Review

Effects of Astaxanthin Supplementation on Oxidative Stress

A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Editor's Choice

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Abstract: A systematic review and meta-analysis was conducted in six databases from 1948 to 2015 to assess the antioxidant activity of astaxanthin in humans. Nine randomized controlled trials were included in the systematic review. Results of meta-analysis revealed a borderline significant antioxidant effect of astaxanthin between the intervention and control groups, with a malondialdehyde-lowering effect for lipid peroxidation ($p = 0.050$). However, the data included here are insufficient. When compared with the baseline in intervention groups, the meta-analysis suggested that astaxanthin supplements significantly decreased plasma malondialdehyde {Standard mean difference (SMD) −1.32 μmol/L [95% CI −1.92, −0.72]; $p < 0.0001$} and isoprostane (SMD −3.10 ng/mL [95% CI −4.69, −1.51]; $p < 0.0001$). However, they increased superoxide dismutase (SMD 1.57 U/mL [95% CI 0.57, 2.56]; $p = 0.002$) and total antioxidant capacity (SMD 0.77 mmol 95% CI [0.12, 1.43]; $p = 0.018$). For dosage subgroup analysis, high dose (≥20 mg/day) of astaxanthin showed significant antioxidant effect (on total antioxidant capacity, isoprostane, and superoxide dismutase, $p < 0.05$). However, low dose (<20 mg/day) showed no significant effect ($p > 0.05$). Further duration subgroup analysis indicated that astaxanthin showed antioxidant effect after a 3-week intervention ($p < 0.001$), whereas this effect was not observed after a 12-week or 3-month intervention (on isoprostane and superoxide dismutase, $p > 0.05$). This review suggested that the antioxidant effect of astaxanthin on humans is unclear.

Keywords: Astaxanthin, meta-analysis, oxidative stress, randomized controlled trial, systematic review

Introduction

Oxidative stress is defined as "the imbalance between the oxidants and the antioxidants favouring the former," resulting in a change for signaling properties of redox and/or molecular damage [1, 2]. Oxidative stress contributes to more than 100 human disorders through DNA, lipid, or protein damage [3]. These human disorders include atherosclerosis [4], cardiovascular diseases [5], cancer [6], diabetes [7], and neurodegenerative diseases [8]. Additionally, a huge amount of in vitro and animal studies indicated that the nutrient antioxidants, such as vitamin C, E, and carotenoids, can combat lipid, protein, or DNA oxidation and defeat chronic diseases [9–14]. However, the evidence from clinical trials on human beings is controversial. Only a few clinical trials have consistently proven the advantages of using nutrient antioxidants to treat chronic diseases [15, 16]. However, others concluded with null or even negative outcomes [17–19]. For example, the protective effect of vitamin E was not been observed in heart outcomes prevention evaluation study, questioning the underlying principles of the antioxidant hypothesis for these micronutrients [17]. Stanner et al. reviewed the epidemiological studies and also failed to show any benefit from the consumption of these nutrient antioxidants by people with cancer and cardiovascular disease [20]. The antioxidant hypothesis has lasted for over 20 years, and still, no conclusion was reached. However, the failure may be due to the limitation of the study design, the oxidative status of subjects, the dosage of supplements, the intervention duration, or the limited studied types of micronutrients [21, 22]. Therefore, more research is needed either by consistently exploring the previously studied micronutrients (such as β-carotene) at different dosages or durations of intervention and on diverse subjects or by exploring novel and potent nutrient antioxidants with different biochemical characteristics [22].

Nowadays, astaxanthin (ASTX) has been studied extensively, because it is a relatively new carotenoid that can purportedly relieve oxidant stress. ASTX is a xanthophyll

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carotenoid that is present in microalgae, fungi, and seafoods; it is a potential antioxidant that can be used to prevent cardiovascular diseases [23]. From a biochemical standpoint, the potentially powerful antioxidant efficacy of ASTX may be due to its conjugated double bonds and polar property. ASTX contains 13 conjugated double bonds, whereas lutein, zeaxanthin, and lycopene contain 10, 11, and 11 conjugated double bonds, respectively [24]. Lee and Min compared the effects of different carotenoids and suggested that the antioxidant effects of the carotenoids are positively associated with the number of the conjugated double bonds [24]. Shimidzu et al. also indicated that more conjugated double bonds in carotenoids contribute to greater singlet oxygen-quenching activity [25]. Furthermore, compared with the nonpolar carotenoids (lycopene and β-carotene), the polar property of ASTX allows strategic placement in cell membranes, protecting the membrane structure as antioxidant [26]. Considering the above-mentioned biological discoveries, ASTX has been shown to possess strong potential as a potent antioxidant.

Furthermore, several animal trials have suggested that ASTX can lower oxidative stress by decreasing 8-hydroxy-2′-deoxyguanosine (8-OHdG) [27] and malondialdehyde (MDA) [28], but it can increase antioxidant enzymes [28, 29]. Clinical trials have also been conducted, but the number of trials is limited. Most of the clinical trials aimed to define the proper dose [30], assess the bioavailability [31], and safety [32] of ASTX on humans. However, according to the clinical trials, the protective effect of ASTX against oxidative stress is still inconclusive.

Carotenoids, such as lycopene, have been studied in systematic reviews and meta-analyses for antioxidant activity [33]. However, ASTX has not been systematically reviewed yet. Thus, the aim of the current review is to assess the impact of ASTX on a variety of parameters, i.e., oxidative stress, including lipid oxidation, protein oxidation, DNA damage, antioxidant enzymes, and other related biomarkers in plasma. Furthermore, we aim to explore the effect of the dosage and duration of ASTX intervention in subgroup analyses.

Methods and materials

Search strategy

Electronic searches were done in CENTRAL (The Cochrane Library) as well as in VOID MEDLINE, EMBASE, ESBCO, Web of Science, and CBM from January 1948 to December 2015. Some related literature was found by further searching through the reference lists of the primary studies and reviews. Trials published in English and Chinese were considered. No other limitation was imposed.

Terms used for OVID MEDLINE (and adopted for other databases) in both subject headings and full fields were:

1. *astaxanthin, haematococcus, carotenoids, xanthophyll;*
2. *antioxidants, DNA damage, protein oxidation, lipid peroxidation, oxidative stress, oxidoreductases, malondialdehyde, oxidation-reduction.*

The search started with the results obtained from using the terms (1) and (2). Then, the term randomized controlled trial was used as the limitation.

Inclusion criteria

Original studies were included if they met the following inclusion criteria: (1) intervention study; (2) randomized controlled trial (RCT); (3) investigated the impact of ASTX on biomarkers of oxidative stress; and (4) presented sufficient information on biomarkers of oxidative stress at baseline and at the end of study in both ASTX intervention and control groups.

The selection of these biomarkers was based on previous research, which specified that lipid peroxidation, protein oxidation, plasma antioxidant, DNA damage, antioxidant enzymes, and antioxidant capacity of plasma can be used as biomarkers to detect oxidative stress [33].

Currently, many markers have been proposed [34]; these markers can be classified as follows:

1. Oxidative damage to lipids: MDA, 4-hydr-oxynonenal, thiobarbituric acid–reactive substances (TBARS), phospholipid hydroperoxides (PLOOH), phosphatidylethanolamine hydroperoxide (PEOOH), and phosphatidylcholine hydroperoxide (PCOOH);
2. Oxidative damage to protein: advanced oxidation protein product (AOPP) and sulphydryl (SH) group;
3. Low-density lipid (LDL) oxidation: LDL lag time and LDL oxidation rate;
4. Product of the free radical oxidation of arachidonic acid: isoprostane (ISP);
5. Plasma antioxidant of lipid oxidation: uric acid (UA);
6. Oxidative damage to DNA: 8-OHdG;
7. Antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and paraoxonase (PON);
8. Plasma antioxidant capability: total antioxidant capability (TAC), total antioxidant status (TAS), total oxidant status (TOS), pro-oxidant-antioxidant balance (PAB), and redox balance.

Exclusion criteria

The following were the exclusion criteria: (1) studies that used food enriched with ASTX or preparations, in which

ASTX is not the main antioxidant; (2) studies that provided no numerical values on baseline; and (3) studies with inadequate details of methodologies or results (even after contacting the authors).

Data extraction

Data were extracted independently by two investigators (DW and HX) using guidelines published by the Cochrane Collaboration [35]. Eligible studies were reviewed and the following data were extracted: (1) first author's name; (2) year of publication; (3) study location; (4) sample size; (5) characteristics of study participants, e.g., age, gender, and body mass index; (6) study design; (7) form of treatment; (8) daily dosage of ASTX; (9) duration of the active treatment phase; and (10) outcome measurement of oxidative stress biomarkers.

Quality assessment

All trials were objectively assessed for the risk of bias by two independent reviewers (DW and HX), as recommended by the Cochrane guideline [35]. Briefly, each paper was assessed using the JADAD scale [36]. The JADAD scale is a 5-point scale for evaluating the quality of randomized trials, in which 3 points or more indicate superior quality. The items, which include allocation sequence, allocation concealment, blinding, and follow-up, were reported in the text.

Statistical analysis

Meta-analysis was conducted with STATA 11.0 software. We focused on all the biomarkers of oxidative stress in each included research and converted them into the same units. Data were extracted as baseline and endpoint means, standard deviations (SDs), and sample sizes of intervention and placebo groups for each oxidative outcome. Only data from the final time point were taken into consideration when tests were performed at multiple time points. The effect was calculated as the difference on the baseline and at the end-trial levels between the intervention and control groups for every dosage. We also extracted data from intervention groups and compared the difference before and after the interventions. One subgroup meta-analysis was conducted to explore whether the treatment effect on oxidative stress levels was associated with the dosage of ASTX (<20 or ≥ 20 mg/day), because we supposed that the influence of phytochemicals dosage on the outcomes was physiologically plausible [37]. In clinical studies, no adverse effect was observed after consumption of ASTX at dosages ranging from 2 mg to 40 mg even for 90 days [38]. Hence, half of the maximum dosage (40 mg/2 = 20 mg) was chosen as the dividing point. The duration of intervention was taken into our second subgroup analysis. We classified the durations into two categories: (1) short term, 21 days (3 weeks); and (2) long term, 84 (12 weeks) or 90 days (3 months). No unified rule is provided to classify the durations; two categories that are supposed to work well for the available data were chosen.

The fixed-effect model and random-effect model were used to combine data. When clinical heterogeneity was sufficient to expect that the underlying treatment effects differed among trials, or if substantial statistical heterogeneity was detected, a random-effect model was adopted to produce an overall summary.

In addition, the robustness of results was explored by sensitivity analysis excluding selected trials with potential risk of bias. Publication bias or small study effect was assessed by fail-safe index [39].

Finally, data not allowed for entry into the STATA software were reported in texts. We listed the supposed factors that might lead to the heterogeneity across studies as follows: (1) characteristic of the participants, which was normal or in a specific state (smoker or the overweight); (2) different design of trials: parallel or repeated measures design.

Results

Search result and description of included studies

The initial screening for potential relevance removed articles whose titles and/or abstracts were irrelevant. Among the 33 full text articles assessed for eligibility, we excluded 8 trials for not focusing mainly on ASTX, 14 trials for not providing specific oxidative stress biomarkers, and 1 trial for not stating explicitly the word "random" in the description of treatment assignment. Two trials [40, 41] focusing on different biomarkers of oxidative stress conducted with the same subjects were included into this meta-analysis as one trial. Characteristics of the nine clinical trials that met the criteria are summarized in Table 1, and the selection procedure is shown in Figure 1.

The nine trials were conducted between 2007 and 2015 and comprised of 436 participants. The trials were conducted in Serbia (two trials) [40–42], Japan [43], Finland [44], China [45], and South Korea (three trials) [46–48]. One trial was conducted by both South Korea and United States [49]. Seven of the nine trials included discrete control group (placebo-controlled or ASTX-free diet controlled)

Table I. Characteristics of include studies.

Study ID location [a]	Study design	Treatment/ Control [b]	ASTX dosage per day	Duration	Participants Characteristic [c]		Sample size	Biomarkers of oxidative stress
Baralic et al. 2013, 2015 [40], [41] Serbia	Randomized	T: ASTX capsules (made from Haematococcu spluvialis)	T: 4mg	90 days	Soccer players, M		Total: 40	TAS, TOS, PAB, UA, PON1, SH groups, TBARS, AOPP, redox balance
	Placebo-controlled	C: placebo (saccharose)	C: 0mg		Age: T: 17.9 ±0.2 ± C: 17.6 ± 0.1		T: 21	
	Parallel trial (2 groups)				BMI: T: 22.4 ± 0.3 ± C: 22.2 ± 0.4		C: 19	
	Double blind							
Djordjevic et al. 2012 [42] Serbia	Randomized	T: ASTX capsules (made from Haematococcus pluvialis)	T: 4mg	90 days	Soccer players, M,		Total: 32	MDA, AOPP, SOD, SH groups, TAS
	Placebo-controlled	C: placebo (sacharose)	C: 0mg		Age: T: 18.1 ± 0.16, C: 17.7 ± 0.6		T: 18	
	Parallel trial (2 groups)				BMI: T: 22.8 ± 1.4, C: 22.7 ± 1.7		C: 14	
	Double blind							
Kim et al. 2011 [46] South Korea	Randomized	T: ASTX capsules (made from Haematococcus pluvialis, soybean oil)	T1:5mg	21 days	T: Smokers	C: Non-smokers	Total : 78	MDA, ISP, SOD, TAC
	Repeated measured	C: n.a.	T2: 20 mg		M/F: 38/1	M/F: 38/1	T1: 13	
	Trial (4 groups)		T3: 40 mg		Age: 21-43	Age: 21-42	T2: 13	
			C: 0 mg		BMI: 24.3 ± 3.27	BMI: 22.6 ± 2.68	T3: 13	
							C: 39	
Nakagawa et al. 2011 [43] Japan	Randomized	T1, T2: ASTX capsules (made of Haematococcus pluviallis, Puresta oil 80 and alive oil.)	T1: 6 mg	84 days	healthy subjects		Total: 30	PCOOH, PEOOH, UA
	Placebo-controlled	C: placebo (maize oil)	T2: 12 mg		F/M: 15/15		T1: 10	
	Parallel trial (3 groups)		C: 0 mg		Age: -69		T2: 10	
	Double blind				BMI: 27.5 ± 2.1		C: 10	
Peng et al. 2011 [45] China	Randomized	T: ASTX capsules (made of Haematococcus pluvialis ± maize oil, and edible gelatin)	T: 40 mg	90 days	healthy subjects		Total: 115	MDA, SOD, GSH-Px activity
	Placebo-controlled	C: placebo (maize oil and edible gelatin)	C: 0 mg		F/M: 62/53		T: 58	
	Parallel trial (2 groups)				Age: 45 -65		C: 57	
					BMI: n.a.			

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Table I. (Continued)

Study ID location [a]	Study design	Treatment/ Control [b]	ASTX dosage per day	Duration	Participants Characteristic [c]		Sample size	Biomarkers of oxidative stress
Choi, Kim et al. 2011 [47] South Korea	Randomized	T: ASTX capsules (made of Haematococcus pluvialis, soybean oil)	T1: 5 mg	21 days	T:Overweight and obese	C:Normal weight	Total: 33	MDA, ISP, SOD, TAC
	Repeated measured	C: n.a.	T2: 20 mg		F/M:20/3	F/M:10/0	T1: 12	
	Trial (3 groups)		C: 0 mg		Age: 25.1 ± 3.7	Age: 26.0±2.34	T2: 11	
	Double-blind				BMI ≥25	BMI :22.8±2.34	C: 10	
Choi, Youn et al. 2011 [48] South Korea	Randomized	T: ASTX capsules (n.a.)	T: 20 mg	84 days	Overweight and obese		Total: 27	MDA, ISP, SOD, TAC
	Placebo-controlled	C: placebo (n.a.)	C: 0 mg		F/M: 23/4		T: 14	
	Parallel trial (2 groups)				Age: 20-55		C: 13	
	Double blind				BMI ≥25			
Karppi et al. 2007 [44] Finland	Randomized Placebo-controlled	T: ASTX capsules (nutrient, fatty acids, ASTX monoesters and amino acids)	T: 8 mg	90 days	Healthy subjects, M,		Total: 39	ISP, PON, UA, LDL lag time
	Parallel trial (2 groups)	C: placebo (microcrystalline cellulose)	C: 0 mg		Age: 19-33			T: 20
	Double blind				BMI: 23.8		C: 19	
Park et al. 2010 [49] USA (South Korean)	Randomized	T: ASTX capsules (made of Haematococcus pluvialis; Con- tain small amounts (<15%) of mixed carotenoids)	T1: 2 mg	56 days	Healthy subjects (College students), F		Total: 42	8-OHdG, ISP
	Placebo-controlled							
	Parallel trial (3 groups)							
	Double blind	C: placebo (n.a.)	T2: 8 mg		Age: 20.2-22.8		T1: 14	
			C: 0 mg		BMI: 16.3-27.5		T2: 14	
							C: 14	

[a]Name of the first author; published year; country where the trial conducted. [b]Intervention arm (ingredients). [c]Characteristic of subjects; Gender (M: male, F: Female); Age (years); BMI: Body Mass Index (kg/m^{2}).
Abbreviations: ASTX, astaxanthin; n.a., not available; T, treatment groups; C, control groups. TAS, total antioxidant status; TOS, total oxidant status; PAB, Prooxidant-antioxidant balance; TAC, total antioxidant capacity; UA,uric acid; SH groups, sulphydryl groups; TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde; ISP, isoprostane; AOPP, advanced oxidation protein product; PEOOH, phosphatidylethanolamine hydroperoxide; PCOOH, phosphatidylcholine hydroperoxide;
SOD, superoxide dismutase; GSH-Px, glutathione activity; PON, PON1, paraoxonase; LDL, low density lipid; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

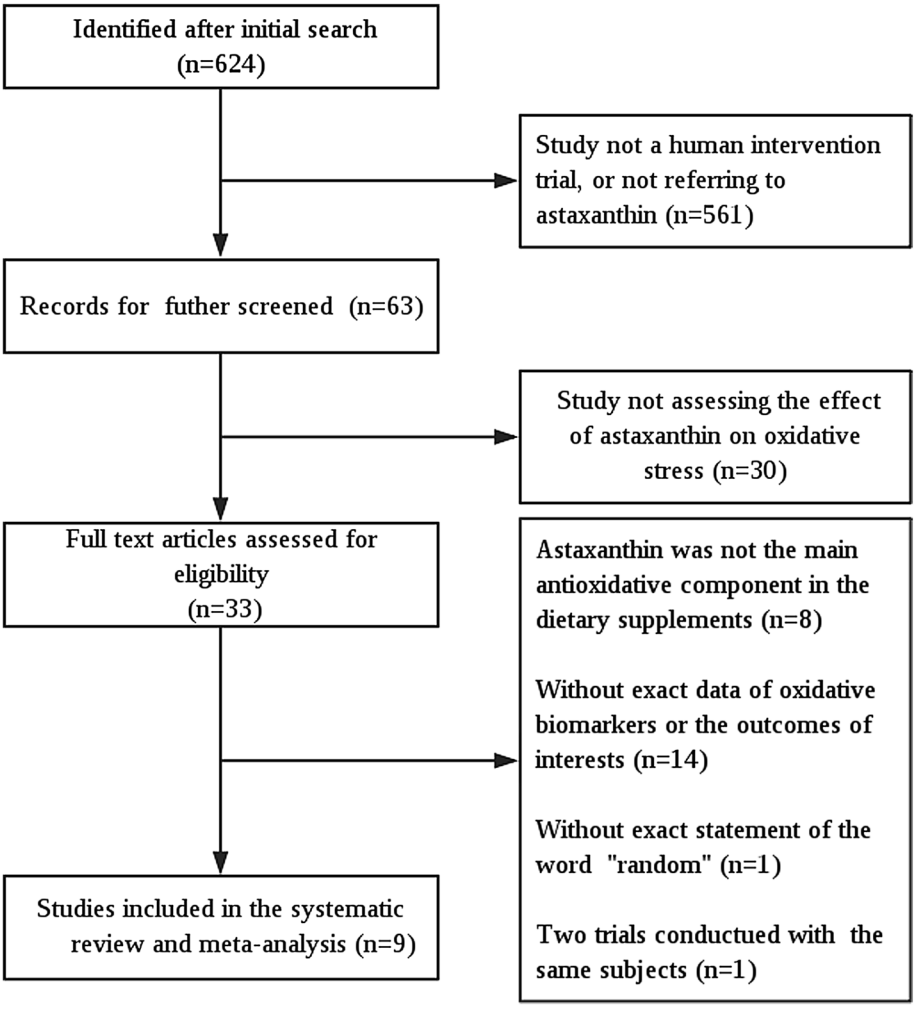

Figure 1. Flow diagram of the study selection procedure.

[40–45, 48, 49], whereas two studies featured a repeated measures design with incomparable characteristics of participants in controlled groups [46, 47] (Table 1).

The sample sizes ranged from 23 to 115 participants. In terms of the intervention arms, all the trials implemented through the use of ASTX capsules, but the treatment dosages were different. The capsuled ASTX was derived from microalgae *Haematococcus pluvialis* [40–43, 45–49]. The capsules used in eight trials contained ASTX as the only effective component on oxidative stress [40–48], whereas, the capsules used in one trial contained small amounts (<15%) of mixed carotenoids [49]. Dosages of ASTX ranging from 2 mg/day to 40 mg/day were administered in the included trials. Duration of supplementation with ASTX ranged from 3 weeks (21 days) to 3 months (90 days).

The participants in the nine trials were adults without specific diseases (diabetes, hypertension, and cardiovascular disease). However, for oxidative damage status, the trial participants were heterogeneous, as follows: included healthy subjects [43–45, 49] without any history of obesity, alcohol abuse, or smoking; and subjects suffering oxidative damage because they are obese or overweight [47, 48], smokers [46], or soccer players [40–42].

All nine included studies [40–49] investigated the antioxidant effect of ASTX on lipid and protein peroxidation products, namely, MDA, TBARS, ISP, and UA. Seven studies [41, 42, 44–48] reported the effect of ASTX on SOD, CAT, GSH-Px, and PON. One study [44] focused on LDL oxidation, specifically LDL lag-time. Five studies [40, 42, 46–48] showed the results of plasma antioxidant capability of TAC, TOS, and PAB. One study [49] focused on DNA damage (8-OHdG).

Quality assessment

The JADAD scores of the included trials reached the standard, which ranged from 1 to 4 [40–49]. Only one study

Table II. Quality assessment of included studies.

Study ID	Description [a]	Compliance	Dietary advice	J.S.[b]
Baralic et al. 2013, 2015 [40], [41]	Randomized; Double blind; C: n.a.; L: 0	Compliance by estimating daily energy and nutrient intake of players.	Instructed to refrain from making any drastic changes in their diet and also to abstain from anti-inflammatory, analgesic drugs throughout the study.	3
Djordjevic et al. 2012 [42]	Randomized; Double blind; C: n.a.; L: 0.	n.a.	Instructed to abstain from any antioxidant supplementation	3
Kim et al. 2011 [46]	Randomized; n.a.; C:n.a.;L:n.a.	Compliance (97.0%) by counting the returned capsules, questioning the subjects and reporting and measuring the plasma ASTX.	Instructed to maintain current and smoking habit and to refrain from taking any vitamins or antioxidant supplements.	1
Nakagawa et al. 2011 [43]	Randomized; Double blind; C: n.a.; L: 0	Compliance (99.5%, 98.1%, 98.7%, 3 groups) by assessing interviews self-reports and returned capsule counts; recording dietary intake, alcohol consumption and physical activity 3 days before each blood collection.	Instructed to maintain usual lifestyle (avoid excessive eating and drinking, intense exercise and lack of sleep).	3
Peng et al. 2011 [45]	Randomized; Placebo (without mentioning "blind"); C: Stratified random sampling; L: 5/120, 4.2%	The subjects who did not follow the prescribed dose were less than 5.	Instructed to maintain usual lifestyle and diet habits	3
Choi, Kim et al. 2011 [47]	Randomized; Double blind; C: n.a.; L: 0	Compliance by counting the remaining ASTX soft capsules, measuring plasma ASTX response, and dietary record.	Instructed to maintain their usual lifestyle and to refrain from taking vitamins, antioxidant supplements and ASTX-rich foods.	3
Choi, Youn et al. 2011 [48]	Randomized; Double blind; C: n.a.; L: 0	Compliance (93.4% and 92.9%, two groups) by counting ASTX capsules.	Instructed to maintain their usual lifestyle and to refrain from taking any vitamins or nutritional supplements.	3
Karppi et al. 2007 [44]	Randomized; Double blind; C:n.a.;L: 2.5%	Compliance by food recordings and measuring plasma ASTX	Instructed not to take any astaxanthin supplementation at baseline and keep their exercise and dietary habits unchanged.	4
Park et al. 2010 [49]	Randomized; Double blind; C: Stratified random sampling; L: 0	Compliance by measuring plasma ASTX and dietary recall	Instructed to consume their normal diets and refrain from eating astaxanthin-rich foods such as salmon, lobster, and shrimp.	4

[a]Randomization; Allocation concealment; Blinding; Loss to follow-up; C, concealment; L, Loss to follow-up; [b]J.S., JADAD score. n.a., not available.

[46] scored 1, because information about the allocation concealment, blinding methods, or follow-up in its content is not available. The remaining eight studies [40–45, 47–49] scored higher than or equal to 3, which could be ranked as "A" level. However, only two trials [45, 49] stated the allocation concealment. The majority of the studies were double-blind, except one study [45], which only stated "placebo" without the description of "blind" in the text. Except for the trial by Kim et a1. [46], the other eight trials [40–45, 47–49] mentioned the exact sample sizes in the of

Table III. Categorised oxidative parameters of each study.

Biomarkers of oxidative stress	Study ID
Lipid oxidation/ peroxidation products	
8-epi-PGF2,8-iso-PGF2, 8-isoprostane(ISP)	Kim et al. (2011) [46] [$p < 0.05$, higher doses (20 mg, 40 mg) groups compared with the lowest one (5 mg)],
	Choi, Kim et al. (2011) [47] ($p < 0.01$, compared with the baseline values in ASTX intervention groups),
	Choi, Youn et al. (2011) [48] ($p < 0.01$),
	Karppi et al. (2007) [44] ($p > 0.05$),
	Park et al. (2010) [49] ($p > 0.05$).
plasma malondialdehyde (MDA)	Kim et al. (2011) [46] ($p < 0.05$, compared with the baseline values in higher doses groups),
	Peng et al. (2011) [45] ($p < 0.01$),
	Choi, Kim et al. (2011)[47] ($p < 0.01$, compared with the baseline values in ASTX intervention groups),
	Choi, Youn et al. (2011) [48] ($p < 0.01$),
	Djordjevic et al. (2012) [42] ($p > 0.05$)
thiobarbituric acid-reactive substances (TBARS)	Baralic et al. (2013) [41] ($p < 0.05$, after 45days, mainly effected by training),
	Djordjevic et al. (2012) [42] ($p > 0.05$)
phospholipid hydroperoxides (PLOOH)	Nakagawa et al. (2011) [43] ($p < 0.05$)
phosphatidyl ethanolamine hydroperoxide (PEOOH)	Nakagawa et al. (2011) [43] ($p < 0.01$, compared with the baseline values in ASTX intervention groups)
phosphatidyl choline hydroperoxide (PCOOH)	Nakagawa et al. (2011) [43] ($p < 0.01$, compared with the baseline values in ASTX intervention groups)
low density lipid (LDL) lag time	Karppi et al. (2007) [44] ($p > 0.05$)
Plasma antioxidant of lipid oxidation	Baralic et al. (2015) [40] ($p > 0.05$),
uric acid (UA)	Nakagawa et al. (2011) [43] ($p < 0.05$, compared with the baseline in ASTX intervention groups),
	Karppi et al. (2007) [44] ($p < 0.05$, compared with the baseline in ASTX intervention groups)
Protein oxidation	
advanced oxidation protein product (AOPP)	Baralic et al. (2013) [41] ($p > 0.05$),
	Djordjevic et al. (2012) [42] ($p > 0.05$)
sulphydryl (SH) groups	Baralic et al.(2013) [41] ($p < 0.05$, compared with baseline values in ASTX intervention groups),
	Djordjevic et al.(2012) [42] ($p > 0.05$)
Plasma antioxidant status or capability	
total antioxidant status (TAS)	Baralic et al. (2015) [40] ($p > 0.05$),
	Djordjevic et al. (2012) [42] ($p < 0.05$)
total oxidant status (TOS)	Baralic et al. (2015) [40] ($p > 0.05$)
pro-oxidant antioxidant balance (PAB)	Baralic et al. (2015) [40] ($p < 0.05$, compared with baseline values in ASTX intervention groups)
redox Balance	Baralic et al. (2013) [41] ($p < 0.001$, compared with baseline values in ASTX intervention groups)
total antioxidant capacity (TAC)	Kim et al. (2011) [46] ($p < 0.05$, compared with the baseline values in all doses),
	Choi, Kim et al. (2011) [47] ($p < 0.01$, compared with the baseline values in ASTX intervention groups),
	Choi, Youn et al. (2011) [48] ($p < 0.01$)
Antioxidant enzyme	
superoxide dismutase (SOD)	Kim et al. (2011) [46] ($p < 0.05$, compared with the baseline values in all dose groups),
	Peng et al. (2011) [45] ($p < 0.01$),
	Choi, Kim et al. (2011) [47] ($p < 0.01$, compared with the baseline values in ASTX intervention groups),
	Choi, Youn et al. (2011) [48] ($p<0.01$),
	Djordjevic et al. (2012) [42] ($p > 0.05$)
glutathione peroxidase (GSH-Px activity)	Peng et al. (2011) [45] ($p < 0.01$)
paraoxonase (PON, PON1)	Baralic.et al. (2013) [41] ($p < 0.05$, compared with the baseline values in ASTX intervention groups),
	Karppi et al. (2007) [44] ($p > 0.05$)
DNA damage	
8-hydroxy-2-deoxyguanosine (8-OHdG)	Park et al. (2010) [49]($p < 0.01$)

$P > 0.05$ means no significant difference with control groups or baseline; $p < 0.05$ or 0.01 means significant difference with control group.

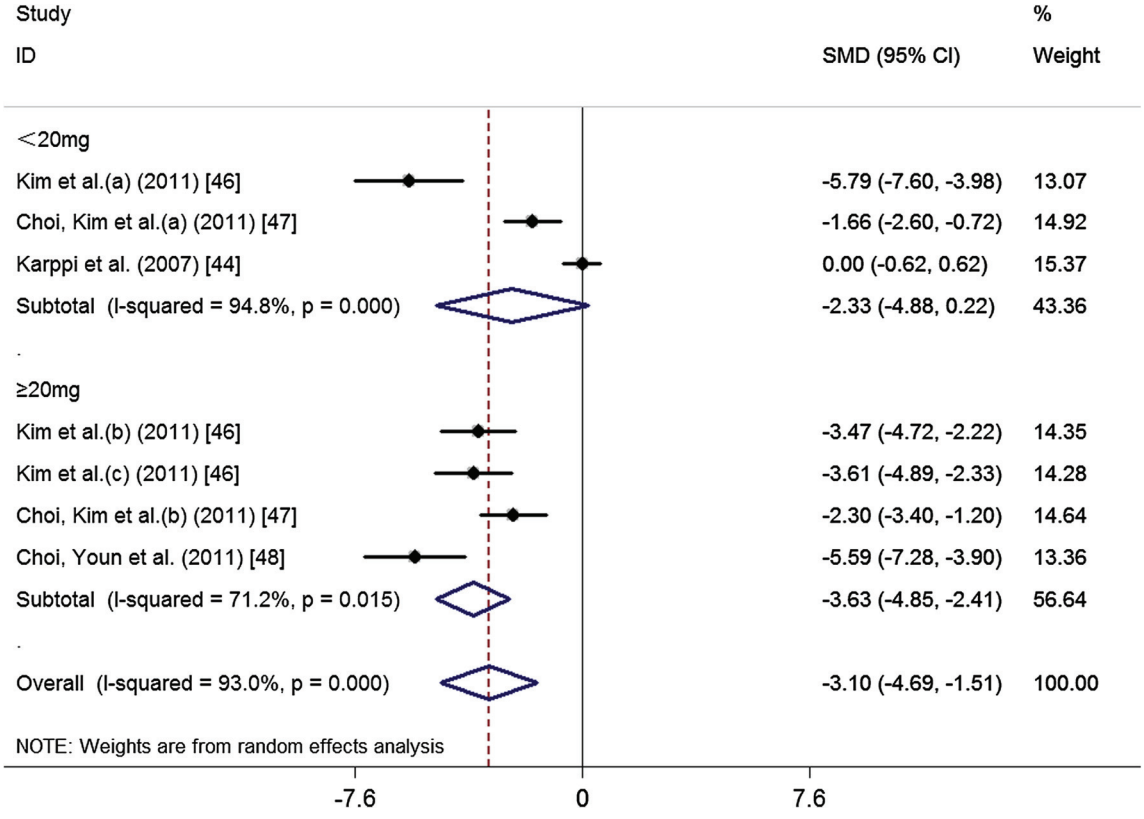

Figure 2. Forest plot: effect of astaxanthin (ASTX) supplementation on plasma isoprostane (ISP) based on the comparison with baseline in intervention groups (subgroup analysis by dosage), presented as ng/mL. SMD, Standard mean difference; CI, confidence interval.

method and result sections; six trials [40–43, 45–47] did not lose any participant, and two trials [44, 45] discussed the details of the loss to follow-up. Most of the trials described the methods for evaluating participant compliance [40, 41, 43–49], including estimating daily energy and nutrient intake of subjects [40, 41, 43, 44, 47–49], counting the returned capsules [43, 46–48], conducting interviews or measuring plasma ASTX [44, 49]; four of the studies provide the adherence rates or amounts of the subjects who did not follow [43, 45, 46, 48]. For dietary advices, seven of the nine studies suggested that the subjects should refrain from having ASTX-rich food, other antioxidants or anti-inflammatory supplementation, and even avoid the supplements which have effect on immune [40–42, 44, 46–49]; seven of them [41, 43–45, 47–49] instructed the volunteers to maintain usual lifestyle and diet habits. All of the studies mentioned the details of dietary advices. Quality assessment results are shown in Table 2.

Oxidative biomarkers assessment

The oxidative stress biomarkers mentioned in the nine included trials are collected and presented in Table 3. The sum of oxidative parameters applied in these studies was nearly 20, covering most of the aspects of oxidative stress. The summary of the results is presented below.

Lipid oxidation/peroxidation products. Lipid oxidation/peroxidation products were evaluated in all of the included studies [41–49].Two trials detected TBARS without clear results [41, 42]. One found no effect ($p > 0.05$), whereas the other one reported the difference between ASTX and placebo groups ($p < 0.05$), which was mainly influenced by training instead of ASTX supplements. Only Nakagawa et al. assessed the value of PLOOH, including PEOOH and PCOOH values ($p < 0.01$, compared with the baseline in ASTX groups) [43]. The total PLOOH levels were lower in the ASTX groups than in the placebo group ($p < 0.05$). Five trials focused on plasma MDA [42, 45–48], whereas ISP was evaluated in five trials [44, 46–49] (Table 3). Data of plasma MDA and ISP were subjected to a meta-analysis.

ASTX intervention and control groups. Three RCTs [42, 45, 48] evaluated the concentration of plasma MDA. The random-effect meta-analysis of data showed a borderline significant decrease of plasma MDA concentrations with ASTX supplementation (SMD −1.15 μmol/L [95% CI −2.30, −0.00]; $p = 0.050$) (the figure is shown in ESM 7). However, given that only three RCTs are qualified to be included in the meta-analysis, data are insufficient for determination.

Comparison with the baseline. The random-effect meta-analysis of data from five trials (eight treatment arms) with a pooled sample size of 152 subjects showed significant effect of ASTX on plasma MDA (SMD −1.32 μmol/L [95% CI −1.92, −0.72], $p < 0.0001$). The treatment effect was apparent when trials were divided into subgroups by ASTX dosage as well (≥20 mg/day, SMD −1.69 μmol/L [95% CI −2.28, −1.09], $p < 0.0001$; <20 mg/day, SMD −0.72 μmol/L, [95% CI −1.41, −0.04], $p = 0.039$). The outcomes were similar in short term (21 days) and long term (84 or 90 days) (short term, SMD −1.16 μmol/L [95% CI −1.54, −0.77]; $p < 0.0001$; long term, SMD −1.55 μmol/L, [95% CI −3.01, −0.08]; $p = 0.038$). No heterogeneity was observed in the short-term group ($I^2 = 0.0\%$; $p = 0.749$), whereas heterogeneity still existed in long-term studies ($I^2 = 93.1\%$; $p < 0.0001$) (figures are shown in ESM 1 and 2).

For ISP, with a total pooled sample size of 96 subjects, the ASTX treatment significantly decreased the concentration of ISP (SMD −3.10 ng/mL [95% CI −4.69, −1.51]; $p < 0.0001$) (Figure 2.). The subgroup meta-analysis of a four-arm trial investigating the effect of ASTX dosage ≥20 mg/day revealed a statistically significant reduction of ISP (SMD −3.63 ng/mL [95% CI −4.85, −2.41]; $p < 0.0001$). In contrast, the subgroup meta-analysis of three-arm trial using ASTX dosage <20 mg/day was not significantly different compared with baseline values (SMD −2.33 ng/mL [95% CI −4.88, 0.22]; $p = 0.073$) (Figure 2.). In the second subgroup analysis of the duration of intervention, ASTX significantly reduced the concentration of ISP in the short-term group (21 days), whereas no effect was shown in the long-term group (84 or 90 days) (short term, SMD −3.23 ng/mL [95% CI −4.44, −2.01]; $p < 0.0001$, long term, SMD −2.74 ng/mL, [95% CI −8.21, 2.74]; $p = 0.327$) (the figure is shown in ESM 3).

No difference was found in control groups of any of the trials. Heterogeneity was high for MDA ($I^2 = 79.5\%$; $p < 0.0001$) but was not explained by different ASTX dosages. To some extent, the duration of intervention contributed to explain the heterogeneity of MDA. However, neither the dosage nor duration of intervention could explain the high heterogeneity of ISP ($I^2 = 93.0\%$; $p < 0.0001$).

Plasma antioxidants of lipid oxidation. UA was investigated in three trials [40, 43, 44]. Except the one directed by Baralic [40], the other two trials reported the differences of UA versus baseline in the treatment groups. However, inverse changes in blood biomarkers were noted, with Karppi et al. [44] indicating an increase in oxidation and Nakagawa et al. indicating a decrease [50]. No other significant effect of ASTX on UA was reported, neither compared with the placebo groups nor baseline. Given the evidence of no heterogeneity among the three studies ($I^2 = 0.0\%$; $p = 0.705$), we used a fixed effects model to calculate the mean difference with a pooled sample size of 119 subjects. In comparison with control groups, a slight decrease on UA in ASTX groups was observed; this decrease is insignificant (SMD −0.01 mg/dL [95% CI −0.37, 0.36]; $p = 0.975$) (figure is shown in ESM 5).

Protein oxidation/oxidation products. In terms of protein oxidation, AOPP was reported in two trials without any significant effect ($p > 0.05$) [41, 42]. These two trials also focused on –SH groups; Baralic et al. found a significant protective effect with the supplementation of ASTX compared with the baseline values [41]. However, no similar evidence was found in the other trails [42] (Table 3).

Plasma antioxidant status or capability. TAS, TOS, PAB, redox balance, and TAC were adopted to assess the total plasma antioxidant status or capability. For TAS, one trial [42] found a significant effect with ASTX treatment ($p < 0.05$). However, the other one [40] reported no effect ($p > 0.05$). In comparison with the baseline values in ASTX groups, PAB and redox balances reportedly showed significant differences ($p < 0.05$) [40, 41] (Table 3). TAC data were considered appropriate for meta-analysis. The random-effect meta-analysis ($I^2 = 72.5\%$; $p = 0.003$) of data from three trials (six treatment arms) [46–48] with a pooled sample size of 76 subjects showed significant effect of ASTX on TAC (SMD 0.77 mmol [95% CI 0.12, 1.43]; $p = 0.018$). However, the subgroup analysis depending on dosage showed no significant effect on the ASTX groups with low dosage (SMD 0.49 mmol [95% CI −1.06, 2.04]; $p = 0.539$) (figures are shown in ESM 6). No significant effect was found for other parameters.

Antioxidant enzyme and DNA damage. Seven trials focused on antioxidant enzymes, including SOD [42, 45–48], GSH-Px [45], and PON [41, 44]. The difference between the intervention and control groups are not statistically significant at a level of $p = 0.055$; data of three RCTs [42, 45, 48] including 90 participants were pooled (SMD 1.09 U/mL [95% CI −0.02, 2.20]). Given the limited data, the results were still uncertain. However, treatment effect was apparent when data obtained before and after the interventions were compared, with 152 subjects [42, 45–48] (SMD 1.57 U/mL [95% CI 0.57, 2.56]; $p = 0.002$) (figures are shown in ESM 4 and 8). When the trials were divided by dosage, the subgroup random effect analysis showed that no significant effect was observed in the low-dose group; however, in the high-dose group, the increase of SOD is found (≥20 mg/day, SMD 1.92 U/mL [95% CI 0.78, 3.06], $p = 0.001$; <20 mg/day, SMD 0.99 U/mL [95% CI −1.41, 3.38], $p = 0.419$). Further subgroup analysis of duration indicated significant increases on SOD in short-term group (21 days). However, ASTX failed to indicate its antioxidant activity in the long-term group (84 or 90 days) (short term, SMD 2.51 U/mL [95% CI 1.99, 3.03], $p < 0.0001$; long term, SMD −2.74 U/mL, [95% CI −1.09,

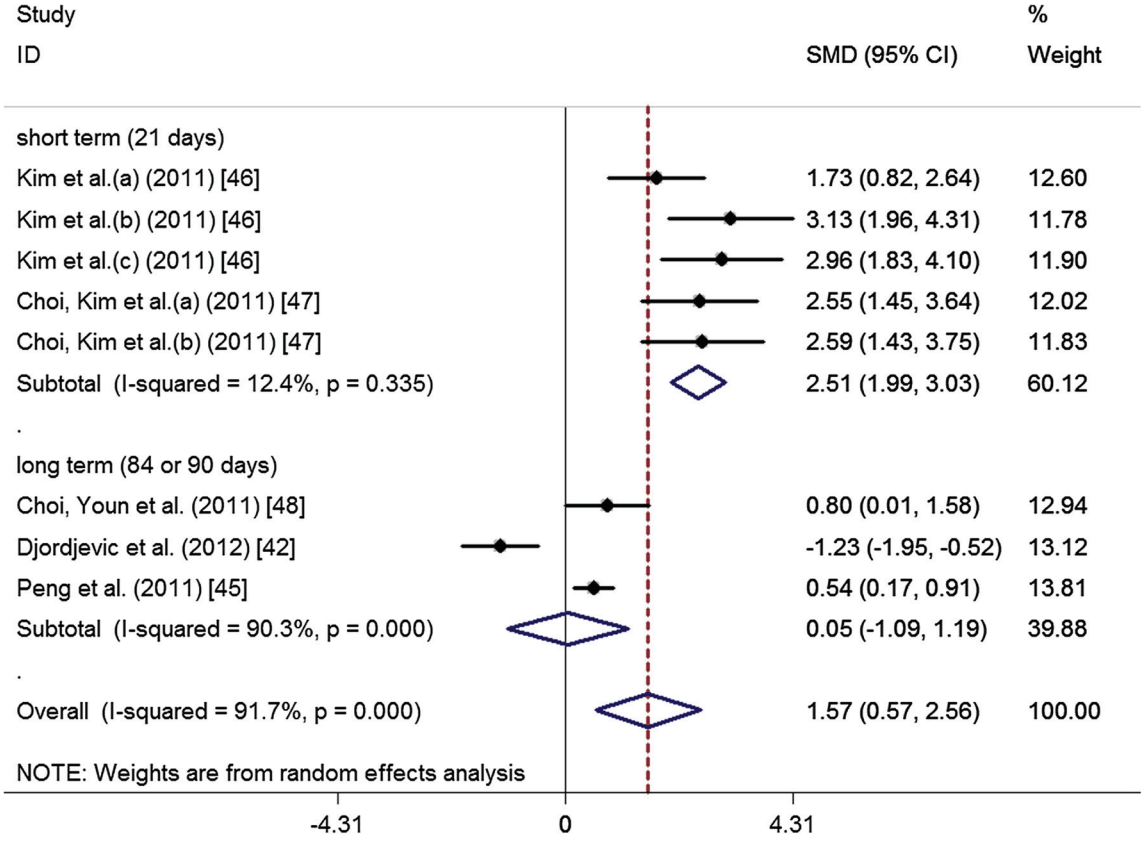

Figure 3. Forest plot: effect of astaxanthin (ASTX) supplementation on plasma superoxide dismutase (SOD) based on the comparison with baseline in intervention groups (subgroup analysis by duration of intervention), presented as U / mL. SMD, Standard mean difference; CI, confidence interval.

1.19], p = 0.937). No heterogeneity was observed in the short-term group (I^2 =12.4%; p = 0.335), whereas heterogeneity still existed in long-term studies (I^2 = 90.3%; p < 0.0001) (Figure 3). Only one trial [45] evaluated GSH-Px activity and reported a significant effect. Karppi et al. assessed PON activity or relative items with no significant effect [51]. Baralic et al. suggested the increase of PON activity compared with baseline (Table 3) [48]. For DNA damage, Park et al. found the decreasing plasma 8-OHdG in ASTX groups versus control groups (p < 0.01) [49] (Table 3).

Sensitivity analyses

Sensitivity analyses were conducted by excluding each study to test the robustness of results. Sensitivity analyses did not alter the results significantly (figures are not shown).

Publication bias

Considering that few studies met the inclusion criteria of our meta-analysis, the publication bias was not visualized by funnel plot. Instead, the fail-safe numbers were conducted to explore the publication bias. At 0.05 and 0.01 significance levels, studies on UA may have publication bias ($N_{fs(0.05)} = -3.8$, $N_{fs0.01} = -3.9$); other $N_{fs0.05}$ or $N_{fs0.01}$ values were consistently greater than the number of studies included in this meta-analysis (table is shown in ESM 9).

Discussion

Our meta-analysis suggests that ASTX may be effective in reducing oxidative stress as ASTX may effectively reduce the total amount of the specific lipid peroxidation (MDA and ISP), enhance plasma antioxidant capability (TAC), and elevate a specific antioxidant enzyme (SOD).

MDA, a decomposition product of peroxidised polyunsaturated fatty acids in membrane lipids, is thought to be one of the most sensitive biological molecules in terms of susceptibility to reactive oxygen species over a long period of time [50, 51]. MDA is one of the most widely used indicators in literature for animal models and clinical trials [45–48, 52–55]; however, the use of MDA as a valid biomarker of lipid peroxidation within human in recent studies is

controversial [56]. Despite the controversy, MDA is still recognized as a relatively good biomarker for the present review. ISP is one of the eicosanoids formed in vivo from free radical-catalyzed oxidation of primarily arachidonic acid. ISP is a frequently used and reliable biomarker of in vivo lipid peroxidation [57, 58]. In our study, daily supplementation with ASTX significantly decreased the plasma levels of MDA and ISP from the baseline level. However, the difference in MDA between the ASTX treatment and control groups was still unclear because of limited data. ASTX is one of the most effective antioxidants against lipid peroxidation among in vitro and in vivo systems [59]. The effect of ASTX in inhibiting radical-initiated lipid peroxidation has been revealed in vitro or in animal models, and ASTX might be several times more active than α-tocopherol in protecting rat mitochondria against lipid peroxidation [60] and human lens epithelial cells against UVB radiation insult [61]. The potent antioxidant activity of ASTX is due to its polar structure, which features ionone rings with the capability to quench free radicals [62].

ASTX might not only eliminate lipid peroxidation but also enhance the antioxidant system. The present review suggested the increase in plasma levels of SOD and TAC with ASTX intake compared with the baseline. SOD is the main in vivo antioxidant enzyme that quenches superoxide anions, and TAC represents a full spectrum of antioxidant activities against various reactive radicals [63]. However, the meta-analysis results of three RCTs did not show significant effect on SOD. This result was different from the result in comparison with the baseline values. The null result might have occurred due to the limited amount of the included RCTs. However, two of the three included trials suggested significant effects [45, 48]. Only Djordjevic et al. showed a negative result [42], which could be due to the subjects in this trial who suffered from prolonged exercise leading to strong oxidative stress in the antioxidant system; therefore, the synthesis result of the meta-analysis was affected. All in all, data were not enough for determination. Thus, the effect of ASTX either on lipid peroxidation or on antioxidant system was still unclear.

Unfortunately, the result based on UA was not confirmed. UA is a final enzymatic product following an oxidant–antioxidant paradox [64]. UA can work as an antioxidant with some protective effects under certain conditions; it may be a pro-oxidant producing other radicals that might lead to oxidative damage. Thus, UA might not be a suitable marker of oxidative stress. Moreover, its unsafe index was relatively low indicating that the evidence may be not reliable. No clear conclusion was found based on UA.

A subgroup analysis was performed to test dosages of ASTX. The effects were more significant if taken in high dosages, which were greater than or equal to 20 mg daily. When the dosages of ASTX were lower than 20 mg, no statistically significant effect was found for ISP, TAC, and SOD, whereas the protective effect was consistently observed in the high-dosage groups. Results suggest that the antioxidant capability of ASTX may be dose-related. However, one trial failed to find any difference between the 5 mg ASTX group and the 20 mg ASTX group on any biomarker or clinical results. However, the plasma concentration of ASTX in the 20 mg group was significantly higher than in the 5 mg group [47]. This phenomenon may be similar with other carotenoids, which may lose their effectiveness as antioxidants at high concentrations [65]. Thus, the dose-related antioxidant effect of ASTX is still unconfirmed. Given the ISP and SOD data, the second subgroup analysis indicated that ASTX showed antioxidant effect in short-term trials (3 weeks), but the effect was not significant when the duration of intervention expanded to long term (12 weeks or 3 months). Controversially, the long-term RCTs included in this review did suggest the antioxidant efficacy of ASTX on other biomarkers, such as MDA, GSH-Px, and TAC [45, 48]. As literature shows, the lack of long-term effect of β-carotene on cardiovascular disease has been suggested [66]. However, given the limited studies on humans, it is still unclear whether ASTX will lose its antioxidant effect as β-carotene in the long term.

More recently, some researchers suggest that antioxidant vitamins, which include vitamins E and C, may have adverse effects on healthy subjects, especially in high doses because of the imbalance of endogenous redox equilibrium [67, 68]. However, the systematic review did not indicate any detrimental effect of ASTX on healthy subjects in doses given. Peng et al., Park et al., and Nakagawa et al. indicated the protective effect of ASTX against oxidative stress on healthy subjects from different aspects [43, 45, 49], whereas Karppi et al. found no antioxidant effect [44]. However, whether ASTX can show antioxidant effects on healthy subjects who are not suffering oxidative damage is unclear. Thus far, no adverse effect or clinical result has been found in the doses administered.

Strengths and limitations

To our knowledge, this is the first systematic review about the effect of ASTX supplementation on oxidative stress based on clinical trials. The included criteria are rigid, as we excluded the trials in which ASTX were not the main antioxidant supplementation to eliminate the interference from other antioxidants. Also, a detailed research protocol with prior specification and evaluation of potential study design considerations without language exclusion was included. Furthermore, subgroup analyses were performed

to explore factors affecting ASTX's antioxidant capability. To completely explore the data, the differences between before and after intervention treatments have been taken into consideration in the meta-analysis as well.

Currently, some biomarkers of oxidative stress used in this review, such as TBARS or MDA, and TAC, have been questioned in human studies [56, 69, 70]. However, no evidence exists to dispute their function as markers. The markers were widely used as important indicators in clinical trials and are relatively accessible. Hence, these controversial biomarkers were still included in this systematic review. Diverse biomarkers of oxidative stress were categorized to analyse the antioxidant activity of ASTX through comprehensive views.

The present systematic review does have its limitations. First, the heterogeneity could not be fully explained by subgroup analyses based on dosage and duration of intervention; this heterogeneity may be caused by other factors, such as the different genders, ages, or the difference in oxidant status of subjects. However, limited trials focusing on the same biomarkers make it challenging to explore all possible factors for the subgroup analyses. Therefore, we were unable to confirm whether ASTX has different antioxidant effects among subjects with different ages, genders, or other characteristics; this result highlights the need for future research. Second, the RCTs involving supplementation with small quantities of other nutrients included might influence antioxidant effects of ASTX. However, the same is true for all the manufactured ASTX supplements. From a practical standpoint, the application of pure ASTX is very limited. Producing ASTX capsules with other essential components such as oils and other nutrients is very common. Thus, this review remains practically valuable for the consumption of lycopene supplements in the real world. Third, the present review includes two repeated-measurement trials with inequivalent control groups [46, 47]. These two trials focused on smokers and the obese or overweight. However, they set normal subjects who did not smoke or with normal weight as control. Therefore, data from control groups in these two trials were not included in meta-analyses. Instead, the differences between pre- and pro-intervention based on the two trials were compared to completely analyse the limited data.

In conclusion, the antioxidant effect of ASTX on humans is still unclear based on this systematic review and meta-analysis of nine trials. However, this review reflects the potential protective effect of supplementation with ASTX on oxidative stress by eliminating the lipid peroxidation/products and enhancing the antioxidant system by inducing an increase in the plasma concentration of SOD. Further, well-designed clinical trials are necessary to provide more evidence.

Electronic supplementary material

The electronic supplementary material is available with the online version of the article at http://dx.doi.org/10.1024/0300-9831/a000497

ESM 1. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma malondialdehyde (MDA) based on the comparison with baseline in intervention groups (subgroup analysis by dosage), presented as μmol/l. SMD, Standard mean difference; CI, confidence interval.
ESM 2. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma malondialdehyde (MDA) based on the comparison with baseline in intervention groups (subgroup analysis by duration of intervention), presented as μmol/l. SMD, Standard mean difference; CI, confidence interval.
ESM 3. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma isoprostane (ISP) based on the comparison with baseline in intervention groups (subgroup analysis by duration of intervention), presented as ng/ml. SMD, Standard mean difference; CI, confidence interval.
ESM 4. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma superoxide dismutase (SOD) based on the comparison with baseline in intervention groups (subgroup analysis by dosage), presented as U/ml. SMD, Standard mean difference; CI, confidence interval.
ESM 5. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma uric acid (UA) based on the comparison between the treatment and control groups, presented as mg/dl. SMD, Standard mean difference; CI, confidence interval.
ESM 6. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on total antioxidant capability (TAC) based on the comparison with baseline in intervention groups (subgroup analysis by dosage), presented as mmol. SMD, Standard mean difference; CI, confidence interval.
ESM 7. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma malondialdehyde (MDA) based on the comparison between the treatment and control groups, presented as μmol/L. SMD, Standard mean difference; CI, confidence interval.
ESM 8. Figure.
Forest plot: effect of astaxanthin (ASTX) supplementation on plasma superoxide dismutase (SOD) based on the comparison between the treatment and control groups, presented as U/mL. SMD, Standard mean difference; CI, confidence interval.

ESM 9. Table.
Fail-safe numbers of each meta-analysis.

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Conflicts of interest
The authors declare no conflict of interests.

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