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Chapter 2

Actions of Glutathione in Chronic Inflammatory Diseases, Including Periodontitis: Dietary Agonists

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Abstract

Glutathione (GSH) plays a critical role in cell signalling and antioxidant defences. It plays a significant role in subjects with periodontitis and associated systemic comorbidities. Its depletion leads to oxidative damage. Some of the prevalent redox reactions and interactions with dietary agonists are addressed.

Glutathione may interact directly with ROS / reactive nitrogen species (RNS); or act as an essential cofactor for GSH S-transferases and glutathione peroxidases. Coordinated actions of GSH and its dependent enzymes which constitute the glutathione system, lead to detoxification of reactive oxygen and nitrogen species (ROS/RNS). Therapeutic interventions aimed at enhancing GSH concentrations *in vivo* include N-acetyl cysteine; activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) by folate supplementation and phytochemicals such as curcumin and resveratrol. An antioxidant defence system comprising a range of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) is active in removing ROS accumulating in cells, in addition to vitamin A, vitamin C, α-tocopherol and plant flavonoids which are available as dietary antioxidants.

Oxidative stress plays an important role in chronic periodontitis (CP), the metabolic syndrome (MetS) and associated conditions. There is a significant correlation between SOD activity, triglycerides, high-density lipoprotein and sVCAM-1 levels. The association between SOD activity and MetS components could be the most significant variable parameter in subjects with MetS; it has potential as a predictive tool to determine the degree of oxidative stress in these subjects. The impact of diabetes mellitus (DM) as a

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risk factor for CP in the context of antioxidant enzyme activity, SOD, glutathione reductase (GR), catalase and the marker of free radical damage, malondialdehyde, favours the role of oxidative stress in both DM and CP.

Dietary non-enzymic antioxidants play an important role in interacting with oxidative stress-inducing mechanisms. Their targeted interaction with the glutathione network results in an enhanced antioxidant profile in chronic inflammatory diseases associated with excessive pro-oxidant activities. Dietary agonists are able to overcome proxidant profiles associated with decreased Nrf2 linked to reduced CAT and GPx m RNA expression . Their administration contribute to coordinated cytoprotective responses in tissues.

Introduction

Redox mechanisms play an important role in periodontitis, affecting tooth supporting structures and in systemic inflammatory diseases. The antioxidant glutathione is synthesized in the body and has protective functions in preventing inflammatory oxidative damage to cells. It aids the detoxification process and optimizes host defences. Stressors could contribute to depleted levels of glutathione which require recycling in order to maintain optimal levels for effective cell function. Redox mechanisms, actions of glutathione, associated enzymic networks and their significance in periodontitis and associated systemic diseases are addressed in this chapter. Dietary agonists that enhance glutathione levels constitute an important aspect of maintaining optimal cellular function. Literature searches for original papers and reviews including systematic reviews over the last 15years were done using Medline, Embase and other search engines. Key words representing these topics were used, to include clinical and scientific publications for the purpose of illustrating the concepts covered.

Metabolic activity and environmental factors generate oxidative stress. The pathophysiology of a wide spectrum of inflammatory diseases is driven by it. The antioxidant response element (ARE) genes are induced in response to cellular responses to reactive oxygen species (ROS). They respond to the transcriptional activator Nrf2 and the repressor Bach1. The development of synthetic small molecules have therapeutic applications in activating the antioxidant network in a protective capacity. ARE-regulated gene activation and the repressor Bach1 are potential targets. The endogenous ligand heme of Bach1, inhibits its binding to ARE. Nrf2-mediated gene expression is thus expressed, including that of heme oxygenase (HMOX1), a well-documented target of Bach1. A synthetic small molecule capable of inducing HMOX1 and inhibiting Bach 1 activity has been demonstrated [1]. It acts as a novel agent in activating the antioxidant response by modulating Bach1 binding to the ARE binding site of target genes.

These mechanisms demonstrate the complexity of intracellular redox equilibrium. The enzyme heme oxygenase 1 (HO-1) is induced by oxidative stress and degrades redox-active heme producing agents. Anti-inflammatory and vasodilatory effects are mediated by HO-1 and it is protective of cellular stresses. The expression of the HO-1 gene HMOX1 is highly inducible by a range of pro-inflammatory stimuli via NF-κB in human endothelial cells. HMOX1 is regulated by the ARE genes, with the transcription factor Bach1 acting as repressor and Nrf2 functioning as an enhancer. A TNFα-inducible endothelial microRNA

miR-155, is predicted to bind to the Bach1 mRNA. Oligonucleotides that mimic miR-155 effectively inhibit Bach1 protein translation resulting in increased expression of HMOX1 mRNA and protein in human endothelial cells [2]. These findings indicate that during inflammation, miR-155 is cytoprotective by enhancing HO-1 expression in endothelial cells; and that elevated HMOX1 expression by TNFα is a result of miR-induced repression of Bach1 and not due to direct induction of HMOX1 via NF-κB. It is relevant that Bach1 repression dominates over Nrf2-mediated HMOX1 transcription. Inactivation of Bach1 is a requirement for induction of HMOX1 [3]. In contrast, it is significant that thioredoxin reductase 1 (TXNRD1) is mediated by Nrf2 and not Bach1. Comparison of expression levels of HOMX1 and TXNRD1 indicated that nuclear accumulation of Nrf2 is not a prerequisite for induction of HMOX1. Inactivation of Bach1 permits Nrf2 already present in the nucleus at basal levels to bind to the HMOX1 promoter and induce HNOX1. Bach1 poses another level of regulation of oxidative stress responses via ARE-dependent genes.

ROS are highly reactive molecules containing oxygen and are the most abundant free radicals in cells. They occur during physiological intracellular metabolism and play crucial roles in cell differentiation, proliferation and host defence responses [4]. However, in excess they could have adverse effects. Oxygen-derived free radicals could cause damage to various cell components including the critical processes of lipid peroxidation, DNA damage and protein oxidation. Polyunsaturated fatty acids are the main components of cell membranes which are particularly vulnerable to free radical damage due to their reactive hydrogen atoms associated with an abundant distribution of double bonds. This results in a predisposition to free radical damage, hydroxyl radicals in particular, resulting in compromised cell membrane permeability and cell dysfunction [5]. DNA damage comprising broken strands, cross-linking, base hydroxylation and base excision is also attributed to ROS. When DNA damage is combined with a deficient apoptotic pathway, it could result in their transformation [6].

Proteins are the main targets of free radicals, resulting in their oxidation. Free radical attack of aromatic acids, cysteine and disulphide bonds results in protein denaturation and enzyme inactivation [5]. Denatured protein derivatives thus formed could perpetuate oxidative damage in other cell components by acting as intermediary agonists [7]. Enzymatic and non-enzymatic antioxidant systems prevail in their actions as free radical scavengers [8]. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) comprise the enzymatic antioxidant system. This is the main defence against ROS in vivo. The two main types of SOD consist of CuZnSOD (SOD1) prevalent in the cytoplasm with Cu and Zn at the active site; and MnSOD (SOD2) found in the mitochondrial matrix with manganese at the active site. They catalyse the reaction that converts superoxide anion radicals to H_2O_2, which is subsequently converted to water and oxygen by CAT or GPx.

CAT is mainly found in peroxisome with iron at its active site; and is one of the most effective redox enzymes [9]. In the absence of this enzyme H_2O_2 would be converted to a hydroxyl radical, one of the most damaging radicals to cells. GPx is a selenium containing enzyme which exerts a protective influence from oxidative damage on cells by eliminating H_2O_2 with oxidation of glutathione. GR subsequently converts the oxidized glutathione to the reduced form. The non-enzymatic antioxidant system which serves as a second defence system against free radicals, protects tissue from oxidative damage. These systems also reinforce the actions of endogenous enzymatic antioxidants by scavenging free radicals in a synergistic capacity [10]. Vitamins C and E are the best known antioxidants in this context.

There are several small molecules present in foods such as phenolic compounds, carotenoids and flavonoids which serve as non-enzymatic antioxidants. These food derived phytochemicals are known as nutraceuticals [11], [12].

Mechanisms of Action of Glutathione

Glutathione (GSH) plays a critical role in cell signalling and antioxidant defences. It may interact directly with ROS / reactive nitrogen species (RNS); or act as an essential cofactor for GSH S-transferases and glutathione peroxidases. Coordinated actions of GSH and its dependent enzymes which constitute the glutathione system, lead to detoxification of ROS and RNS; and electrophiles produced by xenobiotics. Adequate GSH levels are essential for T cell activation, differentiation and optimal functions of the immune system. GSH is an ubiquitous regulator of the cell cycle. It also has critical functions in the brain as an antioxidant, neuromodulator and transmitter; and in enabling survival of neurons. GSH depletion leads to damage as a consequence of nitrosative and oxidative stress, hypernitrosylation, raised levels of proinflammatory mediators and inflammatory capacity. Intracellular signalling networks such as p53, nuclear factor-κB and Janus kinases are rendered dysfunctional, with diminished DNA synthesis and cell proliferation, activation of apoptotic mechanisms and compromised epigenetic regulation of gene expression. There are crucial consequences of GSH depletion on homeostatic control of the immune system, mitochondrial survival, regulation of energy production, oxidative and nitrosative stress pathways [13]. GSH depletion is an integral part of diverse neuroimmune doisorders such as depression, myalgic encephalomyelitis / chronic fatigue syndrome and Parkinson's disease concomitant with increased ROS / RNS and mitochondrial dysfunction. Therapeutic interventions aimed at enhancing GSH concentrations *in vivo* include N-acetyl cysteine; activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) via hyperbaric oxygen therapy; dimethyl fumarate; folate supplementation and phytochemicals such as curcumin, resveratrol and cinnamon.

The universally conserved thioredoxin (TRX) and glutathione (GSH) pathways drive a spectrum of cellular functions involving reversible disulfide formation. These pathways are considered in *Saccharomyces cerevisiae* in the context of their cell compartment-specific actions and mechanisms that address differences in redox states between cell compartments [14]. The cytosol has both TRX and GSH pathways of which the former is dominant and the latter functions as a backup. In the mitochondrial matrix where both pathways are represented, GSH has a major role in redox control. There are areas of intense thiol oxidation in the endoplasmic reticulum (ER) and mitochondrial intermembrane space (IMS); thiol-reductase pathways are attributed to GSH. Mitochondria are insulated from other compartments. Cytosol may be involved in entry and exit of reduced and oxidized GSH between compartments and provide reducing power to the ER and IMS. The mechanisms regulating fluxes of GSH and oxidized glutathione between cytosol, ER and IMS with a possible role for peroxisomes require clarification; in order to propose a model for eukaryotic thiol-redox homeostasis which may be extrapolated to mammals.

The small redox proteins glutaredoxins, reduce disulfides and mixed disulfides between GSH and proteins. *Cyanobacterium Synechocystis* sp. PCC 6803 has three genes coding for

glutaredoxines: ssr2061 (grxA) and slr1562 (grxB) code for dithiolic glutaredoxins; while slr1846 (grxC) code for monothiolic glutaredoxin. Analysis of the expression of these glutaredoxins to stresses such as high light, H_2O_2 and heat shock demonstrate that grxA is induced only by heat while grxC is induced by high levels of light and H_2O_2; and repressed by heat shock [15]. In contrast to these findings, expression of grxB was maintained fairly constant throughout all conditions. Experimentation with mutants demonstrated that grxA and grxC participate in independent pathways while grxA and grxB participate in a common pathway for H_2O_2 resistance. These data indicate that glutaredoxins are essential for stress adaptation in cyanobacteria; although their targets and mechanisms of action need further clarification.

Glutathione and Periodontitis

While several agents such as antioxidant vitamins overcome oxidative stress, glutathione is the most important small molecule antioxidant. It is prevalent in its oxidized and reduced forms as GSSG and GSH respectively. In health, higher levels of GSH are indicated, maintaining a reduced state intracellularly. It plays a significant role in antioxidant defences as a scavenger of free radicals and protects tissues via antioxidant networks, cell metabolism, DNA turnover and repair [16]. Periodontal disease severity may be affected by glutathione depletion [17], [18].

Chronic inflammatory periodontitis is characterized by raised levels of reactive oxygen species (ROS) resulting from an oxidative stress-inducing phenotype seen locally and systemically. The role of sulforaphane (SFN) in restoring cellular glutathione levels and reducing the hyperactivity of circulating PMNs in periodontitis was studied [19]. It was demonstrated that the NADPH oxidase complex is elevated by intracellular glutathione depletion; which could result from lipid raft formation as a result of upregulation of acid sphingomyelinase, regulated by thiol. Primary neutrophils from periodontitis subjects were hyper-responsive to stimuli and showed decreased intracellular glutathione. The main regulator of the antioxidant response, nuclear factor erythroid-2-related factor 2 (Nrf2); is reduced in circulating PMNs from subjects with chronic periodontitis. PMNs expressed a low ratio of reduced- / oxidized glutathione (GSH/GSSG) when compared with healthy controls; with reduced expression of of glutamate cysteine ligase- and modifier subunit mRNAs respectively. All the above parameters were improved by pre-treatment of cells with SFN. These findings indicate that deficient Nrf2-dependent pathways could play an important role in the mechanisms that lead to hyper-responsive neutrophils in chronic periodontitis.

The pathogeneses of chronic inflammatory diseases including periodontitis are driven by oxidative stress. Possible links between oxidative stress markers in saliva and periodontal alveolar bone have been studied [20]. Salivary oxidative stress markers 8-hydroxy-deoxyguanosine (8-HOdG) and malondialdehyde (MDA) levels were significantly higher in periodontitis subjects when compared with controls. Bone loss markers such as C-terminal telopeptide of type 1 collagen (CTX1), matrix metalloproteinases-8 (MMP-8), 25-hydroxy vitamin D3 and osteocalcin were also significantly elevated in chronic periodontitis subjects, with significant positive correlations with clinical parameters of periodontitis. There were significant positive correlations between salivary levels of MDA and CTX1. Salivary

activities for uric acid, total antioxidant capacity (TAC) and glutathione proxidase (GPx) were significantly reduced in periodontitis subjects compared with controls. There were significant negative correlations between uric acid and CTX1; and between MMP-8 and uric acid. There are significant associations between oxidative stress markers and alveolar bone loss in saliva of subjects with periodontitis. These findings confirm a pro-oxidant profile in periodontal disease progression.

A correlation between increased lipid peroxidation (LPO) and oxidative stress has been demonstrated in periodontitis associated with raised levels of MDA and SOD. Total oxidative status (TOS), MDA and SOD levels were studied in periodontitis subjects longitudinally throughout periodontal therapy for its impact [21], in serum, saliva and gingival crevicular fluid (GCF). Levels of TOS and SOD were significantly raised in periodontitis subjects over controls, but only MDA in GCF. Following 16 weeks of periodontal therapy, TOS and SOD levels decreased significantly in serum, saliva and GCF and MDA in GCF. Lipid peroxidation is elevated in periodontits with raised levels of TOS and SOD both locally and systemically. Non-surgical periodontal treatment is effective in restoring antioxidant capacity in periodontitis subjects by modifying the levels of MDA, TOS and SOD locally and systemically. The extent of oxidative stress in peridontitis subjects was assessed by measuring levels of thiobarbituric reactive substances (TBARS), enzymic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GSHPx) and non-enzymatic antioxidants, vitamins E and C; and reduced glutathione (GSH) [22]. It is relevant that periodontitis subjects showed significantly higher levels of TBARS than periodontally healthy subjects. In plasma and gingival tissues enzymatic antioxidants were significantly elevated, while non-enzymatic antioxidants were markedly lower (apart from reduced glutathione in gingival tissue), relative to those in periodontally healthy subjects. Over-production of lipid peroxidation substances causes a disordered, partly compensatory endogenous antioxidant defence system at inflammatory sites, resulting in elevated oxidative stress in subjects with periodontitis.

Finding a functional and reliable molecular marker of periodontal tissue destruction that is sensitive, with good specificity is a great challenge. The utility of molecular markers of soft and hard tissue destruction of the periodontium has been addressed in a systematic review of human studies presenting with such markers in GCF, saliva and serum [23]. Within limits of the scope of the study, no single or consistent combination of markers could adequately disclose periodontal tissue destruction. The most effective source of molecular biomarkers is closely related to bone and soft tissue destruction, requiring objective confirmation; while clinical measurements remain most reliable. An example is demonstrated here. The effects of initial periodontal treatment on GCF and salivary levels of 8-hydroxy-deoxyguanosine (8-OHdG), as a marker of oxidative DNA damage has been evaluated in subjects with chronic periodontitis [24]. There was a significant positive correlation between GCF 8-OHdG and clinical parameters of periodontal disease with a significant reduction following treatment; with not much change in salivary levels. DNA damage and resulting oxidative stress are seen in periodontal pocket tissue of subjects with periodontitis. Periodontal treatment results in resolution of inflammation and oxidative stress, reflected in GCF levels of 8-OHdG which were a more useful biomarker than saliva. 8-Hydroxydeoxyguanisine is an effective marker of periodontal disease severity and treatment responses.

Redox Mechanisms in Smokers with Periodontitis

Smoking is the most significant preventable risk factor for periodontitis. Smoking has a negative impact on chronic inflammatory diseases such as arthritis and inflammatory bowel disease. Periodontal disease is based on a hyper-inflammatory response leading to destruction of tissues of the periodontium. Changes related to the cellular and molecular aspects of the host response and genetic interactions associated with smoking are addressed in the context of local and systemic host responses in periodontitis subjects [25]. An elevated inflammatory response, decreased leukocyte chemotaxis and immunoglobulin production are some of the effects of smoking. Exposure to cigarette smoke products results in increased tissue destructive substances such as ROS, collagenase, serine proteases and proinflammatory cytokines. Epidemiological studies demonstrate the burden of smoking on periodontal health and potential for improvement in response to smoking cessation. Oxidative stress in periodontitis has potential mechanistic links with commonly prevalent systemic diseases such as type-2 DM and atheromatous heart disease in periodontitis subjects. Examination of serum antioxidant concentrations in periodontitis subjects is relevant in this context. Serum levels of vitamin C, bilirubin and total antioxidant capacity (TAOC) show an inverse correlation with periodontitis, increasing with its severity [26]. Higher serum levels of Vitamin C and TAOC are associated with lower odds ratios for severe periodontitis, more pronounced in never-smokers. Reduced relative risk of periodontitis including never-smokers, is associated with raised serum levels of antioxidants. Smoking is a significant factor in contributing to risk of periodontitis. In addition to increasing oxidative stress levels, it is associated with decreased levels of vitamin C and glutathione, and important antioxidant. This is a mechanism whereby smokers could be subjected to increased risk of periodontitis [27].

The effect of smoking status on local and systemic activities of SOD, glutathione peroxidase (GSH-Px) and catalase; and MDA levels were evaluated in periodontitis subjects [28]. The control groups showed the highest gingival activities of SOD, GSH-Px and CAT when compared with periodontitis groups. Serum MDA levels were elevated in all periodontitis groups, over the periodontally healthy non-smoking group, with a significant difference for former smokers with periodontitis. Amongst the periodontitis groups, smokers showed the highest gingival levels of MDA; and compensatory SOD, GSH-Px and catalase activities. These findings indicate that smoking compounds elevated local and systemic levels of MDA, in addition to periodontitis. Decreased local activities of SOD, GSH-Px and catalase seen in periodontitis subjects may be elevated in response to smoking as an adaptive mechanism which may not be adequate to overcome the effects of smoking on periodontal tissues.

The effect of cigarette smoke extract, nicotine and cotinine on neutrophil superoxide production has been investigated. Superoxide generation was detected by lucigenin chemiluminescence. *Fusobacterium nucleatum*, IgG-opsonized *Staphylococcus aureus* and *Escherichia coli* lipopolysaccharide (LPS) acted as pathologically relevant stimuli.There was significant stimulation of superoxide release from neutrophils in response to smoke extract in a dose-dependent manner [29]. Pre-treatment of neutrophils with smoke extract reduced superoxide generation in response to pathologically relevant stimuli, even in the absence of a continued presence of the smoke extract. Simultaneous exposure to both the extract and stimuli resulted in a similar reduction in superoxide production. Neither nicotine nor cotinine

at <10μg/ml induced superoxide release in stimulated or unstimulated neutrophils. These findings indicate that smoking could initiate and maintain oxidative stress at periodontally healthy sites via potential neutrophil-mediated mechanisms; and contribute to disease progression by attenuating host immune responses. Mechanisms involved in peripheral blood neutrophil hyperactivity in chronic and rapidly progressive forms of periodontitis and ROS generation have been addressed in a comprehensive review [30]. It includes environmental factors associated with compromised plasma antioxidant capacity and its influence on periodontal and systemic diseases. A systematic review has demonstrated that smoking cessation improves periodontal clinical parameters, by reducing periodontal pocket probing depths and improving clinical attachment levels following non-surgical periodontal treatment [31]. It is relevant that GCF total antioxidant status was significantly elevated, following periodontal treatment in smokers with periodontitis, while serum total oxidant status was significantly reduced in smokers and non-smokers following periodontal therapy [32]. These findings indicate that non-surgical periodontal therapy is effective in reducing oxidative stress.

Periodontal disease progression and healing responses are affected by tobacco products. In order to elucidate the role of nicotine a significant component of cigarette smoking, on periodontal destruction; its effects on growth, proliferation and protein synthesis were studied in human periodontal ligament fibroblasts (PDLF). At concentrations greater than 2.5mM, nicotine was cytotoxic to human PDLFs. There was significant dose-dependent inhibition of cell proliferation (by 48% and 86% at 50 and 200microM) and protein synthesis (to 44% at 10mM) when compared with controls [33]. In order to elucidate possible mechanisms involved, the antioxidants SOD, catalase, 2-oxothiazolide-4-carboxylic acid (OTZ): a precursor of cysteine that promotes synthesis of GSH; and buthionine sulfoximine (BSO), a cellular GSH synthesis inhibitor, were added to the cultures. It is relevant that OTZ had a protective effect on nicotine-induced cytotoxicity; on the contrary SOD and catalase did not contribute to decreasing the cytotoxicity induced by nicotine. Equally, BSO an inhibitor of GSH synthesis contributed further to nicotine-induced cytotoxicity. These results indicate that thiol depletion could mediate nicotine toxicity in human PDLFs, manifesting as impaired cell growth, proliferation and protein synthesis with concurrent reduced intracellular thiol levels; suggestive of a significant role for nicotine-induced periodontal destruction during cigarette smoking. Agents and mechanisms that enhance glutathione synthesis in human PDLFs may have applications for preventing or attenuating the damaging consequences of cigarette smoking on periodontal disease progression and response to treatment.

The detrimental effects of nicotine on tissues of the periodontium have been demonstrated by several workers. Cigarette smoking contributes to an increased incidence of periodontitis and a poor response to periodontal treatment. The early stress response c-fos gene was studied in human PDLFs following exposure to nicotine, in order to clarify toxicological implications of cigarette smoking at a molecular level [34]. On exposure of quiescent human PDLFs to 2.5mM and 10mM of nicotine for 2h, there were 2.5- and 4.8-fold increases respectively, in the induction of c-fos mRNA expression; peaking at a concentration of 5 mM nicotine at 2h. There is rapid accumulation of the transcript in nicotine treated cells, with a significant signal at 30 minutes of exposure to nicotine, demonstrated by kinetic studies of c-fos mRNA expression. This increase is transient, returning to baseline values of c-fos mRNA expression, comparable to those of control cells in 8h. In order to establish the role of thiol levels for c-fos induction by nicotine, cells were pretreated with the GSH

precursor OTZ (2-oxothiazolide-4-carboxylic acid), to enhance thiol levels; or BSO (buthionine sulfoximine) to deplete GSH. Results show that OTZ pretreatment decreased c-fos mRNA expression; while BSO pretreatment elevated c-fos mRNA expression following exposure to nicotine. Nicotine also caused significant intracellular GSH depletion in a dose-dependent manner at 5mM and 20mM by 22% and 56% respectively. These findings demonstrate that cigarette smoking could induce early reponse stress genes via the c-fos signal transduction pathway; correlating with intracellular thiol levels in human PDLFs.

The oxidative effects of nicotine in human gingival and human oral periosteal fibroblasts were studied, using androgen biomarkers of wound healing and redox status. The antioxidant glutathione (GSH) was used for confirmation of redox responses [35]. Two radiolabelled androgen substrates were used to evaluate the yields of the antioxidant biomarker 5α-dihydrotestosterone (DHT) in order to validate responses to nicotine, GSH and their combinations in a metabolically active model. Nicotine caused significantly reduced yields of DHT, due to down-regulation of 5α-reductase activity, which was overcome by the antioxidant glutathione indicative of modulation of the pro-oxidant effects of nicotine by GSH. These results could be extrapolated to indicate improved healing responses by overcoming the pro-oxidant effects of nicotine. In a similar study, the oxidative effects of nicotine were validated, in human gingival and oral periosteal fibroblasts by demonstrating the responses to hydrogen peroxide (H_2O_2) an established pro-oxidant, for comparison with nicotine, with GSH as an antioxidant, alone and in combination [36]. Radiolabelled androgen substrates were used and the yields of DHT, an antioxidant marker of redox status and wound healing were assayed in response to the agents tested in a metabolically active model. The yields of DHT were significantly reduced by nicotine and H_2O_2. This was overcome by GSH. It is relevant that when nicotine was added to the neutralized combination of H_2O_2 and GSH, decreased yields induced by nicotine were similar to those induced by H_2O_2. The positive effect of GSH was retained. These results indicate a pro-oxidant role for nicotine, considering that oxidative stress mediated by H_2O_2 was overcome by GSH and recurred when nicotine was added. DHT is a sensitive biomarker of oxidate stress which has implications on wound healing.

Potential oxidative effects of glucose, advanced glycation end products (AGE) and nicotine were studied in human gingival fibroblasts, using the antioxidants glutathione (GSH) and insulin-like growth factor (IGF) [37]. Two radiolabelled androgen substrates were used as substrates and assayed for yields of the the oxidative stress marker 5α-dihydrotestosterone (DHT), in response to the agents tested. Significant reduction in the yields of DHT in response to glucose, AGE and nicotine were overcome by GSH. The stimulatory effects of IGF in combination with AGE were enhanced further by the antioxidant effects of GSH. These findings are suggestive of antioxidant effects of GSH and amelioration of oxidative stress responses to glucose, nicotine and AGE. Results from this experimental model may be cautiously extrapolated to redox responses and healing in uncontrolled diabetic smokers.

The impact of diabetes as a risk factor for periodontitis has been studied in the context of antioxidant enzyme activity, namely superoxide dismutase (SOD) glutathione reductase (GR), catalase and the marker of free radical damage, malondialdehyde; in blood and saliva of periodontitis subjects. MDA levels in both periodontitis groups (CP) with (CPDM) and without DM were elevated in comparison with periodontally healthy subject, although the difference between the two periodontitis groups was not significant. There were significant

differences in all enzyme levels between CP and peridontally healthy subjects except blood levels of SOD. Only salivary SOD and GR activities were significantly different in CP and CPDM groups [38]. These findings favour the role of oxidative stress in both DM and CP. Compensatory host mechanism could fail due to excessive free radical influx during periodontitis compounded by the influx from DM contributing to inflammatory overload.

Antioxidant Effects of Glutathione in Systemic Diseases

A range of functions are carried out by glutathione transferases (GSTs) such as detoxification and beyond, with catalytic reactions affecting metabolic pathways and removal of ROS. Based on previous work, GSTM1 and GSTT1 gene polymorphisms and their association with carotid plaque (CP), biochemical parameters of oxidative stress, lipid profile and inflammation have been investigated, in the context of their modulation of atherosclerosis risk. GSTT1 null genotype patients show significantly lower plama lipoprotein levels than the wild-type genotype carriers [39]. Both GST polymorphisms significantly influenced serum IL-6 levels in subjects with CP. Results indicating significant reductions in GSTT1 deletions in subjects with CP, are indicative of a role for GSTs in carotid astherosclerosis and advanced chronic vascular inflammatory disease.

A protective role for the antioxidant enzyme glutathione peroxidase-1 (GPx-1) has been proposed during atherogenesis. A deficiency of GPx-1 accelerates atherosclerosis and increases cellularity of the lesion in ApoE(-/-) mice. The distribution of GPx-1 within the atherosclerotic lesion and mechanisms involved in the increased macrophage numbers seen, require further clarification. Differential expression of GPx-1 in cells within the atherosclerotic lesion and the relationship between deficient GPx-1, macrophage foam cell formation and cellular proliferation were studied [40]. It was demonstrated by in situ-hybridization and immunohistochemistry that both macrophages and to a lesser extent, smooth muscle cells express GPx-1 within atherosclerotic lesions. GPx-1 deficiency resulted in increased foam cell formation, induced by oxidized low density-lipoprotein (oxLDL); and increased proliferative activity of peritoneal macrophages. Proliferation of peritoneal macrophages induced by macrophage colony stimulating factor (MCSF) and ox-LDL in GPx-1 (-/-) ApoE(-/-) mice was mediated by p44/42 MAPK (p44/42 mitogen activated protein kinase), via ERK 1/2 (extracellular-signal regulated kinase1/2) signalling pathway, demonstrated by inhibitors of this signalling pathway. It is relevant that representative effects of GPx-1 deficiency on both macrophage proliferation and MAPK phosphorylation are overcome by the GPx mimic ebselen. These results demonstrate the significance of GPx-1 deficiency on macrophage foam cell formation and proliferation via the p44/42 MAPK (ERK1/2) pathway; underscoring the potential for new therapeutic strategies in managing atherosclerosis.

Subjects with human immunodeficiency virus (HIV) infection are an effective model for demonstration of pro-oxidant mechanisms. These subjects show a significant reduction in levels of enzymes such as glutathione synthase (GSS), glutamate-cysteine ligase-catalytic subunit (GCLC) and glutathione reductase (GSR); responsible for the synthesis of glutathione. These reduced levels of relevant enzymes correlate with reduced intracellular

levels of GSH [41]. GSH capacity in RBCs is a useful marker for a raised level of oxidative stress and immune dysfunction in response to HIV infection. The results support the hypothesis that reduced levels of GSH-synthetic enzymes contribute to compromised levels of GSH in HIV-infected individuals. Considering the role of GSH in combating oxidative stress and improving immune cell function in HIV subjects, supplementary antioxidants could be beneficial in promoting immune cell function and reducing cell damage.

Glutathione peroxidase 3 (GPx3) plays an important role in eliminating hydro- and lipoperoxides from the body. Several single nucleotide polymorphisms (SNP) at the GPX3 gene and altered concentrations have been linked to vascular diseases, but associations between GPX3 and MetS have not been explored. Serum levels of GPX3 and several GPX3 SNPs in Mexican subjects with MetS have been studied [42]. The MetS group demonstrated increased cardiovascular risk and higher serum levels of GPx3, in comparison with controls. Only three of ten GPX3 SNPs screened were polymorphic, with two observed haplotypes, suggestive of tight linkage disequilibrium at this genetic focus. There were no differences for genotype or allele frequencies amongst the observed groups; however rs8177409 (allele T) is significantly linked to cardiovascular risk (odds ratio: 4.5). These findings indicate that serum levels of GPx3 are raised in MetS subjects; and that rs8177409 SNP is associated with cardiovascular risk in the population under study.

Oxidative stress plays an important role in the metabolic syndrome (MetS) and associated conditions. The number of metabolic syndrome components (ischaemic reactive hyperaemia [IRH], plasma levels of soluble vascular cell adhesion molecule-1[sVcam-1], total nitrite, lipid peroxidation products [LPO], hydrogen peroxide [H_2O_2], superoxide dismutase [SOD] and plasma activities of glutathione peroxidase [GPx]), presenting in the subject was correlated with the degree of oxidative stress [43]. sVCAM-1, H_2O_2 and LPO levels are lower in subjects with 2 or 3 MetS components than in those with 4 or 5 parameters. IRH and total nitrite levels are higher in subjects with 2 or 3 MetS componenets than in those with 4 or 5 parameters. SOD and GPx activities are lower in subjects with 2 MetS components than in those with 4 or 5 MetS parameters. There is a significant correlation between SOD activity; and waist circumference, weight, age, triglycerides, high-density lipoprotein and sVCAM-1 levels. MetS subjects with a greater number of MetS components could have greater oxidative stress levels. The association between SOD activity and MetS components could be the most significant variable parameter in subjects with Mets; it has potential as a predictive tool to determine the degree of oxidative stress in subjects with MetS.

Apocyn (4'-hydroxy-3'-methoxyacetophenone) is a commonly used inhibitor of NADPH oxidase; however due to some of its controversial pro-oxidant effects, its applications raise serious concerns. The effects of apocynin on glutathione metabolism being a key intracellular antioxidant, were studied in a well-established rat model of type 2 DM [44]. The effects of apocynin were also compared with those of melatonin. Compared with untreated lean control rats, untreated obese diabetic rats showed increased Nox activity, accelerated generation of hydroxyl free radicals (HFR) and significantly reduced GSH/GSSG ratio, associated with increased glutathione peroxidase (GPx) and reduced γ-glutamylcysteine synthetase (GCS). In the diabetic animals, apocynin treatment attenuated both Nox activity and HFR formation, restored baseline values for the GSH/GSSG (reduced and oxidized glutathione) ratio (due to increased GSH and decreased GSSG levels), normal GPx and slightly increased GCS activity. There was a similar outcome when melatonin was applied to obese diabetic rats. These findings indicate that in the diabetic rat model used in this study, apocynin has a beneficial

effect on renal glutathione homeostasis. The mechanism involves diminished glutathione peroxidase activity which is stimulated excessively during oxidative stress associated with diabetes mellitus.

Teteracyclines have ameliorating antioxidant properties in tissues, including vascular endothelial dysfunction in diabetes mellitus. Low-dose doxycycline (LDD) was used in diabetic rats for 4 weeks. It is relevant that this treatment normalized elevated lipid peroxidase and cellular GSH levels [45]. The diabetes-induced oxidative stress markers MMP-2 and MMP-9 were also normalized in response to LDD. Its antioxidant actions could be effective in vascular disorders amongst DM subjects. Combined treatment with doxycycline has been shown to restore free and total protein thiol levels in experimental diabetes [46]. Ligature-induced periodontitis rats were treated with LDD in order to assess its effects on oxidative stress induced by periodontitis [47]. Gingival tissues were used to assess lipid peroxidation (MDA), the antioxidant enzymes CAT, GPX and SOD, total oxidant and total antioxidant tstatus (TOS, TAS). There was significant inhibition of MDA, reduced TOS, increased TAS and favourable antioxidant enzyme profiles in response to LDD, reinforcing its effective antioxidant properties.

The anti-inflammatory, proanabolic and anti-catabolic, non-antimicrobial actions of tetracyclines are effective in the adjunctive management of inflammatory periodontitis and associated comorbidities. A dysregulated hyperinflammatory immune response in periodontitis could have an autoimmune element in its progression beyond the presence of an initiating antigenic trigger. Its inflammatory pathogenesis and those of the commonly prevalent comorbidities coronary heart disease, DM and arthritis could benefit from adjunctive management with non-antimicrobial chemically modified tetracyclines (CMTs) to curb an over-exuberant inflammatory response. Tetracyclines and their derivatives interact with matrix metalloproteinases (MMPs), tissue inhibitors of MMPs, cytokines and growth factors. They mediate immunomodulation by affecting the sequence of inflammation, cell proliferation and angiogenesis. CMTs maintain anti-inflammatory, anti-apoptotic and anti-proteolytic actions in organs in a range of chronic inflammatory diseases which reinforces their therapeutic scope. Their specific advantageous effects have been demonstrated in experimental models of ischaemia. Some of these actions and mechanisms involved have been addressed in a recent review [48]. Unique non-antimicrobial actions of tetracyclines in a hyper-inflammatory environment have applications in chronic inflammatory disorders. These advantageous effects are relevant to the adjunctive management of periodontitis subjects presenting with commonly prevalent comorbidities mentioned above.

Redox responses of cultured osteoblasts have been studied, in response to bacterial lipopolysaccharides (LPS), glucose (G), glucose-oxidised low density lipoprotein (GLDL) and minocycline (M), using radioactive substrates and the redox marker of wound healing 5α-dihydrotestosterone (DHT), in a metabolically active model [49]. There were significantly decreased yields of the antioxidant marker DHT in response to LPS, G and GLDL, which were overcome by minocycline. These findings demonstrate its potential antioxidant actions in an environment of oxidative stress and may be extrapolated to periodontitis and co-existing risk markers in cardiometabolic diseases. This has implications on the adjunctive anti-inflammatory and antioxidant therapeutic benefits in the management of periodontitis and prevalent comorbidities. There have been significant advances in adjunctive antioxidant therapeutics in the management of these diseases, in the context of oxidative stress being a common denominator. The same workers have demonstrated that IL-6 and CRP alone and in

combination, caused significant reduction in the yields of the antioxidant marker DHT in monolayer cultures of osteoblasts. These effects were ameliorated by doxycycline, resulting in values of DHT similar to those of control incubations in the absence of agents [50]. The oxidative actions of IL-6 and CRP and antioxidant effects of doxycycline are reinforced by the metabolic yields of DHT in respose to the above agents. DHT directly activates androgen receptor proteins which play an important role as redox regulators via direct actions on glutathione S-transferase [51]. It is significant that mouse stem cells pretreated with DHT which elevates levels of the antioxidant enzyme CAT, are able to overcome the oxidative and apoptotic effects of H_2O_2 leading to reduced levels of DHT, cell cycle regulatory proteins and cell viability [52]. In view of these direct antioxidant effects of DHT, the novel metabolically active model used, demonstrates a closer relationship with *in vivo* conditions; which may be applicable in the context of adjunctive therapeutic applications for periodontitis and prevalent comorbidities such as coronary heart disease and arthritis in periodontitis subjects.

There is a significant prevalence of periodontitis subjects presenting with systemic diseases such as coronary heart disease, insulin resistance and arthritis which present with a pro-oxidant inflammatory profile. Changes in gene expression associated with a pro-inflammatory profile and lipid metabolism in response to periodontal pathogens have been shown in animal models, independent of atherosclerotic lesions. A single nucleotide polymorphism of the TNF-α gene is associated with significant attachment loss of teeth in periodontitis subjects with coronary heart disease. Raised levels of the cytokines IL-1, IL-6 and TNF-α associated with chronic low-grade inflammation and insulin resistance is also relevant to the progression of periodontitis. Uncontrolled periodontitis could contribute to the maintenance of systemic inflammatory loading relevant to rheumatoid arthritis (RA), with increased risk of RA in periodontitis subjects. Concepts linking periodontitis and sytemic diseases associated with a pro-oxidant profile and mechanisms involved have been reviewed recently [53]. Weak but consistent associations are seen for surrogate markers of periodontitis, for example tooth loss with multiple systemic conditions. Effective treatment of periodontitis could be beneficial in reducing systemic inflammatory loading and systemic health. Genetic, epigenetic and other subject variables could account for the lack of a consistent cause and effect relationship between diseases with a predominant pro-oxidant profile.

Physiological metabolic activities of cells result in the formation of ROS as by-products. The damaging effects of ROS are protected by superoxide dismutase (SOD), glutathione peroxidase and CAT. ROS may be produced in response to a range of stimuli such as ultraviolet radiation, cigarette smoking, alcohol, ischaemia-reperfusion injury, chronic infections and inflammatory conditions. Disruption of physiological cellular homeostasis by redox signalling could result in tissue damage and susceptibility to certain diseases. Oxidative stress and ROS release could contribute to gastrointestinal diseases such as peptic ulcers, cancers and inflammatory bowel disease [54]. ROS are produced within the gastrointestinal tract. Despite the protective mucosal barrier, ingested substances and pathogens could contribute to inflammatory, immune-mediated responses involving the epithelium of the gastrointestinal tract. Understanding signalling mechanisms initiated by ROS at a cellular level and the host responses to such stimuli, would advance knowledge of disease pathogenesis for the evolution of appropriate treatment strategies.

Redox control in normal human mammary cells is poorly understood. Purified normal human basal mammary epithelial cells maintain low levels of ROS primarily by a relatively

ineffectual glutathione-dependent antioxidant mechanism that utilizes mitochondrial glutathione peroxidase 2. In contrast, matching luminal progenitor cells consume oxygen at a greater rate and contain oxidative nucleotide damage-controlling proteins. They show increased levels of ROS and demonstrate multiple antioxidants independent of glutathione [55]. These luminal progenitor cells are more resistant to H_2O_2, ionizing radiation and to glutathione depletion than basal cells; including those with proliferation and differentiation activity. Distinct mechanisms for control of ROS in subsets of human mammary cells could have functions related to their state of differentiation, with long-term consequences. Oxidative stress plays a significant role in diseases such as atherosclerosis, heart failure and myocardial infarction. Atopic dermatitis characterised by eczema and pruritis is a chronic relapsing inflammatory disease affecting the skin, in response to irritants, environmental and food allergens. Very little is known about the redox status of subjects with atopic dermatitis. Evaluation of malondialdehyde (MDA) a pro-oxidant, the enzymic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and the non-enzymatic antioxidants reduced glutathione (GSH), vitamins A, E and C demonstrated that subjects with atopic dermatitis are more susceptible to ROS damage than healthy controls [56]. There were raised levels of malondialdehyde and decreased enzymatic and non-enzymatic antioxidants in the former. Antioxidants could have potential benefits in atopic dermatitis subjects, which requires further study with larger population groups.

Dietary Agonists

It is relevant that periodontitis is associated with reduced serum micronutrient levels [26]. This could be a consequence of dietary, environmental and genetic factors affecting their absorption, biosynthesis, distribution and actions [57]. Potential beneficial clinical effects of powdered fruit and vegetable juice concentrate on periodontitis subjects have been investigated in a randomized double-blind clinical trial. Dietary intake and biochemical nutritional status were assessed [58]. Results showed that there was improved pocket depth reduction in response to non-surgical periodontal treatment with the dietary supplement as an adjunct when compared with treatment combined with a placebo. It would be pertinent to include dietary recommendations for improved consumption of fruits and vegetables, fibre, oily fish and reduced intake of refined sugar. This would be part of a health message for effective prevention and treatment of periodontitis subjects as suggested in the 2011 European workshop on Periodontology [59]. A dsysregulated, hyperinflammatory immune response in periodontitis subjects resulting in a pro-oxidant profile makes it an important area for antioxidant therapeutics as adjuncts. Dietary measures have potential benefits in this context for better outcome measures in the management of periodontitis and prevalent systemic comorbidities.

Growing interest in the role of oxidative stress in the progression of periodontal disease prompted an assessment of potential oxidant / antioxidant interactions of nicotine with coenzyme Q10 (CoQ), Pycnogenol and phytoestrogens in a cell culture model of osteoblasts and periosteal fibroblasts [60]. Metabolic yields of the antioxidant marker 5α-dihydrotestosterone (DHT) from radiolabelled androgen substrates in response to agents tested, were assayed in a metabolically active model. There were elevated yields of DHT in

response to CoQ, Pycnogenol and phytoestrogens; and significantly reduced yields in response to nicotine, which were overcome by combined incubations with CoQ, Pycnogenol or phytoestrogens. These results indicate that the pro-oxidant effects of nicotine could be reversed by the antioxidants CoQ, Pycnogenol and phytoestrogens, which could have potential applications in an environment of oxidative stress. The role of nutritional antioxidants in periodontal and systemic diseases has been reviewed [61], [62]. There are applications for the role of adjunctive nutritional and therapeutic antioxidants in diseases that present with a distinctly pro-oxidant profile such as periodontitis. Accurate therapeutic targeting to complement conventional periodontal treatment for an effective outcome is exacting. Bioactive phytochemicals play a significant role in decreasing oxidative damage in subjects with MetS with accompanying sub-cellular characteristics of atherogenic dyslipidaemia and a proinflammatory state. Optimally targeted, synergistic therapeutic formulations would be potentially effective as adjuncts in the management of periodontitis and associated systemic comorbidities.

Oxidative stress may be reduced by foods rich in antioxidant micronutrients, such as leafy vegetables, berries, kidney beans, red wine and dark chocolate containing more than 70% cocoa. Complex compounds in nuts, olives and oily fish could slow down gastric emptying time, with fewer less significant spikes in serum glucose in response to vitamin C [63], enhanced by the antioxidant efficacy. Oscillating glucose peaks are more damaging to the redox status of endothelium than mean glucose levels. Post-prandial incremental glucose peaks correlate with carotid intima-media thickness in type 2 diabetes mellitus [64]. Diabetic complications are a consequence of long-term hyperglycaemia and elevated levels of reactive oxygen species (ROS). Honey and ginger have antioxidant properties associated with scavenging ROS. The antioxidant and anti-diabetic effects of gelam honey and ginger alone and in combination were studied in diabetic and non-diabetic Sprague-Dawley rats [65]. In order to obtain a metabolic profile, the parameters measured were glucose triglyceride (TG), SOD, CAT, glutathione peroxidase (GPx), reduced glutathione (GSH): oxidized glutathione (GSSG) ratio and malondialdehyde (MDA). Although a combination of gelam honey and ginger did not show hypoglycaemic potential, the combined treatment significantly reduced levels of MDA; while there was significant elevation of GSH and GSH/GSSG ratio in diabetic rats when compared with controls.

Oxidative stress, a cholesterol-enriched diet and raised cholesterol levels result in raised serum total cholesterol levels (TC) and low density lipoprotein-cholesterol (LDL-C), which contribute to atherosclerosis. Antioxidants play an important role in absorbing free radicals which have damaging consequences in tissues. Protective effects of propolis (a resinous hive product collected from plant sources by honey bees) and thymoquinone (TQ) derived from specific plant seeds have been studied, on early atherosclerotic lesions and serum lipid levels in hypercholesterolaemic rabbits [66]. A cholesterol-enriched diet caused significant increases in serum levels of TC, triglycerides, LDL-C, thiobarbituric acid-reactive substances; and significant decreases in HDL-cholesterol and reduced glutathione levels when compared with controls. When the antioxidants propolis and TQ were administered simultaneously with a cholesterol-enriched diet, there were significant reductions in TC, LDL-C, triglycerides and thiobarbituric acid-reactive substances; and increased levels of HDL-C and glutathione, when compared with the high cholesterol control group. Early atherosclerotic changes represented by endothelial damage and thickened foam cells were seen in the high cholesterol group; the antioxidants propolis and TQ provided protection against damage induced by high

cholesterol. Antioxidant mechanisms associated with the latter could provide protection and minimize the risk of atherosclerosis.

The hypoglycaemic and antioxidant effects of shrimp astaxanthin were studied in the kidney of alloxan-induced diabetic rats. *In vitro* anti-diabetic effects of astaxanthin dissolved in olive oil were compared versus controls in plasma and renal tissue of diabetic rats [67]. Antioxidant enzyme activities including CAT, SOD and non-enzymatic levels of reduced glutathione were significantly decreased in plasma and tissue of diabetic rats compared with controls. The above enzyme activities were significantly improved in response to supplementation with astaxanthin with no additional change in response to olive oil alone. These findings demonstrate that shrimp astaxanthin could play an important role in reducing oxidative damage and pathological changes in diabetic rats, indicating potential use for therapeutic applications. The impact of commercially available green and black tea (GT, BT) beverages on oxidative stress and drug-metabolising enzymes was studied in rats [68], in comparison with de-ionised water. In response to both GT and BT, there were significant increases in hepatic microsomal cytochrome P450 (CYP)1A1 and CYP1A2; and a significant decrease in CYP2C, CYP2E1 and CYP3A enzyme activities. There were lower lipid levels in lungs of rats treated with the GT beverage. Feeding both tea drinks to rats results in modulation of drug-metabolising enzyme activity; and reduced oxidative stress in liver and lungs. The GT beverage was more effective in reducing oxidative stress than the BT beverage.

Nutritional assessment of diet in subjects with MetS and a biochemical analysis of redox levels have been carried out in MetS subjects [69] for comparison with those who did not have MetS. Antioxidant capacity showed a normal range in both MetS and control subjects, with no significant differences in SOD levels between the two groups. Mean glutathione reductase levels were significantly greater in controls than in those with MetS. In the context of trends for oxidative stress markers, isoprostane levels were greater in controls than in MetS subjects and oxidized LDL values were higher in MetS subjects; although the differences were not significant. Trends towards a greater degree of oxidative stress, associated with poorer nutritional and biochemical parameters were seen subjects with MetS in comparison with controls.

There is growing interest in the relationship between diet and ageing. Some antioxidants and restriction of dietary calories have been shown to enhance lifespan in experimental models of ageing. As oxygen is the final electron acceptor in mitochondria, it is essential for aerobic organisms. Excess oxygen could be harmful due to generation of reactive oxygen species (ROS) in a continuous cycle; this could contribute to ageing [70]. An antioxidant defence system comprising a range of enzymes such as SOD, CAT, glutathione peroxidase (GPx) and glutathione reductase (GR) are active in removing ROS accumulating in cells. In addition, vitamin A, vitamin C, α-tocopherol and plant flavonoids which are available as dietary antioxidants also have the capacity to scavenge ROS in cells. They could potentially enhance the lifespan of organisms. In this context, several antioxidants including apple polyphenols, blueberry polyphenols, black rice anthocyanins, tea catechins and theaflavins have been shown to enhance lifespans of fruit flies. Some of these trends may be extrapolated to humans.

Resveratrol has strong antioxidant properties and decreases blood glucose levels which could contribute to minimizing complications associated with DM. The effects of resveratrol on catalase (CAT), glutathione peroxidase (GPx) gene, protein expression, their

phosphorylation states and activities were studied in diabetes-induced rats [71]. There is increased total protein phosphorylation in DM, while mRNA expression, protein levels and their activities were similar. Although there is attenuated transcription of GPx in DM, protein levels and actions were not affected. Administration of resveratrol to DM rats resulted in increased pGPx levels. Increased nucler factor kappa B (NFκB) gene expression in DM, decreased Sirtuin 1(SIRT1) and nuclear factor erythroid 2-related factor (Nrf2); are linked to a decrease in CAT and GPx mRNA expression. Nuclear translocation of redox- sensitive Nrf2 and NFκB in DM could be a compensatory mechanism for a reduction in gene expression of antioxidant enzymes. This is seen as increased nuclear protein levels of Nrf2 and NFκB and reduced cytoplasmic levels of the same. These findings indicate that an increased oxidized state in DM, results in altered cellular phosphoryation and regulation of antioxidant enzymes. Administration of resveratrol also results in coordinated cytoprotective responses in tissues.

Regular consumption of wine, a characteristic feature of the Mediterranean diet has been associated with significant health benefits. The non-alcoholic component contains a wide range of phenolic polyphenols with antioxidant properties. Polphenols found in wine could delay the progression of inflammatory intestinal diseases driven by oxidative stress, particularly due to raised levels in the gut, compared with other tissues.They scavenge ROS and modulate specific genes involved in redox signalling in a milieu of inflammation and also act as antimicrobial agents and prebiotics [72].Wine phenolics have potential as alternative adjuncts for the treatment of inflammatory intestinal diseases. Some of the beneficial effects could partly be attributed to the alcoholic component in view of such effects of ethanol and require clarification order to implement a more solid foundation for their applications.

In view of assertions regarding potential toxic effects of synthetic antioxidants, the poultry industry has been seeking natural antioxidant sources alone or in combination with synthetic antioxidants. The status of antioxidant enzymes, fatty acid profile and serum biochemical profile of broilers was determined in response to no addition (T1) and addition of wheat germ oil (natural α-tocopherol T2), synthetic α-tocopherol (T3), α-lipoic acid (T4), and combinations of α-lipoic acid with natural and synthetic α-tocopherol respectively (T5 and T6) [73]. The dietary supplements used, resulted in improved distribution of saturated and unsaturated fatty acids in breast and leg meat. The fatty acid content was significantly greater in broilers receiving T2 and lower in those receiving T6 in their diet. Total cholesterol and triglyceride levels in serum were lowest in those receiving α-tocopherol and α-lipoic acid. Wheat germ oil containing natural α-tocopherol alone or with a-lipoic acid is more effective than its synthetic counterpart in enhancing antioxidant enzyme levels of superoxide dismutase (SOD), CAT and GR (glutathione reductase). This was accompanied by significantly reduced plasma total cholesterol, LDL and triglycerides; and elevated levels of HDL and plasma protein. A combination of wheat germ oil and a-lipoic acid is effective in improving lipid profiles in this context.

ROS and reactive nitrogen species (RNS) contribute to endothelial dysfunction in advancing age and promote the development of coronary heart disease and DM. The discovery of α-lipoic acid as a catalyst for decarboxylation of pyruvate and α-ketoglutarate has generated interest in its efficacy in protecting mitochondrial dysfunction induced by ROS. Both α-lipoic acid and dihydro α-lipoic acid have potent antioxidant actions and account for the benefits of supplementation with α-lipoic acid. Recent clinical work done on its beneficial effects on endothelial dysfunction and possible mechanisms incolved are reviewed [74]. The

redox status of α-lipoic acid depends on the degree of oxidised status of cellular components. The reducing intracellular environment helps to protect from oxidative damage. Younger healthy subjects are able to synthesise α-lipoic acid in adequate amounts for scavenging ROS and increasing the endogenous antioxidants glutathione, vitamins C and E. With advancing age, a significant decline in α-lipoic acid could lead to endothelial dysfunction. Regulation of gene transcription associated with antioxidant and anti-inflammatory pathways are also attributed to α -lipoic acid in several studies.

Chemical redox properties of α-lipoic acid indicate significant antioxidant actions. α-Lipoic acid and its reduced form dihydrolipoic acid are protective against oxidative stress-induced cell damage from reactive oxygen and nitrogen species (ROS, RNS). The role of nonprotein- bound α-lipoic acid as a physiological antioxidant has been questioned due to reduced and transient levels following oral intake. The micronutrient actions of α-lipoic acid could function in influencing cellular oxidative stress response pathways, which would affect antioxidant levels intracellularly and attenuate pro-inflammatory mechanisms [75]. This mode of action would result in more sustained benefits and resistance of cells to oxidative stress-induced pathologies, rather than act as a transient scavenger of ROS.

Yam (Dioscorea batatas Decne) has been used as a health food for its nutritional and anti-inflammatory effects. ROS, implicated in a wide spectrum of diseases are important precursors of carcinogenesis. The modulatory effect of yam on inflammation and antioxidant status in azoxymethane (AOM)-induced colonic cancer has been studied in male rats [76]. The formation of aberrant crypt foci (ACF), haemolysate antioxidant enzyme activities; and gene expression for inflammatory mediators and antioxidant enzymes in colonic mucosa were quantified. Ingestion of yam prior to carcinogenesis caused significant reduction in ACF formation in response to AOM. Erythrocyte levels of glutathione, glutathione peroxidase (GPx) and catalase (CAT) were elevated when rats were fed yam. It also resulted in up-regulation of Cu/Zn-superoxide dismutase (SOD), Mn-SOD and GPx gene expression in colonic mucosa when compared with the AOM group. Gene expression of the inflammatory mediators NF-κB, inducible nitric oxide synthase , COX-2, TNF-α and IL-1β in colonic mucosa was suppressed in response to the diet supplemented with yam. Enhancing antioxidant defences and modulating inflammatory mediators are some of the mechanisms attributed to yam in the prevention of colonic cancer.

The antioxidant effects of bilberries show beneficial preventive actions against inflammatory bowel disease and colonic cancer. The gastrointestinal tract is a potential target for disease prevention. The commercially available anthocyanin-rich bilberry extract (BE) was compared with four diverse BE-loaded microcapsules systems for their antioxidant properties. Intracellular ROS, oxidative DNA damage and total glutathione (GSH) levels were monitored as markers of antioxidant status. Increased cellular glutathione levels and reduced ROS were demonstrated at high concentrations of BE when incubations were made with BE loaded capsule systems [77]. In addition, there was a positive effect on DNA strand breaks. Biological properties of encapsulated BE were comparable to those of non-encapsulated BE. Both forms of BE appear to have beneficial effects on antioxidant activity in preventing oxidative damage of DNA, reducing intracellular ROS and enhancing cellular total glutathione, under the assay conditions used. Direct beneficial effects of dietary components on the antioxidant actions of the glutathione network have potential for their adjunctive applications in chronic inflammatory diseases in addition to conventional therapy.

Conclusion

Dysregulated immune responses with a hyper-responsive pro-oxidant inflammatory profile characterize periodontitis and associated systemic diseases prevalent in these subjects. The relevance of the glutathione system and mechanisms of their actions and interactions are addressed here, demonstrating significant connections between periodontitis and systemic inflammatory conditions. There are distinct mechanisms involving antioxidant response element (ARE) genes in response to ROS, by cells. The complexity of intracellular redox equilibrium is demonstrated by interactions between ARE genes, the repressor Bach1and their modulation by the expression of transcriptional activator Nrf2- and heme oxygenase genes (HOX1). Induction of the latter by a synthetic small molecule enhances antioxidant activity. Synthetic small molecules have therapeutic applications, in activating the antioxidant network to protect tissues and organ systems against oxidative damage. Similarly, dietary agonists could play an important role in attenuating oxidative stress due to their direct chemical antioxidant properties and by scavenging ROS and RNS. The micronutrient α-lipoic acid and its reduced form are potent antioxidants with beneficial effects on endothelial dysfunction. They also regulate gene transcription associated with antioxidant and anti-inflammatory pathways. Collectively, dietary agonists have advantageous interactions with the antioxidant capacity of the glutathione network. These effects have potential adjunctive therapeutic applications requiring focused and targeted delivery.

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