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ANTIOXIDANT-PROOXIDANT BALANCE IN THE INTESTINE: FOOD FOR THOUGHT 2. ANTIOXIDANTS

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ABSTRACT: This paper further develops the hypothesis presented earlier that the antioxidant-pro-oxidant balance in the intestine is an important determinant of human health. A wide range of evidence in favour of this hypothesis is described. When food is consumed it contains a range of antioxidants including vitamin E, coenzyme Q, carotenoids, vitamin A, ascorbic acid, reduced glutathione, selenomethionine, flavonoids and other polyphenolics. It could also contain some spices, synthetic antioxidants and other compounds possessing antioxidant properties. They balance the prooxidants found in the food. Since we cannot avoid pro-oxidants in our food we need to make sure that they are compensated by consumption of increased levels of natural antioxidants. It would be advantage if our meat and fish meals are served with plenty of vegetables. Various sauces (e.g. tomato sauce) could provide additional antioxidants. Various juices are also good sources of natural antioxidants as well as fruits. If a meal is finished with tea, this will also add to antioxidant potential of the digesta.

KEY WORDS: Antioxidants, Balance, Food, Health, Intestine, Prooxidants

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INTRODUCTION

In the first part of the review (Surai et al., 2004) evidence has been presented showing that average diet contain a range of various prooxidants. They include oxidized polyunsaturated fatty acids and oxysterols which are formed during food processing and cooking. In particular meat and fish meals are considered to be major sources of those prooxidants. Nitrites and nitrates are also common compounds in the food often used as additives for food preservation and other specific purposes. Heavy metal contamination in many cases is related to fish consumption, however, mycotoxin contamination of the food is a worldwide problem. Furthermore persistent organic pollutants represent another potential hazard for human health. Alcohol can also promote lipid peroxidation and immune system of the gastrointestinal tract is considered to also produce free radicals. 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. Fortunately, food also contains a range of antioxidants, which can successfully compensate those prooxidants and maintain healthy gut.

ANTIOXIDANT DEFENCES IN THE GIT

Vitamin E

Vitamin E is main biological chain-breaking antioxidant, found in food in the form of 4 tocopherols and 4 tocotrienols. Biological activity of vitamin E in tissues is mainly due to α-tocopherol, but in food the main form of vitamin E is γ-tocopherol. It is possible that the gut is a special place for γ-tocopherol and tocotrienols to play their antioxidant role. Vitamin E is not stable and is easily oxidized during food processing. Synthetic antioxidants added to the food can inhibit vitamin E oxidation. Vitamin E (α-tocopherol) in the tablet or capsular form is mainly produced in the stable esterified form or as a mixture of tocopherols.

The major vitamin E sources in the diet are vegetable oils (wheat, soybean, sunflower and corn) and some other plant-derived foods. For example, in middle-aged Japanese, vitamin E was mainly of vegetable origin with main contributions coming from spinach, safflower oil and pumpkin (Imaeda et al., 1999). In the UK, the average daily vitamin E intake is 11.7 mg in men and 8.6 mg in women. Similar consumption was reported in the USA and other countries (Weber et al, 1997) with margarines and mayonnaise supplying 23% of total vitamin E consumed. These levels are in the line with the RDA. In fact the Food and Nutrition Board of the Institute of Medicine recently published dietary reference intakes for vitamin E, which is 15 mg for adults being 50% greater than the generous allowance in the 10th edition of Recommended Dietary Allowances published in 1989 (Horwitt, 2001). It has been concluded that, according to the RDA, the intake of antioxidants is adequate in healthy subjects (Diplock et al, 1998). However, the recent data of Bodner et al. (1998) indicate that vitamin E intake in Scotland is 6.6 mg/day for women and 7.3 mg/day for men, comprising only 50% of the RDA and being lower than that in other European countries.

In addition, there are several categories of people whose vitamin E consumption is lower than the RDA. For example it is generally believed that elderly people have inadequate vitamin E consumption (Cid-Ruzafa et al, 1999). Rudman et al (1995) showed that more than 60% of institutionalised elderly people consume less than 50% of the ideal dietary intake of vitamin E.

Vitamin E deficiency is associated with a development of a range of specific diseases involving major tissues of the organism including immune system incompetence, impairment of lipid metabolism, fertility problems and increased susceptibility to common and specific diseases (Machlin, 1991). There are also several clinical conditions where vitamin E deficiency states are described: vitamin E malabsorption syndrome, abetalipoproteinemia, chronic childhood cholestasis, cystic fibrosis, total parenteral nutrition and prematurity (VERIS, 1998). Vitamin E can not be synthesised in the human and its adequate intake relies upon adherence to a well balanced diet (Thurman and Mooradian, 1997).

It has been suggested that by enhancing the intake of vitamin E by fortification of foods or by dietary supplements it may be possible to reduce the risk of many common, yet disabling human diseases (Diplock, 1997). Furthermore, there are many studies suggesting that intake of vitamin E in amounts much higher than RDA are associated with reduced risk of various diseases (Bendich et al, 1997; Weber et al., 1997; Diplock et al, 1998; Chan, 1998) and with enhancement of certain immune responses (Meydani, 1995). During the past decade, the health benefits of vitamin E have been shown in several epidemiological studies (Meydani, 2000). For example, epidemiological evidence shows a lower incidence of infectious disease in subjects with high plasma tocopherol concentrations (Ayres and Mihan, 1978; Nockels, 1979; Chevance et al., 1985). In this respect, Lachance (1996) has shown that the optimal daily antioxidant intakes are 23 mg for vitamin E and 3.2 mg for carotene. It is interesting that about one half of American cardiologists take supplemental vitamin E (Pryor, 2000) even so results of clinical trials with vitamin E supplementation were not as successful as expected.

Vitamin E is considered to be not toxic for humans and a daily dosage of 100-300 mg vitamin E can be considered harmless from a toxicological perspective and therapeutic vitamin E doses start at several hundred mg/day and end at approximately 1,600 mg/day (Kappus and Diplock, 1991). Clearly, vitamin E can be considered as a main contributor to the antioxidant potential of the digesta.

Coenzyme Q

Coenzyme Q, known also as ubiquinone, was discovered in 1957. The name ubiquinone is related to its 'ubiquitous" presence in all cells and the name coenzyme Q reflects the chemical structure of the compound containing one quinone group and 10 isoprenyl units. These two names have been used interchangeably for more than 40 years. Coenzyme Q_{10} (CoQ_{10}) exists both in an oxidised and a reduced form, ubiquinone and ubiquinol, respectively (Overvad et al., 1999). It was found that the ratio of ubiquinol-10 to ubiquinone-10 was about 95/5 in human plasma from healthy donors. A significant increase in the oxidized form (ubiquinone-10) content was observed in plasmas of patients with hepatitis, cirrhosis, and hepatoma when compared with normal subjects, reflecting increased oxidative stress in these patients (Yamamoto and Yamashita, 1997). Therefore the ubiquinol/ubiquinone ratio is considered to be a sensitive marker of oxidative stress and an altered ubiquinol/ubiquinone ratio is the first sign of lipoprotein exposure to oxidative stress (Lagendijk et al, 1997).

The highest concentrations of CoQ_{10} in human tissues are found in heart, liver and kidney (70-110 μg/g) and the lowest concentration (8 μg/g) was detected in the lung. In human plasma CoQ_{10} was found in the range of 0.75-1,00 μg/ml with the total content in the body to be about 1.0-1.5 g (for review see Overvad et al., 1999). A statistically significant circadian rhythm in human plasma CoQ_{10} concentration was demonstrated, but no differences were found in CoQ_{10} concentration between male and female subjects. On the other hand, some racial differences were demonstrated with lower plasma CoQ levels in Caucasian compared to African subjects (Reis et al., 2002). Coenzyme Q is the only fat-soluble antioxidant synthesised in the body. Therefore, tissue CoQ originates from endogenous synthesis and from food. In fact, there are two major forms of CoQ in the human tissues namely CoQ_{10} comprising 95-97% in heart, kidney, liver and spleen and 92% in brain and 87% in lung (Dallner and Sindelar, 2000), the rest of this compound is represented by CoQ_9. In accordance with CoQ_{10} concentration human tissues can be placed in the following descending order: muscle>>heart>kidney> liver>>spleen>>brain> lung (Dallner and Sindelar, 2000).

Contents of CoQ_{10} in foods varied from 157.9 mg/g to below the detection limit with meat, meat products (55%) and fish (9%) to be major sources of this compound (Table 1). Vegetable oils, dairy products, vegetables and fruits plus berries provide 18%, 8%, 6% and 4% of daily CoQ_{10} consumption respectively. In the same foods CoQ_9 is also found varying from 8.5 mg/g (reindeer), 0.4 mg/g (beef and chicken) down to lower levels in other foods. Daily intake of CoQ_{10} was calculated to be 5.4 mg for men and 3.8 mg for women and CoQ_9 intake was shown to be 0.6 mg/day for men and 0.4 mg/day for women (Mattila and Kumpulainen, 2001). It is believed that CoQ is quite stable during food cooking loosing only 15-30% its initial value (Weber et al., 1997a).

CoQ_{10} is characterised by comparatively slow absorption in humans with blood values reaching a maximum after 6 h following intake of 100 mg CoQ_{10} with a secondary peak been shown in the blood 24 h after intake with half-life in the plasma to be about 33 h (Overvad et al., 1999). Potentially, slow absorption of CoQ could be an advantage for antioxidant protection in the GIT. Animal studies showed that the total

Table 1. Contents of Coenzyme Q_{10} in foods, μg/g fresh wt (Adapted from Mattila and Kumpulainen, 2001)

Food	Coenzyme Q_{10}	Food	Coenzyme Q_{10}
Reindeer	157.9	Pea	2.7
Pork heart	126.8	Cauliflower	2.7
Beef heart	113.3	Bean	1.8
Beef liver	39.2	Carrot	1.7
Pork liver	22.7	Tomato	0.9
Beef	36.5	Potato	0.5
Pork ham	20.0	Black currant	3.4
Chicken	14.0	Strawberry	1.4
Egg	1.2	Orange	1.4
Rapeseed oil	63.5	Apple	1.3
Tuna canned	15.9	Orange juice	0.3
Baltic herring	15.9	Yogurt	2.4
Pollack frozen	14.4	Cheese	1.3
Rainbow trout	8.5	Milk (1.5% fat)	0.1

uptake of dietary CoQ supplement comprised only 2-3% (Zhang et al, 1995). However, high consumption of $CoQ_{10}$is associated with a substantial (2.8-fold) increased concentration in human plasma, but tissue response was shown to be much less pronounced. In particular, only liver and spleen responded to CoQ supplementation in rats (Dallner and Sindelar, 2000). Administration of the labelled CoQ_{10} to rats intraperitoneally resulted in an efficient uptake into the circulation, with high concentrations found in spleen, liver, and white blood cells; lower concentrations in adrenals, ovaries, thymus, and heart; and practically no uptake in kidney, muscle, and brain (Bentinger et al., 2003). In liver homogenate most CoQ appeared in the organelles, but it was also present in the cytosol and transport vesicles. In particular mitochondria had a very low concentration of labelled CoQ, which was mainly present in the lysosomes. All organs that took up the labelled lipid also contained water-soluble metabolites (Bentinger et al., 2003).

Some drugs can interfere with CoQ assimilation and metabolism. For example lovastatin and pravastatin (cholesterol-lowering drugs) decreased CoQ_{10} endogenous synthesis (for review Overvad et al., 1999). In general, tissue CoQ_{10}concentrations have been shown to decrease during ageing increasing from birth up to the age of 20-30 years, followed by a decrease to the initial birth level at around age of 80 years (Dallner and Sindelar, 2000). Furthermore decreased CoQ levels have been found in several diseases including cardiomyopathies, degenerative muscle disease and hepatocellular carcinomas (Ernster and Dallner, 1995). Some positive effects of oral CoQ supplementation have been observed in clinical trials and there were no adverse effects of CoQ daily supplements of up to 200 mg for 6-12 months or 100 mg/day for 6 years (Overvad et al., 1999; Singh et al., 1998). A moderate beneficial effect of oral CoQ_{10} supplementation in Parkinson's disease patients has been reported (Muller et al., 2003).

Those findings were used as a justification for CoQ dietary supplementation for humans. On the other hand, it is necessary to underline that CoQ concentrations in the cell are very strictly regulated and tuned to the metabolic needs depending on cell types which are able to synthesise this compound and maintain it in a reduced form. An effective enzymatic mechanism is responsible for the regeneration of ubiquinol (Aberg et al., 1992) with NADH or NADPH being a source of electrons for regeneration of the antioxidant (Beyer, 1994). Lipoamide dehydrogenase belonging to a family of pyridine nucleotide disulfide oxidoreductases and ubiquitous in aerobic organisms also reduces ubiquinone to ubiquinol, the form in which it functions as an antioxidant (Xia et al., 2001). The isolation of cell plasma membrane proteins revealed an NADH-Q oxidoreductase located on the outer plasma membrane surface, which apparently participates in the reduction process (Audi et al., 2003). Most CoQ (>84%) is free in the membrane bilayer comprising a specific CoQ pool (Lenaz, 2001). In fact excessive CoQ incorporation into membrane could potentially cause membrane destabilisation with increased fluidity and permeability with detrimental consequences for membrane functional properties. However, in physiological concentrations CoQ is considered as a membrane stabiliser based on recycling of a-tocopherol radicals (Nohl et al., 2001).

In general, dietary supplementation of CoQ does not affect the endogenous synthesis of CoQ in tissues. However, oxidative stress (physical exercise, thyroid hormone treatment, cold adaptation, vitamin A deficiency, etc.) is associated with increased CoQ synthesis reflecting a cellular adaptation (Ernster and Dallner, 1995). Therefore, CoQ synthesis is considered to be an adaptive mechanism in response to stress conditions when other antioxidants are depleted. For example, in vitamin E and Se deficient rats CoQ concentration elevated and CoQ-dependent reductase system is activated (Navarro et al., 1998).

It is well appreciated that a number of factors are involved in the regulation of the amount and distribution of CoQ in cells and tissues. These factors modify preferentially the biosynthetic mechanism in order to keep up an optimal tissue concentration of the lipid (Turunen et al., 2002). In particular, the amount of substrate provided by the mevalonate pathway is able to both up- and down-regulate the velocity of synthesis. Furthermore, at the translation level, regulation occurs by receptor-mediated ligand binding and appears most clearly upon treatment with hormones and peroxisomal inducers (Turunen et al., 2002). There are specific receptors involved in CoQ synthesis. For example, when involvement of the nuclear retinoid X receptor alpha in CoQ synthesis was investigated it

was shown that in the receptor-deficient liver, the amount of CoQ decreased to half of the control as a result of a significantly lowered rate of biosynthesis (Bentinger et al., 2003).

It is believed that exogenous CoQ, protects cells from oxidative stress by conversion into its reduced antioxidant form by cellular reductases. In particular cytosolic NADPH-CoQ reductase is responsible for cellular CoQ redox cycle as an endogenous antioxidant (Kishi et al., 1999). The plasma membrane oxidoreductase and DT-diaphorase are two such systems, likewise, they are overexposed under oxidative stress conditions (Genova et al., 2003). In addition, the selenoenzyme thioredoxin reductase is an important ubiquinone reductase and can explain how selenium and coenzyme Q, by a combined action, may protect the cell from oxidative damage (Xia et al., 2003). Administration of CoQ_{10} increased plasma and mitochondria levels of CoQ_{10} in rats with a greater magnitude of the increases after 13 weeks than 4 weeks. In particular, CoQ_{10} was primarily incorporated into low-density lipoprotein (Sunesen et al., 2001). A reductive shift in plasma aminothiol status and a decrease in skeletal muscle mitochondrial protein carbonyls were observed after 13 weeks of supplementation (Kwong et al., 2002). Thus, CoQ supplementation resulted in an elevation of CoQ homologues in tissues and their mitochondria, a selective decrease in protein oxidative damage, and an increase in antioxidative potential in the rat.

Possible mechanisms of dietary CoQ_{10} administration on organ function include (Dallner and Sindelar, 2000):

- Direct CoQ organ uptake
- Modulation of signal transduction system
- Influence on organ circulation/vasculature
- Antioxidant effects in the gastrointestinal system
- Effects of CoQ metabolites
- Specific protective effects in GIT

Since CoQ is an essential part of oxidative phosphorylation complex in mitochondria the majority (molar amounts) of endogenous CoQ is found in these organelles. However, exogenous CoQ is usually found in the extramitochondrial fractions including lysosomes and Goldgi vesicles (Dallner and Sindelar, 2000). Catabolic pathways of CoQ have not been fully elucidated and it was shown that two major catabolic derivatives of CoQ in both urine and feces had an intact quinone ring and shortened side chain and they were in conjugated (disulphated or glucuronidated) form (Dallner and Sindelar, 2000). Therefore the majority of CoQ metabolites excreted through the kidney and appeared in the urine. Some metabolites were also present in the feces, which further contained nonmetabolized CoQ, excreted through the bile. Indeed, nonmetabolized CoQ could provide antioxidant protection in small and large intestine. The major metabolites were purified from the urine, and the mass spectrometric fragmentation showed that these compounds, containing the ring with a short side chain, are phosphorylated (Bentinger et al., 2003). Thus, CoQ is metabolized in all tissues, the metabolites are phosphorylated in the cells, transported in the blood to the kidney, and excreted into the urine.

Coenzyme Q function in the cell include (Nohl et al., 2001):

- Proton-motive Q cycle of oxidative phosphorylation in mitochondria
- Electron and proton carrier in plasma membranes, lysosomes and Goldgi vesicles
- Antioxidant in mitochondria and LDL
- NO_2^- reduction to NO in mitochondria

Considering antioxidant function of CoQ it is necessary to mention its unique position in antioxidant system (Ersner and Dallner, 1995; Lass and Sohal, 1998; Stoyanovsky et al., 1995; Crane and Navas, 1997):

- Membrane localisation at sites of lipid peroxidation
- Endogenous synthesis providing an adaptive response to stress conditions
- Enzymatic powerful recycling maintaining CoQ in a reduced active antioxidant form
- Effective synergistic interactions with vitamin E and possibly with other antioxidants enhancing protective activities of the antioxidant system
- Presence in molar amounts in mitochondria, a main source of free radicals in the cell, sufficient to effectively participate in antioxidant defence
- Possibilities of preventing the formation of lipid peroxyl radicals by reducing the initiating perferryl radical
- Possible direct quenching L*
- Direct or indirect eliminating LOO*
- Presence in all parts of the GIT

The presence of high concentrations of CoQ_{10} in all membranes provides a basis for antioxidant action either by direct reaction with radicals or by regeneration of tocopherol and ascorbate. In fact, a protective effect of CoQ against lipid peroxidation was shown in fatty acid emulsions, mitochondria, submitochondrial particles and other model systems (Ersner and Dallner, 1995). CoQ_{10} protects efficiently not only membrane phospholipids from peroxidation but also mitochondrial DNA and membrane proteins from free-radical-induced oxidative damage (Pobezhimova and Voinikov, 2000).

It is generally accepted that stress-induced apoptosis is mediated by the activation of plasma membrane-bound neutral sphingomyelinase with ceramide-dependent caspase activation being an important part of the apoptosis pathway. It was hypothesised that CoQ in the plasma membrane prevents both lipid peroxidation and sphingomyelinase activation resulting in the prevention of ceramide accumulation and caspase 3 activation with a consecutive apoptosis inhibition (Villalba and Navas, 2000). It has also been suggested that CoQ_{10} is a gene regulator and consequently has wide-ranging effects on over-all tissue metabolism (Linnane et al., 2002). In particular it was hypothesised that CoQ_{10} plays a major role in the determination of membrane potential of main sub-cellular membrane systems and that H_2O_2 arising from the activ-

ities of CoQ_{10} acts as a second messenger for the modulation of gene expression and cellular metabolism (Linnane et al., 2002). Evidence for a function in redox control of cell signalling and gene expression is developing from studies on coenzyme Q stimulation of cell growth, inhibition of apoptosis, control of thiol groups, formation of hydrogen peroxide and control of membrane channels (Crane, 2001).

Antioxidant properties of CoQ are directly related to the protection in the gastrointestinal tract. For example, in rats treated *per os* with sodium nitrite increases thiobarbituric-acid reactive substances in small intestinal mucosa and liver were observed. Pre-treatment of nitrite-poisoned rats with CoQ_{10} mitigated lipid peroxidation and increased total antioxidant status in animal blood (Grudzinski and Frankiewicz-Jozko, 2001). The protective effect of administered CoQ_{10} against small intestinal damage caused by ischemia reperfusion was also shown (Matsusaka et al., 1992). In rat intestine, administration of CoQ_{10} normalised a sharp gamma-irradiation-induced inhibition of transformation of phosphatidylcholine from phosphatidylethanolamine (Novoselova et al., 1985). Compared to paired noninflamed mucosa, concentration of CoQ_{10} was significantly decreased n inflamed mucosa (Buffinton and Doe, 1995). The decreased antioxidant defences may severely compromise the inflamed mucosa, rendering it more susceptible to oxidative tissue damage, hindering recovery of the mucosa and return of epithelial cell layer integrity. Therefore, antioxidant and other regulating functions of CoQ_{10} could be extremely important in the GIT.

Carotenoids

Carotenoids comprise a family of more than 600 compounds responsible for a variety of bright colours in fall leaves, flowers (narcissus, marigold), fruits (pineapple, citrus fruits, paprika), vegetables (carrots, tomatoes), insects (ladybird), bird plumage (flamingo, cock of the rock, ibis, canary) and marine animals (crustaceans, salmon) (Pfander, 1992). These pigments provide different colours from light yellow to dark red and when complexed with proteins they can produce green and blue colorations (Ong and Tee, 1992). Yellow, orange and green fruits and vegetables provide a range of carotenoids. β-carotene, α-carotene and β-cryptoxanthin are the major provitamin A carotenoids in human and lutein, zeaxanthin and lycopene are major carotenoids in the diet which are not converted to vitamin A. Biological functions of these natural pigments in relation to animals or humans are not well defined but their antioxidant properties seem to be of major importance. In mixture with other antioxidants they could be much more effective than on their own, and the GIT could be a major place for these compounds to exert their activity. In some conditions, carotenoids can be prooxidants. However, it is well recognised that this possibility is not likely to be the case in physiological conditions, including in the GIT when an array of other antioxidants are present.

Approximately 40 carotenoids are commonly consumed in the U.S. diet and approximately 20 can be detected in human serum and tissues (Cooper et al., 1999). Most nutrition research was concentrated on the six carotenoids found in the highest concentrations in human blood: β-carotene, lycopene, α-carotene, lutein, zeaxanthin and β-cryptoxanthin. The major dietary lutein sources in the human diet are green vegetables and fruits, including spinach, squash, grapes (Sommerburg et al, 1998), broccoli, parsley, peas etc. (Hart and Scott, 1995). Carotenoid consumption and their serum profile vary substantially depending on the origin of the population studied. For example France presents the highest levels of serum lutein and β-carotene and Spain shows the lowest level of β-carotene, along with the highest levels of β-cryptoxanthin (Olmedilla et al, 1997). American women consume approximately 6 mg of total carotenoids per day (Chug-Ahuja et al, 1993), the average daily intake of major carotenoids in Spanish population is 3.5 mg/day (Olmedilla et al, 1997) and in Germany total carotenoid intake amounts to 5.33 mg/day with average lutein intake being 1.91 mg/day (Pelz et al, 1998). Daily consumption of lutein and zeaxanthin in American elderly subjects was 2.7 mg for men and 3.09 mg for women (Tucker et al, 1999). In general, the recommended daily intake of carotenoids can only be achieved by consuming 100-200 g/day of vegetables and fruits with a particularly high carotenoid content (Muller, 1996).

Low lutein consumption reflects low consumption of fresh vegetable and fruits, changes in nutritional habits and use of highly processed food. According to National Health Interview Surveys, the intake of lutein declined among different categories of people in the USA between 1987 and 1992 (Nebeling et al, 1997). There were also significant seasonal differences in plasma carotenoid concentrations in the UK reflecting a higher intake of lutein during the spring compared with summer and autumn (Scott et al, 1996). It is interesting to mention that there is also a high positive correlation of lutein (r=0.889) between maternal plasma concentrations and cord plasma (Yeum et al, 1998) indicating that the nutritional status of mothers is the major determinant of the lutein status of their babies. In addition it has been shown that breast milk is the major source of lutein to the infants (Thurnham et al, 1997). An increased intake of another carotenoid, β-carotene, by lactating women increases the supply of milk β-carotene available to their breast-fed infants (Canfield et al, 1998).

In general carotenoids are not toxic for humans. There is no evidence that conversion of β-carotene to vitamin A contributes to vitamin A toxicity. Probably this process is metabolically regulated. However, extremely high consumption of carotenoids for a long time could cause hypercarotenemia. It can lead to yellowing of the skin which eventually returns to the norm after carotenoid exclusion from the diet. Diabetes mellitus, hypothyroidism, hypothalamic amenorrhoea, anorexia nervosa, liver disease, and some other metabolic disorders can increase carotenoid absorption, leading to hypercarotenemia (Dawson, 2000).

Carotenoid assimilation from the diet varies significantly depending on many various conditions, however, it seems

likely that a substantial proportion of ingested carotenoids could be found in all segments of the digestive tract. Therefore, in combination with other dietary antioxidants carotenoids could promote antioxidant defence in the GIT. Furthermore carotenoid activities related to the promotion of cell differentiation, regulation of cell proliferation and intracellular communication via gap junctions, as well as regulation of the detoxifying enzymes and enhancement of immune system (Surai, 2002) could also be of great importance in the GIT.

Vitamin A

Vitamin A is a generic term combining all-trans-retinol or its esters (acetate, palmitate, etc.), and all-trans-retinal. Most of food products are poor source of vitamin A, however it can be efficiently synthesised in the GIT from carotenoids. Vitamin A is present in foods mainly in the form of esters with long-chain fatty acids. The richest food source of this vitamin is liver. Biological functions of vitamin A are diverse (vision, testicular function, development, bone growth, differentiation, hematopoiesis, pattern formation during embryogenesis, etc.), but its antioxidant properties can be also of importance (Livrea et al., 1996) especially in combination with other antioxidants in the GIT. Intake requirements were calculated to amount to 1000 retinol equivalents (RE) for men, 800 RE for women (Gerster, 1997). One RE is equal to 1 mg retinol or 6 mg b-carotene. Unlike most carotenoids, vitamin A and most retinoids are highly toxic when taken in excessive amounts (Bates, 1995). The US NRC Committee on Dietary Allowances has recommended that adults should not consume more than 7500 retinol equivalents daily (Dawson, 2000).

Ascorbic acid (AA)

Vitamin C is referred to as L-ascorbic acid and its two-electron reduction product dehydro-L-ascorbic acid. Most animal species synthesize AA from glucose, but human subjects are not able to synthesize it. Therefore AA is an essential dietary component playing an important role in many physiological processes and it is a hydrophilic antioxidant functioning in an aqueous environment and possessing high free-radical-scavenging activity. It can participate in vitamin E recycling thus maintaining efficient antioxidant defence. Fresh green fruits and vegetables are good sources of AA. However, during food processing AA is easily oxidized and as a result AA concentration in such foods is substantially decreased. Due to its high reducing potential, in combination with iron ions AA can be a prooxidant. However, it is believed that in physiological conditions and in the GIT ascorbic acid performs mainly its antioxidant functions. In fact ascorbic acid inhibits chemical synthesis of nitrosamines (animal carcinogens) in the gastric contents and there are suggestions that intakes of ascorbic acid much higher than RDA may reduce the risk of such diseases as heart disease and cancer (Hathcock, 1997).

RDA for ascorbic acid for humans is 60 mg/day and the most common adverse effects of high vitamin C intakes (>2 g/day) are gastrointestinal symptoms such as nausea, abdominal cramps, and diarrhea (Hathcock, 1997). After exclusion of the vitamin supplements the symptoms usually disappear within a week or two with no further consequences.

Glutathione

Glutathione (GSH) is the most abundant non-protein thiol in mammalian cells, and is considered to be an active antioxidant in biological systems providing cells with their reducing milieu. Cellular GSH plays a key role in many biological processes and can be synthesised in the human body. GSH is abundantly distributed in the mucosal cells of GIT in man and its highest concentration is found in the duodenum (Loguercio and Pierro, 1999). Cellular GSH plays a key role in many biological processes: the synthesis of DNA and proteins, including cell growth and proliferation, regulation of programmed cell death, immune regulation, the transport of amino acids, xenobiotic metabolism, redox-sensitive signal transduction (Sen and Packer, 2000). Furthermore, GSH thiolic group can react directly with H_2O_2, superoxide anion, hydroxyl radicals, alkoxyl radicals, hydroperoxides (Lenzi et al., 2000; Meister and Anderson, 1983). Therefore, a crucial role for GSH is as free radical scavenger, particularly effective against the hydroxyl radical (Bains and Shaw, 1997), since there are no enzymatic defences against this species of radical. Usually decreased GSH concentration in tissues is associated with increased lipid peroxidation (Thompson et al., 1992). Furthermore in stress conditions GSH prevents the loss of protein thiols and vitamin E (Palamanda and Kehrer, 1993) and plays an important role as a key modulator of cell signalling (Elliott and Koliwad, 1997). Animals and human are able to synthesise glutathione. In addition to body synthetic activity food also provide GSH.

Flavonoids

Flavonoids are low molecular weight polyphenolic substances based on the flavan nucleus. They are widespread in nature, occurring in all plant families, and are found in considerable quantities in fruits, vegetables, grains, cola, tea, coffee, cocoa, beer and red wine (Skibola and Smith, 2000). The list of flavonoids substantially increased from more than 4000 in 1996 (Cook and Samman, 1996) to over 8000 individual compounds known up to date (Pietta, 2000). The major flavonoid classes include flavonols, flavones, flavanones, flavanols (catechins), anthocyanidins, isoflavones, dihidroflavonols and chalcones (Cook and Samman, 1996). Representatives of major groups of flavonols were characterised as having antioxidant properties in vitro and in vivo (Pietta, 2000; Bors et al., 1996; Cook and Samman, 1996; Larson, 1988).

These compounds have received substantial attention in recent years. The major driving forces of research in the field were positive effects of fruits and vegetables on human health and their preventive role in the development of various diseases, especially cancers. The flavonoid content in fruits and vegetables can be as high as 300 mg/kg fresh weight (Ishige et al., 2001). The major problem with antioxidant properties of

these compounds is their low availability from the dietary sources. For example, in human blood or urine polyphenol concentrations was shown to be in a range 1-2 mM (Bell et al., 2000; Lapidot et al., 1998) in comparison to the general concentration of antioxidants in human plasma to be about 1000 mM (Benzi and Strain, 1999). Therefore it has been suggested that the digestive tract is the major site of antioxidant defence afforded by polyphenolic compounds such as flavonoids (Kanner and Lapidort, 2001; Halliwell et al., 2000).

Daily intake of flavonoids varies substantially between different countries and in Asian population and in vegetarians it is the highest. In particular, average daily intake of flavonols in Asian countries comprised about 68 mg and isoflavones 20-240 mg (Skibola and Smith, 2000). In contrast the mean intake of flavonols of the German population was about 11.5 mg, mainly derived from fruits and vegetables, but also from black tea and red wine (Bohm et al., 1998).

Indeed, naturally occurring polyphenolic compounds may play a role in the protective effects of fruits and vegetables against cancers in general, and they appear to have considerable potential as chemopreventive agents against neoplastic changes in the alimentary tract (Gee and Johnson, 2001). In general flavonoids can prevent LDL oxidative modification by scavenging ROS, chelating transition metal ions or inhibiting lipoxygenase and this leads to the prevention of atherosclerosis. For example, a number of studies have shown that consumption of soy is antiatherogenic and that the isoflavones genistein, diadzein and biochanin, which inhibit lipoprotein oxidation in vitro and suppress formation of plasma lipid oxidation products in vivo, are most likely responsible for this effect (Patel et al., 2001).

However, there are no data available on the long-term effects of flavonoid dietary supplementation on humans. A serious problem with flavonoids is that, depending on conditions, they could be antioxidants or prooxidants, antimutagens or promutagens. Therefore unregulated use of flavonoid-containing supplements can have a detrimental effect on human health. For example, the results obtained by Silva et al. (2000) suggest that there is a range of flavonols whose genotoxicity in eukaryotic cells depends on their autooxidation. These flavonols can autooxidize when the pH value is slightly alkaline, such as in the intestine, and therefore can induce genotoxicity in humans. Clearly more research is needed to clarify health benefit and potential dangers of these compounds.

Comparatively low bioavailability and antioxidant potential of various flavonoids could be beneficial for the human providing antioxidant protection in various part of the digestive tract, including the large intestine where levels of other antioxidants would be quite low.

Other poly(phenolics)

Cereal brans contain significant quantities of the phenolic ferulic acid and diferulic acid and their potential health benefits (protection of LDL from oxidative modification and reduction in atherogenesis as well as inhibitory effects on tumor promotion and chemopreventive properties) have been related mostly to their antioxidant activity (Andreasen et al., 2001).

Spices and essential oils

Addition of spices to food is a common procedure in most cultures. The seasonings contribute a pleasant flavour and recently it has been shown that they contain a range of antioxidant compounds and it seems likely that only a small proportion of them have been isolated and identified (Madsen et al., 1997). They include such phenolic diterpenes as carnosoic acid, carnosol, rosmaridiphenol and rosmariquinone from rosemary sage and summer savory. In other spices a range of flavonoids have been identified. In general, spices and herbs have been shown to have over a hundred compounds with high antioxidant activity including 26 active comopounds from the *Labiatae* family, *Rosmarinus officinalis*, *Thymus vulgaris*, *Origanum vulgare* and *O. majorana*, over 40 antioxidative compounds from *Zingiber officinale* and 26 compounds from *Curcuma domestica* (Nakatani, 2000). Spices are effective in prevention food deterioration during storage and this explains why traditional diets in countries with high temperature (India, Thailand, Mexico etc.) are usually rich in spices.

Essential oils from aromatic and medicinal plants have been shown to have antibacterial, antimycotic and antioxidant properties. Recently the essential oils from black pepper, clove, geranium, melissa, nutmeg, oregano and thyme and 33 phytoconstituents have been assessed in vitro (Dorman et al., 2000). All the compounds demonstrated antioxidant capacities superior to the water-soluble a-tocopherol analogue Trolox with the exception of the essential oil melissa and three phytoconstituents. The best results were obtained with clove, oregano and thyme oils and their corresponding phytoconstituents namely eugenol, carvacrol and thymol. Again in the GIT antioxidant properties of various compounds from spices and herbs would contribute to total antioxidant potential.

Selenomethionine

In most diets, the main food sources of Se are cereals, meats and fish (Combs, 2001). Of these, Se in fish is known to have a low availability for animals and human. Selenomethionine (SeMet) represents the major natural form of selenium in feed and food ingredients. It seems likely that SeMet has specific functions on its own beyond being an important source of Se for selenoprotein synthesis. In fact, there is strong evidence that this compound itself can be considered as an antioxidant. When Se as the selenite, SeMet, ebselen or Se-yeast were investigated in an *in vitro* LDL oxidation model, it was shown that SeMet and Se-yeast are powerful *in vitro* and *in vivo* antioxidants (Vinson et al., 1998). Similarly, treatment of lymphocytes with SeMet prior to adding H_2O_2 caused an inhibition in peroxyl radical formation in a manner dependent on SeMet concentration (Sun et al., 1997). Furthermore,

SeMet is considered as a powerful antioxidant protecting against damaging effects of peroxynitrite. For example, it protected human fibroblast lysates from toxic effect of peroxynitrite (Sies et al., 1998). SeMet also protected dihydrorhodamine 123 from oxidation and 4-hydroxyphenylacetate from nitration caused by peroxynitrite while sodium selenite exhibited no effect (Briviba et al., 1996), and protected DNA from single-strand breaks induced by peroxynitrite (Roussyn et al., 1996). Therefore it is most likely that SeMet can contribute to the total antioxidant potential of the GIT.

Recently revised US RDA for Se is 55 mg (Combs, 2001) and in many countries all over the world Se consumption is lower than current RDA. On the other hand, excess selenium intake by animals (more than 4-5 mg/kg dry weight of feed) produce a wide range of adverse effects. However, no adverse effects were observed in a 10 year clinical trial at daily supplemental intake of 200 mg Se in selenized yeast (Hathcock, 1997). For humans it is recommended to restrict Se intake to 400-450 mg/day (Rayman, 2000)

Synthetic antioxidants

Antioxidants in foods may be endogenous origin or may be added externally to preserve their lipid components from peroxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are commonly used in food formulations. However, due to safety concerns, public interest shifted from synthetic to natural antioxidants. As a result mixed tocopherols, herbal extracts such as those of rosemary and sage, as well as tea extracts have been commercialised for food and nutraceutical applications (Shahidi, 2000).

Antioxidant enzymes and proteins

Food derived antioxidant enzymes would be inactivated during thermal food processing. However GIT contains internally-originated antioxidant enzymes SOD, GSH-Px and CAT and they represent an important mechanism of the enterocyte defence from oxidative damage. A specific gastrointestinal GSH-Px (GI-GSH-Px) have been described in 1993 (Chu et al., 1993). The enzymatic properties of this selenoprotein are practically the same as those of cytosolic GSH-Px. The physical properties are also similar; and activity of both enzymes depends on Se supply (Chu et al., 1993). GI-GSH-Px activity was present in both the villus and crypt regions of rat mucosal epithelium and its activity nearly equalled that of classical GSH-Px throughout the small intestine and colorectal segments (Esworthy et al., 1998). GI-GSH-Px could be considered to be a barrier against hydroperoxide resorption (Brigelius-Flohe, 1999). Furthermore, in the gastrointestinal tract there are at least three more selenoproteins including plasma GSH-Px, selenoprotein P and thioredoxin reductase (Mork et al., 1998).

Glutathione and glutathione–dependent enzymes contribute significantly towards intestinal antioxidant defences. In fact, an important peroxide detoxification pathway in the intestine is based on the GSH redox system (LeGrand and Aw, 1998). In this system GSH-Px reduces peroxides at the expense of GSH oxidation. Oxidised glutathione is reduced back to the active form by glutathione reductase utilizing reducing potential of NADPH which is produced in the pentose phosphate pathway. It is known that exogenous GSH provided rat small-intestinal epithelial cells with significant protection against injury induced by t-butyl hydroperoxide or menadione (Lash et al., 1986). Thus, rat small-intestinal epithelial cells can utilize plasma GSH to support intracellular detoxication systems that function in protection against chemically induced injury. On the other hand, decreased intestinal GSH concentration was associated with an increased susceptibility to oxidative injury, metal intoxication and various intestinal pathologies (Iantomasi et al., 1997).

Other antioxidant mechanisms

To adapt to environmental changes and survive different types of injuries, eukaryotic cells have evolved networks of different responses, which detect and control diverse forms of stress. To deal with various aggressive factors including free radicals and toxic products of their metabolism, the gastrointestinal mucosa has a variety of defence mechanisms consisting of functional (mucus-alkaline secretion, mucosal microcirculation), humoral (prostaglandins and nitric oxide) and neuronal (capsaicin sensitive sensory neurones) factors (Tsukimi and Okabe, 2001). Furthermore, oxidative stress at the cellular level is reduced by enzymatic and nonenzymatic antioxidant mechanisms. Recently, new mechanisms of such adaptive defences of the gastrointestinal mucosa at the intracellular level have been characterised. One of these responses, known as the heat shock response is considered to be a universal fundamental mechanism necessary for cell survival under a variety of unfavourable conditions (Santoro, 2000). As mentioned above, intestinal cells are challenged with a great variety of potentially toxic compounds and their protection is a vital part of the strategy to maintain human health. In mammalian cells, the induction of the heat shock response requires the activation and translocation to the nucleus of one or more heat shock transcription factors which control the expression of a specific set of genes encoding cytoprotective heat shock proteins (Santoro, 2000). Therefore heat shock proteins (HSP) function as molecular chaperones in regulating cellular homeostasis and promoting survival. However, if the stress is too high, a signal that leads to programmed cell death, apoptosis, is activated, thereby providing a finely tuned balance between survival and death (Kopecek et al., 2001). In addition to extracellular stimuli, several nonstressfull conditions induce HSPs during normal cellular growth and development. In particular, the HSP family is activated under oxidative stress and provides an important protection against protein denaturation and modifications by capping and refolding, or drives damaged proteins into appropriate proteolytic pathways (Yenari, 2002). In fact HSPs have been

assigned to multiple subcellular sites and implicated in multiple functions ranging from stress response, intracellular trafficking, antigen processing, control of cell proliferation, differentiation, and tumorigenesis (Wadhwa et al., 2002). Therefore in response to environmental or physiological stresses including ethanol and heavy metals cells increase synthesis of HSP (Tsukimi and Okabe, 2001). It has been suggested that the conserved heat shock protein HSP33 functions as a potent molecular chaperone with a highly sophisticated regulation. In fact, at the transcriptional level, the HSP33 gene is under heat shock control; at the posttranslational level, the HSP33 protein is under oxidative stress control (Graf and Jakob, 2002). Therefore redox-regulated chaperone activity of HSP33 specifically protects proteins and cells from the detrimental effects of reactive oxygen species.

ROS-mediated damage has been implicated in the pathophysiology of the gastrointestinal mucosa and HSPs are suggested to play an important role in cytoprotection against oxidative stress-induced injury (Prabhu and Balasubramanian, 2002). For example, the mammalian intestinal epithelial cells respond to heat stress by producing heat shock proteins that provide protection in stress conditions, which would otherwise lead to cell damage or death. The protective effects of HSP are seen in heat stress, infection, and inflammation (Malago et al., 2002). Similarly, glucocorticoid protection of rat intestinal cells against oxidant-induced stress was mediated by HSP72 (Urayama et al., 1998).

The molecular mechanisms of heat shock response-induced cytoprotection are beyond this review. However, they involve inhibition of proinflammatory cytokine production and induction of cellular proliferation for restitution of the damaged epithelium (Malago et al., 2002). HSPs play an important role in gastric mucosal defence under conditions of stress. For example, exposure of rats to restraint and water-immersion stress caused rapid HSP70 mRNA expression and HSP70 accumulation in gastric mucosa and the extent of HSP70 induction inversely correlated to the severity of mucosal damage (Rokutan, 1999). Therefore HSP70 is involved in repair of partially damaged proteins and substantially contributes to protection of the gastrointestinal mucosa against various necrotising factors (Tsukimi and Okabe, 2001).

Heme oxygenase (HO-1), known as HSP 32, can be induced by various stresses. It has been shown that HO-1 induction and the maintenance of its appropriate activity is critical in protecting the intestinal epithelial cells from oxidative injury (Fujii et al., 2003). It is interesting that in the aforementioned experiment HO-1 was markedly induced following LPS treatment in the mucosal epithelial cells in the upper intestine (duodenum and jejunum) but not in the lower intestine (ileum and colon). It seems likely, that there is a delicate interaction between HSPs and other antioxidant defence mechanisms to maintain mucosal integrity and repair of acute mucosal damage.

SITE-SPECIFICITY IN ANTIOXIDANT-PROOXIDANT BALANCE IN THE INTESTINE

Presence of antioxidants in the GIT is an essential factor preventing lipid peroxidation in the stomach, small intestine and colon. In fact total antioxidant activity and activities of SOD and catalase were higher in the rat mucosa/submucosa of the small intestine than in the colon (Blau et al., 1999). It was shown that antioxidant enzymes play a key role in rendering the intestinal mucosal cells resistant to iron induced oxidative damage in rats (Srigiridhar and Nair, 1997). It is interesting that in the rat small intestine, activities of SOD and GSH-Px and lipid peroxidation were not affected by age or strain difference (Jang et al., 2001). On the other hand a differential modulating effect of flavonoids on antioxidant enzyme activities in liver, colon, heart and red blood cells (Breinholt et al., 1999) is of great importance in understanding the antioxidant interactions in the GIT and human tissues. Furthermore naringin, a citrus bioflavonoid, plays an important role in regulating antioxidative capacities by increasing the SOD and catalase activities, up-regulating the gene expressions of SOD, catalase, and GSH-Px, and protecting the plasma vitamin E in high cholesterol-fed rabbits (Jeon et al., 2001). In addition, synergistic effect of different antioxidants is also of great importance. For example, lycopene acts synergistically, as an effective antioxidant against LDL oxidation, with several natural antioxidants such as vitamin E, the flavonoid glabridin, the phenolics rosmarinic acid and carnosic acid, and garlic (Fuhrman et al., 2000). Similarly synergistic interactions between isoflavones and ascorbic acid have been shown (Patel et al., 2001). It is believed that rat intestine and mesenteric lymph possess efficient antioxidant defences against preformed lipid hydroperoxides and (peroxyl) radical mediated lipid oxidation (Mohr et al., 1999).

It was also shown that supplementation of vitamin E alone or in combination with ascorbic acid protects the GIT of Fe-deficient rats against Fe-mediated oxidative damage during Fe repletion (Srigiridhar and Nair, 2000). Since flavonoids are consumed in concentrations usually much higher than other antioxidant compounds, their protective effect during digestion is of great importance. For example, recently it has been shown that plant antioxidants such as flavonoids not only prevented an accumulation of peroxidized lipids but also can switch prooxidant properties of heme-proteins to antioxidant ones (Kanner and Lapidot, 2001). Dietary polyphenols can also modulate in vivo oxidative damage in the gastrointestinal tract of rodents (Giovannelli et al., 2000) supporting the hypothesis that dietary polyphenols might have both a protective and a therapeutic potential in oxidative damage-related pathologies.

The dietary concentration of linoleic acid significantly affected oxidation of pig jejunal mucosa and vitamin E has a protective effect. In particular, a histological study of the jejunal mucosa of pigs showed lower cell desquamation in groups supplemented with a-tocopheryl acetate and a higher cell desquamation was found in the groups fed diets containing

the higher concentration of linoleic acid (Lopez Bote et al., 2001). In the same study in vitro-induced oxidation of jejunal mucosa homogenates was lower in pigs fed diets supplemented with a-tocopheryl acetate. Vitamin E also protects the rat colon from oxidative stress associated with inflammation. In fact, vitamin E supplementation, which resulted in increased colonic vitamin E levels, reduced colonic weight and damage score, prevented lipid peroxidation and diarrhea, reduced interleukin-1 beta levels and preserved glutathione reductase activity and total glutathione levels (Gonzalez et al., 2001)

There are other inducable antioxidant enzymes in the intestine. For example, feeding mice with BHA induces phase II detoxifying enzymes and intestinal and hepatic peroxiredoxin I, a stress-inducible antioxidant, in a manner similar to the induction of glutathione S-transferases (GST, Ishii et al., 2000) and it was suggested that the induction of this antioxidant may be important to protect the cells and tissues against toxic electrophiles and reactive oxygen species. Similarly, in small intestine GST activity changed in response to the different fatty acid supplemented diets and increased as a result of the elevated oxidative stress (Giron et al., 1999).

There are other antioxidant applications in relation to GIT health. For example, irradiation caused increased lipid peroxide and decreased GSH levels in the intestine. Intestinal SOD and GSH-Px activities were increased, but GST activity decreased following irradiation of rats. Selenium and/or vitamin E pre-treatments ameliorated these disturbances in prooxidant-antioxidant balance in the GIT (Mutlu-Turkoglu et al., 2000). This amelioration has been confirmed with histopathological findings. Common mucosal immune responses in orally immunized aged mice were depressed and dietary supplementation with vitamin E restored their mucosal and systemic humoral immune responses to mature adult levels (Enioutina et al., 2000).

When the antioxidant system of the GIT is compromised, various diseases can be observed. For example, in rats at weaning it was shown that early chronic diarrhea and severe protein-energy malnutrition impair the antioxidant defence system in both the small and large intestine (Nieto et al., 2000). Similarly, ulcerative colitis in rats induced by trinitrobenzenesulfonic acid damages the intestinal mucosa and is accompanied by a shift in the antioxidant enzyme activities, and low levels of glutathione (Nieto et al., 2000a).

Indeed, the antioxidant-prooxidant balance in various parts of the intestine would ultimately depend on the level of antioxidants and prooxidants provided with the diet and released by cells themselves as well as on the level of absorption of both antioxidants and prooxidants. Therefore in the stomach the conditions are favourable for lipid peroxidation since major antioxidants and prooxidants are there before absorption or major metabolism, and the pH is also favourable (Kanner and Lapidot, 2001). In the small intestine this balance will be more variable since many antioxidants, mainly vitamins E, A, C and carotenoids will be effectively absorbed. However efficiency of absorption of iron is quite low (Department of Health, 1991) therefore it will be available for stimulation of peroxidation. On the other hand, after lipid absorption, concentrations of substrates of peroxidation will be substantially decreased. Furthermore, various flavonoids will also be available for antioxidant defence. Finally, in the colon/rectum, levels of vitamin E, A, C are low, although various flavonoids and some carotenoids are present (Halliwell et al., 2000) as well as iron, but the lipid concentration would be low. Therefore in each part of the digestive tract there is a possibility of oxidative stress and damage to various biological molecules. Indeed, it seems likely that the protective effects of vegetables and fruits against development of various cancers, especially cancers related to the digestive tract, are based on provision of a variety of antioxidants which are not always well absorbed, thus providing antioxidant protection in the large intestine and preventing oxidative damage and possible mutagenic effect. It is possible to suggest that there is a reason for some antioxidants not to be absorbed completely and in that way providing antioxidant protection in lower parts of the intestine. For example, tocotrienols are common compounds of major food and feed ingredients and possess high antioxidant activity (Surai, 2002). It is well appreciated that they are not well absorbed from the food and feed and would be found in the digesta in colon providing antioxidant protection in the lower intestine. However, this idea needs further investigation.

WHAT SHOULD BE CHANGED TO HAVE A HEALTHY DIET?

Dietary factors are considered to be major contributors to the leading causes of death of Americans, namely coronary heart disease and certain types of cancer (Milner, 2000). Epidemiological findings, supported by animal studies, have led to recommendations that people should consume at least two servings of fruit and three servings of vegetables daily (Diplock et al., 1998) in addition to at least two servings of fish weekly (Krauss et al., 2001). While findings and reports such as these have had an impact on the type and quantity of the food that many of us eat (Margetts et al., 1998) the majority of adults in developed countries fall well short of meeting healthy eating guidelines. At the same time from information provided above it is quite clear that a decreased risk of heart disease and cancers of the breast, prostate, lung, colon and stomach is associated with increased consumption of fruits, vegetables and soy products (Skibola and Smith, 2000). A range of antioxidants can be found in our diet and they are essential part of antioxidant defence in the intestine and further in human tissues. However, it seems most likely that the average diet of Americans or Europeans contains restricted amounts of antioxidant-rich fruits and vegetables and this can at least partly account for the poor health records in some developed countries. Se-deficiency is also a problem of great concern in many countries all over the world.

From information presented above it is clear that in most cases producers and consumers themselves are responsible for increased levels of prooxidants in the diet and ulti-

Table 2. Healthy meals via antioxidant enrichment and decreased lipid peroxidation

	Meat	Egg	Fish
Technological improvement at the producer level			
Vitamin E enrichment	+++	+++	+++
Selenium enrichment	+++	+++	+
Carotenoid enrichment	+	+++	+++
Food preparation			
Cooking oil	Olive	Olive	Olive
Boiling vs frying	+	+++	+
Spices and herbs	+++	+++	+++
Food serving			
With vegetables	+++	+++	+++
Meal composition			
Fruit	+++	+++	+++
Fruit juice	+++	+++	+++
Red wine	+++	+	+
Tea	+++	+++	+++

mately in the GIT. Therefore there is a need for changes in ways our food is produced, prepared, stored and eaten (Table 2). It is possible to improve the situation both at the producer and consumer levels.

At the producer level

- To enrich meats and meat-related food with vitamin E. Indeed there is a great body information accumulated indicating that chicken (Morrissey et al., 1997), turkey (Ahn et al., 1998, Mercier et al., 2001), pork (Buckley et al., 1995), beef (Liu et al., 1995), lamb (Macit et al., 2003) as well as meat from other species (Oriani et al., 2001) can be enriched with vitamin E and this technological solution can substantially decrease lipid peroxidation in meat during processing, storage and cooking. It seems likely that fish enrichment with vitamin E will also be beneficial in terms of prevention of lipid peroxidation (Ruff et al., 2003). Similarly enrichment of eggs with vitamin E can also substantially decrease lipid peroxidation (Surai and Sparks, 2001) and cholesterol oxide formation (Galobart et al., 2002). It is also possible to enrich eggs with vitamin E to such extent that they will provide substantial (daily requirement) amounts of vitamin E which will be beneficial during digestion as well as for general health (Surai et al., 2000; Surai and Sparks, 2001). It is worth mentioning that vitamin E in the egg yolk is in free tocopherol highly available form which could be a major benefit at the level of digestion (Surai, 2002).
- To enrich meat, milk and eggs with organic selenium. This could be beneficial in terms of preventing lipid peroxidation in meat (Surai and Dvorska, 2002; 2002a; Krska et al., 2001) or eggs (Surai and Sparks, 2001, Surai, 2002) but more importantly, selenium is absolutely essential for expression of GI-GSH-Px, the main defence against lipid peroxide absorption in GIT (Brigelius-Flohe, 1999). Selenium is also important for expression of other selenoproteins (e.g. thioredoxin reductase etc.) which play an important role in antioxidant defence in the intestine and in other tissues. In fact, selenium-enriched eggs, meat and milk are already on the market in various countries in the world (Surai, 2002).
- To enrich eggs with carotenoids. These natural antioxidants will be a part of the complex antioxidant defence system interacting with other antioxidants in the GIT and they per se can also provide antioxidant protection (Surai, 2002).
- To improve product storage by decreasing oxygen availability and lipid peroxidation. To minimise storage of cooked products, which are especially susceptible to peroxidation.
- In the fast food restaurants, to change frying oils more often and use olive oil which is less sensitive to peroxidation (Quiles et al., 2002) and to enrich frying oils with natural antioxidants (e.g. tocopherol mixture). It would be advantage to serve fast food with bigger portions of salads and use more sauces providing additional antioxidants. Some other oils (e.g. rapeseed oil) enriched with tocopherols can also be considered to be useful.
- To produce antioxidant-enriched sources, especially for fast food restaurants

Therefore it is possible to provide consumers with a range of animal-derived products with nutritionally modified composition in such a way that they can deliver substantial amount of health-promoting nutrients to improve the general diet and help to maintain good health. In fact, in the UK in main supermarkets (Tesco, Safeway, etc.) Columbus eggs delivering 70% of RDA in Se and vitamin E with a single egg are already available. Therefore, without changing habits and traditions of various populations it is possible to solve problems related to deficiency of various nutrients, in particular selenium. The consumer will go to the same supermarket to buy the same animal-derived products (egg, milk and meat), cook and consume them as usual. The only difference will be in amount of specific nutrients delivered with such products.

At the consumer level:

- To choose olive oil for frying food. This will decrease accumulation of oxidation products (Quiles et al., 2002) and will be beneficial in terms of decreasing omega-6 PUFA consumption and increasing MUFA consumption in accordance with recent health-related findings (Tuck and Hayball, 2002). Rapeseed oil enriched with tocopherols is also an important choice.
- To use more spices and herbs during cooking. This will

decrease oxidation and prevent accumulation of the lipid peroxides.
- To choose boiled eggs instead of fried. If using frying, it is necessary to make sure that duration of the process is minimal.
- To choose antioxidant-enriched eggs, which are already on the market (Surai, 2002).
- To decrease usage of cooked meals after storage.
- To decrease consumption of fast food, prepared by current technology of deep-frying. Use more sauces, which can provide additional antioxidants.
- To make sure that meat meals are served with plenty of vegetables, providing necessary antioxidants.
- To increase vegetable and fruit consumption on everyday basis.

Therefore, since we cannot avoid pro-oxidants in our food we need to make sure that they are compensated by consumption of increased levels of natural antioxidants. For this reason, it would be advantage if our meat and fish meals are served with plenty of vegetables. Various sauces (e.g. tomato sauce) could provide additional antioxidants. Red wine could also add additional flavonoids as a source of antioxidants. Various juices are also good sources of natural antioxidants as well as fruits. If a meal is finished with tea, this will also add to antioxidant potential of the digesta.

All these suggestions are in a line of traditional meals serving in various countries of the worlds. Antioxidants compensate prooxidants and a positive balance in the digestive tract is the first step to healthy life.

All the future exists in the past.

CONCLUSIONS

Recent achievements in biochemistry and molecular biology, together with epidemiological data have changed our thinking about food. It became increasingly clear that our diet plays a pivotal role in maintenance of our health and a disbalanced diet can cause serious health-related problems. It seems likely that antioxidants are among the major regulators of many physiological processes and therefore a balance between antioxidants and prooxidants in the diet, GIT, plasma and tissues is an important determinant of the state of our health. Plants consumed by humans contain thousands of phenolic compounds. Among them the effects of dietary polyphenols are of great current interest due to their higher consumption in comparison to other antioxidants such as vitamin E and their antioxidative and possible anticarcinogenic activities. Realising an importance of the food choice issue fundamental solutions for food improvement should be found and production of functional food is one of them. Therefore, improvement of the diet by balancing essential nutrients via designer/functional food without changing people's food preferences would bring health benefit. In this respect, natural antioxidants have become "a wonder remedy" of the 21st century.

"We are what we eat!"

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