

## Role of quercetin in cardiovascular diseases

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**ABSTRACT:** Cardiovascular disease constitutes a major public health concern in industrialized nations. Over recent decades, a large body of evidence has accumulated indicating that oxidative stress induced free radicals play a critical role in cellular processes implicated in atherosclerosis and many other heart diseases. However a diet high in antioxidants is associated with a reduced risk of cardiovascular disease. The compound quercetin is a dietary antioxidant with a polyphenolic structure that is present in many foods, such as onion, apples, wine and tea. An increased intake of quercetin has been correlated with a decrease in the risk of cardiovascular diseases. Quercetin has been reported to exhibit a wide range of biological and pharmacological effects in animals and man besides its antioxidative and free radical scavenging actions. This paper reviews various steps of oxidative stress mediated atherogenesis and their signaling pathways and also emphasizes the role of quercetin in controlling oxidative stress and reducing the incidence of cardiovascular diseases.

**KEY WORDS:** Oxidative stress, free radicals, cardiovascular disease, atherosclerosis, signaling pathway, Quercetin.

### INTRODUCTION:

Cardiovascular disease (CVD) continues to be the leading cause of death for both men and women, accounting for approximately 40% of all annual deaths. Among the various cardiovascular diseases, coronary artery disease is the single largest killer. World Health Organization (WHO) estimates that 17 million people die of cardiovascular disease annually, of which 7.2 million deaths are attributable to coronary heart disease<sup>1</sup>. In some East, Central, and Southern Africa (ECSA) countries, such as Mauritius and Seychelles, CVD is the leading cause of mortality, responsible for more than one-third of deaths<sup>2</sup>. In Mauritius Death statistics over the past few years show that over 80 % of deaths are due to non communicable diseases. In 2001, 40% of deaths in people of working age were from diseases of the circulatory system, including hypertension however in 2005 approximately 51% of deaths were attributed to cardiovascular diseases<sup>3</sup>. Cardiovascular disease

is a combination of multifactorial risk factors including hypercholesterolemia, hypertension, smoking, diabetes, sedentary life style, consumption of high fat diet, family history of early MI and stress. This may be the result of acute ischemia-reperfusion injury, endothelial damage of hyperhomocysteinemia, as well as chronic oxidative damage secondary to lipid peroxidation<sup>4</sup>.

Over recent decades, a large body of evidence has accumulated indicating that free radicals play a critical role in cellular processes implicated in atherosclerosis. Free radicals are any reactive organic or inorganic molecules with one or more unpaired electrons, commonly formed in the body as a result of metabolic processes which are normally eliminated by the antioxidant line of defense system of the body<sup>5</sup>.

However the free radicals or reactive oxygen species (ROS) that are not destroyed can toxic because of their propensity to react with biological molecules such as lipids, proteins and

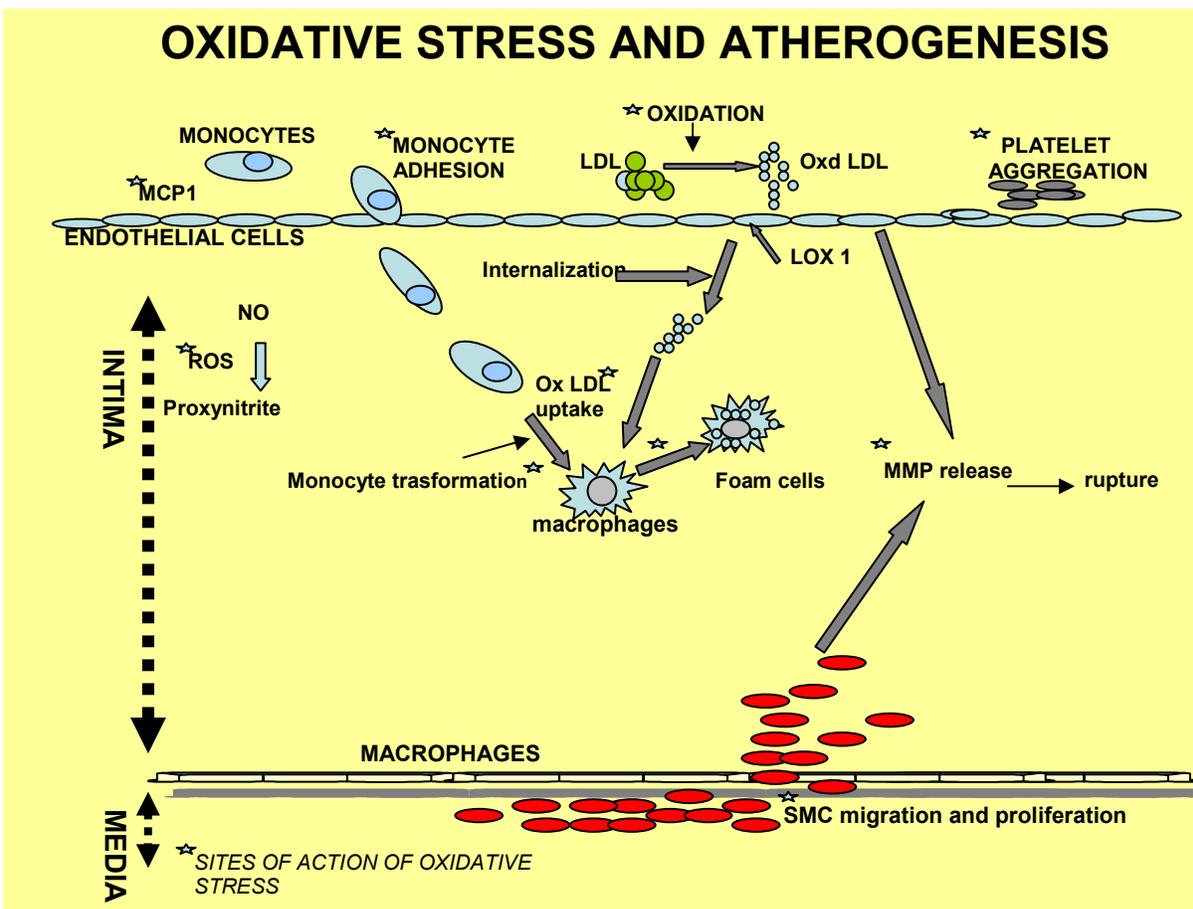
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DNA may cause oxidative-stress induced damage. Excessive production of ROS, outstripping endogenous antioxidant defense mechanisms, is referred to as oxidative stress<sup>6</sup>. Accumulated evidence has shown that reactive oxygen species are also the important mediators of cell signaling events such as inflammatory reactions (superoxides) and the maintenance of vascular tone (nitric oxide). However the overproduction of ROS such as superoxide has been associated with the pathogenesis of variety of diseases including cardiovascular diseases, diabetes, cancers, Alzheimer's disease, retinal degeneration, ischemic dementia and other neurodegenerative disorders and ageing<sup>7,8,9</sup>. Large numbers of studies have been conducted so far, showing the contributive role of quercetin to prevent cardiovascular diseases<sup>10-14</sup>. This

article will review the process of oxidative stress mediated atherogenesis and their signaling pathway through which they relate to the cardiovascular diseases. The role of various antioxidative therapies with more emphasis on "Quercetin"- a flavonoid will be discussed.

**OXIDATIVE STRESS AND ATHEROGENESIS:**

**Figure 1<sup>15</sup>** demonstrates various steps where oxidative stress could be involved in atherogenesis. In general, increased production of ROS may affect four fundamental mechanisms that contribute to atherosclerosis: *endothelial cell dysfunction, vascular smooth muscle cells (VSMC) growth, monocyte migration and oxidation of LDLs (ox LDL)*<sup>16,17</sup>.



**Figure 1<sup>15</sup>:** Oxidative stress affects four fundamental mechanisms that contribute to atherogenesis (I) oxidation of LDL to form ox -LDL (II) endothelial cell dysfunction (increased release of MCP-1, MMPs, increased expression of VCAM-1, ICAM-1 and LOX-1, decreased activity of NO, platelet aggregation) (III) vascular smooth muscle cells migration and proliferation (IV) monocyte adhesion and migration and foam cell development.

**Endothelial cell dysfunction:** Risk factors including hyperlipidemia, hypertension, diabetes, smoking, all are associated with the overproduction of ROS or increase oxidative stress. They are considered to be major factors of the pathogenesis of endothelial dysfunctions and markers of unfavorable cardiovascular prognosis<sup>18</sup>.

Endothelial dysfunction is associated with an increased risk of cardiovascular events. There have been at least eight studies involving over 1500 subjects and all indicate that endothelial dysfunction has prognostic value<sup>19-26</sup>. Endothelial dysfunction is characterized by reduction of the bioavailability of vasodilators, in particular nitric oxide (NO), whereas endothelin-derived contracting factors are increased<sup>27</sup>. This imbalance leads to the impairment of endothelium-dependant vasodilatation which represents the functional characteristics of endothelial dysfunctions. On the other hand, endothelial dysfunctions aside from denoting impaired endothelium dependant vasodilatation also comprises a specific state of endothelial activation, which is characterized by a proinflammatory, proliferative and procoagulatory milieu that favors all the stages of atherogenesis<sup>28</sup>.

**Vascular smooth muscle cells (VSMC) growth:** Proliferation of vascular smooth muscles is a characteristic feature of atherosclerosis and ROS can induce vascular SMC growth. The increase in SMC growth by ROS occurs as a result of stimulation of the expression of fibroblast growth factors (FGF) and fibroblast growth factor receptor-1 (FGFR-1), Insulin like growth factor-1 (IGF-1), Insulin like growth factor -1 receptor (IGF-1R) as well as epidermal growth factor receptor (EGFR)<sup>29-32</sup>. Also there is increasing evidence that ROS generation, via NADPH oxidase activation plays a critical role in Ang II-induced vascular SMC proliferation and hypertrophy<sup>33</sup>. Several studies showed that very large amount of ROS may even induce vascular SMC death by either apoptosis or necrosis<sup>34</sup>.

**Monocyte migration:** Increase adhesion of monocytes to endothelial cells has also been linked to the development and progression of atherosclerosis in humans. One study has shown that the effect of increased concentration of glucose (as in diabetes; important risk factor of CVD) on monocyte adhesion to endothelial cells

is due to increased production of ROS<sup>35</sup>. A number of studies have shown that ROS unregulated the expression of intracellular adhesion molecules-1 (ICAM-1)<sup>31</sup>, vascular cell adhesion molecule-1 (VCAM-1)<sup>36,37</sup>, monocyte chemoattractant protein-1 (MCP-1)<sup>38</sup>, P-selectin<sup>39</sup>, L-selectin<sup>40</sup>, E-selectin<sup>35</sup> and platelet endothelial cell adhesion molecule-1 (PECAM-1)<sup>41</sup> in vascular endothelial cells.

**Oxidation of LDLs (ox-LDL):** A large body of literature has confirmed a central proatherogenic role of ox-LDL in vascular cells<sup>42-45</sup>. Increasing evidence has shown that ox-LDL is not only a marker of oxidative stress, but itself can induce oxidative stress in vascular tissues<sup>46,47</sup>. It has been recently shown that ox-LDL induces proatherosclerotic NADPH oxidase expression and superoxide anion formation in human vascular endothelial cells<sup>48</sup>, and this may be one of the mechanism by which ox-LDL stimulates ROS generation and the resultant endothelial dysfunctions as well as atherosclerosis. Strong evidence in favor of pro-atherosclerotic role for ox-LDL comes from a number of studies demonstrating the noxious effects of ox-LDL on various components of arterial wall. For example ox-LDL causes activation of endothelial cell lining the arterial wall, resulting in the expression of several adhesion molecules that facilitate the adhesion of monocytes/macrophages<sup>42</sup>. Ox-LDL also activates inflammatory cells and facilitates the release of number of growth factors from monocytes/macrophages<sup>43,44</sup>. Vascular SMCs exhibit intense proliferation when exposed to ox-LDL<sup>42</sup> and also enhances the formation of matrix metalloproteinase (MMPs) in vascular endothelial cells and fibroblasts, thus setting the stage wherein oxidative stress leads to rupture of a soft plaque<sup>45</sup>. In addition ox-LDL up regulates the expression of endothelial receptors LOX-1 and other scavenger receptors mainly expressed on macrophage/monocyte. The increased expression of these receptors is responsible for the uptake of ox-LDL and the formation of foam cells, which is an early step in atherogenesis. Angiotensin II (Ang II) enhances the uptake of ox-LDL and biosynthesis of cholesterol in macrophages, leading to the formation of foam cells<sup>49,50</sup>. Angiotensin II up regulates LOX-1 gene and protein expression in cultured human coronary artery endothelial cells and enhances the noxious effects of ox-LDL<sup>49</sup>.

### **OXIDATIVE- STRESS MEDIATED SIGNALING PATHWAY:**

Although the observations discussed above implicate oxidative-stress induced ROS as a stimulus for the expression and release of several pro-atherogenic and inflammatory mediators, little is known regarding the specific intracellular signaling pathways by which ROS act<sup>50</sup>. It is increasingly suggested that signaling in response to pro-atherogenic agents requires ROS<sup>51</sup>. Pro-atherogenic agents comprise a large variety of molecules. For examples cytokines, including tumor necrosis factor- $\gamma$  (TNF- $\gamma$ ), interferon- $\gamma$  (IFN- $\gamma$ ), interleukin-1,6 (IL-1, IL-6), and angiotensin II (Ang II),<sup>52-54</sup> stimulate intracellular generation of ROS. High levels of low-density lipoprotein (LDL), especially in the form of oxidized low-density lipoprotein (ox-LDL), have also been shown to increase intracellular ROS generation<sup>55</sup>. In addition, growth factors, such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) as well as vascular endothelial growth factor (VEGF), and hormones, such as insulin, all greatly induce intracellular ROS generation<sup>56,57</sup>.

Some recent studies suggest that the function and activity of intracellular signals are regulated by ROS. These studies link the cellular oxidative state to specific mitogen-activated protein kinases (MAPKs), such as p38 kinase<sup>49</sup> and to extracellular signal-regulated kinase 1/2 (ERK 1/2),<sup>56</sup> as well as the cJun N-terminal kinase (JNK)<sup>57</sup> signaling pathway. MAP kinase activation regulates cell differentiation, growth and apoptosis through phosphorylation of downstream target proteins and activation of transcription factors. Ang II and endothelin-1 are other activators of MAP kinases in VSMC and are believed to cause vascular remodeling<sup>58,59</sup>. Interestingly, Ang II potently activates ERK1/2 and p38; however, only p38 is sensitive to both inhibition of NADH/NADPH oxidase activity and catalase overexpression in VSMC<sup>60,61</sup>. Vasoactive peptide endothelin-1 stimulated proliferation of human coronary artery smooth muscle cells occurs through the activation of ERK1/2 and transcription factor activator protein-1 (AP-1)<sup>59</sup>. It was also reported that MAP kinase mediated the increase in LOX-1 expression in endothelial cells<sup>62</sup>.

Although MAPK plays a central role in ROS-mediated signal transduction in cardiovascular diseases, other protein kinases are also involved in ROS-mediated changes in vascular cells<sup>63</sup>. Protein tyrosine kinases are among the

intracellular mediators that may be rapidly activated by many stimuli including ROS. Several tyrosine kinases are activated by ROS, and the Src family of kinases has been most frequently described<sup>63</sup>.

Phospholipases may also be activated by ROS<sup>63</sup>. At least three important phospholipases have been shown to be activated by ROS in vascular cells, including phospholipase A<sub>2</sub>,<sup>64</sup> phospholipase C<sup>65</sup> and phospholipase D<sup>66</sup>. Phospholipase A<sub>2</sub> is similarly activated by increase in intracellular calcium caused by ROS. It is important to note that many lipids generated by phospholipase A<sub>2</sub> may themselves generate ROS through the action of monooxygenases. Phospholipase C is a calcium dependent phospholipase that hydrolyzes PIP<sub>2</sub> to generate IP<sub>3</sub> and diacylglycerol. Although there are no published findings to suggest that ROS directly activate phospholipase C, generation of ROS may be an early event in growth factor-dependent signaling and activation of phospholipase C. Phospholipase D has been shown to be stimulated by H<sub>2</sub>O<sub>2</sub>, fatty acid hydroperoxides, and 4-hydroxynonenal in endothelial cells. Further studies are required to elucidate the mechanisms by which phospholipases are activated<sup>63</sup>.

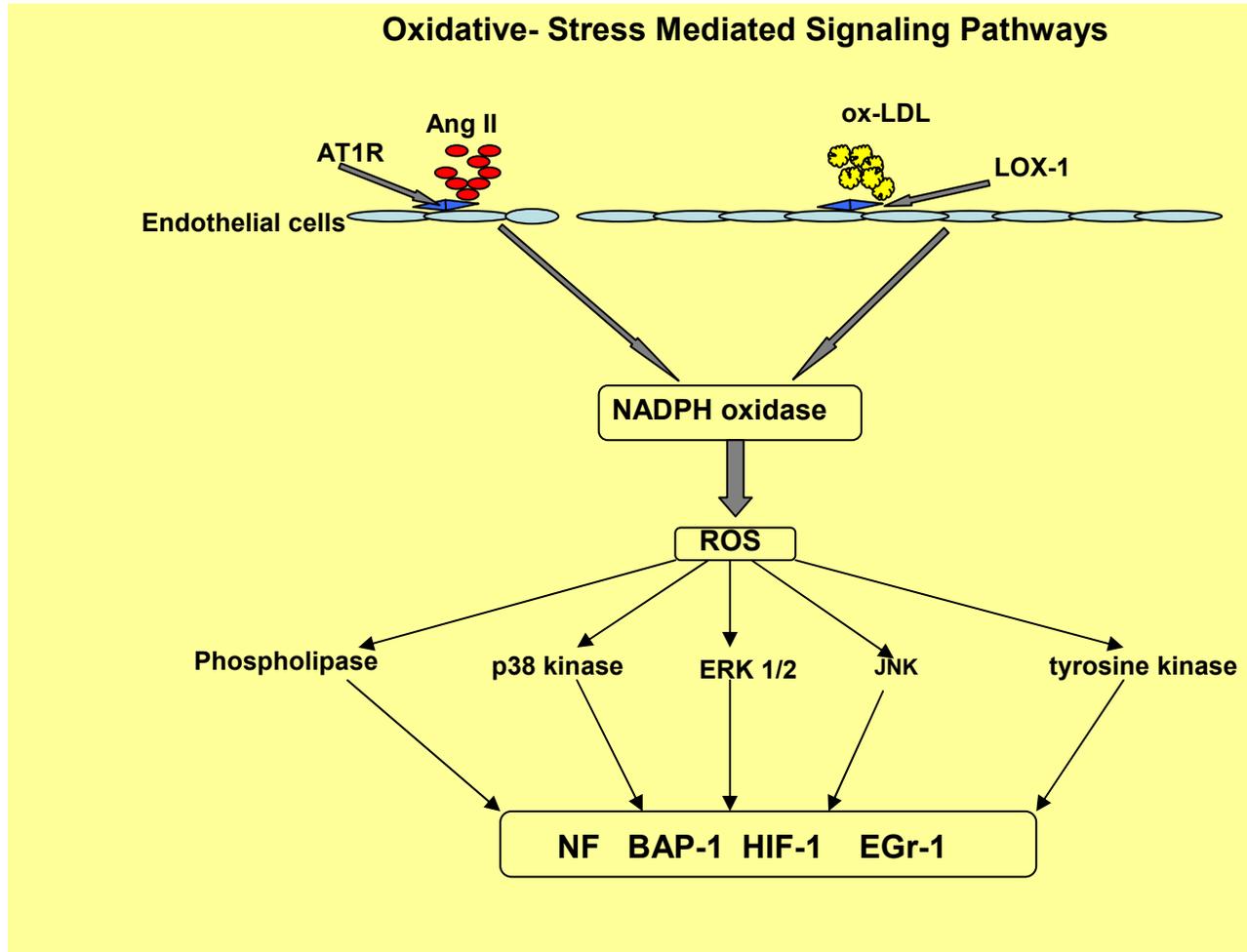
In addition to regulating enzyme activity, ROS participate in signal transduction by generating classic second-messengers (calcium and lipid mediators) that transmit signals to intracellular mediators in both the cytoplasm and nucleus<sup>63</sup>. Other important mediators of the ROS-sensitive signaling pathway are small G proteins. Based on experiments with recombinant p21 Ras *in vitro*, it was shown that ROS directly promote guanine nucleotide exchange on p21 Ras<sup>67</sup>. This leads to an increased population of active p21 Ras-GTP, resulting in increased availability of p21 Ras to its downstream targets<sup>68</sup>.

The final step in the ROS-mediated signaling pathway usually involves the activation of transcription factors, which are proteins that are transported to the nucleus upon activation and trigger target gene expression. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is an inducible transcription factor that is a likely target for ROS signal transduction. The recognition sequence for NF- $\kappa$ B is present in genes, such as E-selectin, VCAM-1 and ICAM-1, whose expression causes monocyte adhesion to endothelial cells.<sup>69</sup>

In addition to NF- $\kappa$ B, activator protein-1 (AP-1) also appears to be activated by ROS. Regulation of AP-1 by ROS has been shown to involve p38 MAPK and ERK1/2 as well as JNK in vascular

SMCs<sup>70</sup>. Other transcription factors such as hypoxia-inducible factor-1 (HIF-1) and early growth response-1 (Egr-1) may also be involved in ROS-mediated pro-atherogenic events in

vascular SMCs and endothelial cells<sup>71,72</sup>. **Figure 2<sup>15</sup>** outlines the ox-LDL and Ang II-induced ROS signaling pathways.



**Figure 2<sup>15</sup>:** Both Ang II and ox-LDL may induce oxidative stress in endothelial cells by activating their specific receptors: AT1R and LOX-1. The increased ROS, produced from NADPH oxidase, may activate phospholipases (PLA2, PLC and PLD), mitogen- activated protein kinases (p38 kinase, ERK1/2 and JNK), and tyrosine kinase leading to the activation of transcription factors such as NF-κB, AP-1, HIF-1 and Egr-1, resulting in the expression of target genes including leucocyte adhesion molecules, metalloproteinases and AT1R as well as LOX-1.

**ANTIOXIDATIVE THERAPIES:**

Given that oxidative stress plays a pivotal role in the pathogenesis of cardiovascular diseases, it is not surprising that antioxidant therapies are one of the most effective and promising strategies against atherogenesis. To date, there are at least five groups of anti-atherosclerotic agents that have exhibited anti-oxidant effects, albeit to

varying degrees<sup>73</sup> (1) probucol; (2) HMG-CoA reductase inhibitors; (3) AT1R blockers and ACE inhibitors; (4) peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands; (5) vitamins C, E and Bioflavonoids.

**Probucol** is a modestly potent LDL-lowering agent with powerful anti-oxidant properties that

effectively inhibits the oxidative modification of LDL independent of its lipid lowering effect and also inhibits VCAM-1 and MCP-1 expression and inhibits human aortic SMC proliferation as well as atherogenesis<sup>74</sup>.

**HMG-CoA reductase** inhibitors, also known as statins, are potent lipid-modifying agents. There is overwhelming evidence from clinical studies that reducing plasma LDL levels with statins results in a markedly lower risk of cardiovascular events related to atherosclerosis<sup>75</sup>. Recent studies in patients with established CAD show that these agents can cause a modest regression of atherosclerotic lesions. It has been suggested that the anti-atherosclerotic effect of statins may be independent of their LDL-lowering effect, among which is their ability to decrease ROS generation.<sup>76, 77</sup>

**AT1R blockers and ACE inhibitors** are widely used to treat patients with hypertension and/or congestive heart failure by blocking the effect of Ang II or its formation. Recent studies show that Ang II is also a strong stimulus for ROS generation and AT1R blockers as well as ACE inhibitors inhibit the expression of pro-atherogenic factors by decreasing ROS production in vascular endothelial cells and in animal models.<sup>78, 79</sup>

**Peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) ligands**, a member of the nuclear receptor super-family, were initially developed to affect glucose and lipid metabolism. These agents, such as rosiglitazone and pioglitazone, are widely used in the treatment of type II diabetes. Recently, PPAR- $\gamma$  ligands have been identified as potent anti-oxidants.<sup>80</sup> By suppressing NADPH oxidase expression and reducing intracellular ROS production, these agents inhibit the expression of several proatherogenic proteins and apoptosis in vascular endothelial cells and SMCs<sup>81, 82</sup>. Experimental studies show that thiazolidinedione, a potent PPAR- $\gamma$  ligand, reduces the size and number of atherosclerotic lesions in the vessel wall by modulating foam cell formation and inflammatory responses of macrophages<sup>83</sup>. A recent study<sup>84</sup> shows a significant vascular protective effect of PPAR- $\gamma$  ligand rosiglitazone in hypercholesterolemic rabbits, most likely due to the attenuation of oxidative stress. This endothelial protective effect of rosiglitazone may reduce leukocyte accumulation in vascular walls and contribute to its antiatherosclerotic effect.

The other ligand, pioglitazone, decreases TNF- $\alpha$ -mediated apoptosis in human coronary artery endothelial cells by reducing ROS formation<sup>85</sup>. Thus, PPAR- $\gamma$  ligands have the potential for the treatment of atherosclerosis and type II diabetes<sup>86</sup>.

**Vitamins E and C** have been demonstrated to reduce the progression of atherosclerosis in animal models<sup>87, 88</sup> but this effect is not consistently observed. Clinical studies show that while these anti-oxidant vitamins do not reduce endpoints related to atherosclerosis, they improve endothelial function by increasing local NO bioavailability and, therefore, endothelium-dependent vasodilation.<sup>89</sup>

**Bioflavonoids**, which are polyphenolic compounds, are believed to be beneficial for the prevention and treatment of atherosclerosis and cardiovascular diseases. Recently, much interest in flavonoids has been generated from the findings of the **French paradox**<sup>90</sup>. This paradox refers to the correlation of a high-fat and high-cholesterol diet with a lower incidence of coronary heart disease found in Mediterranean cultures. It has been shown that the French paradox may be attributable to regular consumption of red wine and that the unique anti-atherogenic effects of red wine reside in the action of polyphenols. Alongside the French paradox, the REGICOR Study has shown another paradox in the Mediterranean area: a high prevalence of cardiovascular risk factors with low incidence of myocardial infarction in the population of Girona, Spain. The antioxidant effects associated with olive oil consumption could explain part of this **Mediterranean Paradox**<sup>91, 92</sup>. Quercetin-derived compound hydroxytyrosol possesses high antioxidant property, decreases the susceptibility of LDL to oxidation<sup>93, 94, 95</sup>.

Many others epidemiological studies also suggest that flavonoid-rich diets high in fruits and/or vegetables reduce the risk of coronary heart disease<sup>96, 97, 98</sup>. Based on a review of more than 250 observational studies fruits and vegetables were reported to be important source of flavonoids, beneficial in the prevention of cancer and cardiovascular disease<sup>99</sup>. Cardiovascular deaths were reduced by 16% (8,000 deaths annually) by increasing the current 250 g of fruits and vegetables per day to 400 g per day in the Dutch general population<sup>99</sup>. In the Zutphen Elderly Study, researchers evaluated the diets of 805 Danish men between the ages of 65 and 84. The subjects were then

followed up for five years. It was found that the men consuming highest flavonoid levels were slightly less than half as likely to have suffered a heart attack during the course of the study<sup>100</sup>. A recent meta-analysis of seven prospective cohort studies with 105,000 individuals indicated that high dietary intake of flavonoids from a small number of fruits and vegetables, tea and red wine are inversely associated with coronary heart disease risk<sup>101</sup>.

Bioflavonoids occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described, and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids<sup>102</sup>.

Quercetin 3, 3', 4', 5, 7-Penta-hydroxyflavon is one of the most widely distributed bioflavonoids (flavonol), which are abundant in red wine, tea, and onions. This is the most abundant flavonol in the diet which possesses biological activities such as antioxidative<sup>103,104</sup>, anticarcinogenic<sup>105,106</sup> and enzyme-inhibiting activities<sup>107,108,109</sup>. However numerous other in vitro studies have revealed diverse biological effects of quercetin, including apoptosis induction, antimutagenesis, protein kinase C (PKC) inhibition, lipoxygenase inhibition, histamine-release inhibition, superoxide dismutase (SOD)-like activity, modulation of cell cycles, angiogenesis inhibition, and inhibition of angiotensin converting enzyme<sup>110</sup>.

It is noteworthy here that quercetin exists rarely as aglycon in foods and plants. It mainly occurs in glycosylated forms and the associated sugar moiety is very often either glucose or rhamnose. However other sugars may also be involved like galactose, arabinose, xylose, or glucuronic acid. Quercetin-3-rutinoside and quercetin-4'-glucoside are the important forms of quercetin in food. Quercetin-3-rutinoside accounts for ~40% of quercetin in black tea<sup>111</sup>, however quercetin-4'-glucoside accounts for ~45% of quercetin in onions<sup>112</sup>. More importantly it is the sugar moiety in quercetin glycosides which maximally affects the bioavailability in humans like the bioavailability of quercetin-3-rutinoside is only 20% of that of quercetin-4'-glucoside<sup>113</sup>. The bioavailability of quercetin-3-glucoside is similar to that of quercetin-4'-glucoside but the glucoside form is more readily absorbed than glycoside forms<sup>113,114</sup>. The mechanism for quercetin absorption is not known. However Hollman et al<sup>113,115</sup> speculated that the intestinal sodium-glucose co-transporter is able to transport glucose attached to quercetin through the intestinal cell. With regard to bioactivity of various forms,

quercetin conjugated with glycosides, glucuronic acid or sulfates also has antioxidant activity in vitro, although the antioxidant activity is lower than that of the quercetin aglycone<sup>116,117</sup>. Fifty percent of ingested quercetin glucosides, is absorbed in the small intestine and subsequently metabolized into isorhamnetin, in the liver and in other organs. The 50% of ingested quercetin which is not absorbed in the small intestine is metabolized by the colonic microflora into quercetin aglycone and phenolic acids which might be absorbed from the colon<sup>116,117</sup>. Only 3% of the ingested quercetin is recovered in urine as aglycone or its conjugates. Metabolites of quercetin may also be biologically important, because they also have antioxidant activity in vitro<sup>117</sup> and might exert antioxidant effects in humans.

### QUERCETIN ACTION ON CARDIOVASCULAR DISEASES:

**Antioxidative action:** Oxidized low density lipoproteins are atherogenic, and are considered to be a crucial intermediate in the formation of atherosclerotic plaques. Oxidized low density lipoproteins cholesterol (LDL cholesterol) is taken up more readily by macrophages, which leads to the formation of foam cells and atherosclerotic plaque<sup>118</sup>. Oxidised LDL has several properties that promote atherogenesis, apart from its rapid uptake into macrophages via the scavenger receptor. An oxidised form of LDL is chemotactic for circulating macrophages and smooth muscle cells and facilitate monocyte adhesion to the endothelium and entry into the subendothelial space and inhibits the release of nitric oxide and the resulting endothelium dependent vasodilation<sup>119,120</sup>. Oxidised LDL can induce arterial wall cells to produce chemotactic factors, adhesion molecules, cytokines, and growth factors that have a role to play in the development of the plaque<sup>121, 122</sup>. Mechanisms that prevent or slow down this chain of events may decrease the risk of coronary heart diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Among all the flavonoids quercetin is the most potent antioxidant whose high reactivity could be associated with the presence of the OH group in B- and the C-ring<sup>123</sup>. **Sim et al** investigated the structure-activity relationship of flavonoid compounds on their antioxidant property and inhibitory effects

against the matrix metalloproteinases (MMP) activity. The effects of several flavonoids; myricetin, quercetin, kaempferol, luteolin, apigenin and chrysin, on the reactive oxygen species scavenging activity and inhibitory effect against the MMP activity were examined. The antioxidant property of quercetin was found to be more than Kaempferol which was correlated with the respective number of OH group on their B-ring<sup>124</sup>.

Infact **Silva et al**<sup>125</sup> has shown that quercetin is an even stronger antioxidant than ascorbic acid, alpha-tocopherol, and flavones. However in another study the antioxidant abilities of several polyphenols were determined, and quercetin was most active among all<sup>126</sup>.

Other studies have also demonstrated that quercetin inhibits lipid peroxidation effectively by scavenging free radicals and/or chelating transition metal ions<sup>127</sup> including superoxide anions<sup>128</sup> singlet oxygen<sup>129</sup> and lipid peroxy-radicals<sup>130</sup>. Quercetin inhibits the iron ion-dependent lipid peroxidation systems by chelating iron ions with the formation of inert iron complexes. Quercetin is able to suppress free radical processes at three stages: the formation of superoxide ion, the generation of hydroxyl radicals in the Fenton reaction and the formation of lipid peroxy radicals<sup>131</sup>.

**Antiplatelets action:** Platelets are involved in atherosclerotic disease development and the reduction of platelet activity by medication reduces the incidence and severity of the disease. Platelet aggregation contributes to both the development of atherosclerosis and acute platelet thrombus formation. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals, which inhibit the endothelial formation of prostacyclin and nitrous oxide. However quercetin directly scavenges free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide<sup>132</sup>. An experimental study in dogs and monkeys showed quercetin to be the most effective inhibitor of platelet aggregation.<sup>133</sup>

In one of the randomized cross-over trials in human designs, a comparative study was done with ten healthy human subjects to evaluate the efficacy of purple grape fruit juice, orange juice or grapefruit on the platelet aggregation. The purple grape juice, which is rich in quercetin content was found to be the potent platelet inhibitor in healthy subjects while the citrus juices showed no effect<sup>134</sup>. Studies have shown that consumption of quercetin inhibits *in vivo*

platelet activation, but the underlying mechanism is still unknown. Since collagen-induced platelet aggregation is associated with a burst of hydrogen peroxide, which in turn contributes to stimulating the phospholipase C pathway, one of the mechanisms could be the inhibition of collagen-induced hydrogen peroxide production, calcium mobilization, and 1, 3, 4-inositol triphosphate formation<sup>135</sup>.

Quercetin interferes with many steps in eicosanoid metabolism. It inhibits phospholipase A2 activity<sup>136</sup> and blocks both the LOX<sup>137</sup> and COX pathways of AA metabolism<sup>138</sup>. At micromolar concentrations ( $\leq 15-40 \mu\text{M}$ ), quercetin inhibits COX-2 and LOX-5 activity<sup>139</sup>. Quercetin decreases platelet aggregation, increases platelet-derived NO release, and decreases superoxide production. In another study semi-synthesized quercetin derivatives--disodium Quercetin-7,4'-disulfate(DQD) inhibited pig platelet aggregation induced by thrombin. Its mechanism was attributed to its inhibitory effect on Ca<sup>2+</sup> influx, intracellular Ca<sup>2+</sup> mobilization, Ca<sup>2+</sup>/PL dependent PKC activity, and actin polymerization<sup>140</sup>. Previously, platelets were considered to be the main cell type involved in the pathogenesis of thrombosis, whereas only a minor role was attributed to leucocytes. However, many investigators recently realized that thrombosis involves multiple cells and the cell interactions further complicating this process<sup>141</sup>. The interaction of platelet and leukocyte may be one of the key factors in the development of thrombotic diseases. Recently quercetin was reported to inhibit leukocyte aggregation and platelet-neutrophil adhesion with higher efficacy than aspirin. It is suggested that quercetin may be developed as an antithrombotic agent<sup>142,143</sup>. However in a double-blind study<sup>144</sup>, healthy men and women were assigned to either a quercetin-supplemented (1 g/d) group or a control group for 28 d. Plasma quercetin was 23-fold higher after quercetin supplementation but did not alter any of the thrombogenic risk factors. Similar observations were made in another randomized crossover study in which volunteers received 220 g onions/d (114 mg quercetin) as one of the treatments for 7 days<sup>145</sup>. *In vitro*, quercetin inhibits platelet aggregation<sup>146</sup> but the levels used ranged from 20 to 500 mmol/L of quercetin, which is several hundred times higher than the plasma levels reached in the human studies. Therefore, it is possible that the inhibitory effect of quercetin on platelet aggregation may require a certain minimum

concentration in the plasma. It is also likely that quercetin elicits its cardioprotective effects via mechanisms other than thrombosis.

**Inhibition of smooth muscle cell proliferation and migration:** It is now apparent that ROS induce endothelial cell damage and VSMC growth which are responsible for cardiovascular remodeling, hypertension, atherosclerosis, heart failure, and restenosis<sup>59,147,148</sup>.

Several lines of evidence indicate that ROS and mitogen-activated protein (MAP) kinases were involved in vascular remodeling under various pathological conditions. Recently, it was also reported that MAP kinases were sensitive to oxidative stress. MAP kinases play an important role in cell differentiation, growth, apoptosis, and the regulation of a variety of transcription factors and gene expressions. Ang II and endothelin-1 are the activators of MAP kinases in VSMC and are believed to cause vascular remodeling. Quercetin inhibited endothelin-1-induced proliferation of VSMC via the inhibition of JNK and p38, thereby suggesting the important role of ROS in MAP kinase activation and MAP kinase-mediated VSMC proliferation by endothelin<sup>70</sup>. During the formation of atherosclerotic lesions, the smooth muscle cells that line the coronary arteries multiply in number and begin to migrate into the interior of those vessels. When human aortic muscle cells are exposed to quercetin, this action is inhibited in a dose-dependent manner. Quercetin works by inhibiting the Mitogen-Activated Protein (MAP) kinase signaling pathway associated with smooth muscle cell migration. In another study, Japanese researchers found that quercetin inhibited the vascular smooth muscle cell hypertrophy seen during the development of coronary artery disease. They hypothesized that quercetin works by inhibiting the activation of a key MAP kinase called JNK(c-Jun N-terminal kinase) by the angiotensin II enzyme. This in turn inhibits the protein synthesis necessary for proliferation and hypertrophy of smooth muscle cells<sup>149,150</sup>.

Quercetin is known to inhibit angiotensin II-induced hypertrophy and serum-induced smooth muscle cell proliferation. However, it is not known whether quercetin exerts similar cardioprotective effects in cells treated with TNF-alpha. Moon et al<sup>151</sup> investigated whether quercetin exerts the multiple suppressive effects on cytokine TNF-alpha-induced human aortic smooth muscle cells (HASMC) or not. Treatment of quercetin showed potent inhibitory effects on the DNA synthesis of cultured

HASMC in the presence of TNF-alpha. These inhibitory effects were associated with reduced extracellular signal-regulated kinase (ERK)1/2 activity and G1 cell-cycle arrest. Treatment of quercetin, which induced a cell-cycle block in G1-phase, induced down-regulation of cyclins and CDKs and up-regulation of the CDK inhibitor p21 expression. Quercetin inhibited TNF-alpha-induced MMP-9 secretion on HASMC in a dose-dependent manner. This inhibition was characterized by down-regulation of MMP-9, which was transcriptionally regulated at NF-kappa B site and activation protein-1 (AP-1) site in the Matrix metalloproteinase-9 (MMP-9 promoter). These findings indicate the efficacy of quercetin in inhibiting cell proliferation, G1- to S-phase cell-cycle progress, and MMP-9 expression through the transcription factors NF-kappaB and AP-1 on TNF-alpha-induced HASMC<sup>151</sup>.

**Anti-hypertensive action:** Spanish researchers evaluated the antihypertensive effects of quercetin in an animal model of essential hypertension. Ten milligram per kilogram (10mg/kg) of quercetin given orally to spontaneously hypertensive rats for five weeks reduced systolic blood pressure by 18%, diastolic blood pressure by 23%, and mean arterial blood pressure by 21%. However quercetin had no effect on the normotensive rats. Quercetin was also found to decrease cardiac and renal hypertrophy, both of which follow hypertension and can lead to heart and kidney failure if left unchecked<sup>141</sup>. Quercetin shows vasodilator effects in isolated aortae stimulated with noradrenaline, KCl or phorbol esters and these effects are independent of the presence of endothelium. Thus, this direct vasodilator effect might contribute to its antihypertensive effects<sup>153,154</sup>. These effects were associated with a reduced oxidant status due to the antioxidant properties of the drug.

In another study quercetin (10 mg/kg) shows both antihypertensive and antioxidant properties in the deoxycorticosterone acetate-salt-hypertensive rats model, where verapamil (20 mg/kg/day) exhibits only antihypertensive effects. In this study, quercetin and verapamil treatments reduced systolic blood pressure of DOCA-salt rats in approximately 67.6 and 63.3% respectively. Both drugs reduced significantly hepatic and renal hypertrophy induced by DOCA-salt administration, while only quercetin prevented cardiac hypertrophy<sup>155</sup>.

In another five weeks experimental study<sup>156</sup>, quercetin treatment reduced systolic blood pressure of Goldblatt (GB) hypertensive rats. It also reduced cardiac hypertrophy and proteinuria developed in GB hypertensive rats. Normalisation of plasma nitrates plus nitrites (NOx), thiobarbituric acid reactive substances (TBARS) concentrations and improvement of the antioxidant defences system in liver were also found to be accompanied with the antihypertensive effect of quercetin. Thus chronic oral treatment with quercetin shows both antihypertensive and antioxidant effects in this model of renovascular hypertension.

#### **Support of mitochondrial function in cardiac cells:**

Inadequate blood supply to a region of the body for a certain period followed by the resumption of blood flow is termed Ischemia-reperfusion. Ischemia-reperfusion results in varying degrees of tissue damage depending on the duration and extent of the hypoperfusion. Myocardial damage induced by ischemia-reperfusion is due, at least in part, to the generation of ROS<sup>149,150</sup>. There have been reports showing a close correlation between the production of ROS and simultaneous consumption of endogenous antioxidants<sup>151,152</sup>. Indirect evidence consistent with this view is the cardioprotective effects of free radical scavengers and antioxidant supplements<sup>161,162,163</sup>. New research finds that rats given oral low dose of quercetin significantly protected against the injury that normally occurs during ischemia and reperfusion<sup>164</sup>. It has been established that mitochondria play a critical role in myocardial recovery from ischemia-reperfusion (I-R) damage, and *in vitro* experiments indicate that quercetin can exert a variety of direct effects on mitochondrial function. Quercetin treatment significantly decreased the impairment of cardiac function following I-R. This protective effect was associated with improved mitochondrial function after I-R. These results indicate that oral low dose quercetin is cardioprotective, possibly via a mechanism involving protection of mitochondrial function during I-R<sup>165</sup>. In another experimentally induced myocardial infarction in dogs by 60-minute occlusion of the coronary artery with the subsequent 24-hour reperfusion, the administration of quercetin solution (50 mg/kg) was found to improve the contractile function of the left ventricular myocardium, decrease the incidence of heart rate and conductivity disorders, limit the ischemic damage area, promote the preservation of the

vessels' integrity, improve the coronary circulation and the prevent of intravascular thrombus formation<sup>166</sup>. Quercetin may also modulate ion channels, and possess structural similarities to several antiarrhythmic voltage-gated sodium channels (VGSC) inhibitors. By inhibiting cardiac voltage-gated sodium channels (VGSC), quercetin may contribute to cardioprotective and antiarrhythmic effects<sup>167</sup>.

**Inhibition of NF-kappa B:** The inflammatory mediator nuclear factor-kappa B (NF-kB), has gathered considerable attention in research circles because of its role in heart disease, kidney disease and other age-related degenerative disorders. One eye-opening study in mice compared the expression of NF-kB in regions of the coronary arteries that are more prone and less prone to the development of atherosclerotic plaques. They found that the NF-kB pathway is primed for activation in the atherosclerosis-prone arterial regions. A high-cholesterol diet activated NF-kB in precisely those regions, and increased expression of genes regulated by NF-kB which are involved in plaque formation and pathology<sup>168</sup>. Some previous data have indicated that quercetin has antiinflammatory property and a possible anti-inflammatory role of quercetin could be related to an interference with the NF-kB signaling pathway, which regulates the expression of various genes involved in inflammation. Recently **Madhvan et al**<sup>169</sup> demonstrated the inhibitory effects of quercetin on NF- $\kappa$ B1 gene modulation. Their data suggested that the inhibitory effects of quercetin on TNF- $\alpha$  production may be mediated by down regulation of NF- $\kappa$ B1 gene expression.

#### **Reducing cardiovascular inflammation:**

Atherosclerosis is more and more being recognized as a chronic inflammatory process associated with enhanced serum levels of inflammation parameters, including in particular C-reactive protein. Cells found in early atherosclerotic lesions are typically inflammatory cells (monocytes/macrophages and T-lymphocytes); and, there is convincing clinical and experimental evidence that formation of reactive oxygen species (ROS) is augmented during this chronic inflammatory process due to an imbalance between synthesis of ROS and neutralizing antioxidative defense mechanisms<sup>170</sup>. Thus the production of reactive oxygen species under pathological conditions may represent an important inflammatory trigger<sup>171</sup>. Inflammation not only promotes

initiation and progression of atherosclerosis but also causes acute thrombotic complications of atherosclerosis. Novel therapeutic strategies specifically targeting inflammation thus bear great potential for the prevention and treatment of atherosclerotic vascular disease. Secretions from mast cells play a central role in the development of inflammatory disorders, and recent research implicates them in cardiovascular inflammation, particularly following stress. Cardiovascular inflammation is now recognized as a key factor in coronary artery disease. Using a new and particularly sensitive blood test, known as high-sensitivity CRP, **Ridker**<sup>172</sup>, has found that elevated levels of inflammation increase the risk of a heart attack by four and one-half times. That strong association makes CRP a far more accurate predictor of heart-attack risk than either cholesterol or homocysteine. Many studies have shown quercetin to inhibit mast cell secretion of inflammatory factors such as histamine, leukotrienes and prostaglandin D2<sup>173</sup>. Several specific flavonoids may be helpful in reducing inflammation, specifically quercetin in a dose of 300-500 mg daily.

Quercetin obtained from numerous dietary sources like onions, apples, fruit juices, and tea is found to be a strong inhibitor of both COX-2 and 5-LOX enzymes involved in the production of the eicosanoids from arachidonic acid<sup>138</sup>. Thus the antioxidant property of Quercetin helps to neutralize free radicals, which promote inflammation.

#### CONCLUSION:

Reactive oxygen species (ROS) contribute to the pathogenesis of cardiovascular diseases including hypertension, atherosclerosis, cardiac hypertrophy and heart failure. Oxidative stress results from excessive generation of ROS that outstrips the antioxidant system. By activating several signal transduction pathways, oxidative stress exerts noxious effects on cells of the vessel wall and leads to the initiation and progression of atherosclerosis culminating in acute events, such as acute coronary syndrome and stroke. Inhibition of ROS generation and function is a potential therapy to attenuate the extent of various cardiovascular diseases.

The hypothesis that dietary antioxidant quercetin lowers the risk of chronic disease has been developed from epidemiologic studies that consistently show that the consumption of whole foods, such as fruit and vegetables, is strongly associated with reduced risk of cardiovascular diseases. Therefore, it is reasonable for scientists

to identify quercetin as a “**magic bullet**”. Most of the cardioprotective actions of quercetin are thought to be due to its antioxidant properties such as metal chelators, free radical scavengers, and chain breaking antioxidants. Even though, one should recognize the complexity of interactions between pro-oxidants and antioxidants. A compound may act as an antioxidant in one oxidative challenge system and be neutral or even pro-oxidant in another, as seen in a study made by **Myara et al**<sup>174</sup>, who demonstrated that quercetin can sequester and reduce the activity of oxidants inducing metals such as iron and copper. But with an anion exchange chromatographic method for testing potential inhibitors of low density lipoprotein oxidation, quercetin was found to have pro-oxidant activity. Altogether, the available evidence indicates that quercetin might be of therapeutic benefit in cardiovascular diseases even though prospective controlled clinical studies are still lacking

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