Biosci. Biotechnol. Biochem., 74 (10), 2137–2140, 2010

JSBA

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Estimated Average Daily Intake of Antioxidants from Typical Vegetables Consumed in Japan: A Preliminary Study

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Received June 8, 2010; Accepted July 3, 2010; Online Publication, October 7, 2010
[doi:10.1271/bbb.100430]

The hydrophilic antioxidant content of 23 vegetables commonly consumed in Japan was assessed by the hydrophilic oxygen radical absorbance capacity (H-ORAC) method to estimate the dietary intake of total antioxidants in Japan. The estimated average H-ORAC value for "typical vegetables" consumed in Japan was 594.3 µmol Trolox equivalent (TE)/100 g. Hence, 2080 µmol TE/d of hydrophilic antioxidants would be ingested when 350 g of vegetables a day are consumed.

Key words: antioxidant; vegetable; hydrophilic oxygen radical absorbance capacity; daily food consumption

Many studies have suggested that antioxidants that are abundant in such foods as vegetables and fruits may contribute to overall health and disease prevention (reviewed in the references[1,2]). For example, quercetin, a major flavonoid in onion, has been studied for its anti-atherosclerotic effect,[3] and resveratrol, a polyphenol in red wine, has been suspected to have cardioprotective and chemopreventive effects.[4] We take such antioxidants in combination from various foods. Although antioxidant-rich foods appear to be beneficial for us, it is still not clear what quantities of daily antioxidants we need to maintain good health. There have been some studies in which the dietary intake of total antioxidants was examined (for Americans[5,6] and for Swedes[7]). Such information is essential for considering the relationship between dietary antioxidants and health, but although there has been no such study for the Japanese. Since eating habits vary considerably between countries, the dietary intake of total antioxidants in Japan needs to be re-evaluated. Hence, in this preliminary study, we assessed the antioxidant contents of vegetables commonly consumed in Japan by the oxygen radical absorbance capacity (ORAC) method, and we estimated the daily intake of antioxidants from these vegetables. The ORAC assay is one of the most widely used antioxidant assays for foods, and it evaluates the respective radical-scavenging activities of water- and lipid-soluble antioxidants in samples by the hydrophilic ORAC (H-ORAC) and lipophilic ORAC (L-ORAC) methods.[8] A previous study has showned that the H-ORAC values of for vegetables were usually much higher than the L-ORAC values,[5] and we therefore focused on the H-ORAC values of vegetables typically consumed in Japan.

We selected the 23 common vegetables shown in Table 1 based on the annual report of the family income and expenditure survey in Japan for 2008, and then attempted to estimate the daily intake of antioxidants from these vegetables. The annual purchase weight of these vegetables accounted for 84.5% of the total weight of purchased raw vegetables. The H-ORAC values for these vegetables are expressed as µmol Trolox equivalents (TE) per gram fresh weight (µmol TE/g) of the edible portion (Table 1). The five vegetables possessing the most potent antioxidative activities were 1. edible burdock, 2. East Indian lotus root, 3. eggplant, 4. edamame, and 5. broccoli. These vegetables were also rich in polyphenols. The H-ORAC value for each vegetable showed a strong positive correlation with the polyphenol content (Fig. 1).

However, these data do not take into account the quantity of each vegetable consumed. We therefore defined "typical vegetables" to reflect the amounts of raw vegetables consumed in Japan based on the annual purchase weight per household for raw vegetables (see the Experimental section for details). The defined composition of 100 g of "typical vegetables" is shown in the second-to-last column of Table 1. The top five vegetables commonly eaten in Japan appeared to be 1. onion tied with cabbage, 3. Japanese radish (daikon), 4. tomato, and 5. potato. The most comprehensive data in this study, *i.e.*, the partial H-ORAC values, are shown in

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Table 1. H-ORAC Values and Polyphenol Contents for Vegetables Consumed in Japan

Sample name	Item No.[a]	H-ORAC (μmol TE/g)[b]	Polyphenol content (mg GAE/g)[c]	"Typical vegetables" consumed in Japan	
				Composition (g/100 g)[d]	Partial H-ORAC (μmol TE/100 g)[e]
Onion, bulb	06153	6.71	0.42	11.5	77.5 (13.0%)[f]
Cabbage, head	06061	3.74	0.37	11.5	43.1 (7.2%)
Japanese radish (daikon), root without skin	06134	3.93	0.20	10.1	39.5 (6.7%)
Tomato, fruit	06182	3.14	0.31	8.4	26.3 (4.4%)
Potato, tuber	02017	6.85	0.50	8.1	55.5 (9.3%)
Carrot, root with skin	06212	5.26	0.32	6.4	33.5 (5.6%)
Cucumber, fruit	06065	1.68	0.15	6.3	10.6 (1.8%)
Chinese cabbage, head	06233	3.10	0.26	6.2	19.1 (3.2%)
Mung bean sprout	06291	6.17	0.41	4.3	26.8 (4.5%)
Crisp lettuce, head	06312	2.61	0.17	4.2	11.0 (1.8%)
Eggplant, fruit	06191	15.55	0.81	3.3	51.4 (8.6%)
Pumpkin, fruit	06046	3.88	0.38	3.2	12.5 (2.1%)
Spinach, leaves	06267	8.20	0.30	2.7	22.1 (3.7%)
Welsh onion (nebuka-negi), leaves, blanched	06226	2.32	0.24	2.4	5.5 (0.9%)
Sweet potato, tuberous root	02006	6.18	0.52	2.3	14.1 (2.4%)
Green sweet pepper, fruit	06245	6.95	0.62	1.6	11.1 (1.9%)
Taro (satoimo), corm	02010	8.33	0.35	1.5	12.9 (2.2%)
Edible burdock, root	06084	52.17	2.36	1.4	75.1 (12.6%)
Broccoli, inflorescence	06263	11.00	0.73	1.4	15.5 (2.6%)
Edamame	06015	13.32	0.59	1.0	13.1 (2.2%)
Shiitake mushroom	08011	2.58	0.24	0.9	2.4 (0.4%)
East Indian lotus root, rhizome	06317	19.57	1.34	0.8	14.8 (2.5%)
Bamboo, shoot[g]	06149	2.06	0.43	0.4	0.9 (0.2%)
Total				100.0	594.3

[a]Item numbers in the Standard Tables of Food Composition in Japan.
[b]The H-ORAC value for each vegetable is expressed as moles of Trolox that are equivalent to the amount of water-soluble antioxidants contained in 1 g of the raw vegetable (μmol Trolox equivalent (TE)/g of edible portion).
[c]The polyphenol contents of each vegetable is expressed as mg of gallic acid that are equivalent to the amount of polyphenols contained in 1 g of the raw vegetable (mg of gallic acid equivalent (GAE)/g of edible portion).
[d]The composition of the "typical vegetables" consumed in Japan was estimated from the annual report on the family income and expenditure survey in Japan for 2008 and the Standard Tables of Food Composition in Japan. Details are shown in the main text.
[e]The partial H-ORAC value indicates the H-ORAC value attributed to each vegetable contained in 100 g of the "typical vegetables." Details are described in the main text.
[f]The values shown in parentheses are contributions to the total H-ORAC value of "typical vegetables."
[g]Bamboo shoots, canned in water, were used because raw bamboo shoots were seasonally unavailable.

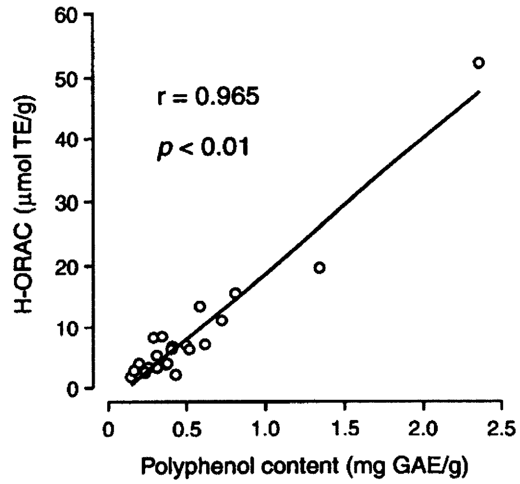

Fig. 1. Relationship between the H-ORAC Value and Polyphenol Content of Typical Vegetables Consumed in Japan.

The correlation was calculated by using a Pearson product moment correlation coefficient (r).

the last column of Table 1. These values indicate the practical antioxidative activity attributable to each vegetable making up 100 g of "typical vegetables" and were calculated as follows:

$$\text{partial H-ORAC } (\mu\text{mol TE}/100\,\text{g}) = \text{H-ORAC } (\mu\text{mol TE/g}) \times \text{composition } (\text{g}/100\,\text{g}).$$

Hence, the top five contributors to the total antioxidative activity of the "typical vegetables" were 1. onion, 2. edible burdock, 3. potato, 4. eggplant, and 5. cabbage. Based on the partial H-ORAC values, the H-ORAC value for "typical vegetables" was calculated to be 594.3 μmol TE/100 g. We measured H-ORAC for a combination of the "typical vegetables" made up from a freeze-dried sample of each vegetable. The actual H-ORAC value for the "typical vegetables" was 644.3 μmol TE/100 g which is almost the same as the calculated value. The difference between the two values may have been due to interaction between the antioxidants making up the "typical vegetables."

The H-ORAC value for the "typical vegetables" was 594.3 μmol TE/100 g. The Japan Ministry of Health, Labour and Welfare administered the national health promotion policy, "Health Japan 21," which recommends consuming at least 350 g of vegetables a day.[9] Hence, 2080 μmol TE of antioxidants would be ingested when 350 g of vegetables are consumed. This amount of antioxidants is equivalent to *ca.* 500 mg of ascorbic acid (vitamin C). Wu *et al.*[5] have estimated in one of the preceding studies that the total H-ORAC intake from vegetables in the United States was 2385 μmol TE/d, this value being consistent with the value obtained in the present study. They[5] also reported that the total H-ORAC intake from fruits in the United States was estimated to be 3174 μmol TE/d. In addition, Rautiainen *et al.*[7] have reported that the total ORAC intake from vegetables and fruits in Sweden was estimated to be 6523 μmol TE/d, and that contributors to the total ORAC intake from all foods were fruits (34.8%), vegetables (21.6%), grain products (19.7%), tea (9.5%), chocolate (4.9%), juice (3.9%) and wine (2.5%). These findings suggest that the contribution of foodstuffs other than vegetables, and especially of fruits, to the total intake of antioxidants may also be considerable in Japan, and that the intake of antioxidants from "typical fruits" in Japan therefore needs to be preferentially estimated.

The vegetables possessing potent antioxidative activities were not necessarily the same as the vegetables contributing substantially to the total antioxidative activity of "typical vegetables" (Table 1). This discrepancy has also been pointed out by Chun *et al.*[6] Dietary antioxidants act cooperatively in our body, and thus the total amount of antioxidants considered from both the antioxidative activity of each vegetable and the daily intake may have an important impact on health. Among the vegetables investigated, edible burdock showed the highest H-ORAC value per weight, and was also a major contributor to the total H-ORAC activity of "typical vegetables," although its intake was relatively low (Table 1). Edible burdock also contained the highest amount of polyphenols, and it has been reported that it contained only the simple polyphenols, chlorogenic acid (38–178 μmol/100 g), ferulic acid (38–100 μmol/100 g) and cinnamic acid derivatives (120–532 μmol/100 g), and did not contain flavonoids.[10] It is rarely eaten as a regular dietary component except in Japan, so the habit of eating edible burdock would be characteristic of the Japanese from the viewpoint of the antioxidant intake.

The results showing that the H-ORAC value for vegetables showed a strong positive correlation with the polyphenol content (Fig. 1) suggests that the antioxidative activities of the "typical vegetables" appear to result mainly from the polyphenols contained. We found in a pilot study that the "typical vegetables" contained the following polyphenols as aglycons: caffeic acid (3.61 mg (20.0 μmol)/100 g), quercetin (2.08 mg (6.9 μmol)/100 g), *p*-coumaric acid (0.80 mg (4.9 μmol)/100 g), ferulic acid (0.57 mg (2.9 μmol)/100 g), genistein (0.12 mg (0.4 μmol)/100 g), and daidzein (0.05 mg (0.2 μmol)/100 g).

The samples used in this study were raw vegetables (uncooked) obtained in one area and in one season, and the values in Table 1 are therefore not representative H-ORAC values for vegetables in Japan. We also only evaluated their H-ORAC values which appeared to contribute more to antioxidative activities of the vegetables than did the L-ORAC values.[5] The data presented here are therefore limited, but they could spotlight one feature of the daily consumption of antioxidants from vegetables in Japan. It is necessary to assess the effects of cooking and area and seasonal variations by measuring both the H-ORAC and L-ORAC values. In addition to this, data concerning foodstuffs other than vegetables, especially fruits, are needed to properly estimate the overall dietary intake of antioxidants in Japan.

Although dietary antioxidants are widely believed to be good for health, there is also evidence to negate the beneficial effects of antioxidant supplements or antioxidant-enriched foods.[11,12] It should be noted that even vitamins and minerals can be harmful in overdoses. Thus, an excessive intake of antioxidants might also be harmful. Foods naturally rich in antioxidants appear good for us at present, but the optimal total amount of antioxidants needed for maintaining good health is still uncertain. To estimate the dietary intake of total antioxidants in Japan, we focused on the H-ORAC values of vegetables typically consumed in Japan in this preliminary study as a first step. We believe that our present and continuing studies will provide basic data for epidemiological studies to clarify relationship between the total antioxidant intake and health.

Experimental

Selection of vegetable samples and definition of "typical vegetables" consumed in Japan. We used the annual report on the family income and expenditure survey in Japan for 2008 (Statistics Bureau; Ministry of Internal Affairs and Communications, Japan) to estimate the consumption of vegetables. This survey showed the annual purchase weight per household of raw vegetables, the weight for 23 kinds of vegetables shown in Table 1 accounting for 84.5% of the total weight of purchased raw vegetables. We therefore selected these 23 vegetables for assessment. The composition of "typical vegetables" consumed in Japan is defined as follows:

$$\text{typical composition (g per 100 g of vegetables)} = [W \times (100 - R)/100]/\left[\sum\{W \times (100 - R)/100\}\right] \times 100$$

where W(g) is the annual purchase weight per household of each vegetable, R(%) is the discarded (or inedible) portion from the Standard Tables of Food Composition in Japan, 5th revised and enlarged ed. (Council for Science and Technology; Ministry of Education, Culture, Sports, Science and Technology, Japan), and $\sum\{W \times (100 - R)/100\}$ is the sum of the estimated annual intake per household of the 23 kinds of vegetables listed in Table 1.

Measurement of H-ORAC. The 23 vegetables shown in Table 1 were commercially purchased in Kumamoto, Japan in June or July 2009, and their edible portions were promptly freeze-dried. The freeze-dried samples were stored at −20 °C. The antioxidants in these samples were then extracted by using an accelerated solvent extraction system (Dionex, ASE-200) as described by Wu *et al.*[13] with some modifications. Briefly, each freeze-dried sample was initially extracted with hexane/dichloromethane (1:1) and then with methanol/water/acetic acid (90:9.5:0.5; MWA). The MWA extracts were diluted with an assay buffer (75 mM KH_2PO_4-K_2HPO_4 at pH 7.4) as necessary, and the H-ORAC values were then measured to reflect the radical-scavenging activities of hydrophilic antioxidants in the samples roughly according to the method of Prior *et al.*[14] Sodium fluorescein and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Trolox was obtained from Aldrich Chemicals (Milwaukee, WI, USA). Trolox calibration solutions (6.25, 12.5, 25, and 50 μM in the assay buffer) were made to obtain a standard curve. An MWA extract, Trolox calibration solution or blank (50 μl), fluorescein (170 μl, 110.7 nM), and AAPH (75 μl, 31.7 mM) were incubated in the assay buffer at 37 °C in a 96-well plate. The fluorescence (Ex. at 485 nm, Em. at 528 nm) was monitored every 2 min for 90 min by a Powerscan HT instrument (DS Pharma Biomedicals, Osaka, Japan). The net area under the curve (AUC) was calculated by subtracting AUC for the blank from that from the sample or standard. The H-ORAC value for each sample was calculated on the basis of the standard curve for Trolox. Data are expressed as moles of Trolox equivalent to the amount of water-soluble antioxidants contained in a sample (μmol TE/g). Each value is the average of the determinations for two separately prepared extracts from one freeze-dried sample of each vegetable to reduce the error associated with extraction and measurement of H-ORAC.

Measurement of the polyphenol content. The polyphenol content was determined according to the method described in International Organization for Standardization (ISO) 14502–1 on the basis of the Folin-Ciocalteu assay[15] with slight modifications. Briefly, a diluted MWA extract or gallic acid standard solution (1.0 ml) was transferred in duplicate to separate plastic tubes. A 1/10 dilution of the Folin-Ciocalteu reagent (Wako Pure Chemical Industries, Osaka, Japan) in water (5.0 ml) was added to each tube. After an interval of 3 to 8 min, a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min, and the absorbance at 765 nm was measured. The concentration of polyphenol in the MWA extract was calculated from the standard curve for gallic acid ranging from 10 to 50 μg/ml. Data are expressed as milligrams of gallic acid equivalents per gram (mg GAE/g). Each value is the average of determinations for two separately prepared extracts from one freeze-dried sample of each vegetable to reduce the error associated with extraction and measurement of the polyphenol content.

Acknowledgments

The authors appreciate helpful discussions with I. Suda, T. Tsushida, and K. Yamasaki. This study was partially supported by a grant from the Ministry of Health, Labour and Welfare of Japan.

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