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Systematic Review

Dietary Biomarkers of Vegetable and Fruit Intake in Asians: An Epidemiological Systematic Review

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Abstract: Accurate estimation of food intake is necessary to clarify the relationship between dietary intake and particular health conditions; however, self-reported assessments often result in estimation errors. In addition, increasing evidence indicates an association between a higher intake of fruits and vegetables and a lower risk of some diseases, and many countries are encouraging their consumption. Biomarkers of vegetable and fruit intake are important tools for objectively estimating dietary intake in nutritional epidemiological studies. To determine the association between vegetable and fruit intake and blood biomarkers, we systematically reviewed relevant literature on Asians. Databases, PubMed, and CiNii Articles were searched for English and Japanese articles. Of the 91 articles retrieved, 4 were selected for review, including 2 cross-sectional studies, 1 longitudinal study, and 1 randomized trial. Our literature review showed that vegetable consumption is positively associated with plasma concentrations of γ-tocopherol, β-cryptoxanthin, α-carotene, β-carotene, lutein, threonate, galactarate, creatine, and ascorbic acid. In comparison, fruit consumption is positively associated with blood concentrations of α-tocopherol, β-cryptoxanthin, α-carotene, β-carotene, lycopene, retinyl palmitate, ascorbic acid, proline betaine, threonate, and galactarate. Therefore, blood β-carotene, β-cryptoxanthin, and ascorbic acid concentrations may be useful biomarkers for predicting vegetable and fruit intake in Asian population.

Keywords: biomarkers; vegetable; fruit; carotenoids; literature review

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1. Introduction

Increasing evidence has revealed that higher consumption of vegetables and fruits reduces the risk of several chronic diseases, including type 2 diabetes [1], hypertension [2], certain cancers, and cardiovascular diseases (CVD) [3]. In a meta-analysis of the association between vegetable and fruit intake and the risk of cardiovascular disease, all-cause cancer, and all-cause mortality, the lowest risk for all cancers was reported at an intake of 600 g/day, and the lowest risk for coronary heart disease, stroke, cardiovascular disease, and all-cause mortality was observed at an intake of 800 g/day [4]. The World Health Organization (WHO) has recommended that adults eat at least 400 g of fruits and vegetables per day to reduce the risk of noncommunicable diseases, including diabetes, heart disease, stroke, and cancer [5]. Therefore, dietary guides in many countries recommend the consumption of fruits and vegetables, some even with quantitative amounts in grams conveyed through graphics, aimed to encourage their consumption [6]. Some examples are listed as follows:

"Eat plenty of vegetables and fruits every day" in South Africa; "Eat vegetables, fruits, and berries frequently (a minimum of 500 g/day, excluding potatoes)" in Finland; "Choose a variety of fruits and vegetables every day" in Bahamas; "Consume three or more servings of vegetables and at least two servings of fruits per day" in Mongolia; and "Consume plenty of vegetables, fruits, and tubers" in China [6]. In Japan, the National Health Promotion Campaign, Health Japan 21 (the second term), and the Japanese Food Guide Spinning Top, modeled after traditional Japanese toys, recommend that adults eat ≥350 g vegetables and 200 g fruits per day [7,8]. However, according to the National Health and Nutrition Survey 2019, vegetable and fruit intake per day in Japanese adults was well below the recommended amounts, at 280.5 g vegetables and 100.2 g fruits [9].

Accurate estimates of dietary intake are necessary to determine the relationship between vegetable and fruit intake and specific health conditions. Conventionally, dietary food consumption has been measured using several methods, including dietary records (DR), 24 h dietary recalls, and food frequency questionnaires (FFQ). However, these methods depend on self-reported estimates of food intake, which are subject to participant biases, such as underreporting of energy intake and memory limitations, and bias derived from the food composition databases used to calculate energy and nutrient intakes [10–13]. In epidemiological studies, these biases significantly affect the accuracy of dietary information.

Minimizing the problem of error in dietary surveys through the use of biomarkers in biological samples such as blood and urine is now being considered. Dietary biomarkers may provide an alternative for assessing dietary intake or as additional indicators for calibrating self-reported dietary intake [14,15]. Blood biomarkers representing fruit and vegetable metabolites have been proposed as objective indicators of fruit and vegetable intake [16]. However, because vegetables and fruits contain and/or produce several bioactive substances, the content of compounds that can serve as biomarkers may vary. A meta-analysis by Pennant et al. showed that the most commonly measured and consistently responding biomarkers with respect to vegetables and fruits are vitamin C and four common carotenoids, alpha (α)- and beta (β)-carotene, β-cryptoxanthin, and lutein, which may be useful biomarkers for objectively measuring general fruit and vegetable intake [17]. Thus, these biomarkers may be important tools for the objective estimation of vegetable and fruit intake in populations in nutritional epidemiological research [17].

Most epidemiological studies on the biomarkers of vegetables and fruits have been conducted in Europe and the United States. Asian food consumption trends differ from those in Europe and the United States. Asia consumes more rice, vegetables, and fish, and fewer potatoes [18]. However, few large-scale epidemiological studies have been conducted in Asian populations, and the association between vegetable and fruit intake and health among Asians has not been well-studied. Therefore, further research on various regional characteristics of individuals is warranted.

This study aimed to conduct a systematic review of the literature to clarify the relationship between vegetable and fruit intake and blood biomarkers in Asians.

2. Materials and Methods

2.1. Protocol

This systematic review was conducted in accordance with the guidelines stipulated in the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [19]. The search terms used to identify relevant articles and the PRISMA criteria for population, exposure, comparisons, and outcomes are shown below.

2.2. Search Strategy

Online databases (PubMed and CiNii Articles, a Japanese academic database from the National Institute of Information) were searched for relevant articles. In PubMed, we used the following strategy based on a published specific query, such as [Title/Abstract (TIAB)] and [Medical Subject Headings (MeSH) Terms]: ("fruit" [TIAB] OR "fruits" [TIAB] OR "fruit" [MeSH Terms] OR "fruit" [TIAB] OR "fruit's" [TIAB] OR "vegetable" [TIAB]

OR "vegetables" [TIAB] OR "vegetables" [MeSH Terms] OR "vegetables" [MeSH Terms] OR ("plant" [TIAB] AND "food" [TIAB]) OR "Plant food" [TIAB]) AND (("dietary" [TIAB] AND "intervention" [TIAB]) OR "dietary intervention" [TIAB] OR "controlled" [TIAB] OR "randomised" [TIAB] OR "random allocation" [MeSH Terms] OR ("random" [TIAB] AND "allocation" [TIAB]) OR "random allocation" [TIAB] OR "randomized" [TIAB] OR ("randomly" [TIAB] AND "assigned" [TIAB]) OR "patient compliance" [MeSH Terms] OR "compliance" [TIAB] OR "compliance" [MeSH Terms] OR ("feeding" [TIAB] AND ("trial" [TIAB] OR "study" [TIAB])) OR "parallel" [TIAB] OR "cross-over studies" [MeSH Terms] OR "Cross-Sectional Studies" [MeSH Terms] OR ("cross-over" [TIAB] AND ("study" [TIAB] OR "Studies" [TIAB])) OR "crossover" [TIAB] OR "cross-over" [TIAB] OR "Cross-Sectional Studies" [TIAB] OR "observational" [TIAB]) AND ((("antioxidant" [TIAB] OR "antioxidants" [TIAB] OR "antioxidant nutrients" [TIAB] OR "antioxidant nutritional" [TIAB] OR "antioxidants vitamins" [TIAB] OR "antioxidative" [TIAB] OR "ascorbic acid" [MeSH Terms] OR "ascorbic" [TIAB]) AND "acid" [TIAB]) OR "ascorbic acid" [TIAB] OR "vitamin c" [TIAB] OR "carotenoid" [TIAB] OR "carotenoids" [TIAB] OR "carotenoid" [TIAB] OR "carotenoid's" [TIAB] OR "carotenoide" [TIAB] OR "carotenoides" [TIAB] OR "carotenoids vitamin" [TIAB] OR "carotenoids" [MeSH Terms] OR "carotenes" [TIAB] OR "xanthophylls" [MeSH Terms] OR "xanthophylls" [TIAB] OR "carotene" [TIAB] OR "beta-carotene" [TIAB] OR "b-carotene" [TIAB]) AND ("blood" [TIAB] OR "blood" [MeSH Terms] OR "plasma" [MeSH Terms] OR "plasma" [TIAB] OR "serum" [MeSH Terms] OR "serum" [TIAB] OR "circulating" [TIAB] OR "biomarker" [TIAB] OR "biomarkers" [TIAB] OR "biomarker" [TIAB] OR "biomarker's" [TIAB]) AND ("English" [Language] OR "Japanese" [Language]) AND ("Japanese" [TIAB] OR "Koreans" [TIAB] OR "Thai" [TIAB] OR "Asians" [TIAB] OR "Asian" [TIAB] OR "Chinese" [TIAB] OR "Burmese" [TIAB] OR "Burmeses" [TIAB] OR "Cambodians" [TIAB] OR "Cambodian" [TIAB] OR "Vietnamese" [TIAB] OR "Vietnameses" [TIAB] OR "mongoloid race" [TIAB] OR "mongoloid race" [TIAB]). In CiNii Articles, we used the following terms: ("biomarker" AND ("vegetable" OR "fruit" OR "food") (in Japanese)). Studies published up until 31 December 2021 were included in the study. The last search date was 3 March 2022.

2.3. PECOS Criteria

The study criteria were defined using the PECOS framework, as follows: "population (P)", human studies in an adult Asian population; "exposure (E)", studies in which biomarkers of vegetable and fruit intake were measured in subjects' blood; "comparison (C)", studies in which biomarkers of vegetables and fruit intake were not measured in subjects' blood; "outcome (O)", the intake of vegetables and fruits and their blood biomarkers; and "study design (S)", interventional studies and observational studies.

2.4. Inclusion Criteria

The inclusion criteria were as follows:

1. Studies involved men and women aged over 20 years in Asia;
2. Studies written in Japanese or English;
3. Studies wherein the intake of vegetables and fruits could be ascertained by weight;
4. Interventional, observational, or studies published before or on 31 December 2021 on biomarkers (vitamin C, α-carotene, β-carotene, β-cryptoxanthin, lutein, and lycopene) of vegetable and fruit intake using blood specimens;
5. Studies on healthy or unhealthy individuals including those with a high risk of CVD and impaired glucose metabolism;
6. Studies wherein health outcomes, including surrogate markers, were measured (e.g., blood cholesterol levels in the case of hyperlipidemia).

2.5. Exclusion Criteria

The exclusion criteria were as follows:

1. Interventional studies wherein the intervention consisted of dietary advice or counseling;

2. Studies that altered dietary profiles (e.g., low-fat diet) through the additional intake of fruits and vegetables. This criterion excluded the possibility that changes in biomarkers were the result of dietary changes in foods other than fruits and vegetables;
3. Interventional studies wherein not all fruits and vegetables were provided or were provided as supplements, juices, or extracts;
4. Studies conducted in children, adolescents, institutionalized older populations, or pregnant or lactating women;
5. Studies conducted in individuals with impaired micronutrient metabolism or vitamin deficiency.

2.6. Data Extraction

Three independent investigators (Y.T., R.I., and A.Y.) extracted data in predefined Excel spreadsheets. Extraction information involved basic article information (language, paper type, publication year, etc.), participant characteristics (sample size, age, survey country, disease status, etc.), study design, lack or presence of dietary intervention, dietary survey methods and other methodological characteristics (plasma biomarkers measured, intervention or observation period, health outcomes, etc.), and general findings.

In primary screening, the title and abstract of each study were checked to ensure that they met the inclusion criteria. In secondary screening, the full text was screened to determine whether it should be included in the review. Two investigators independently conducted screening (R.I. and A.Y.) and quality assessment (Y.T. and N.S.), and disagreements were resolved by consensus or arbitration by other reviewers (Y.T., N.S., and H.T.).

2.7. Quality Assessment

Next, the quality of articles in each study was assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies by the National Heart, Lung, and Blood Institute in 2013 [20]. This tool was designed to align with quality assessment instruments to help reviewers evaluate a study's internal validity. Tailored to specific study designs, these tools identify potential methodological or implementation flaws [20]. The 14 bias risk-check items were judged as *yes*, *no*, or *other* (CD: cannot determine; NA: not applicable; and NR: not reported), and the percentage of *yes* responses out of the 14 items excluding *other* was calculated. This study counted the presence or absence of a research question (one item), selection bias (five items), information bias (five items), confounding bias (one item), causality (two items), and the number of *yes* responses in each category. A high *yes* ratio indicated a lower risk of bias.

3. Results

Figure 1 details the flow diagram of the study selection process. A total of 91 papers were retrieved: 49 from PubMed and 42 from CiNii Articles. However, 66 studies did not meet the selection criteria during primary screening based on title and abstract. Full-text articles were obtained for the remaining 25 studies, which were assessed for eligibility according to the inclusion and exclusion criteria during secondary screening. Accordingly, 22 papers were excluded as they did not meet the following inclusion criteria: criterion numbers 1, 3, 4, and 5 were not met by three, eight, nine, and two studies, respectively. Additionally, 11 studies were excluded as they met the following exclusion criteria: criterion numbers 1, 2, and 3 were met by four, five, and two studies, respectively. Following secondary screening, three papers were included.

One study indicated a relationship between mandarin orange intake and blood β-carotene and β-cryptoxanthin levels in the Japanese population; however, this study was not retrievable using the study's search terms. Therefore, we conducted a manual search of PubMed for nutritional epidemiological articles that reported an association between mandarin orange intake and blood β-carotene and β-cryptoxanthin concentrations in Japanese individuals, published until 31 December 2021. This resulted in the identification of an additional study. In total, four studies were selected for analysis in this review [21–24]. Outlines of the selected studies are presented in Table 1.

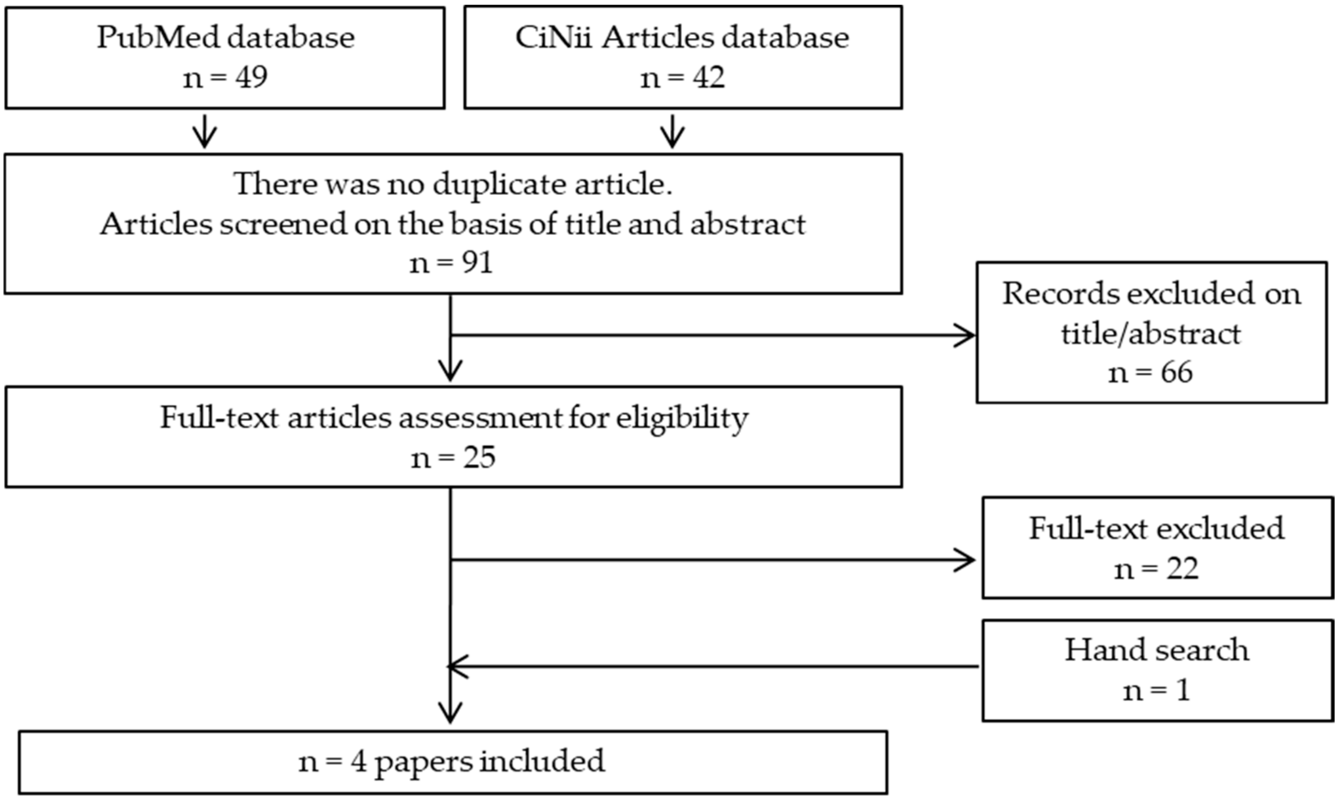

Figure 1. Flow diagram for study identification.

The four selected studies were published between 1998 and 2021. Two studies were cross-sectional [22,23], one was a randomized trial [21], and one was a longitudinal study [24]. Three studies were conducted on local Japanese residents [22–24], and one was conducted on Chinese textile workers [21]. In general, the sample sizes ranged from 27 to 7012, and the ages ranged from 30 to 75 years. The study subjects were both men and women in two studies [22,23], only women in one study [21], and only men in one study [24]. The evidence table showing the included papers is shown in Table 1. The FFQ was used in two studies [21,23], semi-quantitative FFQ in one study [22], and FFQ combined with DR in one study [24].

Table 1. Outlines of the selected studies.

Author	Year	Participants	Study Design	N	Dietary Survey Method	Dietary Factor	Blood Biomarkers	Analytical Methods	Major Findings
Frankenfeld et al. [21]	2012	Textile workers in China	Randomized trial	2031	FFQ	Vegetables and fruits	β-carotene, α-carotene, lycopene, β-cryptoxanthin, lutein + zeaxanthin, retinol, retinol palmitate, α-tocopherol, γ-tocopherol, ascorbic acid	Ascorbic acid: colorimetric method Others: HPLC	• Vegetable consumption was significantly and positively associated with plasma γ-tocopherol and β-cryptoxanthin concentrations. • Fruit intake was significantly and positively associated with plasma concentrations of α-tocopherol, β-cryptoxanthin, lycopene, α-carotene, β-carotene, retinyl palmitate, and vitamin C.
Shibutami et al. [22]	2021	Local residents in Japan	Cross-sectional study	7012	Semi-quantitative FFQ	Vegetables (carotenoid-rich and other vegetables) and fruits	Citrate, creatine, cystine, galactarate, hippurate, lysine, proline betaine, threonate, tyrosine	CE-TOF-MS	• The population's mean intake was as follows: carotenoid-rich vegetables, 78 g/day (10–90th range: 27–146 g/day); other vegetables, 78 g/day (10–90th range: 28–140 g/day); and fruits, 55 g/day (10–90th range: 13–125 g/day). • Notable metabolites in plasma commonly found with the intake of carotenoid-rich vegetables and other vegetables were threonate, galactarate, and creatine. • Plasma proline betaine, threonate, and galactarate were markers for fruit intake.
Sugiura et al. [23]	2004	Local residents in Japan	Cross-sectional study	27	FFQ (Satsuma mandarin and other fruits and vegetables)	Fruits (Satsuma mandarin, mandarin juice, and other fruits) and vegetables (green-yellow and others)	β-cryptoxanthin	HPLC	• Serum β-cryptoxanthin levels in both men and women were correlated strongly with the intake of Satsuma mandarin in the month of blood sampling; however, they did not correlate with the intake of other fruits, green-yellow vegetables, and other vegetables. • Multiple linear regression analysis showed that, in men, the serum β-cryptoxanthin level could be predicted by Satsuma mandarin intake, age, and the month of blood sampling. Conversely, in women, the serum β-cryptoxanthin concentration could be predicted by Satsuma mandarin intake, the month of blood sampling, and age.

Table 1. *Cont.*

Author	Year	Participants	Study Design	N	Dietary Survey Method	Dietary Factor	Blood Biomarkers	Analytical Methods	Major Findings
Tsugane et al. [24]	1998	Local residents in Japan	Longitudinal study	FFQ: 621, Combined with a DR: 203	FFQ combined with a 3-day DR	Vegetables (yellow, green leafy, other, fresh, and pickled vegetables) and fruits	Ascorbic acid	Fluorometric method	• The frequency of consumption of green leafy vegetables by FFQ was significantly associated with plasma ascorbic acid level on the univariate analyses. • Calculated vitamin C intake from fresh vegetables by DR and the amount of consumed fresh vegetables by DR were not predictors of the plasma level of ascorbic acid. • The frequency of fruit consumption by FFQ was the strongest predictor of plasma ascorbic acid among the variables tested.

FFQ, food frequency questionnaire; DR, dietary record; HPLC, high-performance liquid chromatography; CE-TOF-MS, capillary electrophoresis time-of-flight mass spectrometry.

3.1. Relationship between Vegetable Intake and Their Biomarkers

Of the four studies, three showed a positive correlation between vegetable intake and the respective biomarkers [21,22,24]. Frankenfeld et al. [21] reported that vegetable consumption was significantly and positively associated with plasma γ-tocopherol ($p = 0.039$) and β-cryptoxanthin concentrations ($p = 0.046$) in Chinese women. Among healthy Japanese men, the frequency of consumption of yellow (e.g., carrot and pumpkin), green leafy (e.g., spinach), and other vegetables measured by FFQ was not significantly associated with ascorbic acid level in plasma in the univariate analyses, although consumption of green leafy vegetables was statistically significant in the multivariate analysis ($p = 0.02$) [24]. However, both the calculated vitamin C intake from fresh vegetables and the amount of consumed fresh vegetables from the 3-day DR were not predictors of the ascorbic acid level in plasma [24]. Shibutami et al. [22] reported that notable metabolites in the plasma common to the intake of carotenoid-rich vegetables and other vegetables were threonate, a metabolite of ascorbic acid, galactarate, and creatine in capillary electrophoresis mass spectrometry. In contrast, no significant associations were observed between serum β-cryptoxanthin levels and consumption of green–yellow and other vegetables in the Japanese population [23].

3.2. Relationship between Fruit Intake and Their Biomarkers

All four studies indicated a positive correlation between fruit intake and the respective biomarkers [21–24]. For example, Frankenfeld et al. [21] reported that fruit intake in Chinese women was significantly and positively associated with the plasma concentrations of α-tocopherol ($p = 0.009$), β-cryptoxanthin ($p < 0.001$), lycopene ($p < 0.001$), α-carotene ($p < 0.001$), β-carotene ($p = 0.002$), retinyl palmitate ($p = 0.034$), and vitamin C ($p < 0.001$). Among local Japanese residents, serum β-cryptoxanthin levels in both men and women correlated strongly with the intake of Satsuma mandarin in the month of blood sampling ($p < 0.001$) [23]. In addition, Tsugane et al. [24] reported that the average ascorbic acid level in plasma among those in the high fruit consumption category of $\geq$5 days/week was 14 μmol/L higher than in those in the lowest fruit consumption category of $\leq$1 day/week. Furthermore, the frequency of fruit consumption was a stronger predictor of ascorbic acid levels in plasma in healthy Japanese men ($p < 0.001$) than any of the DR measures of foods high in vitamin C [24].

3.3. Article Quality in Each Study Assessment

The search quality of the articles in each study using the National Heart, Lung, and Blood Institute Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies is shown in Table 2 [20]. Assessing the quality of the articles in each study identified that two studies had a *yes* rate of $\geq$65% [21,24] and a low risk of bias. The study with the highest risk of bias had a *yes* rate of 58.3% [22] and a low *yes* for information bias. Two of the four included studies were observational [22,23]; the remaining studies did not examine causal relationships [21,24].

Table 2. Quality assessment of the selected studies.

	Criteria and Questions		Frankenfeld et al. [21]	Shibutani et al. [22]	Sugiura et al. [23]	Tsugane et al. [24]
1	Research question: was the research question or objective in this paper clearly stated?	Research question	Y	Y	Y	Y
2	Study population: was the study population clearly specified and defined?	Selection bias	Y	Y	Y	Y
3	Study population: was the participation rate of eligible persons at least 50%?	Selection bias	Y	Y	NR	Y

Table 2. *Cont.*

	Criteria and Questions		**Frankenfeld et al. [21]**	**Shibutani et al. [22]**	**Sugiura et al. [23]**	**Tsugane et al. [24]**
4	Groups recruited from the same population and uniform eligibility criteria: were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Selection bias	Y	Y	Y	Y
5	Sample size justification: was a sample size justification, power description, or variance and effect estimates provided?	Selection bias	N	N	N	N
6	Exposure assessed prior to outcome measurement: for the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Causality	N	N	N	N
7	Sufficient timeframe to see an effect: was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Causality	N	N	N	N
8	Different levels of the exposure of interest: for exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Information bias	Y	N	N	Y
9	Exposure measures and assessment: were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Information bias	Y	Y	Y	Y
10	Repeated exposure assessment: was the exposure(s) assessed more than once over time?	Information bias	N	N	Y	Y
11	Outcome measures: were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Information bias	Y	Y	Y	Y
12	Blinding of outcome assessors: were the outcome assessors blinded to the exposure status of participants?	Information bias	NA	NA	NA	NA
13	Follow-up rate: was loss to follow-up after baseline 20% or less?	Selection bias	NA	NA	NA	NA
14	Statistical analyses: were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Confounding bias	Y	Y	Y	Y
	Ratio of *YES* * (%)		66.7	58.3	63.6	75.0

The risk of bias and quality of evidence of the accepted articles were assessed using the National Heart, Lung, and Blood Institute Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies [20]. The 14 bias risk-check items were judged as *yes*, *no*, or *other* (CD: cannot determine, NA: not applicable, NR: not reported), and the percentage of *yes* out of the 14 items excluding *other* was calculated. This study counted the presence or absence of a research question (one item), selection bias (five items), information bias (five items), confounding bias (one item), causality (two items), and the number of *yes* in each category. * The ratio of *yes* to the number of items excluding *other* among the 14 items. A higher *yes* ratio indicates a lower risk of bias.

4. Discussion

Our literature review showed that vegetable consumption was positively associated with blood concentrations of γ-tocopherol, β-cryptoxanthin, α-carotene, β-carotene, lutein, threonate, galactarate, creatine, and ascorbic acid. In comparison, fruit consumption was positively associated with blood α-tocopherol, β-cryptoxanthin, α-carotene, β-carotene, lycopene, retinyl palmitate, ascorbic acid, proline betaine, threonate, and galactarate concentrations. Therefore, blood β-carotene, β-cryptoxanthin, and ascorbic acid concentrations may be useful biomarkers for predicting vegetable and fruit intake in the Asian population. According to a systematic review conducted by Pennant et al. [17], groups provided with more fruits and vegetables than controls had increased blood concentrations of vitamin C, α-carotene, β-carotene, β-cryptoxanthin, and lutein, but not lycopene, which is consistent with our results.

Nutritional epidemiological research focuses on clarifying the relationship between food and nutrient intake and specific health conditions. Accurate dietary assessment is essential for estimating nutrient intake, and the use of objective biomarkers may increase the accuracy of this estimation. Moreover, numerous observational studies have indicated that vegetable and fruit intake, as well as β-carotene and folic acid intake, are associated with a reduced risk of chronic diseases, including CVD [3,4,25,26]. However, interventional studies focusing on specific nutrient supplements have not reported any beneficial effects on CVD risk [27,28]. In contrast, interventional studies on increased vegetable intake have shown significant benefits in reducing CVD risk [29,30]. Therefore, using a biomarker as an objective index is crucial for estimating daily vegetable intake.

Assessing habitual intake by using dietary surveys is also important. Evaluating habitual amounts requires long-term memory of food intake from meals, and heterogeneous food groups (such as vegetables) and mixed dishes may interfere with the estimation of amounts and frequencies [31,32]; other possible biases can further complicate analyses [33]. Strassburg et al. [33] compared food consumption using three dietary assessment methods (dietary history, 24 h dietary recall, and weighed food records), and found that food groups perceived as socially desirable, such as fruits and vegetables, had the highest values for diet history interviews. These reports suggest that more objective methods are required for estimating food intake.

Carotenoids are synthesized by plants. Common dietary carotenoids, which are synthesized by plants, include α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene, which are reported as biomarkers of vegetable and fruit intake in this study. Carotenoids are abundant in green and yellow vegetables such as carrots and spinach, and fruits such as Satsuma mandarin and persimmons [34]. Retinol, β-carotene, α-carotene, and β-cryptoxanthin are known sources of vitamin A; these components are used to calculate the vitamin A content in food, known as the retinol activity equivalent [35]. Since vitamin A is stored in large amounts in the liver, despite insufficient vitamin A intake, blood vitamin A levels do not decrease until liver stores fall below 20 μg/g [36]. Ascorbic acid (vitamin C) is an essential dietary component for living organisms and exists in foods as free-form L-ascorbic acid or L-dehydroascorbic acid. It is found abundantly in vegetables such as bell peppers and broccoli and fruits such as kiwifruit and oranges [34]. The concentration of ascorbic acid in plasma is used as an indicator of the vitamin C nutritional status in humans [37].

Cifelli et al. [38] reported a rapid appearance of serum vitamin A that peaked at 12 h after dose administration; subsequently, a rapid disappearance persisted until 5 d after dose administration in healthy participants. Thereafter, the serum vitamin A dose–response curves began to flatten as the curve entered a shallow terminal slope [38]. For β-carotene and zeaxanthin, the plasma half-life for accumulation has been reported to be 6–11 and 12 days, respectively [39,40]. Based on these reports, carotenoids in the blood, such as β-carotene and β-cryptoxanthin, are speculated to be indicators of vegetable and fruit intake, reflected within approximately 5 days after intake. In addition, since carotenoids take a long time (about 50 d) to disappear from the blood, they are a biomarker of habitual intake of vegetables and fruits [21]. Levine et al. [37] reported that ascorbic acid is detectable in the blood within 1 h after ingestion, reaches a maximum blood concentration approximately 2 h later, and disappears from the blood 12 h later. Thus, ascorbic acid cannot be stored in the blood for long periods. When blood ascorbic acid is used as an index of vegetable and fruit intake, the intake is presumed to reflect the previous 24 h.

Numerous studies on biomarkers for estimating vegetable and fruit intake have been reported; however, an internationally harmonized method has not yet been established [41–47].

Standardizing the timing of blood sampling, the type of blood specimen used, and the methods for measuring biomarkers is essential for international harmonization. In non-Asian intervention studies, blood samples were collected before and on the day after a vegetable and fruit intervention, with overnight fasting prior to sampling [41–47]. Both plasma and serum were used in these studies [41–47]. Among the papers in our review, only one provided detailed timing for dietary surveys and blood sampling, three used plasma, and one used serum. Carotenoid levels (α-carotene, β-carotene, lutein, lycopene, and β-cryptoxanthin) in plasma and serum were measured using the HPLC method, and ascorbic acid concentration in plasma and serum was reported using both the fluorescent and HPLC methods as biomarkers for vegetable and fruit intake. In our review, two papers measured carotenoids with HPLC [21,23], and one measured ascorbic acid with the fluorescent method [24]. Each biomarker component for estimating vegetable and fruit intake has a unique pharmacokinetic profile, resulting in different times for blood measurement post-ingestion [21,36–38]. Recent advancements in blood biomarker analysis, such as CE-TOF-MS and LC-MS, have been utilized in numerous studies [48,49]. Future research must adjust for the differences between older and newer techniques. Additionally, individual metabolic variations due to intestinal flora complicate standardization of the relationship between intake and blood markers [50]. Standardizing blood sampling timing, measurement methods for various components of fruits and vegetables, as well as accounting for metabolic products and individual differences could lead to international harmonization of biomarkers for estimating fruit and vegetable intake.

Recent studies on dietary biomarkers, including objective quantification of specific food ingredients and metabolites related to food intake, have been conducted mostly in Western populations [17,51]. Among the studies included in the current review, one Japanese study used metabolome analysis conducted using capillary electrophoresis time-of-flight mass spectrometry [22]. According to this study, plasma metabolites were likely to be associated with long-term food intake of Japanese food groups; the influential compounds were threonate and galactarate for carotenoid-rich vegetables, and proline betaine for fruits. Threonate is a sugar acid derived from threose and an ascorbic acid metabolite. Galactarate (mucate), a sugar acid of galactose, is also found in many foods that contain mucins, such as vegetables and root vegetables. Fruits and vegetables are rich in ascorbic acid [51]. Proline betaine is an osmoprotectant in citrus fruits and a biomarker in human plasma and urine for citrus fruit consumption, including oranges [52,53].

Proline betaine, hesperidin, and naringenin are known biomarkers of citrus fruits [53]. β-cryptoxanthin, abundant in Satsuma mandarin (1800 µg/100 g) [34], has been reported as a biomarker for fruit intake in a Japanese epidemiological study [9]. β-cryptoxanthin has not been reported as a biomarker of fruits in previous studies conducted in Europe and the United States, since Valencia oranges contain less β-cryptoxanthin (130 µg/100 g) than Satsuma mandarin oranges [34]. Satsuma mandarin oranges are one of the most consumed fruits by the Japanese [54]. Therefore, β-cryptoxanthin may be a potential biomarker that reflects fruit intake in the Japanese population.

This bias risk assessment utilized a quality assessment tool for observational cohorts and cross-sectional studies, evaluating selection bias, information bias, measurement bias, and confounding factors [20]. High bias risk was deemed low-quality. Studies focusing on determining causal relationships between exposure and outcome were rated higher. While all selected studies clearly stated their research questions, none addressed causality. The study by Tsugane et al., highly rated, exhibited low selection and information bias, indicating high quality [23]. To clarify the relationship between dietary biomarkers of vegetable and fruit intake in Asians, an overall number of high-quality studies are required.

Our study had some limitations. First, the included articles were observational studies, and the measure of vegetable and fruit intake was from self-reported FFQs and DR; presumably, the vegetable and fruit intake data contained errors. Second, although vegetable and fruit biomarker concentrations in the blood are greatly affected by the time after ingestion, only one of the included papers provided time-related information on the date of the dietary survey and the date of blood sampling [23]. Therefore, quantitative estimation of blood biomarker concentrations and the intake of vegetables and fruits is difficult. In the future, it will be necessary to clarify the relationship between pharmacokinetics, including the blood concentrations of biomarkers, and the intake of vegetables and fruits, as well as their habitual intake.

5. Conclusions

In conclusion, despite the limited available literature, blood β-carotene, β-cryptoxanthin, and ascorbic acid levels are proposed as useful biomarkers for estimating vegetable and fruit intake in nutritional epidemiological studies in Asia. In the future, it will be necessary to accumulate evidence, including the quantitative relationship between vegetable and fruit intake, their biomarkers, and their relationship with health indicators.

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