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Diet Antioxidant Capacity: Relationships to Oxidative Stress and Health

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Abstract

This review will discuss some of the antioxidant assays and biomarkers/measurements used to assess the effect of dietary components on ameliorating *in vivo* oxidative stress. Although the development of dietary antioxidants has become a major component of the dietary supplement market, this review will focus on foods (primarily fruits and vegetables) and their impact on biomarkers of oxidative stress and the ultimate appearance of disease states. Data is reviewed demonstrating the importance of consumption of dietary antioxidants along with meals to prevent postprandial oxidative stress. Epidemiology studies have demonstrated the relationships between dietary antioxidant intake in the form of polyphenolic type compounds and health outcomes. However, additional research is needed in order to clearly define the mechanism(s) of action of the polyphenolics and whether their effects are direct or indirect in nature.

Keywords: Antioxidant, Oxidative stress, Diet.

Introduction

All animals need O_2 for production of energy in mitochondria [1]. When cells utilize oxygen to generate energy, free radicals are created as a consequence of ATP (adenosine triphosphate) production by mitochondria. A free radical is any species containing an unpaired electron. With an unpaired electron in its atomic orbital, the free radical is highly reactive. There are many types of free radicals and non-radical reactive species in living systems; nonetheless, reactive oxygen species (ROS) are the primary reactive species in the human body. When the production of ROS overwhelms antioxidant defenses provided by antioxidants and anti-oxidative enzymes, a status called "oxidative stress" occurs. Oxidative stress leads to cellular damage and may be a causative factor or lead to progression of chronic degenerative diseases, such as cardiovascular disease, cancer and Alzheimer's disease. Nutrient antioxidants (i.e. vitamin C, vitamin E, carotenoids, etc) have defined levels in the diet which are necessary to prevent specific

deficiencies leading to disease states. However, there are another class of compounds found in fruits and vegetables, commonly known as polyphenols, that show antioxidant effects *in vitro*, but whether they have any direct *in vivo* antioxidant effects has been a topic of much debate, recently. In addition to possible direct effects, dietary flavonoids may also have indirect antioxidant effects by stimulating *in vivo* antioxidant systems which may prevent or relieve oxidative stress. For instance, in biological systems glutathione (GSH) is a major nonenzymatic antioxidant involved in the maintenance of redox balance which may ameliorate cellular oxidative damage. At the cellular and molecular levels, redox imbalance causes the activation of redox-sensitive transcription factors that lead to inflammation [2]. Thus, enhanced oxidative stress due to uncontrolled reactive oxygen species is a major factor in both acute and chronic inflammation and inflammatory related diseases, including atherosclerosis and diabetes. To assess the effectiveness of dietary antioxidants *in vivo*, researchers have traditionally used a number of markers of oxidative stress and in the measurements of antioxidant defense including antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidases (GPx) and catalase (CAT) as well as molecules such a albumin, bilirubin and uric acid.

Measures of antioxidant capacity have been developed to assess total antioxidants in fruits and vegetables and other dietary components and to provide another biomarker of oxidative stress in the form of plasma and tissue antioxidant capacity [3 4]. These antioxidant capacity assays can be classified into two types based on their reactions with free radicals: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET) [5]. The majority of HAT-based assays apply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. Assays of this type include: 1) inhibition of induced low-density lipoprotein autoxidation, 2) oxygen radical absorbance capacity (ORAC), 3) total radical trapping antioxidant parameter (TRAP), and crocin bleaching assays. ET-based assays measure the capacity of an antioxidant to prevent the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentrations. ET-based assays include: 1) the total phenols assay by Folin-Ciocalteu reagent (FCR), 2) Trolox equivalence antioxidant capacity (TEAC), 3) ferric ion reducing antioxidant power (FRAP), 4) "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and 5) the diphenyl-1-picrylhydrazyl (DPPH) assay. The total phenols assay by FCR has been used to quantify an antioxidant's reducing capacity and the ORAC assay to quantify peroxyl radical scavenging capacity was used to establish a dataset of antioxidant capacity of fruits and vegetables [6-9]. It has been a desire by many to have a single conversion factor that would relate different antioxidant capacity assays. However, this is not possible due to the very different chemistries, different free radicals involved and reaction mechanisms of the different assays with different antioxidant compounds.

Various antioxidant capacity assays have been used to assess antioxidant capacity of biological samples such as plasma and tissues in animal and human clinical studies. Among them, ORAC is considered particularly biologically relevant in that it uses the peroxyl radical, which is the most common radical found in biological systems. Although the antioxidant capacity assays can be useful for assessing antioxidant capacity of foods and also can be used for plasma and tissues, it must be realized that these are *in vitro* 'test tube' assays and do not necessarily take into account what happens to the dietary antioxidants during absorption and metabolism. Thus, other *in vivo* biomarkers have been used to assess *in vivo* oxidative status and the effectiveness of dietary antioxidants. These biomarkers are usually the stable oxidized products of biological macromolecules. The most commonly used biomarkers include carbonylated proteins, malondialdehyde and isoprostanes from lipids and 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA. In addition, measurements of

antioxidant defenses such as antioxidant enzymes (i.e. SOD, GPx and CAT) as well as non-enzyme antioxidants (i.e. glutathione, vitamin E and ubiquinol) are also widely used to evaluate the *in vivo* redox status [10]. However, evaluation of any single biomarker/measurement or even a few may not truly reflect the overall *in vivo* antioxidant status. This may be one factor leading to seemingly differing conclusions from various antioxidant research studies, because different biomarkers were utilized.

Oxidative Stress, Antioxidants and Disease

Excess ROS can damage cellular lipids, proteins or DNA to impair their normal function. Thus, oxidative stress has been implicated in the etiology of many chronic and degenerative diseases, and it was also believed to play a major role in the aging process [11-13]. The implication of oxidative stress in the etiology of chronic and degenerative diseases suggests that antioxidants, both endogenously and exogenously, are necessary to protect cellular components from oxidative damage.

Oxidative stress and abnormalities in the oxidative defense system may occur in newborn infants. In a study of 30 macrosomic and 30 sex-matched control newborns, plasma ORAC, albumin, vitamin E, SOD, CAT and GPx levels were significantly decreased in macrosomic as compared to control newborns [14]. Excessive weight could be a potential factor contributing to decreased antioxidant capacity and increased oxidative stress in newborns which may lead to abnormalities of the humoral immune defense systems [14].

Multiple sclerosis, a chronic inflammatory disease of the central nervous system, is characterized by loss of myelin, the fatty tissue that surrounds and protects nerve fibers allowing them to conduct electrical impulses. In a cross-sectional study included 33 patients with multiple sclerosis and 24 age and sex matched control subjects, mean serum total antioxidant capacity, measured by a quantitative colorimetric assay kit, was ~30% lower in the multiple sclerosis group of patients compared to the control group of subjects. All patients had definite diagnostic categories (Poser criteria) for multiple sclerosis. The results showed that oxidative stress plays an important role in pathogenesis of multiple sclerosis and suggests that dietary antioxidants may be important in the therapy of multiple sclerosis patients [15].

Antioxidants and Postprandial Oxidative Stress

There is increasing evidence that oxidative stress during the immediate postprandial state may be an important contributing factor to chronic diseases. Although, the results from the postprandial state in short term studies cannot necessarily be extrapolated to long term disease development and outcome with certainty, such studies can provide insight into dietary components that impact oxidative stress and antioxidant status. The role of flavonoids and other phenolic compounds in protecting health and lowering disease risk through their actions in mitigating fed-state metabolic and oxidative stressors is of interest.

In early studies by Prior and coworkers [3], changes in plasma antioxidant capacity (AOC), as determined using ORAC, following consumption of a single meal of berries/fruits (blueberry, dried plum, dried plum juice, grape, cherry, kiwifruit and strawberry) were studied in 5 clinical trials. In two studies with blueberry or grape, additional macronutrients (carbohydrate, fat, protein) were included in the control and treatment meals. Blood samples collected before and after the meal were analyzed for plasma AOC. Consumption of blueberry in 2 studies and of a mixed dried grape powder in another study [12.5, 39.9 and 8.6 mmole Trolox Equivalents (TE) of AOC, respectively] increased plasma hydrophilic AOC AUC. Lipophilic AOC increased following a meal of blueberry containing 12.5 and 39.9 mmole TE of AOC. Consumption of 280 g of cherries (4.5 mmol TE) increased plasma lipophilic-AOC but not hydrophilic-AOC. The plasma AOC in the control groups receiving no dietary antioxidant source, in which additional macronutrients were added, decreased from the postprandial baseline AOC measurement,

indicating development of postprandial oxidative stress conditions. Other investigators have observed increased oxidative stress in response to an oral glucose load [16]. However, the quantity of antioxidants consumed (mmol TE) in the different studies was not directly related to changes in plasma antioxidant status. Differences in amount and components absorbed from different foods may account for some of the lack of a direct correlation between ORAC intake and plasma antioxidant capacity. Factors such as phytochemical profile of the food, vitamin C, carbohydrates or the amount of energy consumed in the meal may alter the response.

Generally, flavonoids are poorly absorbed which has been interpreted that flavonoids may not directly increase serum antioxidant levels. Two theories have been proposed to explain how high fruit intake might increase serum antioxidant capacity. Lotito and Frei [17] found that higher fructose content of fruit causes an increase in serum levels of uric acid, which increased plasma antioxidant capacity. This compound was found to be the more significant contributor to increased serum antioxidant capacity after apple consumption, rather than the phenolic compounds. In another study [18], ascorbate was suggested to be of more significance than phenolic compounds or urate in increasing postprandial serum antioxidant capacity when consuming only strawberries. Thus the fructose-dependent hyperuremic effect, observed after the intake of non-berry fruits [17], is thought not to be responsible for the plasma antioxidant changes following strawberry consumption. Plasma antioxidant capacity increased following consumption of 500 mL of cranberry juice which could largely be accounted for by an increase in vitamin C rather than phenolics. This also may explain the lack of an effect on antioxidant status of a phenolic-rich (primarily anthocyanins) and vitamin C-low blueberry juice [19].

However, in another study, Blacker et al [20] found that consumption of a much lower quantity of blueberries than tested previously reduced postprandial oxidation when consumed with a typical high-carbohydrate, low-fat breakfast. In this study, participants received each of the three treatments over 3 weeks in a cross-over design. Treatments consisted of a high blueberry dose (75 g), a low blueberry dose (35 g) and a control (ascorbic acid and sugar content matching that of the high blueberry dose). Serum ORAC and serum lipoprotein oxidation and serum ascorbate, urate and glucose were measured at fasting, and at 1, 2 and 3 h after treatment consumption. The mean serum ORAC was significantly higher in the 75 g blueberry group than in the control group during the first 2 h postprandially. Serum lipoprotein oxidation lag time showed a significant trend towards protection over the 3 h for both blueberry doses. Changes in serum ascorbate, urate and glucose were not significantly different among the groups. This data demonstrated that the postprandial increase in serum antioxidant capacity is not attributable to the fructose or ascorbate content of blueberries. Blacker et al [20] concluded that it was likely that the effects observed with blueberries are due to the phenolic compounds acting directly or indirectly, to bring about the decreased postprandial oxidative stress.

Cranberry juice consumption (750 mL/day for 2 weeks) did not alter blood or cellular antioxidant status or several biomarkers of lipid status pertinent to heart disease. Similarly, cranberry juice had no effect on basal or induced oxidative DNA damage. These results showed the importance of distinguishing between the *in vitro* and *in vivo* antioxidant activities of dietary anthocyanins in relation to human health [21]. In a recent study [22], contrary to previous studies attributing the protective postprandial effect to fructose and ascorbate, phenolic compounds from oranges contributed directly to the postprandial oxidative protection in serum.

Another important issue is the metabolites of dietary antioxidants that are present in the circulation and the tissues. Some metabolites, especially the catabolites generated by gut microflora from native dietary compounds, may be absorbed at a fairly high concentration and show comparable or even more potent antioxidant activities than their precursors. Urolithin A, which is a major colonic microflora metabolite produced by rats and humans after consumption of ellagitannins, produced significantly elevated plasma ORAC values,

which was associated with high plasma levels of the unconjugated form of urolithin A at 1 hour after oral administration [23]. Significant anti-inflammatory effects were also observed. These observations point out the importance of considering the flavonoid and/or its metabolites and the extent by which it might be conjugated in the absorption process as factors involved in whether an antioxidant response is observed in the blood or other tissues.

Burton-Freeman [24] reviewed the literature on the effect of fruits and their inherent phenolic compounds in human subjects on postprandial lipemia, glycemia/insulinemia and associated events, such as oxidative stress and inflammation. The collected data suggested that consuming polyphenol-rich fruits increases the antioxidant capacity of the blood, and when they are consumed with high fat and carbohydrate 'pro-oxidant and pro-inflammatory' meals, they may counterbalance the negative effects of the high fat/high carbohydrate diets [24]. However, without further long term clinical studies, one cannot necessarily translate increased plasma antioxidant capacity into a potential decreased risk of chronic degenerative disease. Given the content and availability of fat and carbohydrate in the Western diet, regular consumption of polyphenol-rich foods, particularly in conjunction with meals, appears to be a prudent strategy to maintain oxidative balance and health [24].

Biomarkers/measurements Of Oxidative Stress and Dietary Antioxidants

Plasma/serum measures of antioxidant capacity have been useful in the short-term postprandial studies and also in longer term diet intake studies in assessing antioxidant status and oxidative stress. Increasing numbers of studies have reported that an increase in plasma antioxidant capacity is associated with increased intake of fruits and vegetables which are rich in antioxidants [25-27]. In a study by Cao et al., 36 healthy nonsmokers resided in a metabolic research unit and consumed 2 sets of controlled diets and fasting plasma antioxidant capacity, measured as ORAC, and α-tocopherol concentrations were determined on days 1, 6, 11, and 16 of the study. Fasting baseline plasma ORAC was significantly correlated with estimated daily intake of total antioxidants from fruit and vegetables (ave. 5 servings/day) during the previous year [28]. Relative to baseline levels, plasma ORAC in these subjects was significantly increased by consuming 10 servings of fruit and vegetables per day for 10 days or by consuming 10 servings of fruits and vegetables plus 2 servings of broccoli per day. This increase in ORAC could not be explained by any increase in the plasma α-tocopherol concentration [28].

Total antioxidant performance (TAP) [29 30], which is a variation of the ORAC method that measures the ability of both hydrophilic and lipophilic antioxidants to quench radicals and their interactions in plasma/serum [26], was positively associated with intake of fruit and vegetables. The association remained after adjustment for dietary intake of β-carotene and vitamin C, indicating that the association of fruit and vegetable intake with TAP was not solely because of the presence of these well documented antioxidant vitamins [26]. Wang and coworkers [31], using three different antioxidant capacity assays, attempted to determine if plasma antioxidant capacity could effectively predict dietary intake of antioxidants and plasma antioxidant status. Seven-day food records and 12-h fasting blood samples were collected from 40 subjects for dietary and plasma antioxidant assessments. Plasma antioxidant capacity was determined by vitamin C equivalent antioxidant capacity (VCEAC), FRAP and ORAC assays. Plasma antioxidant capacity determined by VCEAC and ORAC had positive correlation with plasma uric acid and total phenolics. However, antioxidant capacity measured by FRAP was correlated only with uric acid. After multivariate adjustment, plasma antioxidant capacity determined by VCEAC was positively associated with dietary intakes of γ-tocopherol, β-carotene, anthocyanins, flavones, proanthocyanidins and dietary antioxidant capacity, as well as with plasma total phenolics, α-tocopherol, β-cryptoxanthin and uric acid. The authors concluded that plasma antioxidant capacity measured by VCEAC reflects both dietary and plasma antioxidants and represents more closely

the plasma antioxidant levels than ORAC and FRAP [31].

A significant progressive increase in fasting plasma TAC and in serum vitamin C concentrations were observed during a 16 day period of consumption of 500 g/d of strawberry in a pilot study of 16 subjects. Enhanced resistance of erythrocytes to hemolysis *in vitro* was also observed with strawberry consumption [32].

Root et al [33] studied whether an increase in combined self-reported fruit and vegetable intake over a 12 week period in a community setting (N = 1000, age 18-85 years, 61% female) was associated with improved multiple markers of inflammatory and oxidant status. Two fasting blood samples were collected separated by 12 weeks. In analyses controlling for other important dietary and lifestyle factors, pro-inflammatory cytokines IL-6 and TNF-α were significantly lower across categories of increasing fruit and vegetable intakes, while plasma FRAP and ORAC were significantly higher and F(2)-isoprostanes were significantly lower. These findings support current guidelines calling for increased fruit and vegetable consumption for improving antioxidant status and lowering inflammation in the prevention of chronic diseases [33].

The effect of coffee consumption for a period of four weeks on antioxidant capacity and lipid peroxidation in 20 healthy volunteers was studied by Correa and coworkers (*17*). Compared with baseline, subjects consuming coffee had an increase of 21 - 26% in plasma total antioxidant status, 13% in CAT, 52 - 75% in erythrocyte superoxide dismutase, and 49 - 62% in erythrocyte GPx. No significant alterations in lipid peroxidation biomarkers (oxidized LDL and 8-epi-prostaglandin (F2α)) were observed. Coffee had antioxidant effects, which may have been due to both chlorogenic acids and Maillard reaction products present in coffee. This data provides an illustration of the need to measure multiple oxidative stress biomarkers that may be important in protecting biological systems and reducing the risk of diseases related to oxidative stress [34].

Plasma total antioxidant capacity (total and perchloric acid (PCA) protein-precipitated plasma oxygen radical absorbance capacity (ORAC) assay) has been shown to be increased within 1 week on a low glycemic index diet in obese male subjects. These changes in antioxidant capacity occurred before changes in other risk factors for cardiovascular disease and diabetes, providing some evidence that oxidative stress may mediate reported effects of glycemic index and hyperglycemia on health [16].

Animal Studies of Dietary Antioxidants and Health Status

Although not exhaustive in terms of literature available, there are studies using animal models of the effects of dietary components on oxidative stress. Strawberry extracts (3 different cultivars) were protective against ethanol-induced gastric mucosa damage in an *in vivo* rat experimental model [35]. The strawberry extracts provided a rich source of anthocyanins and antioxidant capacity. Ethanol caused severe gastric damage and strawberry consumption protected against its deleterious effect. Antioxidant enzyme activities (CAT and SOD) increased significantly after strawberry extract intake and a concomitant decrease in gastric lipid peroxidation was found after 10 days. A significant correlation between total anthocyanin content and percent of inhibition of ulcer index was also found. These effects seem to be associated with the antioxidant activity and phenolic content in the extract as well as with the capacity to promote the action of antioxidant enzymes [35].

Oxidative stress and hypogonadism have been found to link to the increased incidence of cardiovascular disease in males. In a rat model, sham-control and orchidectomized 1 year old male rats were given 27% cranberry juice or 45% cranberry juice. At 120 days after initiation of the study, cranberry juice consumption increased plasma antioxidant capacity and superoxide dismutase activity and reduced nitrate + nitrite and malondialdehyde concentrations in orchidectomized rats. Orchidectomy depressed plasma antioxidant capacity and superoxide dismutase activity, elevated nitrate + nitrite and malondialdehyde in plasma, and increased

triglyceride and cholesterol values in liver and in plasma. The protective effect of cranberry juice from oxidative damage may be mediated by a decrease in nitrate + nitrite and dose-dependent decrease in peroxidation [36].

Red onion fed as peel, or peel plus flesh at 5% of the diet and fed for 4 weeks to rats, was shown to enhance antioxidant defense mechanisms through the induction of plasma SOD and GPx activities and to inhibit liver lipid peroxidation, suggesting that red onion may be able to reduce oxidative stress in rats [37].

Blueberries contain a high level of polyphenols and exhibit strong antioxidant and potential anti-inflammatory effects [6]. $ApoE^{-/-}$ mice fed 1% freeze-dried blueberry powder in the diet developed significantly fewer aortic lesions [38]. Furthermore, some of the antioxidant enzymes were up-regulated by blueberry feeding. In subsequent studies, other potential mechanisms for the anti-atherotic effects of blueberry were studied using a real-time quantitative RT-PCR array with multiple antioxidant/inflammation related genes. The expression of class B scavenger receptor CD36 was reduced by 40% in the aorta from apoE-/ mice fed blueberry for 20 weeks [39]. Serum from blueberry fed $ApoE^{-/-}$ mice was also shown to inhibit signaling pathways leading to the formation of TNF-α and IL-6 [40].

Clinical and Epidemiology Studies: Dietary Antioxidant and Health Outcomes

Increasing numbers of studies have reported that an increase in plasma antioxidant capacity is associated with increased intake of fruits and vegetables which are rich in antioxidants [25-27]. A high fruit, berry and vegetable intake was associated with reduced risk of mortality in middle-aged Finnish men [41], suggesting that diets that are rich in plant-derived foods can promote longevity.

In a 2-month randomized, parallel dietary intervention study of ninety subjects with abdominal obesity (46 in intervention group, 44 in control group), adherence to a Mediterranean-type diet, with emphasis on an increase in foods rich in antioxidants and close dietetic supervision, was shown to increase total dietary antioxidant intake and plasma total antioxidant capacity (ORAC) in this patient population [42]. In a randomized dietary trial (PREDIMED trial), the influence of a Mediterranean dietary pattern, rich in virgin olive oil, on plasma TAC in subjects with high cardiovascular risk was studied. The intervention with the Mediterranean diet was associated with higher levels of plasma antioxidant capacity. Increased plasma TAC was also related to a reduction in body weight after 3 years [43]. In another study [44], higher dietary antioxidant intakes (ORAC) had favorable effects on metabolic disorders and also in preventing subsequent weight and abdominal fat gain during a 3-year follow-up in 1938 subjects from the Tehran Lipid and Glucose Study [44].

Detopoulou and coworkers [45] demonstrated a positive association between dietary antioxidant capacity and adiponectin concentration in free-living, apparently healthy adults from the ATTICA study [310 men (40±11 years) and 222 women (38±12 years)]. However, a negative relation of dietary antioxidant indices with inflammatory markers was observed. These authors proposed an adiponectin-mediated route through which antioxidant-rich foods exert beneficial effects against inflammation and cardiovascular diseases [45].

In a community study (N = 1000, age 18-85 years, 61% female), markers of inflammation (IL-6 and TNF-α) were significantly lower and antioxidant status (plasma FRAP and ORAC) were significantly higher across categories of increasing fruit and vegetable intakes. F_2-isoprostanes were significantly lower across categories of fruit and vegetable intakes [33]. While data were not available on dietary antioxidant intake, the increase of at least 2 servings each of fruits and vegetables could result in an increase of more than 3000 μmole TE per day in total ORAC [3, 6].

Experimental studies suggest that oxidative stress and systemic inflammation are involved in the pathogenesis of ischemic stroke. Consuming a diet with a high total antioxidant capacity has been related to reduced inflammation and increased circulating antioxidants in cross-sectional and randomized intervention studies

[46]. In a study of the relation between dietary total antioxidant capacity and risk of ischemic and hemorrhagic stroke in 41,620 men and women not previously diagnosed with stroke or myocardial infarction, a diet rich in total antioxidant capacity was associated with a reduction in risk for all types of stroke, which was only marginally significant, but when only ischemic stroke cases were considered, a stronger inverse association with dietary TAC was observed [46]. Total antioxidants may play a role in reducing the risk of cerebral infarction but not hemorrhagic stroke, however, a high intake of vitamin E may be positively associated to the risk of brain hemorrhagic events. More focused investigations are needed on how antioxidants may play a role in reducing the risk of cerebral infarction but not hemorrhagic stroke.

Total antioxidant capacity intake in the diet was negatively associated with hypertension in type 2 diabetic patients [47] in a cross-sectional study of 506 type 2 diabetic patients, aged 28-75 years in Tehran. The odds ratios of both systolic (>140 mm Hg) and diastolic blood pressure (>90 mm Hg) decreased across increasing quartiles of total dietary ORAC intake [47].

A small number of epidemiology studies have been published recently in which dietary intake of antioxidant capacity was related to outcomes of some cancers. One of the first studies using data from long term epidemiology studies to relate dietary antioxidant intake and oxidative stress-related carcinogenesis was by Serafini and coworkers [48]. In a large population-based case-control designed study, data were collected through face-to-face interviews with 505 newly diagnosed gastric adenocarcinoma patients and 1116 control subjects to assess dietary habits 20 years before interview. The total radical-trapping antioxidant potential (TRAP) of different plant foods was used to convert food frequency intake into antioxidant potential. Gastric cancer risk in groups exposed to higher levels of oxidative stress (smoking and Helicobacter pylori infection) was also examined. Intake of antioxidant equivalents expressed as TRAP values was inversely associated with the risk of both cardia and distal gastric cancer. Controlling for smoking, the inverse relationship between TRAP values displayed a clearer dose-response pattern. Subjects who had never-smoked with the highest antioxidant intake had the lowest risk of cancer. [48].

In a clinic-based study of 603 incident cases and 1,007 frequency-matched controls, higher antioxidant intake (measured by ORAC) as estimated from a food frequency questionnaire, particularly the lipophilic component, was associated with a lower non-Hodgkin lymphoma risk after accounting for other antioxidant nutrients and vegetable intake [49]. Endometrial cancer risk was studied in a population-based case-control study in New Jersey, including 417 cases and 395 controls [50]. Dietary intake was ascertained using a food-frequency questionnaire, and total antioxidant capacity intake was estimated using the USDA ORAC database and the University of Oslo's Antioxidant Food Database (AFD) and FFQ-derived estimates of intake. Using the ORAC database, after adjusting for major covariates, decreased risks of endometrial cancer was observed for the highest tertile of total phenolic intake compared with the lowest tertile. There was no association for total antioxidant intake based on the AFD, which utilized the FRAP assay to assess antioxidant capacity. There was also no strong evidence for an association with intake of any of the individual antioxidants, however, increasing the total phenolic consumption as reflected in ORAC intake may decrease endometrial cancer risk [50].

An inverse association between selenium consumption from food sources and ovarian cancer risk was observed, while there was little evidence of an association with total antioxidant capacity or any of the other individual antioxidants. Additional research is needed to confirm these findings [51]. In the Health Professionals Follow-Up Study, total antioxidant capacity intake assessed through the FRAP assay was not associated with colorectal cancer [52] In the EPIC cohort, total antioxidant intake, based on the FRAP and TRAP assays, was associated with a reduction in gastric cancer risk [53].

Dietary TAC may have a favorable role in respiratory health, particularly in premenopausal women who have never smoked [54] based upon

a healthy Italian population using FRAP as a measure of TAC and the European Investigation into Cancer and Nutrition Food Frequency Questionnaire was used for dietary assessment. Stratified analysis showed that this relationship between dietary TAC and pulmonary function only occurred in women who were premenopausal/never smokers. In this subgroup, the observed effect of higher FRAP intake was an increased forced expiratory volume equivalent to an improvement in pulmonary age of 3.3 years.

Dietary Intake of Antioxidants Measured as ORAC Value

Sufficient research data to establish definite reference dietary intakes (RDI), as has been done for vitamins A, C, and E, is not possible for total dietary antioxidants (measured by ORAC or any other antioxidant capacity assay). However, some broad recommendations can be made based upon recent epidemiological studies and studies of absorption of antioxidant phytochemicals and their effects on *in vivo* antioxidant status and their ability to prevent postprandial oxidative stress.

Data from food intake records of 30 subjects in a controlled nutrition study indicated that hydrophilic ORAC intake was 7405 units/day/person [1 unit = 1 micromole of Trolox Equivalents (TE)]. Based on data on quantities of foods consumed per day from the USDA's Continuing Survey of Food Intakes by Individuals (1994-1996, 2 days), hydrophilic ORAC intake was calculated to be 5558 units/ day/person. The estimated intake of lipophilic ORAC from this same data was estimated to be 166 units per day [6]. Based on the food consumption data in the NHANES (2001-02) hydrophilic ORAC intake was estimated to be 4650 units per day [6].

The hydrophilic ORAC content of 23 vegetables commonly consumed in Japan was assessed to estimate the dietary intake of antioxidants in Japan. The estimated average hydrophilic ORAC value for "typical vegetables" consumed in Japan was 594.3 µmol TE/100 g. Hence, 2080 units/day of hydrophilic antioxidants would be ingested when 350 g of vegetables a day are consumed [55]. This estimate is similar to that calculated from the USDA food intake data of 2385 units per person per day [6]. However, in data recalculated from an earlier study [28] using the newer food data with the latest ORAC analytical method, ORAC intakes from food frequency questionnaires based upon the previous year was 4360 units of ORAC per day (5 servings fruits and vegetables per day). Using controlled diets providing 10 servings of fruits and vegetables ORAC intake increased to 8600 units of ORAC per day and was increased to 10800 units with the addition of 2 servings of broccoli. However, ORAC intakes were somewhat lower than might be expected because the diets did not contain any of the berries and fruits that are appreciably higher in ORAC [28].

In the Mayo Clinic Case-Control study [49] (603 cases; 1007 controls), dietary hydrophilic ORAC intake was <5266, 5266-8663; 8363-12518; >12519 units/day for the lowest to the highest quartiles, respectively, based upon data from a food-frequency questionnaire. Using the Singapore Chinese Health Study with more than 63,000 women and men aged 45-74 yrs, intake of hydrophilic ORAC from fruits and vegetables was calculated to be 3827 and 1810 units, respectively for a total intake of 5636 units per day [8, 9].

In another large epidemiological study of ORAC intake from Sweden, (Swedish Mammography Cohort, n = 32,561 subjects) [56, 57], total antioxidant capacity intake (ORAC) from the diet was 8537, 10,779, 12,502, 14495, and 18,021 units/day, respectively for each of the quintiles. Thus, ORAC intakes as high as 18,000 units per day are possible from foods if selected properly, but these intakes were only obtained in the top quintile of subjects in the Swedish study. In women from this cohort with a cardiovascular disease-free history, the relative risk for total stroke and cerebral infarction was least in those consuming more than 12,214 units or more of ORAC per day [58]. In women with a cardiovascular disease history, those in the lowest quartile of dietary ORAC (7,444 units ORAC per day) were more likely to have a history of hemorrhagic stroke [58]. A significant trend for decreased risk of endometrial cancer was observed in the population group that was

consuming more that 16,282 units of ORAC per day or more than 2,232 mg gallic acid equivalents of total phenolics [50].

Before any of the data from the large epidemiological studies was available on dietary ORAC intake was available, Prior and coworkers [3] developed a relationship between dietary energy intake and ORAC intake that indicated that 4.6 μmoles TE of antioxidant capacity should be consumed for every Kcal consumed. The basis for proposing a relationship with energy intake was that free radicals are produced in the process of energy metabolism due to inefficiencies in the process. This estimate was considered a minimum as it only accounted for oxidative stress associated with energy metabolism. Other sources of free radicals such as dietary pro-oxidants, cigarette smoke, smog, pesticides, drugs, disease situations, etc would increase the need for antioxidants.

Conclusions

In early studies of dietary antioxidant intake, it has been difficult to differentiate between increased intake of fruits and vegetables and antioxidant intake because an increase in fruit and vegetable consumption is also associated with increased antioxidant intake and also with increased plasma antioxidant capacity. The need for increased vegetable consumption has been known and recommended for many years. Increased fruit and vegetable consumption has also been associated with reduced risk of mortality in men from one study [41]. However, recently with the availability of the USDA ORAC food database and other total antioxidant databases, data has become available on antioxidant intake and relationship with some disease outcomes. Unfortunately, the USDA ORAC food database has been removed from their website. The following statement was provided as a reason for its removal: "USDA's Nutrient Data Laboratory (NDL) removed the USDA ORAC Database for Selected Foods from the NDL website due to mounting evidence that the values indicating antioxidant capacity have no relevance to the effects of specific bioactive compounds, including polyphenols on human health" [59]. From published data presented in this review, it seems clear that their reasoning for removal of the data base was not based upon the recent scientific literature. In studies reviewed in this report, increased dietary intake of total antioxidant capacity has been associated with reduced risk for ischemic stroke, hypertension, gastric cancer, endometrial cancer, and pulmonary function. Furthermore, consumption with antioxidant rich foods can reverse the oxidative stress resulting from consumption of an antioxidant free meal.

Furthermore, from the recent studies, we begin to have better estimates of the quantities of antioxidants we may need in our diet. From 3 different studies in the United States, dietary ORAC intake ranged from 4600 to 7400 units of ORAC per day. In the Singapore Health Study (63,000 participants) dietary ORAC intake was 5600 ORAC units per day [8, 9]. In Sweden, ORAC intake was 8500 in the lowest quintile and 18,000 in the highest quintile [56, 57]. Protection from stroke was observed in individuals consuming greater than 12,200 ORAC units per day and from risk of endometrial cancer with consumption of more than 16,000 ORAC units per day. A greater risk for hemorrhagic stroke was observed with consumption of less than 7,400 ORAC units per day [58]. These data have provided the first definitive data on ORAC intake and the levels that might be needed for improved health outcomes. From the available data, ORAC intakes of at least 12,000 or more should be recommended for reduced risk for the diseases studied and intakes of less than ~7000 units of ORAC per day is likely responsible for increased risk for some diseases. These recommendations are in line with consumption of 7 to 10 servings of fruits and vegetables with some of those foods containing the higher amounts of ORAC. From the available postprandial data on plasma antioxidant capacity, consumption of sources of antioxidants is important with each meal. Because of the difference in the ability of different phytochemicals to quench free radicals, consumption of a mixture of high antioxidant foods would be prudent to provide for this variability.

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