

CAROTENOIDS AND HUMAN HEALTH: COMPARISON OF THE EFFECTS IN HEALTHY POPULATION AND WITH CARDIOVASCULAR RISK. A REVIEW OF THE LITERATURE

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ABSTRACT

Background. Carotenoids are pigments synthesized mainly by plants. Given that humans cannot synthesize them, but they are essential for certain biological functions, carotenoids must be supplied through the diet. Carotenoids can also act as antioxidants, but their bioavailability and effects may vary depending on the food matrix and risk factors of the population that consumes them. **Objective.** To analyze the available scientific evidence regarding the bioavailability of carotenoids and their role in human health, especially on cardiovascular -cv- risk factors, blood lipids, lipoproteins, oxidative stress, glucose intolerance and inflammation, both in healthy and cv risk populations. **Methods.** A critical review of the literature was made from original articles published in PubMed, ScienceDirect and lilacs databases. All articles with the words “carotenoid and clinical trial” found in the title, abstract and / or keywords were included. **Results.** The changes observed after carotenoid supplementation or carotenoid-rich diet, for some lipid, inflammatory and antioxidant biomarkers in both populations (healthy and at cv risk) did not show strong differences. However, it is evident the bioavailability of dietary carotenoids in healthy population may differ than in cv risk population **conclusions.** Although methodological differences among the studies make difficult their comparison, the results suggest that carotenoid consumption could play a role as a preventive strategy than as a co-aid in the treatment of CV risk population.

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CAROTENOIDES Y SALUD HUMANA: COMPARACIÓN DE LOS EFECTOS EN POBLACIÓN SANA Y CON RIESGO CARDIOVASCULAR. REVISIÓN DE LA LITERATURA

RESUMEN

Introducción. Los carotenoides son pigmentos sintetizados principalmente por plantas. Los humanos no pueden sintetizarlos, pero son esenciales para ciertas funciones biológicas, por lo cual deben obtenerlos de la dieta. Los carotenoides pueden también actuar como antioxidantes, pero su biodisponibilidad y efectos pueden variar dependiendo de la matriz alimenticia y los factores de riesgo de la población que los consume. **Objetivo.** Analizar la evidencia científica disponible respecto de la biodisponibilidad de los carotenoides y su función en la salud humana, especialmente sobre factores de riesgo cardiovascular -cv-, lípidos sanguíneos, lipoproteínas, estrés oxidativo, intolerancia a la glucosa e inflamación, tanto en población sana como con riesgo cv. **Métodos.** Se realizó una revisión crítica de la literatura de artículos originales publicados en las bases de datos de PubMed, ScienceDirect, y lilacs. Se incluyeron todos los artículos con las palabras "Carotenoid and clinical trial" en el título, resumen y/o palabras clave. **Resultados.** No hubo diferencias importantes en los cambios observados después del consumo de suplementos de carotenoides o de dietas ricas en carotenoides, para algunos biomarcadores lipídicos, inflamatorios y antioxidantes entre población sana y con riesgo cv. Sin embargo, se evidencia que la disponibilidad de los carotenoides dietarios en población sana puede ser diferente a la de población con riesgo cv. **Conclusiones.** Aunque las diferencias metodológicas entre los estudios hacen difícil su comparación, los resultados sugieren que el consumo de carotenoides podría desempeñar un papel como una estrategia con un enfoque más preventivo que como coadyuvante en el tratamiento de la población con riesgo cv.

Palabras clave: carotenoides, ensayo clínico, salud humana, riesgo cardiovascular.

INTRODUCTION

Carotenoids are pigments synthesized by photosynthetic organisms, and some non-photosynthetic fungi and bacteria. Humans cannot synthesize them, but they are essential compounds for certain biological functions, so they must be supplied through the diet (1). There are currently more than 600 carotenoids in nature, and around 40 carotenoids are consumed regularly in human diet (2). They are found mainly in yellow-orange fruits and vegetables (2). Carotenoids can be classified into two main groups: hydrocarbon carotenoids (e.g. β -carotene and lycopene) composed entirely of hydrogen and carbon; and xanthophylls (lutein, zeaxanthin, canthaxanthin, astaxanthin, fucoxanthin) that contain oxygen besides carbon and hydrogen (3).

Lutein, lycopene, zeaxanthin, β -cryptoxanthin, β -carotene, and α -carotene comprise 60%-70% of the human plasma carotenoid content. Approximately 90% of absorbed carotenoids are found in body tissues, especially in adipose tissue and liver, and 10% in plasma in adults. β -carotene, lutein, lycopene and canthaxanthin are part of the skin (1). Dietary carotenoids also provide provitamin A and have antioxidant properties that may help delay the onset of cardiovascular disease (CVD), cancer, and eye diseases as we age (1,4).

The antioxidant activity of carotenoids is due to their conjugated double bonds that allow the elimination of many free radicals. Epidemiological studies have shown that a high carotenoid diet has been associated with reductions in the risk of developing disorders such as cancer, cataracts, and other degenerative diseases. These compounds have also been reported to mitigate the risk of CVD by reducing oxidative stress and thus prevent oxidation of low-density lipoproteins (LDL) (2).

Nevertheless, oral stability and bioavailability of carotenoids are affected by several factors (4,5). For example, β -carotene or lycopene which are usually lipophilic due to the presence of long unsaturated aliphatic chains, as in some fatty acids, are insoluble in water and susceptible to degradation (e.g., by heat or light). In addition, carotenoids of natural origin usually form complexes with biopolymers, such as proteins and polysaccharides, which restrict their absorption in the human body. The extreme pH environment in the stomach can also result in chemical instability of carotenoids (4,5).

Results from studies evaluating carotenoid effects on humans are controversial. Especially when analyzing different populations, i.e., healthy versus disease populations, it is necessary to evaluate whether carotenoid consumption has a preventive or therapeutic effect. The objective of this review was to analyze the available scientific evidence regarding bioavailability of carotenoids and their role in human health, such as effects on cardiovascular risk factors, lipids, lipoproteins, oxidative stress, markers of glucose intolerance, and inflammation, comparing healthy and cardiovascular risk populations.

METHODOLOGY

A critical review of the literature was made from original articles published in Pubmed, ScienceDirect and LILACS databases. All articles with the keywords “carotenoid AND clinical trial” found in the title, abstract and / or keywords were included. In order to obtain all articles available in the literature about carotenoid or carotenoid-rich food interventions in humans until August 31, 2020, no date range or language filters were included.

Original articles were selected, excluding topic reviews, conferences, clinical guides, and book chapters, and studies conducted in species other than humans, such as rats, fruits, plants, genes, and cell lines. Finally, we excluded studies carried out in populations other than healthy or with cardiovascular risk, such as cancer, macular alterations, gastric problems, among others, obtaining a total of 31 articles suitable for critical review. The article selection process is presented in **Figure 1**.

RESULTS

The selected articles had different methodological approaches according to their main outcome, which makes difficult to compare results even in similar populations (e.g., healthy subjects). **Table 1** shows the main characteristics of each study.

Importance of carotenoid bioavailability and food matrix for intervention studies

Despite the potential health benefits of biologically active carotenoids from fruits and vegetables, their biological properties depend mainly on their bioavailability and bioaccessibility (37). Once foods rich in carotenoid compounds are ingested, carotenoids go through the same stages related to digestion and assimilation as other lipophilic molecules (4).

In general, the bioavailability of plant carotenoids is low (10-65%), because they are affected by the main factors necessary for the digestion process such as pH, digestive enzymes, intestinal microbiota, bile salts; the process of mass transfer from the food matrix, the solubility in the gastric system and by the interactions (of carotenoids) with other molecules such as lipids, carbohydrates and divalent minerals present in the food matrix (38).

In this review, several clinical studies support evidence of how the chemical structure of carotenoids influences bioavailability, as well as the way they are organized in the food matrix. An example of this is lycopene. A study carried out with two types of tomato showed a possible relationship between the size and shape of chromoplasts in this food, with the levels of plasma lycopene. Results from this study showed that lycopene from mandarin tomatoes was 8.5 times more bioavailable than lycopene from red tomatoes; this was correlated with the predominance of cis-lycopene isomers and their presence in chromoplasts in a globular state dissolved in lipids typical of mandarin tomatoes (23).

Another study comparatively evaluated the bioavailability of carotenoids present in calendula flowers and *Chlorella* powder, a single-celled green algae that is easy to grow and which apparently can provide several carotenoids, including (lutein, zeaxanthin, α -carotene and β -carotene) those obtainable from the extract of calendula petals. The results showed the use of *Chlorella* powder could represent a source of lutein that is equivalent to the extract of calendula petals to reduce the risk of chronic diseases, including macular degeneration related to age (14).

Goltz *et al.*, (2012) evaluated how a vegetable salad rich in carotenoids improves their absorption and therefore their bioavailability when accompanied with different amounts and types of oils. The study showed that 20 g of ingested lipids promoted significantly greater absorption compared to both 3 and 8 g of dietary lipids for all species of carotenoids and total carotenoids, and therefore that amount of fat bound to raw vegetables increases plasma concentrations of carotenoids (15).

The formulation of the compounds also plays an important role when it comes to bioavailability, since through this the absorption and arrival of carotenoids into plasma could be improved. A test was carried out in which carotenoids (zeaxanthin, meso-zeaxanthin and lutein) were encapsulated freely and in micelles. It was observed that in a micro micellar formulation they had a higher bioavailability, probably due to better micellization and absorption efficiency (16).

Table 2 shows the impact of encapsulation or protection methods such as tablets, capsules, and liquid gelatin capsules to increase carotenoid bioavailability and/or the percentage of modification (%M) of the parameters studied. The results showed better effects than the results obtained with free carotenoids; although not all results were statistically significant, they are clinically relevant. However, it is not possible to define which encapsulation strategy is the most promising to increase the bioavailability of carotenoids, since the formulations do not describe parameters such as extra coating, capsule shell materials, compression pressure, dilution, hardness, among other parameters that can affect all the factors related to bioavailability and bioaccessibility mentioned above. Nevertheless, encapsulation improves the stability and bioavailability of carotenoid-rich extracts.

Of the articles studied, 21 measured different types of carotenoids by high performance liquid chromatography (HPLC) as a measure of adherence and bioavailability. Of these, 15 articles were performed in healthy population, one of which did not perform statistical analysis, and 3 articles were in cardiovascular risk population. The low availability of carotenoids reported in the latter may be related to the focus of their research, which describes the results based on the changes produced in cardiometabolic markers, while the studies performed in healthy populations sought to establish that the carotenoid treatment was bioavailable, safe and with additional benefits regarding antioxidant status. This has been discussed in the literature, where clinical trials involving healthy volunteers seek to identify safety and tolerability without the interference of concomitant pathological conditions (39).

In controlled intervention studies with fruit and vegetable consumption, differences between healthy and diseased populations were observed. For example, Gibson A.

et al., (2012) who studied healthy population over 12 weeks, reported a percentage change in serum carotenoids of more than 100% (12), while Daniel JA., *et al.*, (2014), who performed the analysis in population at risk (obese with diabetes mellitus 2 - DM2), after 8 weeks of intervention, reported a percentage change in serum carotenoids of only 10% (28). This suggests that in healthy population, carotenoids bioavailability may differ compared to population with risk factors, perhaps the increased inflammatory activity and oxidative stress present in this last population, through the production of reactive oxygen species, may deplete the reserves of carotenoids (antioxidants). In fact, patients with coronary artery disease (CAD) had significantly lower levels of lutein + zeaxanthin, β - cryptoxanthin and β - carotene compared to healthy control subjects (40). Similarly, another study reported significantly lower levels of total antioxidant status and retinol in patients with CAD compared to healthy control subjects (41).

The study by Takagi T, *et al.*, (2020) who evaluated population at risk (obese) after consuming a beverage with a high carotenoid content over 8 weeks, obtained percentages of change above 100% in all the plasma carotenoids evaluated (34), demonstrating that their bioavailability in blood was effective. It is important to note the (middle-aged) subjects included in this study were in the first stage of obesity for Japanese population (body mass index -BMI- above 25 kg/m²), without history of serious diseases or consumption of medications. Therefore, these results may be associated to the inverse association between the bioavailability of antioxidants and levels of inflammatory markers that in this case may not be so elevated. Several studies have described this, concluding that indeed inflammatory markers, BMI and other measures of obesity, are inversely related to the bioavailability of carotenoids in blood (42–44).

Effects of carotenoid consumption on lipids and lipoproteins

For the lipid profile variables, 5 articles evaluating healthy population and 6 in population with cardiovascular risk were included, each one with particularities and special characteristics according to the design (Table 1). Variables such as total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were evaluated for all of them, and in some cases, lipoproteins were also measured.

In healthy populations, Djuric Z, *et al.*, (2009) evaluating the effects of a Mediterranean diet, and Miller R, *et al.*, (2005) evaluating the DASH diet, did not show significant effects ($p > 0.05$) for the lipid profile (6,7). In the study by Kelly D, *et al.*, (2017) no significant differences were observed ($p = 0.561$) between groups in visual performance, which indicates that no effect was evidenced by the intervention. However, significant increases were observed for serum carotenoids and total cholesterol in both control group ($p = 0.003$) and intervention group, who consumed respectively, non-enriched eggs and eggs enriched with carotenoids over 4 weeks ($p = 0.025$) (22). It is important to note that eggs are a rich dietary source of carotenoids, in a highly bioavailable matrix (22). Finally, Goltz S, *et al.*, (2012) reported non-significant results ($p < 0.05$) for the lipid profile, after the consumption of different types of salads (15).

In studies evaluating the effects in cardiovascular risk population, it was observed that in hypertensive patients, the dose-dependent consumption of fruits and vegetables during 8 weeks did not generate a significant effect ($p > 0.05$) on the ratio of total cholesterol and HDL cholesterol, as well as on triglycerides (26). In the same line of dose-dependent fruit and vegetable consumption (1 portion versus 6 portions/day) over 8 weeks, the study by Daniels, *et al.*, (2014) did not show significant results either ($p > 0.05$) in the lipid profile in a population with type 2 diabetes mellitus (28). Similarly, Pivovarova-Ramich O, *et al.*, 2009 evaluating two isocaloric diets (animal protein versus vegetable protein) showed non-significant results ($p < 0.05$) for total cholesterol, HDL cholesterol and triglycerides (33). Interestingly, in the study by Daniels, *et al.*, (2014) when examining the antioxidant activity in the subclasses of HDL, significant results were evidenced for PON1 ($p < 0.006$) and lecithin cholesterol acyl transferase (LCAT) ($p < 0.044$) (28).

Two studies in people with metabolic syndrome where the effects of crocin (a carotenoid from saffron- tablets of 30 mg ($n = 30$) and 15 mg ($n = 30$) consumed over 8 weeks) on the lipid profile were evaluated, did not show significant changes ($p > 0.05$) for any of the lipid variables compare to placebo group ($n = 30$) (30,31). Additionally, a study in 28 obese men who consumed a diet rich in lycopene and lutein for 8 weeks showed no significant changes ($p > 0.05$) in the lipid profile for none of the evaluated interventions (34).

As shown in the articles described no differences in the effects of carotenoid consumption on lipid profile variables between cardiovascular risk and healthy populations were found. However, when analyzing other markers in the lipoprotein particles, beyond their lipid content, other relevant results were observed (28). This coincides with the results of Jung HY, *et al.*, (2016) who found a significant correlation ($p < 0.05$) between the levels of carotenoids and HDL particles (9), in a study evaluating the bioavailability of carotenoids in serum after the consumption of Chlorella powder in healthy subjects. The results observed in these two studies, by Daniels *et al* (2014), and Jung HY, *et al.*, (2019), suggest that lipoprotein assessment and markers associated with them, might represent a better approximation not only to evaluate risk status, but also to evaluate the effects of dietary interventions (e.g. carotenoid consumption) aimed to decreased such risk, compared with the classic lipid variables such as total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (14,45).

It is important to clarify that given these last two studies are so dissimilar in design and in the way in which the effects of carotenoids were evaluated, it is not possible to make a comparison. More in-depth studies are necessary to truly evaluate the effects of carotenoid consumption on cardiovascular risk and its lipid risk factors.

Effects of carotenoid consumption on oxidative stress and antioxidant status

It is expected that blood antioxidant levels increase as a result of the intervention with carotenoids. In fact, some studies reported a significant increase in participants' serum carotenoid levels (see Table 1); however, the effects in antioxidant capacity and/or reduction in oxidative stress markers were not evaluated in all studies. Among the articles that evaluated oxidative stress markers and/ or antioxidant capacity (5

studies), both in healthy populations (3 studies) and populations with cardiovascular risk (2 studies), significant results ($p < 0.05$) were observed for some markers, such as oxidation of low-density lipoprotein (ox-LDL) and antioxidant capacity (6,9,10,31,32).

In healthy participants ($n = 103$) consuming either a typical American (control) diet or the DASH diet rich in fruits and vegetables for 3 months; Miller R, *et al.*, (2005) reported significant reductions in oxidative stress measured by isoprostanes (-226 pg/ml, $p = 0.023$) in the DASH diet group compared to control diet (6). Similarly, change in antioxidant capacity measured by Oxygen radical absorbance capacity (ORAC) was higher in the DASH group, although not significant (143 trolox units/mL, $p = 0.091$). However, there were significant increases in antibodies levels against oxLDL ($p = 0.006$) in the DASH diet group compared to the control diet (net difference of 37 mU / ml); demonstrating that there is a higher level of unbound antibodies against oxLDL and a significant decrease in isoprostane excretion, which in definitively shows an effect on the reduction of oxidative stress mediated by the increase of carotenoids in this population. In another study with healthy subjects ($n = 59$), Stringham N, *et al.*, (2019) observed significant increases in antioxidant capacity (16.7% , $p < 0.05$) in the intervention group consuming lutein, zeaxanthin and meso-zeaxanthin during 6 months, compared to control group (1.6%) (10).

In population with cardiovascular risk, Nikbakht-Jam I, *et al.*, (2015) demonstrated that the pro-oxidant-antioxidant balance (PAB) evaluated in 60 people with metabolic syndrome consuming crocin tablets (30 mg/day) for 8 weeks was significantly reduced (11.7%) compared to the control group (0.7%) ($p = 0.014$) (31). Abedimanesh N, *et al.*, (2019) also reported in 84 CAD patients consuming either crocin (30 mg/day), saffron aqueous extract (30 mg/dL) or placebo during 8 weeks, significant reductions in oxLDL levels in the crocin group compared to placebo ($p = 0.02$), after adjusting by age and sex (32).

According to these results, the effects on antioxidant capacity and oxidative stress markers after carotenoid consumption were similar both in healthy populations and in populations at risk. Nevertheless, when analyzing the results in more detail, it could be observed that the percentage of increase in the antioxidant capacity was greater (about 5%) after the intervention in healthy population than in population at risk. This small percentage difference might be related to the higher levels of oxidative stress observed in subjects with cardiovascular risk compared to healthy subjects (46,47). Therefore, the increase in antioxidant capacity can be evidenced better in a healthy population than in people with cardiometabolic risk factors (48–51). Similarly, the effect of dietary carotenoids on oxidative stress markers e.g., oxLDL, was greater in healthy population than in the population at risk, in the revised studies. However, this has not been observed by other authors evaluating the effects of other antioxidants (i.e. grape seed extract) in metabolic syndrome subjects, in whom it was expected greater changes on these markers, given the oxidative level compromise in this population (52).

Based on this information, it could be concluded that dietary carotenoid effects on markers of oxidative-antioxidant status are evidenced better in healthy population

than in population with cardiovascular risk. However, these results cannot be extrapolated in general, due to methodological limitations, as there were no carotenoid measurements in some of the studies evaluating diseased population (31,32); while in most of the studies involving healthy population, blood carotenoids were measured and significant increases in their levels were observed (see Table 1). Another aspect to consider regarding the better outcomes in the healthy population studies revised here, it could be related with the participants' adherence in the studies, a matter that has been discussed in the literature, where healthy people show better adherence than people who present health alterations (53).

Effects on insulin resistance, blood glucose and glycated hemoglobin

As it has been demonstrated before, by increasing the intake of fruits and vegetables, plasma levels of carotenoids increase (Table 1). However, changes in variables related to glucose metabolism are not always observed after carotenoid consumption. In healthy population, although plasma significant increase in carotenoids ($p < 0.05$) is evident, no statistically significant changes in blood glucose and insulin levels is observed. Djuric Z, *et al.*, (2009), evaluated 69 healthy women consuming either a Mediterranean diet or their own usual diet (control group) for 6 months; at the end of the study the increase in carotenoid levels in the Mediterranean diet group was evident: zeaxanthin in 31%, β -cryptoxanthin in 115%, α -carotene in 151% and β -carotene in 75%; but no statistically significant changes were observed in glucose or insulin levels (7).

In CV risk population, the increase in plasma carotenoids after carotenoid consumption has controversial effects on glucose and insulin levels. Wallace I, *et al.*, (2013) evaluated 89 overweight participants at risk of CVD distributed into three groups, differentiated by daily consumption of fruits and vegetables (2, 4 or 7 servings per day). After 12 weeks of intervention, there were no significant differences between groups in terms of insulin resistance or blood glucose ($p > 0.05$), although there was a significant linear increase in serum lutein levels in all groups (27). Contrarily, Behrouz V, *et al.*, (2020) evaluated 50 participants with type 2 diabetes mellitus (DM2) consuming either 30 mg crocin tablets/day or placebo for 12 weeks. Compared with placebo, crocin group had a significant decrease in blood glucose (13.4%, $p=0.015$), and insulin levels (23.5%, $p = 0.046$), in addition to improve glycated hemoglobin ($p=0.045$). Consequently, the participants showed a significant decreased in insulin resistance measured by HOMA-IR (by 33.3%, $p=0.001$) compared to placebo (35).

These results suggest the increase in plasma carotenoids in healthy population is not associated with significant changes in blood glucose and insulin levels, where there is little room for improvement, while in people with established disease (i.e., DM2), significant changes were evidenced after carotenoid consumption. As expressed before, in a population with greater alterations, the effects of an intervention on these variables could be more evident (54). It has been demonstrated that natural dietary antioxidants ameliorated insulin resistance observed in obese patients, at risk of DM2 or metabolic syndrome, by enhancing the effect of insulin-sensitizing drugs through molecular mechanisms yet to be elucidated (42). In

addition, it has been proposed that antioxidants (e.g., polyphenols, carotenoids) could modulate insulin resistance induced by accelerated ROS production, as seen in obesity/chronic inflammation, resulting in better carbohydrate metabolism (55,56).

Effects of dietary carotenoids on inflammation

From the six studies, included in this revision, evaluating inflammatory markers after carotenoid intervention, 4 studies were performed in healthy population and 2 in CV risk population. From these, only 2 studies in healthy population and one in people at risk had as their main objective to evaluate the effects of carotenoid consumption on inflammation markers. The other 3 studies presented surrogate results. The 6 studies present clear methodological differences regarding intervention time and carotenoid dose.

The most evaluated inflammatory marker was high-sensitivity C-Reactive Protein (hs-CRP). In healthy volunteers, the following studies demonstrated different effects on this marker after carotenoid consumption. Djuric Z, *et al.*, (2009), did not observe significant changes in this marker after carotenoid intervention in healthy population (7). Similarly, Jahns L, *et al.*, (2018) evaluated healthy individuals, with no significant changes ($p > 0.05$) in hs-CRP levels after carotenoid diet for 8 weeks, but significant changes were observed in other inflammatory markers such as interferon α -2, tumor necrosis factor- α , as well as macrophage inflammatory protein-1 β ($p < 0.05$) (11). Hurtado-Barroso S, *et al.*, (2019) reported a significant decrease in hs-CRP and TNF- α levels ($p < 0.05$) after 24h of a single dose of carotenoid-rich meal (tomato sofrito) compared to baseline (8). Stringham N, *et al.*, (2019) also reported significant decreases in IL6 (4.3%) and IL1- β (24%) levels compared with placebo after 6 months of dietary carotenoid consumption (10).

In studies with cardiovascular risk individuals, significant results were only reported by Abedimanesh N, *et al.*, (2019), where after 8 weeks of carotenoid consumption by CAD patients, compared to placebo, beneficial effects were observed in the crocin group by increasing the gene expression of Sirtuin 1 and AMPK, and decreasing the expression of lectin-like oxidized LDL receptor 1 -LOX1- and the pro-inflammatory transcription factor NF- κ B (32). Daniels, *et al.*, (2014) reported no significant changes in hs-CRP after an 8-wk carotenoid-rich diet in obese DM2 patients (28).

The mixed results reported for both healthy and CV risk individuals on inflammatory markers suggest first, the dissimilar methodological approaches to evaluate these markers, which makes difficult to compare results between studies. Second, the need for more studies with similar protocols to have a better understanding of the effects on these markers after carotenoid consumption. Third, the systemic low-grade chronic inflammation characteristic of subjects with CV risk, represent a chronic condition present over years in the participants that it is difficult to ameliorate with short or medium length-interventions with dietary carotenoid (57,58). Although in the acute study by Hurtado-Barroso S, *et al.*, (2019) it was reported a significant decrease in hs-CRP levels, these benefits are not completely due to the lycopene supplied, but also to other bioactive compounds in the given meal (8); as it has been evidenced for other antioxidants (59,60). The results may be associated to a reduction in oxidation and improvement in the ingested lipid profile, i.e., the olive oil

matrix used with the tomato sofrito; as some studies have provided evidence that olive oil might exert beneficial effects on inflammation markers, such as hs-CRP (61).

CONCLUSIONS

Given the diversity of studies and their methodological proposals, as well as their different main objectives to evaluate dietary carotenoid effects, the comparison of results between healthy and CV risk populations is not very conclusive. Changes for some risk biomarkers such as lipid, inflammatory and antioxidant profiles in both populations (healthy and at risk) do not represent strong differences, but it was observed that bioavailability of carotenoids in blood in healthy populations was reported to be higher than in population at risk. Although several factors may influence bioavailability, these results suggest the consumption of carotenoids could play a role as a strategy for prevention than as a co-aid in the treatment of CV risk population. In addition, it is evident from the review that food matrices play a crucial role in the bioavailability of carotenoids and that encapsulation techniques may represent a strategy to improve the stability and bioavailability of carotenoid-rich extracts, especially in diseased population, where bioavailability seems to be low, or the higher oxidative profile observed in this population limit the availability to modulate cardiometabolic markers.

Conflict of interest. The authors declare no conflict of interest.

Author's contribution. Conceptualization: YG-P and JB-A; Articles search and methodology: YG-P; manuscript writing: YG-P, KP-S, NB-T, JZ-M, JQ-Q, JB-A; manuscript review and edition: JB-A and YG-P. All authors have reviewed, discussed, and accepted final version of manuscript.

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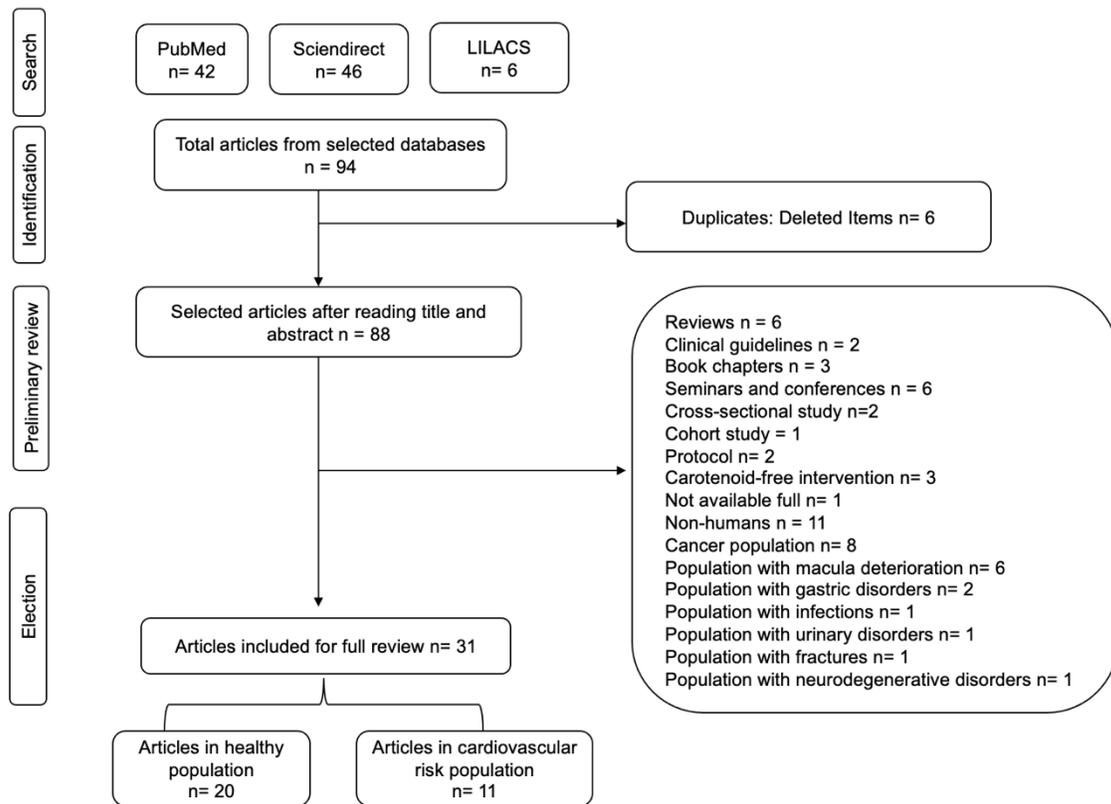


Figure 1. Flowchart for the selection of articles.

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Table 1. General characteristics and main outcome of the selected articles.

Reference	Ref. No.	Design	Intervened population (n)	Control population (n)	Carotenoids supplied (mg/day) or diet	Intervention duration (days)	Significant increase in blood carotenoids	Main outcome
Miller R, et al., 2005	(6)	Parallel	52	51	DASH diet rich in F&V Vs. American diet	90	p=0.001	Decrease in urinary ispropanes (p = 0.023) and increase in ORAC and Ab-oxLDL (p= 0.006) in the DASH diet group compared to control.
Djuric Z, et al., 2009	(7)	Parallel	35	34	Greek-Mediterranean diet with 7–9 F&V servings per day Vs. control non-intervention	180	p <0.05	Increase in total plasma carotenoids by 55% and MUFA, but not changes in blood lipids, triacylglycerol, insulin, glucose or C-reactive protein.
Hurtado-Barroso S, et al., 2019	(8)	Non-experimental	22	0	59.2	1	p <0.05	Decrease in C-Reactive Protein levels (p = 0.010) and TNF-α (p = 0.011).
Beni AA.,2020	(9)	Cross-over	21		12.65	21	not quantified	Protection of lipid peroxidation and improvement in redox environment (p <0.05).
Stringham N, et al.,2019	(10)	Parallel	25	10	13 (low) and 27 (high)	42	p <0.05	IL-1β was significantly decreased compared to placebo in both treatment groups (p <0 .005).
Jahns Let al.,2018	(11)	Non-experimental	38	0	62	56	not quantified	Increase in plasma concentration of interferon α-2 (p = 0.003) and decrease in macrophage inflammatory protein 1 β (p

Healthy population

Cardiometabolic markers

= 0.027) and tumor necrosis factor α ($p = 0.012$).

Gibson A, et al., 2012	(12)	Parallel	39	41	≤ 2 portions/d versus ≥ 5 F&V portions/d	112	$p=0.001$	<p>There was a significant improvement in immune response to anti pneumococcal vaccination, but not to tetanus toxoid.</p> <p>In mandarin tomato juice, a marked 8.5-fold increase in lycopene bioavailability was observed compared to red tomato juice ($p < 0.001$).</p> <p>Better bioavailability in the lutein-enriched chlorella extract group ($p < 0.0001$).</p> <p>20 g of lipids promoted the absorption of carotenoids ($p < 0.05$).</p> <p>The diacetate micromicella formulation (group 4) showed a higher mean response in serum carotenoid concentrations compared to the other active interventions ($p = 0.002$ to 0.019).</p>
Cooperstone J, et al., 2015	(13)	Cross-over	11	10	10.29 (calendula petal extract), 12.35 (regular chlorella powder) and 20.12 (chlorella powder enriched with lutein)	1	not quantified	
Jung HY., et al., 2016	(14)	Parallel	10	6		42	$p < 0.05$	
Goltz S, et al., 2012	(15)	Parallel	29	29	25	7	$p < 0.01$	
Green-Gomez M., 2020	(16)	Parallel	16	16	22 (Group1 y 4), 44 (Group2) and 542 (Group3)	42	$p < 0.05$	

Bioavailability

Kuratsune H, et al., 2010	(17)	Cross-over	17		7.5	42	not quantified	Neurological markers	Improved sleep rhythms (p<0.05).
Power R, et al., 2018	(18)	Parallel	45	46	22	360	p <0.05		Memory score improvement (p=0.009).
Goltz S, et al., 2013	(19)	Cross-over	6		23	35	p <0.05		Increased blood carotenoids (p<0.05).
McGrath M, et al., 2015	(20)	Parallel	10	11	2, 5 or 8 portions F&V/day	28	not quantified	Increased carotenoids	The model containing all carotenoids and vitamin C responded better compared to only with vitamin C (p <0.001) or with the lutein-only model (p = 0.006) for predicting F&V intake.
Aguilar S, et al., 2015	(21)	Parallel	18	22	2.75 or 11	56	not quantified		Consumption of 2.75 mg or 11 mg/day of carotenoids increased similarly the carotenoid status of the skin compared to baseline at week 8 (p< 0.001).
Kelly D, et al., 2017	(22)	Parallel	25	25	140	56	p<0.001		There were no significant changes at the level of the macular pigment between groups consuming carotenoid-enriched eggs or control non-enriched eggs.
Cooperstone J, et al., 2018	(23)	Parallel	12	12	0.640 (β-carotene) and 1.617 (lycopene)	14	p<0.001	Increased carotenoids	Elevated β-Apo-13-carotenone in the blood (p <0.001).
Castro M, et al., 2019	(24)	Parallel	18	20	16.6	28	p <0.05		Total plasma carotenoids increased similarly between groups.
Baswan SM, et al., 2019	(25)	Parallel	29	29	6.523	84	p <0.05		Increased carotenoids in the skin (p<0.05).

Population with cardiovascular risk factors	McCall D, et al., 2009 (26)	Parallel (3 groups)	43, 41	33	1*, 3 or 6 portions/day of F&V	56	Lutein (p=0.002) β-cryptoxanthin (p<0.001)	Cardiometabolic markers	<p>For each 1-portion increase in fruit and vegetable consumption, serum carotenoid increased and there was a 6.2% improvement in blood flow response (p=0.03) in hypertensive subjects.</p> <p>No significant differences were found in insulin resistance between the groups consuming different portions of F&V/day in overweight and CVD risk (≥20% over 10 years) people.</p> <p>Increase in dietary and serum carotenoids improved HDL function by increasing PON1 and LCAT activities in Type 2 diabetic obese subjects (p<0.05).</p> <p>Compared to placebo, carotenoid supplement decreased estradiol levels (p<0.05) and favorably influence serum hormone profiles in men that impact metabolism and body composition.</p> <p>The increase in cholesterol ester transfer protein was not significant between groups after crocin</p>
	Wallace I, et al., 2013 (27)	Parallel (3 groups)	23, 27	26	1-2, 4 or 7 portions/day of F&V	84	Serum lutein (p<0.001)		
	Daniels JA, et al., 2014 (28)	Parallel	40	40	1 or ≥6 portions/day	56	serum α-carotene, β-cryptoxanthin, lutein and zeaxanthin for all comparisons p <0.05		
	Anderson M, et al., 2014 (29)	Parallel	9	10	1200	14	not quantified		
	Javandoost A, et al., 2017 (30)	Parallel	22	22	30	56	not quantified		

Nikbakht-Jama I, et al., 2015	(31)	Parallel	30	30	30	56	not quantified	treatment in subjects with metabolic syndrome. Reduction in serum pro-oxidant-antioxidant balance in the intervened group compared to control (p=0.014), in people with metabolic syndrome consuming crocin.
Abedimanesh N, et al., 2019	(32)	Parallel (3 groups)	28	28	30	56	not quantified	Increase in the gene expression of SIRT1 and AMPK and decrease in the expression of LOX1 and NF-κB (p<0.05) in the crocin group compared with placebo, in patients with coronary artery disease
Pivovarova-Ramich O, et al., 2020	(33)	Parallel	18	19	not quantified	42	alpha-carotene, beta-carotene (p <0.05)	Similar improvements in oxidative stress markers in individuals with type 2 diabetes, consuming a high animal or plant protein diet.
					Sum of carotenoids provided:			
					24.84			
					(GroupA: High Lycopene + High Lutein),			
					21.44			
					(GroupB: High Lycopene + Low Lutein),			
					9.52 (GroupC: Low Lycopene + High Lutein)			
Takagi T, et al., 2020	(34)	Parallel	7	7	and	4 56	p<0.001	Reduction in waist circumference in group B (p=0.02), compared to baseline.

(GroupD: Low
Lycopene +
Low Lutein)

Behrouz V, et al., 2020	(35)	Parallel	25	25	30	84	not quantified	Crocin improved fasting glucose, hemoglobin A1c, and insulin resistance, compared to placebo (p<0.05).
Mohler E, et al., 2011	(36)	Parallel	5 (each group)	8	0,25 to 2 mg/kg/day	39	not quantified	Safety Trans sodium crocetin was safe and well tolerated at the supplied doses.

*1 portion of Fruits and Vegetables (F&V) is defined as 80g and was reported in the studies from: <https://www.nhs.uk/live-well/eat-well/5-a-day/portion-sizes/>.

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Table 2. Impact of delivery system on carotenoid bioavailability and/or percentage of modification (%M)* of biomarkers measured.

Carotenoid	Condition	Delivery system	Trial	Intervention group main report	Reference
Crocina	Encapsulated	30 mg crocin tablets (direct compression method)	<p>Double-blind, placebo controlled.</p> <p>44 patients, 18 to 70 years, with metabolic syndrome defined by the National Cholesterol Education Program (Adult Treatment Panel III).</p> <p>Duration: 8 weeks.</p>	<p>Total Cholesterol = 220.09 ± 55.60 mg/dL (%M: -5,2%).</p> <p>Triglyceride = 160.50 mg/dL (%M: 6,3%).</p> <p>Low-density lipoprotein = 121.00 mg/dL (%M: -26,0%).</p> <p>High-density lipoprotein = 50.00 mg/dL (%M: 35,1%).</p> <p>Fasting blood sugar = 88.50 mg/dL (%M: -4,3%).</p>	Javandoost A, <i>et al.</i> , 2017 (30)
		15 ± 0.8 mg crocin tablets (direct compression method)	<p>60 patients, aged 18–75 years, with metabolic syndrome defined by the International Diabetes Federation (IDF).</p> <p>Participants were randomly allocated to one of two groups (each n=30 subjects): intervention group (13 men, 17 women) and control group (12 men, 18 women).</p> <p>Duration: 8 weeks.</p>	<p>Total Cholesterol = 210.52 ± 52.68 mg/dL (%M: -6,2%).</p> <p>Triglyceride = 132.00 (108–201) mg/dL (%M: -10,5%).</p> <p>Low-density lipoprotein cholesterol = 123.52 ± 48.06 mg/ dL (%M: -19,0%).</p> <p>High-density lipoprotein cholesterol = 49.25 ± 11.05 mg/dL (%M: 27,6%).</p> <p>Fasting blood glucose = 104.52 ± 49.12 mg/dL (%M: -2,1%).</p>	Nikbakht-J, <i>et al.</i> , 2015 (31)

Lutein and zeaxanthin isomers	Encapsulated and free	<p>Crocin (30 mg/day) was filled in 125 mg capsules with corn starch as a vehicle.</p> <p>Free crocin extract (Saffron Aqueous Extract, 30 mg/day). Placebo</p>	<p>Randomized, double-blind, placebo controlled.</p> <p>84 patients with coronary artery disease (male and female aged 40-65 years) without the experience of myocardial infarction, diagnosed by angiography in the past 6 months.</p> <p>Duration: 8 weeks.</p>	<p>Oxidized low-density lipoprotein cholesterol levels:</p> <p>Tablet = 37,82 ± 13,14 mg/dL (%M: -2,54 ±3,30)</p> <p>Free = 48,91 ± 15,15 mg/dL (%M: -0,24±4,79)</p> <p>Monocyte chemoattractant protein-1: Tablet = 162,50 ± 60,21 pg/mL (%M: -3,5 ±4,40)</p> <p>Free = 171,76 ± 45,36 pg/mL (%M: -3,89 ± 4,79).</p>	<p>Abedimanesh N, <i>et al.</i>, 2019 (32)</p>
	Encapsulated	<p>Pills (soft gelatin capsules), with lutein, zeaxanthin, and meso-zeaxanthin suspended in safflower oil.</p>	<p>Double-blind, randomized, placebo controlled.</p> <p>59 subjects were healthy, college-aged (18–25, mean = 21.5 yrs.; 27 male/32 female) non-smokers with a BMI between 18.5 and 27.</p> <p>Duration: 24 weeks.</p>	<p>Lutein in serum proteins =1,25 ± 0,684 µg/mL (%M: 495%).</p> <p>Zeaxanthin isomers in serum proteins = 0,192 ± 0,1,1 µg/mL (%M: 380%)</p>	<p>Stringham NT, <i>et al.</i>, 2019 (10)</p>

Encapsulated

Capsule with macular carotenoids powder (in a Lutein:Meso-Zeaxanthin:Zeaxanthin ratio of 10:10:2)

Parallel group, double-blind, placebo-controlled, block-randomized clinical trial.

Serum Lutein = 0,896±0,584 (%M: 259,8 %).

Serum Zeaxanthin = 0,086±0,050 (%M: 65,4%).

91 subjects aged (mean ± SD) 45.42±12.40 (male= 51.6%) free of retinal disease with ≤0.55 optical density units.

Serum Meso-Zeaxanthin = 0,036±0,040 (%M: 3500%).

Power R, *et al.*, 2018 (18)

Duration: 48 weeks.

* %M (Percentage of modification): (post-trial measurement-pre-trial measurement) / (pre-trial measurement) * 100)

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