieu such as inorganic pyrophosphate, matrix Gla protein, Fetuin A, Osteopontin and Osteoprotegerin, and the major promoters of VC such as endothelin-1, alkaline phosphatase, bone morphogenetic protein (BMP)-2, BMP-4, transforming growth factor (TGF-β) and receptor activator of nuclear factor kappa β ligand (RANKL), favouring the latter (Figure 2) [28].

All the adverse complications of hyperglycaemia in conjunction with OS may weaken the defence mechanisms in the vessel wall and expose the endothelial cell and subsequently the VSMC to promoters of VC. Chronic hyperglycaemia significantly reduces life expectancy by promoting the pathogenesis and progression of CVD, and the processes of ageing [29]. Free radical damage and enhanced OS has been hypothesized as an important pathway of ageing by a cumulative process of OS of the cellular constituents and apoptosis [30].

Hemodynamic forces such as vascular shear stress and

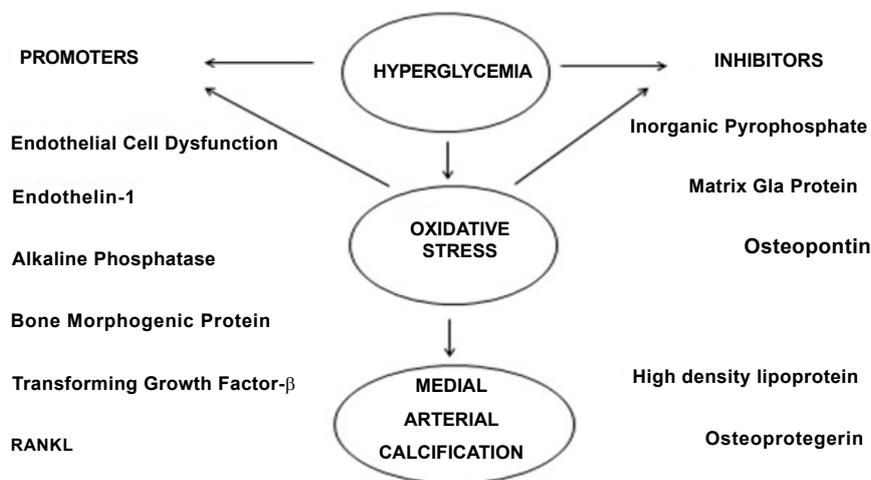


Figure 2: Modulators of Medial Arterial Calcification in Diabetes Mellitus.

compromised compliance as a result of arteriosclerosis have been proposed to increase oxidative stress in the vessel wall [31]. It has been long recognised that atherosclerosis and VC are more likely to manifest in high pressure/wall tension arteries as compared to low pressure vessels such as arterioles and veins [7]. Enhanced laminar shear stress may facilitate processes of atherosclerosis and VC by additional mechanisms such as activation of cellular signalling pathways, pro-inflammatory cytokines, VSMC hypertrophy and differentiation and modulation of extracellular matrix [32]. These adverse processes in the vessel wall may be fuelled by enhanced oxidative stress.<sup>31</sup> As compared to non-diabetics, patients with DM have enhanced burden of vascular disease, with more extensive atherosclerosis, poor compensatory remodelling, greater plaque progression, and decrease in luminal size though with similar girth of the external elastic layer [33].

Enhanced ROS formation, such as hydrogen peroxide, one of the key modulators of processes of atherosclerosis and calcification, has been implicated in these processes and high levels of hydrogen peroxide have been found in calcified vessel wall, providing circumstantial evidence of its involvement [34]. Hydrogen peroxide is one of the most stable of the ROS and has been demonstrated to influence the processes of atherosclerosis and VC through modulation of signalling pathways, mobilisation of pro-calcification proteins such as bone morphogenic protein (BMP) -2 and BMP 4, promoting the processes of phenotypic differentiation of VSMC into calcifying cells and activation of the inflammatory cytokine network such as tumor necrosis factor -  $\alpha$  (TNF -  $\alpha$ ) [9].

Hydrogen peroxide may potentially modulate parallel processes of VC by its influence on other signalling cascades in the vascular lumen [34]. The two major signalling cascades, which are activated by increased oxidative stress ( $H_2O_2$ ) to promote osteoblastic and chondrocytic differentiation in the vessel wall are the Runx2 and the BMP-2 pathways [9]. These two pathways are distinct with each capable of inducing VC on their own, when stimulated [34]. BMP-2 and BMP-4 are members of the transforming growth factor (TGF- $\beta$ ) group of cytokines and are expressed by several cells in the body including endothelial and VSMCs in the vascular lumen and share the same receptor [35].

In physiological state, these BMP-2 and BMP-4 proteins are known to modulate several cellular processes incorporating development of the cardiovascular system, angiogenesis, inflammatory response to vessel wall injury and VC [34]. The expression of BMP-2 may be up regulated by several stimuli such as chronic hyperglycaemia, cytokine activation by TNF- $\alpha$  and enhanced oxidative stress especially with increased levels of  $H_2O_2$ , amongst others [36]. In view of its high vascular permeability and potential adverse effects due to its paracrine action, the levels of  $H_2O_2$  is tightly controlled in the vascular lumen by potent anti-oxidants such as catalase and glutathione peroxidase [37].

In normal conditions, when exposed to pathological stimuli, the VSMCs have inherent potential for phenotypic transformation, proliferation and migration to site of injury to facilitate vasculature repair processes [32]. However, in presence of enhanced vascular damage due to persistent OS and/or hyperglycaemic milieu, the processes of phenotypic differentiation and regulation may be disconcerted, leading to abnormal differentiation and transformation of the VSMC into other mesenchymal lineages such as osteoblasts, chondrocytes and adipocytes, which facilitates processes of atherosclerosis and VC [38]. In addition to NADPH, Xanthine oxidase is a major contributor to increased OS in the vascular lumen and reduced NO levels, especially in type 2 DM subjects [10].

Oxidative stress induced VSMC differentiation is carried out by the action of  $H_2O_2$ , which promotes osteogenic transformation of the calcifying VSMCs, through regulation of the osteogenic transcription factor Runx2 by AKT signalling [9]. Runx2 is one of the major transcription factors in the modulation of osteoblast and chondrocyte differentiation and activity and can potentially induce several osteogenic molecules such as alkaline phosphatase, bone sialoprotein and osteopontin [39]. The manifestation of  $H_2O_2$  induced VSMC calcification through Runx2, in experimental models, is independent of VSMC apoptosis and/or BMP-2 activation [9].

The pathogenesis of VC in DM is multifactorial with different distinct processes working in a disconcerted manner as a result of complex metabolic, cytokine, inflammatory and ageing factors [4]. Chronic hyperglycaemia, on its own, may promote apoptosis of vascular cells (endothelial cells and VSMC) and in conjunction with increased oxidative stress, the impact may be enhanced [40]. The apoptosis of the vascular cells may provide a trigger for TNF-  $\alpha$  stimulation, which induces BMP-2 secretion from the remaining vascular cells [34]. BMP-2 is known to activate the homeobox homolog (Msx2) and Wnt signalling pathways in the vasa-vasorum, the concentric network of blood supply to the large arteries [41]. These pathways promote the process and promoters of mineralisation in the vessel wall, by induction of osteogenic enzymes and matrix proteins at the site of activation, leading to MAC [36]. These pathways may be fuelled by progressive oxidative stress, which may facilitate enhanced progression of VC in subjects with DM [40].

Experimental models have demonstrated a direct apoptotic effect of xanthine oxidase, in addition to increased OS, on the endothelial and VSMC [42]. The high levels of xanthine oxidase in subjects with type 2 DM may reflect the increased oxidant activity, on the background of chronic hyperglycaemia.<sup>10</sup> Recently, it has been demonstrated in experimental models that xanthine oxidase plays a key role in transformation of macrophages into foam cells and thus promote the processes of atherosclerosis and VC [43].

Xanthine oxidase also promotes enhanced production of uric acid, an independent marker of increased cardiovascular mortality in subjects with type 2 DM [44]. Uric acid induced CRP (C - reactive protein) expression in the endothelial and VSMC cells [45], may potentiate the inflammatory component of the vascular disease in type 2 DM, further exacerbating the pro-calcification milieu on the background of chronic hyperglycaemia and OS.

## Current Anti-oxidant Therapies

Given the key role of OS in the initiation and progression of various components of vascular disease such as endothelial cell dysfunction, atherosclerosis and VC, an effective anti-oxidant therapy is vital in the management of vascular complications of DM. Unfortunately, no specific therapy is currently available to fill this void. However, some of the therapies employed in management of co-morbidities of diabetes may indirectly decrease the OS burden as a manifestation of their pleiotropic effect. Some of these therapies such as angiotensin-converting enzyme (ACE) inhibitors [46], angiotensin receptor blockers (ARB) [16] and aldosterone blockers (spironolactone) [47,48], in addition to control of hypertension, may activate eNOS levels in the vascular lumen and thus increase bioavailability of NO. In addition to their known action as potent anti-lipidemic agents, statins also modulate eNOS in the vascular lumen [49]. Benfotiamine, an important agent in the treatment of diabetic neuropathy, may reduce OS by its inhibitory action on ROS formation and activation of eNOS [50].

In addition to the current widely used medications, there are other agents, which are being studied for their efficacy as potential antioxidant agents. Some of these agents such as L-propionyl-carnitine, which has intracellular superoxide scavenging properties, have demonstrated protective effects on DNA damage in the mitochondria, in experimental models [51]. Pentoxifylline, a potent inhibitor of phosphodiesterases and platelet aggregation, has demonstrated significant antioxidant properties in a small cohort of type 2 DM subjects [52]. Bioflavonoids have demonstrated dose-dependent, potent antioxidant, free-radical-scavenging and DNA cleavage properties [53]. Along with the above mentioned agents, reports from small studies have demonstrated significant anti-oxidative activity of alpha-lipoic acid [54], Vitamin C and E [55], which all need to be examined in bigger cohorts. Recently, a number of small studies have reported potent anti-oxidant activity of Allopurinol, in subjects with type 2 DM [56] and those without [57].

### Current Therapies for Vascular Calcification

In spite of high prevalence of VC in chronic diseases such as Diabetes and chronic kidney disease, there are no specific therapies available for treatment of VC. In uncomplicated diabetes subjects with no stigmata of any diabetic complication, the current approach is to maintain good glycemic control, optimum blood pressure and lipid parameters. Improvement in endothelial cell dysfunction by maintaining good glycemic control has been demonstrated to retard the rate of coronary calcification progression in people with type 2 diabetes [58].

ACE inhibitors have been successfully used for control of blood pressure and microalbuminuria in type 2 diabetes, however, their use in prevention of VC is limited due to conflicting evidence from experimental studies with some demonstrating beneficial effects [59] and some not [60]. In people with advanced kidney disease, non-calcific phosphate binders such as lanthanum carbonate has been shown to have beneficial effects on progression of coronary calcification in a recent small study [61], however, another pilot study [62] which examined sevelamer, another preferred non-calcific phosphate binder and Rosuvastatin, did not show any significant benefit on coronary calcification with either of these agents.

In a recently published study [63], Cinacalcet, a calcimimetic, in combination with small dose of vitamin D, demonstrated beneficial effects in prevention of coronary calcification in people on haemodialysis, as compared to vitamin D alone. In another study [64], vitamin D in either high or low dosage had no effect on the rate of progression of coronary calcification. Bisphosphonates may have beneficial effects in prevention of coronary calcification especially in those with abnormal mineral metabolism [65]. Although encouraging, beneficial agents such as lanthanum, Cinacalcet and Bisphosphonates have to be examined in bigger studies and their beneficial effects are limited to patients with advanced kidney disease and abnormal bone/mineral metabolism. As diabetes subjects are known to have VC in early stages with normal mineral metabolism [66], these agents are less likely to be useful in this setting.

### The Therapeutic Potential of Allopurinol

Allopurinol is a potent inhibitor of xanthine oxidase and has been in clinical use for over five decades, primarily in the treatment of gout [67]. On administration, Allopurinol is oxidized by xanthine oxidase to oxypurinol, its active form. At low levels, Allopurinol competitively inhibits xanthine oxidase by acting as a substrate, however, at increased levels, it acts as a non-competitive inhibitor

[68]. The accidental discovery of the role of xanthine oxidase in the production of superoxide, paved the way for more research in this area for development of several other potent xanthine oxidase inhibitors of different classes and the exploration of potential anti-oxidant effect of Allopurinol [69-86]. Apart from its established use in gout and tumor lysis syndrome, the pleiotropic effects of Allopurinol is being explored in a number of conditions [67]. Some of the major studies examining the effects of Allopurinol supplementation in various vascular conditions have demonstrated beneficial effects in human subjects and have been summarised in Table 1 below.

Allopurinol has demonstrated significant cardio-protective potential with significant reduction in arrhythmias, myocardial infarction [87], and a reduction in lipid peroxidation [76], thus improving perioperative recovery, in patients undergoing elective coronary artery bypass surgery. Intra coronary administration of Allopurinol has been shown to improve myocardial efficiency, in subjects with idiopathic dilated cardiomyopathy. Allopurinol has also been shown to improve endothelial dysfunction and associated with improved clinical outcomes and survival [77]. In addition, to cardiovascular conditions, experimental models of cerebrovascular conditions have reported potential therapeutic effects of Allopurinol in amelioration of ischemic cerebral damage with neurological deficits [88], focal cerebral ischemia and hypoxic-ischemic injury [89].

Allopurinol has been reported to normalize endothelial cell dysfunction in a small group of type 2 DM subjects with mild hypertension, by reducing OS [56]. The reduction in OS by Allopurinol is independent of its effect on uric acid [57] and this may provide additional benefit in terms of reducing the OS burden. Increased xanthine oxidase and uric acid expression has been co-localized with lipid particles in atherosclerotic plaque samples [90], which may be circumstantial evidence of their involvement in the processes of calcification. The recent demonstration of the role of xanthine oxidase in the transformation of macrophages into foam cells and development of atherosclerosis, in experimental models [43], opens other plausible avenues for the therapeutic uses of Allopurinol in this setting.

Allopurinol supplementation has been reported to retard the rate of progression of chronic kidney disease and reduction in the overall cardiovascular risk in subjects with kidney disease [85,91]. The potential benefit of Allopurinol was attributed to its amelioration of OS and subsequent reduction in the levels of inflammatory markers. In addition, Allopurinol has recently demonstrated beneficial effects in decreasing microalbuminuria (a state of generalized endothelial cell dysfunction), in a small study of type 2 DM subjects [74]. This finding is important as endothelial cell dysfunction is a key step in the pathogenesis of vascular disease in subjects with type 2 DM. Amelioration of endothelial cell dysfunction has been shown to be independently associated with decrease in the rate of progression of coronary calcification in type 2 DM subjects [58].

In context of vascular disease in DM, xanthine oxidase induced oxidative stress plays an important role in the initiation and progression of endothelial cell dysfunction, atherosclerosis and VC [9]. Though uric acid levels are frequently elevated in these settings [44], its direct role in the pathogenesis of these adverse events is not clear. In view of the major role of xanthine oxidase in the pathogenesis of enhanced oxidative stress in type 2 DM, it is conceivable that specific therapy aimed at negating the effect of this enzyme may help in ameliorating oxidative stress. In this regard, Allopurinol, a potent inhibitor of xanthine oxidase activity, needs further consideration to explore its

Beneficial Effects	Authors	Number of Subjects	Allopurinol Dose (mg)	Study Subjects
<b>Endothelial Dysfunction</b>				
	Butler et al. [56]	23	300	Type 2 Diabetes with mild hypertension
	Dogan et al. [70]	50	900	Normotensive Type 2 Diabetes
	Yiginer et al. [71]	28	300	Metabolic Syndrome
	Guthikonda et al. [72]	28	600	Heavy Smokers
	George et al. [57]	30	300 to 600	Chronic heart failure
	Kanbay et al. [73]	67	300	Asymptomatic hyperuricemia and normal renal function
<b>Proteinuria</b>				
	Momeni et al. [74]	40	100	Type 2 Diabetes
<b>Cardiovascular System</b>				
Myocardial efficiency	Cappola et al. [75]	9	-	Idiopathic dilated cardiomyopathy
Postoperative Recovery	Coghlan et al. [76]	50	-	Undergoing coronary artery bypass grafting
Peripheral blood flow	Doehner et al. [77]	19	300	Hyperuricemic subjects with chronic heart failure
Endothelial progenitor cells	Tousoulis et al. [78]	60	300	Congestive heart failure
Vascular and functional parameters	Greig et al. [79]	74	300	Chronic heart failure
Cardiovascular autonomic neuropathy and Myocardial blood flow				Type 1 Diabetes
Left ventricular mass	Rekhray et al. [80]	66	600	Ischemic heart disease
	Szwejkowski et al. [81]	66	600	Type 2 Diabetes and left ventricular hypertrophy
<b>Cerebrovascular System</b>				
Nitric oxide activity	Dawson et al. [82]	14	300	Type 2 Diabetes
Blood pressure and carotid intima-media thickness	Higgins et al. [83]	80	300	Post ischemic stroke and transient ischemic attack
Inflammatory markers	Muir et al. [84]	50	100-300	Recent ischemic stroke
<b>Chronic Kidney Disease (CKD)</b>				
CKD progression and Cardiovascular risk	Goicoechea et al. [85]	113	100	CKD with and without Diabetes
Endothelial Dysfunction and Left ventricular hypertrophy	Kao et al. [86]	67	300	CKD

**Table 1:** Beneficial effects of Allopurinol in Human Subjects.

potential as an anti-oxidant agent in mitigation of oxidative stress and hence amelioration of progression of VC in type 2 DM subjects.

## Conclusion

Subjects with type 2 DM harbor a great burden of vascular disease resulting in increased risk of cardiovascular and peripheral vascular disease in these subjects as compared to the rest of the population. Enhanced oxidative stress as a result of complex, metabolic, cytokine, inflammatory and ageing factors, on the background of chronic hyperglycaemia, predisposes these subjects to increased risk of vascular damage leading to atherosclerosis and vascular calcification. In the absence of any specific therapy for reduction of oxidative stress, these patients continue to depend on the extended, non-specific, beneficial role of associated therapies of diabetes management such as tight diabetes control, ACE and ARB, statins and benfotiamine. Several small studies have demonstrated significant, clinically important anti-oxidant effects of Allopurinol. In the light of its potential to ameliorate the rate of progression of vascular calcification by a reduction in endothelial cell dysfunction, exploration of potential clinical benefits of Allopurinol in the amelioration of oxidative stress, endothelial cell dysfunction and VC in subjects with type 2 DM may be helpful in this patient group.

## References

- Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H (2001) Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 44 Suppl 2: S14-21.
- Neri S, Signorelli SS, Torrisi B, Pulvirenti D, Mauceri B, et al. (2005) Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: a single-blind, 15-day clinical trial in patients with untreated type 2

diabetes, subjects with impaired glucose tolerance, and healthy controls. *Clin Ther* 27: 1764-1773.

- Singh DK, Winocour P, Farrington K (2011) Oxidative stress in early diabetic nephropathy: fueling the fire. *Nat Rev Endocrinol* 7: 176-184.
- Singh DK, Winocour P, Farrington K (2010) Review: Endothelial cell dysfunction, medial arterial calcification and osteoprotegerin in diabetes. *Br J Diabetes Vasc Dis* 10: 71-77.
- Naidoo DP (2002) The link between microalbuminuria, endothelial dysfunction and cardiovascular disease in diabetes. *Cardiovasc J S Afr* 13: 194-199.
- Schalkwijk CG, Stehouwer CDA (2005) Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin Sci (Lond)* 109: 143-159.
- Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laakso M (1996) Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 16: 978-983.
- Mody N, Parhami F, Sarafian TA, Demer LL (2001) Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radic Biol Med* 31: 509-519.
- Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, et al. (2008) Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J Biol Chem* 283: 15319-15327.
- Kuppusamy UR, Indran M, Rokiah P (2005) Glycaemic control in relation to xanthine oxidase and antioxidant indices in Malaysian Type 2 diabetes patients. *Diabet Med* 22: 1343-1346.
- McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, et al. (2003) Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. *Am J Physiol Heart Circ Physiol* 285: H2290-2297.
- Krenitsky TA, Spector T, Hall WW (1986) Xanthine oxidase from human liver: purification and characterization. *Arch Biochem Biophys* 247: 108-119.
- Hellsten-Westling Y (1993) Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. *Histochemistry* 100: 215-222.

14. Hille R, Nishino T (1995) Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. *FASEB J* 9: 995-1003.
15. Waud WR, Rajagopalan KV (1976) Purification and properties of the NAD<sup>+</sup>-dependent (type D) and O<sub>2</sub>-dependent (type O) forms of rat liver xanthine dehydrogenase. *Arch Biochem Biophys* 172: 354-364.
16. Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, et al. (2002) Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature* 417: 447-452.
17. Zhang Z, Blake DR, Stevens CR, Kanczler JM, Winyard PG, et al. (1998) A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: the role of NADH as an electron donor. *Free Radic Res* 28: 151-164.
18. Radi R, Rubbo H, Bush K, Freeman BA (1997) Xanthine oxidase binding to glycosaminoglycans: kinetics and superoxide dismutase interactions of immobilized xanthine oxidase-heparin complexes. *Arch Biochem Biophys* 339: 125-135.
19. Yu T, Robotham JL, Yoon Y (2006) Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci U S A* 103: 2653-2658.
20. Kehrer JP (2000) The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* 149: 43-50.
21. Ogonowski AA, Kaesemeyer WH, Jin L, Ganapathy V, Leibach FH, et al. (2000) Effects of NO donors and synthase agonists on endothelial cell uptake of L-Arg and superoxide production. *Am J Physiol Cell Physiol* 278: C136-143.
22. El-Remessy AB, Tawfik HE, Matragoon S, Pillai B, Caldwell RB, et al. (2010) Peroxynitrite mediates diabetes-induced endothelial dysfunction: possible role of Rho kinase activation. *Exp Diabetes Res* 2010: 247861.
23. Wang W, Sawicki G, Schulz R (2002) Peroxynitrite-induced myocardial injury is mediated through matrix metalloproteinase-2. *Cardiovasc Res* 53: 165-174.
24. Zweier JL, Broderick R, Kuppusamy P, Thompson-Gorman S, Lutty GA (1994) Determination of the mechanism of free radical generation in human aortic endothelial cells exposed to anoxia and reoxygenation. *J Biol Chem* 269: 24156-24162.
25. Forbes JM, Coughlan MT, Cooper ME (2008) Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 57: 1446-1454.
26. Madamanchi NR, Hakim ZS, Runge MS (2005) Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. *J Thromb Haemostasis* 3: 254-267.
27. Scheidegger KJ, Butler S, Witztum JL (1997) Angiotensin II increases macrophage-mediated modification of low density lipoprotein via a lipoxygenase-dependent pathway. *J Biol Chem* 272: 21609-21615.
28. Guzman RJ (2007) Clinical, cellular, and molecular aspects of arterial calcification. *J Vasc Surg* 45 Suppl A: A57-63.
29. Franco OH, Steyerberg EW, Hu FB, Mackenbach J, Nusselder W (2007) Associations of diabetes mellitus with total life expectancy and life expectancy with and without cardiovascular disease. *Arch Intern Med* 167: 1145-1151.
30. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11: 298-300.
31. Lehoux S (2006) Redox signalling in vascular responses to shear and stretch. *Cardiovasc Res* 71: 269-279.
32. Lehoux S, Castier Y, Tedgui A (2006) Molecular mechanisms of the vascular responses to haemodynamic forces. *J Intern Med* 259: 381-392.
33. Nicholls SJ, Tuzcu EM, Kalidindi S, Wolski K, Moon KW, et al. (2008) Effect of diabetes on progression of coronary atherosclerosis and arterial remodeling: a pooled analysis of 5 intravascular ultrasound trials. *J Am Coll Cardiol* 52: 255-262.
34. Csiszar A, Smith KE, Koller A, Kaley G, Edwards JG, et al. (2005) Regulation of bone morphogenetic protein-2 expression in endothelial cells: role of nuclear factor-kappaB activation by tumor necrosis factor-alpha, H<sub>2</sub>O<sub>2</sub>, and high intravascular pressure. *Circulation* 111: 2364-2372.
35. Csiszar A, Lehoux S, Ungvari Z (2009) Hemodynamic forces, vascular oxidative stress, and regulation of BMP-2/4 expression. *Antioxid Redox Signal* 11: 1683-1697.
36. Al-Aly Z, Shao JS, Lai CF, Huang E, Cai J, et al. (2007) Aortic Msx2-Wnt calcification cascade is regulated by TNF-alpha-dependent signals in diabetic Ldlr<sup>-/-</sup> mice. *Arterioscler Thromb Vasc Biol* 27: 2589-2596.
37. Ardanaz N, Pagano PJ (2006) Hydrogen peroxide as a paracrine vascular mediator: regulation and signaling leading to dysfunction. *Exp Biol Med (Maywood)* 231: 237-251.
38. Iyemere VP, Proudfoot D, Weissberg PL, Shanahan CM (2006) Vascular smooth muscle cell phenotypic plasticity and the regulation of vascular calcification. *J Intern Med* 260: 192-210.
39. Harada H, Tagashira S, Fujiwara M, Ogawa S, Katsumata T, et al. (1999) Cbfa1 isoforms exert functional differences in osteoblast differentiation. *J Biol Chem* 274: 6972-6978.
40. Peiró C, Lafuente N, Matesanz N, Cercas E, Llergo JL, et al. (2001) High glucose induces cell death of cultured human aortic smooth muscle cells through the formation of hydrogen peroxide. *Br J Pharmacol* 133: 967-974.
41. Shao JS, Cheng SL, Pingsterhaus JM, Charlton-Kachigian N, Loewy AP, et al. (2005) Msx2 promotes cardiovascular calcification by activating paracrine Wnt signals. *J Clin Invest* 115: 1210-1220.
42. Li PF, Dietz R, von Harsdorf R (1997) Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. *Circulation* 96: 3602-3609.
43. Kushiyama A, Okubo H, Sakoda H, Kikuchi T, Fujishiro M, et al. (2012) Xanthine oxidoreductase is involved in macrophage foam cell formation and atherosclerosis development. *Arterioscler Thromb Vasc Biol* 32: 291-298.
44. Zoppini G, Targher G, Negri C, Stoico V, Perrone F, et al. (2009) Elevated serum uric acid concentrations independently predict cardiovascular mortality in type 2 diabetic patients. *Diabetes Care* 32: 1716-1720.
45. Kang DH, Park SK, Lee IK, Johnson RJ (2005) Uric acid-induced C-reactive protein expression: implication on cell proliferation and nitric oxide production of human vascular cells. *J Am Soc Nephrol* 16: 3553-3562.
46. Kobayashi N, Honda T, Yoshida K, Nakano S, Ohno T, et al. (2006) Critical role of bradykinin-eNOS and oxidative stress-LOX-1 pathway in cardiovascular remodeling under chronic angiotensin-converting enzyme inhibition. *Atherosclerosis* 187: 92-100.
47. Ogawa S, Kobori H, Ohashi N, Urushihara M, Nishiyama A, et al. (2009) Angiotensin II Type 1 Receptor Blockers Reduce Urinary Angiotensinogen Excretion and the Levels of Urinary Markers of Oxidative Stress and Inflammation in Patients with Type 2 Diabetic Nephropathy. *Biomark Insights* 4: 97-102.
48. Takebayashi K, Matsumoto S, Aso Y, Inukai T (2006) Aldosterone blockade attenuates urinary monocyte chemoattractant protein-1 and oxidative stress in patients with type 2 diabetes complicated by diabetic nephropathy. *J Clin Endocrinol Metab* 91: 2214-2217.
49. Endres M, Laufs U (2004) Effects of statins on endothelium and signaling mechanisms. *Stroke* 35: 2708-2711.
50. Stirban A, Negrean M, Stratmann B, Gawlowski T, Horstmann T, et al. (2006) Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. *Diabetes Care* 29: 2064-2071.
51. Vanella A, Russo A, Acquaviva R, Campisi A, Di Giacomo C, et al. (2000) L-propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. *Cell Biol Toxicol* 16: 99-104.
52. Maiti R, Agrawal NK, Dash D, Pandey BL (2007) Effect of Pentoxifylline on inflammatory burden, oxidative stress and platelet aggregability in hypertensive type 2 diabetes mellitus patients. *Vascul Pharmacol* 47: 118-124.
53. Russo A, Acquaviva R, Campisi A, Sorrenti V, Di Giacomo C, et al. (2000) Bioflavonoids as antioxidants, antioxidants and DNA cleavage protectors. *Cell Biol Toxicol* 16: 91-98.
54. Heitzer T, Finckh B, Albers S, Krohn K, Kohlschütter A, et al. (2001) Beneficial effects of alpha-lipoic acid and ascorbic acid on endothelium-dependent, nitric oxide-mediated vasodilation in diabetic patients: relation to parameters of oxidative stress. *Free Radic Biol Med* 31: 53-61.
55. Farvid MS, Jalali M, Siassi F, Hosseini M (2005) Comparison of the effects of vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. *Diabetes Care* 28: 2458-2464.
56. Butler R, Morris AD, Belch JJ, Hill A, Struthers AD (2000) Allopurinol normalizes

- endothelial dysfunction in type 2 diabetics with mild hypertension. *Hypertension* 35: 746-751.
57. George J, Carr E, Davies J, Belch JFF, Struthers A (2006) High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* 114: 2508-2516.
58. Schindler TH, Cadenas J, Facta AD, Li Y, Olschewski M, et al. (2009) Improvement in coronary endothelial function is independently associated with a slowed progression of coronary artery calcification in type 2 diabetes mellitus. *Eur Heart J* 30: 3064-3073.
59. Ng K, Hildreth CM, Avolio AP, Phillips JK (2011) Angiotensin-converting enzyme inhibitor limits pulse-wave velocity and aortic calcification in a rat model of cystic renal disease. *Am J Physiol Renal Physiol* 301: F959-F966.
60. Tokumoto M, Mizobuchi M, Finch JL, Nakamura H, Martin DR, et al. (2009) Blockage of the renin-angiotensin system attenuates mortality but not vascular calcification in uremic rats: sevelamer carbonate prevents vascular calcification. *Am J Nephrol* 29: 582-591.
61. Kalil RS, Flanigan M, Stanford W, Haynes WG (2012) Dissociation between progression of coronary artery calcification and endothelial function in hemodialysis patients: a prospective pilot study. *Clin Nephrol* 78: 1-9.
62. Lemos MM, Watanabe R, Carvalho AB, Jancikic AD, Sanches FM, et al. (2013) Effect of rosuvastatin and sevelamer on the progression of coronary artery calcification in chronic kidney disease: a pilot study. *Clin Nephrol* 80: 1-8.
63. Ureña-Torres PA, Floege J, Hawley CM, Pedagogos E, Goodman WG, et al. (2013) Protocol adherence and the progression of cardiovascular calcification in the ADVANCE study. *Nephrol Dial Transplant* 28: 146-152.
64. Morosetti M, Jankovic L, Palombo G, Cipriani S, Dominijanni S, et al. (2008) High-dose calcitriol therapy and progression of cardiac vascular calcifications. *J Nephrol* 21: 603-608.
65. Elmariah S, Delaney JA, O'Brien KD, Budoff MJ, Vogel-Claussen J, et al. (2010) Bisphosphonate Use and Prevalence of Valvular and Vascular Calcification in Women MESA (The Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 56: 1752-1759.
66. Singh DK, Winocour P, Summerhayes B, Kaniyur S, Viljoen A, et al. (2012) Prevalence and progression of peripheral vascular calcification in type 2 diabetes subjects with preserved kidney function. *Diabetes Res Clin Pract* 97: 158-165.
67. Pacher P, Nivorozhkin A, Szabó C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 58: 87-114.
68. Pea F (2005) Pharmacology of drugs for hyperuricemia. Mechanisms, kinetics and interactions. *Contrib Nephrol* 147: 35-46.
69. McCord JM, Fridovich I (1968) The reduction of cytochrome c by milk xanthine oxidase. *J Biol Chem* 243: 5753-5760.
70. Dogan A, Yarlioglu M, Kaya MG, Karadag Z, Dogan S, et al. (2011) Effect of long-term and high-dose allopurinol therapy on endothelial function in normotensive diabetic patients. *Blood Press* 20: 182-187.
71. Yiginer O, Ozcelik F, Inanc T, Aparci M, Ozmen N, et al. (2008) Allopurinol improves endothelial function and reduces oxidant-inflammatory enzyme of myeloperoxidase in metabolic syndrome. *Clin Res Cardiol* 97: 334-340.
72. Guthikonda S, Sinkey C, Barenz T, Haynes WG (2003) Xanthine oxidase inhibition reverses endothelial dysfunction in heavy smokers. *Circulation* 107: 416-421.
73. Kanbay M, Huddam B, Azak A, Solak Y, Kadioglu GK, et al. (2011) A randomized study of allopurinol on endothelial function and estimated glomerular filtration rate in asymptomatic hyperuricemic subjects with normal renal function. *Clin J Am Soc Nephrol* 6: 1887-1894.
74. Momeni A, Shahidi S, Seirafian S, Taheri S, Kheiri S (2010) Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. *Iran J Kidney Dis* 4: 128-132.
75. Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, et al. (2011) Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. *Circulation* 104: 2407-2411.
76. Coghlan JG, Flitter WD, Clutton SM, Panda R, Daly R, et al. (1994) Allopurinol pretreatment improves postoperative recovery and reduces lipid peroxidation in patients undergoing coronary artery bypass grafting. *J Thorac Cardiovasc Surg* 107: 248-256.
77. Doehner W, Schoene N, Rauchhaus M, Leyva-Leon F, Pavitt D V, et al. (2002) Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies. *Circulation* 105: 2619-2624.
78. Tousoulis D, Andreou I, Tsiatas M, Miliou A, Tentolouris C, et al. (2011) Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: the impact of inflammatory process and oxidative stress. *Atherosclerosis* 214: 151-157.
79. Greig D, Alcaïno H, Castro PF, Garcia L, Verdejo HE, et al. (2011) Xanthine-oxidase inhibitors and statins in chronic heart failure: effects on vascular and functional parameters. *J Heart Lung Transplant* 30: 408-413.
80. Rekhraj S, Gandy SJ, Szejewski BR, Nadir MA, Noman A, et al. (2013) High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease. *J Am Coll Cardiol* 61: 926-932.
81. Szejewski BR, Gandy SJ, Rekhraj S, Houston JG, Lang CC1, et al. (2013) Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy. *J Am Coll Cardiol* 62: 2284-2293.
82. Dawson J, Quinn T, Harrow C, Lees KR, Weir CJ, et al. (2009) Allopurinol and nitric oxide activity in the cerebral circulation of those with diabetes: a randomized trial. *Diabetes Care* 32: 135-137.
83. Higgins P, Walters MR, Murray HM, McArthur K, McConnachie A, et al. (2014) Allopurinol reduces brachial and central blood pressure, and carotid intima-media thickness progression after ischaemic stroke and transient ischaemic attack: a randomised controlled trial. *Heart* 100: 1085-1092.
84. Muir SW, Harrow C, Dawson J, Lees KR, Weir CJ, et al. (2008) Allopurinol use yields potentially beneficial effects on inflammatory indices in those with recent ischemic stroke: a randomized, double-blind, placebo-controlled trial. *Stroke* 39: 3303-3307.
85. Goicoechea M, de Vinuesa SG, Verdalles U, Ruiz-Caro C, Ampuero J, et al. (2010) Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. *Clin J Am Soc Nephrol* 5: 1388-1393.
86. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, et al. (2011) Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. *J Am Soc Nephrol* 22: 1382-1389.
87. Rashid MA, William-Olsson G (1991) Influence of allopurinol on cardiac complications in open heart operations. *Ann Thorac Surg* 52: 127-130.
88. Akdemir H, Aşık Z, Paşaoğlu H, Karaküçük I, Oktem IS, et al. (2001) The effect of allopurinol on focal cerebral ischaemia: an experimental study in rabbits. *Neurosurg Rev* 24: 131-135.
89. Palmer C, Towfighi J, Roberts RL, Heitjan DF (1993) Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. *Pediatr Res* 33: 405-411.
90. Patetsios P, Song M, Shutze WP, Pappas C, Rodino W, et al. (2001) Identification of uric acid and xanthine oxidase in atherosclerotic plaque. *Am J Cardiol* 88: 188-191.
91. Fleeman N, Pilkington G, Dundar Y, Dwan K, Boland A, et al. (2014) Allopurinol for the treatment of chronic kidney disease: a systematic review. *Health Technol Assess* 18: 1-77.