Oxidative Stress and Antioxidant Therapy in Chronic Kidney and Cardiovascular Disease
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Published by Intech

What Does This Mean?

1. Definitions (from dictionary.com):
   a. CKD: Chronic kidney disease
   b. CVD: Cardiovascular disease
   c. ROS: Reactive oxygen species – a type of free radical

2. Synopsis:
   a. “The progression of CKD or CVD independently exerts synergistic deleterious effects on the other, for example, patients with CKD are more likely to die of CVD than to develop renal failure.”
   b. Oxidative stress is one link between Chronic kidney disease and Cardiovascular disease.
   c. Therapy with antioxidants may help improve these diseases.
   d. Oxidative stress and inflammation are “common themes for causality” of cardiovascular disease.
   e. The links between CKD and CVD are close.
   f. Chronic inflammation is found in CKD and CVD.
   g. Because oxidative stress is linked with inflammation it becomes linked with CKD and CVD.
   h. “Oxidative stress has been implicated in various pathological systems that are prevalent in both CKD and CVD, most importantly inflammation and fibrosis.”
   i. “Antioxidant networks in which there is interplay, crosstalk and synergism to efficiently and specifically scavenge ROS, may also exist. If this is the case, these antioxidant networks could be harnessed to develop polytherapeutic antioxidant supplements to combat oxidant-related pathologies, like CKD and CVD.”
      a. Chronic kidney disease and cardiovascular disease are oxidant-related.
      b. Antioxidants “scavenge” free radicals, thus lowering oxidative stress.
   j. “The role of oxidative stress in upstream transcriptional gene regulation is becoming increasingly recognised.”
   k. Nrf2 plays a role in the defence against oxidative stress.
   l. CKD and CVD are linked to oxidative stress.
   m. “The current state of antioxidant therapies for CKD and CVD is one of promise, but not without controversy.”
n. “Oxidative stress and inflammation are closely interrelated with development of CKD and CVD, and involve a spiralling cycle that leads to progressive patient deterioration.”

See the actual article on the next page.
Chapter from the book *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants*

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1. Introduction

Chronic kidney disease (CKD) and cardiovascular disease (CVD) have major impacts upon the health of populations worldwide, especially in Western societies. The progression of CKD or CVD independently exerts synergistic deleterious effects on the other, for example, patients with CKD are more likely to die of CVD than to develop renal failure. This overlap between CKD and CVD, in part, relates to common etiologies such as diabetes mellitus and hypertension, but important dynamic and bidirectional interactions between the cardiovascular system and kidneys may also explain the occurrence of concurrent organ dysfunction [1]. Cardio-renal syndrome (or reno-cardiac syndrome, the prefix depending on the primary failing organ) is becoming increasingly recognised [2]. Conventional treatment targeted at either syndrome generally reduces the onset or progression of the other [3]. Even though our understanding of various factors and steps involved in the pathogenesis of CKD and CVD and their obvious links has improved, a complete picture of the mechanisms involved is still unclear. Oxidative stress has been identified as one unifying mechanism in the pathogenesis of CKD and CVD [4]. This current chapter gives a brief review of recent literature on the relationship between CKD, CVD and oxidative stress and indicates how, by applying knowledge of the molecular controls of oxidative stress, this information may help improve targeted therapy with antioxidants for these diseases.

2. Pathogenesis of chronic kidney and cardiovascular disease – The links

It is, in fact, very difficult to separate these chronic diseases, because one is a complication of the other in many situations. The development and progression of CKD are closely linked...
with hypertension and dyslipidemia, both causes of renal failure. Diabetic nephropathy is arguably the leading cause of renal failure. CKD, hypertension and diabetes mellitus all involve endothelial dysfunction, a change well known in the development of atherosclerosis and CVD that includes coronary artery disease, heart failure, stroke and peripheral arterial disease [5]. Vascular calcification occurs in progressive atherosclerosis and CVD, but it is also an important part of vascular injury in end-stage renal disease (ESRD), where patients need renal replacement therapy to survive. It is paradoxical that approximately 50% of individuals with ESRD die from a cardiovascular cause [6]. Thus, CKD and CVD patients have closely-linked diseases with increasing morbidity and mortality. Prevention and treatment of these diseases are major aims in health systems worldwide.

The initiating causes of CKD are highly variable, with previously-mentioned hypertension and diabetes being two of the key ones [7]. Epidemiological studies reveal other strong risk factors for CKD, such as a previous episode of acute kidney damage, exposure to nephrotoxins, obesity, smoking, and increasing age [8, 9]. However, no matter the cause, the progressive structural changes that occur in the kidney are characteristically unifying [10]. The characteristics of CKD are tubulointerstitial inflammation and fibrosis, tubular atrophy, glomerulosclerosis, renal vasculopathy, and presence of granulation tissue. Alterations in the glomerulus include mesangial cell expansion and contraction of the glomerular tuft, followed by a proliferation of connective tissue which leads to significant damage at this first point of the filtration barrier. Structural changes that occur in the kidney produce a vicious cycle of cause and effect, thereby enhancing kidney damage and giving CKD its progressive nature. Whilst early pathological changes in the kidney can occur without clinical presentations, due to the high adaptability of the kidney [10], once the adaptive threshold is reached, the progression of CKD is rapid and the development of ESRD imminent. Vascular pathology exacerbates development of CKD, and it is perhaps here that the links with CVD are closest. Hypertension induces intimal and medial hypertrophy of the intrarenal arteries, leading to hypertensive nephropathy. This is followed by outer cortical glomerulosclerosis with local tubular atrophy and interstitial fibrosis. Compensatory hypertrophy of the inner-cortical glomeruli results, leading to hyperfiltration injury and global glomerulosclerosis. Note, however, that although glomerulopathy is an important characteristic of CKD, the incidence of tubulointerstitial fibrosis has the best correlation with CKD development [11]. As such, kidney tubular cells and renal fibroblasts may be the founding cell types in the progressive development of CKD.

The main clinical manifestation of CKD is a loss of glomerular filtration rate (GFR), allowing for staging of CKD with progressively decreasing (estimated) GFR. CKD staging was facilitated by the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) and the Kidney Disease - Improving Global Outcomes (KDIGO), an outcome that highlighted the condition and facilitated its increased diagnosis [12]. The first two stages have normal, or slightly reduced kidney function but some indication of structural deficit in two samples at least 90 days apart. Stages 3-5 are considered the most concerning, with Stage 3 now being sub-classified into Stages 3a and b because of their diagnostic importance. It is thought that stages 2 and 3 should be targeted with prophylactic therapies, such
as lipid lowering drugs or RAS modifiers [13], to minimize the progression of CKD. Table 1 summarises GFR classification and staging for CKD.

<table>
<thead>
<tr>
<th>Stage</th>
<th>GFR*</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90mL/Min</td>
<td>Normal renal function but abnormal urine findings, or structural abnormalities, or a genetic trait indicating kidney disease</td>
</tr>
<tr>
<td>2</td>
<td>60-89mL/min</td>
<td>Mildly reduced renal function, and other findings (as for stage 1) indicate kidney disease</td>
</tr>
<tr>
<td>3A</td>
<td>45-59mL/min</td>
<td>Moderately reduced kidney function</td>
</tr>
<tr>
<td>3B</td>
<td>30-44mL/min</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15-29mL/min</td>
<td>Severely reduced kidney function</td>
</tr>
<tr>
<td>5</td>
<td>&lt;15mL/min or on dialysis</td>
<td>Very severe, or end-stage kidney failure (sometimes called established renal failure)</td>
</tr>
</tbody>
</table>

* Measured using the MDRD formula (MDRD= Modification of Diet in Renal Disease). All GFR values are normalized to an average surface area (size) of 1.73m²

Table 1. Classification and description of the different stages of CKD

Similar to CKD, the initiating causes for CVD are complex. Although exposure to cardiovascular risk factors such as hypertension, dyslipidemia and diabetes mellitus contributes to CVD, obesity, lack of physical exercise, smoking, genetics, and even depression, also play a role [14]. Common themes for causality are oxidative stress and inflammation, be they local or systemic. The prevalence of CVD also has a strong positive correlation with age, with more than 80% of cases of coronary artery disease and 75% of cases of congestive heart failure observed in geriatric patients [14]. Intrinsic cardiac aging, defined as the development of structural and functional alterations during aging, may render the heart more vulnerable to various stressors, and this ultimately favours the development of CVD. In the early stages of CVD, left ventricular hypertrophy and myocardial fibrosis may be seen in many patients [15]. The processes involved in their development, particularly in association with CKD, can be attributed to hypervolaemia, systemic arterial resistance, elevated blood pressure, large vessel compliance, and activation of pathways related to the parathyroid hormone–vitamin D–phosphate axis. Left ventricular hypertrophy and myocardial fibrosis also predispose to an increase in electric excitability and ventricular arrhythmias [16].

Heart failure resulting from CVD may be staged in a system similar to CKD. In its 2001 guidelines, the American College of Cardiology (ACC) and the American Heart Association working groups introduced four stages of heart failure [17]: Stage A with patients at high risk for developing heart failure in the future but no functional or structural heart disorder; Stage B with a structural heart disorder but no symptoms at any stage; Stage C with previous or current symptoms of heart failure in the context of an underlying structural heart problem, but managed with medical treatment; and Stage D with advanced disease requiring hospital-based support, a heart transplant or palliative care. The ACC staging system is
useful in that Stage A may be considered pre-heart failure where intervention with treatment may prevent progression to overt symptoms.

The links between CKD and CVD are so close that it is often difficult to tease out individual causes and mechanisms, given their chronic nature. However, children with CKD present as a particular population without pre-existing symptomatic cardiac disease. This population could also receive significant benefit from preventing and treating CKD and thereby minimizing the forthcoming development of CVD which is a major cause of death in children with advanced CKD. Left ventricular hypertrophy and dysfunction, and early markers of atherosclerosis such as increased intimal-medial thickness and stiffness of the carotid artery, and coronary artery calcification, may develop in children with CKD. Early CKD, before needing dialysis, is the optimal time to identify and modify risk factors and intervene in an effort to avert risk of premature cardiac disease and death in these children [18]. These observations have sparked added interest in the mechanisms of the chronic diseases, and in ways to target these mechanisms with additional therapies, such as antioxidants.

2.1. Inflammation and chronic kidney and cardiovascular disease

The circulating nature of many inflammatory mediators such as cytokines, and inflammatory or immune cells, indicates that the immune system can act as a mediator of kidney-heart cross-talk and may be involved in the reciprocal dysfunction that is encountered commonly in the cardio-renal syndromes. Chronic inflammation may follow acute inflammation, but in many chronic diseases like CKD and CVD, it is likely that it begins as a low-grade response with no initial manifestation of an acute reaction. There are many links with visceral obesity and with increased secretion of inflammatory mediators seen in visceral fat [15]. Proinflammatory cytokines are produced by adipocytes, and also cells in the adipose stroma. The links with oxidative stress as an endogenous driver of the chronic diseases become immediately obvious when one admits the close association between oxidative stress and inflammation. The characteristics of dyslipidaemia (elevated serum triglycerides, elevated low-density lipoprotein cholesterol, and/or low high-density lipoprotein cholesterol) are also often seen in obese patients and these are all recognized as risk factors for atherosclerosis. The links between obesity, inflammation, dyslipidaemia, CKD and CVD also occur through yet another syndrome, metabolic syndrome. An improved understanding of the precise molecular mechanisms by which chronic inflammation modifies disease is required before the full implications of its presence, including links with persistent oxidative stress as a cause of chronic disease can be realized.

3. Oxidative stress and chronic kidney and cardiovascular disease

3.1. Understanding oxidative stress

Oxidative stress has been implicated in various pathological systems that are prevalent in both CKD and CVD, most importantly inflammation and fibrosis. Chronic inflammation is induced by biological (eg. infections, autoimmune disease), chemical (eg. drugs, environ-
mental toxins), and physical factors (eg. lack of physical activity) [19]. The inflammatory cells are then a source of free radicals in the forms of reactive oxygen and nitrogen species, although reactive oxygen species (ROS) are considered the most common. The highly reactive ROS are capable of damaging various structures and functional pathways in cells. In consequence, the presence of inflammatory cells is stimulated by cell damage caused by ROS, creating a cycle of chronic damage that is difficult to break. Oxidative stress arises from alterations in the oxidation-reduction balance of cells. Normally, ROS are countered by endogenous natural defences known as antioxidants, and it is the imbalance between ROS and antioxidants which favours greater relative levels of ROS, thereby giving rise to a state of oxidative stress [20-22]. The simple oxidant “imbalance” theory has now grown to incorporate the various crucial pathways and cell metabolism that are also controlled by the interplay between oxidants and antioxidants [23-27]. The rationale for antioxidant therapies lies in restoring imbalances in the redox environment of cells.

The main ROS are superoxide (O$_2^{•−}$), the hydroxyl radical (OH$^{•−}$) and hydrogen peroxide (H$_2$O$_2$). Mitochondria are considered the major source of ROS, however other contributing sites of ROS generation include the endoplasmic reticulum, peroxisomes and lysosomes [28-30]. Estimated levels of ROS within mitochondria are 5-10 fold higher than cystolic and nuclear compartments in cells [31] due to the presence of the electron transport chain (ETC) within the mitochondrial inner membrane. 1-3% of inspired molecular oxygen (O$_2$) is converted to the most common of the ROS, O$_2^{•−}$ [32, 33], a powerful precursor of H$_2$O$_2$. Although cellular H$_2$O$_2$ is stable in this form, it has the potential to interact with a variety of substrates to cause damage, especially in the presence of the ferrous iron (Fe$^{2+}$), which leads to cleavage and formation of the most reactive and damaging of the ROS, the OH$^{•−}$ [34]. In healthy metabolic cells, the production of the potentially harmful H$_2$O$_2$ is countered by the catalizing actions of mitochondrial or cystolic catalase (CAT) or thiol peroxidases into water and O$_2$. The ETC consists of 5 multi-enzyme complexes responsible for maintaining the mitochondrial membrane potential and ATP generation. Each of these complexes presents a site of ROS generation, however complexes I and III have been identified as primary sites of O$_2^{•−}$ generation [35-38]. ROS generation from mitochondrial complexes increases with age in mice [39]. In humans, Granata and colleagues [40] have demonstrated that patients with CKD and haemodialysis patients display impaired mitochondrial respiration.

Agreement on the role of oxidative stress in the pathogenesis of chronic disease is, however, not complete. Oxidants are involved in highly conserved basic physiological processes and are effectors of their downstream pathways [41, 42]. The specific mechanisms for “oxidative stress” are difficult to define because of the rapidity of oxidant signalling [31]. For example, protein tyrosine phosphatases are major targets for oxidant signalling since they contain the amino acid residue cysteine that is highly susceptible to oxidative modification [43]. Meng and colleagues [25] demonstrated the oxidation of the SH2 domain of the platelet-derived growth factor (PDGF) receptor, which contains protein tyrosine phosphatases, in response to PDGF binding. This may indicate the induction of free radicals in response to receptor activation by a cognate ligand in a process that is similar to phosphorylation cascades of intracellular signalling.
3.2. Endogenous antioxidants – Metabolism or disease modifiers

The production of ROS is usually in balance with the availability and cellular localisation of antioxidant enzymes such as superoxide dismutase (SOD), CAT and glutathione peroxidase (Gpx). In vivo studies have found accumulated oxidative damage occurs from decreased levels of these enzymes rather than increased ROS production [44, 45]. However, adequate levels of both are likely to be vital for normal cell function. Mitochondria possess their own pool of antioxidants to counteract their generation of ROS. Mitochondrial manganese-SOD (Mn-SOD) converts $O_2^\bullet-$ to $H_2O_2$ which is then decomposed to harmless $H_2O$ and $O_2$ by CAT and Gpx [46]. Copper/zinc-SOD (Cu/Zn-SOD) has been implicated in stabilizing $O_2^\bullet-$ within other cellular compartments, especially peroxisomes, and must be considered in maintenance of the redox state of the whole cell [47, 48]. Limited antioxidant actions of Cu/Zn-SOD may also occur within the inter-membrane space [49]. There is no evidence to indicate that glutathione synthesis occurs within mitochondria, however the mitochondria have their own distinct pool of glutathione required for the formation of Gpx [50].

Among the various endogenous defences against ROS, glutathione homeostasis is critical for a cellular redox environment. Glutathione-linked enzymatic defences of this family include Gpx, glutathione-S-transferase (GST), glutaredoxins (Grx), thioredoxins (Trx), and peroxiredoxins (Prx) [51]. Many of these proteins are known to interact with each other, forming redox networks that have come under investigation for their contribution to dysfunctional oxidant pathways. Mitochondrial-specific isoforms of these proteins also exist and include Grx2, Grx5, Trx2 and Prx3 [52-54], which may be more critical for cell survival compared to their cytosolic counterparts [50]. Mitochondrial dysfunction, resulting in depleted ATP synthesis, has the potential to reduce the redox control of glutathione since the rate of glutathione synthesis is ATP-dependent [55]. Intracellular synthesis of glutathione from amino acid derivatives (glycine, glutamic acid and cysteine) accounts for the majority of cellular glutathione compared with extracellular glutathione uptake [56]. Antioxidant networks in which there is interplay, crosstalk and synergism to efficiently and specifically scavenge ROS, may also exist. If this is the case, these antioxidant networks could be harnessed to develop poly-therapeutic antioxidant supplements to combat oxidant-related pathologies, like CKD and CVD.

3.3. Oxidative stress and transcriptional control

The role of oxidative stress in upstream transcriptional gene regulation is becoming increasingly recognised. Not only does this provide insight into the physiological role of oxidative stress, but presents regulatory systems that are possibly prone to deregulation. Furthermore, these sites present targets for pharmacological intervention. Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-dependant transcription factors which have been shown to alter during CKD and CVD [57-59]. They have important roles in the transcriptional regulation of cell differentiation, lipid metabolism, glucose homeostasis, cell cycle progression, and inflammation. There are three PPAR isoforms – $\alpha$, $\beta/d$ and $\gamma$. Peroxisome proliferator gamma coactivator (PGC$\alpha$), in association with PPAR$\gamma$ activation, leads to a variety of cellular protective responses includ-
ing mitochondrial biogenesis [57]. PPARγ regulation in chronic disease is increasingly recognised, with oxidative stress as the unifying initiating feature. Omega-3 polyunsaturated fatty acids (PUFA) reduce inflammation in kidney tubular epithelial cells by upregulating PPARγ [60]. PPARγ activation by pioglitazone reduced cyclo-oxygenase 2 (COX2) expression in smooth muscle cells from hypertensive rats, and upregulated endogenous antioxidants Mn- and Cu/Zn-SOD [61].

Recently, the protective responses of the nuclear factor E2-related factor 2/Kelch-like ECH-associated protein 1 (Nrf2/Keap1)/antioxidant response element (ARE) were noted [62]. Nrf2 is a nuclear transcription factor that is suppressed in the cytoplasm by the physical binding of Keap1 preventing its translocation into the nucleus. Nrf2 is activated by a loss of Keap1 binding by alterations in cellular redox status, such as increased ROS, by-products of oxidative damage, and reduced antioxidant capacity, thereby promoting its transcriptional response at the ARE [63]. The ARE is a vital component of the promoter regions of genes encoding detoxifying, antioxidant, and glutathione-regulatory enzymes such as quinone-reductase, glutathione-peroxidases, glutathione-reductase, thioredoxins and thioredoxin-reductase, peroxiredoxins, gamma-glutamyl cysteine, heme-oxygenase-1 (HO-1), CAT, SOD metallothionein and ferritin [64-67]. Important to note is that by-products of oxidative damage such a 4-hydroxynoneal and J-isoprostanes act as endogenous activators of Nrf2 [68, 69]. Thus, NRF2/Keap1 and the ARE play a crucial role in cellular defence against ROS. Recent pharmacological protocols have allowed the modulation of this pathway to enhance the capabilities of cells to combat oxidative stress and inflammation [70].

3.4. CKD and CVD are unified by oxidative stress

Chronic diseases of the kidney possess various commonalities to chronic disease of the cardiovascular system which can be linked through pathways controlled by oxidative stress, as shown in Figure 1. Vascular, cellular and biochemical factors all contribute. Increased serum uric acid levels (hyperuricaemia) can arise from increased purine metabolism, increasing age and decreased renal excretion, and have harmful systemic effects. Hyperuricaemia is associated with an increased risk for development and progression of CKD. Hyperuricemia is also a risk factor associated with coronary artery disease [71], left ventricular hypertrophy [72], atrial fibrillation [73], myocardial infarction [74] and ischemic stroke [75]. A 20.6% prevalence of hyperuricemia was found in a cross-sectional study of 18,020 CKD patients [76], and a positive correlation was found between serum uric acid and serum creatinine with impaired renal function [77]. Retention of uremic toxins promotes inflammation and oxidative stress, by priming the acute inflammatory polymorphonuclear lymphocytes, activating interleukin (IL)-1β and IL-8 [78] and stimulating the innate immune response through CD8+ cells [79]. Additionally, uric acid synthesis can promote oxidative stress directly through the activity of xanthine oxidoreductase. This enzyme is synthesized as xanthine dehydrogenase, which can be converted to xanthine oxidase by calcium-dependant proteolysis [80] or modification of cysteine residues [81]. In doing so, the enzyme loses its capacity to bind NADH by alterations in its catalytic site and, instead, transfers electrons from O2- thereby generating O2· [82]. However, the role of uric acid in many conditions asso-
ciated with oxidative stress is not clear and there are experimental and clinical data showing that uric acid also has a role in vivo as an anti-oxidant [83].

![Figure 1. Chronic kidney disease and cardiovascular disease are unified by oxidative stress. Mutual risk factors influence the development and progression of CKD and CVD and can either be modifiable (diabetes, obesity, metabolic syndrome, smoking) or non-modifiable (genetic predisposition, increasing age, acute injury). Oxidative stress has been implicated in the majority of initiating factors. The progression of CKD to CVD, or vice versa, is mediated through: (1) inflammation and the release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β) and IL-8 from activated lymphocytes; (2) endothelial dysfunction due to increased retention of uremic toxins, and decreased L-arginine synthesis which causes alterations in nitric oxide (NO) signalling - dyslipidaemia and associated pro-oxidative/inflammatory state lead to increased oxidised-low density lipoproteins (ox-LDL), a major component in the pathogenesis of atherosclerosis; (3) redox perturbations that ultimately underlie oxidative stress due to an imbalance between the production of reactive oxygen species (ROS)/reactive nitrogen species (RNS) and endogenous antioxidants, leading to mitochondrial dysfunction and alterations in redox sensitive pathways such as Nrf2/keap1/ARB.

The kidney is a vital source of L-arginine which is a precursor for nitric oxide (NO). A reduction in renal mass can therefore reduce the production of L-arginine and NO activity. NO is vital for regular vascular endothelial cell function, and decreased amounts have the potential to manifest into CVD [84]. Additionally, oxidized low density lipoprotein (ox-LDL), a by-product of oxidative damage in human blood, plays a pivotal role in the pathogenesis of atherosclerosis [85]. There is also a possible link between CVD and CKD that is regulated by oxidative stress through a functional mitochondrial angiotensin system [86]. Angiotensin type II receptors were co-localised with angiotensin on the inner mitochondrial membrane of human mononuclear cells and mouse renal tubular cells. This system was found to modulate mitochondrial NO production and respiration.

4. Antioxidant therapies in chronic kidney and cardiovascular disease

The current state of antioxidant therapies for CKD and CVD is one of promise, but not without controversy. In vitro studies commonly identify agents that are able to detoxify harmful
oxidants. However, these studies are criticised for their isolated, non-holistic, nature [87, 88].
It is largely the positive pre-clinical results from in vivo studies, usually in rodents, which
drive progress for applicability in chronic human disease, but even these show considerable
discrepancies in translation into patients. Despite the well-documented dysregulated endog‐
eneous oxidant/antioxidant profile in chronic degenerative disorders such as CVD and CKD,
there is still evidence that certain antioxidants have no effect [89-92]. It may first be impor‐
tant to identify patients having an altered oxidative stress profile, since this population pro‐
vides an ideal “intention to treat” cohort. The following trials of antioxidants need then to be
rigorous, identifying not only any positive patient outcomes, but also the underlying mecha‐
nism, and of course any deleterious outcome. Various approaches have been taken to reduce
oxidative stress in models of CKD and accelerated CVD, ranging from reducing oxidant in‐
take in food stuffs [93, 94] to targeted polypharmaceutical compounds. The benefit of rigor‐
ous review of outcome from antioxidant therapies in either CKD or CVD is that the primary
and secondary outcomes related to both can be measured. In the following section, some an‐
tioxidants used for CKD or CVD are reviewed, as shown in Figure 2.

4.1. N-acetylcysteine – An antioxidant with promise

N-acetyl cysteine (NAC) acts as an essential precursor to many endogenous antioxidants in‐
volved in the decomposition of peroxides [95]. NAC attenuates oxidative stress from vari‐
ous underlying causes by replenishing intracellular glutathione stores. Glutathione is
synthesized in the body by three amino acids by the catalysing of intracellular enzymes
gamma-glutamylcysteine synthetase and glutathione synthetase. L-glutamic acid and gly‐
cine are two precursors of glutathione that are biologically and readily available. However,
the limiting precursor to glutathione biosynthesis and the third amino acid, L-cysteine, is
not readily available in a human diet. Although the primary basis for NAC supplementation
is to replenish cellular cysteine levels to maintain intracellular glutathione and thus redox
control, the sulfhydryl-thiol group of L-cysteine is also able to exert direct antioxidant effects
by scavenging free radicals, and NAC may also exert its protective effects against 2,3,5-
tris(glutathion-S-yl)-hydroquinone toxicity. This was demonstrated in isolated renal tubular
epithelial cells, in part by the activation of extracellular signal regulated protein kinase
(ERK) 1/2 [96].

The results of NAC supplementation in kidney disease have been variable and largely de‐
pendent on the type and cause of kidney injury and also the timing of treatment. In cultured
human proximal tubular epithelial cells, NAC reduced lipid peroxidation and maintained
the mitochondrial membrane potential, thereby preventing apoptosis following H2O2 ad‐
ministration [97]. Although NAC had no significant effect on markers of oxidative stress
and inflammation in rats following unilateral ureteral obstruction [98], it reduced kidney
malondialdehyde (MDA) levels in a diabetic mouse model [99]. The treatment of CKD pa‐
tients with NAC with the aim of improving renal function and preventing ESKD has been
largely disappointing, with no evidence of reduction in proteinuria [100, 101]. However,
NAC seems to exert the greatest antioxidant and anti-inflammatory properties when used
against the greatest injury, such as in ESKD patients receiving either haemodialysis or peri‐
tonal dialysis. In those cases, NAC reduced serum 8-isoprostane and the inflammatory cytokine IL-6 [102, 103]. A recent systemic review on antioxidant therapy in hemodialysis patients highlighted NAC as the most efficacious agent in decreasing oxidative stress [104].

The effect of NAC on cardiovascular pathologies is less well investigated than CKD. Crespo et al., (2011) demonstrated in vivo that, although long-term NAC supplementation improved cardiac function, it did not delay progression to cardiomyopathy [105]. Endothelial dysfunction caused by uremic toxins such as indoxyl sulphate induced ROS-dependent expression of the pro-inflammatory and pro-oxidant nuclear factor-κB (NF-κB), which was ameliorated by NAC pre-treatment [106].

**Figure 2.** Cellular sites for antioxidant therapy targets in CKD and CVD. Inflammation, lipid peroxidation and reactive oxygen species (ROS) from mitochondrial, cytoplasmic and extracellular sources contribute to oxidative stress. Vitamin E incorporates into the phospholipid bilayer halting lipid peroxidation chain reactions. Omega (ω)-3 fatty acids displace arachidonic acid in the cell membrane and thus reduce arachidonic acid-derived ROS, but also significantly reduce inflammation and subsequent fibrosis. The cysteine residue of N-acetyl-cysteine (NAC) is a precursor for glutathione (GSH) synthesis, and the thiol group is able to scavenge ROS directly. Bardoxolone exerts transcriptional control by promoting nuclear translocation of Nrf2, facilitating antioxidant response element (ARE) binding that upregulates endogenous antioxidant enzyme activity. Allopurinol inhibits xanthine oxidase-derived ROS and the damaging effects of hyperuricemia. Coenzyme Q₁₀ (CoQ₁₀) enhances the efficacy of electron transport in the mitochondria, thereby reducing mitochondrial-derived ROS – it is also able to directly scavenge ROS. L-carnitine enhances mitochondrial fatty acid synthesis and subsequent ATP production and thereby maintains cell health. L-arginine is a precursor for nitric oxide which restores endothelial function.

4.2. Vitamin E – An established antioxidant with controversial outcomes

Vitamin E, or α-tocopherol, is a lipid-soluble antioxidant that incorporates into the plasma membrane of cells, thereby scavenging free radicals, mainly the peroxyl radical, and halting lipid peroxidation chain reactions [107]. A benefit of α-tocopherol is its ability to restore its antioxidant capacity from its oxidized form following free radical scavenging, and incorporate back into the plasma membrane. Vitamin C (ascorbic acid) is able to directly reduce α-tocopherol [108-110], and intracellular glutathione and lipoic acid can restore α-tocopherol...
indirectly by restoring vitamin C [111]. This is a prime example of a cellular antioxidant network prone to dysregulation. Administration of α-tocopherol to kidney proximal tubular cells in culture decreased cisplatin-induced ROS and increased cell viability [112]. The beneficial effects of α-tocopherol are not limited to its antioxidant properties, and recently attention has focused on its blood oxygenising and endogenous cell signalling functions [113]. Vitamin E foodstuffs primarily consist of α-tocotrienol, an isoform of α-tocopherol which has higher antioxidant efficacy in biological membranes. Despite this, the uptake and distribution of α-tocotrienol is far less than α-tocopherol. Therefore, the basis of vitamin E supplementation is to enhance α-tocopherol levels in cell plasma membranes to prevent lipid peroxidation and resultant oxidative stress. One drawback of α-tocopherol is that it takes several days of pre-treatment to exhibit antioxidant effects [114].

Vitamin E therapy has been extensively researched for renal and cardiovascular benefits in human disease populations. Nevertheless, confounding reports mean there is a lack of consensus as to whether vitamin E therapy induces an overall benefit. It is known that patients with CKD stage 4 display the largest decrease in serum α-tocopherol levels following a progressive decline from stage 1 indicating an increased need for α-tocopherol in the CKD population [115]. Interestingly, within the same cohort of patients, a positive correlation of serum α-tocopherol levels and GFR was found [115]. A large scale trial concluded that vitamin E supplementation to cardiovascular high-risk patients over 4.5 years induced no benefit to cardiovascular outcome [92]. The results from the Selenium and Vitamin E Cancer Prevention Trial (SELECT) are of greater concern. They suggest that vitamin E supplementation significantly increases the risk of prostate cancer for young healthy men [116]. Most studies finding beneficial outcomes of α-tocopherol supplementation have largely focused on the ESKD dialysis populations compared to healthy controls and found a reduced risk of CVD, decreased oxidative stress and increased erythrocyte antioxidants SOD, Gpx and CAT [117-119]. The use of α-tocopherol in CKD patients is not without controversy. Miller and colleagues (2005) concluded that high-dose (≥400 IU/day) vitamin E supplementation may increase all cause mortality which may be due to α-tocopherol displacing gamma-(γ)-tocopherol and delta-(δ)-tocopherol in the body [120]. However, this study was highly criticized owing to a bias in data analysis and numerous methodological flaws [121-130]. The apparent lack of clarity surrounding vitamin E supplementation and associated renal and cardiovascular outcomes appears to stem largely from differences in trial design and failure to specify the form of tocopherol used.

4.3. Coenzyme Q10 - Maintaining mitochondrial health

The heart and kidneys contain the highest endogenous levels of co-enzymes (Co)Q9 and CoQ10 compared to all other organs [131, 132]. This is likely due to the respective reliance on aerobic metabolism and high density of mitochondria in the intrinsic functioning cells from these organs. It is imperative that endogenous CoQ10 levels are maintained to ensure mitochondrial health, and this forms the rationale for CoQ10 therapy. CoQ10 is a fundamental lipid-soluble component of all cell membranes including those enclosing subcellular compartments. The physiological roles of CoQ10 act mostly within the mitochondria where it
has three well-characterised functions: (1) the transfer of electrons from complexes I and II to complex III along the ETC of the inner mitochondrial membrane and subsequent membrane polarisation and ATP generation [133, 134]; (2) the pro-oxidant generation of O$_2^·$ and H$_2$O$_2$ [135, 136]; and (3) the anti-oxidant quenching of free radicals [137]. The continual oxidation-reduction cycle, and existence of CoQ$_{10}$ in three different redox states, explains its actions as an important cellular redox modulator through its pro-oxidant and antioxidant actions. The fully oxidised form of CoQ$_{10}$, or ubiquinone, is able to accept electrons, primarily from NADH, to become fully reduced (ubiquinol - CoQ$_{10}$-H$_2$). The reduced form of CoQ$_{10}$ is able to give up electrons, thereby scavenging free radicals. The intermediate of ubiquinone and ubiquinol is the univalently-reduced ubisemiquinone (Co(4 0-H$^+$) which acts as a pro-oxidant to form O$_2^·$ and, subsequently, H$_2$O$_2$. The major antioxidant role of CoQ$_{10}$ is in preventing lipid peroxidation directly, and by interactions with α-tocopherol [138]. Ubiquinol is able to donate a hydrogen atom and thus quench peroxyl radicals, preventing lipid peroxidation chain reactions. CoQ$_{10}$ and α-tocopherol co-operate as antioxidants through the actions of CoQ$_{10}$-H$_2$ restoring α-tocopheroxyl back to α-tocopherol [109, 139]. However, the reactivity of α-tocopherol with peroxyl radicals far exceeds that of ubiquinol with peroxyl radicals, suggesting that, in vivo, ubiquinols do not act as antioxidants but regenerate the antioxidant properties of α-tocopherols [140]. This is in accordance with in vivo studies investigating the effects of CoQ$_{10}$ supplementation which have primarily found a limited antioxidant capacity. CoQ$_{10}$, acting as a pro-oxidant in all biological membranes including the Golgi, endosome/lysosome systems, as well as mitochondria, has led to much criticism regarding the claimed antioxidant power of CoQ$_{10}$ supplementation in humans [141]. Nonetheless, many in vitro studies demonstrate antioxidant properties of CoQ$_{10}$ in single cells, and benefits of CoQ$_{10}$ supplementation in humans are attributed to its ability to maintain efficient mitochondrial energy metabolism and thus prevent mitochondrial dysfunction, rather than act as a direct cellular antioxidant. CoQ$_{10}$ supplementation in vivo reduced protein oxidation in skeletal muscle of rats but had no effect on mitochondrial H$_2$O$_2$ production in the kidney [142]. However, Ishikawa and colleagues (2011) demonstrated a decrease in kidney O$_2^·$ levels in hemi-nephrectomised rats on a CoQ$_{10}$ supplemented diet, and increased renal function compared with rats on a control diet [143]. Recently, CoQ$_{10}$ supplementation improved left ventricular diastolic dysfunction and remodelling and reduced oxidative stress in a mouse model of type 2 diabetes [144]. CoQ$_{10}$ supplementation in CVD patients also receiving statin therapy is becoming increasingly popular due to the CoQ$_{10}$-inhibitory actions of statins. CoQ$_{10}$ levels decrease with age, but there are no studies measuring endogenous CoQ$_{10}$ levels in CKD or CVD patients and this could prove vital in the identification of population where CoQ$_{10}$ therapy may have beneficial outcomes.

4.4. Omega-3 poly-unsaturated fatty acids – Inflammation and oxidative stress

Inflammation and fibrosis are causes, as well as consequences, of oxidative stress [145, 146]. Direct targeting of inflammatory and fibrotic pathways with more specific modifying compounds presents a way to indirectly decrease oxidative stress in chronic pathologies. Long
chain omega-3 PUFA, including docosahexanoic acid (DHA) and eicosapentanoic acid (EPA), have been investigated in a large range of in vitro and in vivo models and found to possess anti-inflammatory properties. Recently, omega-3 fatty acid treatment of peripheral blood mononuclear cells from pre-dialysis CKD patients reduced the inflammatory markers IL-6, IL-1β, tumor necrosis factor (TNF)-α and C-reactive protein to levels observed in healthy subjects [147]. Although the beneficial effects of EPA/DHA are attributed to their anti-inflammatory properties, they are also known to enhance endogenous antioxidant defence systems such as γ-glutamyl-cysteinyi ligase and glutathione reductase [148]. DHA and EPA incorporate into the phospholipid bilayer of cells where they displace arachidonic acid. Arachidonic acid can generate ROS through the COX2 and xanthine oxidase inflammatory pathways. DHA/EPA administration to renal epithelial cells and macrophages suppresses this pro-inflammatory pathway [149]. Furthermore, chemoattractants derived from EPA are less potent that those derived from arachidonic acid [150, 151]. Recently, in vitro studies determined that EPA and DHA attenuated TNF-α-stimulated monocyte chemoattractant protein (MCP)-1 gene expression by interacting with ERK and NF-κB in rat mesangial cells [152]. Earlier evidence had shown that EPA and DHA inhibit NF-κB expression by stimulating PPARs in human kidney-2 cells in vitro [60]. In vivo studies have now confirmed an improvement in kidney function and structure using EPA/DHA supplementation, with reduced oxidative stress, inflammation and tubulointerstitial fibrosis through the reversal of inflammatory and oxidant pathways [153, 154]. Recently, a highly beneficial outcome of fish oil supplementation was found with heart failure patients with co-morbid diabetes [155]. Clinical studies have found fish oil treatment modulates lipid levels [156, 157], and has anti-thrombotic [158, 159] and anti-hypertensive effects due to its vascular and endothelial actions [160].

4.5. Allopurinol – A xanthine oxidase inhibitor

Allopurinol treatment aims is to inhibit xanthine oxidase to decrease serum uric acid and its associated toxic effects. Allopurinol and its metabolite, oxyipurinol, act as competitive substrates for xanthine oxidase. They enhance urinary urate excretion and block uric acid reabsorption by urate transporters in the proximal tubule, thereby facilitating enhanced uric acid excretion [161-163]. Allopurinol treatment of diabetic mice attenuated hyperuricaemia, albuminuria, and tubulointerstitial injury [164]. Allopurinol may also have antioxidant activities in addition to its enzyme inhibitory activities, by scavenging OH* as well as chlorine dioxide and HOCl [165, 166]. Although later in vivo studies revealed that rat serum obtained after oral administration of allopurinol did not contain allopurinol levels sufficient to scavenge free radicals [167], inhibition of xanthine oxidase-dependent production of NO* and ROS provides allopurinol an indirect mechanism for decreasing oxidative stress in hyperuricaemic CKD patients. Interventional studies of use of allopurinol in renal disease have shown improved uric acid levels, GFR, cardiovascular outcomes and delayed CKD progression. A prospective randomised trial of 113 patients with GFR ≤60 ml/min/1.73m² given allopurinol 100mg/d for 2 years found an increase in GFR of 1.31 ml/min/1.73m² compared to the controls which decreased, and a 71% decreased risk of CVD [168]. Interestingly, Kanbay and colleagues (2007) found that allopurinol at 300mg/d over 3 months improved GFR, uric acid
4.6. Bardoxolone methyl - Targeting the Nrf/Keap1/ARE pathway

A different approach has been investigated by modulating pathways that respond to oxidative stress, rather than targeting ROS by directly increasing endogenous antioxidants. The Nrf2/keap1/ARE pathway presents an exciting target to enhance the oxidant detoxifying capabilities of cells. Bardoxolone methyl [2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO-Me)] is a potent activator of the Nrf2/keap1/ARE pathway and currently shows promise for halting the progressive decline of GFR in type 2 diabetic CKD patients [171, 172]. Bardoxolone methyl is a triterperoid derived from natural plant products that has undergone oleanolic acid-based modification [173]. Its mechanism of action is largely unknown, however, it induces an overall antioxidative protective effect with anti-inflammatory and cytoprotective characteristics [174, 175]. Bardoxolone methyl administered to mice ameliorated ischemia-reperfusion induced acute kidney injury by Nrf2-dependant expression of HO-1 and PPARγ [176]. Its mechanism may also reside in regulating mitochondrial biogenesis given the involvement of PPARγ. A large international study evaluating the full scale of bardoxolone methyl’s effects on CKD progression is in progress, the results of which could determine if bardoxolone methyl should become a standard treatment in renal disease patients. Concurrent benefits to CVD will undoubtedly also be measured.

4.7. L-Carnitine – Improving cardiovascular health in dialysis

Carnitine is an essential cofactor required for the transformation of free fatty acids into acylcarnitine and its subsequent transport into the mitochondria for β-oxidation [177]. This underlies its importance in the production of ATP for cellular energy. Acylcarnitine is also essential for the removal of toxic fat metabolism by-products. Carnitine is obtained primarily from food stuffs, however it can be synthesised endogenously from the amino acid L-lysine and methionine [177]. L-carnitine supplementation primarily benefits ESRD patients on hemodialysis and their associated cardiovascular complications, especially anemia. This is primarily due to the well-described decrease in serum free carnitine in maintenance hemodialysis patients compared to non-dialysis CKD and healthy patients [178]. L-carnitine supplementation offsets renal anemia, lipid abnormalities and cardiac dysfunction in hemodialysis patients [179]. Left ventricular hypertrophy regressed in hemodialysis patients receiving 10mg/kg of L-carnitine immediately following hemodialysis for a 12 month period. [180]. Other measures of cardiac morbidity such as reduced left ventricular ejection fraction and increased left ventricular mass also significantly improved following low dose L-carnitine supplementation [181]. Benefits to the peripheral vasculature have also been demonstrated by L-carnitine through a mechanism thought to involve an associated de-
crease in homocysteine levels [182]. Interestingly, oxidative stress is a major characteristic of hemodialysis patients [183].

As well as the physiological role of L-carnitine in mitochondrial fatty acid synthesis, oxidant reducing capabilities have also been demonstrated and may underlie the health benefits of L-carnitine therapy in CKD and CVD. L-carnitine infusions significantly improved blood urea nitrogen (BUN) and creatinine levels in a 5/6 nephrectomy model of CKD with a concomitant increase in plasma SOD, Gpx, CAT and GSH, and decrease in the oxidative stress marker malondialdehyde [184]. Ye et al., (2010) suggest that L-carnitine attenuates renal tubular cell oxidant injury and subsequent apoptosis by reducing mitochondrial-derivedROS [97]. They suggest that this anti-apoptotic mechanism may also explain the demonstrated reduction in morbidity from cardiomyopathies in L-carnitine supplemented hemodialysis patients.

4.8. L-Arginine - Maintaining endothelial function

The premise of L-arginine supplementation is to maintain NO signalling and thereby maintain vascular endothelial cell function. L-arginine is a physiological precursor to NO and its availability and transport determine the rate of NO biosynthesis. CKD patients most often present with atherosclerosis, thromboembolic complications, and endothelial dysfunction, primarily due to altered endothelium-dependant relaxation factors [185]. It is believed that the impaired NO synthesis, common in CKD individuals, contributes significantly to their disease pathogenesis [186]. L-arginine synthesis occurs in the liver and kidney, with the kidney functioning to maintain homeostatic plasma levels since the liver processes NO from the diet [187]. The addition of L-aspartic acid or L-glutamic acid with L-citrulline and argininosuccinic acid synthase as the rate determining enzyme forms L-arginine [188]. The proximal tubular cells account for the majority of kidney NO synthesis [189, 190], thus kidney damage and atrophy, a primary corollary of CKD, results in decreased synthesis of L-arginine. The majority of research demonstrates decreased levels of NO production in CKD and CVD patients [191-193]. However, some research suggests NO activity increases [194, 195]. These disparate findings highlight the need to measure L-arginine levels in patients before commencing L-arginine supplementation. Rajapaske et al. (2012) demonstrated impaired kidney L-arginine transport and a contributing factor to hypertension in rats, irrespective of an underlying renal disease [196]. During a state of oxidative stress, L-arginine supplementation was shown to decrease MDA, myeloperoxidase and xanthine oxidase and increase glutathione in both heart and kidney tissue from rats [197]. As such, L-arginine supplementation represents an approach to restoring a dysregulation of NO signalling and subsequent endothelial dysfunction in both chronic kidney and heart diseases.

4.9. Combination antioxidants

Compounds commonly used to alleviate oxidative stress exhibit different antioxidant actions, and so there exists the potential for different antioxidants to work together to improve whole cell and organ function through a targeted polypharmaceutical approach to decrease oxidative stress. However, most clinical studies investigating the effects of combination anti-
oxidants have demonstrated confounding results. Mosca et al., (2002) demonstrated that daily intake of NAC 100mg, L-carnitine 100mg, selenomethionine 0.05mg, α-tocopherol 10mg, CoQ_{10} 100mg and α-lipoic acid 100mg successfully increased plasma CAT, Gpx and total antioxidant capacity whilst decreasing lipid peroxides and ROS generation by lymphocyte mitochondria [198]. However, this trial only included healthy participants and cannot be extrapolated to the CKD and CVD populations.

In a murine model of diabetic nephropathy, a major cause of CKD with associated CVD, the beneficial effects of NAC, L-ascorbic acid (vitamin C) and α-tocopherol were demonstrated [199]. Daily supplementation for 8 weeks decreased lipid peroxidation, BUN, serum creatinine and blood glucose, mainly due to a reduction in the inflammatory response induced by hyperglycemia. In comparison, a prospective trial investigating oral supplementation of mixed tocopherols and α-lipoic acid in stage 3 and 4 CKD patients has revealed disappointing results. Over 2 months, supplementation did not reduce biomarkers of oxidative stress (F₂-isoprostanes and protein thiol concentration) or inflammation (CRP and IL-6). The short period of time (2 months) of the intervention may explain this result and longer trials need to be carried out. The inclusion of vitamin E in these interventions has polarized discussion on the outcomes, because of its negligible benefits when cardiovascular outcomes were measured [91, 92, 200] and also because of contraindications, discussed previously. Despite this, long-term treatment in with the antioxidants vitamin C, vitamin E, CoQ_{10} and selenium has been shown to reduce multiple cardiovascular risk factors [201]. Recently, multiple antioxidants in combination with L-arginine have shown promise in animal models of CKD and associated CVD. Korish (2010) has demonstrated in a 5/6 nephrectomy CKD model that L-arginine improved the effects of L-carnitine, catechin and vitamins E and C on blood pressure, dyslipidemia, inflammation and kidney function [84].

5. Conclusion

CKD is a progressive disease with increasing incidence, having very little success in current conventional therapies once CKD reaches stage 4. Stages 2 and 3 are best to target to slow or stop further development of the disease. There is an almost inseparable connection between CKD and CVD, with many patients with CKD dying of the cardiovascular complications before renal failure reaches its fullest extent. Oxidative stress and inflammation are closely interrelated with development of CKD and CVD, and involve a spiralling cycle that leads to progressive patient deterioration. Given the complex nature of oxidative stress and its molecular pathways, antioxidants may need to be given as a polypharmacotherapy to target each aberrant pathway, with the aim of reducing the burden of these chronic diseases. It is vital for the progression of antioxidant therapy research in CKD and CVD that measures of oxidative stress are compared with pathophysiological outcome in the diseases, especially in connection with antioxidant therapies that may be delivered with or without more conventional CKD therapies.
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References


