Original Research

Plasma Antioxidant Capacity Changes Following a Meal as a Measure of the Ability of a Food to Alter *In Vivo* Antioxidant Status

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Key words: Oxygen radical absorbance capacity ($ORAC_{FL}$), blueberries, grapes, kiwifruit, strawberry, cherry, dried plums, antioxidant capacity

Objective: Determine 1) if consumption of a meal of different fruits or berries increases plasma hydrophilic (H-) or lipophilic (L-) antioxidant capacity (AOC) measured as Oxygen Radical Absorbance Capacity ($ORAC_{FL}$); 2) if including macronutrients in the meal alters postprandial changes in AOC; and 3) if preliminary recommendations can be developed for antioxidant intake.

Methods: Changes in plasma AOC 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 with 6–10 subjects per experiment. 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 AOC.

Results: Consumption of dried plums or dried plum juice did not alter either the H- or L-AOC area under the curve (AUC). Consumption of blueberry in 2 studies and of mixed grape powder [12.5 (Study #1), 39.9 (Study #4) and 8.6 (Study #5) mmole Trolox Equivalents (TE) AOC, respectively] increased hydrophilic AOC AUC. L-AOC increased following a meal of blueberry containing 12.5 mmole TE AOC (Study #1). Consumption of 280 g of cherries (4.5 mmol TE AOC) increased plasma L-AOC but not H-AOC. The AOC in the control groups in which additional macronutrients (Studies #4 and #5) were added decreased from the postprandial baseline AOC measurement.

Conclusion: We have demonstrated that 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 increased plasma AOC into a potential decreased risk of chronic degenerative disease. Preliminary estimates of antioxidant needs based upon energy intake were developed. Consumption of high antioxidant foods with each meal is recommended in order to prevent periods of postprandial oxidative stress.

INTRODUCTION

Fruits and vegetables contain numerous phytochemicals that have antioxidant capacity (AOC) [1,2]. However, AOC can vary by more than 50-fold in different foods [1]. 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 [3–8] and a

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Abbrevations: AAPH = 2,2'-azobis(2-amidino-propane) dihydrochloride, AOC = antioxidant capacity, AUC = area under the curve, $ORAC_{FL}$ = oxygen radical absorbance capacity, ROS = reactive oxygen species, TE = Trolox equivalents, DP = dried plum, DPJ = dried plum juice.

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recent study indicated that increased consumption of fruit during childhood was associated with a lower odds ratio for cancer as an adult [9]. 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. A recent study [4] suggested that dietary intake of antioxidants measured as total radical-trapping antioxidant potential (TRAP) was inversely associated with risk of cancer of both the cardia and distal stomach [4]. Increased dietary intake of selected classes of flavonoids (isoflavone, anthocyanidins, flavones and flavonols) was associated with a reduced risk of colorectal cancer [10].

However, the measurement of high AOC in foods may or may not be an 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. In the five studies to be reported, changes in plasma AOC, measured by the Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) assay, were used as an indicator of AOC. The studies were conducted at 4 different institutions involving different groups of investigators, so there are some differences in experimental design. Four of the 5 studies were a randomized crossover design with 6 or 7 subjects per study. In 2 studies (#2 and #3), a control treatment was not included because we did not observe any significant changes in plasma AOC during the period of sampling following the initial sample if only water was consumed. The objectives of the studies to be described were to address the question of whether changes in AOC following a meal can be used to assess the potential for a particular food component to alter *in vivo* antioxidant status and to provide estimates of dietary antioxidants necessary to prevent postprandial oxidative stress.

SUBJECTS AND METHODS

Chemicals and Apparatus

Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), fluorescein (Na salt) (FL) and ascorbic acid were obtained from Aldrich (Milwaukee, WI). 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Randomly Methylated β-Cyclodextrin (Trappsol®) (Pharm Grade) (RMCD) was obtained from Cyclodextrin Technologies Development Inc. (High Springs, FL). Other solvents were purchased from Fisher (Fair Lawn, New Jersey). Plasma $ORAC_{FL}$ was measured as described previously [11]. Fluorescent measurements for $ORAC_{FL}$ assay were obtained from one of two microplate readers (FLUOstar Galaxy or Optima, BMG Labtechnologies, Duraham, NC).

Subjects and Study Design

Human subjects were recruited for five studies which were conducted in different institutes with collaborators. Written informed consent was obtained from each study participant according to the appropriate human studies review committee. All study participants were considered in good health based upon a medical history questionnaire, physical examination and normal results of clinical laboratory tests. All of the subjects fulfilled the following eligibility criteria: 1) no history of cardiovascular, hepatic, gastrointestinal, or renal disease; 2) no alcoholism; 3) no antibiotic or supplemental vitamin and/or mineral use at least 4 weeks prior to the start of the study; and 4) no smoking. Candidates were excluded if they were in poor health, obese, regularly used nutritional supplements, medications, alcohol or recreational drugs. All studies except Study #2 were a randomized crossover design.

Study #1 (Blueberry, Dried Plum)

Six healthy women (65–70 y, 66.2 $\pm$ 1.1 kg) were recruited to participate in this study. The study protocol was approved by the Human Investigation Review Committee of Tufts University and the New England Medical Center. Subjects were admitted to the Metabolic Research Unit at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts 24 h before the day of sampling. In the evening before the day of sampling, subjects were fasted overnight. In the morning of the sampling day, an intravenous catheter was inserted into one forearm. A 15 mL heparin blood sample (zero baseline sample) was obtained from each fasting subject, following which the subjects were given one of five dietary treatments: 1) Control, 315 mL water; 2) 315 mL of dried plum juice (DPJ); or 3) dried plums (DP) (131 g blended in 315 mL water); 4) 94.5 g frozen wild blueberries (low dose) blended in 315 mL water, or 5) 189 g wild blueberries (high dose) blended in 315 mL water. Blueberries were provided by the Wild Blueberry Association of North America and were obtained from a composite batch of blueberries representing the major clones of wild blueberries found in the northeast U.S. in one production year. DP and DPJ were provided by the California Dried Plum Board. The particular treatments were selected because chlorogenic acid or its isomers were major phytochemicals in blueberries, DP and DPJ; however, only blueberries contained anthocyanins which are a major contributor to the AOC of blueberries. The nutrient and selected phytochemical intake is presented in Table 1. The experiment was a randomized crossover design with an interval of at least two-weeks between each treatment. Following the zero time blood sample, additional blood samples (15 mL) were

Table 1. Composition and Amounts Consumed in Meals Containing Blueberries (BB), Dried Plums (DP), Dried Plum Juice (DPJ), Cherry, Kiwifruit, Strawberry (SB), and Red Grape

Component	Study #1			Study #2		Study #3	
	BB	DP	DPJ	Cherry	Kiwifruit	SB	R.Grape
Nutrient Composition[1]							
Kcal,/100g	57	240	71	32	61	32	69
Total Carbohydrate, g/100 g	14.5	63.9	17.5	7.7	14.7	7.7	18.1
Fat, g/100g	0.33	0.38	0.03	0.30	0.52	0.30	0.16
Protein, g/100 g	0.74	2.18	0.61	0.40	1.14	0.67	0.72
Amount Consumed, g	189	131	315	280	300	300	280
Energy, kcal	108	314	224	90	183	96	193
Total Carbohydrate, g	27.4	83.7	55	21.6	44.1	23	50.7
Fat, g	0.6	0.5	0.1	0.1	1.6	0.9	0.4
Protein, g	1.4	2.9	1.9	1.1	3.4	2.0	2.0
Chlorogenic acid, μmol	1042	37	71	ND[3]	ND[3]	ND[3]	ND[3]
3-*O*-Caffeoylquinic acid, μmol	0	340	493	ND[3]	ND[3]	ND[3]	ND[3]
4-*O*-Caffeoylquinic acid, μmol	0	190	339	ND[3]	ND[3]	ND[3]	ND[3]
Anthocyanins, mg	690	0	0	342[4]	0[4]	63[4]	75[4]
Proanthocyanidins, mg	657	0	0	23.0[5]	11.1[5]	435[5]	171[5]
Flavan-3-ols, mg	97.7[1]	0[1]	0[6]	24.3[1]	1.1[1]	13.5[1]	6.4[1]
Flavanones, mg	0[1]	0[1]	0[6]	0[1]	0[1]	0.8[1]	0[1]
Flavones, mg	0.4[1]	0.01	0[6]	0[1]	3.4[1]	0[1]	3.6[1]
Flavonols, mg	18.0[1]	2.4[1]	ND[3]	7.7[1]	0[1]	4.8[1]	3.9[1]
Total Phenolics, mg gallic acid equiv.	1530	1835	1102	456	ND[3]	ND[3]	ND[3]
$ORAC_{FL}$, mmol TE	12.5	10.8	6.7	4.5	12.5	1.7	4.2

[1] Food composition obtained from USDA National Nutrient Database [29].

[2] Low dose of blueberries was 50% (94.5 g) of the high dose which is shown in the table.

[3] ND = not determined.

[4] Data from Wu et al. [38].

[5] Data from Gu et al., [16].

[6] Estimated based upon content in dried plums.

collected at 1, 2, and 4 h after consumption of the treatment. The consumption of water was not limited during the collection period. Other foods or beverages were not allowed.

Study #2 (Cherries)

Ten healthy women (BMI $<$ 30 kg/m^2, 29.9 $\pm$ 6.1 y) were recruited and accepted into study #2. The study was approved by the Human Subjects Review Committee of the University of California, Davis. The clinical portion of this study was conducted at the USDA Western Human Nutrition Research Center, University of California, Davis, CA in May, during California's fresh cherry season.

To partially standardize and limit intake of antioxidants prior to the experimental cherry dose, subjects were asked to refrain from consuming fruits and vegetables or their juices, tea, or wine for two days prior to the cherry dose. Fresh sweet Bing cherries (ORAC = 16.1 μmol TE/g, fresh weight (FW)) were obtained from O.G. Packing, Stockton CA and were stored at 4 °C until fed. The subjects were fed 280 g of depitted cherries (about 45 cherries) after an overnight fast (See Table 1 for nutrient composition) and were required to consume all of the cherries within 10 minutes. Blood and urine samples were taken prior to the dose, and at 1.5, 3, and 5 h postdose. Subjects were allowed to leave the clinical unit after the 1.5 and 3 h post-dose blood draws but were required to return within ten minutes of the next scheduled blood draw, and avoid consumption of any food or drink except from an 8 oz bottle of water given after the 1.5 h draw. The subjects were scheduled over 6 days and a 70 g portion of the fed cherries was taken at each feeding day and frozen at −70 °C until analysis for antioxidant and polyphenol content.

Study #3 (Grapes, Kiwifruit, Strawberries)

Seven healthy women (BMI $<$ 30 kg/m^2, 18–40 y) were recruited and accepted for study #3. Subjects were expected to refrain from fruits, vegetables, juice, tea and wine consumption for two days prior to the dosing visit. Dietary treatments in this study included two servings of either red Crimson Seedless' grapes (280 g), Hayward' kiwifruit (300 g) or Seascape' strawberries (300 g) (See Table 1 for nutrient composition and intake). The Crimson Seedless' red grapes, Seascape' strawberries, and Hayward' kiwifruits were purchased from commercial shippers in Fresno, Watsonville, and Marysville, California, respectively, transported to Davis within 3 hours in an air-conditioned car and stored at 0 C and 90–95% relative humidity until used in the study.

These were fed one week apart to the study participants in a randomized crossover design. Blood samples were taken after

an overnight fast before fruit ingestion and at 1.5, 3.0 and 4.5 h after fruit ingestion. Calculated nutrient intake is presented in Table 1. AOC ($ORAC_{FL}$) was 15.0, 41.8 and 5.8 μmol TE/g FW for grapes, kiwifruit and strawberries respectively. This provided for an AOC intake of 4.2, 12.5 and 1.7 mmoles TE for red grapes, kiwifruit, and strawberries, respectively (Table 1).

Study #4 (Blueberries)

Six women (43.8 ± 3.8 y, 80.9 ± 16.2 kg) were recruited and participated in this experiment. This study protocol was approved the by University of Maine Human Studies Review Committee. The experiment used a randomized crossover design. Three of the participants consumed 1 cup of blueberries daily as part of their regular meals for a period of 14 days. On the last day, 3 control subjects and 3 treatment subjects that had consumed blueberries came to the Eastern Maine Regional Medical Center for blood sampling. In the morning of the sampling day, an intravenous catheter was inserted into one forearm. A 15 mL heparin blood sample (zero baseline sample) was obtained from each fasting subject, following which the subjects were given either the control drink or a blueberry drink made from a powder of freeze dried whole wild blueberries. The composition and nutrients provided by the breakfast meals are shown in Table 2. Blood samples were obtained before and at 1, 2 and 4 h after the meal. Following a 2 week wash out period, subjects were then switched to the opposite treatment and those subjects who were controls during the previous period consumed 1 cup of blueberries for the following 14 days after which all subjects returned to the Medical Center for blood sampling. The freeze dried blueberry powder (1.12 g/kg BW) provided 1424 mg anthocyanins and 39.9 mmol TE of $ORAC_{FL}$ (Table 2). Wild blueberries were provided by the Wild Blueberry Association of North America.

Study #5 (Grapes)

Six women (46.3 ± 5.6 y, 63.5 ± 9.9 kg) were recruited and accepted to participate in this experiment. This study protocol was approved by the University of Arkansas Medical Sciences Human Studies Review Committee.

On the evening before the day of sampling, subjects were fasted overnight. In the morning of the sampling day, an intravenous catheter was inserted into one forearm. A 15 mL heparin blood sample (zero baseline) was obtained from each fasting subject, following which the subjects were given one of two dietary treatments (Table 2): 1) Control meal (C) or 2) control meal plus freeze dried grape powder. Treatments were given on 2 occasions separated by 2 weeks in a random crossover design. The grape powder was prepared from a mixture of table grapes by the CA Grape Commission. The nutrients provided by the breakfast meals are shown in Table 2. The freeze dried grape powder provided 53 mg anthocyanins and

Table 2. Composition of Breakfast Meal and Nutrient Intake in 2 Clinical Studies

Item	Study #4		Study #5	
	Control	Blueberry Powder	Control	Grape Powder
Ingredient				
Coconut milk, %	3.66	1.15	1.60	1.60
ProMod Powder[1], %	2.56	0.80	1.94	1.12
Polycose, %	7.30	0.00	12.96	0.00
Cream, Coffee creamer, %	12.18	3.83	5.76	5.34
Sugar, %	3.66	0.00	6.92	0.00
Water, %	70.64	73.95	70.80	70.74
Grape or Blueberry Powder, %	0.00	20.28	0.00	21.20
Nutrient Composition[2]				
Kcal,/100 g	103.7	103.5	117.3	117.5
Total Carbohydrate, g/100 g	20.5	20.5	22.3	22.3
Fat, g/100g	1.2	1.2	1.7	1.7
Protein, g/100 g	2.4	2.4	2.0	2.0
Amount Consumed				
Kcal	483.5	471.9	423.8	411.6
Total Carbohydrate, g	93.5	95.6	80.5	78.0
Fat, g	5.4	5.3	6.1	6.0
Protein, g	11.1	10.8	7.2	7.0
Grape or blueberry powder, g	-	92.5 ± 7.3	-	74.2 ± 13.9
Anthocyanins, mg	-	1424	-	53
Phenolics, mg	-	5046	-	1312
Flavans (catechin), mg	-	-	-	78
Resveratrol, mg	-	-	-	1.4
$ORAC_{FL}$, mmol TE	-	39.9	-	8.6

[1] ProMod protein supplement powder, Ross Nutrition, Abbott Laboratorios.

[2] Composition from USDA National Nutrient Database [29].

8.6 mmol TE of $ORAC_{FL}$ (Table 2). Blood samples were obtained before and at 1, 2 and 4 h after the meal.

Sample Preparation and Antioxidant Assay

Blood was collected into evacuated tubes with heparin as an anticoagulant. The blood was immediately centrifuged at 4°C to separate red cells and aliquots of the plasma were frozen at −70 °C for later analysis. AOC was determined in the foods and blood plasma using the hydrophilic and lipophilic $ORAC_{FL}$ method [11,12]. All antioxidant capacity data were calculated based upon Trolox as a standard and expressed as Trolox Equivalents (TE).

Analysis of Phytochemicals and Nutrients

Analysis of phytochemicals in the foods were conducted using an HP 1100 HPLC (Hewlett-Packard, Palo Alto, CA) coupled with a diode array detector, florescent detector and Esquire 3000 Ion Trap Mass Spectrometer (MS) (Bruker Daltonics, Billerica, MA). Concentrations of anthocyanins were measured in blueberries as described by Wu et al. [13,14] and proanthocyanins as described by Gu et al [15,16]. Chlorogenic acid and its isomers were separated and quantitated using HPLC conditions similar to that for anthocyanins except that the wavelength monitored was 316 nm [17]. Total phenolics were measured by the Folin-Ciocalteu assay based upon modification of the method of Singleton and Rossi [18]. Content of other nutrients was based upon published data, information provided by the manufacturer, or from the USDA Nutrient or Flavonoid Database [29].

Data Analysis

The net area under the plasma curve (AUC) for AOC was calculated, using mathematical functions in SigmaPlot (Systat Software Inc., Richmond, CA), based upon the change in plasma AOC from the zero baseline reading. Statistical differences were determined using paired t-tests.

RESULTS

The intake of the nutrients, major phytochemicals and antioxidant capacity of the dietary components in five studies are presented in Tables 1 and 2. In terms of well-known dietary antioxidants, chlorogenic acid and its isomers predominate in DP and DPJ. Detailed analyses of the phytochemical content of DP have been reported previously [17,19]. Chlorogenic acid was also a major component in blueberries, along with anthocyanins and proanthocyanidins [13,14,16]. Sweet cherry also contains anthocyanins and proanthocyanidins as major antioxidants [13,14,16]. Phenolic compounds including anthocyanins and other polyphenols were major antioxidants in grape. Anthocyanins, proanthocyanidins, and other flavan-3-ols are present in significant quantities in strawberry and red grape (Table 1). Flavonoids are noticeably absent in kiwifruit except for some proanthocyanidins and flavan-3-ols.

Table 3. Net Area under the Plasma Curve (AUC) of the Change in Plasma Hydrophilic or Lipophilic $ORAC_{FL}$ during a period of 4– 5 h Following a Meal in Human Subjects from 5 Different Clinical Studies

Study No.	Treatments	$ORAC_{FL}$ Intake, mmoles	Energy Consumed, Kcal	Hydrophilic ORAC AUC[1]	Total AUC[2]	AUC/dose[3]	Lipophilic ORAC AUC[1]
#1	Control	0	0	−11 ± 100	0	-	88 ± 102
	Low BB	6.3	54	−25 ± 102	28	4.5	−20 ± 54
	High BB	12.5	108	278 ± 48**	385	31	355 ± 183**
	Dried Plum (DP)	10.8	314	56 ± 103	367	34	−52 ± 17
	Dried Plum Juice (DPJ)	6.7	224	86 ± 155	308	46	105 ± 89
#2	Cherry	9.2	90	−258 ± 171†	−169	−18	587 ± 115‡
#3	Kiwifruit	12.5	183	484 ± 121‡	665	53	−18 ± 46
	Strawberry	1.7	96	110 ± 70	205	121	10 ± 85
	Red Grapes	4.2	193	5 ± 159	196	47	−5 ± 54
#4	Control	0	484	−451 ± 247	-	-	ND[4]
	Blueberry	39.9	467	171 ± 177*	633	16	ND[4]
#5	Control	0	424	−447 ± 92	-	-	ND[4]
	Mixed Grape	8.6	407	229 ± 100**	632	73	ND[4]

[1] AUC expressed as μmol TE/L · h

[2] Total adjusted AUC observed assuming that 1 Kcal energy intake produces a decrease in plasma AUC of 0.99 μmol TE/L · h. Assumes consumed antioxidants overcame negative effects of consumed energy plus producing the net AUC measured as hydrophilic ORAC.

[3] Area under curve per dose defined as μmol TE/L · h per mmol TE of antioxidant capacity consumed ($ORAC_{FL}$ Intake, mmoles).

[4] Not determined.

* Significantly different from control ($p < 0.09$) using paired t test.

** Significantly different from control ($p < 0.05$) using paired t test.

† Difference from zero approached statistical significance ($p < 0.10$).

‡ Significantly different from zero ($p < 0.05$).

In study #1, both hydrophilic and lipophilic AOC increased significantly following the high dose of blueberries (Table 3, Fig. 1) [11,12]. Plasma hydrophilic AOC increased ($p < 0.05$, Paired t test) at 1 h after the meal and then declined to below the baseline by 4 h after the meal. Lipophilic $ORAC_{FL}$ increased at 2 h ($p < 0.05$, Paired t test) and remained above baseline at 4 h after the meal. The time at which the maximal increase in lipophilic $ORAC_{FL}$ was observed varied by individual; 2 individuals reached the highest level at each of the times of 1, 2 and 4 h after the meal.

Plasma AOC (hydrophilic and lipophilic $ORAC_{FL}$) did not change after a meal containing DP (Table 3, study #1), DPJ (Fig. 1) or the low dose of blueberries (Table 3, Study #1) compared to levels in control subjects that consumed only 315 mL of water at zero time. AOC consumed from the high dose of blueberries was nearly twice that of the DPJ and 15% higher than that from DP. Chlorogenic acid or isomers predominate in DP. However, it appears that very little chlorogenic acid or its isomers were absorbed. We were unable to detect any of these compounds or potential metabolites in plasma or urine (data not presented) following the meal in which the intakes were in the range of 500–1000 μmoles of chlorogenic acid or its isomers (Table 1).

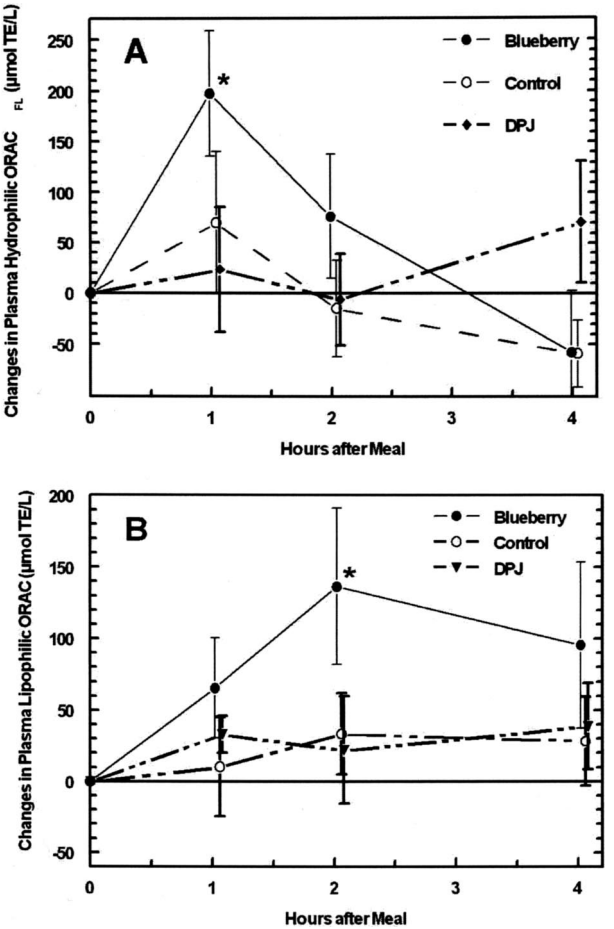

Fig. 1. Changes in hydrophilic (A) and lipophilic (B) $ORAC_{FL}$ following a control meal or one containing blueberries (189 g frozen) or dried plum juice (DPJ) (315 mL) (Study #1) (*$p < 0.05$ using paired t test). Data for blueberry treatment from (11). See Table 3 for net AUC for each treatment.

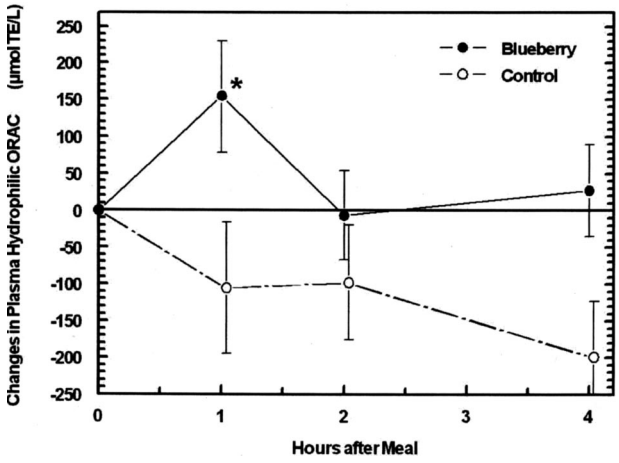

Fig. 2. Changes in hydrophilic $ORAC_{FL}$ following a control meal or one containing blueberries (92.5 g freeze dried powder) ((Study #4) (*$p < 0.05$ using paired t test). See Table 3 for net AUC for each treatment.

Anthocyanins, the other major class of antioxidant compounds in blueberries, could not be detected in plasma following the high blueberry meal [20] and urinary excretion of the anthocyanins was quite low [20]. Lowbush blueberry contains a high concentration of total anthocyanins, but distributed among some 27 different anthocyanins [14] with no particular anthocyanin predominating; delphinidin galactoside and glucoside are highest in concentration [14]. Total anthocyanin intake in the meal in study #4 was calculated to be 1.4 g which along with other phenolics provided 39.9 mmol TE AOC from the blueberry powder. A significant increase in plasma AOC AUC was observed (Table 3, Fig. 2). In study #1, consumption of 12.5 mmoles TE of AOC from blueberries produced an increase in the hydrophilic AOC AUC (Table 3, Fig. 1) but a dose of 6.3 mmole TE from blueberry (Study #1) did not produce a significant change in plasma AOC (Table 3). The relationship between AOC intake from blueberries and Total AUC (see Table 3) (Studies #1 and #4) was defined by a logarithmic relationship as follows:

$$Y = -499 + 315\ ln\ X,\ r_{xy} = 0.97, \text{ where}$$

$$Y = \text{Adjusted Total AUC (umol TE/L} \cdot \text{h)};$$

$$X = \text{Antioxidant Capacity consumed (mmoles TE)}$$

This relationship indicates that at least for blueberries that the plasma response increases with AOC consumed but at a diminishing rate.

In studies #2 and #3, Bing sweet cherries (280 g), red grapes (280 g), kiwifruit (300 g), or strawberries (300 g) were given to

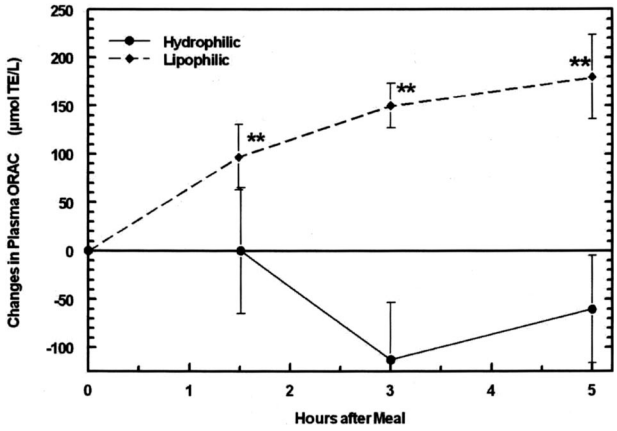

Fig. 3. Changes in hydrophilic and lipophilic $ORAC_{FL}$ following a meal of cherries (Study #2) (**$p < 0.01$ using paired t test). See Table 3 for net AUC for each treatment.

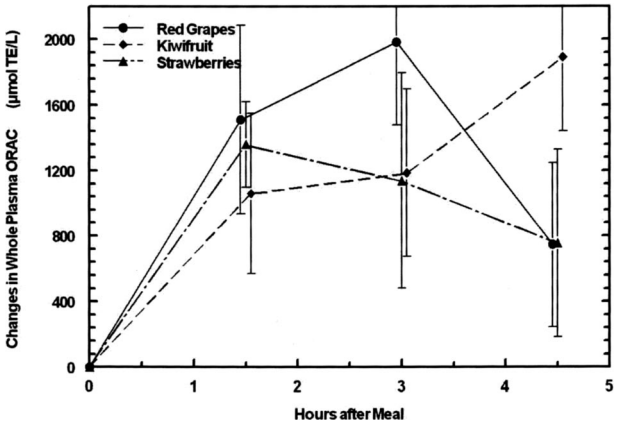

Fig. 4. Changes in whole plasma $ORAC_{FL}$ following meal of red grapes, kiwifruit or strawberries (Study #3). See Table 4 for the net change in AUC. Increases in AUC were significant for all treatments ($p < 0.05$).

subjects in a single meal without any added macronutrients. The key phytochemicals in the Bing sweet cherries have been documented previously [21] (Table 1). Hydroxycinnamates comprised the largest class of phenolics, representing some 42% of the total phenolics of 163 mg/100 g [21]. The next largest fraction of phenolics was anthocyanins at 23%. In these two studies, no blood samples were obtained as control' in which no fruit was consumed. Based upon results from study #1, the assumption was made that plasma AOC would not change in the 5 h during which blood sampling occurred in the absence of any food consumption.

Following cherry consumption, there was a significant increase in the lipophilic AOC AUC (Table 3) which was represented by a consistent rise in plasma lipophilic AOC (Fig. 3). Following consumption of cherries, hydrophilic AOC decreased at 2 h (Fig. 3) and remained below the zero time baseline at 4.5 h giving a net negative AUC which approached significance ($p < 0.10$) (Table 3).

In Study #3, kiwifruit was the only fruit that produced a significant ($p < 0.05$) increase in hydrophilic AOC AUC (Table 3). Neither kiwifruit, strawberry or red grape produced a significant change in lipophilic AOC AUC. The mixed grape meal in Study #5 produced an increase in the hydrophilic AOC AUC (Table 3, Fig. 6). The AOC intake from the mixed grape (8.6 mmoles TE) was lower than with blueberries (12.5 and 39.9 mmoles TE) in studies #1 and #4 that also increased plasma AOC. With grapes, it is less clear what phytochemicals might be effective in increasing plasma AOC. Grapes contain low levels of anthocyanins, but contain resveratrol which blueberry does not have (Table 1).

DISCUSSION

Sample Preparation and Plasma AOC

The usual method for assaying hydrophilic or lipophilic AOC involves extraction and removal of the protein. In study #3, $ORAC_{FL}$ of the whole plasma was also measured following the meal. When the whole plasma was assayed without extraction, a significant increase in AOC following the meal was observed with all three fruits (kiwifruit, strawberry and red grape, Fig. 4). Correspondingly, there was a significant increase in the AOC AUC (Table 4) which amounted to a 7–9.5% increase over baseline. For studies of this nature, we have assumed that it is the small molecular hydrophilic components in the plasma that are absorbed that would lead to an increase in plasma AOC following a meal. The data with whole plasma suggests that it might not be that simple, i.e. these compounds may interact with protein and perhaps not be completely extracted during the extraction process such that not all of the hydrophilic AOC is being measured following extraction. Another possible reason may be that the phytochemicals may cause an increase in the concentration of antioxidant proteins or other large molecular antioxidants. The increase in the AUC above baseline for deproteinized hydrophilic $ORAC_{FL}$ was 12.6% for kiwifruit. Even though the AOC response was smaller with whole plasma (8.4%), it was more consistent between individual subjects than with the deproteinized AOC. Further studies are needed to confirm whether indeed use of the

Table 4. Change in Net AUC of Whole Plasma $ORAC_{FL}$ Following a Meal of Kiwifruit, Strawberries or Red Grapes (Study #3)

Treatment	$ORAC_{FL}$ Intake, mmoles	Whole Plasma $ORAC_{FL}$ Area Under Curve (AUC[1])	Increase over Baseline, %
Kiwifruit	12.5	4787 ± 1738**	8.4
Strawberry	1.7	4314 ± 1153**	7.0
Red Grapes	4.2	5796 ± 1619**	9.5

[1] Expressed as µmol TE/L · h

** Significantly different from zero baseline ($p < 0.05$)

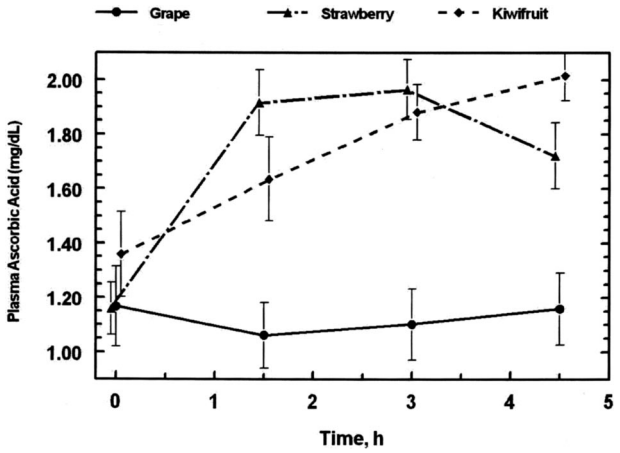

Fig. 5. Changes in plasma Vitamin C following meal of red grapes, kiwifruit or strawberries (Study #3). The change over time for strawberry and kiwifruit was significant ($p < 0.05$).

whole plasma may provide a better measure of changes in plasma AOC.

The reasons for the lack of response of red grape in study #3 compared to the relatively large response resulting from the mixed grape treatment in study #5 is not readily apparent, other than the total AOC consumed was 8.6 mmol TE in study #5 compared to 4.2 mmol TE in study #3.

The limited data available on the phytochemical composition of kiwifruit indicates that the flavonoid content is relatively low (Table 1), however, it is known to contain β-carotene, vitamin E and considerable vitamin C. Plasma ascorbate increased significantly in the 4.5 h after consumption of both kiwifruit and strawberry (Fig. 5). Collins and coworkers [22] demonstrated a significant AOC of kiwifruit *ex vivo* and *in vitro,* not attributable entirely to the vitamin C of the fruit. Consumption of kiwifruit has also been shown to increase the resistance of DNA to oxidative damage induced by H_2O_2 in isolated lymphocytes *ex vivo,* in comparison with lymphocytes collected after a control drink of water [22].

Mechanisms of Plasma AOC Increase

Indirect Effects. In addition to the antioxidant polyphenolics in food, both vitamin C and uric acid have been shown to contribute significantly to the AOC measured in plasma [23,24]. The increase in plasma AOC in humans after apple consumption in a study by Lotito and Frei [24] was shown to be due mainly to the well-known effect of fructose on urate production, not apple-derived antioxidant flavonoids [24]. Uric acid was not measured in these studies, so we do not know whether changes in plasma uric acid might account for any of the observed changes in AOC. Fructose has been known to increase plasma urate levels due to rapid fructokinase-mediated metabolism to fructose 1-phosphate [25–28] leading to a decrease in hepatic ATP and inorganic phosphate and thus AMP degradation to urate. However, DP (~12.5 g/100g) and red grapes (~8 g/100g) have higher fructose levels than apples (~6 g/100g) [29], but the amount of fructose that would have been consumed from DP (16.4 g) and red grapes (22.4 g) in this study was only 11–35% of that consumed from apples (63.9 g) in the study of Lotito and Frei [24]. Thus, one would not expect much of an elevation in urate. Small increases in urate would not likely account for all of the increase in plasma AOC [23]. In a previously published study [23], consumption of 1) strawberries (240 g), 2) ascorbic acid (1.25 g), 3) spinach (294 g) or 4) 300 mL of red wine phenolics (RWP) produced a significant increase in the AOC from baseline (Time zero) of a perchloric acid extract of serum using the ORAC assay. The AUC (μmol TE/L · h) was 187 ± 86, 252 ± 52, 366 ± 154, and 183 ± 57 for strawberries, ascorbic acid, spinach and RWP, respectively, compared to a control meal (23 ± 103). Significant increases in plasma urate concentrations were observed following strawberry and spinach consumption, but the magnitude of the changes were not calculated to be sufficient to account for the changes in plasma AOC.

Direct Effects. The increase in plasma vitamin C following strawberry and kiwifruit consumption (Fig. 5) indicates at least a part of the increase in AOC may be accounted for by vitamin C. Low apparent absorption of phytochemicals such as anthocyanins and chlorogenic acid and/or metabolism to compounds which may have lower AOC may account for the limited *in vivo* antioxidant response from foods containing these phytochemicals. In rats given 250 μmol/day, the recovery of chlorogenic acid in urine was low (0.8%, mol/mol), and the total urinary excretion of caffeic acid liberated by hydrolysis of chlorogenic acid accounted for <0.5% (mol/mol) of the dose ingested [30]. Microbial metabolites accounted for 57.4% (mol/mol) of the chlorogenic acid intake indicating that the bioavailability of chlorogenic acid depended largely on its metabolism by the gut microflora [30]. Metabolites of dietary phenolics may have a lower AOC than their parent compounds [31]; therefore, the contribution of dietary phenolics to antioxidant activity *in vivo* might be lower than expected from *in vitro* tests. Chlorogenic acid is also found in coffee and black tea [32]. Recently, intake of coffee containing 108 to 1341 mg (305–3780 μmoles) of chlorogenic acid was shown to increase the ferric-reducing ability of plasma (FRAP) in healthy women but these levels of intake are quite high [33].

Although changes in AOC were observed following the blueberry meals in studies #1 and #4, the AOC intake required was quite high. Mazza et al [34] gave a blueberry powder containing 1.20 g of total anthocyanins (42% of the total phenolics) which appeared to prevent a mean decrease in serum AOC as experienced in the control group following the consumption of a high-fat meal (as determined by TEAC assay) in human subjects. Nineteen of the 25 anthocyanins present in the blueberries were detected in human blood serum [34]. However, because of either low apparent absorption or rapid disappearance of anthocyanins from the blood, it appears that measurable changes in plasma hydrophilic AOC may not be

observed unless 100–200 g (0.6–1.2 cups) of blueberries are consumed.

The increase in plasma lipophilic AOC observed with blueberry in study #1 (Table 3, Fig. 1) [34] and with cherries in study #2 is somewhat surprising given that berries and fruits are generally low in lipophilic antioxidants [1]. The phytochemicals in cherries and blueberries that might account for this observation are not known. The response seems to be more sustained following consumption of cherries compared with blueberries since by 4 h after the meal with blueberries the response had started to decline, but was still increasing slightly at 5 h after cherry consumption.

Recommendations for Dietary AOC Intake

Because subject to subject response can be quite variable and the relative response generally is small, calculation of the area under the plasma AOC curve provides a convenient means of evaluating treatment differences and smoothing some of the effects of variability at individual sampling time points. One of the advantages of this experimental approach is that one can assess the net effects of the digestion/absorption process on antioxidant components that appear in the peripheral circulation.

Plasma AOC decreased in the two studies where the control drink mix that was consumed contained additional macronutrients (Table 3, Studies #4 and 5), but no decrease was observed if only water was consumed (Table 3, Study #1). The decline in plasma AOC 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 [35]. 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 [36]. Oxidative stress occurs when excess reactive oxygen species (ROS) overwhelm the endogenous antioxidant systems. A decline in plasma AOC may indicate that the antioxidant defense systems have not been able to keep up with the oxidative challenge. In two control groups of subjects (Figs. 2 and 6), the net change in AUC following a meal containing just the macronutrients (484 and 424 kcal) was -451 ± 247 and -447 ± 92 for an average decrease in plasma antioxidant capacity of 0.99 units of AOC AUC (unit = μmol TE/L · h) per kcal of energy intake in the absence of any dietary antioxidants. This argues strongly for the need to include high antioxidant foods in each and every meal in order to prevent this redox imbalance.

In Table 3, the hydrophilic ORAC AUC was adjusted based upon the observed decrease in AOC AUC with energy intake.

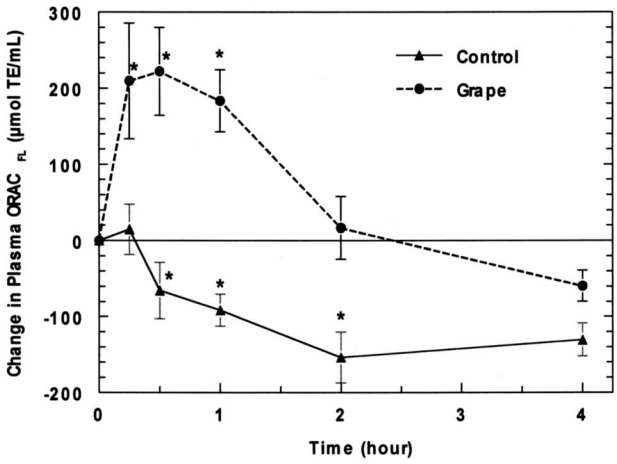

Fig. 6. Changes in hydrophilic $ORAC_{FL}$ following a control meal or one containing freeze dried grape powder (74.2 g) (Study #5). (*$p < 0.05$ using paired t test). See Table 3 for net AUC for each treatment.

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 ORAC intake to obtain the AUC/dose. Based upon the response observed from 8 fruits in this study and 3 foods in a previous study [23], an average of the AUC/dose was calculated (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 [23].

For a meal of 700 kcal, antioxidants would be needed to overcome a projected decline in AOC AUC of 693 units. Assuming foods provide an average of 54 μmol TE/L · h per mmol TE of AOC in protection, 12.2 mmol TE of dietary AOC would be needed to prevent a projected transient postprandial period of oxidative stress. However, a more reasonable assumption is that the free radical and oxidative stress results primarily from that energy that is inefficiently utilized in the mitochondria, which has been estimated to be ~25% [37] of the overall energy from carbohydrate which is lost and not converted to ATP [37]. If this is the case, then 3.2 mmol TE of AOC or about 1.5 servings of antioxidant containing fruits and/or vegetables would be required in order to prevent a postprandial oxidative stress situation. The average serving of fruits and/or vegetables has been calculated to provide approximately 2.2 mmol TE per serving [1]. If we assume that there is a linear relationship between energy intake and the need for dietary antioxidants, one can estimate dietary antioxidant needs based upon dietary energy intake (Fig. 7). For an individual consuming 2500 kcal per day, AOC needs are estimated to be 11.5 mmol TE. Projected intakes of 5–15 mmol TE per day of antioxidant capacity as $ORAC_{FL}$ are certainly within the realm of achieving with selection of appropriate high antioxidant foods (Table 5); however, the intake range can be quite large based upon the 2 examples provided in Table 5, which are both

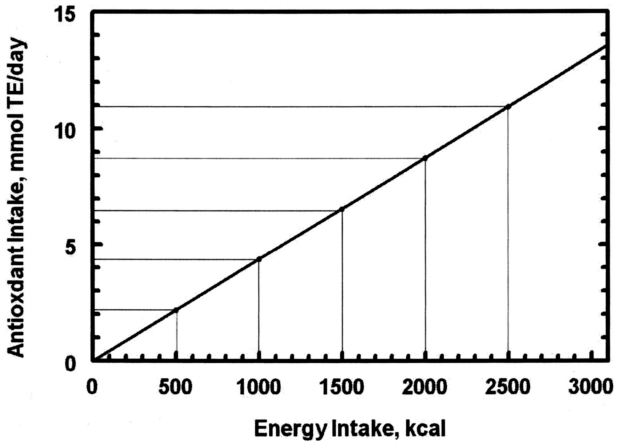

Fig. 7. Estimated antioxidant intake required (mmol/day) to prevent postprandial oxidative stress relative to energy intake (kcal). Relationship calculated as follows: Y = ((EI * DAUC)/MAUC) * IENU = ((EI*0.99/57)*0.25 = EI * 0.00461 where: Y = Antioxidant intake (mmol TE/day based upon $ORAC_{FL}$); EI = Energy Intake, kcal; MAUC = Mean Area Under Curve (μmol TE/L · h) per dose of fruit or berry in mmol Trolox Equivalents (TE) for 13 different foods tested in 6 clinical trials; DAUC = Decline in plasma AUC per kcal energy consumed in the absence of dietary antioxidants (0.99 μmol TE/L · h); and IENU = Inefficiency in utilization of dietary energy (Assumed to be 25%) [37].

Table 5. Examples of AOC Intake from 9 Servings of Cereal, Fruits, and Vegetables Selected from Foods with Relatively High and Relatively Low AOC[1]

Food	Serving Size	High ORAC Intake, μmol TE	Low ORAC Intake, μmol TE
Oat bran	2 servings	2120	-
Strawberry	1 Cup	5938	-
Orange	1 Fruit	2540	-
Plum	1 Fruit	4118	-
Spinach	12 Cup	1056	-
Broccoli	12 Cup	2621	-
Lettuce, Red leaf	4 leaves, 68 g	1213	-
Beets	12 Cup	1886	-
Corn flakes	1.5 Cup	-	708
Honeydew Melon	1 Cup, 170 g	-	410
Peaches, canned	12 Fruit	-	411
Watermelon	1 Cup, 152 g	-	216
Cucumber	12 Cup	-	74
Lettuce, iceberg	4 leaves	-	144
Carrot, cooked	46 g	-	171
Peas, canned	12 Cup	-	326
TOTALS, μmol TE		21,492	2,460

[1] Data selected from Wu et al. [1]

calculated on the basis of 9 servings of cereals, fruits and vegetables. These estimations of the AOC needs would not consider the added amounts needed if other oxidant stressors were present such as dietary pro-oxidants, disease situations, cigarette smoke, drugs, etc. Estimates of "normal" antioxidant intake, based upon USDA's Continuing Survey of Food Intake by Individuals (1994-1996), was 5.6 mmol TE [1]. More recently we have calculated intake from food consumption data of NHANES 2001-02 and arrived at a value of 4.7 mmol TE (unpublished data).

Limitations to the Concept of Determining Recommended Levels of AOC Intake

The approach presented 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. Based upon the data available, it seems clear that increases in plasma AOC are not directly proportional to the *in vitro* AOC. Other methods of analysis of plasma AOC would not likely alter this conclusion, since we have observed a similar pattern of change following a meal using other methods of analysis (FRAP, TEAC) [23]. Thus, any recommendation cannot be based solely upon *in vitro* analysis of food $ORAC_{FL}$. 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.

In the approach presented, we have assumed that the decline in AOC is linear with energy intake, however, at this point, 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 calorie sources (fat, vs carbohydrate vs 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, likely will not demonstrate as large of a further decrease postprandially as someone in the "normal" range, but may be more responsive to an antioxidant meal.

Although a number of assumptions are inherent in these calculations, 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.

CONCLUSIONS

Results from these 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. We have demonstrated that 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 decreased risk of chronic degenerative disease.

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