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Antioxidant therapy effectiveness: an up to date

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Abstract. – Antioxidants are chemical compounds that giving an electron to free radical molecules convert them to an harmless configuration, avoiding damaging chain reaction, which can involve lipids, proteins enzymes carbohydrates, DNA, cell and nuclear membranes up to the cell death. Being either exogenous or endogenous they are addressed to prevent the oxidation induced damage, a process that causes damage in all tissues through free radicals chemical reactivity.

A lot of natural, nutraceutical or chemical compounds are being actually marketed with a lot of different claims and are prescribed by doctors or sold over the counter. Unfortunately, in the medical literature many heterogeneous published articles support the use of this class of drugs, but most of them cannot be compared or pooled to achieve statistical significance of effectiveness.

Our review aims at defining the state of the art of antioxidant therapy, with specific reference on the evidence based clinical use.

Key Words:

Antioxidant, Reactive oxygen species, Free radicals, Natural and nutraceutical compounds, Human diseases.

Introduction

Oxidative stress is due to the imbalance of the body's scavenging ability to face free radical species. The two main types of free radical species are reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS seem to be an important factor involved in endothelial dysfunction, diabetes, atherosclerosis and ischemia. RNS have been associated with arthritis, diabetes, degenerative neuronal diseases, cancer and atherosclerosis (Table I). Under physiological conditions, overproduction of ROS and RNS and their neutralization is prevented by the activity of endogenous anti oxidative defense system (AOS): it encloses enzymes like superoxide dismutase; catalase, glutathione peroxidase, and other antioxidant- re-generating enzymes such as gluthatione reductase; dehydroascorbate reductase and glucose-6 phosphate dehydrogenase, that maintains reduced NADPH; hydrophylic scavengers like urate ascorbate gluthatione, flavonoids; lipophilic scavengers, like tocopherols, carotenoids and ubiquinone.

The great majority of antioxidants are supplied with the diet and enclose polyphenols, lipoic and ascorbic acid carotenoids, resverartrol, epigallocathechin-3-0-gallate, lycopene, quercetine, genstein, ellagic acid, ubiquinone and indole-3 carbinole. In fact, in the biological systems, the normal processes of oxidation produces highly reactive free radicals and each of this administered compounds is involved in the physiological redox balance preventing damage to the tissues.

Several antioxidants and their mechanisms of action.

A growing body of evidence indicates that oxidative stress plays an important role in the pathogenesis of many clinical conditions involving cardiovascular diseases, liver diseases, lung diseases, gastrointestinal disorders, neurological disorders, muscle damage, diabetes, and aging. Aim of this review is to update the results of clinical trials with antioxidants, in the most common disease areas.

Cochrane Library Experience on Antioxidants (2006-2008)

The issue of antioxidants has been largely investigated by the Cochrane Library Authors: a meta analysis of antioxidant uses in clinical trials, followed by further updates on clinical inves-

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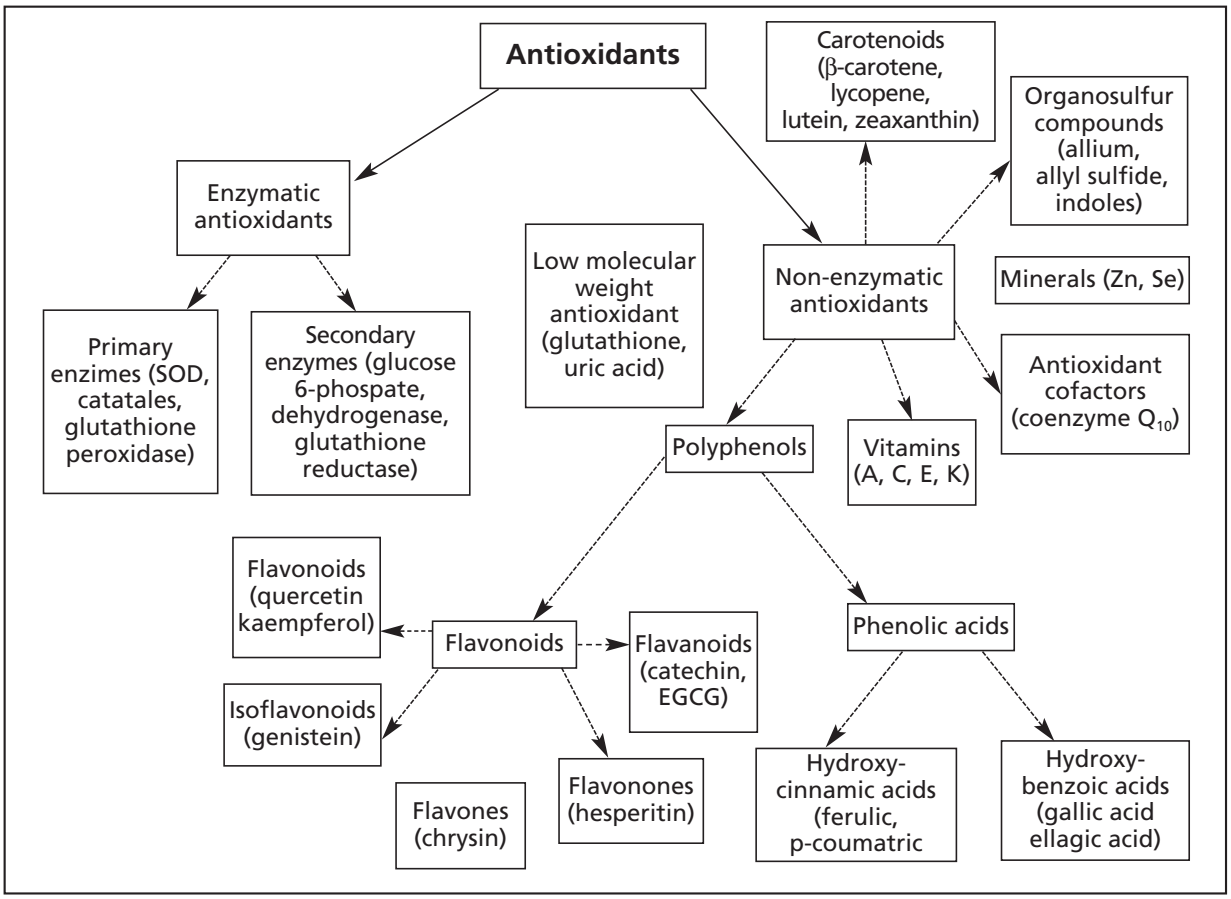

Figure 1. Antioxidants classification.

Table I. Several antioxidants and their mechanisms of action.

Antioxidant	Mechanism of action
13C	Inhibition of DNA-carcinogen adduct formation; suppression of free radical production
Anthocyanin	Reduces inflammatory mediators; protect cells form UV-induced DNA damage
Catalase	Decomposes H_2O_2 to molecular oxygen and water
Coenzyme Q_{10}	Inhibition of lipid peroxidation; reduces mitochondrial oxidative stress
Curcumin	Inhibits eicosanoid synthesis; acts as free radical scavenger; inhibits lipid peroxidation; induces glutathione S-transferase
Ellagic acid	Scavenging of H_2O_2; stimulation of glutathione-S-transferase
Epigallocatechin-3-O-gallate	Metal chelation; scavenging H_2O_2, OH and singlet oxygen; tocopherol regeneration
Genstein	H_2O_2 scavenging
Glutathione	Intracellular reducing agent
Lycopene	Trapping of singlet oxygen
N-acetyl cysteine	Scavenging of H_2O_2 and peroxide; deacetilation of precursor for glutathion synthesis
Naringin	H_2O_2 scavenging
Quercetin	H_2O_2 scavenging, one of the potent antioxidant among polyphenols
Superoxide dismutase	Dismutation of superoxide to H_2O_2
Vitamin C	Scavenging of superoxide anion by forming semidehydroascorbate radical which is subsequently reduced by glutathione
Vitamin E	Direct scavenging of superoxide; upregulation of antioxidant enzymes; inhibition of lipid peroxidation

tigations are reported. The Cochrane Library is a very good source of quite a good number of systematical reviews on antioxidants and their effects. They enclose all the major randomized clinical trials involving the use of antioxidant supplements versus no intervention or placebo. Unfortunately a great number of poorly designed studies with low numbers of participants and short-term endpoints, lead to unconclusive. drawbacks.

Mortality: Primary or Secondary Prevention

Bjelakovic et al.[1] investigated the effect of antioxidants supplements such as beta-carotene, vitamin A, vitamin C, vitamin E, and selenium versus placebo or no intervention, on mortality in primary or secondary prevention randomized clinical trials. The Authors collected 67 randomized trials including 232,550 participants. 47 trials including 180,938 participants had low risk of bias. Twenty-one trials included 164,439 healthy participants. Forty-six trials included 68,111 participants with various diseases (gastrointestinal, cardiovascular, neurological, ocular, dermatological, rheumatoid, renal, endocrinological, or unspecified). Overall, the antioxidant supplements had no significant effect on mortality in a random-effects meta-analysis (relative risk [RR] 1.02, 95% confidence interval [CI] 0.99 to 1.06), but significantly increased mortality in a fixed-effect model (RR 1.04, 95% CI 1.02 to 1.06). In meta-regression analysis, the risk of bias and type of antioxidant supplement were the only significant predictors of inter-trial heterogeneity. In the trials with a low risk of bias, the antioxidant supplements significantly increased mortality (RR 1.05, 95% CI 1.02 to 1.08). When the different antioxidants were assessed separately, analyses including trials with a low risk of bias and excluding selenium trials found significantly increased mortality by vitamin A (RR 1.16, 95% CI 1.10 to 1.24), beta-carotene (RR 1.07, 95% CI 1.02 to 1.11), and vitamin E (RR 1.04, 95% CI 1.01 to 1.07), but no significant detrimental effect of vitamin C (RR 1.06, 95% CI 0.94 to 1.20). Low-bias risk trials on selenium found no significant effect on mortality (RR 0.91, 95% CI 0.76 to 1.09). This trials collection not supports the use of antioxidant supplements for primary or secondary prevention. Vitamin A, beta-carotene, and vitamin E seem to increase mortality and future randomized trials could evaluate the potential effects of vitamin C and selenium for primary and secondary prevention.

Lirussi et al.[2] evaluated the antioxidants (vitamin A, carotenoids, vitamin C, vitamin E, selenium) effects versus placebo in patients affected by fatty liver disease (NAFLD) or non-alcoholic steatohepatitis. The trials have been collected on the basis of methodological quality with which they were realized. All statistical analysis were considered of significant level with a P value equal to 0.05. This systematic review involves 6 clinical trials and none of the trials reported any death. Treatment with antioxidant supplements showed a significant, though not clinically relevant, amelioration of aspartate aminotransferase levels, but not of alanine aminotransferase, as compared to placebo or other interventions. Gamma-glutamyl-transpeptidase was decreased, albeit not significantly, in the treatment arm. Radiological and histological data were too limited to draw any definite conclusions on the effectiveness of these agents. Adverse events were non-specific and of no major clinical relevance. There is insufficient data to either support or refute the use of antioxidant supplements for patients with NAFLD. Accordingly to the Authors, it may be advisable to carry out large prospective randomized clinical trials on this topic.

Amyotrophic Lateral Sclerosis (SLA)

Orrell et al.[3] referred the effects of antioxidants supplements in amyotrophic lateral sclerosis (SLA) affected patients. 23 studies have been collected for consideration whose nine were randomized trials (inclusion criteria) and only two studies used a predetermined primary outcome measure: survival at 12 months treatment. However, sufficient data were available from four studies to allow analysis of this outcome measure, and a meta-analysis was performed. In the individual studies no significant effect was observed for vitamin E 500 mg twice daily; vitamin E 1 g five times daily; acetylcysteine 50 mg/kg daily subcutaneous infusion; or a combination of L-methionine 2 g, vitamin E 400 International Units, and selenium 0.03 mg three times daily. No significant effect on the primary outcome measure was observed in a meta-analysis of all antioxidants combined. No significant differences were demonstrated in any of the secondary outcome measures. There is insufficient evidence of efficacy of individual antioxidants, or antioxidants in general, in the treatment of people with amyotrophic lateral sclerosis and

there is no contraindications, as well. One study reported a mild positive effect, but this was not supported by adequate analysis.

Multiple Sclerosis

Farinotti et al.[4] assessed if a dietary regimens including polyunsaturated fatty acids (PUFAs) and vitamins might improve outcomes in people with multiple sclerosis (MS). The main outcome of this study was that PUFAs did not have a significant effect on disease progression, measured as worsening of Disability Status Scale. Omega-6 fatty acids (11-23 g/day linoleic acid) had no benefit in 75 relapsing remitting (RR) MS patients (progression at two years: relative risk (RR)=0.78, 95% CI [0.45 to 1.36]) or in 69 chronic progressive (CP) MS patients (RR=1.67, 95% CI [0.75 to 3.72]. Linoleic acid (2.9-3.4 g/day) had no benefit in CPMS (progression at two years: RR=0.78, 95% CI [0.43 to 1.42]). Slight decreases in relapse rate and relapse severity were associated with omega-6 fatty acids in some small studies, however these findings are limited by the limited validity of the endpoints. Omega-3 fatty acids had no benefit on progression at 12 months in 14 RRMS patients or at 24 months in 292 RRMS patients (RR=0.15, 95% CI [0.01 to 3.11], p= 0.22 at 12 months, and 0.82 95% CI [0.65 to 1.03], p=0.08, at 24 months). In conclusion the Authors underline that PUFAs seem to have no major effect on the main clinical outcome in MS (disease progression), and does not substantially affect the risk of clinical relapses over 2 years. They emphasized also the need for more clinical trials.

Alchoolic Liver Disease

Rambaldi et al.[5] collected all articles regarding SAMe and alcoholic liver diseases to determine if S-adenosyl-L-methionine may benefit patients with this kind of diseases. The Authors identified nine randomized clinical trials including a heterogeneous sample of 434 patients with alcoholic liver diseases. The methodological quality regarding randomization was generally low, but 8 out of 9 trials were placebo controlled. Only one trial including 123 patients with alcoholic cirrhosis used adequate methodology and reported clearly on all-cause mortality and liver transplantation. No significant effects of SAMe were detected on all-cause mortality (relative risks (RR) 0.62, 95% confidence interval (CI) 0.30 to 1.26), liver-related mortality (RR 0.68, 95% CI 0.31 to 1.48), all-cause mortality or liver transplantation (RR 0.55; 95% CI 0.27 to 1.09), or complications (RR 1.35, 95% CI 0.84 to 2.16), but the analysis is based mostly on one trial only. SAMe was not significantly associated with non-serious adverse events (RR 4.92; 95% CI 0.59 to 40.89) and no serious adverse events were reported. The Authors concluded that they cannot support or discard the use of SAMe for patients with alcoholic liver diseases and that they need more long-term, high-quality randomized trials on SAMe.

Selenium in Oncology Treatments

Dennert et al.[6] summarized the results or the investigation of selenium as a remedy against chemotherapy, radiotherapy or surgery, in oncologic patients. After searching for all randomized-controlled trials of selenium mono-supplements in cancer patients undergoing tumor specific therapy such as chemotherapy, radiotherapy or surgery, only 2 trials have been included, a randomized controlled trial with 60 participants at the beginning of the study investigating secondary lymphoedema and an ongoing trial with preliminary results of 63 participants investigating radiotherapy induced diarrhoea as a secondary outcome. Both trials had drawbacks with regard to study quality and reporting. The trial on secondary lymphoedema reported a decreased number of recurrent erysipela infections in the selenium supplementation group compared to placebo. However, results must be interpreted with caution and cannot be generalized to other populations. The ongoing trial on radiotherapy associated diarrhoea preliminarily reported a lower incidence of diarrhoea in patients receiving selenium supplementation concomitant to pelvic radiation, however, no data were presented. Publication of final results must be awaited to discuss these findings in detail. No randomized controlled trials were found studying the effect of selenium supplementation on other therapy-associated toxicities or quality of life or performance status in cancer patients. The Authors conclude there is insufficient evidence at present that selenium supplementation alleviates the side effects of tumor specific chemotherapy or radiotherapy treatments.

Eye Age Related Macular Disease

Evans et al.[7] study's had the aim to examine the evidence as to whether or not taking vitamin or mineral supplements prevents the development of AMD. This review included all randomized trials comparing an antioxidant vitamin and/or

mineral supplement (alone or in combination) to control. Only studies where supplementation had been given for at least one year have been included. 3 randomized controlled trials were included in this review (23,099 people randomized). These trials investigated alpha-tocopherol and beta-carotene supplements. There was no evidence that antioxidant vitamin supplementation prevented or delayed the onset of AMD. The pooled risk ratio for any age-related maculopathy (ARM) was 1.04 (95% CI 0.92 to 1.18), for AMD (late ARM) was 1.03 (95% CI 0.74 to 1.43). Similar results were seen when the analyses were restricted to beta-carotene and alpha-tocopherol. The Authors conclude that there is no evidence to date that the general population should take antioxidant vitamin and mineral supplements to prevent or delay the onset of AMD.

Pregnancy and Pre-eclampsia

Rumbold et al.[8] study's had the objective to determine the effectiveness and safety of any antioxidant supplementation during pregnancy and the risk of developing pre-eclampsia and its related complications. The Authors collected all randomized trials comparing one or more antioxidants with either placebo or no antioxidants during pregnancy for the prevention of pre-eclampsia and trials comparing one or more antioxidants with another. 10 trials, involving 6533 women, were included in this review, five trials were rated high quality. For the majority of trials, the antioxidant assessed was combined vitamin C and E therapy. There was no significant difference between antioxidant and control groups for the relative risk (RR) of pre-eclampsia (RR 0.73, 95% confidence intervals (CI) 0.51 to 1.06; nine trials, 5446 women) or any other primary outcome: severe pre-eclampsia (RR 1.25, 95% CI 0.89 to 1.76; two trials, 2495 women), preterm birth (before 37 weeks) (RR 1.10, 95% CI 0.99 to 1.22; five trials, 5198 women), small-for-gestational-age infants (RR 0.83, 95% CI 0.62 to 1.11; five trials, 5271 babies) or any baby death (RR 1.12, 95% CI 0.81 to 1.53; four trials, 5144 babies). Women allocated antioxidants were more likely to self-report abdominal pain late in pregnancy (RR 1.61, 95% CI 1.11 to 2.34; one trial, 1745 women), require antihypertensive therapy (RR 1.77, 95% CI 1.22 to 2.57; two trials, 4272 women) and require an antenatal hospital admission for hypertension (RR 1.54, 95% CI 1.00 to 2.39; one trial, 1877 women). The Authors conclude that this review does not support routine antioxidant supplementation during pregnancy to reduce the risk of pre-eclampsia and other serious complications in pregnancy.

Cardiovascular Risk Profile

EJ Brunner et al.[9] study's had the aim of assessing the effects of providing dietary advice to achieve sustained dietary changes or improved cardiovascular risk profile among healthy adults. The Authors collected all randomized studies with no more than 20% loss to follow-up, lasting at least 3 months involving healthy adults comparing dietary advice with no advice or minimal advice. Trials involving children, trials to reduce weight or those involving supplementation were excluded.38 trials with 46 intervention arms (comparisons) comparing dietary advice with no advice were included in the review. 17,871 participants/clusters were randomized. Twenty-six of the 38 included trials were conducted in the USA. Dietary advice reduced total serum cholesterol by 0.16 mmol/L (95% CI 0.06 to 0.25) and LDL cholesterol by 0.18 mmol/L (95% CI 0.1 to 0.27) after 3-24 months. Mean HDL cholesterol levels and triglyceride levels were unchanged. Dietary advice reduced blood pressure by 2.07 mmHg systolic (95% CI 0.95 to 3.19) and 1.15 mmHg diastolic (95% CI 0.48 to 1.85) and 24-hour urinary sodium excretion by 44.2 mmol (95% CI 33.6 to 54.7) after 3-36 months. Three trials reported plasma antioxidants where small increases were seen in lutein and β-cryptoxanthin, but there was heterogeneity in the trial effects. Self-reported dietary intake may be subject to reporting bias, and there was significant heterogeneity in all the following analyses. Compared to no advice, dietary advice increased fruit and vegetable intake by 1.25 servings/day (95% CI 0.7 to 1.81). Dietary fiber intake increased with advice by 5.99 g/day (95% CI 1.12 to 10.86), while total dietary fat as a percentage of total energy intake fell by 4.49 % (95% CI 2.31 to 6.66) with dietary advice and saturated fat intake fell by 2.36 % (95% CI 1.32 to 3.39). Authors conclude that dietary advice appears to be effective in bringing about modest beneficial changes in diet and cardiovascular risk factors over approximately 10 months but longer term effects are not known.

Neonatal Growth Under Parenteral Nutrition (PN)

Soghier et al.[10] study's had the aim to determine the effects of supplementing parenteral nu-

trition with cysteine, cystine or its precursor N-acetylcysteine on neonatal growth and short and long-term outcomes. The Authors selected all randomized trials that examined the effects of cysteine, cystine or N-acetylcysteine supplementation of neonatal PN were reviewed. Predetermined outcome variables included growth, nitrogen retention, mortality, morbidity secondary to oxidation injury, bone accretion, acidosis, liver disease, and cysteine levels. 6 trials fulfilled entry criteria. The majority of patients in these trials were preterm. Five small trials evaluated short-term cysteine supplementation of cysteine-free PN. One large multicenter RCT evaluated short-term N-acetylcysteine supplementation of cysteine-containing PN in extremely low birth weight infants (≤ 1000 g). The Authors reported the following outcomes: primary outcomes – Growth was not significantly affected by cysteine supplementation (evaluated in one quasi-randomized trial) or by N-acetylcysteine supplementation (evaluated in one RCT). Nitrogen retention was significantly increased by cysteine supplementation (studied in four trials) (WMD 31.8 mg/kg/day, 95% confidence interval +8.2, +55.4, n = 95, including 73 preterm infants); secondary outcomes – plasma levels of cysteine were significantly increased by cysteine supplementation but not by N-acetylcysteine supplementation. N-acetylcysteine supplementation did not significantly affect the risks of death by 36 postmenstrual weeks, bronchopulmonary dysplasia (BPD), death or BPD, retinopathy of prematurity (ROP), severe ROP, necrotizing enterocolitis requiring surgery, periventricular leukomalacia, intraventricular hemorrhage (IVH), or severe IVH. No data were available on other outcomes. The Authors concluded that available evidence from RCTs shows that routine short-term cysteine chloride supplementation of cysteine-free PN in preterm infants improves nitrogen balance, but there is insufficient evidence to assess the risks of cysteine supplementation, especially regarding metabolic acidosis, which has been reported during the first two weeks of cysteine chloride administration.

Melatonin and Cognitive Impairment or dementia

Jansen et al.[11] tried to assess the evidence of clinical efficacy and safety of melatonin in the treatment of manifestations of dementia or cognitive impairment (CI). The Authors collected all relevant, randomized controlled trials in which orally administered melatonin in any dosage was compared with a control group for the effect on managing cognitive, behavioural (excluding sleep), and/or affective disturbances of people with dementia of any degree of severity. 3 studies met the inclusion criteria. This review revealed non-significant effects from the pooled estimates of MMSE cognitive, and ADAS-cognitive change scores. Individual study estimates for treatment effect demonstrated a significant improvement for 3 mg melatonin compared with placebo in behavioural and affective symptoms as measured by the ADAS non-cognitive scale in a study of 20 patients, and the Neuropsychiatric Inventory (NPI) following treatment with 2.5 mg/day (SR) melatonin, but not with 10 mg/day (IR) melatonin in a larger study of 157 patients. Authors concluded that there is insufficient evidence to support the effectiveness of melatonin in managing the cognitive and non-cognitive sequelae of dementia.

A study to assess the role and clinical efficacy of alpha lipoic acid in the treatment of dementia was of Sauer J, Tabet N, Howard R: Alpha lipoic acid for dementia. (Cochrane Database Syst Rev 2004, Issue 1. Art. No.: CD004244. DOI: 10.1002/14651858.CD004244) One of the main articles related to the theme of antioxidants we have found in Cochrane Library was that on the therapeutic use of Ginkgo Biloba for the treatment of age-related macular degeneration by JR Evans (Evans JR. Ginkgo Biloba extract for age-related macular degeneration. Cochrane Database Syst Rev 1999, Issue 3. Art. No.: CD001775. DOI: 10.1002/14651858.CD001775).

Ginkgo is used in the treatment of peripheral vascular disease and 'cerebral insufficiency'. It is thought to have several potential mechanisms of action including increased blood flow, platelet activating factor antagonism and prevention of membrane damage caused by free radicals. Vascular factors and oxidative damage are thought to be two potential mechanisms in the pathology of age-related macular degeneration (AMD). The Authors aim was to determine the effect of Ginkgo Biloba extract on the progression of AMD.0

The Authors searched the Cochrane Central Register of Controlled Trials (CENTRAL). All randomized trials where Ginkgo Biloba extract had been compared to control in people with AMD were included. Two published trials were identified by the Authors identified but, unfortunately these trials were small (20 and 99 participants) and short. The overall conclusion of this

review was that current research has not answered the question as to whether Ginkgo Biloba is of benefit to people with AMD and that further research is needed.

Sauer et al searched the Specialized Register of the Cochrane Dementia and Cognitive Improvement Group (CDCIG) on 14 July 2005 using the terms 'alpha lipoic acid' and 'thioctic'. All double-blind randomized placebo-controlled trials examining the efficacy of alpha lipoic acid in dementia were included in this review. The Authors observed the absence of randomized double-blind placebo-controlled trials investigating ALA for dementia. The Authors concluded that until data from trials become available for analysis, ALA cannot be recommended for people with dementia.

Neurodegenerative Diseases

The brain sensivity to oxidative stress seems to be due to the high fatty acids content of nervous system and neuron high metabolic activity that expose the brain to free radicals and iron overload damage. Free radical species seem to be involved in the pathogenesis of Parkinson's disease in which we can observe decreased levels of antioxidant enzyme activity. Evidence of oxidative stress in the form of increased lipid peroxidation and oxidation of DNA bases is seen in the substantia nigra (the area of the brain affected in Parkinson's disease). Similar increased lipid peroxidation and oxidation of DNA and proteins have also been seen in Alzheimer's, the most important of neurodegenerative disorders in which brain cells, damaged by naturally occurring reactive oxygen species (ROS), have been observed. It has been suggested that Alzheimer disease may be linked to diet and that reduced AD risk is associated with diets high in antioxidants. However, whether this oxidative damage causes neurodegeneration or might be a consequence has not been detected.

Alzheimer Disease

Dowling et al.[12] to test whether the addition of melatonin to bright-light therapy enhances the efficacy in treating rest-activity (circadian) disruption in institutionalized patients with Alzheimer's disease (AD), developed a randomized controlled trial involving fifty subjects (mean age 86) with AD. Experimental subjects received 1 hour of morning light exposure (or = 2,500 lux in gaze direction) Monday to Friday for 10 weeks and 5 mg melatonin (LM, n=16) or placebo (LP, n=17) in the evening. Control subjects (n=17) received usual indoor light (150-200 lux). Nighttime sleep variables, day sleep time, day activity, day: night sleep ratio, and rest-activity parameters were determined using actigraphy. The Authors reported the following results: linear mixed models were employed to test the primary study hypotheses. No significant differences in night-time sleep variables were found between groups; at the end of the intervention. The LM group showed significant improvement in daytime somnolence as indicated by a reduction in the duration of daytime sleep, an increase in daytime activity, and an improvement in day-night sleep ratio. The LM group also evidenced a significant increase in rest-activity rhythm amplitude and goodness of fit to the cosinor model. In conclusion light treatment alone did not improve nighttime sleep, daytime wake, or rest-activity rhythm and light treatment plus melatonin increased daytime wake time and activity levels and strengthened the rest-activity rhythm. The Authors evidenced that future studies should resolve the question of whether these improvements can be attributed to melatonin.

Gray et al.[13] tried to examine whether use of vitamins C or E alone or in combination was associated with lower incidence of dementia or AD. The study involves 2969 participants aged 65 and older. Over a mean follow-up ± standard deviation of 5.5 ± 2.7 years, 405 subjects developed dementia (289 developed AD). The use of vitamin E was not associated with dementia (adjusted hazard ratio (HR)=0.98, 95% confidence interval (CI)=0.77–1.25 or with AD (HR=1.04; 95% CI=0.78–1.39). No association was found between vitamin C alone (dementia: HR=0.90, 95% CI=0.71–1.13; AD: HR=0.95, 95% CI=0.72–1.25) or concurrent use of vitamin C and E (dementia: HR=0.93, 95% CI=0.72–1.20; AD: HR=1.00, 95% CI=0.73–1.35) and either outcome. In conclusion, the use of vitamin E and C, alone or in combination, did not reduce risk of AD or overall dementia over 5.5 years of follow-up. The current evidence did not support recommending use of antioxidant vitamin supplements for prevention of dementia in older adults.

Analyzing these clinical studies we observe the use of antioxidants melatonin, vitamins C and E in the treatment of Alzheimer disease. The first trial shows that light treatment plus melatonin seem to elevate patients activity levels but, because of the small number of subjects involved, it cannot be considered sufficient to assess the real

effects and benefits of this type of treatment. The second clinical trial involves a large number of subjects and strongly evidences that the use of vitamin E and C alone or in combination do not reduce risk of AD. On the basis of these studies we can say that an antioxidant therapy is really far to bring important results in the treatment of this neurodegenerative disorder.

Parkinson Disease

Weber et al.[14] reviewed the use of antioxidants and other supplements for the prevention and treatment of Parkinson's disease (PD). The Authors considered three main antioxidants or supplements for use in the prevention or treatment of PD: tocopherol, CoQ_{10}, and glutathione. These agents have been studied because of their potential to alter the course of 2 common theories of PD pathogenesis: free radical generation and mitochondrial complex-1 deficiency. The literature search revealed 3 large clinical studies of tocopherol (2 observational, 1 prospective randomized), 4 trials of CoQ_{10}, and 1 study of glutathione. With the exception of the large observational studies with tocopherol and one study of CoQ_{10} that enrolled 80 patients, each of the other studies retrieved included fewer than 30 patients and were conducted for 3 months or less. Antioxidant supplementation, in particular tocopherol, did not appear to alter the course of PD. However, in 2 of the studies of CoQ_{10} and in the study of glutathione, a small but statistically significant improvement in PD symptoms was observed. This review emphasized how antioxidant supplements appear to have a limited role in the prevention or treatment of PD. Of those reviewed here, CoQ_{10} appeared to provide some minor treatment benefits.

Medeiros et al.[15] developed a randomized, double blind, placebo-controlled study to evaluate the effect of melatonin on sleep and motor dysfunction in PD. The Authors studied 18 patients with motor dysfunction assessed by UPDRS II, III and IV prior to treatment. Subjective sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI) and daytime somnolence by the Epworth Sleepiness Scale (ESS). Full polysomnography (PSG) was performed in all subjects. Patients were then randomized to receive melatonin (3 mg) or placebo one hour before bedtime for four weeks. All measures were repeated at the end of treatment. On initial assessment, 14 patients (70%) showed poor quality sleep (PSQI 6) and eight (40%) excessive daytime sleepiness (ESS 10). Increased sleep latency (50%), REM sleep without atonia (66%), and reduced sleep efficiency (72%) were found on PSG. 8 patients had an apnea/hipopnea index greater than 15 but no severe oxygen desaturation was observed. Sleep fragmentation tended to be more severe in patients on lower doses of levodopa (p=0.07). Although melatonin significantly improved subjective quality of sleep (p=0.03) as evaluated by the PSQI index, PSG abnormalities were not changed. Motor dysfunction was not improved by the use of melatonin.

The two previously described studies analyze respectively the effects of tocopherol, CoQ_{10}, glutathione and melatonin in the treatment of Alzheimer disease. The first study reviews eight clinical trials concluding that the antioxidants had very limited effect on AD in particular CoQ_{10}. The second trial gives evidence of a positive effect of melatonin in the improvement of quality of sleep but not in motor dysfunction. These trials enrolled e very limited number of patients and the follow up was too short; thus, further and better constructed studies are required to reach more reliable results and a final judgement.

Asthma

Asthma is an inflammatory airways process reducing the airflow in and out of the lungs. Asthmatics are exposed to continuous oxidative stress and antioxidant supplements are supposed to ease the asthma symptoms.

Patel et al.[16] wanted to assess the independent associations of some nutrients (vitamin C, manganese etc.) with asthma trough a study involving 515 adults with physician-diagnosed asthma and 515 matched controls whose dietary data had been obtained from seven-day food diaries. In this study 51.5% of the population reported zero consumption of citrus fruit, but, people who consumed >46.3 g/day had a reduced risk of diagnosed and symptomatic asthma (OR adjusted 0.59, CI 0.43-0.82, and 0.51, 0.33-0.79, respectively). In nutrient analysis, dietary vitamin C and manganese were inversely and independently associated with symptomatic asthma (adjusted OR per quintile increase 0.88, 0.77-1.00, vitamin C and 0.85, 0.74-0.98, manganese) but only manganese was independently associated with diagnosed asthma (0.86, 0.77-0.95). Adjusted plasma vitamin C levels were significantly lower in symptomatic cases (54.3 μmol/L) than in controls (58.2 μmol/L, p=0.003). The Authors con-

clude that diet may be a modifiable risk factor for the development of asthma.

Shaheen et al.[17] described a randomized, double blind, placebo-controlled trial to assess the efficacy of selenium supplementation in adults with asthma taking inhaled steroids at baseline. 197 participants were randomized to receive either a high-selenium yeast preparation (100 microg daily, n=99) or placebo (yeast only, n=98) for 24 weeks. Linear regression was used to analyse the change in outcome between the two treatment arms with the following results: there was a 48% increase in plasma selenium between baseline and end of trial in the active treatment group but no change in the placebo group. Concluding the study evidenced that selenium supplementation had no clinical benefit in adults with asthma, the majority of whom were taking inhaled steroids.

Wood et al.[18] study's investigated changes in asthma and airway inflammation resulting from a low antioxidant diet and subsequent use of lycopene-rich treatments. 32 asthmatic adults were enrolled and submitted to a low antioxidant diet for 10 days and then started a randomized, crossover trial involving 3 × 7 day treatment arms (placebo, tomato extract (45 mg lycopene/day) and tomato juice (45 mg lycopene/day)). With a low antioxidant diet, plasma carotenoid concentrations decreased, Asthma Control Score worsened, %FEV and %FVC decreased and %sputum neutrophils increased. Treatment with both tomato juice and extract reduced airway neutrophil influx. Treatment with tomato extract also reduced sputum neutrophil elastase activity. The authors conclude that dietary antioxidant consumption modified clinical asthma outcomes and that changing dietary antioxidant intake should be contributing to rising asthma prevalence. Lycopene-rich supplements should be further investigated as a therapeutic intervention.

These clinical trials analyze a possible use of vitamin C and manganese, selenium, lycopene respectively as a possible treatment in asthma disease. The rising evidence is that diet, vitamin C and manganese especially, seem to have an important role in asthma development instead of selenium that seems not to have a relevant clinical effect. Lycopene should be further investigated.

Pregnancy Pre-Eclampsia

Pre-eclampsia occurs usually after 20 pregnancy weeks affecting both the mother and the unborn baby. The disease is characterized by high blood pressure and proteinuria. The main symptoms are: swelling, sudden weight gain, headaches, changes in vision, generalized vasoconstriction, increased vasoactivity, reduced perfusion to organs and platelet activation. There is substantial evidence that preeclampsia clinical patterns arise from changes of the maternal vascular endothelium. Dysfunction in the antioxidant defenses has been suggested as an important factor in the pathogenesis of pre-eclampsia contributing to endothelial dysfunction. Supplementing women with antioxidants during pregnancy may help to counteract oxidative stress and thereby prevent or delay the onset of pre-eclampsia.

Poston et al.[19] study's tried to evaluate whether vitamin C and vitamin E supplements could reduce the risk of pre-eclampsia. Through a randomized, placebo-controlled trial, 2410 women identified as at increased risk of pre-eclampsia from 25 hospitals were assigned to receive 1000 mg vitamin C and 400 IU vitamin E (alpha tocopherol; n=1199) or placebo (n=1205) daily from the second trimester of pregnancy until delivery. Of 2404 patients treated, the Authors analyzed 2395 (99.6%). The incidence of pre-eclampsia was similar in treatment placebo groups (15% [n=181] vs 16% [n=187], RR 0.97 [95% CI 0.80-1.17]). These Authors concluded that the high-dose antioxidants are not justified in pregnancy because supplementation with vitamin C and vitamin E didn't prevent pre-eclampsia in women at risk in contrast with a Cochrane meta-analysis of antioxidant supplements for prevention of pre-eclampsia that suggested a modest benefit even if included studies in which micronutrients other than antioxidants were used.

Spinnato et al.[20] analyzed whether antioxidant supplementation reduced the incidence of preeclampsia in high risk patients through a randomized, controlled, double-blind clinical trial involving women 12-19 week of gestation. The daily treatment consisted of both vitamin C (1,000 mg) and vitamin E (400 International Units) or placebo. Analyses were adjusted for clinical site and risk group (prior preeclampsia, chronic hypertension, or both). Outcome data for 707 of 739 randomly assigned patients revealed no significant reduction in the rate of preeclampsia (study drug, 13.8% [49 of 355] compared with placebo, 15.6% [55 of 352], adjusted risk ratio 0.87 [95.42% confidence interval 0.61-1.25]). There were no differences in mean gestational age at delivery or rates of perinatal mortality, abruptio placentae, preterm delivery, and

small for gestational age or low birth weight infants. Among patients without chronic hypertension, there was a slightly higher rate of severe preeclampsia in the study group (study drug, 6.5% [11 of 170] compared with placebo, 2.4% [4 of 168], exact *P*=11, Odd's Ratio 2.78, 95% confidence interval 0.79-12.62). This trial failed to demonstrate any benefit of antioxidant supplementation in reducing the rate of preeclampsia among patients with chronic hypertension or prior preeclampsia.

Mehendale et al.[21] investigated whether free radical-mediated membrane lipid peroxidation should be implicated in the pathogenesis of pre-eclampsia using a sample of 55 healthy pregnant women and 60 pre-eclamptic women recruited at Bharati Medical Hospital, Pune, India. The Authors concluded that pre-eclamptic women showed reduced total omega-3 fatty acids ($p<0.05$), increased omega-6:omega-3 ratio ($p<0.05$), higher oxidative stress ($p<0.05$), and lower antioxidant ($p<0.05$) levels. Similar trends were also observed in cord samples. Reduced antioxidants and increased oxidative stress leading to impaired essential polyunsaturated fatty acid levels should be a key factor in the development of pre-eclampsia and it suggested that supplementation of antioxidants along with polyunsaturated fatty acids, particularly omega-3 fatty acids, should be useful in the management of pre-eclampsia.

Rumbold et al.[22] searched all randomized trials involving the use of one or more antioxidants vs placebo or no antioxidants during pregnancy for the prevention of pre-eclampsia, and trials comparing one or more antioxidants with another. They collected 10 trials including 6533 women. The majority of trials regard the use of combined vitamin C and E therapy. The Authors noted no significant difference between antioxidant and control groups for the relative risk (RR) of pre-eclampsia (RR 0.73, 95% confidence intervals (CI) 0.51 to 1.06; nine trials, 5446 women) or any other primary outcome: severe pre-eclampsia (RR 1.25, 95% CI 0.89 to 1.76; two trials, 2495 women), preterm birth (before 37 weeks) (RR 1.10, 95% CI 0.99 to 1.22; five trials, 5198 women), small-for-gestational-age infants (RR 0.83, 95% CI 0.62 to 1.11; five trials, 5271 babies) or any baby death (RR 1.12, 95% CI 0.81 to 1.53; four trials, 5144 babies). Women allocated to antioxidants were more likely to self-report abdominal pain late in pregnancy (RR 1.61, 95% CI 1.11 to 2.34; one trial, 1745 women), require antihypertensive therapy (RR 1.77, 95% CI 1.22 to 2.57; two trials, 4272 women) and require an antenatal hospital admission for hypertension (RR 1.54, 95% CI 1.00 to 2.39; one trial, 1877 women). The Authors found no reduction in pre-eclampsia, high blood pressure or preterm birth with the use of antioxidant supplements. When antioxidants were assessed separately, there were insufficient data to be clear about whether there was any benefit or not, except for vitamin C and E. The current evidence did not support the use of antioxidants to reduce the risk of pre-eclampsia or other complications in pregnancy.

The previously described studies involved the use of vitamin C, vitamin E and omega- 3 fatty acids supplements as a possible treatment or to prevent pre-eclampsia. Vitamin C and vitamin E seem not to prevent pre-eclampsia instead supplementation of antioxidants along with polyunsaturated fatty acids, particularly omega-3 fatty acids, may be useful in the management of this pathology.

Cancer

Cancer seems to be straightly linked to oxidative damage. A very great number of studies to assess a possible relationship between cancer and diet have been performed. Exposure to various environmental factors, including tobacco smoke and radiation, can also lead to free radical formation. In humans, the most common form of free radicals is oxygen. When an oxygen molecule (O_2) becomes electrically charged it tries to take off electrons from other molecules, causing damage to the DNA. This damage may become irreversible and lead to cancer. The main investigations between antioxidants and cancer were the following:

Almushatat et al.[23] studied the relationship between lipid soluble antioxidant vitamins, lipid peroxidation, disease stage and the systemic inflammatory response in healthy subjects (n = 14), patients with benign prostate hyperplasia BPH (n = 20), localized (n = 40) and metastatic (n = 38) prostate cancer. Prostate cancer patients had higher concentrations of malondialdehyde (0.05) and lower circulating concentrations of lutein (0.05), lycopene (0.001) and beta-carotene (0.05). Patients with metastatic prostate cancer compared with patients having localized disease seemed to have a higher Gleason score (0.01) and had more hormonal treatment, but lower concentrations of PSA (0.05), alpha-tocopherol (or=0.05), retinol (0.01), lutein (0.05) and ly-

copene (0.01). In the prostate cancer patients, PSA was correlated with the concentrations of the lipid peroxidation product, malondialdehyde (rs=0.353, *p*=0.002). C-reactive protein was not correlated with the vitamin antioxidants nor malondialdehyde. In contrast, there was a negative correlation between malondialdehyde concentrations and both lutein (rs=–0.263, *p*=0.020) and lycopene (rs=–0.269, *p*=0.017). The previous described results indicated that lower concentrations of carotenoids, in particular lycopene, reflected disease progression rather than the systemic inflammatory response in patients with prostate cancer.

Kirsh et al.[24] evaluated the association between intake of antioxidants from foods and supplements and the risk of prostate cancer among men in the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. At baseline, trial participants completed a 137-item food frequency questionnaire that included detailed questions on 12 individual supplements. Cox proportional hazards models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs). All statistical tests were two-sided. The Authors reported the following results: 1338 cases of prostate cancer among 29 361 men during up to 8 years of follow-up were identified; there was no association between prostate cancer risk and dietary or supplemental intake of vitamin E, beta-carotene, or vitamin C; among current and recent (i.e., within the previous 10 years) smokers, decreasing risks of advanced prostate cancer (i.e., Gleason score; or = 7 or stage III or IV) were associated with increasing dose (RR; 400 IU/day versus none = 0.29, 95% CI = 0.12 to 0.68; P trend = .01) and duration (RR; or = 10 years of use versus none = 0.30, 95% CI = 0.09 to 0.96; P trend = .01) of supplemental vitamin E use; beta-carotene intake at a dose level of at least 2000 microg/day was associated with decreased prostate cancer risk in men with low (below the median of 4129 microg/day) dietary beta-carotene intake (RR = 0.52, 95% CI = 0.33 to 0.81); among smokers, the age-adjusted rate of advanced prostate cancer was 492 per 100,000 person-years in those who did not take supplemental vitamin E, 153 per 100,000 person-years in those who took more than 400 IU/day of supplemental vitamin E, and 157 per 100,000 person-years in those who took supplemental vitamin E for 10 or more years; among men with low dietary beta-carotene intake, the age-adjusted rate of prostate cancer was 1122 per 100,000 person-years in those who did not take supplemental beta-carotene, and 623 per 100,000 person-years in those who took at least 2000 microg/day of supplemental beta-carotene. These results lead the Authors to conclude that this study do not provide strong support for population-wide increase of high-dose antioxidant supplementation for the prevention of prostate cancer but vitamin E supplementation in male smokers and beta-carotene supplementation in men with low dietary beta-carotene intakes were associated with reduced risk of this disease.

Reid et al.[25] investigated the association between selenium supplementation and prevalent and incident colorectal adenomas and CRC detected during the Nutritional Prevention of Cancer trial follow-up. 1,312 recipients were randomized to 200 mcg of selenized yeast of matching placebo but only 598 underwent endoscopic screening (flexible sigmoidoscopy or colonoscopy) for CRC sometime during the follow-up period, which ended in February 1, 1996. There was no colorectal screening performed at baseline. Of those screened, 77% were male (with a mean age of 62.8 years), 42% were former and 25% were current smokers. Adenomas were classified as prevalent (identified at the first endoscopic examination post-randomization during the follow-up period) or incident (identified at the second or subsequent examination). Ninety-nine prevalent and 61 incident adenomas were ascertained. Logistic regression odds ratios (OR) and 95% confidence intervals (CI) were calculated, adjusting for age, gender and smoking status. For prevalent adenomas, there was a suggestive but non significant decrease in risk associated with selenium treatment (OR = 0.67, 95% CI = 0.43-1.05). Subjects in the lowest tertile of baseline selenium (OR = 0.27, 95% CI = 0.09-0.77) and current smokers (OR = 0.27, 95% CI = 0.11-0.66) had significant reductions in risk. The OR for incident adenomas was 0.98 (95% CI = 0.57-1.68). These results evidenced that selenium supplementation was associated with a significantly reduced risk of prevalent adenomas, but only among subjects with either a low baseline selenium level or among current smokers.

Hercberg et al.[26] tested whether supplementation with a combination of antioxidant vitamins and minerals could reduce the risk of skin cancers (SC) performed within the framework of the Supplementation in Vitamins and Mineral Antioxidants study, a randomized, double-blinded, placebo-controlled, primary prevention trial test-

ing the efficacy of nutritional doses of antioxidants in reducing incidence of cancer and ischemic heart disease in the general population. French adults (7876 women and 5141 men) were randomized to take an oral daily capsule of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg beta-carotene, 100 μg selenium, and 20 mg zinc) or a matching placebo. The median time of follow-up was 7.5 y. A total of 157 cases of all types of SC were reported, from which 25 were melanomas. Because the effect of antioxidants on SC incidence varied according to gender, men and women were analyzed separately. In women, the incidence of SC was higher in the antioxidant group [adjusted hazard ratio (adjusted HR) = 1.68; p=0.03]. Conversely, in men, incidence did not differ between the 2 treatment groups (adjusted HR = 0.69; p=0.11). Despite the small number of events, the incidence of melanoma was also higher in the antioxidant group for women (adjusted HR = 4.31; p=0.02). The incidence of non melanoma SC did not differ between the antioxidant and placebo groups (adjusted HR = 1.37; p=0.22 for women and adjusted HR = 0.72; p=0.19 for men). This study suggests that antioxidant supplementation affects the incidence of SC differentially in men and women.

Weinstein et al.[27] investigated if serum alpha-tocopherol or intake of vitamin E (eight tocopherols and tocotrienols) was associated with prostate cancer risk with up to 19 years of follow-up in the alpha-Tocopherol, beta-Carotene Cancer Prevention Study cohort. Of the 29,133 finnish male smokers (aged 50 to 69) the Authors have recruited into the study, 1,732 were diagnosed with incident prostate cancer between 1985 and 2004. Baseline serum alpha-tocopherol was measured by high-performance liquid chromatography and the components of vitamin E intake were estimated based on a 276-item food frequency questionnaire and food chemistry analyses. Proportional hazard models were used to determine multivariate-adjusted relative risks (RR) and 95% confidence intervals (95% CI). Higher serum alpha-tocopherol was associated with reduced risk of prostate cancer (RR, 0.80; 95% CI, 0.66-0.96 for highest versus lowest quintile; P trend = 0.03) and was strongly and inversely related to the risk of developing advanced disease (RR, 0.56; 95% CI, 0.36-0.85; P trend = 0.002). The inverse serum alpha-tocopherol-prostate cancer association was greater among those who were supplemented with either alpha-tocopherol or beta-carotene during the trial. There were no associations between prostate cancer and the individual dietary tocopherols and tocotrienols. Concluding, these results evidenced that higher prediagnostic serum concentrations of alpha-tocopherol, but not dietary vitamin E, was associated with lower risk of developing prostate cancer, particularly advanced prostate cancer.

Bardia et al.[28] to estimate the association between antioxidant use and primary cancer incidence and mortality and to evaluate these effects across specific antioxidant compounds, target organs, and participant subgroups, developed a systematic review searching multiple electronic databases from their dates of inception until August 2005 to identify eligible randomized clinical trials. Random effects meta-analyses estimated pooled relative risks (RRs) and 95% confidence intervals (CIs) that described the effect of antioxidants vs placebo on cancer incidence and cancer mortality. The reported results were the following Twelve eligible trials, 9 of high methodological quality, were identified (total subject population, 104,196). Antioxidant supplementation did not significantly reduce total cancer incidence (RR, 0.99; 95% CI, 0.94-1.04) or mortality (RR, 1.03; 95% CI, 0.92-1.15) or any site-specific cancer incidence. Beta carotene supplementation was associated with an increase in the incidence of cancer among smokers (RR, 1.10; 95% CI, 1.03-1.10) and with a trend toward increased cancer mortality (RR, 1.16; 95% CI, 0.98-1.37). Selenium supplementation was associated with reduced cancer incidence in men (RR, 0.77; 95% CI, 0.64-0.92) but not in women (RR, 1.00; 95% CI, 0.89-1.13, value for interaction, .001) and with reduced cancer mortality (RR, 0.78; 95% CI, 0.65-0.94). Vitamin E supplementation had no apparent effect on overall cancer incidence (RR, 0.99; 95% CI, 0.94-1.04) or cancer mortality (RR, 1.04; 95% CI, 0.97-1.12). This review evidenced that beta carotene supplementation appeared to increase cancer incidence and cancer mortality among smokers, whereas vitamin E supplementation had no effect; selenium supplementation might have anticarcinogenic effects in men and thus requires further research.

Gramignano et al.[29] realize a study to assess if L-carnitine (LC) supplementation is able to improve fatigue symptoms in patients with cancer. The Authors tested the efficacy and safety of LC supplementation in a population of patients who had advanced cancer and developed fatigue, high blood levels of reactive oxygen species, or both.

As outcome measures they evaluated fatigue and quality of life in relation to oxidative stress, nutritional status, and laboratory variables, mainly levels of reactive oxygen species, glutathione peroxidase, and proinflammatory cytokines. 12 patients who had advanced tumors (50% at stage IV) at different sites were enrolled (male-to-female ratio 2:10, mean age 60 y, range 42-73). Patients were only slightly anemic (hemoglobin 10.9 g/dL) and hemoglobin levels did not change after treatment. LC was administered orally at 6 g/d for 4 wk. All patients underwent antineoplastic treatment during LC supplementation. The Authors obtained the following results: Fatigue, as measured by the Multidimensional Fatigue Symptom Inventory–Short Form, decreased significantly, particularly for the General and Physical scales, and for quality of life in each subscale of quality of life in relation to oxidative stress. Nutritional variables (lean body mass and appetite) increased significantly after LC supplementation. Levels of reactive oxygen species decreased and glutathione peroxidase increased but not significantly. Proinflammatory cytokines did not change significantly. The Authors concluded that the improvement of symptoms with respect to fatigue and quality of life in relation to oxidative stress may be explained mainly by an increase in lean body mass, which may be considered the most important nutritional or functional parameter in assessing the cachectic state of patients. In this view, fatigue with related symptoms can well be considered an important constituent of cancer-related anorexia cachexia syndrome.

Mantovani et al.[30] recently carried out an open-labeled phase II study with the aim to test in a population od patients with advanced cancer with CACS/OS the efficacy and safety of an integrated treatment based on diet with high polyphenols content, antioxidant treatment (alpha lipoic acid plus carbocysteine lysine salt plus vitamin E plus vitamin A plus vitamin C), pharmaconutritional support enriched with n-3 polyunsatured fatty acids (eicosapentaenoic acid, docosahexaenoic acid), medroxyprogesterone acetate, and a selective cyclooxygenase-2 inhibitor (celecoxib). The results of this phase II study demonstrate that the treatment administered was effective in inducing a significant increase not only of total body weight (p=0.031) but also LBM (p=0.024). There was a significant decrease of proinflammatory cytokines interleukin [IL]-6 (p=0.015). The decrease of IL-6 after treatment was the only variable significantly correlated with LBM. This finding further strengthens the role of proinflammatory cytokines in the pathophysiology of CACS/OS. The significant increase of leptin after treatment ($p \leq 0.0001$) confirms its inverse association with proinflammatory cytokines. Based on the results previously described a phase III randomized study was started in february 2005 as a multicenter trial involving 10-12 italian oncology departments with the aim of testing the safety and efficacy of an integrated approach of CACS/OS to improve objective clinical symptoms such as LBM and subjective symptoms such as functioning and QOL. This study has the aim of enstablishing which is the most effective/safe treatment of CACS/OS in terms of amelioration of "key" variables of CACS/OS. The study is under way, and up until November 2006, 115 patients have been enrolled. The ultimate goal should be to translate the results obtained in patients with advanced cancer into a prevention trial in a population of individuals at risk of developing CACS/OS.

Analyzing the previous reported studies and reviews we can see that most antioxidants have been investigated to assess a possible beneficial effects in cancer treatment. Different studies (Almushatat et al.; Weinstein et al.) evidence that carotenoids seem to be involved in prostate cancer progression; instead the Kirsh V.A. et al. trial report results that suggest that vitamin E supplementation in male smokers and beta-carotene supplementation in men with low dietary beta-carotene intakes were associated with reduced risk of this disease.

Selenium supplementation seems to be associated with a significantly reduced risk of prevalent adenomas in subjects with a low baseline selenium level or among current smokers subjects.

Hercberg S. tests whether supplementation with vitamic C, vitamin E, beta-carotene. Selenium and zinc could reduce the risk of skin cancers (SC) reporting that in women, the incidence of SC was higher in the antioxidant group and in men, incidence did not differ between the 2 treatment groups suggesting that antioxidant supplementation affects the incidence of SC differentially in men and women.

Cardiovascular Diseases

Many studies evaluated the antioxidant impact on reducing cardiovascular disease risk but no evidence supported this hypothesis. Oxidation of low-density lipoprotein (LDL) is important in the

development of fatty buildups in the arteries. Atherosclerosis, is a process due to the oxidation of low-density lipoprotein (LDL) and cholesterol and inflammatory debris (atheromas or atherosclerotic plaques) infiltrating the arterial sheaths leading to heart attacks and strokes or peripheral vascular diseases. It was thought that LDL cholesterol lipoprotein oxidation and its biological effects could be prevented by using antioxidant supplements. However, more recent clinical trials have failed to demonstrate a beneficial effect of antioxidant supplements. The following studies describe the state of the art:

Berhendt et al.[31] in their placebo controlled study studied the effect of anti-oxidant vitamins C and E in 40 cardiac transplant recipients according to normal or abnormal coronary endothelial vasomotor function at baseline to assess if vitamin C (500 mg twice a day) and vitamin E 400 (IU twice a day) for 1 year retarded the progression of cardiac transplant associated arteriosclerosis (TxAA). The antioxidants effect was related to the change in intimal index using intravascular ultrasound (IVUS). In the 21 placebo administered patients the increase in intimal index was greater in the presence vs absence of endothelial dysfunction (11 ± 3% vs 5 ± 1%, $p<0.05$). Among patients with endothelial dysfunction (n = 21) the intimal index increased 11 ± 3% with placebo, but decreased −1 ± 2% with vitamins ($p=0.002$). Among patients with normal endothelial function (n = 14), the intimal index increased 5 ± 1% with placebo and 1 ± 1% with vitamins ($p<0.05$). The Authors concluded that endothelial dysfunction indicates rapid TxAA progression and that anti-oxidant vitamins reduce disease progression in patients with normal or abnormal endothelial function with a particular benefit in patients with endothelial dysfunction.

Hatzigeorgiou et al.[32] evaluated the association between antioxidants and advanced coronary atherosclerosis. The study involved 865 patients aged 39-45 years without known coronary artery disease. The Authors used the Block Dietary Questionnaire to assess antioxidant intake. Coronary atherosclerosis was identified by measuring coronary artery calcification using electron beam computed tomography. At the end of this study vitamin supplements were used by 56% of the participants, and the mean (±SD) daily intake (dietary plus supplemental) of vitamins A, C, and E were 1683 mg (±1245), 371 mg (±375), and 97 mg (±165), respectively. The Authors concluded there was no significant correlation between coronary artery calcification score and individual vitamin or total antioxidant vitamin intake. The highest quartile of vitamin E was positively associated with calcification (odds ratio=1.77; 95% confidence interval, 1.02-3.06). Antioxidant vitamin intake seemed not to be significantly related to coronary artery calcification, implying that there is no effect on the development of early coronary atherosclerosis. High doses of vitamin E should be associated with increased risk of calcified atherosclerosis.

Inatomi et al.[33] performed a study to investigate the efficacy of edaravone, a powerful neuroprotective free radicals scavenger, in patients with cardioembolic stroke. The patients treatment was based on the use of intravenous infusion of edaravone (ED group, n=141) for 7 days, and was compared with a historical-controlled cohort of similar patients (control group, n=114). The Authors reported an early improvement between day 0 and day 10 (defined as change in National Institutes of Health Stroke Scale: NIHSS) which was seen more frequently in mild patients among the ED group than in the control group (change in NIHSS +2 vs. -2, respectively, $p=0.013$). Similar efficacy was not seen in the moderate to severe (NIHSS 7) patients. Independent patients (modified Rankin Scale; or = 2) 6 months after the onset were likely to be less frequent in the ED than the control group (28% versus 41%; $p=0.066$). This study results suggested that edaravone should only be effective in mild patients with cardioembolic stroke.

Ullegaddi et al.[34] tested whether supplementary antioxidants with or without B-group vitamins administered after acute ischemic stroke, enhanced antioxidant capacity or mitigate oxidative damage. 96 acute ischemic stroke patients were randomized to receive either daily oral 727 mg vitamin E and 500 mg vitamin C (n=24), or B-group vitamins ((5 mg folic acid, 5 mg vitamin B(2), 50 mg vitamin B(6), and 0.4 mg of vitamin B(12); n = 24), both vitamins together (n = 24), or no supplementation (n = 24) for 14 days.

After subdividing all patients for stroke subtype and age, blood samples were obtained at day 7 and day 14 (before treatment) for measurements of plasma or blood vitamin status, plasma total antioxidant capacity (TAOC), malondialdehyde (MDA), tHcy (Total homocysteine (tHcy) is the sum of several circulating homocysteine (Hcy) species that can be measured in plasma or serum and C-reactive protein (CRP). This study evidenced that supplementation with antioxidant vit-

amins and B-group vitamins separately or together significantly increased the plasma concentration of vitamin C, E, pyridoxal phosphate B_6, red blood cell folate, and improved a measure of B_2 status, compared with the control group. Plasma TAOC increased significantly in the antioxidant treatment groups compared with the non significant decline seen in the control group. tHcy (total homocysteine) concentrations decreased in subjects who received B-group vitamins and the control group compared with the rise seen in those who received antioxidants alone. There was a significant reduction in plasma MDA concentration in the 3 treatment groups, in contrast to the increase seen in the control group; however, the changes were most evident in antioxidant groups. CRP concentrations (a marker of tissue inflammation) were significantly lower in the 3 treatment groups compared with the control group. There were no additive or synergistic effects of antioxidants and B-group vitamins together on any outcome measure.

In conclusion, this study evidenced that antioxidants supplementation with or without B-group vitamins enhances antioxidant capacity, mitigates oxidative damage, and may have an anti-inflammatory effect immediately postinfarct in stroke disease.

Yesilbursa et al.[35] assessed the effect of N-acetylcysteine (NAC) treatment on oxidative stress, infarct size, and left ventricular (LV) function, as adjunct therapy in myocardial infarction (MI). Patients with acute MI where divided into 2 groups to receive either 15 g NAC infused over 24 h (n = 15) or no NAC (n = 15), combined with streptokinase. Peripheral venous blood was serially sampled to measure creatine kinase (CK)-MB levels. Plasma malondialdehyde (MDA) level was measured at admission and after 4 and 24 h. Echocardiography was performed within 3 days of MI and after 3 months. At admission, plasma MDA levels were not different between the groups. In the NAC-treated patients plasma MDA levels decreased, whereas in the nontreated NAC patients MDA levels increased at 4 and 24 h (0.01 and 0.001, respectively). Left ventricular ejection fraction was higher (0.05) and LV end-systolic and end-diastolic diameters were lower (0.001) in patients receiving NAC on day 3. Left ventricular wall motion score index was significantly lower in patients treated with NAC on day 3 (0.05). Left ventricular diastolic parameters were not different whether patients were treated with NAC or not. No difference in reduction of infarct size was detected between the groups according to CK-MB levels. This study conclusion evidenced that administration of NAC in combination with streptokinase significantly diminished oxidative stress and improved LV function in patients with acute MI.

Botha et al.[36] assessed the efficacy of Nitric Oxide (NO) in reducing neutrophil infiltration and associated injury if administered from the very onset of reperfusion in clinical lung transplantation. For this randomized study were enrolled 20 bilateral sequential lung transplant patients to receive 20-ppm inhaled NO (NO group) or a standard anesthetic gas mixture (control group) from the onset of ventilation. Bronchoalveolar lavage was performed immediately prior to implantation and after 30 minutes of reperfusion and analyzed for inflammatory cytokine levels and free radical surrogates. Primary graft dysfunction (PGD) scoring was performed prospectively for 72 hours post-transplant. The Authors obtained that the prophylactic administration of NO during the first 30 minutes of reperfusion had no significant effect on the development of Grade II to III PGD (5 of 10 in NO group and 7 of 10 in control group, p=0.36) or gas exchange (area under the curve: 429 ± 296 vs 336 ± 306; p=0.64) in the NO and control groups, respectively. Pulmonary neutrophil sequestration, as measured by the transpulmonary arteriovenous neutrophil difference, was not influenced by the administration of NO. Prophylactic NO did not significantly alter the concentration of interleukin-8, myeloperoxidase or nitrotyrosine during transplantation. In conclusion the Authors could not demonstrate a significant effect of inhaled NO during the first 30 minutes of reperfusion in the prevention of neutrophil injury and primary graft dysfunction after lung transplantation.

Cook et al.[37] examined the individual effect of ascorbic acid (vitamin C) on CVD. The Women's Antioxidant Cardiovascular Study tested the effects of ascorbic acid (500 mg/d), vitamin E (600 IU every other day), and beta carotene (50 mg every other day) on the combined outcome of myocardial infarction, stroke, coronary revascularization, or CVD death among 8171 female health professionals at increased risk in a 2 × 2 × 2 factorial design. The study involved 40 years or older recipients with a history of CVD or 3 or more CVD risk factors and were followed up for a mean duration of 9.4 years, from 1995-1996 to 2005. A total of 1450 women experienced 1 or

more CVD outcomes. There was no overall effect of ascorbic acid (relative risk [RR], 1.02; 95% CI, 0.92-1.13 [P = .71]), vitamin E (RR, 0.94; 95% CI, 0.85-1.04 [*p*=0.23]), or beta carotene (RR, 1.02; 95% CI, 0.92-1.13 [*p*=0.71]) on the primary combined end point or on the individual secondary outcomes of myocardial infarction, stroke, coronary revascularization, or CVD death. A marginally significant reduction in the primary outcome with active vitamin E was observed among the prespecified subgroup of women with prior CVD. There were no significant interactions between agents for the primary end point, but those randomized to both active ascorbic acid and vitamin E experienced fewer strokes (*p* value for interaction, 0.03). Conclusively, there were no overall effects of ascorbic acid, vitamin E, or beta carotene on cardiovascular events among women at high risk for CVD.

Hozawa et al.[38] investigated the effect of carotenoids on atherosclerotic risk factors. The association of circulating carotenoids with inflammation, oxidative stress, endothelial dysfunction, and smoking were the main factors analyzed. Black and white men and women in the Coronary Artery Risk Development in Young Adults study aged 18 to 30 years from 4 US cities, were investigated over 15 years. The Authors included 2048 to 4580 participants in analyses of the sum of serum alpha-carotene, beta-carotene, zeaxanthin/lutein, and beta-cryptoxanthin concentrations and of lycopene at year 0 and at year 7. The year 0 sum of 4 carotenoids was inversely associated (0.05) with year 0 leukocyte count (slope per sum carotenoid SD, –0.17); year 7 fibrinogen (slope, –0.10); year 7 and year 15 C-reactive protein (slope, –0.12 and –0.09); and year 15 F(2)-isoprostanes (slope, –13.0), soluble P-selectin (slope, –0.48), and soluble intercellular adhesion molecule-1 (sICAM1; slope, –5.1). Leukocyte counts and sICAM1 and F(2)-isoprostane concentrations had stronger associations in smokers than in nonsmokers, and sICAM1 concentrations were higher in the highest carotenoid quartile in smokers than in the lowest carotenoid quartile in nonsmokers. Superoxide dismutase was positively associated with the sum of 4 carotenoids (slope, 0.12; p< 0.01). Lycopene was inversely associated only with sICAM1. The year 7 carotenoid associations with these markers were mostly similar to those at year 0. Based on the previous data, the Authors concluded that circulating serum carotenoids were associated, some interactively with smoking, with some benefits as to markers of inflammation, oxidative stress, and endothelial dysfunction.

Macao et al.[39] study's showed an increase in oxidative stress associated with the progression of the severity of Chagas' disease. Components of the antioxidant system and oxidative biomarkers present in the blood were measured in the same chronic chagasic patients (n=40), before and after vitamin E (800 IU/day) and vitamin C (500 mg/day) supplementation for 6 months. Antioxidant enzymes and contents of reduced glutathione in erythrocytes and plasma thiobarbituric acid-reactive substances (TBARS) contents were analyzed in four groups of patients in different stages of chronic Chagas heart disease (n=10 each group, groups I, II, III, and IV) according to the Los Andes classification. After the combined vitamin supplementation, TBARS and protein carbonyl levels were decreased in plasma, whilst red cell GSH contents were increased in group I. The vitamin E contents found in the plasma were inversely related to the severity of the disease. No differences in gamma-glutamiltransferase activities were detected but the myeloperoxidase levels were decreased in patients at the initial stages, whilst seric nitric oxide levels were increased in groups II and III. After the antioxidant supplementation, CAT activity was increased in group II, GPx activity was increased in group I, GR activity was increased in groups I and II, whilst the GST activity was decreased in groups II, III and IV. The results indicated that the antioxidant supplementation was able to counteract the progressive oxidative stress associated with the disease. In conclusion the Authors concluded that perspectives for the treatment of Chagas' disease might include an antioxidant therapy in order to attenuate the consequences of oxidative insult related to this disease.

Plantinga[40] evaluated the effect of short-term combined treatment with the antioxidants vitamins C and E on endothelial function, arterial stiffness, and oxidative stress in untreated essential hypertensive patients. 30 male essential hypertensive recipients were involved in this randomized, double-blind, placebo-controlled crossover study to receive either vitamin C (1 g) and vitamin E (400 IU) or placebo for 8 weeks. Endothelium-dependent response was assessed as flow-mediated dilation (FMD) of the brachial artery. Arterial stiffness was assessed as central pulse wave velocity (PWV) and augmentation index (AIx). Plasma markers of oxidative stress

and antioxidant status were measured. The Authors obtained the following results: after vitamin supplementation, FMD was significantly improved, central PWV was significantly reduced, while AIx tended to decrease, plasma vitamin levels and antioxidant capacity increased significantly,levels of oxidative stress decreased and changes in central PWV were related to changes in levels of oxidative stress. On the basis of the previously described results the Authors concluded that combined treatment with vitamins C and E has beneficial effects on endothelium-dependent vasodilation and arterial stiffness in untreated, essential hypertensive patients and that effect is associated with changes in plasma markers of oxidative stress.

Shinke, T. et al.[41] investigated if the antioxidant vitamin C might help to reverse hyporesponsiveness to beta-adrenergic stimulation and improve myocardial efficiency in patients with heart failure (HF) after myocardial infarction (MI). 19 patients with mild to moderate HF due to previous MI (mean left ventricular [LV] ejection fraction 39%) were investigated with conductance and coronary sinus thermodilution catheters. Left ventricular contractility, expressed as E(es), the slope of end-systolic pressure-volume relationship, and mechanical efficiency, expressed as the ratio of LV stroke work (SW) to myocardial oxygen consumption (MVO2), were measured in response to the intravenous infusion of dobutamine (4 microg/kg per min) before (Dob) and during (Dob + Vit C) the infusion of vitamin C (2.0-g bolus injection and subsequent 50-mg/min infusion through the jugular vein) (vitamin C group, n = 10). The infusion of vitamin C augmented the E(es) response to dobutamine by 20% ± 8% (Dob 2.1 ± 0.3, Dob + Vit C 2.5 ± 0.4 mm Hg/mL) and the SW/MVO2 response by 21% ± 5% (Dob 36% ± 3%, Dob + Vit C 43% ± 4%). In the control group (n = 9), E(es) and SW/MVO2 were measured in response to dobutamine before (Dob) and during (Dob + vehicle) the infusion of saline. No difference in E(es) or SW/MVO2 was observed between Dob and Dob + vehicle (E(es): Dob 2.1 ± 0.2, Dob + vehicle 2.1 ± 0.2 mm Hg/mL per square meter, *p* = nonsignificant) (SW/MVO2: Dob 35% ± 4%, Dob + vehicle 33% ± 4%, *P* = nonsignificant). The previous data brought the Authors to conclude that the administration of the antioxidant vitamin C is able to enhance the contractile response to dobutamine and improve myocardial efficiency in patients with HF.

Singh et al.[42] described the outcome of 5 weeks of 100 mg/d or 200 mg/d gamma-T supplementation on thrombotic markers such as platelet reactivity, lipid profile and the inflammation marker C-reactive protein (CRP). The Authors developed a double blinded paralles study involving 14 healthy subjects consuming 100 mg/day while 13 consume 200 mg/d of gamma-Tocopherol and 12 received placebo (soybean capsules with less than 5 mg/d gamma-T). Fasting pre and post dose blood samples were analyzed. Blood gamma-T concentrations increased significantly (0.05) relative to dose during the intervention period. Both groups receiving active ingredients showed significantly lower platelet activation after supplementation (0.05). Subjects consuming 100 mg/d gamma-T had significantly decreased LDL cholesterol, platelet aggregation and mean platelet volume (MPV) (0.05). Little effect of gamma-T was observed on other parameters. The Authors conclude that gamma-T supplementation seems to have a permissive role in decreasing the risk of thrombotic events by improving lipid profile and reducing platelet activity.

Suda et al.[43] investigated the anti-edema effect of edaravone by magnetic resonance imaging in six patients with extensive hemispheric ischemic stroke. T(2) relaxation time in the infarct core, the boundary zone of the infarct, and the noninfarcted hemisphere were calculated, and T(2) mapping was performed before and after edaravone administration. Edaravone administration significantly decreased the mean T(2) relaxation time in the boundary zone of the infarct from 121.5 ± 9.2 (mean ± standard deviation) to 114.5 ± 9.9 msec (p=0.008), but not in the core from 142.3 ± 13.4 to 142.2 ± 18.5 msec (p=0.97) or the noninfarcted hemisphere from 93.0 ± 3.7 to 93.1 ± 3.8 msec (p=0.91). The T(2) subtraction map clearly demonstrated shortened T(2) relaxation time in the boundary zone of the infarct. The results from this study indicated that edaravone can salvage the boundary zone of the infarct and is a useful cytoprotective anti-edema agent.

Vincent et al.[44] explored whether 3 months of lipoic acid (LA) supplementation improved walking tolerance and delayed claudication pain onset in peripheral arterial disease (PAD). The Authors designed a randomized, double-blind, controlled study at General Clinical Research Center enrolling 28 participants (15 men, 13 women) with PAD (ankle brachial index range

0.9-0.4, mean age 73.2 ± 1.6 years). LA (600 mg/day) or placebo were administered for 3 months. The parameters observed were walking tolerance assessed by 6-minute walk test distance, 4-meter walk time, initial claudication pain time (ICT) and distance (ICD), and peak claudication pain. Serum was assessed for inflammation (C-reactive protein [CRP]) and oxidative stress (lipid hydroperoxides) as potential mechanisms for changes in walking tolerance. The study gave the following results: ICT increased 34.4% and 15%, ICD was reduced by 40.5% and 18%, and peak claudication pain ratings were reduced by 93% and 7% in LA and placebo groups, respectively. Although the improvements in peak pain and ICT achieved significance within the LA group (both 0.05), the interactions of group by time were not found to be significant (0.05). Oxidative stress and CRP measures were not different between groups by month 3 (0.05). There were no serious side-effects associated with the LA. Basing on the previous results the Authors concluded that LA seem to relieve exercise pain but longer and larger trials are needed to determine long-term effects of LA alone or combined with other interventions on PAD symptoms.

Milman et al.[45] tested the hypothesis that vitamin E could reduce cardiovascular events in diabetic patients with the Haptaglobulin (Hp) 2-2 genotype, a subgroup that comprises 2% to 3% of the general population. 1434 DM individuals were randomized to vitamin E (400 U/d) or placebo. The primary composite outcome was myocardial infarction, stroke, and cardiovascular death. At the first evaluation of events, 18 months after initiating the study, the primary outcome was significantly reduced in individuals receiving vitamin E (2.2%) compared with placebo (4.7%; p=0.01) and led to early termination of the study. The Authors concluded that Vitamin E supplementation appears to reduce cardiovascular events in individuals with DM and the Hp 2-2 genotype.

Ischemia-Reperfusion (IR) Injury

Pleiner et al.[46] tested if IR(-induced endothelial dysfunction) could be prevented by administration of the antioxidant vitamin C. Twenty-six healthy male subjects and eight male patients with peripheral arterial disease (PAD) were enrolled in this randomized placebo-controlled study. Forearm blood flow (FBF) measurements in response to the vasodilators acetylcholine (ACh; endothelium-dependent agonist) or nitroglycerin (NTG; endothelium-independent) were performed before and after forearm ischemia for 20 min. FBF responses were reassessed during reperfusion with intra-arterial co-administration of 24 mg/min vitamin C or placebo. In six volunteers responses to the NO-synthase inhibitor N-monomethyl-L-arginine (L-NMMA) were also assessed before and after ischemia with and without vitamin C. The study gave the following results: ACh-induced vasodilatation was blunted in subjects receiving placebo after reperfusion (0.05 versus baseline); administration of vitamin C completely prevented impaired responsiveness; NTG-induced vasodilatation was not affected by reperfusion or vitamin C and this finding was consistent in patients with PAD and impaired endothelial function, where local vitamin C infusion restored FBF reactivity to ACh before and after IR injury (0.05 versus baseline); blunted L-NMMA responses seen during reperfusion could be completely reversed by vitamin C. The previous resumed data leaded the Authors to conclude that IR-induced vascular injury can be prevented by administration of antioxidants.

Rodrigo et al.[47] evaluated if vitamins C and E are associated with a decrease in BP (blood pressure) in patients with EH. A randomized double-blind placebo-controlled clinical trial was conducted in 110 men with grade 1 EH (35-60 years of age without obesity, dyslipidaemia and diabetes mellitus, non-smokers, not undergoing vigorous physical exercise, without the use of any medication and/or high consumption of fruit and vegetables). Participants were randomly assigned to receive either vitamins C+E [vitamin C (1 g/day) plus vitamin E (400 international units/day)] or placebo for 8 weeks. Measurements included 24 h ambulatory BP and blood analysis of oxidative-stress-related parameters in erythrocytes (GSH/GSSH ratio, antioxidant enzymes and malondialdehyde) and plasma [FRAP (ferric reducing ability of plasma)], and levels of 8-isoprostane, vitamins C and E were measured at baseline and after treatment. Following administration of vitamins C+E, patients with EH had significantly lower systolic BP, diastolic BP and mean arterial BP and higher erythrocyte and serum antioxidant capacity compared with either placebo-treated patients with EH or the patients with EH at baseline prior to treatment. BP correlated positively with plasma 8-isoprostane levels and negatively with plasma FRAP levels in the vitamins C+E- and placebo-treated groups. In

conclusion, the present study supported the view that oxidative stress is involved in the pathogenesis of EH, and that enhancement of antioxidant status by supplementation with vitamins C and E in patients with EH is associated with lower BP, and this might be endorsed n the clinical practice

Tardif et al.[48] investigated the effects of AGI-1067m (succinobucol a recent antixodant related to the proertes of probucol) on coronary atherosclerosis. The Authors designed a placebo-controlled randomized trial to assess the effects of AGI-1067 280 mg qd started before percutaneous coronary intervention (PCI) and administered for 12 months after PCI on atherosclerosis progression as assessed by coronary intravascular ultrasound (IVUS). Among patients with IVUS examinations considered technically adequate both at baseline and follow-up upon central laboratory assessments (n=232), plaque volume was not significantly modified with placebo (least squares mean change: –0.4 mm(3), p=0.85 versus baseline), but was significantly reduced by –4.0 mm(3) at end of treatment in the AGI-1067 group (p=0.001 versus baseline, p=0.12 versus placebo). LDL-cholesterol varied by –9% and +4% in the placebo and AGI-1067 groups, respectively (0.05 between groups), and HDL-cholesterol was reduced by 1% with placebo and 14% with AGI-1067 (0.05 between groups). Plasma myeloperoxidase was reduced by 6% with AGI-1067 (0.05) but hs-CRP was not significantly different between groups. Basing on this study results the Authors could conclude that atherosclerosis regression (–4.0 mm(3)) was observed in patients treated with AGI-1067, although this was not significantly different from placebo. The anti-inflammatory effect of AGI-1067 was supported by reduced levels of myeloperoxidase.

Concluding from the previously described studies we can observe that anti-oxidant vitamins C and E seem to reduce disease progression in patients with normal or abnormal endothelial function with a particular benefit in patients with endothelial dysfunction and have beneficial effects on endothelium-dependent vasodilatation and arterial stiffness in untreated, essential hypertensive patients. This effect is associated with changes in plasma markers of oxidative stress.

Edaravone showed some effectiveness in mild patients with cardioembolic stroke.

NAC administration in combination with streptokinase significantly diminished oxidative stress and improved LV function in patients with acute MI.

Ascorbic acid, vitamin E, or beta carotene have no effects on cardiovascular events among women at high risk for CVD.

Gamma-tochoferol supplementation seem to have a permissive role in decreasing the risk of thrombotic events by improving lipid profile and reducing platelet activity.

Vitamin C is also able to enhance the contractile response to dobutamine and improve myocardial efficiency in patients with HF and has been observed its importance in preventing IR-induced vascular injury. AGI-1067 showed some anti-inflammatory effect and seem to play an important role in atherosclerosis regression.

Chronic Obstructive Pulmonary Disease (COPD)

Oxidative stress seems to have an important role in COPD pathogenesis because of oxidative inactivation of antiproteinases, epithelial injury, increasing in number of neutrophils in the pulmonary microvasculature, and gene expression of proinflammatory mediators. Antioxidant depletion or deficiency in antioxidants may contribute to oxidative stress. Airflow limitation seem to be related to dietary deficiency of antioxidants and so dietary supplementation may constitute a possible treatment in COPD disease.

Cerda et al.[49] investigated the effects of antioxidant polyphenol-rich pomegranate juice (PJ) supplementation for 5 weeks on patients with stable COPD. The Authors realized a randomized, double-blind, placebo-controlled trial involving 30 patients with stable COPD randomly distributed in two groups (15 patients each). Both groups consumed either 400 ml PJ daily or matched placebo (synthetic orange-flavoured drink) for 5 weeks. Trolox Equivalent Antioxidant Capacity (TEAC) of PJ, blood parameters (14 haematological and 18 serobiochemical), respiratory function variables, bioavailability of PJ polyphenols (plasma and urine) and urinary isoprostane (8-iso-PGF(2alpha)) were evaluated. The daily dose of PJ (containing 2.66 g polyphenols) provided 4 mmol/l TEAC. None of the polyphenols present in PJ were detected in plasma or in urine of volunteers. The most abundant PJ polyphenols, ellagitannins, were metabolized by the colonic microflora of COPD patients to yield two major metabolites in both plasma and urine (dibenzopyranone derivatives) with no TEAC. No differences were found (0.05) between PJ and placebo groups for any of the parameters evaluated (serobiochemical and haemato-

logical), urinary 8-iso-PGF(2alpha), respiratory function variables and clinical symptoms of COPD patients. The Authors results suggested that PJ supplementation adds no benefit to the standard COPD therapy.

Ochs-Balcom et al.[50] had the objective to investigate the association between antioxidant nutrients and markers of oxidative stress with pulmonary function in persons with chronic airflow limitation. The Authors designed a cross-sectional study exploring the association of antioxidant nutrients and markers of oxidative stress with forced expiratory volume in the first second (FEV1%) and forced vital capacity (FVC%).The study included 218 persons with chronic airflow limitation recruited randomly from the general population of Erie and Niagara counties, New York State, USA. The study evidenced the following results: after adjustment for covariates, multiple linear regression analysis showed that serum beta-cryptoxanthin, lutein/zeaxanthin, and retinol, and dietary beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, vitamin C, and lycopene were positively associated with FEV1% (0.05, all associations); serum vitamins beta-cryptoxanthin, lutein/zeaxanthin, and lycopene, and dietary beta-cryptoxanthin, beta-carotene, vitamin C, and lutein/zeaxanthin were positively associated with FVC% (0.05, all associations); erythrocytic glutathione was negatively associated with FEV1%, while plasma thiobarbituric acid-reactive substances (TBARS) were negatively associated with FVC% (0.05). The previously described results lead the Authors to conclude that an imbalance in antioxidant/oxidant status is associated with chronic airflow limitation, and that dietary habits and/or oxidative stress play contributing roles.

Dal Negro et al.[51,52] realize a study with the aim to investigate the anti-oxidant effects of erdosteine, a recent drug currently used in chronic obstructive pulmonary disease (COPD) for its rheological activity.

Two groups of 10 persons matched for sex, age and cigarette consumption entered a controlled, double blind, parallel groups study. They were randomized to receive erdosteine 600 mg daily or placebo for 10 days. IL-6; IL-8; TNF-α were measured in bronchial secretions, after 4, 7, and 10 days of erdosteine or placebo; e-NO and both ROS and 8-Isoprostane in blood were also measured at the same experimental times.

ANOVA: a t-test with Bonferroni correction; p=0.05 was accepted.

Blood ROS and IL-8 in bronchial secretions dropped significantly following erdosteine starting from day 4 (both $p \leq 0.02$), while 8-isoprostane drop was significant only after day 10 ($p \leq 2$), and the e-NO decrease proved evident but not significant. No significant changes were observed in the placebo group.

The Authors concluded that erdosteine affects substantially some pro-inflammatory cytokines specifically involved in oxidative stress in current smokers with mild COPD. Effects appeared differently time-dependent. Further long-term studies are needed to confirm these pilot data and to assess their long-term clinical relevance.

The previously described clinical studies evidence that PJ supplementation add no benefit to COPD and underline that an imbalance in antioxidant/oxidant status is associated with chronic airflow limitation. Also dietary habits and/or oxidative stress seem to play contributing roles.

Diabetes Type I and II

Diabetes is a blood glucose metabolism impairment subdivided in type 1, and type 2. Type 1 is due to the body's inability to produce insulin. Insulin breaks down blood glucose so it can be used by human body. Type 2 diabetes is the most common one in which the body can produce the insulin needed, but cells do not respond to it, making it ineffectual. The major cause of Type 2 diabetes is obesity. Diabetes is a very serious condition, as it can lead to severe illness and even death. Variations in blood sugar level, too low and too high (hypoglycemia and hyperglycemia), can lead to atherosclerosis (fatty deposit buildup), neuropathy (loss of nerve function), retinopathy (eye disease and leading cause of blindness) and nephropathy (kidney damage).

In diabetic patients oxidative stress reach high levels probably because of hyperglycemia.

This seem to be due to superoxide anions production by the cell glucose metabolisms able to damage oxidative blood balance. This damage can lead to proteins glycation and 02, $H2O_2$ release. Also it seems that low density lipoproteins (LDL) diminishes their resistance against oxidation especially in type I diabetes.

Costacou et al.[53] investigated the effect of serum antioxidants and total antioxidant reserve (TAR) on coronary artery disease (CAD) incidence in type 1 diabetes. Subjects were identified from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) cohort, a 10-year prospective study of childhood-onset type 1 dia-

betes. Mean age at baseline was 28 and diabetes duration 19 years. Coronary artery disease was defined as physician-diagnosed angina, confirmed MI, stenosis (or=50%), ischemic electrocardiogram (ECG), or revascularization. Controls were gender, age, and diabetes duration (± 3 years) matched with cases. Samples and risk factors used in analyses were identified from the earliest exam prior to incidence in cases (54 cases, 67 controls). None of the antioxidant measures (alpha-tocopherol, gamma-tocopherol, retinol, TAR) showed protection against incident CAD overall. However, a protective effect of alpha-tocopherol against CAD was observed among antioxidant supplement users (HR=0.22, 95% CI=0.10-0.49) and in renal disease (HR=0.46, 95% CI=0.23-0.91). Despite similar alpha-tocopherol concentration, there was no protective effect among nonusers of antioxidant supplements. High alpha-tocopherol levels among patients with renal disease and in those using vitamin supplements were associated with lower CAD risk in type 1 diabetes.

Endo et al.[54] assessed the role of probucol and statins to suppress oxidative stress in diabetic patients. This study analyzed the effects of probucol and the statin atorvastatin on urinary 8-hydroxy-2'deoxyguanosine (8-OHdG) levels in diabetics with hypercholesterolemia. The randomized and open study had been performed on a total of 36 patients with type 2 diabetes and hypercholesterolemia. These patients were randomly assigned to a probucol group (500 mg/day, n = 18) or an atorvastatin group (10 mg/day, n = 18). During three months, total- and LDL-cholesterol decreased significantly in both groups. LDL-cholesterol was significantly lower in the atorvastatin group than probucol group. HDL-C decreased significantly in the probucol group and did not change in the atorvastatin group. 8-OHdG decreased significantly in both groups after 3 months; 12.4 ± 7.5 to 8.1 ± 4.2 ng/mg/Cr in the atorvastatin group (0.05) and 12.3 ± 8.8 to 6.8 ± 2.6 ng/mg/Cr in the probucol group (0.05), and these changes did not differ significantly between the two groups. But, in patients with high 8-OHdG levels (more than 10 ng/mg/Cr) before administration, urinary 8-OHdG decreased significantly from 19.5 ± 4.9 to 9.2 ± 3.4 ng/mg Cr (0.01) in the atorvastatin group, and from 19.7 ± 8.2 to 6.67 ± 2.2 ng/mg Cr (0.01) in the probucol group. Urinary 8-OHdG was significantly lower in the probucol group than in the atorvastatin group after the second and third months of administration (0.05). The Authors concluded that probucol and atorvastatin both reduce systemic oxidative stress and that probucol might be the more clinical useful in patients with strong oxidative stress.

Kamenova[55] developed a study with to determine the effect of oral administration of alpha-lipoic acid on insulin sensitivity in patients with type 2 diabetes. The study involved 12 patients (mean ± SD; age 52.9 ± 9.9 yrs; body mass index 33.9 ± 7.4 kg/m^2) which were treated with oral alpha-lipoic acid, 600 mg twice daily over a period of 4 weeks. twelve subjects with normal glucose tolerance served as a control group in terms of insulin sensitivity (Is). Is was measured by a 2h manual hyperinsulinaemic (insulin infusion rate-40 mU/m^2 body surface area/min) euglycaemic (blood glucose kept at 5 mmol/l) clamp technique and expressed as a glucose disposal rate (M) and insulin sensitivity index (IsI). The treatment period bring the following results: Is of diabetic patients was significantly increased, M from 3.202 ± 1.898 to 5.951 ± 2.705 mg/kg/min (mean ± SD), and IsI from 4.706 ± 2.666 to 7.673 ± 3.559 mg/kg/min per mIU/l x 100 (mean ± SD). The difference was not statistically significant between the Is of diabetic patients after alpha-lipoic acid therapy and control subjects. The Authors concluded that short-term oral alpha-lipoic acid treatment increased peripheral insulin sensitivity in patients with type 2 diabetes mellitus.

Agrawal et al.[56] to assessed if Cilostazol, a selective inhibitor of PDE3, with a protective effect on endothelium after ischemic vascular damage, through production of nitric oxide (NO). in hypertensive type 2 diabetic patients. After informed consent, 30 patients received Cilostazol (100 mg) twice daily orally as add-on therapy. At 1 month follow-up, 26 patients in control group and 22 patients in Cilostazol group completed the trial and particular parameters were re-evaluated. The results were: the mean age and duration of diabetes were 55 ± 7 years and 8 ± 6 years, respectively; at follow-up, the Cilostazol group showed significant (0.001) decrease in hsC-reactive protein (23.6%), erythrocyte sedimentation rate (38.7%), total leukocyte count (12.6%), plasma malondialdehyde (17.6%), HbA1c (0.17%, p=0.002) and increase in serum albumin (11.9%), blood reduced glutathione (3.5%) from baseline; UKPDS 10 years risk of coronary heart disease decreased by 6% (p=0.002); the control group did not show significant improvement in inflammatory profile, ox-

idative status and HbA1c. After the described evidences the Authors concludes that inflammatory and oxidative stress is high in hypertensive type 2 diabetic patients and that Cilostazol reduces these factors as well as coronary heart disease risk in diabetes mellitus.

Ceriello et al.[57] performed a study involving 36 type 1 diabetic patients and 12 control subjects were enrolled. The diabetic patients were divided into three groups. The first group was treated for 24 h with insulin, achieving a near normalization of glycemia. After 12 h of this treatment, vitamin C was added for the remaining 12 h. The second group was treated for 24 h with vitamin C. After 12 h of this treatment, insulin was started, achieving a near normalization of glycemia for the remaining 12 h. The third group was treated for 24 h with both vitamin C and insulin, achieving near normalization of glycemia. The same protocols were performed after 1 month of telmisartan or placebo. The treatments evidence the following results: neither normalization of glycemia nor vitamin C treatment alone was able to normalize endothelial dysfunction or oxidative stress; combining insulin and vitamin C normalized endothelial dysfunction and decreased oxidative stress to normal levels; telmisartan significantly improved basal endothelial function and decreased nitrotyrosine plasma level; in patients treated with telmisartan, a near normalization of both flow-mediated vasodilation and oxidative stress was achieved when glycemia was normalized, whereas adding vitamin C infusion did not show further effect on endothelial function or nitrotyrosine plasma levels. Such results brought the Authors to the conclusion that combining the normalization of glycemia with an antioxidant is possible to normalize endothelial function in type 1 diabetic patients and that telmisartan works as an antioxidant like vitamin C.

Neyestani et al.[58] developed a study with the aim of evaluating the antioxidant effects of lycopene in physiological doses and its possible effects on the immune response in patients with Type 2 diabetes mellitus (T2DM). The Authors enrolled 35 patients with T2DM of both sexes aged 54 ± 9 yr in a double-blind placebo-controlled clinical trial conducted for 2 months. After a 2-week lycopene-free diet washout period, patients were allocated to either lycopene supplementation group (10 mg/day) (no.=16) or placebo group (no.=19), which were age- and sex matched. Patients were instructed to keep their diet and physical activity as unchanged as possible. With the previous described treatment they obtained that dietary intake of energy and body weight did not change and taht the ratio of serum total antioxidant capacity (TAC) to malondialdehyde (MDA) increased significantly in the lycopene group compared to the placebo group (p=0.007). Though a statistically significant increase in serum concentrations of lycopene (0.001) was not accompanied by enhanced delayed-type hypersensitivity response, a significant negative correlation was found between serum levels of lycopene and immunoglobulin (Ig)G (r=–0.338, p=0.008). Interestingly, variations of serum levels of lycopene directly correlated with those of IgM (r=0.466, p=0.005). There was an insignificant decrement in serum anti-oxidized LDL IgG levels in the lycopene group. These results emphasize that lycopene (probably by increasing TAC and inhibiting MDA-LDL formation) may attenuate T cell-dependent adaptive (pro-atherogenic) immune response. The Authors concluded also that with enhancement of innate immunity and hence prevention of ox-LDL uptake by macrophage and foam cell formation, lycopene should be effective in prevention of long-term diabetic complications, notably cardiovascular disease.

Stranges et al.[59] evaluated the effect of long-term selenium supplementation on the incidence of type 2 diabetes. 1202 persons seen in dermatology clinics who did not have type 2 diabetes at baseline have been involved in a randomized, double-blind, placebo controlled trial to receive oral administration of selenium, 200 microg/d, or placebo. During an average follow-up of 7.7 years (SD, 2.7), type 2 diabetes developed in 58 selenium recipients and 39 placebo recipients (incidence, 12.6 cases per 1000 person-years vs. 8.4 cases per 1000 person-years, respectively; hazard ratio, 1.55 [95% CI, 1.03 to 2.33]). The lack of benefit of selenium supplementation on the incidence of type 2 diabetes persisted in analyses stratified by age, sex, body mass index, and smoking status. An exposure-response gradient was found across tertiles of baseline plasma selenium level, with a statistically significantly increased risk for type 2 diabetes in the highest tertile of baseline plasma selenium level (hazard ratio, 2.70 [CI, 1.30 to 5.61]). From the previous results the Authors conclude that selenium supplementation does not seem to prevent type 2 diabetes and it may increase risk for the disease.

Tessier et al.[60] study, has the objective of evaluate the effects of the administration of two dosages of vitamin C (Vit-C) (0.5 and 1g/day, vs. placebo) in elderly patients with type 2 diabetes mellitus on the intracellular levels of Vit-C and glutathione, and on the lipid peroxidation markers and vitamin E (Vit-E) content of low-density lipoprotein (LDL) and on LDL susceptibility to gamma radiolysis-induced peroxidation. Thirty-six patients were randomized into three groups. In patients on 0.5 g Vit-C/day versus the placebo group, a significant increase in cellular reduced glutathione level was observed (0.60 ± 0.26 vs. 0.33 ± 0.27). In patients on 1g Vit-C/day versus placebo, a significant increase was also observed in cellular reduced glutathione (0.93 ± 0.70 vs. 0.33 ± 0.27), in Vit-C (5.66 ± 2.00 vs. 2.72 ± 1.88) and in vitamin E content of LDL (1.98 ± 0.38 vs. 1.48 ± 0.40). The following results have been observed: no change was observed in either group in basal levels of lipid peroxidation markers and in the susceptibility of LDL to peroxidation provoked by gamma-radiolysis. In conclusion, Vit-C has a dose-dependent effect on the cellular contents of antioxidants and on vitamin E content of LDL in elderly patients with type 2 DM. These changes are not sufficient to decrease the LDL susceptibility to peroxidation.

Vossler et al.[61] study's had the purpose of evaluating the impact of DEX (dexlipotam: R-Lipoic acid) on endothelial function in patients with type 2 diabetes (DM2) and to estimate the safety and tolerability of DEX. DEX 960 mg and DEX 1,920 mg were investigated in DM2 patients (114 diabetic recipients were randomized to the three study groups) over a period of 4 weeks using a randomized, placebo-(PLA) controlled, double-blinded study with 3 parallel groups. The marker of arterial function after 4-week therapy with DEX was the maximum percentage change versus baseline in the flow-mediated dilation of the brachial artery (FMD) after reperfusion. This study evidenced the following results: DEX was safe and well tolerated; dyspepsia appeared to be the most relevant side effect of DEX treatment; systolic (p=0.078) and diastolic blood pressure (p=0.059) tended to be lower in patients treated with DEX at a dose of 1,920 mg; there were no significant differences in FMD between the placebo- and the DEX-treated groups; in patients with poorer glucose control (HbA_{1c}; 6.5% Hb), FMD increased significantly after 4-week treatment with DEX: PLA –1.51 ± 2.98%, DEX 960 mg +1.22 ± 3.22, p=0.027, DEX 1,920 mg +1.47 ± 3.78, p=0.012; the magnitude of the mean change compared to placebo was 2.73% (DEX 920) and 2.98% (DEX 1,920) in patients with HbA_{lc} > 7.5% Hb (DEX 960, p=0.007, DEX 1,920, p=0.032); the effects of treatment were usually statistically significant in subgroups with more severe vascular stress (longer duration of disease, pretreatment history, higher LDL-C, higher blood pressure). On the basis of these results the Authors concluded that DEX therapy appears to reduce endothelial dysfunction in DM2, especially in men with long history of DM2 and having poor glucose control and these findings will be useful in patient selection in future prospective clinical trials with drugs to treat vascular stress.

Concluding alpha-tocopherol seems to have a protective effect against CAD in type 1 diabetes recipients. There is clinical evidence that Probucol and Atorvastatin both reduce systemic oxidative stress and that probucol might be the more clinical useful in patients with strong oxidative stress. Oral alpha-lipoic acid treatment increased peripheral insulin sensitivity in patients with type 2 diabetes mellitus. Cilostazol seems to reduce inflammatory and oxidative stress in hypertensive type 2 diabetic patients as well as coronary heart disease risk in diabetes mellitus. Combining the normalization of glycemia with an antioxidant (telmisartan or vitamin C) it is possible to normalize endothelial function in type 1 diabetic patients. Lycopene may be effective to prevent long-term diabetic complications, notably cardiovascular disease instead selenium supplementation does not seem to prevent type 2 diabetes and it may increase risk for the disease.

Liver Diseases

The liver is the largest organ in the body. Its main functions are: metabolize substances in the blood in preparation for excretion, synthesis of most essential proteins, production of bile and regulation of nutrients such as glucose, cholesterol, and amino acids. Liver inflammation is can be causes by an increment in liver free radicals. Aspecific and not infectious inflammation and hepatitis damage might be expressed or emphasized by oxidative damage. Normally the liver uses internally generated antioxidants to neutralize the toxin-produced free radicals But when the liver antioxidants are low, due to alcohol or chronic drug use, damage from free radicals increases, resulting in inflammation and the formation of scar tissue (fibrosis). So it is important to maintain a constant supply of antioxidants and an healthy

lifestyle (abstaining from all alcohol and avoiding environmental toxins) to reduce the strain on the liver. Alcohol lowers the liver's levels of antioxidants, including vitamin E and S-adenosyl-L-methionine making the liver vulnerable. In addition, alcohol lowers glutathione, an important internal antioxidant. Because heavy drinkers consume a substantial number of calories as alcohol, they consume less vitamin- and mineral-rich food than they otherwise might, exacerbating alcohol-induced nutritional deficiencies. These include low levels of vitamin C, riboflavin, zinc, pyridoxine (vitamin B_6), and vitamins.

Phillips et al.[62] designed a randomized clinical trial comparing antioxidant and corticosteroids treatments. One hundred and one patients were randomized either to receive corticosteroids or a novel antioxidant cocktail with a primary endpoint of 30-day mortality. The following results were evidenced: at 30 days there were 16 deaths (30%) in the corticosteroid treated group compared with 22 deaths (46%) in the antioxidant treated group (p=0.05); the odds of dying by 30 days were 2.4 greater for patients on antioxidants (95% confidence interval 1.0-5.6); a diagnosis of sepsis was made more frequently in the AO group (p=0.05), although microbiologically proven episodes of infection occurred more often in the CS group (0.01). The survival advantage for corticosteroid treated patients was lost at 1 year of follow-up (p=0.43). This study has shown that corticosteroids in the form of prednisolone 30 mg daily are superior to a broad antioxidant cocktail in the treatment of severe alcoholic hepatitis.

Gabbay et al.[63] to assess the safety and efficacy of antioxidant therapy for patients with chronic hepatitis C virus (HCV) infection enrolled one hundred chronic HCV infection patients failed in interferon treatment to receive randomly either combined intravenous and oral antioxidants or placebo, or oral treatment alone. The primary end points were liver enzymes, HCV-RNA levels and histology. The following were evidenced: combined oral and intravenous antioxidant therapy [Patients in the treatment group received a combination of seven different anti-oxidants orally at the appropriate dose (glycyrrhiza capsules, 500 mg, bid; schizandrae capsules, 500 mg, tid; ascorbate capsules, 2000 mg, tid; L-glutathione capsules, 150 mg, bid; silymarin capsules, 250 mg, tid; lipoic acid capsules, 150 mg, bid; d-alpha tocopherol, 800 IU/d)] was associated with a significant decline in ALT levels in 52% of patients who received antioxidant therapy vs 20% of patients who received placebo (p=0.05); histology activity index (HAI) score at the end of treatment was reduced in 48% of patients who received antioxidant therapy vs 26% of patients who received placebo (p=0.21); HCV-RNA levels decreased by 1-log or more in 28% of patients who received antioxidant therapy vs 12% who received placebo (p=NS); in part II of the trial, oral administration of antioxidants was not associated with significant alterations in any of the end points. The Authors concluded that antioxidant therapy had a mild beneficial effect on the inflammatory response of chronic HCV infection patients who are non-responders to interferon and that combined antiviral and antioxidant therapy may be beneficial for these patients.

Irshad et al.[64] had the aim to explore the possibility of HCV core induced lipid changes and ensuing oxidative liver damage in these liver diseases. The Authors studied a total number of 130 patients including 50 patients with acute viral hepatitis (AVH), 30 with chronic hepatitis (CH), 30 with hepatic cirrhosis and 20 patients with fulminant hepatic failure (FHF). Sera from all these patients were analyzed for hepatitis viral markers and HCV core protein using EIA assays. Sera/plasma from them were simultaneously analyzed for total cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL), apolipoprotein A-1 and B, and also for antioxidants. This study evidenced the following results: analysis of data demonstrated the presence of viral hepatitis B, C and E infections in these case; hepatitis A and D infections were absent in all the patients; when data on lipid and lipoprotein were analyzed in relation to HCV core expression, we could not observe a significant change in the serum level of total cholesterol, triglyceride, LDL, HDL, apolipoprotein A-1 and apoprotein B in core positive patients as compared to core negative cases. However, lipoprotein (a) [Lp(a)] level was significantly reduced in core positive patients as compared to core negative cases. Furthermore, analysis of superoxide dismutase (SOD), Total antioxidant (TAO) and Uric Acid in these patients demonstrated only a minor change in SOD and TAO levels in relation to HCV core, though at the same time, Uric Acid was found raised in all the groups. These observations clearly indicated that core expression did not bring a significant change in serum level of lipids, lipoprotein and apoproteins. Similarly, HCV core expression also

did not show a major change in SOD and TAO levels suggesting an insignificant impact of core on oxidative stress during liver diseases.

Stewart et al.[65] investigated whether antioxidant therapy, alone or in combination with steroids, improved survival in patients with acute alcoholic hepatitis. Patients with a severe alcoholic hepatitis were divided by sex and steroid use, and then randomized. The active group received N-acetylcysteine for one week, and vitamins A-E, biotin, selenium, zinc, manganese, copper, magnesium, folic acid and Coenzyme Q daily for 6 months. The trial was double blinded and placebo controlled. The primary end-point was mortality within 6 months. The following results were evidenced: thirty-six (20 male, 16 female; mean discriminant function (DF) 86.6) received active drug, and 34 (18 male, 16 female; mean DF 76.4) received placebo; 180-day survival was not significantly different between patients receiving drug and placebo (52.8% vs. 55.8%, p=0.699, this was not affected by stratification for steroid use or sex); the only predictors of survival in multivariate analysis were initial bilirubin (p=0.017), white cell count (p=0.016) and age (p=0.037); treatment allocation did not affect survival in multivariate analysis (p=0.830). The Authors concluded that antioxidant therapy, alone or in combination with corticosteroids, didu't improve 6-month survival in severe alcoholic hepatitis.

Schemmer et al.[66] designed a randomized controlled double blind single center clinical trial with two study arms comprising a total of 40 patients to assess the effects of a single preoperative dose of melatonin before major liver resection. Primary endpoints include the determination of safety and tolerance of the regimen as well as clinical parameters reflecting pathophysiological functions of the liver. Furthermore, data on clinical outcome (infectious and non-infectious complications) will be collected as secondary endpoints to allow a power calculation for a randomized clinical trial aiming at clinical efficacy. Based on experimental data, this ongoing clinical trial represented an advanced element of the research chain from bench to bedside in order to reach the highest level of evidence-based clinical facts to determine if melatonin can improve the general outcome after liver resection.

Combined antiviral and antioxidant therapy seems to be beneficial for the treatment of HCV infection. Instead antioxidant therapy, alone or in combination with corticosteroids, does not improve the condition of patients affected by severe alcoholic hepatitis.

Pancreatitis

Free radicals can be generated by intracellular anaerobic activity followed by re-oxygenation and, combined with protein and lipid molecules leading to tissue and cells damage and death. Oxidative stress is involved in acute pancreatitis and has been demonstrated that oxygen-derived free radicals are generated during acute pancreatitis. A lot of clinical trials have been done to assess if antioxidants supplementation could fill the antioxidants depletion that has been observed during acute pancreatitis.

Kirk et al.[67] developed a randomized, double-blind, placebo-controlled crossover trial with the aim of determine the efficacy of a combined antioxidant preparation in the management of chronic pancreatitis (CP). Patients with confirmed chronic pancreatitis (N = 36) were randomized to receive treatment with either Antox, which contains the antioxidants selenium, betacarotene, L-methionine, and vitamins C and E, or placebo for 10 weeks. Each group of patients then switched to receive the alternative treatment for a further 10 weeks. Markers of antioxidant status were measured by blood sampling, whereas quality of life and pain were assessed using the SF-36 questionnaire. Nineteen patients completed the full 20 weeks of treatment. Treatment with Antox was associated with significant improvements in quality of life in terms of pain (+17 antioxidant vs. –7 placebo), physical (+9 vs. –3) and social functioning (+8 vs. –7), and general health perception (+10 vs. –3). On the basis of the previously described results the Authors concluded that treatment with antioxidants may improve quality of life and reduce pain in patients suffering from chronic pancreatitis.

Romagnuolo et al.[68] with the aim of determine if allopurinol decreases the rate of pancreatitis (PEP), randomized 586 subjects, 293 to each arm (patients referred for ERCP to 9 endoscopists at 2 tertiary centers) to receive either allopurinol 300 mg or identical placebo orally 60 minutes before ERCP, stratified according to high-risk ERCP (manometry or pancreatic therapy). The primary outcome (PEP) was adjudicated blindly; pancreatitis was defined according to the Cotton consensus, and evaluated at 48 hours and 30 days. Secondary outcomes included severe PEP, length of stay, and mortality. The trial was terminated after the blinded (midpoint) interim analy-

sis, as recommended by the independent data and safety monitoring committee. The study evidenced the following results: the crude PEP rates were 5.5% (allopurinol) and 4.1% (placebo), (p=0.44; difference = 1.4%; 95% confidence interval, –2.1% to 4.8%); the Mantel-Haenszel combined risk ratio for PEP with allopurinol, considering stratification, was 1.37 (95% confidence interval, 0.65-2.86); subgroup analyses suggested non significant trends toward possible benefit in the high-risk group, and possible harm for the remaining subjects; logistic regression found pancreatic therapy, pancreatic injection, and prior PEP to be the only independent predictors of PEP. On the basis of the previously described results the Authors concluded that allopurinol does not appear to reduce the overall risk of PEP but further study is required.

The previously described studies evidence the use of Antox (selenium, betacarotene, L-methionine, vitamin C, and vitamin E), which seems to improve quality of life and reduce pain in patients suffering from chronic pancreatitis and allopurinol which not appear to reduce the overall risk of PEP. Further studies will be required.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and sometimes shows multisystem involvement.

Reactive oxygen species seem to play an important role in the development of rheumatoid arthritis in humans. In fact during the inflammatory rheumatic processes reactive oxygen and nitrogen species that can react with lipid protein and nucleic acids are producted.

This evidence lead to the possibility of using antioxidants to counteract the formation of these damaging species.

Here are summarized some studies regarding the clinical trials related to the use of antioxidant supplementation to assess their efficacy in RA treatment.

Canter et al.[69] tried to systematically review the evidence from randomized clinical trials (RCTs) for the effectiveness of the antioxidant vitamins A, C, E or selenium or their combination in the treatment of arthritis. The Authors performed a systematic search of computerized databases from inception to September 2006 for relevant RCTs, application of pre-defined inclusion/exclusion criteria and independent data extraction by two Authors. Methodological quality was assessed using the Jadad scale. The searches identified 20 unique RCTs meeting the inclusion criteria: 11 in inflammatory arthritis and 9 in osteoarthritis (OA). The studies included are generally of poor quality. They fall into three main clusters: selenium for rheumatoid arthritis (n = 5); vitamin E for inflammatory arthritis (n = 5) and vitamin E for OA (n = 7). One RCT suggests superiority of vitamin E over placebo and three RCTs suggest equivalence between vitamin E and diclofenac in the treatment of inflammatory arthritis. In OA, four RCTs compared vitamin E with placebo. Two shorter-term studies were positive and two longer-term studies were negative. Two further RCTs suggest equivalence between vitamin E and diclofenac in the treatment of OA. Findings for selenium, vitamin A and a combination product in inflammatory arthritis and for vitamin A, and a combination product in OA were negative. An isolated positive result for vitamin C in OA is of doubtful clinical significance. The Authors reached the following conclusions: clinical trials testing the efficacy of vitamin E in the treatment of OA and inflammatory arthritis had been methodologically weak and have produced contradictory findings; there is presently no convincing evidence that selenium, vitamin A, vitamin C or the combination product selenium ACE is effective in the treatment of any type of arthritis.

De Pablo et al.[70] had the aim to compare antioxidants and other novel and traditional cardiovascular disease (CVD) risk factors in participants with rheumatoid arthritis (RA) and non-RA controls in a large population sample. The Third National Health and Nutrition Examination Survey (NHANES-III) was a cross-sectional population survey in which subjects ages > or=60 underwent a musculoskeletal examination. RA subjects were defined as those who who met > or=3 of 6 available 1987 American College of Rheumatology (ACR) criteria. Non-RA subjects were defined as those who met no ACR criteria. The Authors performed univariate and multivariate analyses of the association between RA and each novel and traditional cardiovascular disease (CVD) risk factor in RA versus non-RA subjects. The sample included 5,302 subjects ages > or=60, with 131 (2.5%) RA and 4,444 (84%) non-RA participants. A total of 727 subjects were excluded. These were the results from this clinical trial: plasma levels of antioxidants alpha-carotene, beta-cryptoxanthin, lutein/zeaxanthin, and lycopene were significantly lower in RA subjects compared with non-RA subjects in multivariate analysis adjusting for poten-

tial confounders; compared with non-RA participants, RA subjects were more likely to have increased C-reactive protein (CRP) levels in multivariate analysis adjusting for potential confounders. RA and non-RA participants had similar prevalence of traditional CVD risk factors and previous CVD. Basing on the described results the Authors conclude that in this large population study, RA subjects had similar prevalence of previous CVD and traditional CVD risk factors as controls. Among novel CVD risk factors, plasma carotenoid levels were significantly lower and CRP level was significantly higher in RA compared with non-RA subjects after adjustment for potential confounders. The Authors also underlined that further clinical trials should evaluate whether these differences account for the observed increased incidence of CVD in individuals with RA.

Analysing the these studies there is no evidence of a beneficial effect of selenium, vitamin A, vitamin C or the combination product selenium ACE in the treatment of any type of arthritis.

Kidney Diseases

Oxidative stress in dialysis patients is a common finding. Renal sources for ROS are activated macrophages, vascular cells, and various glomerular cells. ROS may affect cells of the host organism, especially at sites of inflammation, in addition to playing a role in the defense system against other agents. This effect plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, which can contribute to proteinuria and other conditions. ROS are also thought to contribute to the pathogenesis of ischemia reperfusion injury in the kidney. This suggests that the kidney may be particularly susceptible to oxidative stress.

Also chronic renal failure has been associated with oxidative stress. Serum sulfite, sulfate, cysteine, homocysteine, cysteine sulfinic acid, and gamma-glutamylcysteine are elevated in patients on hemodialysis, suggesting an accelerated catabolism of sulfur-containing amino acids or a reduced elimination of sulfite/sulfate, or both. The increase of oxidative stress is on of the major risk factors in patients with chronic kidney disease (CKD), especially under dialytic treatment.

Ong-ajyooth et al.[71] tried to assess whether dietary supplementation with the antioxidant agent, vitamin E, reduces renal damage in patients with IgA nephropathy. Twenty-eight patients with idiopathic IgA nephropathy were supplemented with vitamin E 400 mg/day for 6 months. Antioxidant enzymes, glutathione, plasma malondialdehyde (MDA), and renal function were studied after 3 and 6 months therapy. The result of this study showed high plasma MDA and significant reduction after therapy (1.15 ± 0.45 VS 0.86 ± 0.30 microM, 0.0001). The RBC vitamin E was also elevated statistically significantly (5.07 ± 2.42 VS 15.70 ± 3.37 microM, 0.001). Glutathione peroxidase activities were decreased (38.52 ± 15.53 VS 23.97 ± 7.63 U/gHb, p < 0.001). Glutathione was also decreased (44.80 ± 9.70 VS 32.45 ± 6.74 mg/dl, p < 0.05) but there were no changes in red cell catalase and superoxide dismutase activities. Creatinine clearance, proteinuria, urine N-acetyl glucosaminidase and beta2-microglobulin also showed no improvement. These results leaded the Authors to conclude that the particular group of IgA nephropathy patients with low vitamin E level and high oxidative stress had significant reduction of oxidative stress after vitamin E therapy.

Mastalerz-Migas et al.[72] investigated the role of lovastatin or of hypolipemic diet on oxidative stress in hemodialyzed patients. The Authors addressed the issue by measuring the total antioxidant status (TAS) and the level of 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidative DNA damage metabolite, in the serum. The study group consisted of 71 patients. They were divided into 3 groups: treated with lovastatin (20 mg/day, n=30), with a hypolipemic diet alone (n=28), and untreated controls (n=13). Serum levels of TAS and 8-OHdG (8-hydroxy-2-deoxyguanosine) were determined. Blood samples were collected at the beginning of the study and then after a 6 months' therapy. We found that the level of 8-OHdG decreased considerably only in the lovastatin-treated group; the decrease was from 15.6 ± 8.1 to 12.5 ± 4.8 ng/ml (p=0.04). In the other two groups changes in 8-OHdG were insignificant. The level of TAS increased significantly in the lovastatin-treated group from 1.28 ± 0.20 to 1.37 ± 0.116 mmol/l (p=0.011), decreased in the diet-treated group from 1.55 ± 0.14 to 1.45 ± 0.11 mmol/l (p=0.007), and remained unchanged in the untreated group (1.42 ± 0.11 vs. 1.40 ± 0.12 mmol/l). The Authors conclude that lovastatin, but not hypolipemic diet alone, has antioxidant effect in hemodialyzed patients. However, the determinants of the antioxidant effect of statins in patients with chronic renal failure are unclear and their resolution would require alternative study designs.

Sahin et al.[73] studied N-acetylcysteine (NAC), in cardiovascular events prevention improving oxidative stress on endothelial cells in patients with chronic kidney-disease (CKD). High-resolution Doppler ultrasound of brachial artery before and after 6 weeks of oral NAC (2 × 600 mg) medication was used on 30 uremic patients (age 40 ± 12 years, 6 males) on hemodialysis (HD). Also, 13 healthy controls (35 ± 9 years, 5 males) were included in the study. Reactive hyperemia following 5 min forearm ischemia was accepted as endothelium-dependent vasodilatation (flow-mediated dilatation; FMD) and compared to endothelium-independent vasodilatation in response to sublingual glyceril trinitrate (GTN). The following results have been reported: patients on HD had lower DeltaFMD (0.28 ± 0.17 vs. 0.41 ± 0.11, 0.05) and FMD% (7.5 ± 5.05 vs. 11.33 ± 2.95, 0.05) than the controls. Baseline DeltaGTN and GTN% were similar in two groups. NAC treatment significantly increased the DeltaFMD (0.41 ± 0.11, 0.001 vs. baseline) and FMD% (10.59 ± 3.22, 0.01 vs. baseline) of patients on HD, while it had no effect on DeltaGTN and GTN%. These results suggest that NAC treatment could improve the ED by preventing the reduction of FMD in patients on HD.

Al-Awadi K. A. et al.[74] conducted a randomized clinical trial to determine whether ESWL (extracorporeal shock wave lithotripsy) produces ischaemia and reperfusion injury in the kidneys and whether oral administration of antioxidants reduces the degree of short-term renal injury in patients treated with ESWL. The study included 120 patients with renal stones (1-3 cm in size) treated with ESWL. The patients were divided into three groups-patients in group A (n = 39) served as a control group and were not given any antioxidants; patients in group B (n = 41) were given two capsules of antioxidants "Nature Made r" (Antioxidants used Each "Nature Made r" antioxidant capsule (Pharmavite Corporation, Mission Hills, CA, USA) contains high levels of the following antioxidants, Vitamin A (as -carotene) 10,000 i.u., Vitamin C 250 mg, and Vitamin E 200 i.u., and mineral supplements like zinc 7.5 mcg and selenium 15 mcg. The capsule was chosen because it contains more than the daily recommended allowances of most of the antioxidants) 2 h before ESWL, and 2 and 8 h after ESWL; and patients in group C (n = 40) were given two capsules of the antioxidants 2 and 8 h after ESWL. Double "J" stents were inserted in patients before treatment with ESWL. Blood and urine samples were obtained from all patients just before the start of treatment with ESWL, and at 2 and 24 h and on 7th and 28th day after ESWL. Serum levels of malondialdehyde (MDA), alpha-tocopherol, cholesterol, albumin and ascorbic acid, and alpha-tocopherol/cholesterol ratio were determined. Urinary levels of albumin and beta(2) microglobulin were also determined as measures of renal tubular injury. At 24 h after ESWL, patients given antioxidants (groups B + C) had significantly reduced mean serum concentration of MDA (0.001); higher levels of serum ascorbic acid (0.001) and serum albumin (0.001); lower alpha-tocopherol/cholesterol ratio, lower urinary albumin and beta(2)microglobulin levels compared with patients who did not receive antioxidants (group A). These findings suggested that treatment with ESWL generates free radicals through ischaemic/reperfusion injury mechanism, and that oral administration of antioxidant may protect these patients from short term renal injury caused by ESWL.

The previously described studies analyze the effects of antioxidants on kidney diseases. Vitamin E therapy seems to reduce oxidative stress in IgA nephropathy patients. Another antioxidant, lovastatin, is effecive in hemodialyzed patients.

There is evidence that NAC treatment can improve the ED by preventing the reduction of FMD in patients on HD and that oral administration of antioxidant may protect patients from short term renal injury caused by ESWL.

Natural Antioxidants and Self-Prescription Use

We are living in the world wide web age where a search for treatments for a wide range of diseases shows immediately a lot of alternative and conventional treatment. Websites describe "natural" treatment basing on anecdotal cases of success without putting the stress on the importance of dosages and not describing possible side effects and adverse outcomes.

There is an ocean of information available on Western herbs, Chinese herbs, Ayurvedic herbs, vitamins and all kinds of supplements but often the information given are not precise and complete enough to help the consumer to do a correct and conscious use of the antioxidant supplements. Furthermore, the chemical characteristic of each compound, influences it's absorbtion and

Table II. Role of free radicals in various diseases.

Disease	Free radicals effetcs in the pathology
Acute lung injury, acute respiratory distress syndrome, inflammation and hyperoxia	ROS-mediated inflammation and endothelial dysfunction
Age-related mecular degeneration	Photochemical reaction in the oxygen-rich environment of the outer retina lead to the liberation of cytotoxic (ROS)
Aging	Cell damage and metabolic abnormalities
Alzheimer's disease	Amyloid peptide and advanced glycation end products ROS-mediated neurotoxicity to hippocampal cells and the synaptosomal membranes
Atherosclerosis	Superoxide-mediated endothelial dysfunction, activation of macrophages
Autoimmune's disease	ROS-mediated inflammation and tissue destruction
Cancer	ROS-mediated gene mutations (modification of pyridine and purine bases) and post-translational modifications leading disruption of cellular processes
Diabetes	ROS accelerated formation of advanced glycation end products (AEGs) superoxide accelerated endothelial dysfunction
Hypertension	ROS-mediated vascular smooth muscle cell proliferation, oxidant production via NADH/NADPH oxidase and endothelial dysfunction
Hungtington's disease	ROS-mediated transcriptional dysregulation and mitochondrial impairment
Kidney disease	ROS-mediated inflammation and endothelial dysfunction
Liver disease	ROS-mediated DNA damage with evolution to cancer ROS-mediated inflammation and tissue destruction
Myocardial infarction	ROS driven ischemic reperfusion injury and myocyte necrosis and/or apoptosis
Pancreatitis	ROS-mediated inflammation and tissue destruction
Parkinson's disease	ROS-mediated mitochondrial dysfunction
Pre-eclampsia	ROS-mediated endothelial dysfunction

bioavailability, often neutralizing any clinical benefit; for this reason, proper formulation, toxicity awareness, and clinical trials are needed, in order to optimize the use of the products.

Looking at the scientific literature published in the last 3 years in MEDLINE we have observed that there are increasing reports of self poisoning by antioxidants.

One of the most frequently abused is selenium because of its supposed beneficial effects in the treatment of a variety of illnesses. A fatal case of acute selenium poisoning in a 75-year-old man that after reading on the Internet about a possible role of selenium in prostate cancer, ingested 10 g of sodium selenite and despite of intensive care treatment, underwent cardiac arrest and died 6 hours after ingestion. Other poisoning cases have been reported emphasizing the risk of Internet self prescription and destroying the myth that natural therapies are inherently safe.

In conclusion, we can say that self-prescription can be safe for some supplements, whose benefits are well known and with few side effects. Everyone ought to consult a qualified expert, especially if specific disease has to be faced with antioxidants and drugs. Supplements should be generally more useful in addition to satisfying whole foods diet and regular exercise.

Conclusions

Free radicals cause oxidative damage to cells and DNA, which can be reduced by antioxidants. Antioxidant nutrients appear to play an important role in protection against various disorders.

Oxidative stress in humans is observed by determining and analyzing products of oxidative damage and by investigating the human body tissues ability to fight against this oxidation. The

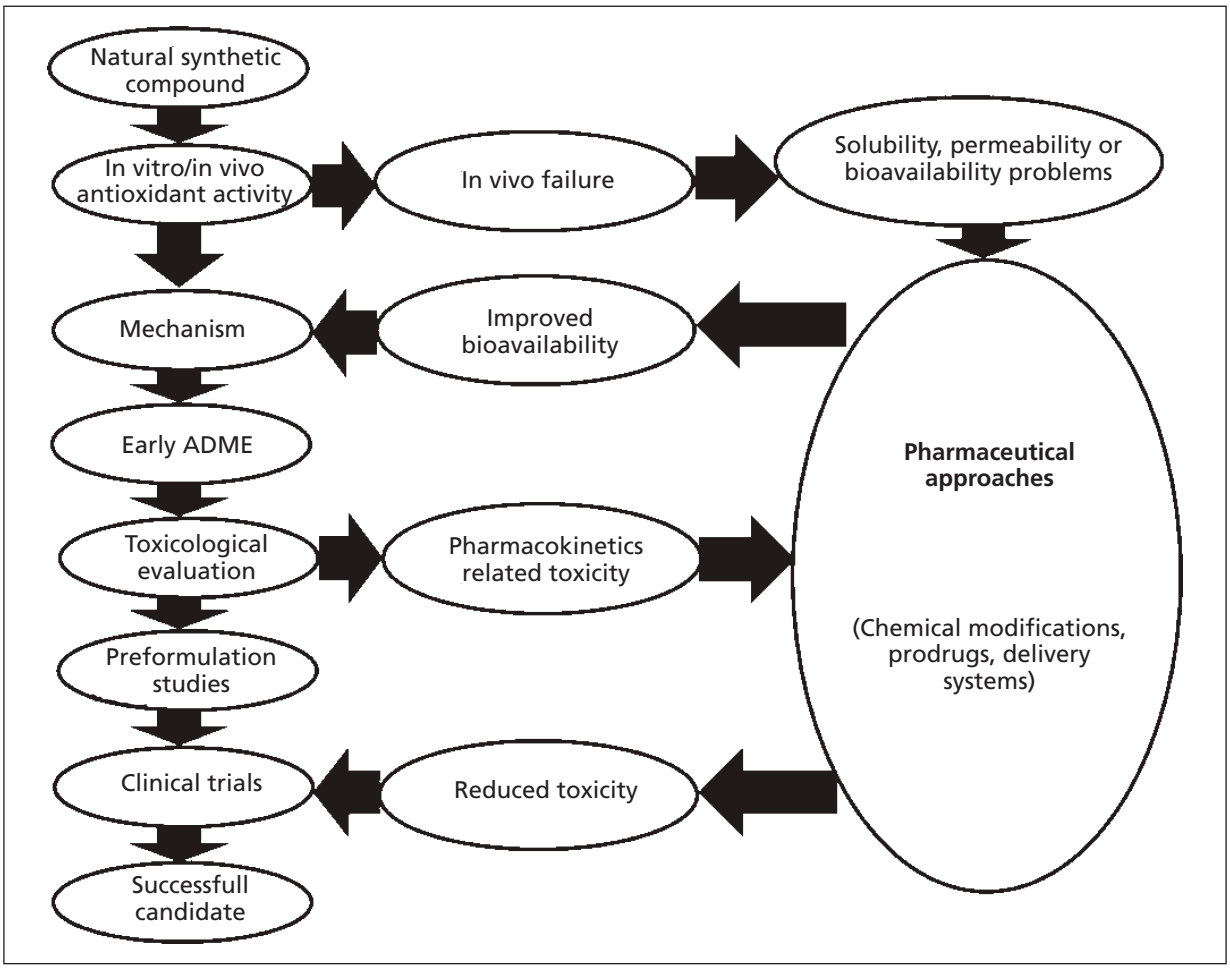

Figure 2. Role of pharmaceutics in development of antioxidants.

previously described studies have explored the impact of different antioxidant treatments to improve some free-radicals induced or enhanced diseases. Unfortunately, many of them, either due to the small patients sample size, with uncontrolled admissions and treatment criteria, or to relevant bias of the clinical studies, failed to give us, precise informations, on effectiveness and practical advantage in taking antioxidants.

Notwithstanding, some indications to antioxidant treatment reached the evidence, and recommendations for a fruits and vegetables rich diet, even if we don't know exactly the benefits definite role provided by antioxidant supplements in disease prevention, and good health maintainance.

An other very relevant focus in the advancement of our knowledges, is the measure of the blood free radicals as well as it's antioxidant properties. In fact, an easy dosage of these parameters by means of point of care instruments, will be of unvaluable help, to detect the role of any specific antioxidant treatment in some specific diseases, relating the emerging clinical data, with laboratory detected levels. We reviewed carefully this instrument area, and our choice was addressed to FORD test who, in comparison with other even more cumbersome devices, was reliable and very easy to be used. FORD (Free Oxygen Radicals Defence) is a colorimetric test based on the ability of antioxidants present in plasma to reduce a preformed radical cation. The principle of the assay is that at an acidic pH (5.2) and in the presence of a suitable oxidant solution (FeCl3), 4-Amino-N,Ndiethylaniline, the FORD chromogen, can form a stable and colored radical cation.

Antioxidant molecules (AOH) present in the sample which are able to transfer a hydrogen atom to the FORD chromogen radical cation, re-

duce it quenching the color and producing a decoloration of the solution which is proportional to their concentration in the sample.

The " Point of care phylosophy", with such a diagnostic device will discriminate at the best the high risk of oxidative damage on sick or healthy individuals, monitoring with precise lab parameters the clinical situation at the baseline and in the follow up, of a medical prescription. The problem of the individual bioavailability of each antioxidant molecule ought to be monitored during it's administration, with a pre-post measure of the oxidative balance, with the FORD instrumentation, in order to achieve the evidence of the oxidative background related to the outcome of specific symptoms and disease

Summarizing, the interest in the topic of oxidative stress is growing up and the measurement and monitoring of free radicals levels and oxidative stress is entering more and more in clinical chemistry laboratories and in medical practice. Because an easy, cheap and reliable method of oxidative stress monitoring is available, epidemiological studies will be encouraged,and the role of nutrition and targeted antioxidant therapy will be better defined.

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