1-B)、運動トレーニングにより耐糖能の改善が認められたが、ビタミン C の摂取によりそれが妨げられることはなかった。

OGTT 実施後、さらに 1 週間の運動トレーニングを行った後に解剖し、各群の血液生化学検査を行った (表 2)。過去 1～2 週間の血糖コントロールの指標であるグリコアルブミン濃度は、糖尿病ラットにおいて、運動トレーニングにより健常ラットと有意差のないレベルまで低下したが、ビタミン C の大量摂取は無影響であった。血中 TG については、糖尿病ラットにおいて、運動トレーニングの影響は認められなかったが、ビタミン C の摂取により減少した。HDL コレステロールについては、糖尿病により上昇し、運動トレーニングにより健常レベルまで低下した。ビタミン C 摂取の影響は認められなかった。LDL コレステロールについては、運動トレーニングの影響はなく、ビタミン C の摂取により増加した。遊離脂肪酸については、糖尿病ラットにおいて、運動とビタミン C 摂取の交互作用が認められ、運動を行わなかったラットがビタミン C を大量摂取すると遊離脂肪酸が有意に増加した。

血中グリコアルブミンの結果については、糖尿病により上昇した食後血糖値が、運動トレーニングによる耐糖能改善効果により低下し、ビタミン C の大量摂取がそれを妨げることはなかったと解釈できる。一方、血中脂質 (TG、HDL、LDL、遊離脂肪酸) の結果については、合理的な説明が困難である。ストレプトゾトシンを用い 1 型糖尿病ラットを作成すると、血中脂質異常が起こり、TG、HDL コレステロール、LDL コレステロールが、総じて健常ラットと比較して増加することが報告されている [4、5]。しかし、今回作成した糖尿病ラットは 2 型であり、空腹時血糖は正常である (図 1-A)。このラットでは、減少したインスリンで血糖値を正常に保つため、様々な代替経路で代謝が行われるため、結果の解釈が困難となっている可能性が考えられた。糖尿病ラットの、血中 TG、LDL コレステロール、遊離脂肪酸レベルについては、ビタミン C の大量摂取の影響が認められたが、これが再現性のある現象なのか、また、糖尿病の運動療法の効果に影響を及ぼすかどうかについては、さらなる検討が必要であろう。

D. 結論

ビタミン C の大量摂取が、運動トレーニングによる耐糖能の改善を妨げると言う先行研究 [2] を受け、2 型糖尿病モデル動物を用いて検討を行った。運動トレーニングにより、耐糖能の改善が認められたが、これがビタミン C の大量摂取により打ち消されることはなかった。従って、ビタミン C の大量摂取により、2 型糖尿病に対する運動療法の効果が妨げられることはないことが示唆された。

E. 研究発表

1. 論文発表

なし

2. 学会発表

なし

F. 知的所有権の取得状況

1. 特許取得

なし

2. 実用新案登録

なし

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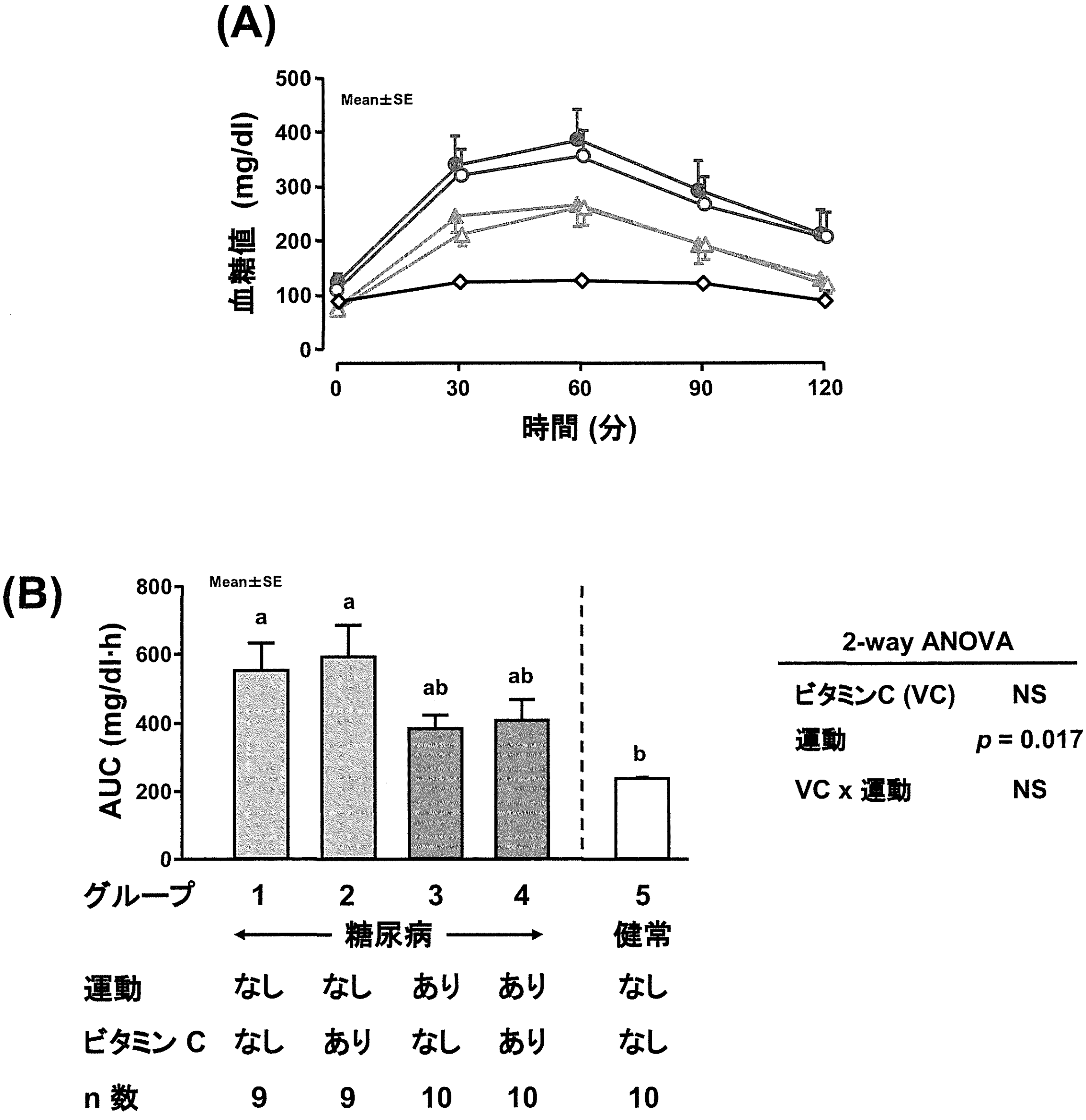

グループ	1	2	3	4	5
	← 糖尿病			→	健常
運動	なし	なし	あり	あり	なし
ビタミン C	なし	あり	なし	あり	なし
n 数	9	9	10	10	10

2-way ANOVA	
ビタミンC (VC)	NS
運動	$p = 0.017$
VC x 運動	NS

図 1. 経口糖負荷試験 (OGTT)

(A) タイムコース: グループ 1 (○)、グループ 2 (●)、グループ 3 (△)、グループ 4 (▲)、グループ 5 (◇)

(B) 曲線下面積: 異なる文字間には有意差あり (Tukey-Kramer 法、$p < 0.01$)

表 1. 実験スケジュール

日数	運動群	非運動群
1	トレーニング 1	休み
2	トレーニング 2	休み
3	トレーニング 3	休み
4	トレーニング 4	休み
5	休み	休み
6	休み	休み
7	トレーニング 5	休み
8	トレーニング 6	休み
9	トレーニング 7	休み
10	トレーニング 8	休み
11	トレーニング 9	休み
12	休み	休み
13	トレーニング 10	休み
14	トレーニング 11	休み
15	休み	休み
16	トレーニング 12	休み
17	トレーニング 13	休み
18	トレーニング 14	休み
19	休み	休み
20	休み	休み
21	トレーニング 15	休み
22	トレーニング 16	休み **OGTT**
23	休み **OGTT**	休み
24	休み	休み
25	トレーニング 17	休み
26	休み	休み
27	休み	休み
28	トレーニング 18	休み
29	トレーニング 19	休み
30	トレーニング 20	休み
31	トレーニング 21	**解剖**
32	**解剖**	**解剖**

運動トレーニングは傾斜をつけないトレッドミルで実施
(速度:25 m/分、時間:60 分/日)
運動トレーニングの 4 時間前に、ビタミン C を強制経口投与 (750 mg/kg)

表 2. 血液生化学検査

	グループ 1	グループ 2	グループ 3	グループ 4	グループ 5	2-way ANOVA		
糖尿病・健常	糖尿病	糖尿病	糖尿病	糖尿病	健常			
運動トレーニング	なし	なし	あり	あり	なし			
ビタミン C 投与	なし	あり	なし	あり	なし	運動	VC	運動×VC
n 数	9	9	10	10	10			
グルコアルブミン (%)	4.1 ± 0.6 ^{a}	4.1 ± 0.6 ^{a}	2.9 ± 0.5 ^{ab}	2.8 ± 0.4 ^{ab}	1.8 ± 0.0 ^{b}	$p = 0.02$	NS	NS
TG (中性脂肪) (mg/dl)	62.4 ± 8.5 ^{a}	31.8 ± 6.3 ^{b}	45.6 ± 5.3 ^{ab}	28.2 ± 4.9 ^{b}	45.2 ± 8.1 ^{ab}	NS	$p < 0.01$	NS
HDL コレステロール (mg/dl)	31.6 ± 1.0 ^{ab}	32.1 ± 1.5 ^{b}	26.6 ± 0.9 ^{ac}	26.7 ± 1.5 ^{ac}	26.2 ± 1.1 ^{c}	$p < 0.01$	NS	NS
LDL コレステロール (mg/dl)	5.3 ± 0.2 ^{ab}	6.0 ± 0.4 ^{bc}	5.2 ± 0.1 ^{ab}	7.3 ± 0.6 ^{c}	5.0 ± 0.0 ^{ab}	NS	$p < 0.01$	NS
遊離脂肪酸 (mEq/L)	1.4 ± 0.1 ^{ab}	3.0 ± 0.3 ^{c}	1.3 ± 0.1 ^{ab}	1.0 ± 0.1 ^{a}	1.8 ± 0.2 ^{b}	$p < 0.01$	$p < 0.01$	$p < 0.01$

平均 ± 標準誤差を示す。異なる上付き文字間には有意差あり (Tukey-Kramer 法、$p < 0.05$)

NS = not significant ($p > 0.05$)

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Jun Takebayashi Tomoyuki Oki Jun Watanabe Koji Yamasaki Jianbin Chen Maki Sato-Furukawa Megumi Tsubota-Utsugi Kyoko Taku Kazuhisa Goto Teruki Matsumoto Yoshiko Ishimi	Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods	Journal of Food Composition and Analysis	29	25-31	2013
竹林 純、沖 智之、渡辺 純、山崎 光司、陳 健斌、古川（佐藤）麻紀、坪田（宇津木）恵、卓 興鋼、後藤 一寿、松本 輝樹、石見 佳子	日本において一般的に食されている野菜・果物の親水性抗酸化能およびこれらの食品からの親水性抗酸化物質一日摂取量の推算	ビタミン	87	274-276	2013 (印刷中)

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Original Research Article

Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods

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Food analysis
Food composition

ABSTRACT

The sum of hydrophilic antioxidants of 23 vegetables and 13 fruits commonly consumed in Japan was evaluated by a modified hydrophilic-oxygen radical absorbance capacity (H-ORAC) method. The "typical vegetable" and "typical fruit" in Japan contained hydrophilic antioxidants that are equivalent to 6.95 and 12.23 μmol of Trolox per g of the edible portion, respectively, on average. Hence, the daily intake of hydrophilic antioxidants from vegetables and fruits was estimated to be 4423 μmol Trolox equivalent (TE)/d based on data of the National Health and Nutrition Survey in Japan. However, the biological significance of these antioxidant values has not yet been clarified. To address this issue, our data will provide a foundation for high-quality epidemiological studies aimed at elucidating the relationship between daily intake of antioxidants and health. In addition, the comparison of the results of the H-ORAC assay with those of polyphenol content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was also studied. The H-ORAC values had a strong positive correlation with polyphenol contents ($r = 0.956$), and were 1.0–18.2-times higher than the antioxidant capacities evaluated by the DPPH assay.

1. Introduction

There is growing evidence that reactive oxygen species (ROS) are related to many diseases and that foods rich in antioxidants may contribute to overall health and disease prevention (Lotito and Frei, 2006; Osawa and Kato, 2005). There is a wide variety of different types of antioxidants in foods, and these antioxidants occasionally act coordinately in the body. Therefore, the efficacy of dietary antioxidants needs to be investigated not only from the viewpoint of individual antioxidants but also from the viewpoint of the sum of antioxidants contained in foods, that is, the concept of "antioxidant capacity" (AOC) of foods. Valtueña et al. (2008) found in a human trial that food selection based on AOC of foods can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. Thus, not only individual antioxidants but also AOC of foods may be helpful to improve dietary quality for maintenance of good health.

Several observational epidemiologic studies showed significant associations between AOC intake and health (Brighenti et al., 2005; Psaltopoulou et al., 2011; Puchau et al., 2009, 2010; Serafini et al., 2002), whereas other studies showed no significant association (Devore et al., 2010; Mekary et al., 2010). One possible reason for this discrepancy in results is that appropriate AOC databases were

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not used; i.e., the databases contained data for a limited number of food items and/or AOC values of foods marketed in a foreign country. Hence, to obtain more conclusive evidence from epidemiological studies, an AOC database of higher quality is required.

An oxygen radical absorbance capacity (ORAC) method is one of the most widely used methods for evaluating AOC due to known and unknown antioxidants present in tested foods (Prior et al., 2005). Peroxyl radical-scavenging activities of water-soluble and lipid-soluble antioxidants in samples are evaluated by the hydrophilic ORAC (H-ORAC) and lipophilic ORAC (L-ORAC) methods, respectively (Prior et al., 2003). Rautiainen et al. (2008) reported that more than half of ORAC intake from all foods in Sweden is attributed to vegetables and fruits. In vegetables and fruits, H-ORAC values are usually much higher than L-ORAC values (Wu et al., 2004a).

The final goal of this study is to elucidate the relationship between daily intake of antioxidants and health. For this purpose, a reliable analytical method is needed to establish an appropriate AOC database of foods for epidemiological studies. Recently, the analytical precision of the H-ORAC method was improved (Watanabe et al., 2012). This improved H-ORAC method is one of the few analytical methods for evaluating AOC of foods validated by an interlaboratory test in accordance with internationally harmonized protocol (Horwitz, 1995). Based on this reliable method, an antioxidant database of foods suitable for the Japanese population has been developed. This database will enable high-quality epidemiological studies concerning daily intake of antioxidants and health in Japan. In our preliminary note (Takebayashi et al., 2010), H-ORAC values of 23 vegetables commonly consumed in Japan have been reported and the daily intake of hydrophilic antioxidants from these vegetables in Japan has been estimated for the first time to our knowledge. However, the sample size for each vegetable was very limited ($n = 1$), and thus representative values and variances of the H-ORAC values for each vegetable were unknown. In addition, foods other than vegetables, especially fruits, appear to contribute to the daily AOC intake. Hence, in this study, H-ORAC values of 23 vegetables and 13 fruits from 3 independent samples each were obtained and daily intakes of hydrophilic antioxidants from these vegetables and fruits in Japan were estimated.

2. Materials and methods

2.1. Selection of vegetable and fruit samples

The most consumed vegetables and fruits in Japan were selected for this study according to the annual report on the family income and expenditure survey in Japan for 2005–2009 (Statistics Bureau; Ministry of Internal Affairs and Communications, Japan). This survey showed annual purchase weights per household for raw vegetables and fruits, and the weight for 23 vegetables shown in Table 1 and 13 fruits shown in Table 2 accounted for 83.4% and 84.5% of the total weights for purchased raw vegetables and fruits, respectively. Percentage of intake for each vegetable was calculated as follows:

$$\text{Percentage of intake}\,(\%) = \frac{[W \times (100 - R)/100]}{[\sum\{W \times (100 - R)/100\}]} \times 100,$$

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 – 2010 – (Council for Science and Technology; Ministry of Education, Culture, Sports, Science and Technology, Japan), and $\sum\{W \times (100 - R)/100\}$ (g) is the sum of the estimated annual intake per household of the 23 vegetables listed in Table 1. Percentage of intake for each fruit was calculated in the same manner.

2.2. Chemicals

Sodium fluorescein, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), Folin-Ciocalteu reagent, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Trolox was obtained from Aldrich Chemicals (Milwaukee, WI, USA). All other reagents were of analytical grade.

2.3. Food sample preparation

The 23 vegetables and 13 fruits shown in Tables 1 and 2 were independently purchased in raw form in 3 prefectures of Japan (more details on where and when the samples were collected are shown in the footnotes of Tables 1 and 2). In each case, most of the samples were acquired from one local market, but some samples were bought on the Internet due to unavailability. At least 2 units and 200 g in total for each vegetable and fruit were collected. These samples were cooled on crushed ice before cutting to avoid loss of antioxidants by enzymes such as polyphenol oxidases, and inedible portions were removed. Then, sample size was reduced by sub-sampling, and the edible portions were snap frozen by liquid nitrogen and were promptly freeze-dried. The freeze-dried samples were milled until homogeneous by using a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and stored at −20 °C. The antioxidants in the freeze-dried samples were then extracted by using an accelerated solvent extraction system (Dionex, ASE-200) as described by Wu et al. (2004b) with some modifications. Briefly, each freeze-dried sample was first subjected to extraction with hexane/dichloromethane (1:1) followed by extraction with methanol/water/acetic acid (90:9.5:0.5; MWA), and then the H-ORAC assay, measurement of polyphenol content, and DPPH assay were performed using the same MWA extracts.

2.4. H-ORAC assay

The interlaboratory precision of the original H-ORAC method (Prior et al., 2003) has been shown to be insufficient (Watanabe et al., 2010), and some methodological improvements to assure satisfactory interlaboratory precision have been made (Watanabe et al., 2012). In this study, this improved H-ORAC method was used. Briefly, the MWA extracts were diluted 10-fold with an assay buffer (75 mM KH_2PO_4–K_2HPO_4 at pH 7.4) and then further diluted with the assay buffer containing 10% MWA. Trolox calibration solutions (6.25, 12.5, 25 and 50 μM in the assay buffer containing 10% MWA) were made to obtain a standard curve. The diluted MWA extract, Trolox calibration solution or blank (35 μl), fluorescein (115 μl, 110.7 nM in the assay buffer), and AAPH (50 μl, 31.7 mM in the assay buffer) were incubated in the assay buffer at 37 °C in a 96-well plate sealed with a plastic film. 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 for the sample or standard, and the H-ORAC value for each sample was determined by comparison of net AUC for the sample with that for the Trolox standard. More details are shown in the reference (Watanabe et al., 2012). Data are expressed as moles of Trolox equivalent to the amount of water-soluble antioxidants contained in a sample, that is, μmol Trolox equivalent (TE) per gram fresh weight of the edible portion (μmol TE/g). Each value is the average of determinations for two separately prepared extracts

Table 1
H-ORAC values for vegetables commonly consumed in Japan.

Sample name	Item No.[a]	Percentage of intake (%)[b]	H-ORAC (μmol TE/g)[c]							Contribution (%)[g]	Polyphenol content (mg GAE/g)[h]	DPPH radical scavenging capacity (μmol TE/g)[i]
			1[d]	2[e]	3[f]	Average	SD	RSD (%)	Max/Min			
Onion, bulb	06153	11.2	6.71	13.17	12.28	10.72	3.50	32.7	2.0	17.4	0.61	0.59
Cabbage, head	06061	11.1	3.74	3.88	2.40	3.34	0.82	24.6	1.6	5.3	0.27	1.04
Japanese radish (daikon), root without skin	06134	10.1	3.93	2.93	4.78	3.88	0.92	23.8	1.6	5.6	0.16	0.59
Tomato, fruit	06182	8.6	3.14	3.43	2.86	3.14	0.29	9.1	1.2	3.9	0.28	1.18
Potato, tuber	02017	8.0	6.85	7.72	5.53	6.70	1.10	16.5	1.4	7.7	0.46	1.22
Cucumber, fruit	06065	6.6	1.68	1.54	1.69	1.63	0.08	5.2	1.1	1.5	0.15	0.30
Carrot, root with skin	06212	6.4	5.26	1.46	3.35	3.36	1.90	56.6	3.6	3.1	0.22	0.74
Chinese cabbage, head	06233	6.1	3.10	3.33	1.96	2.80	0.74	26.4	1.7	2.4	0.22	0.82
Mung bean sprout	06291	4.4	6.17	6.05	5.57	5.93	0.32	5.3	1.1	3.7	0.45	0.46
Crisp lettuce, head	06312	4.3	2.61	1.09	3.02	2.24	1.02	45.4	2.8	1.4	0.15	0.54
Eggplant, fruit	06191	3.4	15.55	28.62	38.79	27.65	11.65	42.1	2.5	13.4	1.39	8.26
Pumpkin, fruit	06046	3.2	3.88	2.78	3.17	3.28	0.56	17.0	1.4	1.5	0.32	0.97
Spinach, leaves	06267	2.8	8.20	10.59	7.29	8.69	1.70	19.6	1.5	3.5	0.35	0.75
Welsh onion (nebuka-negi), leaves, blanched	06226	2.4	2.32	2.72	2.79	2.61	0.26	9.8	1.2	0.9	0.26	0.57
Sweet potato, tuberous root	02006	2.2	6.18	5.28	7.33	6.26	1.03	16.4	1.4	2.0	0.51	2.47
Taro (satoimo), corm	02010	1.7	8.33	7.15	14.83	10.10	4.14	41.0	2.1	2.4	0.46	2.47
Green sweet pepper, fruit	06245	1.6	6.95	7.15	11.83	8.64	2.76	32.0	1.7	2.0	0.84	4.14
Edible burdock, root	06084	1.5	52.17	68.85	77.19	66.07	12.74	19.3	1.5	13.9	3.18	19.37
Broccoli, inflorescence	06263	1.3	11.00	18.74	18.55	16.10	4.41	27.4	1.7	3.1	0.98	3.59
Edamame	06015	1.1	13.32	14.75	8.82	12.29	3.09	25.2	1.7	1.9	0.61	1.74
Shiitake mushroom	08011	1.0	2.58	3.06	2.94	2.86	0.25	8.8	1.2	0.4	0.21	0.62
East Indian lotus root, rhizome	06317	0.8	19.57	27.18	21.60	22.78	3.94	17.3	1.4	2.5	1.64	12.76
Bamboo, shoot[j]	06149	0.5	2.06	4.51	8.39	4.99	3.19	64.0	4.1	0.4	0.64	0.34

[a] Item numbers in the Standard Tables of Food Composition in Japan.

[b] The percentage of intake for each vegetable was estimated from the data in the annual report on the family income and expenditure survey in Japan for 2005–2009 and in the Standard Tables of Food Composition in Japan – 2010. Details are shown in Section 2.

[c] 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).

[d] The samples were commercially purchased in Kumamoto, Japan in June or July of 2009. The H-ORAC values presented in this column are from the reference (Takebayashi et al., 2010).

[e] The samples were commercially purchased in Nagoya, Japan in November or December of 2009 or January 2010.

[f] The samples were commercially purchased in Ibaraki, Japan in August 2010.

[g] The estimated contribution of each vegetable to hydrophlic AOC intake from vegetables. Details are described in Section 2.

[h] The polyphenol content for 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 gallic acid equivalent (GAE)/g of edible portion). The average values from 3 independent samples each are given. For details see Supplementary Data (Table S1).

[i] The DPPH radical scavenging capacity for each vegetable is expressed as μmol 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). The average values from 3 independent samples each are given. For details see Supplementary Data (Table S3).

[j] Bamboo shoots, canned in water, were used because raw bamboo shoots were seasonally unavailable.

from one freeze-dried sample of each vegetable and fruit to reduce the error associated with extraction and measurement of H-ORAC.

2.5. Calculations of the averaged H-ORAC values for "typical vegetable" and "typical fruit" consumed in Japan and contribution of each vegetable and fruit to hydrophilic AOC intake

To obtain the averaged H-ORAC value for "typical vegetable" consumed in Japan, the H-ORAC values for 23 vegetables were weighted-averaged considering their consumptions as follows:

Averaged H-ORAC value for "typical vegetable" (μmol TE/g)

$$= \sum \{\text{H-ORAC} \times P/100\},$$

where H-ORAC (μmol TE/g) is the H-ORAC value for each vegetable, and P (%) is the percentage of intake for the corresponding vegetable.

The contribution of each vegetable to intake of hydrophilic antioxidants from vegetables was calculated as follows:

$$\text{Contribution}\,(\%) = \text{H-ORAC} \times P/\text{Avg. H-ORAC},$$

where Avg. H-ORAC (μmol TE/g) is the averaged H-ORAC value for "typical vegetable".

The averaged H-ORAC value for "typical fruit" and contribution of each fruit were calculated in the same manner.

2.6. Measurement of polyphenol content

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 (ISO, 2005) with slight modifications. Briefly, the MWA extract was diluted more than two-fold with water. The diluted MWA extract, gallic acid solution or blank (1.0 ml) was dispensed into tubes. A 1/10 dilution of the Folin-Ciocalteu reagent in water (5.0 ml) was added to each tube. After an interval of 3–8 min, a sodium carbonate solution (7.5%, w/v, 4.0 ml) 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 and fruit to reduce

Table 2
H-ORAC values for fruits commonly consumed in Japan.

Sample name	Item No.[a]	Percentage of intake (%)[b]	H-ORAC (μmol TE/g)[c]							Contribution (%)[g]	Polyphenol content (mg GAE/g)[h]	DPPH radical scavenging capacity (μmol TE/g)[i]
			1[d]	2[e]	3[f]	Average	SD	RSD (%)	Max/Min			
Banana	07107	20.5	8.69	8.61	5.14	7.48	2.03	27.1	1.7	12.5	0.62	3.56
Apple	07148	20.0	16.89	ND[j]	19.14	18.02	–	–	1.1	29.5	0.85	4.26
Satsuma mandarin, juice sacs, early ripening type	07028	19.5	13.28	12.93	14.49	13.57	0.82	6.0	1.1	21.6	0.77	1.72
Japanese pear	07088	7.6	1.89	ND	1.28	1.58	–	–	1.5	1.0	0.12	0.32
Strawberry	07012	5.7	34.32	29.79	36.30	33.47	3.34	10.0	1.2	15.7	2.29	15.74
Japanese persimmon (kaki), nonastringent	07049	4.9	7.54	4.75	4.62	5.64	1.65	29.2	1.6	2.3	0.74	5.48
Watermelon	07077	4.9	1.84	1.79	1.98	1.87	0.10	5.3	1.1	0.7	0.14	0.30
Grape	07116	4.0	3.23	4.21	4.21	3.88	0.57	14.6	1.3	1.3	0.29	1.21
Grapefruit, juice sacs	07062	3.3	17.23	21.28	15.89	18.13	2.80	15.5	1.3	4.9	1.01	3.38
Peach	07136	3.1	28.59	ND	17.96	23.28	–	–	1.6	5.9	0.92	5.71
Melon, open culture	07135	2.8	2.80	1.86	2.99	2.55	0.60	23.7	1.6	0.6	0.28	1.03
Kiwifruit	07054	2.0	6.45	7.66	9.02	7.71	1.28	16.6	1.4	1.3	0.90	5.78
Valencia orange, juice sacs	07041	1.7	18.91	21.21	20.26	20.13	1.16	5.8	1.1	2.8	1.24	3.43

[a] Item numbers in the Standard Tables of Food Composition in Japan.

[b] The percentage of intake for each fruit was estimated from the data in the annual report on the family income and expenditure survey in Japan for 2005–2009 and in the Standard Tables of Food Composition in Japan – 2010. Details are shown in Section 2.

[c] The H-ORAC value for each fruit is expressed as moles of Trolox that are equivalent to the amount of water-soluble antioxidants contained in 1 g of the raw fruit (μmol Trolox equivalent (TE)/g of edible portion).

[d] The samples were commercially purchased in Kumamoto, Japan in August or September of 2009.

[e] The samples were commercially purchased in Nagoya, Japan in November or December of 2009 or January 2010.

[f] The samples were commercially purchased in Ibaraki, Japan in August or September of 2010.

[g] The estimated contribution of each fruit to hydrophlic AOC intake from fruits. Details are described in Section 2.

[h] The polyphenol content for each fruit is expressed as mg of gallic acid that are equivalent to the amount of polyphenols contained in 1 g of the raw fruit (mg gallic acid equivalent (GAE)/g of edible portion). The average values from 2 or 3 independent samples each are given. For details see Supplementary Data (Table S2).

[i] The DPPH radical scavenging capacity for each fruit is expressed as μmol of Trolox that are equivalent to the amount of water-soluble antioxidants contained in 1 g of the raw fruit (μmol Trolox equivalent (TE)/g of edible portion). The average values from 2 or 3 independent samples each are given. For details see Supplementary Data (Table S4).

[j] ND means not determined.

the error associated with extraction and measurement of polyphenol content.

2.7. DPPH assay

The DPPH radical scavenging capacity was measured by the method described previously (Oki et al., 2003) with a slight modification. Briefly, the MWA extracts were diluted two-fold with a 10% methanol aqueous solution and then further diluted with a 50% methanol aqueous solution. Trolox calibration solutions (40, 80, 120 and 160 μM in a 50% methanol aqueous solution) were made to obtain a standard curve. The diluted MWA extract, Trolox calibration solution or blank (100 μl), a 200-mM 2-morpholinoethanesulphonic acid (MES) buffer (pH 6.0) (50 μl), and a DPPH solution (50 μl, 0.8 mM in ethanol) were pipetted to each well of a 96-well plate. After the reaction mixture had been allowed to stand for 20 min at 30 °C, its absorbance at 520 nm was measured (Multiskan JX, Thermo Labsystems Oy, Vantaa, Finland). The DPPH radical scavenging capacity was expressed as moles of Trolox equivalent per gram (μmol TE/g). Each value is the average of determinations for two separately prepared extracts from one freeze-dried sample of each vegetable and fruit to reduce the error associated with extraction and measurement of DPPH radical scavenging capacity.

3. Results and discussion

3.1. H-ORAC values of vegetables and fruits commonly consumed in Japan

The H-ORAC values, which reflect the sum of hydrophilic antioxidants contained in samples, for the 23 vegetables are shown in Table 1. The values ranged from 1.63 to 66.07 μmol TE/g. The relative standard deviation (RSD) of H-ORAC values from 3 independent samples each ranged from 5.2 to 64.0%, and their max/min ratios ranged from 1.1 to 4.1. The five vegetables possessing the most potent antioxidative activities were edible burdock, eggplant, East Indian lotus root, broccoli and edamame in that order. The averaged H-ORAC value for "typical vegetable" in Japan was obtained by weighted averaging the H-ORAC values for vegetables evaluated according to their consumption as shown in Section 2. As a result, the averaged H-ORAC value for "typical vegetable" was calculated to be 6.95 μmol TE/g. Then the contribution of each vegetable to intake of hydrophilic antioxidant from vegetables was calculated as shown in Section 2. The top five vegetables contributing to hydrophilic AOC intake were onion, edible burdock, eggplant, potato and Japanese radish (daikon) in that order. Of them, onion, potato and Japanese radish (daikon) had medium H-ORAC values, but they were frequently consumed. The contributions of edible burdock and eggplant were due to their high H-ORAC values rather than their percentages of intake.

The H-ORAC values for the 13 fruits ranged from 1.58 to 33.47 μmol TE/g (Table 2). The RSD of H-ORAC values from 3 independent samples each ranged from 5.3 to 29.2%, and their max/min ratios ranged 1.1–1.7. The five fruits possessing the most potent antioxidative activities were strawberry, peach, Valencia orange, grapefruit and apple in that order. The weighted average of H-ORAC values for "typical fruit" was calculated to be 12.23 μmol TE/g. The top five fruits contributing to hydrophilic AOC intake were apple, Satsuma mandarin, strawberry, banana and peach in that order. Of them, apple, strawberry, and peach possessed both high H-ORAC values and intake frequencies, whereas the H-ORAC values for Satsuma mandarin and banana were medium levels, but their consumption frequencies were high.

3.2. Estimation of daily intake of hydrophilic antioxidants from vegetables and fruits in Japan

On average, the "typical vegetable" and "typical fruit" consumed in Japan were estimated to contain hydrophilic antioxidants that are equivalent to 6.95 and 12.23 μmol of Trolox per g, respectively. In the recent National Health and Nutrition Survey in Japan (Ministry of Health, Labour and Welfare, Japan), daily intakes of vegetables (including vegetables, potatoes, pulses, and mushrooms) and fruits for adults (≥20 years old) were 427 and 119 g/d, respectively, on the 5-year average (2004–2008). From these data, daily intake of hydrophilic antioxidants was estimated to be 4423 μmol TE/d (2967 μmol TE from vegetables and 1456 μmol TE from fruits). On the other hand, the Japanese government recommends consumption of at least 350 g of vegetables per day (Health Japan 21 (Udagawa et al., 2008)) and 2 servings (ca. 200 g) of fruit per day (Japanese Food Guide Spinning Top (Yoshiike et al., 2007)). Hence, if 350 g/d of "typical vegetable" and 200 g/d of "typical fruit" are consumed, 4879 μmol TE/d of hydrophilic antioxidants (2432 μmol TE from vegetables and 2447 μmol TE from fruits) would be ingested.

In this way, the daily intake of hydrophilic antioxidants from vegetables and fruits was estimated to be 4423 μmol TE/d in Japan based on the data of the National Health and Nutrition Survey. This value is about 10% lower than the value based on the government recommendations. However, it should be emphasized here that this does not mean a deficiency of hydrophilic antioxidants in Japan, because hydrophilic antioxidants are contained in other foods, such as tea beverages and soy foods, that are consumed frequently in Japan. Also, it is unclear whether 4423 μmol TE of hydrophilic antioxidants from vegetables and fruits is "not enough" or "just enough" to maintain health. Further accumulation of experimental data as well as epidemiological studies are required to clarify the appropriate daily AOC intake.

3.3. Relationships of H-ORAC values with polyphenol contents and DPPH radical scavenging capacities of vegetables and fruits

Since polyphenolic compounds contained in vegetables and fruits appeared to be major contributors to H-ORAC, the relationship between H-ORAC values and polyphenol contents was investigated. It was found that H-ORAC values for the vegetables and fruits had a strong positive correlation with polyphenol contents ($r = 0.956$, Fig. 1), suggesting that the antioxidative activities of the vegetables and fruits evaluated result mainly from their contained polyphenolic compounds. Wu et al. (2004a) reported that there was a strong positive correlation between H-ORAC values and polyphenol contents in vegetables and fruits, in accordance with our results, though the correlation was not high in all of the foods analyzed (including vegetables, fruits, nuts, spices and grain-based foods). The averaged values of polyphenolic contents of the vegetables and fruits are shown in Tables 1 and 2 (the full results are shown in Tables S1 and S2 in Supplementary data). The weighted average of polyphenol contents for "typical vegetable" and "typical fruit" were calculated to be 0.43 and 0.75 mg GAE/g, respectively, and the estimated daily intake of polyphenolic compounds would be 272 mg GAE/d (183 mg GAE from vegetables and 89 mg GAE from fruits) in Japan based on the data of the National Health and Nutrition Survey.

The DPPH assay is an in vitro assay widely used to measure AOC for foods (Prior et al., 2005). To evaluate the comparability between the H-ORAC assay and the DPPH assay, the relationship between H-ORAC and DPPH radical scavenging capacities was also investigated. It was found that H-ORAC values for the vegetables and fruits had a positive correlation with DPPH radical scavenging capacities ($r = 0.894$, Fig. 2), but the results of the H-ORAC assay were 1.0–18.2-times higher than the results of the DPPH assay. This large variance in the ratio between two values made it impossible to estimate the H-ORAC values of foods from the results of the DPPH assay (and perhaps other antioxidant assays). The average values of the DPPH assay of the vegetables and fruits are shown in Tables 1 and 2 (the full results are shown in Tables S3 and S4 in Supplementary data). The weighted average of DPPH radical scavenging capacities for "typical vegetable" and "typical fruit" were calculated to be 1.56 and 3.67 μmol TE/g, respectively.

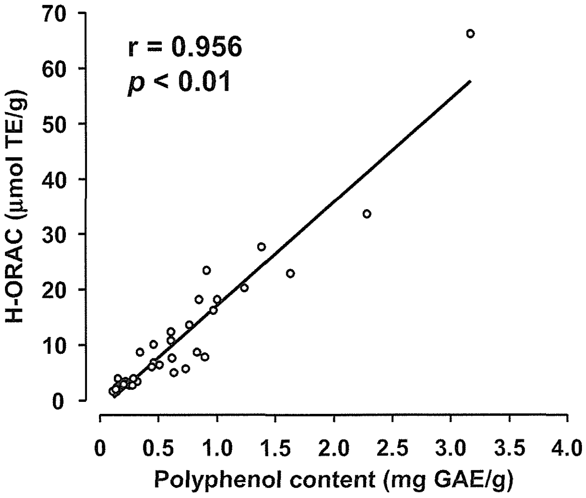

Fig. 1. Relationship between H-ORAC value and polyphenol content of vegetables and fruits commonly consumed in Japan.
Closed circle: vegetable; open circle: fruit; solid line: regression line. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

3.4. Comparison of the results of the present study with results of previous studies

The H-ORAC values obtained in this study were roughly consistent with the H-ORAC values reported by the United States

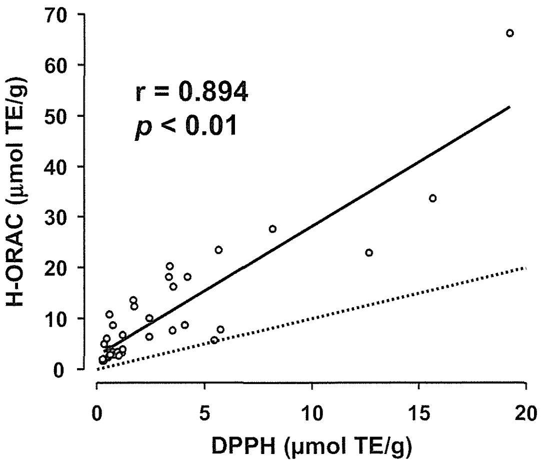

Fig. 2. Relationship between H-ORAC and DPPH values of vegetables and fruits commonly consumed in Japan.
Closed circle: vegetable; open circle: fruit; solid line: regression line; dotted line: $y = x$. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

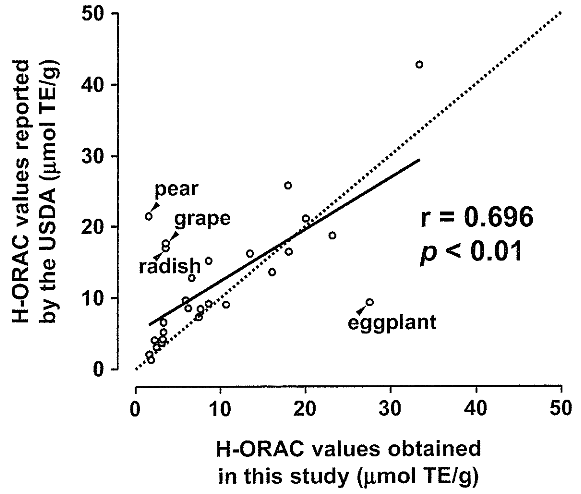

Fig. 3. Comparison of H-ORAC values in this study with those reported by USDA. Closed circle: vegetable; open circle: fruit; solid line: regression line; dotted line: $y = x$. The correlation was calculated by using a Pearson product moment correlation coefficient (r).

Department of Agriculture (USDA) (published on the web: http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/ORAC/ORAC_R2.pdf: accessed December 2011) ($r = 0.696$, Fig. 3). However, some foods, including radish, eggplant, pear and grape, showed large differences in H-ORAC values between Japan and the USA. This may be due to difference in cultivars on the marketplaces. In addition, some foods commonly consumed in Japan, such as Chinese cabbage, Welsh onion, edible burdock, Edamame, shiitake mushroom, East Indian lotus root, bamboo shoot and Japanese persimmon, are not included in the USDA's ORAC database. Of them, edible burdock and East Indian lotus root are rarely eaten as regular dietary components in countries other than Japan, but they appear to be major contributors to daily hydrophilic AOC intake in Japan (Table 1). Hence, a suitable ORAC database for foods consumed in Japan should be established for accurate estimation of AOC intake in the Japanese population.

There have been several reports on daily AOC intake (Chun et al., 2005; Pellegrini et al., 2007; Rautiainen et al., 2008; Wu et al., 2004a; Yang et al., 2011). Wu et al. (2004a) calculated H-ORAC intake from vegetables and fruits in the USA to be 5558 μmol TE/d (2385 μmol TE/d from vegetables and 3174 μmol TE/d from fruits). Rautiainen et al. (2008) estimated the total ORAC (H-ORAC + L-ORAC) intake from vegetables and fruits in Sweden to be 6523 μmol TE/d. These values are generally in accordance with the value estimated in the present study (4423 μmol TE/d). Daily AOC intake was also estimated in other studies (Chun et al., 2005; Pellegrini et al., 2007; Yang et al., 2011), but antioxidant databases used in those study were not based on the ORAC assay. Thus, a direct comparison is impossible because antioxidant capacity of foods differs greatly depending on the assay used.

3.5. Issues and perspectives of this study

In this study, the sum of hydrophilic antioxidants contained in raw vegetables and fruits commonly consumed in Japan was focused, but there are some limitations in the data presented here. (1) Each of the foods also contains a certain amount of lipophilic antioxidants. Thus, the sum of lipophilic antioxidants needs to be measured by the L-ORAC method in a future study. (2) ORAC data for foods other than vegetables and fruits are important. Rautiainen et al. (2008) showed that contributors to the total ORAC intake from all foods in Sweden were fruits (34.8%), vegetables (21.6%), grain products (19.7%), tea (9.5%), chocolate (4.9%), juice (3.9%) and wine (2.5%). Fukushima et al. (2009) suggested that beverages, especially coffee and green tea, contributed greatly to the consumption of polyphenols in the Japanese diet. Furthermore, soy beans and soy bean products were not examined in the present study, but they are consumed frequently in Japan and contain isoflavones, which have potent ORAC activities (Rüfer and Kulling, 2006). (3) The effects of cooking and combinations of ingredients need to be clarified. Yamaguchi et al. (2001) and Jiménez-Monreal et al. (2009) studied changes in AOC of several vegetables during cooking and showed that AOC can change positively or negatively after cooking. Taking the above issues into consideration, a more comprehensive ORAC database is needed for estimation of overall daily intake of antioxidants.

4. Conclusions

The H-ORAC values for 23 vegetables commonly consumed in Japan ranged from 1.63 to 66.07 (average 6.95) μmol TE/g. The H-ORAC values for 13 fruits ranged from 1.58 to 33.47 (average 12.23) μmol TE/g. The daily intake of hydrophilic antioxidants from vegetables and fruits in Japan was estimated to be 4423 μmol TE/d (2967 μmol TE from vegetables and 1456 μmol TE from fruits). This value is generally in accordance with the daily ORAC intakes reported in earlier papers. The H-ORAC values for the vegetables and fruits evaluated had a strong positive correlation with polyphenol contents ($r = 0.956$), suggesting that polyphenolic compounds are major contributors to H-ORAC. The H-ORAC values correlated with DPPH radical scavenging capacities ($r = 0.894$), but the results of the H-ORAC assay were 1.0–18.2-times higher than the results of the DPPH assay. The H-ORAC values obtained here were roughly consistent with those reported by the USDA ($r = 0.696$), but some foods showed largely different H-ORAC values or are not included in the USDA's ORAC database. To elucidate the relationship between daily intake of antioxidants and health, continued research is needed on establishing a more comprehensive AOC database including L-ORAC values, other foods and effects of cooking.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2012.10.006.

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研究論文紹介

日本において一般的に食されている野菜・果物の親水性抗酸化能およびこれらの食品からの親水性抗酸化物質一日摂取量の推算*

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Hydrophilic antioxidant capacities of vegetables and fruits commonly consumed in Japan and estimated average daily intake of hydrophilic antioxidants from these foods

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近年，がん，糖尿病といった生活習慣病，アルツハイマー病など種々の疾病の発症および増悪に活性酸素・フリーラジカルが関与していることが明らかとなってきた．このような活性酸素・フリーラジカルを消去し，健康を維持・増進するため，野菜，果物などの食品に豊富に含まれる抗酸化物質に大きな関心が寄せられている．我々は，通常の食事から複数の抗酸化物質を組み合わせて摂取している．そのため，個々の抗酸化物質について個別に研究するだけでなく，抗酸化物質の総量を反映する食品の抗酸化能（Antioxidant Capacity：AOC）に着目した研究が必要である．現在までに，AOC を指標として用い，抗酸化物質の総摂取量と健康に関する疫学研究が幾つか報告されているが，AOC の高い食事が健康維持に有用であることを示す研究[1)-5)]がある一方，無関係とする研究[6)7)]もある．研究結果が一致しない原因の一つとして，研究に用いた食品の抗酸化能データベースが不適切であり，食品数が限られていたり，名称は同一だが品種が異なる海外の食品の分析結果をそのまま引用しているため，AOC 摂取量を正確に推算できていない可能性が考えられる．抗酸化物質摂取の健康影響を疫学研究で明らかにするためには，調査対象者に合致した食品の抗酸化能データベースを構築することが重要である．

今回紹介する論文は，上記の背景を踏まえ，我が国において一般的に食されている野菜・果物に含まれる親水性抗酸化物質の総量を hydrophilic-oxygen radical

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absorbance capacity (H-ORAC) 法を用いて数値化したものである．H-ORAC 法は食品中の親水性抗酸化物質のペルオキシルラジカル消去活性を評価する方法であり，AOC 評価法として広く用いられている[8]．信頼性の高いデータベースを構築するためには，分析法の標準化 (standardization) が重要である．近年，著者らは H-ORAC 法の分析精度向上に成功しており，その妥当性を室間共同試験で確認している (Method Validation, 標準作業手順書に従って，誰がどこで分析しても測定結果が一定の範囲内に収まることを実証)[9]．今回の測定はこの改良 H-ORAC 分析法で行った．

総務省が実施している家計調査[10]には，23 種類の野菜と 13 種類の果物の購入重量が記載されており，その割合は全野菜・果物の購入重量の実に 8 割以上を占める．このことから，家計調査に具体名が示された野菜・果物を日本において一般的に食されているものとし，その H-ORAC 値を測定した (図 1)．その結果，野菜ではなす，ごぼう，れんこんに，果物ではいちご，もも，オレンジに高い抗酸化能が認められた．野菜・果物別に摂食量を考慮して H-ORAC 値を加重平均すると，野菜の H-ORAC 値は平均 6.95 μmol Trolox-equivalent (TE) /g であり，果物のその値は平均 12.23 μmol TE/g であった．加重平均値への各野菜・果物の寄与度を図 2 に示す．寄与度の高い野菜はたまねぎ，ごぼう，なす，果物はりんご，みかん，いちごであった．これらのうち，たまねぎ，みかんなどではそれらの

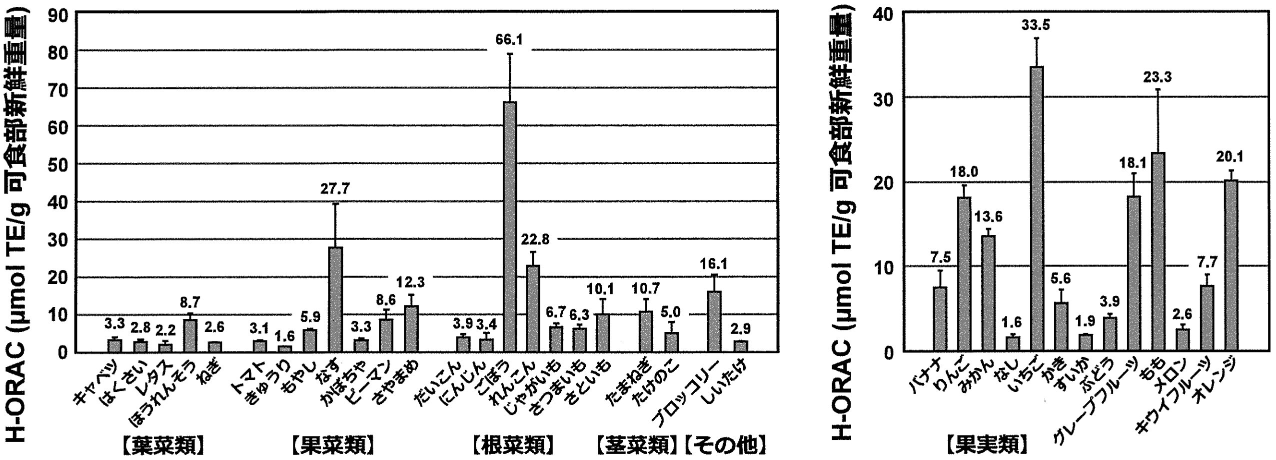

図 1　日本において一般的に食される野菜・果物の親水性抗酸化能

H-ORAC 法を用いて各野菜・果物の抗酸化能を測定した．結果は，可食部 1 g に含まれる親水性抗酸化物質と等しい抗酸化活性を示す Trolox のモル数で表した (μmol Trolox-equivalent (TE) /g)．3 地域で購入した検体の分析結果 (平均 ± 標準偏差) を示した．

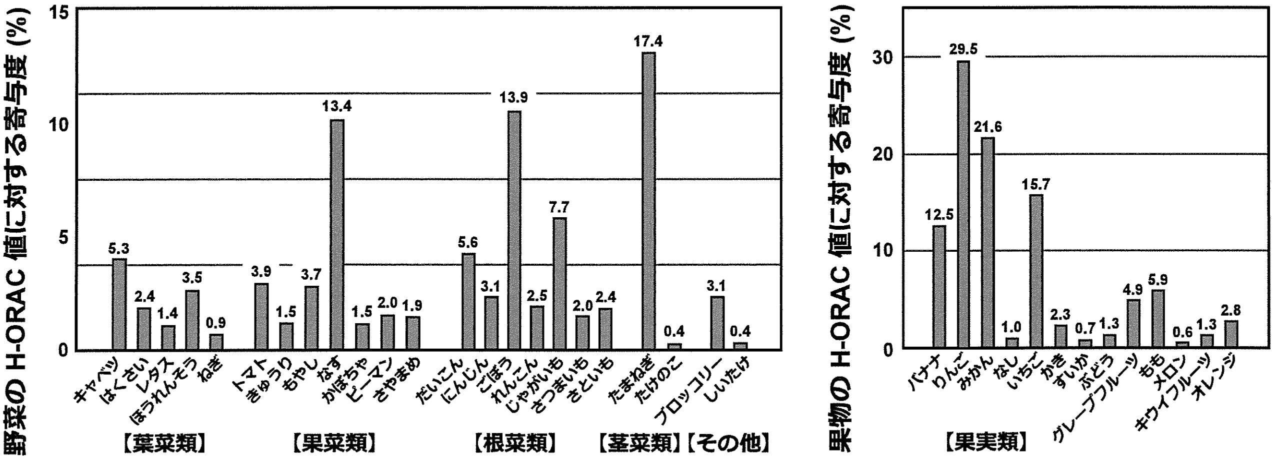

図 2　野菜・果物の平均 H-ORAC 値への各野菜・果物の寄与度

H-ORAC値は中程度であるが摂食量が多いことが，またごぼう，なすなどではそれらの摂食量は多くないけれどH-ORAC値が高いことが，寄与度が高い理由であった．

国民健康・栄養調査(2005～2009年)[11)]によると，野菜(野菜類＋いも類＋豆類＋きのこ類)および果物の一日摂取量はそれぞれ427および119 gである．したがって，野菜・果物からの親水性AOC一日摂取量は4423 μmol TE(野菜から2967 μmol TE，果物から1456 μmol TE)であると推算された．なお，この4423 μmol TEという数字であるが，この量の親水性抗酸化物質が健康の維持・増進に充分なのか，さらなる摂取を必要とするのか，あるいは健康とは無関係なのか，現時点では不明である点に注意する必要がある．抗酸化物質摂取の健康影響を明らかにするためには，食品の抗酸化能データベースをさらに充実させ，高精度の疫学研究を実施することが必須である．

最後に，著者らが食品のORAC値に着目して研究している意図を説明したい．食品のORAC値については，以前からその生体に及ぼす影響について多くの問題点が指摘されている[12)]．著者らも，個々の食品や食品由来成分について，そのORAC値のみを指標としてその生体に及ぼす影響を論ずるのは不適切だと認識している．しかし，著者らが対象としているのは，単一の食品や抗酸化成分ではなく，日々の食事に含まれる全抗酸化物質であり，疫学的アプローチでその健康に及ぼす影響を明らかにしたいと考えている．疫学研究において，食品の機能性を測るための最も基本的な指標はその重量であろう．野菜・果物は，種類によって当然生体に及ぼす影響が異なるが，それらの総摂取重量が種々の生活習慣病の罹患率と逆相関することを示す疫学研究は数多く，野菜・果物の摂取が推奨される強固なエビデンスとなっている．*In vitro*での食品のORAC値は，直接*In vivo*での抗酸化活性を示すものではないが，野菜・果物の摂取重量と同様に食生活の質を測る指標の一つである．AOCの高い「食生活」が健康に寄与するかを明らかにすることが，著者らの研究意図である．

(平成25.2.28受付)

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