Effects of Oral Carotenoids on Oxidative Stress: A Systematic Review and Meta-Analysis of Studies in the Recent 20 Years

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Carotenoids protect organs, tissues, and cells from the damaging action of singlet oxygen, oxygen radicals, and lipid peroxides. This systematic review was sought to evaluate the influence of oral carotenoids on antioxidant/oxidative markers, blood carotenoids levels, and lipid/lipoprotein parameters in human subjects. A comprehensive review of relevant literature was conducted in PubMed, Web of Sciences, and the Cochrane library, from 2000 to December 2020. Randomized controlled trials, case-controlled trials, or controlled trials were identified. A total of eighteen trials were included, with the target populations being healthy subjects in 16 studies, athletes in 1 study, and pregnant women in 1 study. The meta-analysis results showed that carotenoids complex supplementation significantly increased the levels of antioxidative parameters ferric-reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) [standardized mean difference (SMD) = 0.468; 95% CI: 0.159–0.776, \( p = 0.003 \); SMD = 0.568; 95% CI: 0.190–0.947, \( p = 0.003 \)] and decreased the blood triglyceride (TG) level (SMD = −0.410, 95% CI: −0.698 to −0.122, \( p = 0.005 \)). Oral carotenoids supplement significantly increased the blood levels of \( \beta \)-carotene (SMD = 0.490, 95% CI: 0.123–0.858, \( p = 0.009 \)), \( \alpha \)-tocopherol (SMD = 0.752, 95% CI: 0.020–1.485, \( p = 0.044 \)), and the intaking durations were 8 weeks. The levels of antioxidative enzymes and other lipid/lipoprotein parameters were not different between subjects receiving carotenoids and controls (\( p > 0.05 \)). In conclusion, our systematic review showed that the carotenoids complex is beneficial for alleviating potential oxidative stress via interacting with free radicals or decreasing blood TG levels. The intaking duration of carotenoids should be 8 weeks to reach enough concentration for function.

Keywords: carotenoids, oxidative stress, antioxidants, lipid/lipoprotein, age-related disease

INTRODUCTION

Oxidative stress has been implicated in the etiology of several chronic diseases, namely, cardiovascular disease (1), type 2 diabetes (2), neurodegenerative disease (3), some cancers (4–6), and also involved in the aging process and age-related diseases (ARDs) (7). Aging is an unavoidable biological phenomenon affecting all multicellular organisms (8). Various hypotheses have been put
forward to explain the molecular reasons for aging (9, 10). In damage theories, reactive oxygen species (ROS) is considered to lead to cumulative DNA, protein, and lipid damages, which play a prominent role in the pathogenesis and progression of aging (11). Accumulation of ROS leads to inflammation, cellular dysfunction and cell death, and mitochondrial dysfunction. Decline in mitochondrial function, the oxidative stress response in aging, and accumulation of aberrant proteins

FIGURE 1 | Flowchart of literature search and selection of studies.
may contribute to ARD (12). What is more, a specific form of oxidative stress called photo-oxidative stress (13) which is induced by UV exposure can cause a common external aging, i.e., photoaging (14). Uncontrolled production of ROS is implicated in vascular injury and oxidative stress participates in antioxidant mechanisms in the development and progression of atherosclerosis (15). ROS can also promote tumor formation by inducing DNA mutations and pro-oncogenic signaling pathways; oxidative stress is an important factor in both the tumor development and responses to anticancer therapies (16).

In this regard, carotenoids are of particular interest (12). Carotenoids act as electron-transport agents and play crucial roles in protecting organs, tissues, and cells from the damaging action of singlet oxygen, oxygen radicals, and lipid peroxides (17). Experimental studies have demonstrated that they reduce chemical-induced neoplasia (18), improve erythrocyte antioxidant status (19), and protect tissues from UV-related damage (20). According to chemical structure, carotenoids were generally classified as pure hydrocarbon carotenoids called “carotenes” (such as lycopene, α-carotene, β-carotene and β-cryptoxanthin) and carotenoids containing one or several oxygen functions known as “xanthophylls” (such as lutein, zeaxanthin) (21). Some precursors during biosynthesis such as lycopene also belong to carotenoids (21). Nowadays, carotenoids are the most numerous and widespread group of hydrophobic pigments mainly in fruits and vegetables (22). High fruit and vegetable consumption is linked with changes in specific antioxidant markers or early-disease indicators, for example, cholesterol oxidation products, plasma antioxidant capacity, oxidized DNA base damage, etc. (23–28). It has recently been hypothesized that carotenoids cannot be biosynthesized by humans and animals de novo, but can be derived from their food and feed, respectively (21).

To the best of our knowledge, the systematic investigation of the relationship between diary carotenoids and redox markers or lipid/lipoprotein parameters was lacking. The current systematic review was conducted to evaluate the influence of diary carotenoids on antioxidant/oxidative stress, blood carotenoids levels, and lipid/lipoprotein parameters, in human subjects. The

### TABLE 1 | Fundamental information of subjects.

| References               | Country | Sex (male/female) | Age (years) | Body mass index (kg/m²) | Weight (kg) |
|--------------------------|---------|------------------|-------------|-------------------------|-------------|
| Zhuang et al.            |         |                  |             |                         |             |
| Duthie et al. (29)       | UK      | 9/12             | 48.3 (5.6)  | 26.6 (3.9)              | 20.2 (2.3)  |
| Ryu et al. (30)          | Korea   | 22/11            | 48.2 (1.4)  | 23.9 (0.6)              | 20.1 (2.3)  |
| Turner et al. (31)       | USA     | 0/34             | 23.0 (4.0)  | 20.4 (2.9)              | 61.4 (11.5) |
| Miyazawa et al. (32)     | Japan   | 3/3              | 58.0 (7.0)  | 23.4 (3.4)              | 59.3 (10.4) |
| Ma et al. (33)           | China   |                  | T1: 10.0 (0.6); |                         |             |
|                         |         |                  | T2: 18.6 (1.2); |                         |             |
|                         |         |                  | T3: 65.3 (9.0) |                         |             |
|                         |         |                  | C1: 10.1 (0.7); |                         |             |
|                         |         |                  | C2: 18.6 (0.8); |                         |             |
|                         |         |                  | C3: 24.4 (3.2) |                         |             |
| Jacob et al. (34)        | Spain   | 12               | 23.0 (2.0)  | 21.5 (2.8)              | 70.8 (1.2)  |
| Briviba et al. (35)      | Germany | T1: 21/0; T2: 21/0 | 24.1 (2.4); |                         |             |
| Concentrate et al. (36)  | USA     | 31               | 25.3 (3.4)  | 24.0 (3.9)              | 24.5 (3.1)  |
| Tauler et al. (37)       | Spain   | 8/0              | 23.3 (2.0)  | 24.5 (1.3)              |             |
| Aust et al. (38)         | Germany | T1: 12; T2: 12   |             | (18.0–25.0)             |             |
| Hininger et al. (39)     | France  | 0/33             | 29.0 (3.4)  | 23.3 (7.4)              |             |
| Upritchard et al. (40)   | Netherlands | 1/20 | 35.0–70.0 | 22.8 (76.9) |             |
| Nelson et al. (41)       | USA     | T1: 13; T2: 15; T3: 13 | (18.0–25.0) |             |
| Kiokias and Gordon (42)  | UK      | 31               | 71.2 (5.5)  | 22.47 (3.0)             |             |
| Heinrich et al. (43)     | Germany | T1: 12; T2: 12   |             | 80.0 (11.3)             |             |
| Schmidt et al. (44)      | USA     | 21               | 22.5 (3.9)  | 82.3 (8.7)              |             |
| Stahl et al. (45)        | Germany | 9                | 26.0–67.0   |                     |             |
| Stahl et al. (46)        | Germany | 10               | 20.0–57.0   |                     |             |
### TABLE 2 | Intervention and measurement parameters of all included studies classified by intervention type.

| References | Treatment (T) | Control (C) | Measurement |
|------------|---------------|-------------|-------------|
| **Carotenoids complex** | | | |
| Ryu (33) | 5.0 g chlorella (13.0 mg lutein, 0.25 mg zeaxanthin, 1.2 mg β-carotene, 0.85 mg α-carotene) (daily, 4 weeks) | Placebo (daily, 4 weeks) | TG (µmol/L), HDL (µmol/L) |
| Miyazawa (32) | 8.0 g chlorella (22.9 mg lutein, 5.0 mg β-carotene) (daily, 2 months) | 0.0 g chlorella (daily, 2 months) | HDL (mg/dL), TG (mg/dL), lutein (µmol/L), zeaxanthin (µmol/L), α-carotene (µmol/L), β-carotene (µmol/L), lycopene (µmol/L), α-tocopherol (µmol/L) |
| Ma (33) | T1 (children): 2,000.0 IU retinol, 1.0 mg β-carotene, 100.0 mg α-tocopherol, 300.0 mg ascorbic acid, 200.0 µg selenium (daily, 2 months); T2 (young people): 3,000.0 IU retinol, 1.5 mg β-carotene, 200.0 mg α-tocopherol, 500.0 mg ascorbic acid, 400.0 µg selenium (daily, 2 months); T3 (old people): 3,000.0 IU retinol, 1.5 mg β-carotene, 200.0 mg α-tocopherol, 500.0 mg ascorbic acid, 400.0 µg selenium (daily, 2 months) | C1 (children): placebo (daily, 2 months); C2 (young people): placebo (daily, 2 months); C3 (old people): placebo (daily, 2 months) | TG (µmol/L), HDL (µmol/L), GPx (nKat/109 cells), CAT (K/109 cells), SOD (pKat/109 cells) |
| Concentrate (36) | 7.5 mg β-carotene, 234.0 mg vitamin C, 45.0 IU vitamin E, 420.0 mg folate, 60.0 mg calcium (daily, 11 weeks) | Placebo (microcrystalline cellulose) (daily, 11 weeks) | ORAC (µmol/L), β-carotene (µmol/L), lutein (µmol/L), lycopene (µmol/L) |
| Tauer (37) | 250.0 mg Vitamin E, 15.0 mg of β-carotene (daily, 90 days), 1.0 g vitamin C (daily, 16–90 days) | Placebo (lactose) (daily, 90 days) | SOD (pKat/109 cells), CAT (K/109 cells), GPx (nKat/109 cells) |
| Aust (38) | T1: 9.8 mg lycopene, 0.8 mg phytoflueone, 1.0 mg phytoene, 0.4 mg β-carotene (daily, 12 weeks); T2: 8.2 mg lycopene, 3.2 mg phytoflueone, 4.6 mg phytoene, 0.4 mg β-carotene (daily, 12 weeks) | 10.2 mg lycopene, 0.0 mg phytoflueone, 0.0 mg phytoene, 0.0 mg β-carotene (daily, 12 weeks) | lycopene (µmol/L), β-carotene (µmol/L) |
| Hiringer (39) | 60.0 mg Vitamin C, 4.8 mg β-carotene, 10.0 mg vitamin E, 1.4 mg thiamin, 1.6 mg riboflavin, 15.0 mg niacin, 6.0 mg pantothenic acid, 200.0 mg folic acid, 1.0 mg cobalamin, 15.0 mg Zn, 87.5 mg Mg, 100.0 mg Ca (daily, 2 months) | Placebo (daily, 2 months) | β-carotene (µmol/L) |
| Upritchard (40) | T1: 43.0 mg vitamin E, 0.22 mg lutein, 0.06 mg lycopene, 0.06 mg α-carotene, 0.11 mg β-carotene (daily, 11 weeks); T2: 111.0 mg vitamin E, 0.63 mg lutein, 0.18 mg lycopene, 0.14 mg α-carotene, 0.28 mg β-carotene (daily, 11 weeks) | 1.3 mg vitamin E (daily, 11 weeks) | HDL (µmol/L), TG (µmol/L), α-tocopherol (µmol/L), lutein (µmol/L), α-carotene (µmol/L), β-carotene (µmol/L), lycopene (µmol/L), FRAP (µmol/L) |
| Nelson (41) | T1: one food group (11.0 mg β-carotene, 6.0 mg lutein, 0.6 mg zeaxanthin, 10.0 mg lycopene) (daily, 5 weeks); T2: 2 antioxidant capsule (2.4 mg β-carotene, 6 mg lutein/zeaxanthin, 0.5 mg lycopene) (daily, 5 weeks); T3: 2 antioxidant tablet (4.0 mg β-carotene, 4.0 mg lutein/zeaxanthin) (daily, 5 weeks) | Placebo (daily, 5 weeks) | lutein (µmol/L), zeaxanthin (µmol/L), lycopene (µmol/L), α-carotene (µmol/L), β-carotene (mmol/L), α-tocopherol (µmol/L), ORAC (µmol/L) |
| Kiokias (42) | 4 capsules (1.0 g) (fish oil), 24.6 mg tomato extract, 6.3 mg palm oil carotene extract, 2.0 mg marigold extract, 3.7 mg paprika extract, 3.7 mg bixin (daily, 6 weeks) | Placebo (daily, 5 weeks) | ORAC (µmol/L) |
| Heinrich (43) | T1: 24.0 mg β-carotene, soybean oil, 8.0 mg lycopene (daily, 12 weeks); T2: 8.0 mg β-carotene, 8.0 mg lycopene, 8.0 mg lutein, 8.0 mg vegetable oil (daily, 12 weeks) | Soybean oil (daily, 12 weeks) | β-carotene (µmol/L), lutein (µmol/L), lycopene (µmol/L) |
| Schmidt (44) | 20.050.0 IU β-carotene, 330.0 mg ascorbic acid, 650.0 IU α-, β-, γ-, δ-tocopherols, 167.0 g selenium, 13.2 mg catechin, 500.0 µg lutein, 100.0 µg lycopene, 181.0 µg N-acetyl-1-cysteine, 5.0 mg pomegranate extract, 100.0 mg vegetable blend concentrate (lutein, β-carotene, α-carotene, lycopene) (daily, 24 days) | Placebo (daily, 24 days) | FRAP (moles trolox equivalents/ml), ORAC (moles trolox Equivalents/ml), α-carotene (µg/ml), β-carotene (µg/ml), lutein (µg/ml), lycopene (µg/ml), α-tocopherol (µg/ml), zeaxanthin (µg/ml) |

(Continued)
TABLE 2 | Continued

| References      | Treatment (T)                                                                 | Control (C)                                                                 | Measurement                                                                 |
|-----------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Stahl (45)      | 40.0 g tomato paste, 10.0 g olive oil, 16.0 mg lycopene, 0.5 mg β-carotene 0.1 mg lutein (daily, 10 weeks) | 10.0 g olive oil (daily, 10 weeks)                                        | α-tocopherol (µmol/L), α-carotene (µmol/L), β-carotene (µmol/L), lutein (µmol/L), lycopene (µmol/L) |
| Stahl (46)      | Carotenoid, vitamin E (daily, 12 weeks)                                       | 25.0 mg total carotenoids, 25.0 mg carotenoids, 13.0 mg all-trans-β-carotene, 10.5 mg 9-cis β-carotene, 0.3 mg other cis isomers β-carotene, 0.75 mg α-carotene, 0.18 mg cryptoxanthin, 0.15 mg zeaxanthin, 0.12 mg lutein (daily, 12 weeks) | β-carotene (µmol/L), α-tocopherol (µmol/L) |
| Fruits/vegetables |                                                                                   |                                                                            |                                                                            |
| Duthie (23)     | 480.0 g diverse fruits, vegetables, fruit juices (daily, 12 weeks)               | 3 or fewer portions fruits and vegetables (daily, 12 weeks)                  | FRAP (µmol Fe(II)/L), SOD (U/g Hb), CAT (U/g Hb), GPx (U/g Hb), HDL (µmol/L), TG (µmol/L) α-tocopherol (µg/ml), β-carotene (µg/ml), α-carotene (µg/ml), lycopene (µg/ml) |
| Turner (31)     | 0.5 mg vitamin A, 200.0 g orange-fleshed sweet potatoes (daily, 6 days/week, 3 weeks)  | 200.0 g white-fleshed sweet potatoes, 2 corn-oil capsule (daily, 6 days/week, 3 weeks) | β-carotene (µmol/L), α-carotene (µmol/L), lycopene (µmol/L), α-tocopherol (µmol/mmol lipid) |
| Jacob (34)      | 500.0 ml tomato juice (41.8 mg lycopene, 90.0 mg vitamin C) (daily, 2 weeks)       | 500.0 ml tomato juice (870.0 mg vitamin C) (daily, 2 weeks)                 | lycopene (µmol/L), β-carotene (µmol/L), lutein (µmol/L), tocopherol (mg/L), FRAP (µmol/L) |
| Briviba et al. (35) | T1: 0.8 (0.2) servings vegetables, 1.1 (0.4) servings fruits (total 1.9 (0.5) servings) (daily, week 1–4) 4.3 (0.6) servings vegetables, 3.5 (0.5) servings fruits (total 7.8 (1.1) servings) (daily, weeks 4–8); T2: 0.9 (0.2) servings vegetables, 1.0 (0.3) servings fruits (total 1.9 (0.4) servings) (daily, week 1–4) 2.8 (0.4) servings vegetables, 1.9 (0.3) fruits servings (total 4.6 (0.7) servings) (daily, weeks 4–8) | 1.0 (0.3) servings vegetables, 1.0 (0.4) servings fruits (total 2.0 (0.6) servings) (daily, week 1–4) 1.0 (0.5) servings vegetables, 1.0 (0.4) servings fruits (total 2.0 (0.8) servings) (daily, weeks 4–8) | Lutein (nM), zeaxanthin (nM), α-carotene (nM), β-carotene (nM), lycopene (nM) |

ORAC, oxygen radical absorbance capacity; TG, triglyceride; HDL, high density lipoprotein; FRAP, ferric-reducing ability of plasma; SOD, superoxide dismutase; CAT, catalase.

FIGURE 2 | The methodological quality assessment of included studies based on risk of biases presenting as percentages across all included studies.
study will add valuable evidence of carotenoids on improving disease caused by oxidative stress.

**MATERIALS AND METHODS**

**Search Strategy**

A systematic review of the literature was conducted in the following databases: PubMed, Web of Sciences, the Cochrane library, and CNKI from 2000 to December 2020. The searching strategies were interventions (“antioxidant” or “carotenoid” or “carotene” or “lutein” or “lycopene” or “zeaxanthin” or “tocopherol” or “cryptoxanthin” or “canthaxanthin”), outcomes (“oxidative stress” or “ferric-reducing ability of plasma (FRAP)” or “oxygen radical absorbance capacity (ORAC)” or “superoxide dismutase (SOD)” or “catalase (CAT)” or “glutathione peroxidase (GPx)” or “lipid” or “lipoprotein” or “high density lipoprotein (HDL)” or “triglyceride (TG)”), and study designs (“random” or “control”). In addition, a hand-searching of the citation lists and the articles of the relevant publications were performed to identify other potentially eligible studies.

**Study Selection**

The clinical trials with the following criteria were included in this systematic review: (1) randomized controlled trials (RCTs), case-controlled trials, and controlled trials; (2) human subjects being the target population without age limitation; (3) studies relating to oxidative stress, lipid/lipid-protein, or carotenoids level; and (4) treatment group receiving oral carotenoids supplementation (single carotenoid, carotenoids complex, or dietary botanical carotenoids) and control group (placebo, no treatment, or other treatment). Studies that lack necessary information were excluded.

**Data Extraction and Quality Assessment**

Characteristics of eligible studies were extracted using a predesigned collection form. The data extracted included: The first author’s name, publication year, country, subject numbers, gender, age, body mass index (BMI), weight, intervention, and outcome.

The methodological quality of each included study was assessed using the “the Cochrane Collaboration Risk of bias tool.” The assessment covers the following biases: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias.

Two independent authors (Chengfei Zhuang and Yan Sun) did the above tasks of literature searching, study selection, data extraction, and risk of bias assessment. Upon any disagreement, a third author (Yan Wu) was resorted to reach a consensus.

**Statistical Analysis**

All the statistical analyses were performed using STATA 13.0 software (Stata Corporation, College Station, Texas, USA).
Moreover, RevMan V.5.3 software (Cochrane Collaboration, Oxford, UK) was used for risk of bias assessment. Standardized mean difference (SMD) with 95% CI was used to express the comparison results of two groups, based on the inverse variance method and Cohen statistics. When an outcome was measured in 2 or more studies, the pooled estimate was made. When outcomes were measured at 2 or more time points in the included studies, only data of baseline and final time point were extracted and the change levels of treatment and control group were compared. Subanalysis by supplements and time points was undertaken if there were enough studies to conduct a separate meta-analysis. The data were considered the significant difference between treatment and control groups when the $p < 0.05$. The results of the meta-analysis were presented as forest plots. Heterogeneity was determined using $p$-value and $I^2$ statistics. The $I^2 > 50\%$ with a $p < 0.05$ was denoted to be significant heterogeneity between the studies, and a random-effects model was adopted, or else, a fixed-effects model would be used. If obvious clinical heterogeneity existed, though statistical heterogeneity was not detected, a random effects model would be adopted. Publication bias was scrutinized using Egger’s test with a $p < 0.1$ being representative of significant publication bias.

RESULTS

Literature Search

A total of 13,812 potentially relevant articles were found in the initial search, and 11,991 articles were excluded by removing the duplications and screening the titles and/or abstracts. Full-text evaluations were conducted for the remaining 134 articles, and 104 of these articles were excluded for not meeting inclusion criteria. Eventually, 18 articles were included in our analysis (29–46). The details of the step-by-step trial's identification and selection process are given in Figure 1.

Characteristic of Included Studies and Subjects

The effects of carotenoids were evaluated by oxidative stress parameters, carotenoid level, and lipids or lipoprotein levels. Trials were conducted in 9 countries and published between 2000 and 2017. The sample size ranged from 12 to 99 subjects and...
FIGURE 5 | Meta-analysis result showing that carotenoids complex significantly increased oxygen radical absorbance capacity (ORAC) concentration comparing to control group.

age ranged from 10.0 to 71.77 years old. BMI ranged from 18 to 27.8 kg/m² and weight ranged from 20.2 to 82.3 kg. The target populations were healthy subjects in 16 studies, athletes in 1 study (29), and pregnant women in 1 study (30). Table 1 presents the fundamental information of all the subjects.

The treatment durations are mainly 4–12 weeks, with the shortest duration being 2 weeks and the longest duration being 12 weeks. The tested carotenoids in blood were β-carotene, α-carotene, lutein, lycopene, zeaxanthin, and α-tocopherol. The interventions of 14 trials were carotenoids complex, 4 trials were fruits/vegetables, and 0 trial was single carotenoid. The formulations of carotenoids were capsules or tablets and fruits/vegetables were juice or servings. The doses of carotenoids complex were characterized into low dose (<20 mg), medium dose (≥20 mg, <50 mg), and high dose (>50 mg). Among the studies for meta-analysis, both the low and medium doses of carotenoids complex were applied in trials measuring HDL and TG and, thus, subgroup analyses stratified by doses were conducted, simple medium dose of carotenoids complex was seen in trials with FRAP measurement, and simple low doses were found in trials with other measured parameters. Of all included studies, only one study applied high doses of carotenoids complex and did not participate in meta-analysis as the necessary data were unavailable. Table 2 shows the detailed interventions and measurements of all the included studies.

Quality Assessment

All the trials except for 1 trial (32) used an appropriate method of random sequence generation. The majority of trials (15/18) were assessed as low risk of bias for allocation concealment. One trial did not explain the detailed process of blinding, 10 trials were classified as low risk of blinding of participants and personnel (performance bias) and 11 trials had a low risk of blinding of outcome assessment (detection bias). One trial explained the unclear risk of incomplete outcome data (attrition bias). Risks of bias are shown in Figures 2, 3.

Meta-Analysis Results and Descriptive Analysis Results

Two Antioxidative Capability Parameters

There were respective 5 and 3 trials that compared change levels of FRAP and ORAC between the treatment and control groups. Both the FRAP and ORAC concentrations were significantly increased in the group receiving carotenoids compared with the control groups (SMD = 0.371; 95%
### TABLE 3 | Meta-analysis/subgroup analysis and publication bias of included studies.

| Measurement index | Number of study | SMD    | 95% CI    | p     | Heterogeneity | Model | Publication bias |
|-------------------|-----------------|--------|-----------|-------|---------------|-------|------------------|
| **Antioxidative capability parameters** |                 |        |           |       |               |       |                  |
| FRAP              | Overall         | 5      | 0.371     | 0.113 | 0.629         | 0.005* 0.000% 0.407 Fixed −1.06 0.368 |
|                   | Fruits/vegetables | 2     | 0.143     | −0.331 | 0.616        | 0.554 0.000% 0.896 Fixed −1.06 0.368 |
|                   | Carotenoids complex (MD) | 3     | 0.468     | 0.159 | 0.776         | 0.003* 26.0% 0.259 Fixed −1.06 0.368 |
|                   | Carotenoids complex (LD) | 3     | 0.568     | 0.190 | 0.947         | 0.003* 40.9% 0.184 Fixed −1.61 0.354 |
| **Antioxidative enzymes** |                 |        |           |       |               |       |                  |
| SOD               | Overall         | 3      | 0.237     | −0.673 | 1.147        | 0.200 67.1% 0.048 Random 0.55 0.680 |
|                   | Fruits/vegetables | 1     | −0.043    | −0.629 | 0.542        | 0.885  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  − −  -
Subgroup analysis of β-carotene showing a higher β-carotene level in fruits/vegetables group than in placebo group at week 8.

CI: 0.113–0.629, \( p = 0.005 \); SMD = 0.568; 95% CI: 0.190–0.947, \( p = 0.003 \) (Figures 4, 5). Subgroup analysis of FRAP showed that medium-dose carotenoids complex significantly increased antioxidative capability comparing to control (SMD = 0.468; 95% CI: 0.159–0.776, \( p = 0.003 \)), while supplements of fruits/vegetables did not significantly increased antioxidative capability (\( p > 0.05 \)) (Table 3).

Three Antioxidative Enzymes Parameters

The changes from posttreatment to the baseline of SOD, CAT, and GPx were compared between treatment and control groups. No significant differences were seen for all the parameters (\( p > 0.05 \)) neither pooled results nor subgroup results were stratified by different forms of oral carotenoids (Table 3).

Blood Levels of Seven Carotenoid Levels Indexes

In all, 14 trials compared β-carotene levels between the treatment and control groups. Meta-analysis of 11 trials showed that significant difference was seen at week 8 and the difference came from fruits/vegetables (SMD = 0.665, 95% CI: 0.232–1.098, \( p = 0.003 \)) (Figure 6), as the fruits/vegetables treatment group produced higher β-carotene level than the control group (Table 3). The other 3 trials that had no deserved data for meta-analysis also showed that the treatment groups had higher β-carotene levels than the control groups (\( p = 0.003 \); Table 4).

There were 7 trials comparing α-tocopherol levels between the treatment and control groups. The pooled results of 6 trials showed significant differences (\( p < 0.05 \)) and the subgroup analysis presented that supplement of low-dose carotenoids complex contributed to the difference (SMD = 1.314, 95% CI: 0.520–2.107, \( p = 0.001 \)) (Figure 7) (Table 3). One trial showed that no significant differences were present between the orange-fleshed sweet potatoes treatment group and the white-fleshed sweet potatoes control group (\( p > 0.05 \); Table 4).

The meta-analyses results showed that the levels of other 5 kinds of carotenoid, i.e., α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin were not different between treatment and control groups (\( p > 0.05 \)) (Table 3). One trial that had no sufficient data for meta-analyses showed that remarkable changes of α-carotene, lutein, or zeaxanthin were, respectively, seen between the low-dose carotenoids complex group and the placebo group (\( p < 0.05 \); Table 4).

Lipid or Lipoprotein Parameters

The changes from posttreatment to the baseline of HDL and TG were compared between treatment and control groups.
were the most abundant consumed carotenoids, and β-carotene and lycopene were the most abundant consumed carotenoids biochemically (47). In recent years, carotenoids complex and dietary botanical carotenoids (especially in fruits/vegetables) were more welcomed than a single form of carotenoid. The current systematic review, not only an updated systematic review of the previous one but specially reported carotenoids complex and dietary botanical carotenoids rather than single carotenoids. Besides, we added antioxidative markers and lipid/lipoprotein parameters that may be influenced by oral carotenoids, and these important markers were not analyzed in the previous systematic review.

In the aspect of antioxidative effects, FRAP and ORAC are standardized indexes measuring the antioxidant capacity of nutraceutical or dietary supplement industries (48). FRAP follows a single electron transfer mechanism and ORAC follows a hydrogen atom transfer mechanism. Antioxidant phenols directly interact with free radicals by electron or hydrogen atom donation to fulfill antioxidative capacity (49). For example, Shanely et al. (50) showed that watermelon puree was associated with increases in plasma antioxidant capacity measured by FRAP and ORAC. In our meta-analysis, pooled results showed that oral carotenoids supplementation significantly increased the blood levels of FRAP, and subgroup analysis indicated that the antioxidative capacity attributed to carotenoids complex but not fruits/vegetables. The pooled results of studies measuring ORAC also showed higher antioxidative capacity after intaking the carotenoids complex. One previous uncontrolled study demonstrated that levels of single antioxidants in food do not necessarily reflect their total antioxidant capacity which also depends on the synergic and redox interactions among the different molecules present in the food (51). This theory may explain the relatively high antioxidative capacity of the carotenoids complex. In the current study, we also assessed

The pooled results showed that no significant differences were seen of any parameters (p > 0.05). The subgroup analysis, stratified by different forms of carotenoids, showed that medium-dose carotenoids complex significantly decreased the blood TG level (SMD = −0.410, 95% CI: −0.698 to −0.122, p = 0.005) (Figure 8) (Table 3). The trial that had no sufficient data for meta-analyses showed that remarkable changes of HDL or TG were, respectively, seen between the low-dose carotenoids complex group and placebo group (p < 0.05; Table 4).

**Publication Bias (Table 3)**
Egger’s regression tests showed that most indexes had no evidence of publication bias (p > 0.05), with the exception of α-tocopherol (t = 4.01, p = 0.016) and TG (t = −5.89, p = 0.004) (Table 3).

**DISCUSSION**
A systematic review conducted in 2015 assessed that a range of single carotenoids among which β-carotene and lycopene were the most abundant consumed carotenoids, and β-carotene and α-carotene were the most commonly assessed carotenoids biochemically (47). In recent years, carotenoids complex and dietary botanical carotenoids (especially in fruits/vegetables) were more welcomed than a single form of carotenoid. The current systematic review, not only an updated systematic review of the previous one but specially reported carotenoids complex

### TABLE 4 | Descriptive analyses of included studies that had no sufficient data for meta-analyses.

| Measurement index | Intervention | References | Outcome |
|-------------------|-------------|------------|---------|
| **β-carotene**    | Fruits/vegetables | Turner (31) | Plasma β-carotene increased 250% in the orange-fleshed sweet potatoes group, and the mean change in plasma β-carotene (0.306 ± 0.070 mmol/L) was different from that in the white-fleshed sweet potatoes group (p < 0.001). |
|                   | Carotenoids complex (HD) | Ma (33) | Significant increases of plasma β-carotene after 2 months supplementation in all three supplemented groups compared with the respective placebo groups (p < 0.001). |
|                   | Carotenoids complex (LD) | Hninger (39) | Significant higher level of plasma β-carotene in the supplemented group at 9 months of gestation, comparing to placebo group (p < 0.05). |
| **α-carotene**    | Carotenoids complex (LD) | Ryu (30) | Remarkable changes of α-carotene between two groups (Chlorella 163.6%; placebo 15%; p < 0.0001). |
| **Lutein**        | Carotenoids complex (LD) | Ryu (30) | Remarkable changes of lutein two groups (Chlorella 89.6%; placebo −1.7%; p < 0.0001). |
| **Zeaxanthin**    | Carotenoids complex (LD) | Ryu (30) | Remarkable changes of zeaxanthin between two groups (Chlorella 89.6%; placebo −1.7%; p < 0.0001). |
| **α-tocopherol**  | Fruits/vegetables | Turner (31) | No significant differences between orange-fleshed sweet potatoes group and white-fleshed sweet potatoes group. |
| **HDL**           | Carotenoids complex (LD) | Ryu (30) | Remarkable changes of HDL (Chlorella 4.0%; placebo < betwep = 0.023) compared with placebo. |
| **TG**            | Carotenoids complex (LD) | Ryu (30) | Remarkable changes of TG (Chlorella 4.0%; placebo sweet pp = 0.023) compared with placebo. |

HDL, high density lipoprotein; TG, triglyceride; LD, low dose (<20 mg); MD, medium dose (≥20 mg, <50 mg); HD, high dose (≥50 mg).
the levels of antioxidative enzymes in blood before and after carotenoid supplements. The antioxidative enzymes play protective effects against active and massive oxidative attacks due to the ability to decompose ROS (48). For example, SOD can catalyze superoxide into oxygen and hydrogen peroxide (52), and CAT and GPx can neutralize the hydrogen peroxide by decomposing it into molecular oxygen and water (53, 54). Our meta-analysis showed that oral intake of carotenoids, no matter carotenoids complex or fruits/vegetables, did not influence the blood levels of these antioxidative enzymes. The current results indicated that oral carotenoids play antioxidative roles via interacting with free radicals by electron or a hydrogen atom (direct action) but not via promoting antioxidative enzymes (indirect action).

Given the relationships between some botanical antioxidants (for example, polyphenol compounds and phytochemicals) and vitagenes network in health benefit (for example, neuroprotection) (3, 55), we should not ignore such potential indirect mechanism of carotenoids. The generation of ROS is involved in the regulation of cellular stress response mechanisms and is a highly regulated process under the control of vitagenes (55, 56). Vitagenes are redox-sensitive genes coding for redox proteins (55, 56). These proteins control a complex network of intracellular signaling pathways relevant to life span and preservation of cellular homeostasis under stress conditions (3). Hormetric dose responses are mediated by endogenous cellular defense pathways (3). Modulation of endogenous cellular defense mechanisms represents a therapeutic intervention in oxidative stress-related diseases (3). Antioxidants including carotenoids may play their protective role through a hormetic-dependent activation of vitagenes (3).

High-density lipoprotein (HDL) is plasma lipoproteins that are macromolecular assemblies of proteins and lipids (57). A review put forward that high levels of lipid oxidation products in HDL appear to associate with the prevention of atherosclerosis (58). In our meta-analysis, the supplement of various carotenoids did not change the level of HDL in the blood. Interestingly, the blood TG level was significantly decreased by the carotenoids complex. According to a previous report, a high level of TG could aggravate oxidative stress, then drove mucosal inflammation and increased mucosal barrier permeability, thus promoting colitis (59). High TG levels also cause prediabetic neuropathy through oxidative-nitrosative stress (60). These results demonstrated that oral intake of carotenoids, especially carotenoids complex, may have protective effects of some oxidative stress relating diseases via decreasing TG levels.
After being orally taken, carotenoids are liberated from the food matrix, absorbed into the bloodstream, and then they will exert biological effects (61). Some factors may influence the bioactivities of carotenoids. For example, different isomers of lycopene have discriminatory bioaccessibility. The cis-isomer in tissues exceeds that in foods, and the greater bioaccessibility contributes to the enrichment in tissues, as compared with all-trans isomers (62). Besides, serum concentrations of most carotenoids decrease abiding by first-order kinetics, with plasma half-life between 26 and 76 days (about 4 and 10 weeks) (63). In our systematic review, the blood levels of 6 carotenoids were tested, and α-carotene, β-carotene, and α-tocopherol got positive findings. The blood β-carotene level was not increased until 8 weeks of fruits/vegetable supplement. In addition, the carotenoids complex supplement produced a high level of α-tocopherol after week 4. These results reflect that the intake duration of carotenoids should be long enough to reach enough concentration for function.

This systematic review has several advantages. Different from other systematic reviews, the current one especially, examined the effects of carotenoid supplantations on oxidative stress in vivo. We first demonstrated that the carotenoids complex had more advantages over fruits/vegetables. All potential studies, namely, RCTs, case-controlled trials, and controlled trials, were included to obtain as much information as possible, and the methodological quality of each study was relatively high. Nevertheless, the following limitations need further research. The measurement assessment of some types of carotenoids, such as α-carotene and zeaxanthin, was limited to only one time point. We were unable to state whether carotenoids have different antioxidative effects among subjects with variable physiologic states. Besides, origins (natural or synthetic) and administration methods (for example, chlorella crushed or not?) may influence the effects of carotenoids. We cannot provide the important information owing to unavailability.

In conclusion, this systematic review showed that the carotenoids complex is beneficial for alleviating potential oxidative stress via interacting with free radicals or decreasing blood TG levels. The intaking duration of carotenoids should be 8 weeks to reach enough concentration for function. Intake of carotenoids nutrition may have huge potentials for disorders/diseases relating to oxidative stress.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**AUTHOR CONTRIBUTIONS**

CZ: conceptualization and methodology. CZ, JZ, and YD: literature search. CZ, YS, and JY: formal analysis and investigation. CZ and YW: writing—original draft preparation and writing—review and editing. YW:
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.754707/full#supplementary-material
oral carotenoids on oxidative stress

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