ANTIOXIDANT-PROOXIDANT BALANCE IN THE INTESTINE: FOOD FOR THOUGHT

1. PROOXIDANTS

Katie P. Surai, Peter F. Surai, Brian K. Speake and Nick H. C. Sparks

Avian Science Research Centre, Animal Health Group, SAC, Auchincruive, Ayr, KA6 5HW, Scotland

[Received: April 9, 2003; Accepted: June 22, 2003]

ABSTRACT: This paper presents the hypothesis that the antioxidant-pro-oxidant balance in the intestine is an important determinant of human health. When food is consumed it contains a range of potentially dangerous substances including oxidized polyunsaturated fatty acids, oxysterols, iron and copper ions, nitrates and nitrates. It is likely that food also contains traces of mycotoxins, heavy metals, persistent organic pollutants and alcohol. The data presented in this review clearly indicate that most of foods are contaminated with one or several of those potentially dangerous substances in various concentrations. In fact most of those compounds are considered to be potent pro-oxidants. Even if they are present in the food in low concentrations a combination of them could be a potential source of free radical production in the gastrointestinal tract. Immune system of the gut, if activated, could also produce free radicals. In such a situation antioxidant compounds of the food including vitamin E, coenzyme Q, carotenoids, vitamin A, ascorbic acid, reduced glutathione, selenomethionine, flavonoids and other polyphenolics could have a protective effect. Furthermore, antioxidants synthesised in the body (antioxidant enzymes, glutathione, coenzyme Q, etc.) could further improve protection in the gut.

KEY WORDS: Antioxidants, Balance, Food, Health, Intestine, Prooxidants
somes), the $H_2O_2$ removal from the cell is very much dependent on GSH-Px activity.

It is important to remember that superoxide radical or hydrogen peroxide are not major damaging molecules in biological systems, but rather precursors of the more damaging hydroxyl radical ($OH^*$). Therefore the action of SOD prevents the reaction of superoxide radical with biological molecules, or from participation in the production of more powerful radicals by donating an electron, and thereby reducing $Fe^{3+}$ and $Cu^{2+}$ to $Fe^{2+}$ and $Cu^{+}$, as follows:

$$O_2^\cdot - + Fe^{3+}/Cu^{2+} \rightarrow Fe^{2+}/Cu^{+} + O_2$$

Further reactions of $Fe^{2+}$ and $Cu^{+}$ with $H_2O_2$ are a source of the hydroxyl radical ($OH^*$) in the Fenton reaction:

$$H_2O_2 + Fe^{2+}/Cu^{+} \rightarrow *OH + OH^* + Fe^{3+}/Cu^{2+}$$

The sum of reactions of superoxide radical with transition metals and transition metals with hydrogen peroxide is known as the Haber-Weiss reaction.

The strategy of antioxidant defense also includes maintenance of transition metals in binding to proteins forms. Therefore, the first line of antioxidant defense includes also metal-binding proteins such as transferrin, lactoferrin, haptoglobulin, hemopexin, metallothionenin, ferritin, myoglobin, ceruloplasmin, ceruloplasm, etc. However, this first level of antioxidant defense in the cell is not sufficient to completely prevent free radical formation and lipid peroxidation and some free radicals do escape through the preventive first level of antioxidant safety screen. Therefore the second level of defense consists of chain-breaking antioxidants: vitamin E, ubiquinol, carotenoids, vitamin A, ascorbic acid, uric acid, glutathione and thioredoxin systems and some other antioxidants.

Chain-breaking antioxidants inhibit peroxidation by keeping the chain length of the propagation reaction as small as possible. Therefore, they prevent the propagation step of lipid peroxidation by scavenging peroxyl radical intermediates in the chain reaction:

$$LOO^* + Toc \rightarrow Toc^* + LOOH$$

(LOO* is lipid peroxyl radical; Toc - tocopherol, Toc* - tocopheroxyl radical, LOOH - lipid hydroperoxide)

Vitamin E, the most effective natural free radical scavenger identified to date, is the main chain breaking antioxidant in the cell. However, hydroperoxides produced in the reaction of vitamin E with the peroxyl radical, are still toxic and if not removed, impair membrane structure and functions (Gutteridge and Halliwell, 1990). As shown above, lipid hydroperoxides are not stable and in the presence of transition metal ions can decompose producing new free radicals and cytotoxic aldehydes (Diplock, 1994). Therefore hydroperoxides have to be removed from the cell in the same way as $H_2O_2$, but CAT is not able to react with these radicals and Se-dependent GSH-Px can deal with these potentially toxic compounds converting them into nonreactive products (Brigelius-Flohe, 1999) as follows:

$$ROOH + 2GSH \rightarrow ROH (non-toxic) + H_2O + GSSG$$

Thus, vitamin E performs only half the job in preventing lipid peroxidation by scavenging free radicals and forming hydroperoxides. The second part of this important process of antioxidant defense is due to Se-GSH-Px.

However, even the second level of antioxidant defense in the cell is not able to prevent lipid peroxidation and some biological molecules are damaged. In this case, the third level of defense is based on systems that eliminate damaged molecules or repair them. This level of antioxidant defense includes lipolytic (lipases), proteolytic (peptidases or proteases) and other enzymes (DNA repair enzymes, ligases, nucleases, polymerases, proteinases, phospholipases and various transferases).

Since maintaining the integrity of the genome is of the vital
importance, living organisms have evolved a range of systems that can recognise, signal the presence of, and repair the various forms of DNA damage. These systems include a number of enzymatic processes ranging from base recognition and excision to ligation of DNA strands. In particular, DNA glycosylases recognise a damaged purines and pyrimidines and hydrolyse the bond linking the abnormal base to the sugar-phosphate backbone (Halliwell and Gutteridge, 1999); the 5'-apurinic endonucleases process strand breaks, sites of base loss, and the products of DNA glycosylase/apurinic lyase action. DNA polymerase fills in the one-nucleotide gap with the correct base. DNA ligases complete the repair process by sealing the 3' end of the newly synthesised stretch of DNA to the original portion of the DNA chain (Cardozo-Pelaez et al., 2000; Wallace et al., 1997; Croteau and Bohr, 1997). It is believed that most damaged or inappropriate bases in DNA are removed by excision repair, while a minority are repaired by direct damage reversal (Krokan et al., 2000). Furthermore, programmed cell death (apoptosis) is also involved in maintenance of the genetic integrity by removing genetically altered cells. The importance of these DNA repair systems is confirmed by the fact that defects in these can result in cell death and hypersensitivity to endogenous or environmental mutagens (Jackson, 1999). Therefore removing mutagenic lesions in DNA is a vital task for repair systems. In general, the repair DNA damage mechanisms in bacteria are well defined, whereas in higher eukaryotes the genes and proteins responsible for repair await further investigation (Croteau and Bohr, 1997). Therefore, DNA damages by free radicals are repaired by specific enzymatic systems and, for example, selenomethionine can induce a DNA repair response (Seo et al., 2002).

Furthermore, protein-bound methionine residues are sensitive to oxidation with a formation of methionine sulf oxide residues. Therefore methionine sulfoxide reductase (MSR) is responsible for repairing this modification (Moskovitz et al., 2001) and MSR is shown to be selenoprotein (Bar-Noy and Moskovitz, 2002). On the other hand, proteasomes can catabolise damaged proteins producing amino acids which are consequently used as a building material for newly synthesised proteins (Dunlop et al., 2002). For example, selenomethionine non-specifically incorporated into various proteins (when available from the diet) could be released as a result of proteasome action and used for additional synthesis of active selenoproteins in stress conditions.

All these antioxidants are operating in the body in association with each other forming an integrated antioxidant system. The co-operative interactions between antioxidants in the cell is vital for maximum protection from the deleterious effects of free radicals and toxic products of their metabolism. For example, it is well established that vitamin E is the major antioxidant in biological membranes, a "head quarter" of antioxidant network. However it is usually present there in low molar ratios (one molecule per 2000-3000 phospholipids) but vitamin E deficiency is difficult to induce in adult animals. It is probably due to the fact that oxidised vitamin E can be converted back into the active reduced form by reacting with other antioxidants: ascorbic acid, glutathione, ubiquinols or carotenoids (Figure 2). This figure demonstrates a connection of antioxidant defence to the general body metabolism.

As a result of antioxidant action of vitamin E, tocopheroxyl radical is formed. This radical can be reduced back to an active form of tocopherol by coupling with ascorbic acid oxidation. Ascorbic acid can be regenerated back from the oxidised form by recycling with glutathione which can receive a reducing potential from NADPH, synthesised in the pentose phosphate cycle of carbohydrate metabolism. Enzymes involved in vitamin E recycling are as follows: 1. Thioredoxin reductase, a selenium dependent enzyme; 2. Glutathione reductase, a FAD containing enzyme dependent on a riboflavin provision; 3. Glucose-6-phosphate dehydrogenase (as an important part of pentose phosphate pathway, transketolase is thiamine dependent). Due to incomplete regeneration (the efficiency of recycling is usually less than 100%) in biological systems, the antioxidants have to be obtained from the diet (vitamin E and carotenoids) or synthesised in the tissues (glutathione).
(the pentose phosphate cycle is the major producer of reducing equivalents in the form of NADPH) and shows involvement of other nutrients in this process. For example, dietary protein is a source of essential amino acids for glutathione synthesis, riboflavin is an essential part of glutathione reductase, niacin is a part of NADPH and Se is an integral part of thioredoxin reductase. At the same time thiamine is required for transketolase in the pentose phosphate pathway.

Thus, a major finding in recent years is the possibility of direct or indirect vitamin E recycling from its oxidised radical form by means of ascorbate (Chan et al., 1991; Chan, 1993), glutathione (Niki et al., 1982; Chan, 1993), cysteine (Mukai et al., 1990), ubiquinols (Frei and Packer, 1993; Chan, 1993), lipoic acid (Packer, 1998), estrogens (Mukai et al., 1990), carotenoids (Palozza and Krinsky, 1992; Bohm et al., 1997) and potentially quercetin and catechins (Pietta, 2000), anthocyanins (Frank et al., 2002) and rosemary extracts (Madsen et al., 1997). Enzymatic regeneration of α-tocopherol has been also described (Mague et al., 1989; Kagan et al., 1998). The rate of reduction of phenoxyl radical in the membrane decreased in the order of ascorbic acid>cysteine>glutathione (Niki, 1996). The rate of regeneration, or recycling, of the vitamin E radicals that form during their antioxidant action may affect both its efficiency in antioxidant action and its lifetime in biological systems and the greater recycling activity is associated with increased efficiency of inhibition of lipid peroxidation (Packer, 1995). Whether all these regeneration reactions take place in vivo await investigation. Due to incomplete regeneration (the efficiency of recycling is usually less than 100%) in biological systems, the antioxidants have to be obtained from the diet (e.g., vitamin E and carotenoids) or synthesised in the tissues (glutathione).

Therefore the antioxidant protection in the cell depends not only on vitamin E concentration and location, but also relies on the effective recycling. Indeed, if the recycling is effective then even low vitamin E concentrations are able to maintain high antioxidant protection in physiological conditions. For example, this could be demonstrated using chicken brain as a model system. Indeed, our data (Surai, 2002) indicate that the brain is characterised by extremely high concentrations of long chain polyunsaturated fatty acids predisposing this tissue to lipid peroxidation. Furthermore, brain contains much lower levels of vitamin E than other body tissues. However, in fresh chicken brain, levels of products of lipid peroxidation are very low, which could be a reflection of an effective vitamin E recycling by ascorbic acid which is present in this tissue in comparatively high concentrations. Antioxidant recycling is the most important element in understanding mechanisms involved in antioxidant protection against oxidative stress. Therefore, regeneration, or recycling, of the vitamin E radicals may affect both its antioxidant efficiency and its lifetime in biological systems. As can be seen from data presented above the antioxidant defence includes several options (Surai, 2002):

- Decrease localised oxygen concentration;
- Prevention of first-chain initiation by scavenging initial radicals (SO D, GSH-Px and catalase);
- Binding metal ions (metal-binding proteins);
- Decomposition of peroxides by converting them to non-radical, non-toxic products (Se-GSH-Px);
- Chain-breaking by scavenging intermediate radicals such as peroxyl and alkoxy radicals (vitamins E, C, glutathione, uric acid, ubiquinol, bilirubin etc.);
- Repair and removal of damaged molecules.

Additional defensive mechanisms responsible for maintenance of physiological metabolism in stress conditions include (Surai, 2002):

- Antioxidant recycling mechanisms;
- Redox-signalling and gene expression with an additional synthesis of important antioxidant molecules;
- Stress-protein synthesis (e.g. heat shock proteins);
- Apoptosis (can remove damaged cells and restrict mutagenesis).

**THE GASTROINTESTINAL TRACT (GIT) AS A MAJOR SITE OF ANTIOXIDANT ACTION**

As mentioned above some antioxidants, such as antioxidant enzymes and glutathione can be synthesised in the body, but the diet is the major provider of nutrients possessing antioxidant properties directly (vitamin E, vitamin A, carotenoids, ascorbic acid, flavonoids etc.) (Table 1) or essentials for the synthesis of antioxidant enzymes: Se is an essential part of a range of selenoproteins performing antioxidant functions (GSH-Px, thioredoxin reductase, selenoproteins P and W, etc.); Mn is an integral part of mitochondrial Mn-SOD; Zn and Cu are integral parts of cytosolic Cu,Zn-SOD; Fe is an integral part of catalase.

When food is consumed and appears in the stomach and then in the small intestine, it contains a range of antioxidants but may also contain a range of potentially dangerous substances (Figure 3). To keep a balance between antioxidants and prooxidants in the intestine is a very important task in which diet plays a crucial role. In general, prooxidants which can be found in the digestive tract could be summarised as follows.

**PROOXIDANTS IN THE GIT**

**Peroxidized PUFAs**

It is believed that in modern food technology low-level oxidation of lipids in meat, poultry and milk during storage and processing is practically unavoidable (Cohn, 2002). Lipid stability in meat and meat products depends on many factors, including species, muscle type, the amount and type of fat in the diet, the nutritional status of the animal at slaughter, the presence of absence of disease or infection and the type of processing to which the meat is subjected (Morrisey et al., 1998). Most fried meat products contain lipid peroxides in various concentrations. If cooked meat is stored, the level of
Deleterious changes in foods caused by lipid oxidation include loss of flavour, development of off-flavours, loss of colour and nutrient value, and the accumulation of toxic compounds which are detrimental to human health. In fact all foods that contain polyunsaturated fatty acids are susceptible to oxidation but especially affected are foods which are dehydrated, subjected to high temperatures or cooked and subsequently stored. Specific examples of compounds which are of health concern include lipid peroxides, malondialdehyde, and several cholesterol oxidation products (Addis, 1986). Lipid oxidation in foods is initiated by free radical and/or singlet oxygen mechanisms which lead to autocatalytic free radical reactions. These autoxidation reactions cause the breakdown of lipid and the formation of a wide array of oxidation products. Secondary products of lipid autoxidation can be absorbed and may cause oxidative stress (Kubow, 1992). Lipid oxidation yields a very complex group of by-products that include hydroxy and dihydroxy fatty acids, hydroperoxides, volatile aldehydes, and alkyd olefinic radicals that contribute to the flavor deterioration of foods and that are implicated in biological oxidation and can cause oxidative stress (Frankel, 1987). Oxidised lipids are absorbed in the digestive tract (Staprans et al., 1999) and incorporated into membrane phospholipids altering their structure and properties (H. ayam et al., 1994; Staprans et al., 1994). In animal models it has been shown that oxidised lipids in the diet can suppress growth (Lin et al., 1989; Calabotta and Shermer, 1985), reduce vitamin E level in tissues increasing their susceptibility to lipid peroxidation (Sheehy et al., 1994), increase tissue protein oxidation (H. ayam et al., 1997) and increase the number of aberrant crypts in the intestine (Yang et al., 1998). In particular, in rats consuming thermally oxidised corn oil, increased concentrations of lipid peroxides were observed in the liver and kidney, in association with a decreased growth rate, food and protein efficiency ratio (Nwanguma et al, 1999; Lopez-Varela et al., 1995). The gastrointestinal epithelium of swine and chickens responded to oxidant stress imposed by oxidized fat by increased enterocyte turnover and the gut associated immune system was compromised (Dibner et al., 1996).

Furthermore, the consumption of oxidized fats caused diarrhea, liver enlargement, growth depression and histological changes in tissues of experimental animals (Andia and Street, 1975; Cutler and Schneider, 1973; Koshio et al., 1994; Shibata et al., 1992). Chylomicrons isolated from subjects consuming oxidized fat are more susceptible to lipid peroxidation ex vivo, suggesting that at least some hydroperoxy fatty acids are absorbed (Vine et al., 1998). Hated oils also showed potent teratogenic actions in experimental animals (Indart et al., 2002).

One of the biggest contributors to the consumption of lipid peroxides are fast foods, since the typical American consumes approximately three hamburgers and four servings of fries per week (Saguy and Dana, 2003). They could provide a significant amount of potentially hazardous peroxides as well as trans fatty acids. The percentage of fat from fast foods and ethnic foods increased from 1 in 1965 to 11% in 1996 (Dobarganes and Marquez-Ruiz, 2003). In particular oils which are used for deep-fat frying (e.g. chips and French fries preparation) are heated to very high temperature and decomposition products are formed (Chang et al., 1978). In fact most of the oxidised fats in foods come from fats and oils heated at high temperature in particular from frying fats (Dobarganes and Marquez-Ruiz, 2003). During frying, the

Table 1: Natural food sources of some antioxidants (Adapted from Shahidi, 1997)

<table>
<thead>
<tr>
<th>Compound/Property</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E (tocopherols &amp; tococtrielenols)</td>
<td>Oilseeds, vegetable oils, nuts, whole grains, cereals, margarine etc.</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Fruits and vegetables, berries, citrus fruits, green papers</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Dark leafy vegetables, carrots, sweet potatoes, tomatoes, apricots, citrus fruits, kale etc.</td>
</tr>
<tr>
<td>Flavonoids/isoflavonoids</td>
<td>Fruits and vegetables, oilseeds, berries, peppers, citrus fruits, tomatoes, onions etc.</td>
</tr>
<tr>
<td>Phenolic acids/derivatives</td>
<td>Oilseeds, cereals, grains, etc.</td>
</tr>
<tr>
<td>Catechins</td>
<td>Green tea, berries, certain oilseeds, etc.</td>
</tr>
<tr>
<td>Extracts/essential oils</td>
<td>Green tea, rosemary, sage, clove, oregano, thyme, oat, rice bran etc.</td>
</tr>
</tbody>
</table>
oil undergoes three deleterious reactions: hydrolysis caused by water, oxidation, and thermal alteration caused by oxygen and heat (Saguy and Dana, 2003). These reactions cause the formation of polymerization products, of which over 400 have been identified (Paul and Mittal, 1997). Furthermore, decomposition products which are formed as a result of reactions between food ingredients and oil comprise another large group of potentially toxic compounds (Takeoka et al., 1996). It is generally accepted that oxygen plays a major role in the deterioration of the oil during frying and selective absorption may occur, enriching the food product with breakdown oil compounds (Saguy and Dana, 2003). Products of lipid oxidation formed in the food depend on the temperature. For example, at low or moderate temperature hydroperoxides are the major products formed while in high temperature treated products secondary oxidised triacylglycerol monomers and polymers are more common compounds (Dobarganes and Marquez-Ruiz, 2003). Therefore food frying in fast food restaurants may be problematic due to lengthy oil exposure to extreme conditions and the lack of adequate oil replenishment and discarding. In particular a significant number of oils and fats from fast-food outlets contain more than 25% newly formed compounds (Dobarganes and Marquez-Ruiz, 2003).

It is well known that lipid peroxidation is associated with the formation of a wide range of secondary aldehyde products such as n-alkanals, trans-2-alkenals, 4-hydroxy-trans-2-alkenals and MDA (Lynch et al., 2001). While linoleic, gamma-linolenic and arachidonic acids found in different foods were precursors of hexanal, propanal was the dominant aldehyde formed from the breakdown of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids (Shahidi, 2001). For example, propional, pentenal, hexanal and 4-hydroxynonenal were the primary aldehydes formed during lipid oxidation in beef (Lynch et al., 2001). Those products are shown to be comparatively stable and can readily diffuse into cells causing toxicological effects (Szweda et al., 1993). Therefore prolonged frying caused a substantial rise in MDA concentration (Saguy and Dana, 2003) which is shown to be toxic and mutagenic. Furthermore, MDA can damage proteins and phospholipids by covalent bonding and cross-linking (Aubourg, 1993). Rats fed a diet containing MDA suffered from retarded growth, irregular intestinal activities, enlarged liver and kidneys, anaemia and low serum and liver vitamin E (Esterbauer, 1993). Similarly, the results of Raza et al. (2002) showed that 4-hydroxynonenal (HNE), a reactive by-product of lipid peroxidation, caused mitochondrial oxidative stress leading to a decrease in the GSH pool and increased membrane lipid peroxidation.

It is necessary to underline that heat treatment of the food can also cause heterocyclic amine formation. For example, approximately 20 heterocyclic amines of high mutagenesis were isolated and identified from protein-rich foods (Saguy and Dana, 2003). Furthermore, mutagenic activity was found in beef cooked in regular domestic conditions (Felton et al., 1997). It has been shown that heterocyclic amines can cause oxidative stress leading to DNA adduct formation and oxidative DNA damage (Murata and Kawanishi, 2002). Therefore heterocyclic amines formed during the cooking of meat and fish and possessing mutagenic, genotoxic and carcinogenic properties can be detected in beefburgers, steaks, pork ribs (Knize et al., 1998) and Italian sausages (Abdulkarim and Smith, 1998).

Acrylamide in food products-chiefly in commercially available potato chips, potato fries, cereals, and bread was deter-
mined by liquid chromatography-tandem mass spectrometry in thirty food samples (Becalski et al., 2003). Concentrations of acrylamide varied from 14 ng/g (bread) to 3700 ng/g (potato chips) and the WHO estimates the average consumer ingests about 0.8 mg/kg body weight daily (Mitika, 2002). It is necessary to take into account that acrylamide caused an increase in lipid peroxidation and decrease in glutathione contents and activity of glutathione-S-transferase in the rat liver in a dose dependent manner (Srivastava et al., 1983). From data presented above it is clear that lipid peroxidation in the food is an important source of toxic products! and a potential source of free radicals in the human digestive tract.

Oxysterols

Cholesterol oxidation products (oxysterols) are commonly found in foods of animal origin and they are formed during food processing, storage and cooking. In fact, more than 60 cholesterol oxides have been identified (Schroepfer, 2000; Savage et al., 2002). Recent findings suggest that oxysterols may modulate cytotoxicity by exerting effects on the induction of apoptosis. Furthermore, the generation of an oxidative stress may be a significant event occurring during 7-beta-oxysterol-induced apoptosis (O’Callaghan et al., 2002) simultaneously with a significant decrease in GSH levels and an increase in the activity of SOD and caspase-3. When cytotoxicity of various oxysterols was examined in a human mononcytic blood cell line (O’Callaghan et al., 2001), it was shown that they are cytotoxic and the mode of cell death was by apoptosis. However, mode of cytotoxic action of various oxysterols varied substantially, since some oxysterols induced apoptosis without changes in the GSH concentration or the SOD activity in the U937 cells. It is interesting that 7-ketocholesterol is considered to induce apoptosis and enhance superoxide radical production. In fact the substantial overproduction of superoxide radical was obtained with 7-beta-hydroxycholesterol and 7-ketocholesterol (Miguet-Alfonsi et al., 2002). Overproduction of superoxide radical was always correlated with enhanced lipid peroxidation and vitamin E was capable to significantly counteract apoptosis and oxidative stress induced by 7-beta-hydroxycholesterol and 7-ketocholesterol. Furthermore, oxidation of triglyceride-rich lipoproteins was significantly greater in rabbits fed oxidized cholesterol compared to the pure cholesterol-fed animals (Vine et al., 1998). Oxysterols can also replace cholesterol in membranes, perturbing permeability, stability and other membrane properties (Guardiola et al., 1996). This property could be extremely important in relation to enterocyte membranes in the intestine.

Sources of cholesterol oxides in the diet are represented mainly by processed animal-derived products including egg products, dairy products and meat products. For example, fresh egg yolk contains negligible levels of cholesterol oxide (Paniangy et al., 1995), however, spray-dried egg yolk powder is a rich source of cholesterol oxides. Similarly, fresh meat is almost free of cholesterol oxides but cooked meat products are considered to be a substantial source of cholesterol oxides in the diet (Savage et al., 2002). Cholesterol was oxidised in meat samples during household cooking and the rate of oxidation differed according to the cooking time and cooking temperature (Pie et al., 1981). In general, cholesterol oxide consumption could be substantial in many populations. For example, the average diet in the Netherlands provides about 1 mg of 7-beta-hydroxycholesterol and 0.5 mg of cholesterol-7-epoxide per day (Van de Bovenkamp et al., 1988). It is necessary to underline that certain oxidation products of cholesterol are carcinogenic (Pearson et al., 1983). Therefore, cholesterol oxides are associated with various animal-related foods and they are cytotoxic and potentially can be involved in lipid peroxidation in the intestine.

Iron ions

Iron is recognised as an essential nutrient and its reference nutrient intakes (RNI) in the UK are 8.7 mg/day for men and postmenopausal women and 14.8 mg/day for premenopausal women with USA RDA being 8 mg/day and 18 mg/day respectively (Kelly, 2002). However, iron absorption from a diverse diet has been shown to be about 15% (Department of Health, 1991). The main problem with iron nutrition is its reactivity and possible involvement in free radical generation. Iron ions are considered to catalyze the formation of the hydroxyl radical and accelerate the decomposition of lipid hydroperoxides (Davies and Slater, 1987; Donovan and Menzel, 1978) and to stimulate lipid peroxidation (Braughler et al., 1986, Minotti and Aust, 1987). In fact the potential risks of an excess intake of iron, associated with an increase in free radical generation were first proposed more than 40 years ago (Richmond, 1959). Numerous compounds, including heme and nonheme iron, have been reported to act as catalysts of lipid oxidation in food systems (Pearson et al., 1983). In fact muscle tissue is rich source of iron bound to proteins in the form of myoglobin. Furthermore, meat can also contain some hemoglobin residues from residual blood and iron-containing myoglobin. Post-slaughter events such as early post-mortem pH drop, carcass temperature and tenderising techniques such as electrical stimulation are involved in disruption of cellular compartmentalization and release of catalytic metal ions (Morrisssey et al., 1998). The next step of lipid peroxidation in the meat is associated with meat handling, processing, storage and cooking. It is widely believed that during heat treatment of the meat products nonheme iron is released from high molecular weight sources such as haemoglobin, myoglobin, ferritin and haemosiderin becoming the major pro-oxidant in cooked meat (Pearson et al., 1983; Morrisssey et al., 1998).

Iron fortification of various foods could represent another source of potentially dangerous free iron in the digestive tract. Fortification of cereal products with Fe has been in practice for a number of years throughout the developing world. In fact thirty countries throughout the developing world now have a range of products fortified with iron including wheat flour, maize flour, milk, rice and weaning foods (Danton-Hill...
allowable daily intakes for nitrites and nitrates are 8 and 220 mg/person per day, respectively (Joint FAO/WHO Expert Committee on Food Additives, 1974). In addition to added nitrate and nitrite to the food as preservatives, vegetables (e.g., lettuce, potato, etc.) comprise a major source of nitrates (Cammack et al., 1999). Therefore, humans are subjected to significant nitrate and nitrite levels in foods and water, as well as those formed in vivo. In fact, nitrates and nitrites formed from nitrogenous sources by micro-organisms in saliva and intestine, are considered to be the major source of human exposure under physiological conditions (Chow and Hong, 2002).

It is generally accepted that nitrate is concentrated in the saliva and rapidly converted to nitrite by facultative anaerobic bacteria. Benjamin et al. (1994) showed that nitrite is converted to NO under the highly acidic conditions (pH 3) which occur in the lumen of the stomach. It was observed that the generation and accumulation of NO from typical nitrite concentrations found in biological tissues increases 100-fold when the pH falls from 7.4 to 5.5 (Zweier et al., 1999). Therefore nitrate and nitrite can generate NO* radical by either direct disproportionation or reduction under the acidic and highly reduced conditions (Chow and Hong, 2002). Moreover, NO* can be a source of another reactive free radical peroxynitrite (ONOO-), which is 1000 times more oxidizing than H2O2 and has half-life in solution about 1–2 seconds (Van Dyke, 1997). It is well accepted that peroxynitrite is a potent cytotoxic agent (Pryor and Squadrito, 1995; Squadrito and Pryor, 1998). Peroxynitrite can also affect antioxidant system by changing activities of antioxidant enzymes. In fact peroxynitrite has been shown to inhibit the activities of catalase (Keng et al., 2000), GSH-Px (Padmaja et al., 1998) and promote lipid peroxidation (Shi et al., 1999). Moreover, it appears that ONOO can oxidize a variety of essential molecules (e.g., sulfhydryls, thiols, ascorbate, proteins, DNA) and trigger injurious processes, including lipid peroxidation (Patel et al., 1999, Hogg and Kalynaraman, 1999). On the other hand, peroxynitrite may be decomposed forming highly reactive hydroxyl radical (•OH) and nitrogen dioxide (Beckman et al., 1990; Augusto et al., 1994).

The amount of nitrates and nitrites in the diet vary substantially. However, in combination with other prooxidants in the digesta they can be involved in free radical formation and lipid peroxidation.

Nitrites and nitrates
Nitrite is consumed in the diet, through vegetables and drinking water and it is also added to meat products as a preservative (Cammack et al., 1999). In fact, nitrates and nitrites, are common antimicrobial agents used in the food industry and have been used for centuries as preservatives in a number of red meat, poultry, and fish products. Nitrite contributes to the flavour, reacts with myoglobin producing the characteristic pink colour of cured meat and inhibits the growth of food spoilage bacteria (Cammack et al., 1999). The currently allowable daily intakes for nitrites and nitrates are 8 and 220 milligrams, respectively (Committee on Food Additives, 1974). In addition to added nitrate and nitrite to the food as preservatives, vegetables (e.g., lettuce, potato, etc.) comprise a major source of nitrates (Cammack et al., 1999). Therefore, humans are subjected to significant nitrate and nitrite levels in foods and water, as well as those formed in vivo. In fact, nitrates and nitrites formed from nitrogenous sources by micro-organisms in saliva and intestine, are considered to be the major source of human exposure under physiological conditions (Chow and Hong, 2002).

It is generally accepted that nitrate is concentrated in the saliva and rapidly converted to nitrite by facultative anaerobic bacteria. Benjamin et al. (1994) showed that nitrite is converted to NO under the highly acidic conditions (pH 3) which occur in the lumen of the stomach. It was observed that the generation and accumulation of NO from typical nitrite concentrations found in biological tissues increases 100-fold when the pH falls from 7.4 to 5.5 (Zweier et al., 1999). Therefore nitrate and nitrite can generate NO* radical by either direct disproportionation or reduction under the acidic and highly reduced conditions (Chow and Hong, 2002). More importantly, NO* can be a source of another reactive free radical peroxynitrite (ONOO-), which is 1000 times more oxidizing than H2O2 and has half-life in solution about 1–2 seconds (Van Dyke, 1997). It is well accepted that peroxynitrite is a potent cytotoxic agent (Pryor and Squadrito, 1995; Squadrito and Pryor, 1998). Peroxynitrite can also affect antioxidant system by changing activities of antioxidant enzymes. In fact peroxynitrite has been shown to inhibit the activities of catalase (Keng et al., 2000), GSH-Px (Padmaja et al., 1998) and promote lipid peroxidation (Shi et al., 1999). Moreover, it appears that ONOO can oxidize a variety of essential molecules (e.g., sulfhydryls, thiols, ascorbate, proteins, DNA) and trigger injurious processes, including lipid peroxidation (Patel et al., 1999, Hogg and Kalynaraman, 1999). On the other hand, peroxynitrite may be decomposed forming highly reactive hydroxyl radical (•OH) and nitrogen dioxide (Beckman et al., 1990; Augusto et al., 1994).

The amount of nitrates and nitrites in the diet vary substantially. However, in combination with other prooxidants in the digesta they can be involved in free radical formation and lipid peroxidation.

Heavy metals
Agricultural uses of phosphate fertilizers and sewage sludge and industrial uses of cadmium have been identified as a major cause of widespread dispersion of the metal at trace levels into human foodstuffs. Approximately, 1/3 of cadmium dietary intake is attributed to the ingestion of animal products, while plant products provide the remaining 2/3 (Nasreddine and Parent-Massin, 2002). Recently it has been calculated that daily cadmium intake to be 30 µg being within the safe intake level of 70 µg per day recommended by WHO/FAO (Satarug et al., 2003). The dietary intakes of lead...
for European countries vary between 16 µg/day (Spain) and 280 µg/day (Italy) and intakes of arsenic and mercury comprise 38-286 µg/day and 0.7-13.5 µg/day respectively (Nasreddine and Parent-Massin, 2002). Mercury is a common contaminant of fish. For example, fish with a total mercury content between 0.5 and 1.5 ppm include fresh and frozen tuna, swordfish and shark. Mercury levels in freshwater fish vary, but in general bass, pike, muskellunge and walleye have high levels (Wooltorton, 2002).

It is well known that heavy metals can cause oxidative stress and stimulate lipid peroxidation. For example, cadmium increased lipid peroxidation in liver, kidney, and testes of rats and reduced metallothionein and total sulphhydryl in liver and kidney (Khandelwal et al., 2002). In renal tubular epithelial cells of rats significant decrease in activities of GSH, GSH-Px, SOD and increased MDA formation were observed as a result of the treatment with lead and cadmium (Wang et al., 2002). In fact prooxidant effect of cadmium is much more complex. Cadmium depletes GSH and protein-bound sulphhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide, hydroxyl radicals, and hydrogen peroxide (Stohs et al., 2001). These reactive oxygen species result in increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression, and apoptosis. Enhanced production of nuclear factor-kappaB and activation of protein kinase C occur. Furthermore, the p53 tumor suppressor gene is involved in the cascade of events associated with the toxicities of these cations (Stohs et al., 2001). On the other hand, reduced glutathione (GSH) and α-tocopherol offer significant protection against cadmium toxicity in rats by diminishing oxidative stress via raising GSH concentration and reducing lipid peroxidation (Singh and Rana, 2002).

It is believed that lead can alter certain membrane bound enzymes and may cause oxidative stress. For example, exposure of HepG2 cells to lead ions decreased cell viability and stimulated lipid peroxidation of cell membranes decreasing the fluidity in the polar surface of cell membranes (Chen et al., 2002). Levels of lipid peroxidation products such as malondialdehyde, conjugated diene and hydroperoxide were increased in liver, lung and kidney of lead-treated rats. Administration of exogenous antioxidants in the lead treated animals significantly reduced the prooxidant effect of the toxicant (Upasani et al., 2001). Consumption of lead in drinking water by rats imposed oxidative stress increasing lipid peroxidation in peripheral blood mononuclear cells and liver (Ercal et al., 2000).

Mercury is also a strong prooxidant able to increase lipid peroxidation and decrease GSH content in liver of Swiss albino mice. In serum of HgCl2 treated mice there was significant elevation in glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities but significant decline in the alkaline phosphatase activity (Sharma et al., 2002). Similarly, mercury treatment enhanced lipid peroxidation in kidney, testis and epididymus of rats (Mahboob et al., 2001). Mercury can interact with other potential prooxidants. For example, mercury or selenite when injected alone, did not alter hepatic or renal lipid peroxidation in mouse, however, simultaneous exposure to these compounds significantly increased lipid peroxidation (Farina et al., 2003).

Heavy metal concentrations in major food sources are quite low, however, in combination with other prooxidants they potentially can be involved in generation of free radicals and causing oxidative stress in the GIT.

Persistent organic pollutants

Persistent organic pollutants (POPs) comprise a class of chemicals that are among the most insidiously dangerous compounds and it includes many organochlorine pesticides. Examples of persistent organic pollutants found in food include dioxins, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and some pesticide chemicals (Jensen et al., 2001). Recently, typical daily diets were constructed for four regions in the US, reflecting foods typically eaten in each region. The data were then analysed to determine the number and concentrations of POPs residues found in each food item for each of these daily meal plans. Analysis of the data showed that POPs residues are present in virtually all categories of food, including baked goods, fruit, vegetables, meat, poultry and dairy products (Schafer and Kegley, 2002). For example DDT and its metabolites were found in 21% of samples tested in 1998 and 22% in 1999 and dieldrin was found in 10% of samples tested in 1998 and 12% in 1999. Therefore a typical daily diet in the western US could potentially deliver 66 “hits” (hit is defined as the occurrence of one POP chemical in a single food item) of POPs exposure (Schafer and Kegley, 2002). Residues of organochlorine pesticides were detected in 21% of total diet samples in Spain (Lazarro et al., 1996) with eggs, legumes, fish and meat being major sources of those contaminants. About 60% of the samples of organic vegetables in the USA are found to contain pesticides and contaminated with organochlorine insecticide residues (Benbrook, 2002). Market samples (60) of six seasonal vegetables were monitored during 1996-1997 in India to determine the magnitude of pesticidal contamination by insecticide residues representing four major chemical groups i.e. organochlorine, organophosphorous, synthetic pyrethroid and carbamate. The tested samples showed 100% contamination with low but measurable amounts of residues (Kumari et al., 2002). In 1998, oranges, peaches, carrots and spinach were analysed for 20 pesticides in the EU and about 32% of samples contained residues of pesticides at the levels legally permitted in specific food items and 2% samples exceeded that level (Nasreddine and Parent-Massin, 2002). Similarly in 1999 residues of pesticides were found in melons (32% samples), peppers (24%), wheat grains (21%) and cauliflower (17%; Nasreddine and Parent-Massin, 2002). On the other hand, organochlorines can cause oxidative stress. For example, the adverse effect of organochlorine pesticide methoxychlor on the male reproductive system was shown to be due to
induction of oxidative stress in testis (Latchoumycandane and Matthur, 2002; Latchoumycandane et al., 2002). Similarly, a pesticide hexachlorocyclohexane (HCH) compromised antioxidant defence and induced oxidative stress in rat cerebral hemisphere (Sahoo et al., 2000).

Dieldrin exposure caused depolarization of mitochondrial membrane potential in a dose-dependent manner, stimulated generation of ROS and significantly increased lipid peroxidation (Kitazawa et al., 2001). The effect of the cyclodiene organochlorine pesticides aldrin, dieldrin and endosulfan was assessed on CHO-K1 cultures. Unlike oxidised glutathione, the content of total glutathione declined significantly after exposure to cyclodiene insecticides. Membrane leakage and peroxide production were significantly enhanced by the pesticides (Bayoumi et al., 2001). Recent experimental studies indicate that dieldrin-induced production of reactive oxygen species, depletion of hepatic antioxidant defences (particularly α-tocopherol), and peroxidation of liver lipids (Stevenson et al., 2001). Furthermore, dieldrin-induced oxidative stress could be associated with modulation of gene expression. Oral administration of DDT to rats dose-dependently increased thiobarbituric acid reactive substance levels in serum after 8 wk of treatment. In addition, such DDT exposure markedly suppressed the humoral immune response as assessed by anti-sheep RBC antibody titres (Koner et al., 1998). Simultaneous treatment with ascorbic acid (100 mg/kg) markedly attenuated the effects of DDT on lipid peroxidation and humoral immune suppression indicating the possible involvement of free radicals in organochlorine-induced immunotoxicity. Similarly, in chickens hydrogen peroxide and lipid peroxide formation significantly increased in kidney, lung and intestine as a result of their treatment with DDT (Hatolkar and Pawar, 1995). The results of Hassoun et al. (1993) demonstrated that the four structurally dissimilar polyhalogenated hydrocarbons (lindane, DDT, chlordane and endrin) could produce oxidative stress in rats with a significant increases in hepatic lipid peroxidation (lindane, DDT, chlordane and endrin) could produce oxidative stress in rats with a significant increases in hepatic lipid peroxidation and DNA damage. In general, in all those studies POPs concentrations tested were much higher than in contaminated food, however, if they are present in combination with each other or with other prooxidants their detrimental effects could be multiplied. Even if the levels of those are in the accepted limits, they are still able to participate in lipid peroxidation. Therefore, persistent organic pollutants represent an important health hazard for humans and they also can be involved in promotion of lipid peroxidation in the GIT.

**Mycotoxins**

At least 25% of world's grain production is contaminated with mycotoxins, which are a worldwide problem (Fink-Gremmels, 1999). For example, aflatoxins are considered to be unavoidable contaminants of food, since they cannot be prevented or eliminated by current agricultural practice (Peralca and Domijan, 2001). The intake of aflatoxin M1 from milk was calculated to vary from 0.1 ng/person/day in Africa up to 12 ng/person/day in Far Eastern countries (Creppy, 2002). Milk and dairy products purchased at Egyptian markets and breast milk from lactating mothers were analysed. Three of 15 cow's milk samples were found positive for AFM1 with mean value 6.3 μg/kg. Twenty percent of hard cheese samples contained detectable levels of AFM1 and one sample from ten contained AFB1 and AFG1. For breast milk, two of ten samples were positive for AFM1 with mean value 2.75 μg/kg, while 3 of 10 samples were positive for OA (Ei-Sayed et al., 2000). In Turkey, the incidence of AFM1 in cheese was 89.5% with the highest concentration to be 810 ng/kg (Huseyin and Sonal, 2001). From fifty four samples of fresh full cream and skimmed milk, powdered milk, yoghurt and infant formula collected in Kuwait, 28% were contaminated with AFM1 with 6% being above the maximum permissible limit of 0.2 μg/L (Srivastava et al., 2001).

Therefore the chance of getting traces of mycotoxins in our diet are very high. For example, the results of survey of 313 UK retail foods and 153 UK cereal samples showed that ochratoxin A was detected in 25% samples with 27 samples containing OA at concentrations above 4 ng/g (Atkins and Norman, 1998). OA was found in a number of samples of feed and food from various countries with its detection in human blood (Peralca and Domijan, 2001). In fact, ochratoxin can be detected at levels greater than 0.1 ppb in more than 90% of human and swine blood samples in central European countries (Petzinger and Weidenbach, 2002). Ochratoxin has been found in human blood samples in number of countries in cool temperate areas of the Northern Hemisphere (Creppy, 2002). When blood analyses were performed in Scandinavian blood donors the mean plasma levels of OA was 0.18 μg/L in Oslo and 0.21 μg/L in Visby (Thuvander et al, 2001). In particular in Croatia OA consumption was estimated to be 0.4 ng/kg body weight (Peralca and Domijan, 2001). Ochratoxin in combination with aflatoxin showed a synergistic toxicity (Campbell et al., 1983). For ochratoxin the elimination half life in human was calculated to be 840 hours (Creppy, 2002). In the UK composite duplicate diet samples from 50 individuals and corresponding plasma and urine samples were obtained over 30 days. Average intake of OA varied in a range of 0.24-3.54 ng/kg body weight/day and OA was detected in all plasma samples and in 92% of urine samples (Gilbert et al., 2001).

In Germany the absolute OA intake was approximately 28.7-290.8 ng/day in 1996-1999 and the calculated total daily intake of OA varied between 0.9 ng/kg body weight in Germany and 4.6 ng/kg body weight in Italy (Petzinger and Weidenbach, 2002). It is necessary to mention that OA altered both barrier and absorption function of the intestinal epithelium causing intestinal injuries, including inflammation and diarrhea (Maresca et al., 2001). Consumption of mouldy sorghum or maize containing high levels of fumonisins B1 caused an outbreak a human disease in India involving gastrointestinal symptoms (Creppy, 2002). Estimated intake of fumonisins in Europe, Far East, Latin America, Middle East and Africa comprises (mg/kg body weight) 0.2, 0.7, 1.0, 1.1
Many outbreaks of acute human disease involving gastrointestinal upset and diarrhoea have been resisted in Asia due to consumption of Fusarium-contaminated grains (Creppy, 2002). DON was detected in various food products (bread, breakfast cereals, beer, baby and infant foods) in Europe and Northern America (Creppy, 2002). It is interesting that inhibition of protein synthesis and induction of apoptosis are the main mechanisms of DON toxicity in intestinal cells (Maresca et al., 2002). When 88 commercially available samples of wheat-based breakfast cereals were randomly collected from different supermarkets in Lisbon, 72.8% samples contained levels of DON between 103 and 6040 µg/kg with mean level of 754 µg/kg (Martins and Martins, 2001). Patulin, a common contaminant of apples and apple products, is shown to affect the barrier function of the intestinal epithelium by inducing epithelial cell degeneration, inflammation, ulceration and hemorrhages (Mahfoud et al., 2002). In general, main mycotoxin contaminants of the food including AFB1, fumonisins, T-2 toxin, DON, zearalenone and ochratoxin are shown to compromise antioxidant system and stimulate lipid peroxidation in vivo and in vitro (Surai, 2002).

Mycotoxins are considered to be unavoidable contaminants of the most food and feed ingredients and they are potent prooxidants. Therefore even in comparatively low concentrations which are lower than officially allowed limits they still represent an important source of free radical generation in the GIT.

**Alcohol**

Exposure of the intestinal mucosa to ethanol leads to morphological injuries and impairs the absorptive capacity creating an oxidative stress in the small intestine (Kaur et al., 1998). The diet seems to affect this process since enrichment of the rat diet with fish oil increased the detrimental effect of alcohol. The in vivo formation of the alpha-hydroxyethyl free radical metabolite of ethanol has been demonstrated (Knecht et al., 1990). Free radical formation in ethanol-treated rats has been detected (Knecht et al., 1995). In the study of Reinke et al. (1997) the spin trapping method was used to assess formation of free radical intermediates in vivo before and after acute alcohol administration to rats. Ascorbyl radicals and spin adducts of dietary alcohol or endogenous compounds, such as lipids, were detected with higher frequency in bile from alcohol-fed rats than in corresponding samples from rats fed control diets. Furthermore, formation of lipid radicals was enhanced after acute alcohol administration. Liver microsomes from alcohol-fed rats also metabolized ethanol to the 1-hydroxyethyl radical at higher rates than controls. During ethanol metabolism the antioxidant system is compromised, with reduced levels of vitamin E (Kawase et al., 1989), selenium (Aaseth et al., 1980; Dworkin et al., 1985) and reduced glutathione (Trimble et al., 1993). Therefore it has been suggested that the underlying mechanism of intestinal epithelial barrier dysfunction induced by EtOH is due to oxidative injury to the cytoskeleton (Banan et al., 2000). This hypothesis includes following points:

- Ethanol causes iNOS up-regulation
- Activation of the iNOS enzyme leads to intracellular increases in NO and ONOO that causes oxidative stress oxidising cellular proteins such as tubulin.
- Tubulin oxidation causes disruption in the microtubule cytoskeleton, leading to a loss in integrity of the intestinal barrier, and predispose the organism to inflammation.

Therefore, alcohol can be involved directly or indirectly in the free radical production and lipid peroxidation in the intestine. Furthermore its effects on intestinal immune system and on inflammation in particular are further investigated. It seems likely that alcohol in combination with other prooxidants can be a source of free radicals in the GIT.

**Immune system**

The immune system is considered to be an important source of ROS in the human body (Sural, 2002) and intestinal immunity is not an exception. Indeed, the intestinal tract is considered to represent the largest immune organ of the human body responding to the challenge of bacteria or food antigens by production of ROS (Halliwell et al., 2000). Mucosal surfaces covered by a layer of epithelial cells represent the most critical interface between the organism and its environment since the mucosal interstitia of the intestine is continuously exposed to large amounts of dietary and microbial antigens. Therefore epithelial cells engage in cross talk with luminal bacteria and their products and produce mediators and signals that are key components of host innate and acquired mucosal immunity (Maser and Kagnoff, 2002). The mucosal immune system is a first line of defense against foreign antigens, including microbial and dietary antigens and under normal circumstances it employs tightly regulated dynamic mucosal intra- and interets consisting of inductive (e.g. Peyer’s patch) and effector (e.g. intestinal lamina propria) tissues and maintains an appropriate immunological homeostasis between the host and mucosal environments (Kiyono et al., 2001). Therefore the mucosal immune system has evolved efficient mechanisms to distinguish potentially pathogenic from nonpathological antigens. For example, the mucosal immune compartment must be able to choose the appropriate effector function (e.g., tolerance vs. clearance) necessary to deal with each encountered antigen whether it be innocuous or pathogenic in nature (Laroux et al., 2001).

However, abrogation of these mucosal defence mechanisms may alter immunological homeostasis in the gastrointestinal tract and induce pathological changes including chronic active inflammation, mucosal atrophy and tissue injuries (Nagura et al., 2002). It is important to stress that under inflammatory conditions in the intestine the maintenance of the epithelial barrier could be broken.

Diet is a potent mechanism for altering the environment of cells of most organs, particularly the gastrointestinal tract. Nutrient metabolism provides an essential stimulus for the
induction, differentiation, and maintenance of the mucosal immune system (Cunningham-Rundles, 2001). Therefore changes in diet, through the composition of the lumen environment, alter the expression of genes encoding for proteins that signal to the mucosal immune system (Sanderson and Naik, 2000). In fact enterocytes act as immune cells having receptors for bacterial products and expressing a variety of molecules on their surface responsible for interaction with immunocytes within the intestine (Sanderson, 2001). Macrophage inflammatory protein 2 (MIP-2) is a chemokine that attracts neutrophils, and its secretion from intestinal epithelial cells is enhanced by inflammatory stimuli such as interleukin 1-beta and the production of MIP-2 by epithelial cells is responsible for increase leukocyte migration into the intestine (Ohtsuka et al., 2001). In particular, increased neutrophil migration into the intestine and activation of myeloperoxidase were shown to be a result of induced inflammatory bowel disease-like colitis in transgenic mice (Ohtsuka and Sanderson, 2003). Inflammatory responses involve the recruitment of specific leukocyte subsets. In this process non-pathogenic, resident bacteria have a specific role in the pathogenesis of inflammation in the small intestine releasing butyrate, a normal bacterial metabolite, and modulating chemokine secretion by epithelial cells (Ohno et al., 1997). Therefore, epithelial cells, apart from their participation in absorptive, digestive and secretory processes perform more than a passive barrier function and are directly involved in immune processes (Tlaskalova-Hogenova et al., 1995). Moreover, in specific pathological conditions enterocytes could become a target of mucosal immune factors. For example, the intestinal immune response to enteric antigens includes production of T helper-1-type cytokines which may affect the gut epithelium directly and/or activate a resident macrophage to release large amounts of proinflammatory mediators: cytokines as well as reactive oxygen species (ROS) and nitric oxide (NO). The result is the recruitment of additional leukocytes and subsequent tissue injury (Laroux et al., 2001).

Macrophage migration inhibitory factor (MIF) inhibits macrophage migration and has pleiotropic activities on immune and inflammatory responses, cell growth, and glucose metabolism. MIF is produced by T cells, macrophages, and endothelial cells. It has been shown recently that human intestinal epithelial cells are a major source of MIF (Maaser et al., 2002). It has been also suggested that paracrine factors of intestinal epithelium increased the phagocytic capacity of intestinal monocytes/macrophages to be ready for immune and inflammatory responses (Kanzato et al., 2001). A characteristic feature of inflammation is the concomitant peroxidation of lipids and formation of bioactive lipid peroxidation products, some of which are considered to be potent chemottractants, pro-inflammatory eicosanoids and other signalling molecules. Recent studies demonstrated a principal role for myeloperoxidase (MPO), a heme protein secreted by activated neutrophils, monocytes, and some macrophages, in promotion of oxidant stress at sites of inflammation. It has been shown that this enzyme serves as a major enzymatic catalyst of lipid peroxidation by formation of ·NO-derived oxidants (Zhang et al., 2002). Furthermore, in response to various stimuli intestinal epithelial cells can produce nitric oxide (Witthoft et al., 1998) and gastric surface mucous cells possessed a phagocyte NADPH oxidase-like system and secreted abundant superoxide anion (Rokutan, 1999).

It is necessary to stress that the nutrient requirement to maintain a highly active immune system in the digestive tract could be quite high. In fact different sources of injury to the intestinal mucosa (nutritional, infectious or allergic) act via a common mechanism of cell-mediated immune damage and nutrient repletion is required for restoration of immune function (Cunningham-Rundles, 2001). In particular, the immunomodulating properties of natural antioxidants (Surai, 2002) could be of great advantage for the intestinal immunity. Furthermore, intestinal epithelium can modulate the level of immune activity in the mucosal immune system according to the environment of the intestinal lumen (Sanderson, 1999). Data presented above indicate that intestinal immune system can generate free radicals in response to various antigens including microbes and some food allergens.

Physicochemical environment of the gastrointestinal tract depends on many factors with diet, bacterial metabolites and body secretion being major determinants (Sanderson, 1999). There is a delicate balance between the environment of the lumen and epithelial cell functionality and dietary factors are responsible for gene expression in the intestine and its adaptation. In this regard, oxidative stress could cause changes in this balance affecting absorption of nutrients. Even if each of those lipid peroxidation promoters is present at a very low concentration, their combination could be much more powerful. For example, as mentioned above lipid hydroperoxides can produce peroxy radicals in presence of iron or copper ions and once chain reaction of lipid peroxidation started many other food-derived PUFAs can be oxidized. It was calculated that pH and temperature, as well as presence of oxygen in stomach could be favourable for lipid peroxidation (Kanner and Lapidot, 2001). Fortunately, our diet contains a range of antioxidants, which can prevent or decrease lipid peroxidation in the digestive tract.

CONCLUSIONS

Relationship between food and human health has attracted attention of scientists for many years. It is now widely accepted that fruits and vegetables are important dietary components responsible for maintenance of good health. However, molecular mechanisms of protective effects of fruits and vegetables have not been fully elucidated. One of the attractive ideas is that various antioxidant compounds of fruits and vegetables are responsible for prevention of oxidative damage in digestive tract. In fact, antioxidant-prooxidant balance in digestive tract is considered to be a major determinant of human and animal health.

Data provided above indicate that average diet contains a
range of various prooxidants. They include oxidized polyunsaturated fatty acids, oxysterols, nitrates, nitrites, heavy metals, mycotoxins, persistent organic pollutants, alcohol, etc. In many cases those contaminants are found in the food in low or very low concentrations, however, their various combinations in the food could be an important source of free radical production in the gut. Therefore an effective antioxidant protection in the gastrointestinal tract is needed to maintain gut health and this protection is based on food derived antioxidants.

ACKNOWLEDGMENTS
We are grateful to the Scottish Executive Environment and Rural Affairs Department for financial support and to the WPSA for the Research Award to PFS.

CONFLICT OF INTEREST DISCLOSURE: There is no conflict of interest.

REFERENCES


