Quercetin, a member of the flavonoids family, is one of the most prominent dietary antioxidants. It is ubiquitously present in foods including vegetables, fruit, tea and wine as well as countless food supplements and is claimed to exert beneficial health effects. This includes protection against various diseases such as osteoporosis, certain forms of cancer, pulmonary and cardiovascular diseases but also against aging. Especially the ability of quercetin to scavenge highly reactive species such as peroxynitrite and the hydroxyl radical is suggested to be involved in these possible beneficial health effects. Consequently, numerous studies have been performed to gather scientific evidence for these beneficial health claims as well as data regarding the exact mechanism of action and possible toxicological aspects of this flavonoid. The purpose of this review is to evaluate these studies in order to elucidate the possible health-beneficial effects of the antioxidant quercetin. Firstly, the definitions as well as the most important aspects regarding free radicals, antioxidants and oxidative stress will be discussed as background information. Subsequently, the mechanism by which quercetin may operate as an antioxidant (tested in vitro) as well as the potential use of this antioxidant as a nutraceutical (tested both ex vivo and in vivo) will be discussed.

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1. Free radicals and reactive species

1.1. Origin and terminology

Free radicals are reactive molecules due to the presence of one or more unpaired electron(s). They are formed in the human body either as an essential mediator in vital processes including neurotransmission and inflammatory reactions, or as a byproduct that does not have a role in the actual process. In aerobic life forms, the reduction of oxygen is of special interest. This reduction comprises binding of most of the oxygen to hydrogen to give water, a process involved in the oxidative phosphorylation. However, a small part of the oxygen (approximately 1–3%) is only partly reduced during this redox reaction (Magder, 2006). As a result, free radicals or other reactive species, that can either oxidize other compounds or easily form radicals, will arise. These partly reduced forms of oxygen are collectively described as reactive oxygen species (ROS). Similarly, reactive nitrogen species (RNS) are continuously produced. Physiologically important ROS include singlet oxygen (1O2), superoxide (O2−), hydrogen peroxide (H2O2), hydroxyl radical (·OH), ozone (O3), and hypochlorous acid (HOCl). Examples of physiological important RNS are nitric oxide (NO−) and peroxynitrite (ONOO−). An overview of reactive oxygen and nitrogen species is given in Table 1 (Kohen and Nyska, 2002).

It should be noted that not all ROS and RNS are equally reactive. Some compounds, such as H2O2, O2− and NO− react in vivo relatively selectively with only a few biological molecules, whereas for example the radical ·OH is a very reactive ROS that will react instantaneously with virtually any molecule it encounters (Galli et al., 2005). The reactivity of the other ROS and RNS lies in between these extremes (Halliwell, 1994, 1996a; Sies, 1997). Another difference between reactive oxygen species and reactive nitrogen species is the site of their reactivity; free radicals will react almost instantaneously at the site of their formation, whereas non-radical ROS such as H2O2 might also pass biological membranes and in that way spread their reactivity and possible toxicity.

1.2. Beneficial effects

As mentioned above, in the human body ROS and RNS are produced that display several crucial physiological functions, including smooth muscle relaxation, metabolism of xenobiotics and the respiratory burst to kill invading micro-organisms (Baldridge and Gerard, 1993; Bast, 1986; Bast et al., 1991; Moncada et al., 1991).

Smooth muscle vasodilatation depends on the release of a relaxing factor from the endothelium of blood vessels. This factor, initially referred to as the endothelium-derived relaxing factor (EDRF), appeared to be the nitric oxide radical (NO−) (Moncada et al., 1989). This radical is produced by nitric oxide synthases (NOS) out of l-arginine. NO− will activate guanylate cyclase that forms cyclic guanosine monophosphate (cGMP), which may lead to muscle relaxation (Arnold et al., 1977; Miki et al., 1977; Moncada et al., 1991). Muscle relaxation can be stopped by the reaction of NO− with O2−, that is formed locally in the endothelium of the blood vessels (Gryglewski et al., 1986).

Numerous compounds are metabolized in the liver into more polar compounds by cytochrome P-450 (Bast, 1986). The reactive oxygen generated in the active site of this enzyme can oxidize virtually any endogenous compound or xenobiotic, including chemically very inert compounds as benzene (Gilette et al., 1957; Bast, 1986).

During infections, ROS such as O2− and HOCl are generated as lethal weapon to kill invading micro-organisms. The explosive production of ROS by phagocytic leukocytes (i.e. neutrophils, eosinophils, monocytes and macrophages) is called the respiratory burst (Baldridge and Gerard, 1933; Bast, 1984). Important enzymes that are involved in this inflammatory reaction include NADPH oxidase, the inducible form of NOS and myeloperoxidase.

NADPH oxidase is dormant in resting phagocytic cells but comes into action when the cell is activated by invading micro-organisms (Bast, 2000). This enzyme initiates the oxidant generation of the respiratory burst by forming O2−. The inducible form of NOS is expressed on phagocytes and, upon stimulation, will form NO− (Marletta, 1989; Steur et al., 1990; Hevel et al., 1991; Weinberg et al., 1995). O2− and NO− themselves are not very reactive towards micro-organisms. On the contrary, the product formed out of both radicals, i.e. ONOO−, is very cytotoxic (Huie and Padmaja, 1993). Furthermore, the bactericidal activities of the phagocytes are boosted by the formation of the highly reactive HOCl out of H2O2 and chloride, a reaction catalyzed by the enzyme myeloperoxidase (Hampton et al., 1998; Klebanoff, 2005). The H2O2 necessary for this reaction is formed by the dismutation of superoxide, either spontaneously or catalyzed by the enzyme superoxide dismutase (McCord and Fridovich, 1988; Fridovich, 1998).

1.3. Damaging effects

ROS and RNS react readily with practically all bio-molecules, including DNA, RNA, proteins, carbohydrates and lipids, thereby damaging the attacked molecule (Diplock et al., 1998). The reaction with these molecules often starts with the subtraction of a hydrogen atom from the attacked molecule, thereby converting the unpaired electron into a more stable electron-pair. Alternatively, an electron instead of a hydrogen atom might be transferred. From an electrochemical point of view, the hydrogen or electron donating molecule is oxidized. Consequently, free radicals and reactive species are often called (pro)‐oxidants.

An important target of oxidation by ROS and RNS are the polyunsaturated fatty acids present for example in the cell membrane (Halliwell and S., 1993). Initiation of this reaction involves the subtraction of a hydrogen atom from the attacked fatty acid, thereby leaving an unpaired electron on the lipid. This newly-formed lipid radical undergoes molecular rearrangement to increase its stability (Gutteridge, 1995) and will then rapidly react with oxygen, thereby creating a peroxy radical. Subsequently, this peroxy radical will create a lipid hydroperoxide as well as a new lipid radical by subtracting a hydrogen atom from a second fatty acid and so on. Prolongation of this chain reaction, referred to as lipid peroxidation, takes place by continuously passing the unpaired electron from one molecule to another (Halliwell and S., 1993). Termination of this chain reaction may occur upon (i) the consumption of one of the two reactants, i.e. the fatty acids or the oxygen, (ii) the formation of a relatively unreactive radical or (iii) the reaction of two radicals that will combine to form a non-radical pair. Products formed during lipid peroxidation include 4-hydroxy-2-alkenals and the three-carbon compound malondialdehyde (MDA) (Halliwell and S., 1993). By reacting with DNA bases, MDA can cause mutagenic lesions that may be involved in the pathology of various diseases (Spiteller, 2001).

### Table 1

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive oxygen species (ROS)</td>
<td>Hydrogen peroxide, H2O2</td>
</tr>
<tr>
<td>Superoxide, O2−</td>
<td>Hypochlorous acid, HOCl</td>
</tr>
<tr>
<td>Hydroxyl, HO−</td>
<td>Peroxynitrite, ONOO−</td>
</tr>
<tr>
<td>Peroxyl, RO2</td>
<td>Alkyl peroxyxinitrites, ROONO−</td>
</tr>
<tr>
<td>Alkyl peroxyxinitrites, ROONO−</td>
<td></td>
</tr>
</tbody>
</table>
The initiation and prolongation of lipid peroxidation are schematically depicted in Fig. 1 and illustrate how free radicals may damage bio-molecules.

Oxidative damage caused by ROS and RNS will lead, among others, to DNA lesions (Knaapen et al., 1999; Spencer et al., 2000; Waris and Ahsan, 2006), function loss of enzymes (Haenen et al., 1987; Sastre et al., 2000), disturbed signaling over the cell (Yorimitsu et al., 2004; Kim et al., 2006; Shen and Liu, 2006) and eventually even necrotic cell death or apoptosis (Stangel et al., 1996; Diplock et al., 1998; Kim et al., 2006; Shen and Liu, 2006). Consequently, damage induced by reactive species is often suggested to play a role in the pathophysiology of various diseases, including diabetes (Mehta et al., 2006), cancer (Valko et al., 2006) and lung diseases such as chronic obstructive pulmonary disease (Boots et al., 2003a) and the interstitial lung diseases sarcoidosis (MacNee, 2001; Kanoh et al., 2005) and idiopathic pulmonary fibrosis (Rahman et al., 1999).

1.4. Link between reactive species and inflammation

Besides directly or indirectly damaging various bio-molecules, reactive species are also involved in inflammation. A key factor in this process is the production of cytokines, i.e. glycoproteins secreted by various immune cells including macrophages, neutrophils and helper T lymphocytes (Parkin and Cohen, 2001). Cytokines may exert either pro- or anti-inflammatory activities. ROS are capable of inducing the production of various cytokines via activation of transcription factors such as nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1) (Schreck et al., 1991; MacNee, 2001; Rahman, 2002; Rahman et al., 2002). In vitro studies, using both macrophages and alveolar and bronchial epithelial cells, have demonstrated that oxidants can initiate the production of inflammatory mediators like interleukin(IL)-8 and NO (Antonicelli et al., 2000). The activation of NF-κB by ROS is mediated by the breakdown of its inhibitor part, i.e. IκBα, which causes the normally inactive transcription factor to become active.

Contradictory, a few studies have reported that (cytokine-induced) NF-κB activation can also be inhibited by pre-treatment with or simultaneous exposure, either acute or chronic, to a specific ROS, i.e. H₂O₂ (Flescher et al., 1998; Lahdenpohja et al., 1998; Korn et al., 2001; Moodie et al., 2004). Inhibition by H₂O₂ could be adaptively, resulting in a reduced ROS-forming activity that is not capable of effective IκB phosphorylation (Lahdenpohja et al., 1998). Alternatively, this inhibition may be associated with a promotion of apoptosis resulting from too high levels of damage, induced by the presence of both elevated ROS and inflammatory cytokines levels (Moodie et al., 2004). This would be inline with the fact that only intermediate levels of oxidative stress are capable of activating NF-κB, whereas high levels will induce apoptosis (Halliwell and Gutteridge, 1999). This apparent paradox can be seen as a biphasic response of NF-κB to oxidative stress.

In summary, it can be stated that, although some studies display ambiguous results, ROS appear to induce NF-κB activation. Different outcomes of studies regarding this induction may be the result of variables including the cell type, culturing conditions, antioxidant defense present, the level of oxidative stress or other inducers and time frames involved (Moodie et al., 2004).

An important cytokine induced by ROS via NF-κB is tumor necrosis factor α (TNFα). On its turn, TNFα itself can also mediate the activation of NF-κB by e.g. increasing the production of ROS (Kaul and Forman, 1996). This occurs via stimulation of the radical production during both the mitochondrial oxidative phosphorylation (Flohe et al., 1997; Ginne-Pease and Whisler, 1998; Chandel et al., 2000) and the inflammatory burst (Kitagawa et al., 1988; Ferrante, 1992; Kaul and Forman, 1996). Furthermore, TNFα can also enhance the transcription of other cytokines, including IL-8 (Baggiolini and Clark-Lewis, 1992; Schoonbroodt and Piette, 2000). This feed forward mechanism, depicted in Fig. 2, amplifies both the TNFα- and the NF-κB-mediated
Table 2
Enzymatic and non-enzymatic antioxidants

<table>
<thead>
<tr>
<th>Enzymatic antioxidants</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutases (SOD)</td>
<td>$2 \text{O}_2^- + 2H^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$</td>
</tr>
<tr>
<td>Catalases</td>
<td>$2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}$</td>
</tr>
<tr>
<td>Glutathione peroxidases (GPx)</td>
<td>$2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{H}_2\text{O}$</td>
</tr>
<tr>
<td>$2 \text{GSH} + \text{ROOH} \rightarrow \text{GSSG} + \text{ROH} + \text{H}_2\text{O}$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-enzymatic antioxidants</th>
<th>Hydrophilic</th>
<th>Hydrophobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione (GSH)</td>
<td>α-Tocopherol (vitamin E)</td>
<td></td>
</tr>
<tr>
<td>Ascorbate (vitamin C)</td>
<td>Caretenoids</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>Ubiquinol-10</td>
<td></td>
</tr>
</tbody>
</table>

Effects. Final result of this amplification might be cytotoxic levels of cytokines and ROS, resulting in apoptosis.

2. Antioxidants

Fortunately, the human body comprises an elaborate antioxidant defense system to protect cellular compounds from damage induced by free radicals, ROS and other reactive species. An antioxidant has been defined as “any substance that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate” (Sies, 1993; Halliwell, 1995). The antioxidants that react directly with radicals or other reactive species to prevent cellular compounds from becoming oxidized can be subdivided into enzymatic and non-enzymatic antioxidants (Table 2).

Enzymatic antioxidants react with reactive species and are subsequently efficiently recycled. In fact, the enzyme functions as a catalyst. Consequently, only small amounts of these enzymes are needed to offer protection. Important antioxidant enzymes are superoxide dismutases, catalase and glutathione peroxidases (Diplock et al., 1998).

Non-enzymatic antioxidants can be divided into hydrophilic and hydrophobic antioxidants. Hydrophobic antioxidants include α-tocopherol (vitamin E), caretenoids, and ubiquinol-10 and are mostly present in lipoproteins and membranes. Hydrophilic scavengers include glutathione, ascorbate and uric acid. They can predominantly be found in cytosolic, mitochondrial and nuclear aqueous compartments (Chaudiere and Ferrari-Iliou, 1999). The most important endogenous hydrophilic antioxidants that contribute to the total antioxidant defense are GSH, ascorbic acid and uric acid.

### 2.1. Antioxidant interplay

In contrast to the antioxidative enzymes, non-enzymatic antioxidants act by directly scavenging free radicals. During this process, the antioxidants donate an electron or proton to a radical, thereby forming a relatively stable product out of the scavenged radical. Consequently, the antioxidant itself becomes oxidized during this reaction (Bast and Haenen, 2002; Haenen and Bast, 2002). Since only the reduced form of the antioxidant can exert scavenging capacities, the oxidized form of the antioxidant, generated during its protective actions against free radicals, has to be converted back into its reduced status. Moreover, the oxidized form of the antioxidant often comprises a radical that, due to some residual activity of its parent compound, might still cause damage to vital cellular targets (Bast and Haenen, 2002; Haenen and Bast, 2002). Therefore, the body contains a distinct network of antioxidants that can chemically reduce each other, thereby diminishing the reactivity of the formed antioxidant radical and regaining the reduced antioxidant for the defense against reactive species. In this way, antioxidants act in synergy to destroy reactive species (Chaudiere and Ferrari-Iliou, 1999; Vertuani et al., 2004; Blomhoff, 2005).

An example of this antioxidant interplay is shown in Fig. 3. The lipid peroxyl radical (LOO$^\cdot$), formed during lipid peroxidation, becomes scavenged preferably by vitamin E. During this reaction, vitamin E becomes oxidized into vitamin E$^\cdot$. Vitamin E$^\cdot$ is toxic and as antioxidant useless to the antioxidant defense system. The reaction of vitamin E$^\cdot$ for example GSH results in the reduction of vitamin E and the concurrently oxidation of GSH into GSSG. In this way, vitamin E is preserved for the defense system at the expense of GSH. Alternatively, ascorbate is also known to regenerate vitamin E out of its radical (Machlin and Bendich, 1987). Recycling GSH or ascorbate for the network may occur by a reaction of their oxidized forms, i.e. GSSG or DHA, with another antioxidant.

This antioxidant interplay is of special importance since each antioxidant exerts preferentially scavenging activities towards specific free radicals or reactive species at specific compartments. It is known, for example, that LOO$^\cdot$ is preferably scavenged by vitamin E while HOCI reacts predominantly with GSH. The interplay between all antioxidants provides an intricate shield that offers optimal defense by the appropriate antioxidant against a particular reactive species at any site throughout the body (Machlin and Bendich, 1987; Chaudiere and Ferrari-Iliou, 1999). Interestingly, it has been shown that exogenous antioxidants such as the flavonoids can take over the LOO$^\cdot$ scavenging role of vitamin E in this exogenous antioxidant network (van Acker et al., 2000).

3. Oxidative stress

In normal situations, the endogenous antioxidant network as described earlier provides sufficient protection against reactive species such as ROS and RNS (Bast et al., 1991). However, when an imbalance between the production of and protection against reactive species occurs in favor of the production, a situation called oxidative stress arises (Fig. 4). Oxidative stress may result in increased oxidative damage and can be caused either by an overproduction of free radicals and ROS or by an impairment of the endogenous antioxidant defense system (Halliwell, 1993; Chaudiere and Ferrari-Iliou, 1999). Due to the intricate antioxidant network, as described earlier, a deficiency in one compound may also affect the...
efficacy of others. This could result in a greater loss of protection against reactive species than would be expected based on the deficiency of only one antioxidant.

As mentioned earlier, oxidative stress is associated with various diseases and, as could be anticipated due to the link between ROS and inflammation, in most of these diseases elevated inflammation is also implicated (MacNee, 2001).

4. Antioxidant therapy

Due to their ability to scavenge free radicals and reactive species, thereby reducing oxidative stress and associated damage, various health claims have been made regarding the use of exogenous, dietary antioxidants (Halliwell, 1996b; Diplock et al., 1998). As a result, numerous studies have been performed to examine the possible beneficial health effects of antioxidant supplementation. However, most of these studies have been conducted with healthy volunteers, i.e. people with a sufficient antioxidant shield and no substantial oxidative stress. Therefore, it is not surprising that the outcome of these studies was often rather disappointing (Gaziano et al., 1995; McCall and Frei, 1999; Meagher et al., 2001; Moller et al., 2004; Duthie et al., 2006). Additionally, more direct beneficial health effects of antioxidant supplementation can be expected in patients suffering from a disease that is actually associated with increased levels of oxidative stress, such as diabetes, coronary heart diseases or the chronic lung diseases idiopathic pulmonary fibrosis and sarcoidosis (Newman, 2005). In these patients, antioxidant levels are impaired and empowering their antioxidant shield via supplementation could, therefore, result in reduction of the elevated oxidative stress present and thus in improvement of their clinical status.

Moreover, free radicals and ROS are also involved in various other processes such as inflammation (MacNee, 2001; Rahman, 2002), cell-to-cell communication (Lee and Lee, 2006), atherosclerotic plaque formation (Madamanchi et al., 2005; Soccio et al., 2005), angiogenesis (Cave et al., 2006; Lee and Lee, 2006), impairment of receptor functions (Aslan and Ozben, 2003) and DNA lesions (Cave et al., 2006; Waris and Ahsan, 2006). Therefore, it can be expected that reducing the reactive species load, by strengthening the antioxidant defense with an exogenous antioxidant, will also mitigate these processes. This might be of especial importance in diseases of which the pathology is linked to several of these other processes as well, including sarcoidosis, atherosclerosis and cancer. In such diseases, tackling only one of the underlying processes will most likely not result in an optimal treatment, as can be exemplified by the fact that treatment of sarcoidosis with only immuno-suppressive agents like glucocorticoids fail to be completely efficacious (Baughman and Lower, 2005). More effect can be expected from treatment with multi-target compounds, such as antioxidants, that are capable of tackling various processes at the same time.

Consequently, the use of exogenous antioxidants to support the treatment of these diseases, or maybe even cure them, has gained a lot of interest recently (Vertuani et al., 2004). However, most studies regarding the use of antioxidants in chronic diseases have measured the effect of the supplementation on markers of oxidative stress or of

### Table 3

A selection of clinical studies, performed during the last few years, investigating the effect of antioxidant supplementation on the clinical status of patients suffering from diseases related to oxidative stress

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Dosing regime</th>
<th>Disease</th>
<th>End point(s)</th>
<th>Antioxidant effect(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAC</td>
<td>12 weeks, 3×600 mg/day; combined with low maintenance corticosteroids</td>
<td>Pulmonary fibrosis</td>
<td>Pulmonary function tests</td>
<td>Improvement of pulmonary function tests</td>
<td>Behr et al. (1997)</td>
</tr>
<tr>
<td>NAC</td>
<td>24 months, 3×600 mg/day; added to standard therapy</td>
<td>IPF</td>
<td>Lung functions including FEV1 and DLCO</td>
<td>Slower deterioration of lung functions</td>
<td>Demedts et al. (2005)</td>
</tr>
<tr>
<td>NAC</td>
<td>10 weeks, 600 mg/day</td>
<td>COPD</td>
<td>Lung functions including FEV1 and inflammatory parameters including IL-8</td>
<td>No effect on lung functions, reduction of some inflammatory parameters including IL-8</td>
<td>Cerda et al. (2006)</td>
</tr>
<tr>
<td>NAC</td>
<td>72 mg/kg i.v. as a bolus, later 72 mg/kg over 12 h</td>
<td>Pre-treatment of cardiac surgery patients</td>
<td>Neutrophil-mediated inflammation and lung injury</td>
<td>Reduced neutrophil influx and elastase activity, possible protection against lung injury</td>
<td>De Backer et al. (1996)</td>
</tr>
<tr>
<td>Fatty acids, vitamins C and E</td>
<td>180 mg EPA + 120 mg DHA, 400 IU vit E and 3000 mg vit C daily, added to standard therapy</td>
<td>Schizophrenia</td>
<td>Positive and negative symptoms of the disease, severity of side-effects of routine treatment</td>
<td>Reduced positive and negative symptoms of schizophrenia, reduction of side-effects of routine treatment</td>
<td>Sivrioglu et al. (2007)</td>
</tr>
<tr>
<td>Vitamins C and E</td>
<td>30 days, 1200 mg and 600 mg/day respectively, added to standard therapy</td>
<td>Acute myocardial infarction</td>
<td>Primary endpoint is composed out cardiac mortality, new myocardial infarction, VT/VF; asystole and shock/pulmonary edema, Incidence of second primary cancers and cancer-free survival</td>
<td>Increase of the occurrence of second primary cancers and a reduced cancer-free survival</td>
<td>Jaxa-Chamiec et al. (2005)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>3 years, 400 IU/day</td>
<td>Head and neck cancer</td>
<td>Pre-hypertensive and stage 1 hypertensive patients</td>
<td>No effect on lung functions, reduction of side-effects of routine treatment</td>
<td>Bairati et al. (2005)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4 weeks, 730 mg/day</td>
<td>BP and oxidative stress parameters in blood and urine</td>
<td>BP and oxidative stress parameters in blood and urine</td>
<td>Reduction of BP, no effects on oxidative stress parameters</td>
<td>Edwards et al. (2007)</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>5 weeks, 2.66 g/day</td>
<td>COPD</td>
<td>Clinical symptoms and respiratory function tests</td>
<td>No effect</td>
<td>Omenn et al. (1996)</td>
</tr>
<tr>
<td>Flavonol-rich cocoa</td>
<td>6 weeks, 444 mg/day</td>
<td>Coronary artery diseases</td>
<td>Vascular function parameters</td>
<td>No effect</td>
<td>Farouque et al. (2006)</td>
</tr>
<tr>
<td>Garlic pears</td>
<td>2 months, 250 mg/day</td>
<td>Essential hypertension patients</td>
<td>Oxidative stress parameters and BP</td>
<td>Reduction of oxidative stress parameters, mild reduction of BP</td>
<td>Dhawan and Jain, (2005)</td>
</tr>
<tr>
<td>α-lipoic acid</td>
<td>3 days, 600 mg/day</td>
<td>Diabetes</td>
<td>Oxidative stress in plasma and NF-κB activity</td>
<td>Improvement of walking tolerance, delay of claudication pain onset</td>
<td>Hofmann et al. (1999)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>1 week, 30 mg/day</td>
<td>Exercise-induced asthma</td>
<td>Post-exercise reduction of lung function FEV1</td>
<td>Protection against this post-exercise reduction in FEV1</td>
<td>Neuman et al. (2000)</td>
</tr>
</tbody>
</table>

NAC = N-acetyl cysteine; IPF = idiopathic pulmonary fibrosis; COPD = chronic obstructive pulmonary disease; FEV1 = forced expiratory volume/s; DLCO = diffuse lung capacity for CO; IL = interleukin; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; BP = blood pressure; AIDS = acquired immunodeficiency syndrome; NF-κB = nuclear factor-κB.
one of the other processes mentioned earlier. Only in relatively few studies the effects of antioxidant supplementation on the clinical status of patients have been investigated so far. Table 3 highlights a selection of these studies, all performed during the last few years in various diseases associated with elevated oxidative stress.

Antioxidants tested vary from (i) endogenous antioxidants such as vitamins C and E (Jaxa-Chamiec et al., 2005; Sivrioglu et al., 2007) to (ii) precursors of endogenous antioxidants such as N-acetyl cysteine (NAC) (De Backer et al., 1996; Behr et al., 1997; Demedts et al., 2005; Cerda et al., 2006) to (iii) exogenous antioxidants including α-lipoic acid (Hofmann et al., 1999; Vincent et al., 2007), polyphenols as a group (Omenn et al., 1996) or quercetin alone (Edwards et al., 2007) and even (iv) exogenous antioxidant food supplements such as flavonol-rich cacao (Farouque et al., 2006) or garlic pearls (Dhawan and Jain, 2005). The diseases of interest include mostly chronic diseases including COPD (Cerda et al., 2006), pulmonary fibrosis (Behr et al., 1997; Demedts et al., 2005), diabetes (Hofmann, 1999) and cancer (Bairati et al., 2005). However, some studies have also investigated the effect of antioxidant supplementation on acute diseases such as acute myocard infarction (Jaxa-Chamiec et al., 2005). Although most of these supplementation studies display health-beneficial effects of the antioxidant supplementation, some show no (Omenn et al., 1996; Farouque et al., 2006) or even adverse health effects (Bairati et al., 2005). Clearly, more research is needed to optimize the use of specific antioxidants in diseases associated with oxidative stress.

Important aspects that should be considered in such research to justify the supplementation of antioxidants include their scavenging capacities, their possible place in the antioxidant network and their bio-availability. In other words, the effectiveness of antioxidant therapy relies on whether the appropriate scavenger will attain intracellular concentrations that are high enough to fill the existing defect in the endogenous antioxidant network. Moreover, the possible toxicity of antioxidants, when administered in high levels that exceed their daily intake by far, has to be considered too, as can for instance be derived from the toxic effects of high doses of antioxidant β-carotene found in two clinical trials with heavy smokers and asbestos workers (Albanes et al., 1995; Omenn et al., 1996; Pryor et al., 2000).

5. Possible candidates for antioxidant therapy: flavonoids

A group of antioxidants that is often suggested to be good candidates for antioxidant therapy due to their potential role in supporting health are the flavonoids. Flavonoids are a class of naturally occurring polyphenolic compounds, ubiquitously present in photosynthesising cells (Saito, 1974; Salunkhe et al., 1982). Over 5000 different naturally occurring flavonoids have already been identified and the list is still growing (Middleton and Kandaswami, 1993; Shahidi and M., 1995). Flavonoids are present in fruits, vegetables, nuts and plant-derived beverages such as tea and wine (Kuhnau, 1976; Hertog et al., 1993). Additionally, many antioxidant supplements and herb-containing medicaments contain high doses of flavonoids.

Most flavonoids share a common three-ring structure, depicted in Fig. 5, of which rings A and B are aromatic and ring C is heterocyclic. The variation in the heterocyclic C ring forms the basis of the division of the flavonoids in various subclasses, i.e. the flavones, isoflavones, flavonols, flavanols, flavanones, anthocyanidins and chalcones (Scalbert and Williamson, 2000).

The total amount of flavonoids consumed in the Netherlands is estimated at several hundreds of mg/day (Hertog et al., 1993a). The Dutch intake of flavones and flavonols is determined as 23–24 mg/day and quercetin, the main flavonol present in our diet, represents 70% of this intake (Hertog et al., 1993a; de Vries et al., 1997). The molecular structure of quercetin is given in Fig. 6. Quercetin, commonly found in apples, onions and green tea (Scalbert and Williamson, 2000), occurs mainly as glycoside, i.e. a sugar group such as glucose, galactose, rhamnose, rutinose or xylose is bound to one of the hydroxyl groups of the flavonol (Havsteen, 1983; Middleton and Kandaswami, 1993).

6. Quercetin

6.1. Absorption, metabolism and bio-availability

Because of the hydrophilic character of its glycosides, only quercetin without a sugar group, i.e. the aglycon, was initially suggested to be taken up in the gastro-intestinal tract by passive diffusion (Kuhnau, 1976; Griffiths, 1982). However, a study with human ileostomy volunteers showed not only that quercetin glycosides can indeed be absorbed in the small intestine, but also that this absorption surpasses that of the aglycon by far, i.e. 52% of the glycosides was absorbed versus 24% of the aglycon (Hollman et al., 1993).
performed to provide support for the alleged beneficial health effects of flavonoids. Epidemiological studies performed so far support a beneficial role for flavonoids in the lung, but they do not provide conclusive evidence for beneficial health effects of a high flavonoid or quercetin intake in diseases concerning other organs, including various forms of cancer. Consequently, more accurate epidemiological research is necessary to elucidate the overall beneficial health effects of flavonoids.

6.3. Antioxidant: in vitro studies

6.3.1. Beneficial effects: ROS scavenging

Quercetin has been shown to be an excellent in vitro antioxidant. Within the flavonoid family, quercetin is the most potent scavenger of ROS, including $O_2^-$ (Hanasaki et al., 1994; Cushnie and Lamb, 2005), and RNS like NO$^\cdot$ (van Acker et al., 1995; Haenen and Bast, 1999) and ONOO$^-$ (Haenen et al., 1997; Heijnen et al., 2001). These antioxidant capacities of quercetin are attributed to the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging, i.e., the catechol group in the B ring and the OH group at position 3 of the AC ring (Heijnen et al., 2002). Moreover, quercetin is suggested to substantially empower the endogenous antioxidant shield due to its contribution to the total plasma antioxidant capacity which is 6.24 times higher than the reference antioxidant trolox, whereas for example the contribution of both vitamin C and uric acid virtually equals that of trolox (Arts et al., 2004).

6.3.2. Beneficial effects: anti-inflammatory

Quercetin is known to possess strong anti-inflammatory capacities (Read, 1995; Orsolic et al., 2004). Several in vitro studies using different cell lines have shown that the flavonoid is capable of inhibiting LPS-induced cytokine production. For instance, quercetin inhibits LPS-induced TNF-α production in macrophages (Manjeet and Ghosh, 1999) and LPS-induced IL8 production in lung cells (A549) (Geraets et al., 2007). Moreover, in glial cells it was even shown that quercetin can inhibit LPS-induced mRNA levels of two cytokines, i.e. TNF-α and IL-1α (Bureau et al., 2008). In a microglial–neuronal coulture, this effect of the flavonoid resulted in a diminished apoptotic neuronal cell death induced by microglial activation (Bureau et al., 2008).

A possible explanation for these anti-inflammatory effects of quercetin may be found in the interplay between oxidative stress and inflammation. ROS are not only involved in the occurrence of oxidative stress, but also in the promotion of inflammatory processes via activation of transcription factors such as NF-κB and activator protein (AP)-1 which induce the production of cytokines like TNF-α (MacNee, 2001; Rahman, 2002). Consequently, scavenging ROS would not only prevent the occurrence of oxidative stress but also help mitigate inflammation. Indeed, it has already been shown that quercetin can inhibit the production as well as the gene expression of TNF-α via modulation of NF-κB in human peripheral blood mononuclear cells (Nair et al., 2006). A possible mechanism behind this modulation was reported to be the inhibition of the degradation of the inhibitory part (κB-κB) of this transcription factor (Peet and Li, 1999).

6.3.3. Beneficial effects: miscellaneous

Furthermore, it has been shown in vitro that quercetin also possesses anti-bacterial (Lee et al., 2003), anti-coagulative (Bucki et al., 2003), anti-bacterial (Cushnie and Lamb, 2005), anti-atherogenic (de Whalley et al., 1999; Perez-Vizcaino et al., 2006), anti-hypertensive (Duarte et al., 2001; Perez-Vizcaino et al., 2006) and anti-proliferative effects.
properties (Kuo, 1996; Orsolic et al., 2004; Orsolic et al., 2004; Gulati et al., 2006). Furthermore, quercetin is reported to directly modulate the gene expression of enzymes involved in biotransformation (Walle et al., 1995; Pacifici, 2004; Schwarz et al., 2005; Moon et al., 2006) and to inhibit cell proliferation by interacting with estrogen binding sites (Piantelli et al., 1995; Caltagirone et al., 1997). Altogether, these studies indicate that quercetin may exert health-beneficial capacities via various damage modulating effects. However, most of these studies have been performed with immortalized or cultured cell lines only and are thus not easy to extrapolate to the in vivo human situation.

6.3.4. Toxic effects: quinone formation

It is known that during its antioxidative activities, quercetin becomes oxidized into various oxidation products (Fig. 7). The two-electron oxidation of quercetin yields the oxidation product quercetin-quinone, denoted as QQ, that has four tautomeric forms, i.e. an ortho-quinone and three quinonemethides (Fig. 8) (Jorgensen et al., 1998; Awad et al., 2002; Boots et al., 2003b). It has been well described that oxidation products like semiquinone radicals and quinones display various toxic effects due to their ability of arylating protein thiols (Kalyanaraman et al., 1987; Ito et al., 1988; Monks et al., 1992; Metodiewa et al., 1999). Indeed, QQ is very reactive towards thiols and can instantaneously form an adduct with GSH, the most abundant endogenous thiols (Galati et al., 2001; Awad et al., 2002). This adduct is called GSQ and its formation cannot be prevented by ascorbate (Fig. 7) (Boots et al., 2003b). Furthermore, it has been shown that the enzyme DT-diaphorase (NQO1), which is reported to protect against quinone-induced toxicity by reducing quinones to hydroquinones, plays no

![Fig. 7. Oxidation of quercetin, followed by the possible reaction of its major oxidation product QQ with glutathione (adapted from Awad et al., 2002).](image-url)
substantial role in the protection against QQ either. In other words, GSH seems to be the main reactant of QQ. However, their product GSH is not stable; it rapidly dissociates into GSH and QQ with a half life of 2 min (Boots et al., 2005). It has been shown that, as long as the GSH concentration is high, it will offer protection against QQ by trapping it as GSQ (Boots et al., 2005). However, when the GSH concentration is low, the dissociated QQ will react with other thiol groups, e.g. protein sulfhydryls. This might imply that the “protection” offered by GSH against QQ might only be transient; GSH protects against QQ by scavenging it at the time and site of formation, but ultimately GSH will transfer the quinine to other thiols (Boots et al., 2005). The binding of QQ to these other thiols may lead to toxic effects such as increased membrane permeability (Yen et al., 2003) or, as previously shown for other quinones (Kalyanaraman et al., 1987; Boots et al., 2002), altered functioning of enzymes that contain a critical SH-group (Fig. 8). Such QQ-induced toxicity has been shown in various in vitro studies and has recently been defined as the quercetin paradox, i.e. the conversion of quercetin into a potential toxic product while offering protection by functioning of enzymes that contain a critical SH-group (Fig. 8). Such QQ to these other thiols may lead to toxic effects such as increased membrane permeability (Yen et al., 2003) or, as previously shown for other quinones (Kalyanaraman et al., 1987; Boots et al., 2002), altered functioning of enzymes that contain a critical SH-group (Fig. 8).

6.3.5. Toxic effects: genotoxic

Quercetin has also been reported to display genotoxic effects in vitro. Interestingly, these mutagenic effects of quercetin are predominantly shown in bacteria and are suggested to require quinone formation as mediators as well (Vriesen et al., 1990; Jurado et al., 1991; Rueff et al., 1995; Silva et al., 2000). In mammalian cells and experimental animals, conflicting data regarding the capacity of the flavonoid to induce DNA lesions and mutations are reported. On the one hand, induction of chromosomal aberrations and single strand breaks combined with point mutations are shown in respectively hamster ovary (Silva et al., 2000) and mouse lymphoma cells (Meltz and MacGregor, 1981). On the other hand, quercetin supplementation in either mice (Jin et al., 2006) or rats with aorta restriction (Jalili et al., 2006) could protect against benzo[a]pyrene-induced DNA damage and was suggested, respectively, to protect against the development of lung cancer (Jin et al., 2006) and to attenuate cardiac hypertrophy (Jalili et al., 2006). Clearly, more research is required to explain the discrepancy observed between the in vitro and in vivo quercetin genotoxicity studies in order to further elucidate the possible genotoxic effects of the flavonoid for man.

6.4. Nutraceutical: in vivo studies

Until now, very few studies have been performed to examine the in vivo antioxidative effects of quercetin. In vivo quercetin supplementation for 28 days (1 g a day) in healthy volunteers resulted in a significantly increased plasma quercetin concentration, but did not show any beneficial health effects regarding risk factors for coronary diseases (Conquer et al., 1998). This lack of effect might be explained by the fact that this study was performed in healthy volunteers who display only relatively low levels of oxidative stress and are, therefore, not in need of extra antioxidative defense. Another quercetin supplementation in healthy volunteers (11 mg/day for 28 days via a quercetin-rich fruit juice) also increased the plasma quercetin concentration as well as the total plasma antioxidant capacity (Wilms et al., 2005). However, no in vivo oxidative stress markers or other parameters to measure in vivo health effects were included in this study. Interestingly, the fact that this supplementation did cause a 41% reduction of ex vivo-induced oxidative damage (P<0.07) might contribute to the rationale for supplementing antioxidants only in situations of elevated oxidative stress.

To our knowledge, there have only been two studies regarding the in vivo effect of quercetin in patients suffering from a disease, which is associated with oxidative stress and other damaging processes like inflammation, so far.

The first study examined the effect of in vivo quercetin supplementation on the blood pressure of both pre-hypertensive and stage 1 hypertensive patients (Edwards et al., 2007). Four weeks of 730 mg of quercetin daily caused a significant reduction in systolic, diastolic and mean arterial pressures in stage 1 hypertensive patients. Remarkably, no alterations in blood pressure were found in the pre-hypertensive patients (Edwards et al., 2007), suggesting that beneficial health effects of antioxidants such as quercetin can indeed be mostly expected when basal levels of damage are increased. The fact that no effects in markers of oxidative stress could be measured in the plasma or urine after this supplementation seems contradictory at first. However, it should be taken into consideration that the levels of oxidative stress measured in both patient groups as well as in normotensive controls were the same. This clearly indicates that there was no elevated oxidant stress present in this cohort and therefore it doesn’t seem strange that quercetin could not exert its antioxidative capacities (Edwards et al., 2007). Consequently, it is reasonable that another mechanism, such as limiting the production of angiotensin II, is underlying the observed health-beneficial effects of quercetin (Edwards et al., 2007).

The second study investigating the in vivo health effects of quercetin has been performed in patients suffering from sarcoidosis, a chronic inflammatory lung disease of which the exact cause is still unknown (Baumgarten et al., 2003). The inflammatory aspect of this disorder is characterized by increased levels of pro-inflammatory cytokines such as TNFα and IL-8 (Moller, 1999). Additionally, the pathology is also associated with oxidative stress, as can be deduced from increased levels of biomarkers of oxidative damage such as exhaled ethane (Kanoh et al., 2005) and both oxidized proteins and 8-isoprostane in the broncho-alveolar lavage fluid (BALF) of sarcoidosis patients (Lenz et al., 1996; Montuschi et al., 1998). Furthermore, it has recently been shown that the total antioxidant capacity in the blood of sarcoidosis patients is reduced by 75% compared to that of healthy controls (Boots et al., submitted for publication). This study has been conducted in non-smoking, untreated sarcoidosis patients. After 4+500 mg quercetin in 24 h the antioxidative defense system of the patients had been improved, as was indicated by an increased total antioxidant capacity. Moreover, markers of both oxidative stress and...
inflammation had been reduced as well as ex vivo LPS-induced cytokine levels. Interestingly, the effects of the quercetin supplementation appeared to be more pronounced when the basal levels of the oxidative stress and inflammation markers were higher (Boots et al., submitted for publication). In other words, the extent of the beneficial effects of quercetin appears to be mainly dependent on the individual level of damage present in the patient. Again, these findings indicate that beneficial effects of antioxidant supplementation can merely be foreseen in people with enhanced oxidative stress and inflammation and thus not in healthy subjects. Unfortunately, the effect of the supplementation on clinical parameters and symptoms has not been measured in this study. Consequently, it is not possible yet to make any statement regarding the health-beneficial effects of quercetin in sarcoidosis, although the present study does provide an excellent rationale for it.

7. Conclusions

The flavonoid quercetin has been proven to be an excellent antioxidant that also possesses anti-inflammatory, anti-proliferative and gene expression changing capacities in vitro (Fig. 9). Until now, only its antioxidative and anti-inflammatory effects have been shown in vivo as well. Interestingly, these two effects of quercetin appear to be more pronounced when the basal levels of respectively the occurring oxidative stress and inflammation are high. This indicates that the use of quercetin supplementation is especially fruitful in people suffering from a disease that is associated with both processes, such as hypertension and sarcoidosis.

Up to date, toxic effects of quercetin could only be observed in vitro (Fig. 9). These effects are most likely associated with the formation of possible toxic products upon oxidation of quercetin during its ROS scavenging activities. The most important oxidation product of quercetin is its ortho-quione, denoted as QQ. QQ is highly thiol reactive and reacts almost instantaneously with GSH or, in the absence of this thiol, with protein sulfhydryl groups, thereby impairing the function of several critical enzymes. Consequently, during in vivo quercetin supplementation care should be taken of the possible toxicity of its metabolites. Especially in a chronic disorder, when supplementation has to proceed over a much longer time period, the safety, tolerability and efficacy of (long term) quercetin supplementation remain to be established.

8. Implications

As stated above, the dose-dependent safety of the (long term) use of quercetin in vivo should be examined in more detail before any recommendation regarding the use of this flavonoid as a nutraceutical can be made. Since it can be expected that antioxidant therapy will be mainly applied in patients suffering from chronic diseases that are associated with ongoing damage, chronic use of such supplementation will most likely be required. Up to date, there are no data available regarding the safety of long term use of high dosages of antioxidants in general and quercetin in particular. As described above, quercetin becomes oxidized while exerting its antioxidative capacities and potentially toxic oxidation products are formed. The long term cytotoxic effects of these oxidation products in vivo should be monitored in detail in order to evaluate the safety of long term quercetin supplementation. This can be achieved, for example, by screening the health status of cells that are expected to be exposed to the flavonoid the most, i.e. blood and lung cells. Alternatively, the thiol reactivity of these oxidation products can be quantified by, for example, measuring the activity of various enzymes of which it is known that they can be affected by the oxidation products of quercetin, i.e. calcium ATPase of glutathione transferases. Prevention of toxicity, induced by the thiol reactive oxidation products, might be achieved by supplementing quercetin together with a dithiol that is capable of capturing, and thus detoxifying, these products.

Optimal effects of any supplementation can only be expected when the compound that needs to be supplemented is administered in the most appropriate way. For quercetin, various ways of supplementing are possible including a pure supplement or a diet intervention using a food component with a high quercetin content. A supplement usually contains only the aglycon form of quercetin, whereas a food component normally comprises high amounts of various quercetin derivatives that might have a better biological availability than the aglycon itself. Another advantage of a dietary supplementation versus a ‘conventional’ supplement might be a better compliance, especially in long term use. A good compliance is mandatory in chronic diseases. Conversely, a disadvantage of using the diet is that the intake of alimentary antioxidants is relatively low, i.e. ~100 mg a day versus up to several grams a day using an antioxidant supplement. As a result, the plasma quercetin concentrations achieved by a quercetin supplement are usually higher compared with the plasma levels obtained by a dietary intervention. Consequently, larger beneficial health effects might be expected when supplementation is applied via a supplement instead of via the diet. However, in order to make a balanced overall comparison between both ways of supplementation, more research is necessary.

References


