Antioxidant Capacity and Polyphenolic Components of Teas: Implications for Altering *In Vivo* Antioxidant Status[1] (44376)

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Abstract. **The Oxygen Radical Absorbance Capacity (ORAC) assay was used to determine the total antioxidant capacity of tea. Green and black teas (n = 18) had a mean antioxidant capacity of 761.1 ± 85.3 μmol Trolox Equivalents (TE) per g dry matter. However, their antioxidant capacity varied from 235 μmol to over 1526 μmol Trolox equivalents (TE)/g dry matter, and total phenolics ranged from 32 to 147 mg/g in different commercial teas. One tea phenolics extract had an antioxidant capacity of 4796 μmol TE/g dry matter and 625 mg total phenolics/g. On a dry matter basis, an antioxidant capacity of 761 μmol TE/g is considerably higher than any of the other fruits and vegetables measured in our laboratory. However, since dry tea is not consumed directly, brewing conditions may influence the final antioxidant capacity in the tea as consumed. We tested both green and black teas by placing one tea bag (1.95 g) in 150 ml (5 oz.) of boiling water. In the first brewed cup, approximately 84% of the total antioxidant activity was solubilized within the first 5 min of brewing. An additional 13% of the antioxidant activity was extracted into the second glass of 150 ml with an additional 5 min of brewing. At the dilutions obtained after the first brewing, the tea as consumed would contain approximately 8.31 μmol TE per ml. This total antioxidant capacity compares to other drinks from fruits and vegetables that had antioxidant capacity values ranging from 1.6 to 15 μmol TE/ml. At these antioxidant levels, consumption of 150 ml of tea could make a significant contribution to the total daily antioxidant capacity intake. (−)-Epicatechin and (+)-catechin, two components from tea, had an antioxidant capacity of 2.36 and 2.49 μmol/μmol or 8.13 and 8.58 μmol/mg, respectively. In 16 tea samples we observed a mean of 10.0 ± 0.6 μmol TE/mg total phenolics. Tea can be an important source of what has been referred to as "nonnutrient" antioxidant phytochemicals. However, with the variation that exists in antioxidant capacity with various tea preparations, measures of antioxidant capacity intake are critical to the study of intake and health outcomes and/or biomarkers of health outcomes.** [P.S.E.B.M. 1999, Vol 220]

The free radical hypothesis of aging suggests that age-related changes occur as a result of an increasing inability to cope with oxidative stress that occurs throughout the life span. Oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the former, resulting in oxidative damage to molecules such as DNA, lipids and proteins. After life-long free-radical insult on an organ that already shows increased vulnerability to oxidative stress, functional deficits are observed. Indeed, one of the primary efforts in aging research is to investigate putative changes in repair processes, as well as in the antioxidant defenses. As is well known (see Refs. 1–4 for reviews), free radicals can arise from both extrametabolic (e.g., pollution, radiation, toxins, etc.) and metabolic sources. Among the most significant biological sources of free radicals are those that lead to O_2-derived superoxide (O_2^-) from electron transport associated with mitochondrial membranes. In this case, the conversion of oxygen to water requires electron transfer. Among the products formed by

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these reactions are the hydroperoxyl radical (HO_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical($OH^{\cdot}$), which is potentially the most damaging pro-oxidant in cellular systems. Other cellular sources of free radical generation are those of microsomal and nuclear membranes. These membranes contain electron transport systems, cytochromes P-450 and b_5, which may produce free radicals. Fortunately, during normal biological functioning there are a number of antioxidant defenses that remove excess superoxides and H_2O_2. These include superoxide dismutase, catalase, and peroxidase. Peroxidase appears to be associated with membranes and participates in retarding membrane lipid peroxidation. In addition, there also are low- molecular-mass antioxidants such as glutathione (GSH), vitamin E, and ascorbic acid (AA). Since organisms do not scavenge free radicals with 100% efficiency, the repair of oxidative damage in DNA, proteins, and lipids is extremely important. Thus, a wide variety of enzymes and antioxidants exist to aid in this repair (see Refs. 1–4 for review).

One hypothesis that has been advanced is that the protection provided by fruits, vegetables, and drinks such as tea and red wine against the diseases of aging (i.e., cancer and cardiovascular diseases), can be attributed to a large class of antioxidant phytochemicals contained in these foods and drinks. These phytochemicals are primarily simple phenolics, as well as a group of polyphenolic flavonoids. Flavonoids are diphenylpropanes that commonly occur in plants (more than 4000 flavonoids have been found) and are generally considered to have antioxidant activities. The immediate family members of flavonoids include flavones and isoflavones, and the 2,3-dihydroderivatives of flavone, namely, flavanones. Flavanones undergo a series of transformations affecting the heterocyclic C ring to give rise to other family members of flavonoids, including anthocyanins and catechins (5). Catechins and a few of the flavonols are the principle flavonoid compounds found in tea (6). Flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic properties (see Ref. 7 for review). The flavonoid compounds are generally considered nonessential dietary nutrients. Flavonoids have been shown to have strong antioxidant activity *in vitro* using several different systems of measurement: 1) the Oxygen Radical Absorbance Capacity (ORAC) assay (8); 2) the Trolox Equivalent Antioxidant Capacity (TEAC) assay (9–11); and 3) protection of lipoproteins from oxidation (12).

Assessment of Antioxidant Capacity

The ORAC assay developed recently by Cao *et al.* (13, 14) provides an effective way to evaluate the potential antioxidant capacity of various phytochemicals and biological samples. In this assay, β-phycoerythrin (β-PE) or R-PE is used as an indicator protein, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water soluble vitamin E analog) as a standard. One unit of antioxidant capacity in the ORAC assay is defined as the equivalent amount of antioxidant capacity as 1 μmol of Trolox (Trolox Equivalents, TE). The ORAC assay, like other assays that have been developed, does not provide information on individual antioxidants contained in a sample of mixed antioxidants, but represents the total antioxidant capacity of the sample. The ORAC method is unique to other similar methods for two reasons. First, the ORAC assay system uses an area-under-curve (AUC) technique for quantitation of the data and thus combines both inhibition time and inhibition degree of free radical action by an antioxidant into a single quantity (11). Secondly, different free radical generators or oxidants can be used in the ORAC assay. This is important because the measured antioxidant activity of a biological sample depends upon which free radical or oxidant is used in the assay (15).

Antioxidant Capacity of Flavonoids

In general for flavonoid compounds, the more OH substitutions, the stronger the $ORAC_{ROO\cdot}$ activity (8). Weak $ORAC_{ROO\cdot}$ activity (0.2–0.6 TE) has been observed for flavones with single OH substitutions on the 3, 6, 2′, or 4′ position and in flavanones with single OH substitutions in the 7, 2′, 3′, 4′, or 7′ position. A flavone with a single OH substitution on the 5 position, however, has undetectable $ORAC_{ROO\cdot}$ activity, whereas a flavanone with a single OH substitution on the 6 position has an $ORAC_{ROO\cdot}$ activity of 1.36 TE, even stronger than Trolox (8).

The flavonoids that contain multiple OH substitutions have very strong antioxidant activities against peroxyl radicals. For example, ORAC of kaempherol, luteolin, quercetin, Myricetin, fustin, eriodictyol and taxifolin were 2.67, 3.57, 3.29, 4.32, 3.91, 3.41, and 3.59 TE, respectively, (Table I) (8), whereas α-tocopherol, ascorbic acid, β-carotene, GSH, uric acid, and bilirubin were reported to have ORAC values of 1.0, 0.52–1.12, 0.64, 0.68, 0.92, and 0.84 TE, respectively (8,10,11). This observation for flavonoids means that the stoichiometric factor (i.e., the number of peroxyl radicals trapped per molecule antioxidant) of these flavonoids is about 6–9, since the stoichiometric factor of Trolox is 2. The flavonols (kaempferol, quercetin, and Myricetin) and their glycosides may account for 0.5%–2.5% wt/wt, as aglycone, of teas (16, 17).

Catechins including the gallocatechins represent the major polyphenolic constituent of green teas (17) and make up as much as 30% wt/wt of dissolved solids. The four most common catechins are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) (17). Gallocatechin gallate (GCG) is present in much smaller quantities. The three catechins that we measured for ORAC had antioxidant capacities of ≈ 2.4 μmol TE/μmol (Table I), lower than some of the flavonols, but much higher that the common nutrient antioxidants, such as vitamins C or E. Lin *et al.* (18) measured ORAC on several of the tea catechins and found values just over 1 unit

Table I. Antioxidant Capacity of Simple Polyphenols, Catechins, and Other Polyphenolics Found in Tea.

Flavonoid	Molecular Weight (g/mol)	ORAC (μmol/μmol)	ORAC (μmol/mg)
Simple Polyphenols			
Gallic acid	170.1	1.74	10.23
Chlorogenic acid	354.3	3.00	8.47
Catechins			
(−)-epicatechin	290.3	2.36	8.13
(+)-catechin	290.3	2.49	8.58
gallocatechin gallate	458.4	2.43	5.30
Flavones/Flavanones			
Kaempherol (3,4′,5,7-tetrahydroxyflavone)	286.2	2.67	9.33
Luteolin (3′,4′,5,7-tetrahydroxyflavone)	286.2	3.57	12.49
Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone)	318.2	4.32	13.57
Quercetin (3,3′,4′,5,7-pentahydroxyflavone)	302.2	3.29	10.87

for EGCG, EGC, and ECG. However, ORAC of epicatechin and catechin were 0.5 or less (18), and gallic acid had a value of ≈ 1 equivalent to Trolox. These values are lower than what we have observed (8, 14) because of differences in the definition and calculation of one unit of antioxidant capacity. Several investigators have demonstrated antioxidant properties of tea components in various food and lipid model systems (19–24). Yen and Chen (22) concluded that the antioxidative properties of tea extracts may explain the antimutagenicity of tea extracts.

Antioxidant Capacity of Teas Relative to Other Drinks and Fruits and Vegetables

Data from our laboratory represent the first attempt to measure total antioxidant capacity of fruits and vegetables using the ORAC procedure (15, 25). The antioxidant capacity of common fruits and vegetables, and drinks including green and black teas, commercial fruit juices, and wines were measured with the automated ORAC assay using a peroxyl radical generator ($ORAC_{ROO\cdot}$). Based upon the dry weight of the edible portion, total antioxidant activity of the fruits and vegetables we evaluated was less that 200 μmole TE/g (15, 25). The antioxidant capacity of 18 commercial teas averaged 761.1 ± 85.3 μmol TE/g with a large range in antioxidant capacity from 236 to 1526 μmole TE/g (Table II). These samples included both green and black teas. Lin *et al.* (18) also observed considerable variation in both yields of solids in tea water extracts and the amount of (−)-epigallocatechin-3-gallate. With the variability between samples, there were no clear differences in antioxidant capacity of green and black teas, although black teas seemed to have a higher antioxidant activity. However, this may simply be a result of the particular commercial teas analyzed as we do not have data on tea from the same source processed in the two different methods for preparing green and black teas. Average total phenolics in 16 different commercial tea samples was 79.7 ± 10.3 (Range: 32–147 mg/g). The ORAC to phenolic ratio ranged from 4.63 to 14.3 with a mean of 10.0 ± 0.6 μmol TE/mg (Table II). However, as we consider possible *in vivo* effects on health outcomes, it is not only the concentration but also the amount (and quality) of antioxidant capacity consumed in a normal serving and also the amount absorbed or the bioavailability. Both of these concepts will be dealt with later.

Absorption and Bioavailability of Tea Polyphenolics

Data on the bioavailability of polyphenolics from tea or any of the fruits and vegetables are extremely limited. Sensitive instrumentation for the detection of flavonoids and their metabolites in plasma and biological samples has been a limitation. Nakagawa and Miyazawa (26) recently reported that tea catechin is absorbed from the digestive tract into the rat and human body, with the maximal levels of EGC observed 30 min after a single oral dose of 56 mg in the rat. Marked increases in EGC were observed in the human 60 min after administration of 97 mg EGC in a single oral supplement following 10 hr of fasting. Da Silva *et al.* (27) administered (−)-epicatechin to rats and found in plasma at 1 hr after administration, that the glucuronide and glucuronide-sulfate conjugates in both the free and O-methylated form were present. After 6 hr, the plasma concentration of total EC metabolites decreased, and the remaining conjugates were mostly present as the O-methylated form. However, they were able to demonstrate that the EC metabolites, particularly the conjugates in the free form, possess antioxidant activity in blood plasma.

Table II. Antioxidant Capacity and Phenolic Content of Commercial Teas.[a]

Type of Tea	ORAC, μmol TE/g			Total Phenolics, mg/g			ORAC/Phenolics		
	Mean ± SEM	Range	(*n*)	Mean ± SEM	Range	(*n*)	Mean ± SEM	Range	(*n*)
Black tea	1100.8 ± 142.7	478–1526	(6)	129.3 ± 7.2	109–147	(5)	9.5 ± 0.3	8.0–10.4	(5)
Green tea	608.1 ± 224.7	236–1197	(4)	71.7 ± 30.9	32–133	(3)	7.5 ± 1.4	4.6–9.0	(3)
Herbal/berry	582.8 ± 90.2	255–965	(8)	51.7 ± 7.2	21–76	(8)	11.3 ± 0.7	8.8–14.3	(8)
Overall means	761.1 ± 85.3	236–1526	(18)	79.7 ± 10.3	32–147	(16)	10.0 ± 0.6	4.6–14.3	(16)
Tea extract	4796	—		625	—		7.7	—	

[a] Means ± SEM of different commerical preparations of tea. The herbal/berry teas were not indicated as being either black or green teas.

Thus, it is clear that tea flavonoids are absorbed, but the relative amounts that are absorbed have not been determined definitively.

Dietary Intake of Antioxidant Capacity/ Flavonoids from Tea Relative to Other Dietary Sources

Kuhnau (28) estimated that the average intake of all flavonoids combined was approximately 1 g/day. These results have been widely quoted; however, more recent results of Hertog *et al.* (29, 30) suggest that these values may be approximately five-fold higher than the typical intake in a Western population. Hertog and co-workers (29, 30) calculated that the average intake was approximately 25 mg/day for the flavonols, quercetin, kaempherol, myricetin, apigenin and luteolin. Some of these large differences may be accounted for by the much improved analytical techniques based upon high performance liquid chromatography (31) that are currently available. The most important of these five flavonols among 4112 adults was quercetin, with a mean intake of 16 mg/day (29). Tea (48% of total intake), onions (29%), and apples (7%) were the most important sources of flavonoids in the Zutphen Elderly Study. Rimm *et al.* (32) in a study of 34,789 male health professionals in the United States used the flavonoid database of Hertog *et al.* (29, 30) and found that the mean intake of quercetin, kaempherol, and myricetin, three primary flavonoids, were 15.4, 3.6, and 0.9 mg/day, respectively. The main sources of these flavonoids were tea (25%), onions (25%), apples (10%) and broccoli (7%). On a milligram-per-day basis, the intake of flavonoids exceeded that of β-carotene and vitamin E. However, we have separated as many as 120 electroactive peaks in foods with high ORAC activity (33). Thus, these later estimates likely underestimate total intake of polyphenolic flavonoids.

We have determined from analysis of food frequency responses in 36 subjects consuming five servings of fruits and vegetables per day that total antioxidant capacity intake, as measured by ORAC, was 1670 μmol TE per day (34). From data in the continuing food intake survey conducted by the USDA, the estimated intake of ORAC was approximately 1200 μmol TE per day. This estimate seems reasonable since in the general population, the number of servings of fruits and vegetables normally consumed is closer to 3 servings per day rather than the 5 per day that we observed in our study group. The relationship between ORAC intake and plasma antioxidant capacity was a curvilinear response (34) with an increase in plasma antioxidant activity that plateaus above an intake of 4000 μmol TE per day.

Plasma antioxidant capacity has been shown to increase following consumption of a drink containing 3700 μmol TE of antioxidant capacity from either strawberries, spinach, red wine, or vitamin C (35). Fasting plasma ORAC also has been shown to increase significantly within 11 days of changing from the consumption of 5 (1670 μmol TE) to 10 servings of fruits and vegetables (3200–3800 μmol TE) (31). Some individuals were observed to consume as much as 6000 μmol TE from self-selected diets. Data from these studies point to dietary levels of antioxidant capacity in excess of 3500 μmol TE needed to impact total plasma antioxidant capacity and thus to influence those health outcomes that are affected by total antioxidant status. From the overall relationship between antioxidant capacity and total phenolic content in foods (Fig. 1), an intake of 3500 ORAC units would equate to a total phenolic intake of approximately 422 mg.

Tea in the diet can make a significant contribution to the total phenolic and antioxidant capacity intake. After water, tea is the most widely consumed beverage in the world today (17). Currently in the United States, per capita consumption of tea is approximately 340 g/yr which produces 35–40 liters of beverage (17). However, in order to estimate this contribution to total antioxidant capacity, a measure of phenolic and antioxidant capacity of brewed tea as consumed is needed. We undertook some experiments to obtain this information (Tables II and III). With a weight of tea per tea bag of 1.95 g and with an extraction efficiency of 84% in 5 min into 150 ml (5 oz.) hot water (Table III) and an antioxidant capacity of 761 μmol TE/g, the antioxidant capacity of the tea drink would be 8.31 μmol TE/ml. Using an average of 761 μmol TE/g or 8.3 μmol TE/ml, per capita daily consumption of antioxidant capacity from tea could range from 796 to 910 μmol TE/day, which is about 60% of the antioxidant capacity that might be provided in the average U.S. diet from fruits and vegetables. Adding together the contribution of tea and fruits and vegetables from our estimates, the per capita daily consumption of tea could provide about 36% of the total antioxidant capacity. This compares to the data of Rimm *et al.* (32) who found that tea contributed 25% of the total of three flavonols. In order to consume 3500 μmol TE intake, 421 ml of tea containing 761 μmol TE/g(8.3 μmol TE/ml) would need to be ingested. However, if the antioxidant capacity of the tea were only 236 μmol TE/g(2.6 μmol TE/ml), then 1346 ml of tea would have to be consumed to achieve 3500 μmol TE intake per day. At this point it is not clear what accounts for the variability in antioxidant capacity of different tea preparations; genetic background, growing and processing conditions can all contribute to variability in antioxidant activity.

The working hypothesis that dietary antioxidant capacity needs to be increased to 3500 μmol TE/day or more is supported further by analysis of responses in clinical studies that included tea as a dietary component. Phenolic intake and estimated ORAC intake from three of these studies are summarized in Table IV (33–35). In one study (36), the estimated ORAC intake from green tea was 4710 μmole TE/day, and a significant increase in the total antioxidant capacity of plasma was observed. However, with only 1577 μmole TE intake per day from black tea, no increase in plasma antioxidant capacity was observed (36). In neither tea treatment was an effect observed on serum lipid con-

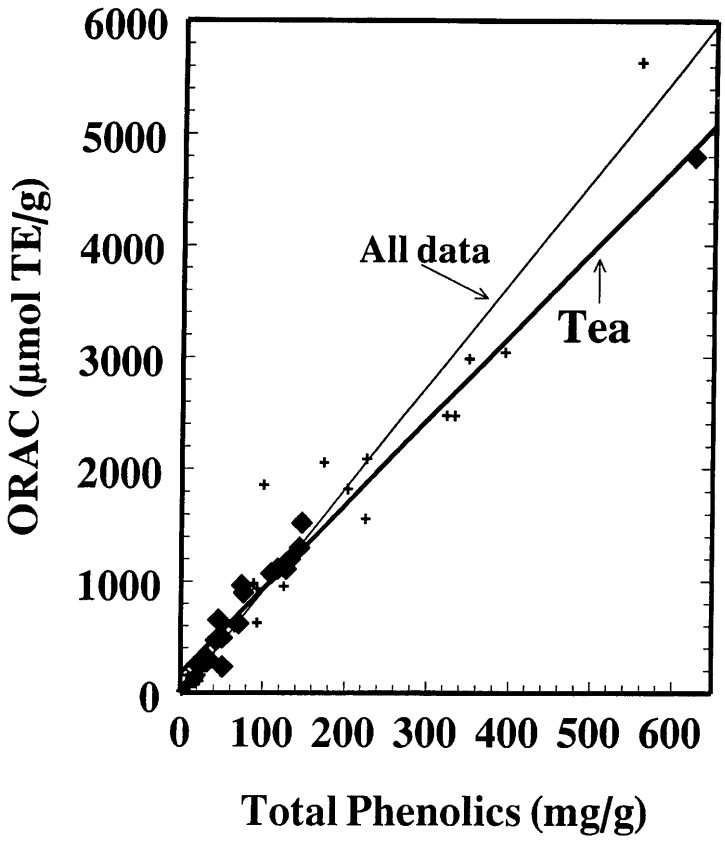

Figure 1. Linear Relationship of Antioxidant Capacity (ORAC) (μmol Trolox Equivalents/g)(Y) to Total Phenolics (mg/g)(X) in Teas. The regression equation for the tea samples is: Y = 175.2 + 7.54X (r_{xy} = 0.991; n = 16). The regression equation for a total of 104 samples including blueberries, cereals, and other berry and antioxidant extracts is: Y = −13.6 + 9.2X (r_{xy} = 0.983; n = 104).

centrations, resistance of LDL to oxidation *ex vivo,* or markers of oxidative damage to lipids *in vivo.*

In a second study over a 4-week period (37) during which participants consumed five cups of tea containing the polyphenolics listed in Table IV with an estimated ORAC content of 2707 μmole TE, the lag time in LDL oxidation was increased from 54 to 62 min. This increase was observed in spite of much lower plasma flavonoid concentrations than were used in an *in vitro* study to bring about the same effect on the delay of LDL oxidation.

Princen *et al.* (38) (Table IV) concluded that consumption of black or green tea (six cups/day) had no effect on plasma lipids or LDL oxidation *ex vivo* and no sparing effect on plasma antioxidant vitamins. Intake of a high dose of isolated green tea polyphenols decreased plasma vitamin E, but had no effect on LDL oxidation *ex vivo.* Estimated ORAC intake for these three treatments was 2235, 798, and 5153 μmol TE, respectively. Based upon ORAC, it is surprising that consumption of the green tea polyphenol extract did not alter measures of antioxidant status. Sufficient polyphenols were consumed such that *in vivo* antioxidant status should have been increased; however, the extraction procedures may have altered the antioxidant capacity of the polyphenols.

Serafini *et al.* (39) observed a significant increase in plasma antioxidant capacity as assayed by the TRAP assay following a single bolus quantity (300 ml) of either black or green tea. The antioxidant capacity of green tea was five times greater than black tea as measured by TRAP. Sufficient information was not provided to compute an estimated ORAC of the teas consumed. In another study, rabbits receiving either black or green tea in the drinking water had a 13% and 15% prolongation of the lag phase of LDL oxidation whereas vitamin E increased the lag phase by 63% with a concomitant decrease in the LDL oxidation rate (40).

Summary

Data from numerous *in vitro* test systems clearly indicate that catechins and other flavonoid-type compounds in teas can function as strong antioxidants. However, the ef-

Table III. Antioxidant Capacity and Phenolics Extracted from Tea into First and Second Cups of Boiling Water.

Item	ORAC[a] (%)	Phenolics[b] (%)
First cup	83.5 ± 1.6	91.7 ± 0.9
Second cup	13.3 ± 1.4	8.3 ± 0.09
Remaining	3.1 ± 0.8	0.0

Note. The quantity of tea in tea bags was weighed and extracted for 5 min into 150 m of boiling water. Then the tea bag was placed into a second cup of boiling water for another 5 min. The material remaining after these extractions was further extracted with 100% acetone. The total was determined as the sum of amounts present in the three extractions. The amount of antioxidant activity (ORAC) and phenolics extracted during the first two periods was expressed as a percentage of the total.

[a] Data presented as means ± SEM of 18 commercial tea sources.

[b] Data presented as means ± SEM of 16 commerical tea sources.

Table IV. Daily Intakes of Catechins (mg/day) and Estimated ORAC (μmol TE/day) from Tea in Three Clinical Studies.

Item	Study #1[a]		Study #2[b]		Study #3[c]		
	Green[d]	Black[f]	Tea[e]	Tea extract	Green[f]	Black[f]	Extract[g]
Epicatechin, mg	15.5	2.5	38	72.4	28.4	21.2	79.2
Epicatechin gallate, mg	6.5	2.2	—	9.6	24.4	20.8	114.8
Epigallocatechin, mg	50.2	5.8	101	33.2	79.0	8.3	124.7
Epigallocatechin gallate, mg	58.2	3.5	215	221.1	103.0	24.0	288.0
Catechin, mg	—	—	—	—	3.6	2.6	15.9
Gallocatechin, mg	—	—	—	—	19.5	2.4	30.6
Gallocatechin gallate, mg	—	—	—	—	26.0	0.0	37.8
Theaflavin, mg	—	—	11	—	0	11.4	0
Theaflavin digallate, mg	—	—	4	—	—	—	—
Est. ORAC intake (μmol TE/day)	4710	1577	2707	2467	2235	798	5153

[a] Source: Ref. 38.
[b] Source: Ref. 39.
[c] Source: Ref. 40.
[d] Increased total antioxidant capacity of plasma following treatment.
[e] Prolonged lag time before LDL oxidation.
[f] No effect observed on any parameter measured *in vivo*.
[g] Decreased plasma vitamin E compared to control group.

fectiveness of tea and its components in altering *in vivo* antioxidant status is a function of the concentrations and amounts consumed. Variability in antioxidant capacity of different teas can likely be impacted by growing conditions, harvest time, and processing methods. Clear differences in antioxidant capacity between green and black teas were not observed in different commercial preparations. Consumption of in excess of 3000 μmol TE of antioxidant activity (expressed as μmol TE in the ORAC assay) seems to be necessary to impact *in vivo* antioxidant status. To insure this level of intake, consumption of 8–10 cups of tea daily would be required.

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