Distribution and Major Sources of Flavonoid Intakes in the Middle-Aged Japanese Women

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Summary

We estimated the intake of individual flavonoids in a cross sectional study and clarified the major sources contributing to the flavonoid levels in the middle-aged Japanese women by a 24-h weighed dietary record study. The subjects included in the study were 516 free-living women. Each subject completed a 24-h weighed dietary record and received a health check-up. We used the Functional Food Factor database for estimating the intake of 5 major flavonoid intakes, i.e. flavan-3-ols, isoflavones, flavonols, flavanones and flavones. The mean intake of flavan-3-ols, isoflavones, flavonols, flavanones and flavones was 1277, 216, 58, 31 and 15 μmol/d, respectively. The richest source of flavan-3-ols was green tea. The 3 major food sources of isoflavone were the processed soy foods and those of flavonol were the onion, moroheiya (nalta jute) and Japanese radish leaves. Grapefruit and citrus fruit juices were the major sources of flavanones, and tsurumurasaki (malabar spinach), green pepper and grapefruit were the main sources of flavone. Furthermore, analysis of sub-samples from middle-aged Japanese women indicated that there may be an association between flavonoid intake and the levels of oxidized LDL, which might be related to the incidence of cardiovascular diseases.

Key Words: 24-hour weighed dietary record, functional food factor database, flavonoids, oxidized LDL, cardiovascular diseases

Introduction

Several epidemiological studies show that flavonoid intake might decrease the risk of coronary heart disease (CHD) in elderly people [1–4]. It has been suggested that flavonoids scavenge free radicals, chelate metal ions and prevent the oxidation of low density lipoprotein (LDL) in vitro. Oxidized LDL plays an important role in the formation of atherosclerotic plaques. Animal studies [5, 6] and some human intervention studies [7, 8] have reported that flavonoids delay the lag time of LDL to oxidation.

Fruits and vegetables are the main sources of flavonoids. Flavonoids have 2 aromatic rings that are bound by an oxygenated heterocyclic ring. On the basis of their chemical structure, they are divided into several subclasses: flavones, flavonols, flavanones, flavan-3-ols, anthocyanins and isoflavones. In our diet, flavones and flavonols are found in leaf vegetables. Flavanones are mainly found in citrus fruits. Tea and cacao are the richest sources of flavan-3-ols. Soy and soy products are the main sources of isoflavones [9].

In the 1970’s, Kuhnau estimated the flavonoid intake to be
up to 1 g/d [10]. More recently, some studies in Western countries have assessed the subclasses of flavonoid intakes with the food frequency questionnaire (FFQ). These studies estimated the total flavonoid intake to be 1–200 mg/d in Western countries [11, 12]. However, there are few reports that assessed the intake of other flavonoid subclasses, besides isoflavones, in Japan [13, 14]. Therefore, we estimated the intake of individual flavonoids, namely, flavones, flavonols, flavanones, flavan-3-ols and isoflavones, in a cross sectional study. Furthermore, we clarified major food sources contributing to the flavonoid levels in middle-aged Japanese women by a 24-h weighed dietary record (DR) study in order to understand of weighed DR-FFQ relationship.

Methods

Survey sample and brief methodology

This cross-sectional study was carried out from 1996 to 2005 in the northern part of Japan. This study included 569 female volunteers, who agreed to participate in the study and provided their written informed consent. The study design was approved by the ethical committee of the Tokyo University of Agriculture. The subjects were asked to record the weight of all the food they ingested for 1 d as well as cooking methods adopted on that day. The DRs were checked the next day through interviews with trained dieticians.

The anthropometric data and blood samples for biochemical analysis were obtained on the same day as dietary recording. Anthropometric data, including the height, weight, and blood pressure were measured. Casual blood pressure was measured in the sitting position after a 5-min rest by the auscultatory method. The body mass index (BMI, kg/m²) was calculated from the height and weight.

After an overnight fast, blood samples were collected and used for biochemical analysis of total cholesterol (TC), triacylglycerol (TG) and high density lipoprotein cholesterol (HDL-C). Biochemical analysis was performed with a clinical analyzer (Hitachi 7250, Hitachi, Tokyo, Japan). The low density lipoprotein cholesterol (LDL-C) concentration in the plasma was calculated using the Friedewald formula. Other measurements obtained by a health questionnaire included lifestyle variables such as the smoking status, alcohol intake and use of medication.

We examined the association between the flavonoid intake and oxidized LDL levels in the sub-samples. The oxidized LDL in 39 postmenopausal subjects was measured in 2006. The monoclonal antibody DLH3 was used to quantify the concentration of oxidized LDL in the plasma. This assay was carried out using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer’s instructions (MX, Kyowa Medex, Tokyo, Japan). Other biochemical analysis and DRs were collected in a similar manner as mentioned above. Normal LDL-C levels were noted in the case of 25 subjects, and 14 subjects had hyper LDL cholesterol. Subjects with an LDL-C concentration of >140 mg/dl, were divided into 2 groups on the basis of the oxidized LDL levels in the plasma: a low plasma oxidized LDL group and high plasma oxidized LDL group.

Each recorded food item was coded, and the consumption dose and frequency were determined. Food nutrients were calculated with the Standard Tables of Food Composition in Japan (5th revised edition).

The intake of flavonoids was estimated with the Functional Food Factor (FFF) database [15–17]. This database calculated 44 polyphenols, 6 carotenoids, and 7 sulfur compounds. However, we estimated the intake of major flavonoids (flavan-3-ols, epicatechin, epigallocatechin and catechin: isoflavones, daidzein and genistein: flavonols, quercetin, kaempferol, myricetin and fisetin: flavones, luteolin and apigenin, and flavanones, hesperetin and narinigenin). In the FFF database, the values of epicatechin gallate and epigallocatechin gallate are converted into nongallate type values.

For this analysis, we excluded women who did not fill in the health questionnaire and those with an implausibly high (>4000 kcal) or low (<1000 kcal) total energy intake. After these exclusions, 514 (91%) women were included in the analysis.

Statistics

The mean intake with standard deviation (SD) and median intake with the interquartile range are presented for each flavonoid. The percentage intake of individual flavonoids from different food sources was also presented.

Data manipulation and statistical analysis of the data were conducted using MS Excel and the statistical package for social sciences (SPSS) for Windows version 12.0. Differences in flavonoid intake among the groups were compared using Wilcoxon’s rank sum tests.

Results

Anthropometric data and biochemical analysis data are shown in Table 1, and the mean energy and nutrient intake are shown in Table 2. The percentage of subjects who did not consume flavan-3-ol was 22.5%; flavonol, 8.3%; flavanone, 56.2% and flavone, 89.1%. All study participants consumed isoflavone from soy products (data not shown). The average intake of flavonoids is shown in Table 3.

Table 4 shows the important sources of flavonoid. The main food sources of flavonol were onion, moroheiya (nalta jute) and Japanese radish leaves; those of isoflavone were soy processed food, such as tofu, natto and miso; that
Table 1. Anthropometric and plasma biochemical characteristics of this study population

|          | AVE | SD  | 10  | 50  | 90  |
|----------|-----|-----|-----|-----|-----|
| Age (years) | 58  | 10  | 45  | 59  | 71  |
| HT (cm)   | 152 | 6   | 145 | 152 | 159 |
| BW (kg)   | 54  | 8   | 45  | 53  | 65  |
| BMI (kg/m²) | 23  | 3   | 20  | 23  | 28  |
| Body fat (%) | 31  | 6   | 23  | 30  | 38  |
| Systolic BP (mmHg) | 125 | 17  | 104 | 126 | 146 |
| Diastolic BP (mmHg) | 74  | 10  | 62  | 74  | 86  |
| TG (mg/dL) | 97  | 58  | 45  | 79  | 167 |
| TC (mg/dL) | 222 | 39  | 173 | 219 | 271 |
| HDL-C (mg/dL) | 62  | 15  | 45  | 60  | 83  |
| LDL-C (mg/dL) | 141 | 35  | 98  | 137 | 185 |

HT, height; BW, body weight; BMI, body mass index; BP, blood pressure; TG, triacylglycerol; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

Table 2. Energy, nutrients and foods intake in middle aged Japanese women

|          | AVE | SD  | 10  | 50  | 90  |
|----------|-----|-----|-----|-----|-----|
| Energy (kcal) | 1898 | 412 | 1382 | 1880 | 2416 |
| Protein (%E) | 16  | 3   | 12  | 16  | 20  |
| Fat (%E)   | 23  | 6   | 16  | 23  | 32  |
| Carbohydrate (%E) | 60  | 7   | 51  | 61  | 70  |
| Fiber (g)  | 19  | 7   | 12  | 18  | 27  |
| β-carotene (µg) | 4919 | 3529 | 1355 | 4177 | 9365 |
| α-tocopherol (mg) | 8   | 4   | 4   | 8   | 13  |
| Vitamin C (mg) | 126 | 60  | 59  | 116 | 207 |
| Beans (g)   | 91  | 80  | 4   | 73  | 200 |
| Fruits (g)  | 169 | 162 | 0   | 135 | 359 |
| Colored vegetables (g) | 178 | 130 | 38  | 156 | 348 |
| Other vegetables (g) | 222 | 116 | 85  | 204 | 384 |

Table 3. Distribution of flavonoid intake (µmol/day) among middle aged Japanese women

|          | AVE | SD  | 10  | 50  | 90  |
|----------|-----|-----|-----|-----|-----|
| Flavonols |     |     |     |     |     |
| quercetin | 58.4| 62.7| 0.5 | 39.3| 141.5|
| kaempferol | 52.4| 58.6| 0.0 | 35.3| 131.7|
| myricetin | 5.8 | 14.0| 0.0 | 0.0 | 19.4 |
| rutin | 0.0 | 0.3 | 0.0 | 0.0 | 0.0  |
| fisetin | 0.0 | 0.1 | 0.0 | 0.0 | 0.0  |
| Isoflavones | 215.7| 147.3 | 50.4 | 191.6 | 407.1  |
| genistein | 123.4| 84.7 | 28.5 | 109.9 | 237.0  |
| daidzein | 92.3 | 63.9 | 20.0 | 81.1 | 176.5  |
| Flavanonols | 1277| 1403 | 0   | 1009 | 2884  |
| (−)-epicatechin | 287 | 315  | 0   | 229  | 658   |
| (−)-epigallocatechin | 984 | 1095 | 0   | 795  | 2224  |
| catechin | 5.6 | 12.9 | 0.0 | 0.0 | 22.0  |
| Flavones | 30.5 | 145.8 | 0.0 | 7.9  |
| hesperidin | 15.5 | 107.9 | 0.0 | 0.0  |
| narigenin | 15.0 | 90.0 | 0.0 | 0.0  |
| luteolin | 15.0 | 51.6 | 0.0 | 20.0 |
| apigenin | 13.0 | 51.7 | 0.0 | 0.0  |

However, the miso intake did not differ in each category.

We conducted a stratified analysis of subjects with LDL cholesterol concentrations >140 mg/dL. Twenty five subjects showed normal LDL-C level (normal group) and 14 subjects exhibited hyper LDL-cholesterolemia. The subjects with hyper LDL-cholesterolemia were divided into 2 groups based on the oxidized LDL levels in the plasma, namely, the low oxidized LDL group and high oxidized LDL group. The level of oxidized LDL in the case of the low oxidized LDL group was similar to that in the case of the normal group. Table 6 shows the anthropometric measurements, serum lipids levels, energy and energy-adjusted nutrient intakes. In physical characteristics, the height was significantly lower in the high oxidized LDL group than in the normal and low oxidized LDL groups. The waist circumference was higher in the high oxidized LDL group than in the normal group. TC was significantly higher in the high oxidized LDL group than in the low oxidized LDL group. HDL-C was lower in the low oxidized LDL group than in the normal group. LDL-C was significantly higher in the low and high oxidized LDL groups than in the normal group.

The energy intake was significantly lower in the low oxidized LDL group than in the high oxidized LDL group. The energy-adjusted nutrient intake did not differ among the 3 groups.

Fig. 1 shows the energy-adjusted antioxidant vitamins and quercetin intake. There were no significant differences
in the antioxidant vitamins between the low and high oxidized LDL groups. The quercetin intake was higher in the low oxidized LDL group than in the high oxidized LDL group. Fig. 2 shows correlation between oxidized LDL and quercetin intake. In subjects with hyper LDL cholesterol, there was a significant inverse correlation between the intake of quercetin and the oxidized LDL levels, but this was not the case in normal LDL-C subjects.

Discussion

In the present study, we assessed the mean intake of total flavonoids in middle-aged Japanese women with the Functional Food Factor database. Of the total flavonoid, the mean intake of flavan-3-ols was the highest followed by isoflavones, flavonols, flavanones and flavones. The intake of flavonols in this study was similar to that reported in an Australian study (50 μmol/d, 15.3 mg/d, in the case of women with ages ranging from 45 to 64 years) [18]. Arts et al. reported that the average flavan-3-ol intake was 160 μmol/d (50 mg/d) in 6200 Dutch men and women aged 1 to 97 years old [19]. In our study, flavan-3-ol levels were 10 times higher than those in previous study. The flavan-3ol levels were higher in green tea than in black tea [20]. The flavanone intake in our study populations was 31 μmol/d; this was lower than that in the case of a previous report from U.S.A., where the values ranged from 30 to 100 μmol/d (10 to 30 mg/d) [21]. The consumption of flavones was close to 0 μmol, and other reports showed the same values. Flavones are much less common as compared to the flavonoids present in vegetables and fruits. Soy products are rarely consumed in Western countries. The isoflavone intake in these countries was ranged 0 to 10 μmol/d (< 1 mg/d) [12]. In this study, all subjects consumed soy products and the isoflavone intake was 20 times higher than in the Japanese population than in populations in Western countries.

Additionally, we demonstrated the sources of flavonoid in the diet of middle-aged Japanese women. The major dietary sources of total flavonoids were tea, onions and soy products in our study population. The results of this study differed from that of previous studies because the Japanese traditional diet includes many soy products such as tofu, natto and miso paste. In the Long Island Breast Cancer Study Project (LIBCSP), the major dietary sources of total flavonoids intake were tea, citrus fruit juices and citrus fruits [12]. Similarly, Ok Kyoung Chun et al. reported that the main sources of flavonoid intake were tea, citrus fruit juices, wine and citrus fruits in the National Health and Nutrition Examination Surveys (NHANES) study [21].

The main sources of flavan-3-ols and flavanones, namely, tea or citrus fruits and citrus fruits juices, were similar to those reported in the previous study by Ock Kyoung Chun et al. [21]. The main sources of isoflavones are soy products. Several soy products, such as natto, tofu and miso were sources of isoflavone and contributed to up to 80% of the isoflavone intake in our study population. Quercetin, which is the main flavonol, commonly occurs in various food sources. It is abundantly present in onion, which is the main source of flavonol intake in Dutch diets [19]. Our results were similar to those of the previous study. The major sources of flavonol intake were not only onion but also leafy vegetables, such as moroheiya (nalta jute), Japanese radish leaves, komatsuna and okra. Flavones composed less than 1% of the total flavonoids ingested by participants in this study. Intake of flavones is much less compared to other flavonoids present in fruits and vegetables [22]. Parsley and

| Percentage contributed | Cumulative percentages contributed |
|------------------------|----------------------------------|
| Malabar spinach        | 79.5 (%)                         |
| Green pepper           | 12.3 (%)                         |
| Grapefruits            | 3.6 (%)                          |
| Parsley                | 2.2 (%)                          |
| Celery                 | 1.1 (%)                          |
| Onion                  | 58.8 (%)                         |
| Mroheya                | 15.9 (%)                         |
| Radish leaves          | 5.6 (%)                          |
| Komatsuna              | 4.8 (%)                          |
| Okra                   | 4.3 (%)                          |
| Grapefruits            | 38.6 (%)                         |
| Orange juice           | 27.9 (%)                         |
| Mandarin orange juice  | 11.8 (%)                         |
| Mandarin orange        | 8.9 (%)                          |
| Orange                 | 6.3 (%)                          |
| Tea                    | 98 (%)                           |
| Apples                 | 1.9 (%)                          |
| Peach                  | 0.2 (%)                          |
| Plum                   | 0.1 (%)                          |
| Strawberry             | 0.1 (%)                          |
| Tofu                   | 42.2 (%)                         |
| Natto                  | 28.7 (%)                         |
| Miso                   | 16.3 (%)                         |
| Fried tofu             | 2.6 (%)                          |
| Kinako                 | 2.1 (%)                          |

Table 4. Contribution of food stuff to flavonoid intakes by 24 h weighed dietary record in middle aged Japanese women.
The 24-h weighed DR method was used as a gold standard.
estimated isoflavone intakes from FFQ included in 8 major contributors of isoflavone intake and DR and measured the blood and urine isoflavone in from 215 subjects. The mean intake estimated from the FFQ, DR, serum concentrations and urine excretion in the case of daidzein were 71 μmol/d (18 mg/d), 59 μmol/d (15 mg/d), 120 nmol/L, 170 μmol/L, respectively, and in the case of genistein were 115 μmol/d (31 mg/d), 85 μmol/d (23 mg/d), 475 nmol/L, 14 μmol/L, respectively. Correlations between the daidzein intake values obtained from the FFQ and those from DR, serum and urine excretion were 0.64 (p<0.05), 0.31 (p<0.05) and 0.43 (p<0.05), respectively. They also demonstrated that a shorter version of the FFQ with 3 items showed a similar correlation as that with 8 items. In Western countries, a modified FFQ was used to estimate the daily intake of quercetin and naringenin [24]. The original questionnaire specified 38 food stuffs that were quercetin sources and 16 food stuffs as naringenin sources. The estimated mean intakes of quercetin and naringenin were 97 μmol/d (29 mg/d) and 214 μmol/d (58 mg/d), respectively. The mean urinary excretion of quercetin was 0.2 μmol/d (60 μg/d) and that of naringenin was 2 μmol/d (560 μg/d). The correlation between FFQ and urinary excretion of quercetin and naringenin was 0.82 (p<0.05) and 0.25 (p<0.05), respectively. Two original FFQs for assessing some flavonoid intakes have sufficient levels for epidemiologic studies.

Epidemiologic studies have indicated that the flavonoid intake is inversely associated with CHD. The Zutphen Elderly study demonstrated that catechin intake was significantly associated with reduced risks of ischaemic heart disease and CHD mortality, and there were 68% reductions in the mortality risk [25]. On the other hands, recently published data in a large prospective cohort of women from the Nurses’ Health Study does not support an inverse association between flavonol intake and CHD risk [26]. Epidemiological studies have not yet provided a consensus of the association between flavonoid intake and CHD diseases.

Our results showed that high intake of quercetin was associated with lower levels of circulating oxidized LDL in postmenopausal women who had an LDL-C concentration >140 mg/dL. Oxidized LDL is thought to play an important role in atherosclerosis. In fact, oxidized LDL has been recognized as a risk factor for CHD [27]. Pistavas et al. showed that the traditional Mediterranean diet, which includes antioxidant vitamins and phenolic compounds, is associated with lower levels of circulating oxidized LDL and higher total antioxidant capacity [28]. The traditional Mediterranean diet might help in decreasing the levels of circulating oxidized LDL. Recently published data indicated that an association exists between the antioxidant capacity of flavan-3-ols and suppression of oxidized LDL via intervention study. Baba et al. conducted a study in humans and reported that the cocoa polyphenol, which contains flavan-3-

Fig. 1. Energy adjusted antioxidant vitamins and quercetin intakes in the three groups.

*Normal group vs The high oxidized group (p<0.05).

#Normal group vs The low oxidized LDL group (p<0.05).

Fig. 2. Correlation between oxidized LDL and energy-adjusted quercetin intake

to estimate the total flavonoid intake in this study population. This is the most reliable method for estimation of the absolute intake of nutrients compared to any other methods of dietary assessment. By the 24-h weighed DR method, 5 major contributors of each flavonoid were about up to 90% of subclasses of flavonoid intakes. Estimating the total flavonoid intake and the major food sources contributing to the intake of each flavonoid sub-class was the first step in the understanding the DR-FFQ relationship. FFQ is used for estimating dietary nutrient intakes in epidemiologic studies. This method is cost effective and can assess diet over long periods of time. Yamamoto et al. adopted the FFQ to assess the isoflavone intake in the Japanese population [23]. The FFQ provides results that are both valid and reproducible, and hence, it can be used in epidemiological studies.
ol, might increase the resistance against LDL oxidation [29]. It has been suggested that the antioxidant capacity of flavonoids might contribute to their potentially protective role in the suppression of LDL oxidation.

Quercetin has antioxidant capacities in vitro according to the 2,3-double bond in the C ring and the 4-oxo function and belongs to hydroxyl groups in positions 3, 5, 7, 3', 4'. [30]. Furthermore, quercetin conjugates also have antioxidative efficacies. Quercetin is converted to the conjugated metabolites by phase II enzyme. The major quercetin metabolites are the quercetin-3-glucuronide and quercetin 3'-glucuronide in human and rat plasma [31, 32]. It is likely that quercetin-3-glucuronide has an effect of antioxidant capacity in blood plasma LDL due to retaining its catechol structure in positions 3' and 4' [31]. In addition, quercetin-3'-O-methylquercetin delayed the copper induced oxidation of human plasma LDL [7]. Quercetin conjugates are about half to two-thirds antioxidant activity of the aglycone [7, 31]. Moreover, quercetin conjugates retain some biological effects in vivo in human intervention studies [33]. Our results suggested that quercetin could have a potentially protective role in suppression of LDL oxidation regardless of the effect of antioxidant vitamins. Therefore, a high consumption of quercetin may have a protective effect for chronic diseases such as CHD and CVD due to LDL oxidation.

There are some limitations to this study. First, although we estimated the flavonoid intake by the weighed DR method, which is a gold standard method, our sample study population was small. Second, because our study population included only middle-aged women, we have not considered age or sex-related variations in flavonoid intake. Third, we have not taken into account the seasonal variations in flavonoid intake. Seasonal variations play an important part in the Japanese diet. Since this factor influence the vegetable and fruit intake in Japan, the food sources of flavonoids would differ with the seasons. Fourth, flavonoid intake is provisional. Our data might have overestimated the flavonoid intake. Contents of flavonoids in foods are influenced by storage conditions, processing and cooking methods. Frying and microwaving cause 30% to 70% flavonoid degradation [34, 35]. By boiling, about 30% loss quercetin glycosides transfer to the boiling water [36]. Additionally, the flavonoid contents of food differ between the inner parts and outer parts of foods. Our Functional Food Factor database has taken these factors into consideration. Therefore, we estimated the flavonoids in the case of fresh-food intake by using the DR method. Finally, the values of epicatechin and epigallocatechin are included in non-gallate type in the FFF database. Tea contains epicatechin gallate and epigallocatechin gallate [20]. Therefore, the FFF database should distinguish catechins from garic acid esters of catechins.

In conclusion, we assessed the flavonoid intake and demonstrated the major food sources contributing to the levels of flavonoid in middle-aged Japanese women. The total flavonoid intake was higher in our study population compared to that in previous reports, and the sources of total flavonoid differed from those in Western countries. This is the first step towards understanding the DR-FFQ relationship in case of Japanese middle-aged women. Furthermore, analysis of the sub-samples from middle-aged Japanese women indicated that there may be an association between flavonoid intake and oxidized LDL, which might be related to the incidence of CHD and CVD.

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