Antioxidant Status In Vivo: The Case for Regular Consumption of Antioxidant Rich Fruits and Vegetables

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Abstract
Since metabolism of energy is a major source of reactive oxygen species, the quantity of dietary antioxidants needed may be related to energy consumption. Antioxidant status in vivo can be altered by diet, but the postprandial response is dependent upon factors such as 1) antioxidant capacity (AOC) of food, 2) amount consumed, 3) type of phytochemicals and their content in the foods, 4) absorption/metabolism of the dietary antioxidants in the body and 5) the fructose content particularly of fruits and berries. The ability of different foods to prevent postprandial oxidative stress varies due to these and perhaps other factors. We have demonstrated that consumption of certain berries and fruits, such as blueberries, mixed grape and kiwifruit, was associated with an increased plasma AOC in the postprandial state, and consumption of an energy source of macronutrients containing no antioxidants was associated with a decline in plasma AOC. High antioxidant berries, which have high levels of anthocyanins, are not as effective in vivo as antioxidants as some other foods, apparently because of the poor stability and/or absorption of anthocyanins. In order to prevent periods of postprandial oxidative stress, increased consumption of high antioxidant foods is needed, and perhaps more important, antioxidant containing foods need to be consumed in conjunction with carbohydrate and other sources of energy in each meal.

INTRODUCTION
Fruits and vegetables contain numerous phytochemicals that have antioxidant capacity (AOC) (Cho et al., 2004; Wu et al., 2004a). However, AOC can vary by more than 50-fold in different foods (Wu et al., 2004a). Numerous epidemiology studies have indicated that increased consumption of fruits and vegetables is associated with a decreased risk for a number of diseases associated with aging (De Stefani et al., 2000; Hirvonen et al., 2001; Joshipura et al., 1999, 2001; Rissanen et al., 2003; Serafini et al., 2002), and a recent study indicated that increased consumption of fruit during childhood was associated with a lower odds ratio for cancer as an adult (Maynard et al., 2003). The components within fruits and vegetables which might be responsible for these associations are largely unknown. The antioxidant effects of polyphenols and other compounds have been suggested as one among many possibilities. One research study (Serafini et al., 2002) suggested that dietary intake of antioxidants measured as total radical-trapping antioxidant potential (TRAP) was inversely associated with the risk of cancer of both the cardia and distal stomach (Serafini et al., 2002). Increased dietary intake of selected classes of flavonoids (isoflavone, anthocyanidins, flavones and flavonols) has been associated with a reduced risk of colorectal cancer (Rossi et al., 2006). Epidemiology studies such as these are inherently difficult and expensive to conduct, and it is difficult to link directly any particular components in foods with health outcomes.

The objective of this review is to provide evidence and background for developing some preliminary recommendations for the consumption of antioxidants and provide a...
discussion of factors that impact making these recommendations.

**POSTPRANDIAL OXIDATIVE STRESS**

Another approach to understanding the role of antioxidant components in fruits and vegetables is to look at the short term effects of consumption of antioxidants on in vivo antioxidant status. In recent human clinical studies (Prior et al., 2007), postprandial plasma antioxidant capacity was shown to decrease following consumption of a drink mix that contained macronutrients (carbohydrate, protein, lipid) (450 kcal), but no antioxidant sources. The decline in antioxidant capacity postprandially may indicate that the antioxidant defense systems were not able to adapt sufficiently to protect against the oxidative challenge presented by the meal. Other investigators have demonstrated that reactive oxygen species produced following consumption of glucose (Aljada et al., 2006; Ghanim et al., 2007; Mohanty et al., 2000), lipid (Mohanty et al., 2002; Patel et al., 2007) and protein (Mohanty et al., 2002) can induce postprandial oxidative stress. In these studies, oxidative stress was assessed not only by plasma antioxidant capacity, but with other indicators of oxidative stress such as malondialdehyde (MDA), lipid hydroperoxides, 8-isoprostaglandin F₂α (F(2)-isoprostanes), uric acid and whole blood glutathione (Aljada et al., 2006; Weinbrenner et al., 2004; Wiswedel et al., 2004). The question that naturally follows is whether consumption of foods high in antioxidant capacity can prevent postprandial oxidative stress.

Postprandial plasma antioxidant status depends on factors such as the intake and consumption of compounds that are either oxidants or antioxidants and the sparing effect of some antioxidants on other antioxidants that have higher redox potentials (Natella et al., 2002a). Postprandial oxidative stress, often associated with lipemia, hypertriglyceridemia or hyperglycemia, is well recognized in the scientific literature (Anderson et al., 2006; Bloomer et al., 2007; Prior et al., 2007; Ruano et al., 2005; Weinbrenner et al., 2004). A number of different food sources have been studied that have protective effects against postprandial oxidative stress.

Aljada et al. (Aljada et al., 2004, 2006) demonstrated that glucose (75 g) intake leads to an acute increase in the generation of reactive oxygen species (ROS) by leukocytes and an increase in plasma thiobarbituric acid-reacting substances. The superoxide radical (O₂⁻) produced as a result of glucose metabolism can cause the activation of the key pro-inflammatory transcription factor, nuclear factor κB (NF-κB). Glucose intake also induces an increase in TNF-α mRNA which is probably mediated through an enhanced NF-κB binding activity to TNF-α gene promoter sites in mononuclear cells in healthy human subjects (Aljada et al., 2006). The increase in ROS with hyperglycemia and the related proinflammatory and prothrombotic effects in vivo may promote atherosclerosis in the long term. Accumulating evidence indicates that oxidative stress plays a major role in the initiation and progression of cardiovascular dysfunction associated with diseases such as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease, and chronic heart failure (Taniyama and Griendling, 2003). Thus, measures are warranted that will prevent or lower the postprandial production of ROS.

The measurement of high AOC in foods is an excellent starting point, but may or may not be an accurate indication of the potential for altering in vivo antioxidant status. The bioactive phytochemicals in foods have varying bioavailability and may influence biological processes through direct antioxidant effects, or indirectly through various signaling pathways, cytokines, receptors, etc that may protect the cell from free radical damage. Alternatively, these phytochemicals may impact cellular processes that are completely independent of antioxidant mechanisms. Thus, it is important to understand whether sufficient quantities of antioxidant phytochemicals can be absorbed in a form that might alter in vivo antioxidant status.

Wiswedel et al. (Wiswedel et al., 2004) examined whether an oxidative stress-mediated increase in plasma F(2)-isoprostanes could be counteracted by a flavanol-rich cocoa beverage. Consumption of a cocoa drink low in flavanols caused a slight increase
in the plasma concentrations of F(2)-isoprostanes 4 h after intake (+23%) which may have been attributable to postprandial oxidative stress. This increase did not occur following consumption of a cocoa drink containing high amounts of flavanols. The difference in F(2)-isoprostanes 2 and 4 h after intake of the high flavanol versus low flavanols cocoa drink became statistically significant when the intake was combined with physical exercise.

Oxidative stress, associated with postprandial lipemia, has been shown to contribute to endothelial dysfunction, which can shift hemostasis to a more thrombogenic state; and phenolics in olive oil have been shown to protect the endogenous antioxidant defense mechanisms in the postprandial state (Weinbrenner et al., 2004). Furthermore, a virgin olive oil with high amounts of phenolic compounds was found to change the postprandial hemostatic profile to a less thrombogenic state in hypercholesterolemic subjects (Ruano et al., 2005). Bogani et al. (Bogani et al., 2007) evaluated changes in inflammatory thromboxane B2 [(TXB(2)) and leukotriene B4 [LTB(4)] and oxidative stress markers (urinary hydrogen peroxide levels and serum antioxidant capacity), after a fat-rich meal administered to 12 normolipemic, healthy subjects. A significant decrease in inflammatory markers, namely TXB(2) and [LTB(4)], occurred at 2 and 6 h following consumption of extra virgin olive oil; a concomitant increase in serum AOC was also observed. The authors suggested that the protective effect observed was due to the antioxidant phenolic components, primarily hydroxytyrosol and oleuropein, in the extra virgin olive oil (Bogani et al., 2007). High fat meals, as well as cigarette smoking, can induce oxidative stress (Bloomer et al., 2007). Young cigarette smokers were observed to have an exaggerated oxidative stress response to feeding, as well as exaggerated hypertriglyceridemia, compared to non-smokers (Bloomer et al., 2007).

Caloric intake in the form of orange juice did not induce either oxidative or inflammatory stress, likely due to its content of antioxidant flavonoids and/or ascorbic acid (Ghanim et al., 2007). Consumption of proanthocyanidin-rich grape seed extract (GSE) by healthy volunteers increased plasma antioxidant capacity in the postprandial phase where the meal was a “fast-food” based meal (Natella et al., 2002a). Consumption of red wine has also been shown to increase plasma antioxidant status and decrease oxidative stress as indicated by plasma MDA (Micallef et al., 2007; Ventura et al., 2004). Consumption of coffee has also been shown to increase plasma antioxidant capacity (Natella et al., 2002b). Tea, which is one of the most consumed drinks, consistently increases blood antioxidant capacity (Rietveld and Wiseman, 2003). In terms of protection of biomolecules, the evidence from consumption of green and black tea is strongest for protecting DNA from oxidative damage.

Although only a portion of the work reported in the literature on postprandial oxidative stress is cited above, there are a number of antioxidant foods that may be effective in preventing postprandial oxidative stress. What has not been fully appreciated until recently is what happens to the antioxidant compounds during digestion and absorption is critical to whether an in vivo antioxidant effect is observed. If extensive metabolism occurs, the metabolites may or may not have any antioxidant capacity. Methylation or glucuronidation of hydroxyl groups on the B-ring of flavonoids is a common pathway of metabolism, a process which will likely decrease the antioxidant capacity, in which case there may not be any antioxidant response in vivo in plasma or other tissues.

**TRANSLATING IN VITRO FOOD ANTIOXIDANT CAPACITY DATA INTO MEANINGFUL IN VIVO INTAKE INFORMATION**

The working hypothesis and assumption has been that foods with a high antioxidant capacity in vitro will translate into a large response in vivo in antioxidant capacity. Thus, consumption of foods high in antioxidant capacity would be advantageous and information on antioxidant capacity of foods is important.

Antioxidant capacity, as determined by ORAC<sub>FL</sub>, or many of the other measures of antioxidant capacity have been expressed as micromoles of Trolox Equivalents (TE) per g
or 100 g of fresh weight or dry weight of the food. Trolox, which is a water soluble analogue of vitamin E, is used as the common standard for all analyses. However, the amount of AOC consumed may depend more on the serving size than on the actual concentration in the food. Thus, the concentration data has been used to arrive at an estimate of intake using serving size.

For the in vitro studies, the same ORACFL methodology is used except that the antioxidant capacity in a blood plasma sample is determined, and the change in that value after a meal containing different foods is determined. The area under the plasma curve represents the extent to which the food was able to impact one measure of in vivo antioxidant status.

The variation in antioxidant capacity among different fruits and vegetables varies considerably. In selected fruits analyzed by Wu et al. (Wu et al., 2004a,b), total antioxidant capacity ranged from 1.4 to 94.6 µmol Trolox equivalents /g fresh weight. Of all the fruit samples, berries, plums and some varieties of apples had the highest antioxidant capacity with cranberry and lowbush blueberry having the highest, while ORACFL of melons was relatively low. Those fruit samples with the highest antioxidant capacity also have the highest anthocyanin content (Schauss et al., 2006; Wu et al., 2004a, 2006). The range of total ORACFL in different fresh vegetables is not as large as the total ORACFL range among fruits (5 to 20 µmol TE/g FW). Fruits, in general, have about twice the levels of antioxidant capacity as vegetables.

Antioxidants in foods can be classified into two groups based upon their solubility in polar (hydrophilic) or nonpolar (lipophilic) solvents. Hydrophilic ORACFL of fruits and vegetables accounts for 90% or more of the total ORACFL measured using the peroxyl radical. Those vegetables which have the highest lipophilic antioxidant capacity generally are those that have a dark green color, suggesting that the green pigments or lipophilic components associated with these pigments may be responsible for their higher lipophilic ORACFL values. The range observed for lipophilic ORACFL was 0.1 to 6 µmol TE/g FW with most foods less than 1 µmol TE/g FW. Spinach, avocado, broccoli, asparagus, and lettuce (excluding iceberg variety) contain higher lipophilic antioxidant capacity than most other vegetables. Cranberry, raspberry and blackberry contained more than 1 µmol TE/g FW, which was much higher than other berry samples with similar hydrophilic ORACFL values. Much less is known about in vivo responses to the consumption of many of the lipophilic antioxidant components in fruits and vegetables, although consumption of blueberries or cherries produced an increased plasma lipophilic AOC (Prior et al., 2007).

Considerable emphasis has been placed upon ‘concentrations’ of antioxidants in foods; however, it may be more important to think in terms of amounts typically consumed rather than absolute concentrations. Consideration of serving size will take into account concentration, but also moisture content which greatly affects the ‘concentration’ value. In the data from Wu et al., (Wu et al., 2004a), foods were assigned a common serving size based upon sizes used in the USDA Nutrient Database and were divided into four groups based upon the ranges of their ORACFL content per serving. These groupings, basically by quartile, were from 0-499, 500-999, 1000-1999 and 2000-14,000 µmol TE, respectively. Most of the foods in the highest ORACFL group were fruits, particularly berries. Spices samples were not listed because of their small and variable serving size, but some of the spices could make a significant contribution to antioxidant intake even with the small serving size because of their extremely high concentrations on antioxidant capacity.

**Estimation of Total Daily ORAC Intake of Fruits and Vegetables**

Since fruits and vegetables are the major antioxidant sources in our daily diet, estimation of daily antioxidant capacity intake from these foods has been calculated based upon the quantities of foods consumed per day using the USDA’s Continuing Survey of Food Intakes by Individuals, 1994-96 (2 days). The estimated hydrophilic ORAC intake was 5558 and the intake for lipophilic oxygen radical absorbance capacity using
flourescein as probe (ORACFL) was 166 µmol TE per day, respectively (Prior et al., 2007; Wu et al., 2004a). The ORACFL intake calculations, based upon food consumption data from NHANES 2001-2002, averaged 4650 µmol TE per day. Vegetables contributed more of the lipophilic components and fruits more of the hydrophilic antioxidant components. From the NHANES data, beverages made up ~40% of the total antioxidant capacity consumed. On an individual basis, these numbers will vary considerably from this average depending upon the number of servings of fruits and vegetables consumed daily. If we assume that the average number of servings of fruits and vegetables consumed daily in the U.S. is 2.5, then an average serving would contain about 2200 µmol TE. Thus, if an individual consumed nine servings per day as is recommended, total intake could be 20 mmol TE per day.

Recommendations for Dietary Antioxidant Capacity Intake

Data currently available that can be used to arrive at some recommendations for antioxidant capacity intake is limited. Since metabolism of energy is a major source of ROS, we have suggested that the quantity of antioxidants needed will be related to energy consumption (Prior et al., 2007). A decrease in plasma AOC of 1 unit (µmol Trolox equivalents/L•hr) per kcal energy consumed was observed following a meal containing no dietary antioxidants (Prior et al., 2007). The decline in plasma antioxidant capacity following a carbohydrate meal might be expected since production of significant free radicals occurs during the metabolism of carbohydrate and the utilization of oxygen. The oxygen molecule is capable of accepting an additional electron to create superoxide, a more reactive form of oxygen. Ubisemiquinone species, generated in the respiratory chain, donate electrons to oxygen and this provides a constant source of superoxide radicals (Raha and Robinson, 2000).

Data obtained following a meal containing various fruits or berries have been used to develop some estimates of antioxidant requirements. The response in plasma antioxidant capacity following the consumption of different fruits is presented in Figure 1 (Adapted from Prior et al., 2007). Responses in hydrophilic and lipophilic antioxidant capacity differ considerably among grape, blueberry, kiwi and cherry (Fig. 1). The area under the curve (AUC) for plasma hydrophilic antioxidant capacity has been adjusted for the amount of ORACFL in the fruit and the energy that was consumed. Conditions differed between the different studies which may account for some of the variability in response. Only the blueberry with meal and the grape (mixed) were consumed with a meal that contained other macronutrients (carbohydrate, lipid and protein) but no other antioxidants. In the other treatments, the fruit was consumed as the only component of the meal. However, each of the treatments were adjusted based upon the energy that was consumed using our observed decrease in plasma antioxidant capacity of 1 unit (µmol Trolox equivalents/L•hr) per kcal energy consumed. Based upon the energy consumed from each food, the expected decline in plasma AOC AUC was calculated and added onto the observed AUC to obtain the Total AUC. The total AUC was divided by the ORACFL intake to obtain the AUC/dose. Based upon the responses observed (Cao et al., 1998; Prior et al., 2007) for 11 different foods, an average AUC/dose was calculated of 54 µmol TE/L•h per mmol TE of AOC consumed. Vitamin C, which could be considered a readily absorbed positive control, produced a larger increase in the AOC AUC of 124 µmol TE/L•h per mmol TE of AOC consumed (Cao et al., 1998).

Assuming that there is a linear relationship between energy intake and the need for dietary antioxidants, we previously estimated dietary antioxidant needs based upon dietary energy intake to be 4.61 mmol Trolox equivalents (TE) per 1000 kcal energy intake (Prior et al., 2007). For an individual consuming 2500 kcal per day, AOC needs are estimated to be 11.5 mmol TE. Dietary intakes of 5-15 mmol TE per day of antioxidant capacity as ORACFL are certainly within the realm of achieving with selection of appropriate high antioxidant foods. These estimates of AOC needs would not consider the additional amounts needed if other oxidant stressors were present such as dietary pro-oxidants, disease situations, cigarette smoke, drugs, etc.
At this point in time, any approach for determining recommended levels of AOC intake has definite limitations, largely because of the limited data that is currently available to fully develop a model. It appears from the data available that increases in plasma AOC are not directly proportional to the in vitro AOC. Other methods of analysis of plasma AOC will not likely alter this conclusion, since we have observed a similar pattern of change following a meal using other methods of analysis (FRAP, TEAC) (Cao et al., 1998). Thus, any recommendation cannot be based solely upon in vitro analysis of food ORACFL. However, as we learn more about the absorption/metabolism of different classes of antioxidant phytochemicals in different foods, one may be able to predict a response based upon food AOC and a measure of relative absorption efficiency. It seems clear from the data available that the high antioxidant berries which have high levels of anthocyanins are not as effective in vivo as antioxidants as some other foods, apparently because of the poor stability and/or absorption of anthocyanins (Wu et al., 2004c, 2005).

In the approach presented, we have assumed that the decline in AOC is linear with energy intake; however, data are only available for a single quantity of energy consumed. In addition, it is not clear whether the postprandial decline in AOC is the same for all sources of calories (fat versus carbohydrate versus protein). Furthermore, the postprandial decline may also depend upon the baseline antioxidant status. In normal situations, the changes that are observed in plasma AOC are likely ±20% of “normal” and in diseased situations, it might be as much as ±50% of “normal”. An individual with a low baseline AOC will likely not have as large of a decline postprandially as someone in the “normal” range, but may be more responsive to an antioxidant meal and may require more antioxidants to return to a “normal” range.

CONCLUSIONS

Although a number of assumptions are inherent in these recommendations, this represents one of the first attempts to quantitate dietary antioxidant needs. The conclusion that seems apparent from these studies is that in order to prevent periods of apparent postprandial oxidative stress, increased consumption of high antioxidant foods is needed, and perhaps more important, antioxidant containing foods need to be consumed in conjunction with carbohydrate and other sources of energy in each meal. Results from clinical studies clearly demonstrate that the antioxidant status in vivo can be altered by diet, but the response is dependent upon factors such as 1) AOC of food, 2) amount consumed, 3) type of phytochemicals and their content, 4) absorption/metabolism of the dietary antioxidants in the body and perhaps 5) the fructose content particularly of fruits and berries. Consumption of certain berries and fruits, such as blueberries, mixed grape and kiwifruit, was associated with increased plasma AOC in the postprandial state and consumption of an energy source of macronutrients containing no antioxidants was associated with a decline in plasma AOC. However, without further long term clinical studies, one cannot necessarily translate increases in plasma AOC into a potential for decreased risk of chronic degenerative disease.

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Literature Cited


Fig. 1. Plasma hydrophilic (A) and lipophilic (B) antioxidant capacity following a meal containing different fruits. For the plasma hydrophilic antioxidant capacity (A), the area under the plasma curve (AUC) was normalized for the amount of energy and antioxidant capacity (ORAC FL) consumed. Mixed grape and blueberry (w/meal) were consumed with a meal containing additional sources of carbohydrate, lipid and protein with a total energy content of 450 Kcal. Other treatments were consumed following blending with water. Adapted from: Prior et al. (2007).