**Residual renal and cardiovascular disease risk in conventionally-treated patients with type 2 diabetes: the potential of non-traditional biomarkers and treatments related redox metabolism**

**Short Title: Early detection of micro- and macrovascular complications of type 2 diabetes and its prevention**

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**Abstract**

Diabetes is a leading cause of chronic kidney disease (CKD) in the developed world. Promoters of the progression of kidney disease include the traditional profile of cardiovascular risk factors. However, the development of CKD and vulnerability to end-stage renal disease (ESRD) is highly variable. Determinants of the susceptibility to ESRD may include non-traditional risk factors such as gene-environment interactions, socio-geographic factors and/or treatment strategies.

We review the conflicting clinical relevance of studies implicating pathways related to oxidative stress. These pathways are strongly implicated in the phenotype of some groups of high-risk patients and could assume importance in clinical care. Recent clinical trial evidence has shown that newer glucose-lowering agents also have beneficial effects on reducing the incidence of renal dysfunction and cardiovascular events in high-risk patients. Research is required to identify which patients will benefit most from newer approaches to managing diabetes. Understanding the relationship of non-traditional risk factors to renal and cardiovascular disease could help clinicians targeting new therapeutic approaches in the management of type 2 diabetes.

**Key words:**

Type 2 diabetes, chronic kidney disease, cardiovascular disease, pathogenesis, racial/ ethnic, endothelium, oxidative stress, treatment targets.

**Introduction:**

Chronic Kidney Disease (CKD) is an increasing public health issue. CKD-related deaths rose 82.3% in the last two decades and is ranked 18th in the list of causes of global deaths (1) The world-wide prevalence of CKD was estimated at 8-16% independent of age, sex, ethnic group and comorbidity (2). Predictive modelling suggests that 47.1% of 30-year-olds will develop CKD during their lifetime with 4.4% reaching ESRD (3).

Diabetes is a leading cause of CKD (4). The pathophysiology of diabetic kidney disease relates to a combination of microvascular changes within the kidney that occur due to a complex interplay of metabolic, haemodynamic and cellular changes. Initial hyper-filtration with a subsequent decline in glomerular filtration rate (GFR) - with and without progressive albuminuria - is associated with excessive deposition of extracellular matrix, glomerular basement membrane thickening, podocyte loss and raised intra-glomerular pressures (5). These changes are linked to increases in reactive oxygen species (ROS) and a large experimental evidence base supports the role of inflammatory and oxidative stress in the development the haemodynamic and metabolic disturbances associated with diabetic kidney disease (6).

**Background**

Reducing sugars such as glucose react non-enzymatically with amino groups in proteins, lipids and nucleic acids to form Schiff bases that can rearrange to form Amadori products and ultimately advanced glycation end products (AGEs) (7). Under hyperglycaemic conditions found in diabetes mellitus, accelerated non-enzymatic glycation occurs which promotes the accumulation of AGEs and reactive oxygen species (ROS). Hyperglycaemia-induced ROS production is associated with reduced activity and/or production of endogenous antioxidant enzymes - catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) - that occurs in diabetes mellitus (8). A consequence of the imbalance between ROS production and the capability to detoxify these intermediates is an upregulation of transcription factors such as NF-kB and recruitment of pro-inflammatory cytokines and chemokines, resulting in renal oxidative stress and a reduced availability of the vasodilator nitric oxide (9). This pathway promoting inflammation, renal fibrosis and endothelial dysfunction with haemodynamic disturbances accounts for the pathophysiological changes occurring in diabetic kidney disease and the progression to ESRD.

Reno-protective measures such as blood pressure control and the specific, beneficial effect of systemic inhibition of the renin-angiotensin-aldosterone system (RAAS) has led to a reduction in the incidence of ESRD in non-diabetic kidney disease (10). However, these interventions together with strict glycaemic control have not brought about similar sized reductions for diabetic kidney disease which remains the leading cause of ESRD in the Western World. The sizeable residual risk for ESRD in both non-diabetic and diabetic kidney disease may be related to an association with obesity (11).

Progression of CKD to ESRD differs by gender and race, with female gender and non-Caucasian race being at higher risk (12-15). Some of the difference in kidney disease progression is explained by the relatively higher rates of hypertension and proteinuria in the patients of African origin, but is unrelated to variations in access to healthcare before dialysis (16). In the United Kingdom Prospective Diabetes Study, in which patients were followed-up from the diagnosis of type 2 diabetes, racial heritage was found to be an independent determinant of renal dysfunction (17). And, it has been reported that inflammatory stress, has a stronger relationship than albuminuria with early CKD in patients with diabetes of African heritage, compared with other heritage groups (18)

**Early detection of diabetic kidney disease using biomarkers: current and evolving strategies**

Microalbuminuira was identified as a surrogate marker for progressive renal disease in patients with type 1 diabetes nearly 4 decades ago (19). Historically, microalbuminuria defined as urinary albumin excretion rate of 30 – 300mg/24hour was considered the earliest marker of diabetic kidney disease, with persistent microalbuminuria or macroalbuminuria (>300mg urinary albumin/24hour) used to predict progression to ESRD and in patients with type 2 diabetes also predicted mortality (20). Whilst there is robust evidence that it is a marker of glomerular and tubular injury occurring as a result of diabetes there are several limitations to using this as a biomarker to detect the onset and progression of kidney disease. ESRD can develop in the absence of albuminuria and significant renal damage with loss of glomerular filtration can occur prior to the onset of albuminuria. It has been estimated that 1:3 patients with diabetes at risk of CKD may not have increased urinary protein excretion (21). Furthermore the evolution of microalbuminuria is variable: regression to non-albuminuria can take place irrespective of ongoing renal risk factors and damage; similarly, it can remain unchanged or progress to overt proteinuria. In a follow-up study of the Diabetes Complications and Control trial, the 10 year cumulative progression to macroalbuminuria and regression to normoalbuminuria was 28% and 40% respectively (22). Various factors other than progression of kidney disease can influence the amount of urinary albumin present, such as obesity, exercise, diet, smoking and inflammation. Together, these observations suggest that microalbuminuria may represent an initial reversible phase of kidney damage.

There are limitations to using serum creatinine alone to measure and estimate the risk of renal disease although plasma creatinine-based equations to estimate glomerular filtration are now widely used in clinical practice. More accurate estimates of glomerular filtration can be assessed by measuring renal clearance of both exogenous markers and endogenous markers but are not used in routine clinical practice. However, urinary albumin excretion still retains the most clinical utility - together with estimates of glomerular filtration - for risk stratification of the individual patient for progressive renal disease and feature in many of today’s guidelines (23). In summary, the risk of progressive renal disease in diabetes is not necessarily associated with a reversal of microalbuminuria and neither does its absence preclude the risk of its onset. These limitations suggest that different models of progressive renal disease risk in diabetes, that include non-traditional risk markers are required to improve the sensitivity and specificity of predicting CKD and ESRD (24). Several reviews have summarised the potential of new plasma and urinary biomarkers (25-28) (Figure1) which reflect either tubular or glomerular function, or measure inflammation. Given these comprehensive reviews ours will focus on the role and clinical utility of markers of oxidative stress.

**Oxidative stress in patients with type 2 diabetes at high-risk of developing kidney disease**

As previously mentioned, oxidative stress plays a key role in the propagation of cellular injury that occurs in DN. Thus there has been interest in finding a marker to identify those patients with increased oxidative stress in an effort to target potential anti-oxidant treatment towards this group of patients. One such emerging marker of oxidative stress is urinary 8-hydroxydeoxy-guanosine (8-OHdG). 8-OHdG is produced as a result of oxidative DNA damage initiated by ROS and is excreted unchanged in the urine, making it a sensitive marker of oxidative stress (29)

There are conflicting results from studies on the use of urinary 8-OHdG as a biomarker of microvascular damage: some authors found a positive correlation between glycated haemoglobin (HbA1c) and 8-OHdG as well as raised levels of 8-OHdG in those with diabetes as opposed to those without and in relation to increased urinary albumin in those with diabetes compared to those with normal urinary urinary albumin excretion (29-32)

Hinoki et al found that urinary 8-OHdG was the strongest predictor of diabetic nephropathy when corrected for known risk factors (33). Kanauchi reported an association between increasing 8-OHdG and biopsy-proven tubulo-interstitial injury around the same time (34). There was also limited evidence to support an association between increased 8-OHdG and macrovascular damage, as measured by increased vascular stiffness (35) IMT and increased CHD risk score (30). Others have questioned the value of these findings, pointing out that 8-OHdG provided no earlier detection of diabetic kidney disease than urinary albumin and was unable to predict those at risk of progressing to overt diabetic kidney disease (32). A recent review concluded that the question of whether 8-OHdG could be used as a risk stratification tool but whether it added further value in addition to existing tests remained to be answered in large, long-term cohort studies (36). None of the array of emerging markers are sensitive and specific enough in comparison to established biomarkers to identify early diabetic kidney disease and predict progression to CKD in type 2 diabetes. However, these studies suggest that a systemic marker of oxidative stress provides some insight in to changes that are occurring in both the micro- and macrovascular beds which is likely to be expressed through a detrimental effect of the endothelium to maintain an appropriate vascular tone which precedes the irreversible target organ damage.

**Endothelium**

The endothelium regulates vascular function - in response to compounds such as acetylcholine, adenosine diphosphate, and bradykinin and physiological stimuli such as shear stress - through the release of constrictor mediators such as thromboxane and endothelin-1, and dilator mediators such as prostacyclin, endothelium derived hyperpolarizing factor and most importantly, nitric oxide (NO).

NO is the primary source of reactive nitrogen species that is produced in almost all tissues and organs (37). It is synthesized from its precursor the amino acid L-arginine and molecular oxygen through the action of a family of enzymes known as the NO synthases (NOS) (38) which requires Ca2+/calmodulin, FAD, FMN, and tetrahydrobiopterin as cofactors (Figure2). The NOS system consists of three distinct isoforms, neuronal (nNOS or NOS1), inducible (iNOS or NOS2), and endothelial (eNOS, NOS3 or cNOS) which are encoded by the three genes NOS1, NOS2, and NOS3, respectively. NO bioavailability refers to a three-step process - normal NO synthesis, NO bioactivity, and NO action - that guarantees normal endothelial function.

**Decreased NO production**

This is a consequence of reduced arginine production. In pathophysiological conditions such as diabetes, eNOS is reported to be impaired and plasma arginine levels are reduced. However, in *in vitro* studies, diminished endothelial function can be restored by the addition of L-arginine (39-42).

The most important endogenous NOS inhibitor is asymmetric dimethylarginine (ADMA) – a naturally occurring analogue of L-arginine which is present in the human circulation (Figure2). There is a renewed and growing interest in ADMA and its pathways because of it is considered a key factor causing, and a potential biomarker for endothelial dysfunction (43, 44). Several clinical studies have demonstrated that plasma concentrations of ADMA are elevated in patients with micro- and macro-vascular disease such as; hypertension (45), diabetes (46), chronic heart failure (47, 48, 42), and CKD (49). Some authors consider that ADMA is the missing link that could explain the co-occurrence of cardiovascular and kidney disease (50).

**Increased NO inactivation**

Hyperglycaemic conditions induce superoxide anion (O2−•) which leads to an acceleration in the production of advanced glycation end-product production and further superoxide production (Figure2). The reaction of NO with superoxide anions forms the potent oxidant peroxynitrite (ONOO−). The latter, decomposes to OH-, nitrogen dioxide gas (NO2.) and nitronium ion (NO2+) (51,52). NO2 may react with H2O2 to form OH- and HNO3. Also, ONOO- causes eNOSuncoupling a process that renders eNOS dysfunctional and produces superoxide rather than NO. (53,54)

The antioxidant enzyme superoxide dismutase (SOD) catalyses the conversion of O2**.-** to H2O2 and O2. It was shown that treatment of rabbit aorta with diethyldithiocarbamate (DETC) - an inhibitor of the extracellular SOD (Cu/Zn-SOD) - would increase superoxide levels and eliminates endothelium dependent vasodilatation indicating that normal NO bioactivity requires adequate scavenging of the superoxide radical (55,56).

**NO bioavailability**

Endothelial dysfunction occurs in many groups of patients with increased cardiovascular risk, including hypertension, renal failure, diabetes and in subjects with a family history of vascular disease. NO inhibits leukocyte migration, platelet activation and vascular smooth muscle cell proliferation which are properties consistent with an anti-atherogenic role (57-61).

Additionally, a clear role has been established for NO as a vasodilator of peripheral and renal vasculature. It regulates both the afferent and the efferent arteriole of the glomerulus, increasing GFR and renal vascular resistance (48,57). NO also facilitates pressure natriuresis, preserves medullary perfusion, decreases tubuloglomerular reabsorption and modulates renal sympathetic nerve activity (62).

**Endothelial function in kidney disease and diabetic nephropathy**

Endothelial function assessed by different indices was found to be impaired in patients at all stages of CKD in comparison to individuals without CKD. This observation could be due to decreased availability of the substrate, L-arginine or to increased ADMA. Plasma NO levels were reported to be decreased in patients with CKD compared with healthy normotensive non-diabetic patients were decreased with increasing degrees of renal dysfunction whilst ADMA was associated with the progression of renal failure (63)*.* Basal NOS activity in neutrophils is impaired in ESRD patients on haemodialysis independently of L-arginine availability (61). Furthermore, endothelial function assessed by flow mediated dilatation (FMD) of the brachial artery suggests that endothelium-dependent response to shear stress is decreased in patients with CKD and increased systolic blood pressure (64).

Diabetic eNOS knockout mouse studies have reported that deficiency of NO is an important susceptibility factor in the development of diabetes-related renal injury.(Takahashi, Harris 2014?) Intriguingly, patients with diabetic nephropathy show an upregulated glomerular and cortical eNOS which is related to different degrees of vasculopathy. Glomerular eNOS was reported to be strongly increased among different degrees of proteinuria and lower in macroalbuminuric patients than microalbuminuric (66, 67). Moreover, urine NO metabolites (15N-nitrate) were shown to be increased in type 1 diabetic subjects with normal urinary albumin excretion than in control subjects following intravenous administration of L-[15N]2-arginine. (68). This correlated with a marker of lipid peroxidation urinary isoprostane production and NO synthesis in diabetic subjects. These findings reinforce the involvement of oxidative stress with endothelial dysfunction.

**Endothelial function and ethnicity**

Genetic association studies and meta-analyses report an association between eNOS gene polymorphism and the development of DN, however there is a divergence in the pattern of association when comparing patients of different ethnic groups. 894T eNOS is negatively associated with diabetic kidney disease in Caucasian populations but it is positively associated with diabetic kidney disease in non-Caucasian population (69,70).

Moreover, racial differences in the activity of the NO pathway have also been described. Healthy young individuals of African-Caribbean origin compared to Caucasians have reduced NO-mediated dilatation of the forearm vasculature as measured by decreased acetylcholine response (71). In addition, Stein et al found that intra-arterial administration of endothelium-dependent agonist and endothelium-independentagonists, isoproterenol, sodium nitroprusside, and methacholine resulted in an increase of 3.7-fold, 3.6-fold, and 5.0-fold in the forearm blood flow in black normotensive Americans, while, there was a 7.5-fold, 5.2-fold, and 6.9-fold increase in forearm blood flow in white Americans (72). Moreover, it has been shown that angiotensin II infusions in healthy African Americans and age-matched Caucasians caused a significantly blunted vasoconstrictor response in the African Americans compared to Caucasians (73). Another investigation assessed whether there were racial difference of endothelium-dependent vasodilation response in leg blood flow after exposure to graded intra-femoral arterial infusions of the endothelium-dependent vasodilator methacholine chloride. African American subjects exhibited reduced endothelium-dependent vasodilation compared with Caucasians (74). Although, these data imply that responses to vasodilatory stimuli are abnormal in Black patients, it remains unknown if racial differences in the NO pathway exist in, or contribute to the development of disease states and if oxidative stress is involved.

Mels et al reported that South African men in comparison to Caucasian South African men with increased blood pressure and ACR have higher synthesis of NO as assessed by L-arginine and L-citrulline levels and increased NO inhibitors (ADMA and Symmetric dimethylarginine (75). This led the investigators to conclude that African men have a favourable NO synthesis but this is counteracted by increased NO inactivity. In addition, African men exhibited an upregulation of redox markers and ADMA was inversely related to GPx activity and GPx/SOD ratio. Together, these data suggest that patients of non-Caucasian origin have compromised endothelial function related to oxidative stress.

**Ethnic differences in renal vascular function in type 2 diabetes**

We Investigated changes in renal vasodilatation and glomerular filtration in response to the experimental stimulus of an amino acid infusion in African-Caribbean, Indo-Asian and Caucasian subjects with type 2 diabetes and microalbuninuria. Under euglycaemic conditions, glomerular filtration rate, and renal plasma flow (RPF) were calculated from the clearance of inulin and para-aminohippurate (PAH) respectively. Urinary clearance of the metabolites of NO (NOx) were measured before and after the infusion. Renal haemodynamic responses and urinary NOx were reduced in the combined African-Caribbean and Indo-Asian (African-Asian) group compared with the Caucasian group. Compared with the African-Asian group, Caucasian patients exhibited a greater increase in GFR, RPF and higher clearance of NOx. These data suggest that African-Asian type 2 diabetic patients have a reduced vasodilator capacity of the renal vascular bed compared to Caucasians that might be secondary to diminished NO-bioactivity (76). These findings are consistent with other data showing that black subjects have reduced renal perfusion and enhanced vasoconstrictor tone compared with matched white control subjects and may be of relevance to differences in renal disease progression (77). Further investigations of the differential responses to the inhibition of NOS by l-Ng-monomethyl-l-arginine under euglycemic conditions revealed a significant fall in renal blood flow and rise in systolic blood pressure, which is related to an increase in renal vascular resistance in African-heritage group (78). A potential explanation of these observations could be related to differences in oxidative stress reducing the bioavailability of NO.

**Amelioration of blunted vascular reserve**

Therapeutic interventions directed towards improvement of NO production in addition to management of other risk factors may prevent development of endothelial dysfunction and enable better management of patients with type 2 diabetes who are at increased risk of CKD. In the African-American Heart Failure Trial (A-HeFT), 1050 African American patients received either the specific NO-generating combination of isosorbide dinitrate and hydrallazine or placebo. The primary composite outcomes of death from any cause, a first hospitalization for heart failure, and change in the quality of life was approaching half of that in the placebo group, with patients aged ≥65 years experiencing the greatest survival benefit (79,80). Together, these studies suggest that factors related to NO bioavailability and/or increased oxidative stress may account for the differences in vascular phenotypes.

**Antioxidant interventions**

There is robust experimental evidence for the role of oxidative stress and inflammation in the development of kidney disease but clinical trials of antioxidant treatment to prevent progression of CKD / ESRD have not shown clear benefit. However, the renal and cardio-protective effects of drugs of the HMG Co-A reductase, angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker classes are partly mediated via their indirect anti-oxidant and anti-inflammatory actions (81).

A Cochrane review of antioxidant interventions in patients with and without diabetes, including Bardoxolone, Vitamin E, recombinant human SOD, coenzyme Q and N-acetyl-cysteine reported that while there was no clear reduction in CVD or all-cause mortality, there was an increase in creatinine and eGFR, with reduced risk of ESRD in people with CKD taking antioxidants. (? 82Jun, Venkataraman et al. 2012). A more recent review in patients with diabetes, without CKD included 15 trials, with a total of 4345 participants (of which 3654 participants were from a single trial) using a range of antioxidant treatments, found that the interventions tended to reduce albuminuria - suggesting a beneficial effect on early renal damage - but no effect on renal function as measured by GFR (83).

Bardoxolone methyl is an oral antioxidant inflammatory modulator and exerts its anti-oxidant and anti-inflammatory effects by activating nuclear factor-erythroid-2-related factor 2 (Nrf2) and supressing NF-kB (84). In the initial randomised, placebo-controlled trial, patients with type 2 diabetes and CKD received varying doses of Bardoxolone to assess the changes from baseline of eGFR at 24 and 52 weeks (85). There was a significant, maximal improvement in eGFR of 11.24 mls/min/1.73m2 with the 75 mg od dose of the agent; mild-moderate side-effects, mainly consisting of muscle cramps were reported. A follow-up trial, the BEACON trial, was designed to assess whether bardoxolone methyl could slow progression to or even prevent ESRD and cardiovascular death within a population with CKD4 (85). The trial was terminated early due to an increased rate of cardiovascular events (death from cardiovascular causes and hospitalization or death from heart failure) in those receiving bardoxolone, while it did not prevent ESRD from developing (86)

**Endogenous antioxidant pathways:**

Antioxidants such as vitamin C, vitamin E, probucol, tiron, or N-acetylcysteine directly scavenge and inactivate ROS rather than interfering with expression and function of oxidant and antioxidant enzymes (87).

Concentrations of Vitamin E, C and various minerals have been shown to be reduced in patients with diabetes, potentially contributing to the increased susceptibility to oxidative stress damage and has previously been proposed as a mechanism to explain the differing risk profiles for CKD and ESRD between populations. Free radicals derived from oxygen react with a variety of biological molecules, resulting in peroxidation. Of particular relevance to diabetic complications is the role lipid peroxidation plays in atherogenesis (88). Vitamin E, is the principle lipid-soluble antioxidant and plays a crucial role in protecting cell membranes from lipid peroxidation, where it serves as a chain-breaking antioxidant and lipid peroxyl radical scavenger (89). Our previous work showed that Vitamin E levels were lower, while lipid hydroperoxide levels was higher in patients of African origin; lower levels of Vitamin E was also shown to be an independent predictor of deteriorating plasma creatinine (90). In rodent models Vitamin E supplementation prevented histological changes of kidney damage (91) whilst limited observational studies in humans seemed to show an association between increased micronutrient intake and decreased CKD risk (92).

Experimental and clinical observational studies have shown a reduced vascular risk in relation to relatively high intakes of dietary and/or supplemental Vitamin E (93). However, earlier clinical trials failed to show cardiorenal protective benefit from Vitamin E supplementation (94-97). In the HOPE study, 400IU Vitamin E given for an average of 4.5 years had no effect on cardiovascular or renal outcomes in older (>55years) patients with either CV disease or coronary risk factors (96). Other small interventional studies did show a benefit in reducing albuminuria, a sign of early renal damage and cardio-vascular complications in diabetes, especially when specific high risk groups are targeted. One such high-risk group is based on the presence of differing Haptoglobin-1 and Haptoglobin-2 genotypes. Haptoglobin is an antioxidant and acts by reducing the oxidative activity of haemoglobin. The anti-oxidant activity of these genotypes vary: protein products of Hp2-2 are weaker antioxidants, with data showing that patients with this genotype, as opposed to Hp1-1 or Hp2-1, have worse cardiovascular disease outcomes. Patients with Hp2-2 genotypes are at increased risk derive benefit from Vitamin E supplementation which is not the case in individuals with the Hp2-1 genotype (98-99).

Vitamin E has also been shown to be effective in several secondary prevention trials in high-risk groups. Patients receiving dialysis have higher levels of oxidative stress and rates of cardiovascular death than those not receiving dialysis (100). Others have found that in this high-risk group of patients that supplementation with high-dose Vitamin E reduced the composite cardiovascular disease end-point and myocardial infarction 2-fold compared with placebo (101).

In the CHAOS trial, patients had angiographically proven coronary artery disease randomised to Vitamin E experienced almost a 50% reduction in cardiovascular death non-fatal myocardial infarction (102). Differences between this trial and other similar secondary prevention trials, such as the GISSI trial in which no effect of Vitamin E on cardiovascular outcome was found (103) have been attributed to different doses of Vitamin E administered, different diets of the study groups and in prevalence of genetic polymorphism of NO synthase affecting the susceptibility to the antioxidant effects of Vitamin E (88).

In a rodent model of type 2 diabetes, treatment with tocotrienol-rich fractions improved glycemic status, serum lipid profile and renal function in association with restoration of anti-oxidant enzyme activity (104). These data suggest that, oxidative stress promotes renal and vascular damage that may be ameliorated by an antioxidant intervention in those patients at highest risk of their development.

Previously, we found GPx activity to be lower in patients with type 2 diabetes of African heritage compared with Caucasians. Others have shown that low GPx activity is associated with an accelerated development of vascular lesions in both experimental rodent models, and patients with diabetes (105-106). However, data are limited on the role of GPx activity or its regulation with respect to the development or progression of renal disease in patients with diabetes.

The effect of antioxidant supplementation to prevent or slow progression to ESRD in patients with diabetes have failed to show conclusive benefits, in spite of robust pre-clinical data. Possible reasons behind this could be that very early diabetic kidney disease cannot routinely be detected by currently used biomarkers. Changes to creatinine and eGFR occur late and are influenced by a variety of confounding factors. Most studies measured effect of antioxidants by using albuminuria, creatinine, low levels of GFR and proteinuria. More selective and/or sensitive biomarkers could detect earlier and more subtle changes upstream of target organ damage in selected high risk groups of patients.

The PREVENT trial will provide new information on whether progression of the early stages of CKD, is related to, or modified by, oxidative stress and/or host antioxidant defence mechanisms in type 2 diabetes. Type 2 diabetes mellitus will be diagnosed according to WHO criteria. Eligible patients will have hypertension 3 consecutive sitting blood pressure readings >140 systolic and/or diastolic 90 mmHg without treatment or receiving treatment for known hypertension) and early CKD defined as an eGFR > 45 and <90 mL/min/1.73 m2and/or urinary albumin:creatinine ratio >3 mg/mmol. Ethnic origin will be self-determined as white, Caucasian and non-Caucasian (African, Caribbean or Indo-Asian). An equal number of eligible patients will be randomized to receive, either, active selenium (200 μg once daily) or its placebo and/or vitamin E (400 IU once daily) or its placebo in a 2 × 2 factorial design.

Patients will be excluded if they have a history of cardiovascular disease, defined as having a clinical record of ischaemic heart disease (angina, myocardial infarction, coronary artery revascularization and or heart failure), peripheral vascular disease (intermittent claudication or peripheral artery revascularization) or cerebrovascular disease (transient ischaemic episodes or stroke), a history of malignancy or any other life threatening illness, current pregnancy, systolic blood pressure >200 mmHg, diastolic blood pressure >160 mmHg, haemoglobin A1c > 86 mmol/mol (10 %), significant renal impairment (eGFR < 45 mL/min 1.73 m2) and nephrotic range urine protein excretion (total protein excretion rate >3 g/day or albumin:creatinine ratio >300 mg/mmol).

PREVENT is sufficiently powered to examine early changes in GFR which is its primary outcome. Secondary outcome measures will include a number of biomarkers related to the activity of endogenous antioxidant activity with treatment induced changes to determine whether they have any predictive power. This study will assist our understanding of the mechanisms of actions of existing and novel drugs and may yield biomarkers that can be used to monitor drug response. It is notable that cardiovascular outcome trials (CVOT) of sodium glucose transport inhibitors and glucagon-like 1 polypeptide agonists in patients with type 2 diabetes at high risk for cardiovascular events show benefit across the spectrum of renal function independently of modification of traditional risk factors such as blood pressure, body weight and glycaemic control (107,108) Emerging experimental data based on these CVOTs suggests glucose-dependent amelioration of oxidative stress limits lipotoxic and smooth muscle damage involved in the cardiorenal disease pathway (109-111). Whether the manipulation or activation of endogenous antioxidant pathways affects similar pathways remains to be determined.

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