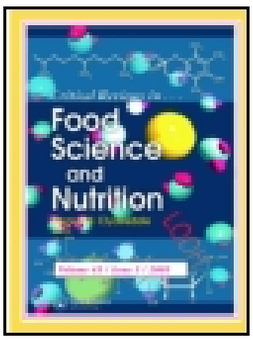

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Effectiveness of nanoscale delivery systems on improving the bioavailability of lutein in rodent models: a systematic review

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CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
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REVIEW

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Effectiveness of nanoscale delivery systems on improving the bioavailability of lutein in rodent models: a systematic review

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ABSTRACT

Lutein, a potent antioxidant and the main macular pigment that protects the macula from light-initiated oxidative damage, has low bioavailability. Various nanoscale delivery systems have been developed for improving its bioavailability. This systematic review aims to evaluate the effectiveness of nanoscale delivery systems on improving lutein bioavailability in rodent models. Using EBSCOhost and PubMed, a total of eleven peer-reviewed articles published from 2000 to 2020 were identified. Plasma lutein concentration, pharmacokinetic parameters, including maximum concentration (C_{max}), area under curve (AUC), and time to reach the maximum concentration (T_{max}), and lutein accumulation in organs were extracted to evaluate the bioavailability of lutein using nanoscale delivery methods as compared with unencapsulated or raw lutein. Various nanoscale delivery systems, including polymer nanoparticles, emulsions, and lutein nanoparticles, significantly improved the bioavailability of lutein, as evidenced by increased plasma lutein concentrations, C_{max}, or AUC. Additionally, five out of seven studies observed enhanced accumulation of lutein in the liver and the eyes. Polymer nanoparticles and emulsions improve the dispersibility and stability of lutein, thus lutein might be more accessible in the small intestine. Lutein nanoparticles shortened the T_{max}. Further studies are warranted to evaluate the effectiveness of nanoscale delivery systems on improving the functionalities of lutein.

KEYWORDS

Bioavailability; carotenoids; emulsions; lutein; nanocarrier; nanoparticles

Introduction

Lutein (Figure 1), a xanthophyll carotenoid commonly found in kale, spinach, and eggs, plays an important role in disease prevention and treatment (Ribaya-Mercado and Blumberg 2004). Lutein acts as a potent antioxidant due to its polarity and number of conjugated double bonds (Ochoa Becerra et al. 2020; Medhe, Bansal, and Srivastava 2014). It is particularly vital for eye health, as it is the main macular pigment that accumulates in the photoreceptors and macula area of the eyes (Landrum and Bone 2001). This macular pigment helps improve visual functions, e.g., visual acuity and contrast sensitivity, and delay the progression of age-related macular degeneration (AMD), by filtering blue light and scavenging light-initiated reactive oxygen species (Liu et al. 2015). Additionally, lutein was reported to be the most abundant carotenoid in the brain of infants and children (Vishwanathan et al. 2014) and involved in their cognitive development (Lieblein-Boff et al. 2015). Moreover, it was reported that in diabetic patients, lutein suppresses the transcript of redox sensitive factors in immune systems induced by high glucose levels, thus decreasing their susceptibility to infections (Muriach et al. 2008). Last but not least, several observational studies revealed the protective effects of lutein on certain cancers, such as breast and lung cancer, and on cardiovascular diseases (Ribaya-Mercado and Blumberg 2004).

Despite its important physiological functions, the absorption efficiency or bioavailability of lutein is known to be low (Lienau et al. 2003), mainly due to the hydrophobic nature of this carotenoid. Lutein is mostly confined in the lipid phase of the food, making it difficult to diffuse into the aqueous digesta and reach the intestinal cell epithelium for absorption in the digestive tract (Kopec et al. 2017). In addition, the presence of conjugated double bonds in lutein implies its high reactivity in lipid oxidation, which limits its stability during processing, storage, and cooking. Therefore, it is necessary to develop effective techniques for lutein to improve its stability and bioavailability.

Diverse nanoscale delivery systems have been developed for improving the water dispersibility, stability, and bioavailability of bioactive food components and nutraceuticals (McClements 2015). The use of appropriate nanoscale delivery systems can enhance the efficacy of bioactive food compounds, especially the lipophilic ones (Rehman et al. 2020; Steiner, McClements, and Davidov-Pardo 2018; Bhat et al. 2020; Mardani et al. 2020). Desirable nanoscale delivery systems for lipophilic guest compounds include emulsions (e.g., microemulsions, emulsions, nanoemulsions, multiple emulsions, and multilayer emulsions), solid lipid nanoparticles,

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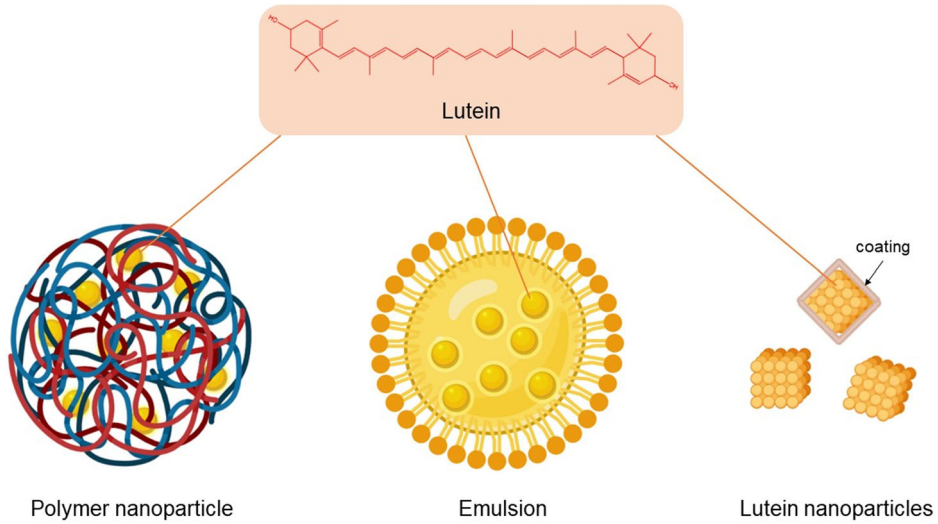

Figure 1. The structure of lutein and three types of nanoscale delivery systems applied in the studies reviewed in this article.

liposomes, biopolymer nanoparticles, microgels, and inclusion complexes to name but a few (McClements 2015). Most widely used for lipophilic bioactive compounds are oil-in-water (O/W) emulsions (Figure 1), consisting of an oil phase carrying the lipophilic compound and being dispersed as small spherical droplets in an aqueous phase. Polymer nanoparticles (Figure 1), especially those constituted by amphiphilic proteins and polysaccharides, can be used to carry lipophilic bioactive compounds. In addition, the lipophilic bioactive compound aggregates can be processed into nanoparticles (Figure 1) by antisolvent precipitation with or without the use of coating materials and stabilizers. These nanoscale delivery systems have been extensively studied in in vitro systems, and their effects on the solubility and bioaccessibility of carotenoids were previously reviewed (Focsan, Polyakov, and Kispert 2019; Soukoulis and Bohn 2018). Evaluation in in vivo models, e.g., animal models, is also increasing due to the promising effect of various nanoencapsulation techniques on improving the bioavailability of lipophilic bioactive compounds, including lutein. Yet, no systematic review has been conducted to evaluate the effectiveness of various nanoscale delivery systems on improving the bioavailability of lutein.

Since lutein is an important carotenoid for human health and the effort to improve its bioavailability is critical in formulating food and nutrition products, the present systematic review aims to assess the effectiveness of existing nanoscale delivery systems on the bioavailability of lutein in rodent models. Such information will help inform the food and nutraceutical industries about the potential applicability of these techniques in optimizing lutein intake and preventing or treating related diseases.

Methods

Literature search strategy

This protocol was registered with PROSPERO (#CRD42020168592). The PRISMA flow diagram of the literature search process is shown in Figure 2 (Moher et al. 2009). EBSCOhost and PubMed were the databases used. For EBSCOhost, seven databases were included, which were Academic Search Premier, CINAHL Plus with Full Text, Cochrane Central Register of Controlled Trials, Cochrane Methodology Register, Health Source: Nursing/Academic Edition, Cochrane Clinical Answers, and MEDLINE. The searching model Boolean/Phrase was used with all databases selected. Keywords used in the literature search were "lutein" AND "nanoencapsulation OR emulsion OR nanoparticles OR liposomes OR nanocarriers OR nanoemulsion OR microemulsion OR micelles AND bioavailability OR bioaccessibility OR bioactivity." Key terms for searching the nanoscale delivery systems were chosen based on previous literature (McClements 2015). Only peer-reviewed articles published from 2000 to 2020 and written in English were considered.

Inclusion/exclusion criteria for eligibility

In order for a study to be included in this systematic review, lutein should be ingested via oral dosing or being incorporated in the diet. To confirm the formation of nanoencapsulated lutein, included studies should provide the characterization of nanostructures, such as microscopic photographs and the measurement of particle size. To evaluate the effects of the nanoscale delivery system on the bioavailability of lutein, the study had to measure at least the plasma concentration of lutein at a given time point after lutein intake/administration. In addition, at least one control group using unencapsulated lutein, such as pure lutein or physical mixture of lutein and other components, had to be included. In vitro studies, studies employing delivery techniques that do not meet the definition of nanoscale nutrient delivery systems, and studies that administered a mixture of nutrients as the treatment were excluded.

Data extraction

Relevant information was extracted from the articles independently by the authors (YZ, LK, and LT). Extracted data are (1)

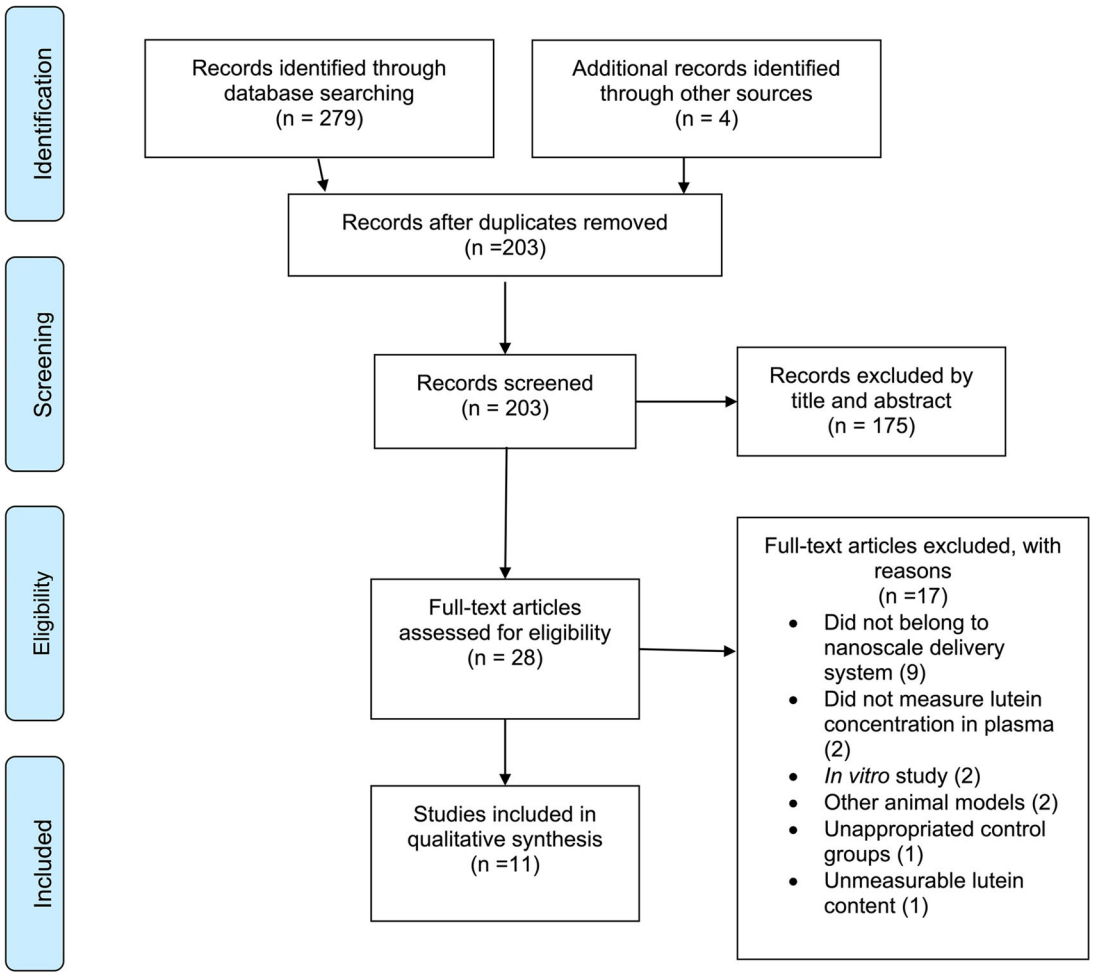

Figure 2. Search strategy flow diagram for research evaluating the effectiveness of nanoscale delivery system on improving the bioavailability of lutein in rodent models.

characteristics of the nanoscale delivery system, including the carrier, the particle size, and the encapsulation efficiency/loading capacity, (2) animal experimental design, including characteristics of the rodent model, the control group(s), the dosage and administration method of lutein, and the time points for blood and/or organ collection, and (3) measured outcomes, including plasma lutein concentrations, lutein pharmacokinetic parameters, and/or lutein concentrations in various organs. Pharmacokinetic parameters reported in the studies include: maximum concentration (C_{max}), which is defined as the highest concentration of the compound of interest in plasma and/or in targeted organs after single dose administration; area under the concentration-time curve (AUC), an important parameter of the compound's bioavailability that reflects the exposure of the compound over time after administration; time to reach the maximum concentration (T_{max}), defined as the time taken to reach C_{max}; and half-life ($T_{1/2}$), which refers to the time required to reduce the initial dose in body by half (Urso, Blardi, and Giorgi 2002; Gidal et al. 2017). All the extracted data is shown in Tables 1–3.

Quality assessment

The Systematic Review Center for Laboratory Animal Experimentation risk of bias (SYRCLE's RoB) tool (Hooijmans et al. 2014) was used for quality assessment (Supplementary material Table S1). This tool, modified from the Cochrane RoB tool, includes 10 questions to assess possible biases in animal studies, including bias of selection, performance, detection, attrition, and reporting (Hooijmans et al. 2014). Two authors (YZ and LT) individually assessed the quality of articles, and any inconsistency was discussed with the third author (LK).

Results

Study identification and characteristics

After removing duplicates, a total of 203 articles were identified from databases and reference lists (Figure 2). Upon reviewing their titles and abstracts, 28 articles were subject to full-text review to determine their eligibility for inclusion. Finally, 11 articles met the inclusion criteria and were included in this systematic review. Based on the specific nanoscale delivery technique used, the 11 articles are grouped into three categories (Figure 1): studies using polymer nanoparticles (6 studies), studies using emulsions (1 study), and studies using lutein nanoparticles produced by antisolvent precipitation and spray drying technology (4 studies). The study conducted by (Sato et al. 2018) evaluated

Table 1. Effect of polymer nanoparticle carriers on lutein bioavailability in rodent models.

	Technique (Treatment group)			Experiment design				Outcomes[c]	
Study	Carriers[a]	Particle Size (nm)	EE/LC[b] (%)	Rodent Model	Control	Dosing (mg/kg BW)	Blood/tissue collection time	Plasma response	Organ response
Arunkumar, Prashanth, and Baskaran (2013)	Chitosan	80–600 (Mean: N/A)	EE: 85 ± 1	Male mice (n = 6/group)	Mixed micelles	Single dose 0.91	8 h	C: 53.5% ↑ ($p < 0.05$)	Liver: C: 53.9% ↑ ($p < 0.05$) Eye: C: 62.8% ↑ ($p < 0.05$)
Toragall, Jayapala, and Vallikannan (2020)	CHI-OA-ALG	40–160 (Mean:125)	N/A	Weanling Wister rats (n = 6/group)	Mixed micelles	Single dose 1.24	14 d	Single dose: C: 128.3% ↑ ($p < 0.01$)	N/A
Arunkumar et al. (2015)	PLGA-PEG	80–500 (Mean: 200)	EE: 88 ± 2 LC: 5.15 ± 0.05	Male lutein-deficient mice (n = 5/group/ time point)	Mixed micelles	Single dose 0.91	24,81,22,448 h	C_{max} :1.8-fold ↑ ($p < 0.05$) AUC: 5.4-fold ↑ ($p < 0.05$) T_{max}: no sig. difference	N/A
Ranganathan et al. (2019)	PLGA-PL	140 ± 6	EE: 90 ± 2 LC: 6.23 ± 0.03	Male Lutein-deficient mice (n = 5/group/ time point)	Mixed micelles	Single dose 0.91	24,81,22,448 h	C_{max}: 3.6- fold ↑ ($p < 0.05$) AUC: 3.9- fold ↑ ($p < 0.05$) T_{max}: no sig. difference	Liver: C_{max}: 2.5-fold ↑ AUC: 2.9-fold ↑ ($p < 0.05$) Eye: C_{max}: 3.4-fold ↑ AUC: 3.1-fold ↑ ($p < 0.05$)
Kamil et al. (2016)	PLGA	124 ± 4	EE: 52 ± 3	Male lutein-deficit Fischer rats (n = 8/group/ time point)	Free lutein	Single dose 10	0.17, 0.5, 0.83, 1.17, 2.17, 4.17 h.	C_{max}: 52.1-fold ↑ ($p \leq 0.05$) AUC: 77.6- fold ↑ ($p \leq 0.05$) T_{max}: no sig. difference	Liver: C_{max}: 3.6-fold ↑ ($p \leq 0.05$) Spleen: $C_{max:}$ 19.1-fold ↑ ($p \leq 0.05$) Fat depot: C_{max}: 3.9- fold ↑ ($p > 0.05$) Lung: C_{max}: 1.21-fold ↑ ($p > 0.05$)
Sato et al. (2018)	PVP	154.3 ± 3.9	N/A	Male Wistar rats (n = 10/group)	Powder lutein	Single dose 2.5	12,34,56,81,01,224 h. Organs at 4 and 24 h. Lymph cannulation experiment: Lymph collected every 30 mins from 0 up to 9 h, and every 60 minutes from 9 h to 12 h.	C: 100-, 80-, and 75- fold ↑ at 2, 5, 10 h (P value not reported)	*At 4 h:* Liver*: C:* 4.5-fold ↑ SI: C: 17-fold ↑ Kidney & Spleen: no sig. difference *At 24 h:* SI: C:20-fold ↑ Lymph: C: 2.3-fold ↑ Mass: 4.2-fold ↑ ($p < 0.05$ for all)

[a]PLGA, poly lactic-co-glycolic acid; PEG, polyethylene glycol; PL, phospholipid; CHI-OA-ALG, chitosan-oleic acid-sodium alginate, PVP, polyvinylpyrrolidone.
[b]EE, encapsulation efficiency; LC, loading capacity; N/A, not available.
[c]C, lutein concentration; C_{max}, maximum concentration; AUC, area under the concentration-time curve; T_{max}, time to reach the maximum concentration; SI, small intestine. The outcomes show the changes in the treatment group compared with the control group(s).

Table 2. Effect of emulsion carriers on lutein bioavailability in rodent models.

	Technique (Treatment group)			Experiment design				Outcomes[c]	
Study	Carriers[a]	Particle Size (nm)	EE/LC[b] (%)	Rodent Model	Control	Dosing (mg/kg BW)	Blood/tissue collection time	Plasma response	Organ response
Sato et al. (2018)	Soybean oil	336.9 ± 93.6	N/A	Male Wistar rats (n = 10/group)	Powder lutein	Single dose 2.5	12,34,56,81,01,224 h. Organs at 4 and 24 h. Lymph cannulation experiment: Lymph collected every 30 mins from 0 up to 9 h, and every 60 minutes from 9 h to 12 h.	C: 10–30 ng/mL ↑ ($p < 0.05$)	*At 4 h:* Liver: C: 1.5-fold ↑ ($p < 0.05$) SI: C: 15-fold ↑ ($p < 0.05$) Kidney & Spleen: no sig. difference *At 24 h*: Liver, SI, Kidney, Spleen: no sig. difference Lymph: C: 2.5-fold ↑ ($p < 0.05$) Mass: 4.2-fold ↑ ($p < 0.05$)
Murillo et al. (2016)	MCT	254.2	N/A	Male Guinea pigs (n = 8/group)	Powder lutein	Lutein was incorporated into daily diet: 14	After 6 weeks	C: 2.4-fold ↑ ($p < 0.001$)	Liver: 1.5-fold C ↑ ($p < 0.001$) Eyes and adipose: no sig. difference

[a]MCT, Medium-chain triglyceride
[b]EE, encapsulation efficiency; LC, loading capacity; N/A, not available.
[c]C, lutein concentration; SI, small intestine. The outcomes show the changes in the treatment group compared with the control group(s).

Table 3. Effect of lutein nanoparticles on lutein bioavailability in rodent models.

	Technique (Treatment group)			Experiment design				Outcomes[b]	
Study	Carriers	Particle Size (nm)	EE/LC (%)[a]	Rodent Model	Control	Dosing (mg/kg BW)	Blood/tissue collection time	Plasma response	Organ response
Zhang et al. (2015)	Starch coating	214.7	N/A	Sprague–Dawley rats (n = 6/group)	Powder lutein	Single dose 100	12, 46, 81, 01, 22, 632 h	C_{max}: 1.9-fold ↑ AUC: 1.4-fold ↑ T_{max}: 1.3 h ↓ (no p value reported)	N/A
Liu et al. (2017)	Film	377.9 ± 32.1	N/A	Sprague–Dawley rats (n = 9/group)	Lutein solution and raw lutein loaded on film	Single dose 10	0.083, 0.25, 0.5, 0.75, 1, 35, 81, 224 h	*Compare with lutein solution:* C_{max}: 5.5-fold ↑ ($p < 0.05$) AUC: 2.1- fold ↑ ($p < 0.05$) T_{max}: 2.25 h ↓ ($p < 0.05$) *Compare with raw lutein loaded on film:* C_{max}: 2.8-fold ↑ ($p < 0.05$) AUC: 1.6- fold ↑ ($p < 0.05$) T_{max}: 0.04 h ↓ ($p > 0.05$)	N/A
Chang et al. (2018)	N/A	159.5	N/A	Sprague–Dawley rats (n = 6/group)	Powder lutein	Single dose 50	5, 15, 30, 45 min, and 1, 35, 81, 224 h	C_{max}: 3.2- fold ↑ ($p < 0.05$) AUC: 2.3-fold ↑ ($p < 0.05$) $T_{1/2}$: 6.3-fold ↓ ($p < 0.05$) T_{max}: no sig. difference	N/A
Wu et al. (2019)	N/A	164.1 ± 4.3	N/A	Experiment 1: Sprague–Dawley rats (n = 6/group) Experiment 2: Kunming strain mice (n = 3/group/time point)	Power lutein ester	Single dose of lutein ester 50	Experiment 1: blood collected at 5, 15, 30, and 45 min, and 1, 23, 46, 81, 224 h. Experiment 2: Organs collected at 0.25, 0.5, 1, 2, 6, and 24 h	C_{max}: 2.5-fold ↑ ($p < 0.001$) AUC: 1.4-fold ↑ ($p < 0.05$) T_{max}: 1.75 h ↓ ($p < 0.05$)	Heart: C_{max}: 2.1-fold ↑ AUC: 1.7-fold ↑ $T_{max:}$ 1.5h ↓ Liver: C_{max}: 1.6-fold ↑ AUC: 1.9-fold ↑ $T_{max:}$ 1.5h ↓ Spleen: C_{max}: 2.0-fol↑ AUC: 1.9-fold ↑ $T_{max:}$ 1h ↓ Lung: C_{max}: 1.4-fold ↑ AUC: 1.3-fold ↑ $T_{max:}$ 1h ↓ Kidney: C_{max}: 2.3-fold ↑ AUC: 1.3-fold ↑ $T_{max:}$ 5h ↓ Brain: C_{max}: 1.5-fold ↑ AUC:1.7-fold ↑ $T_{max:}$ 1.5h ↓ Eye: C_{max}: 2.3-fold ↑ AUC: 2.3-fold ↑ $T_{max:}$ 1.5h ↓ ($p < 0.05$ for all data)

[a]EE, encapsulation efficiency; LC, loading capacity; N/A, not available.
[b]C_{max}, maximum concentration; AUC, area under the concentration-time curve; $T_{1/2}$, half-life; T_{max}, time to reach the maximum concentration. The outcomes show the changes in the treatment group compared with the control group(s).

both polymer nanoparticles and nanoemulsion technique on lutein bioavailability. The units of lutein dosage in the original articles were all converted to mg/kg BW for comparison.

Of the 11 studies, 7 used rat, including Sprague–Dawley (Zhang et al. 2015; Wu et al. 2019; Chang et al. 2018), Wistar (Toragall, Jayapala, and Vallikannan 2020; Sato et al. 2018; Liu et al. 2017), and Fischer (Kamil et al. 2016) rat, three used mouse (Arunkumar, Prashanth, and Baskaran 2013; Arunkumar et al. 2015; Ranganathan et al. 2019), and one used Guinea pig (Murillo et al. 2016) as the animal model. Lutein was incorporated in the diet for daily consumption in 1 study (Murillo et al. 2016), while the other 10 studies used a single dose of lutein via oral administration. Eight studies used a lutein dosage between 0.91 and 14 mg/kg BW (Arunkumar, Prashanth, and Baskaran 2013; Arunkumar et al. 2015; Ranganathan et al. 2019; Sato et al. 2018; Toragall, Jayapala, and Vallikannan 2020; Kamil et al. 2016; Zhang et al. 2015; Wu et al. 2019; Liu et al. 2017), while three studies used a relatively larger dosage at 50 or 100 mg/kg BW (Wu et al. 2019; Zhang et al. 2015; Chang et al. 2018). The study duration varied from 8 h to 6 weeks, but most studies were conducted within 2 days. In the 10 studies involving a single lutein dose, plasma lutein concentrations were measured at various time points, and thus can be regarded as pharmacokinetic studies. Of these ten, seven calculated lutein pharmacokinetic parameters based on the plasma lutein kinetic curve (Zhang et al. 2015; Kamil et al. 2016; Chang et al. 2018; Wu et al. 2019; Arunkumar et al. 2015; Ranganathan et al. 2019; Liu et al. 2017). Seven studies additionally measured lutein concentrations in various organs (Ranganathan et al. 2019; Arunkumar, Prashanth, and Baskaran 2013; Toragall, Jayapala, and Vallikannan 2020; Kamil et al. 2016; Sato et al. 2018; Wu et al. 2019; Murillo et al. 2016).

Polymer nanoparticles

Six of the 11 studies used polymer nanoparticles as lutein carriers (Table 1). Four of these six are from the same research group (Arunkumar, Prashanth, and Baskaran 2013; Arunkumar et al. 2015; Ranganathan et al. 2019; Toragall, Jayapala, and Vallikannan 2020). In this series of studies, chitosan, alginate, poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), and their blends were used as polymeric carriers. Chitosan is a positively charged polysaccharide obtained through deacetylation of chitin, which is extracted from the exoskeletons of arthropods, such as crustaceans (e.g., crabs, lobsters and shrimps) and insects (Elieh-Ali-Komi and Hamblin 2016). Alginate is a negatively charged polysaccharide isolated from algae (Chan, Lee, and Heng 2002). Both chitosan and alginate are natural biopolymers and widely used in foods and nutraceutical applications (Elieh-Ali-Komi and Hamblin 2016; Friedman et al. 2013). PLGA, PEG, and PVP are all synthetic biodegradable polymers and are permitted to use as food additives and in pharmaceutical formulations (Vroman and Tighzert 2009; Arunkumar et al. 2015).

In the earliest report published by this research group, chitosan was first hydrolyzed to reduce its molecular weight and then used to encapsulate lutein by co-precipitation (Arunkumar, Prashanth, and Baskaran 2013). The size of nanoparticles obtained ranged from 80 to 600 nm, and the encapsulation efficiency (EE) was 85% ± 1%. Male mice (Swiss albino, IND-CFT (1 C)) was randomly assigned to two groups (n = 6/group), which received a single dose of lutein (0.91 mg/kg BW) encapsulated by chitosan nanoparticles or a mixed micelle of lutein at the same dosage as the control group. At 8 h after dose administration, mice were euthanized with blood, liver, and eyes collected. Results showed that the concentration of lutein in the plasma, liver, and eye of the chitosan nanoparticle group was increased by 53.5%, 53.9%, and 62.8%, respectively, compared to the control group ($p < 0.05$).

Toragall, Jayapala, and Vallikannan (2020) reported the use of chitosan-oleic acid-sodium alginate (CHI-OA-ALG) nanoparticles as the lutein carrier. The two oppositely charged polysaccharides formed nanoscale polyelectrolyte complex aggregates through gelation and the use of oleic acid could facilitate the loading and delivery of lutein. The particle size of the CHI-OA-ALG nanoparticles ranged from 40 to 160 nm. Weanling Wistar rats were randomly assigned into two groups to receive either a single oral dose of lutein-loaded CHI-OA-ALG or a lutein micelle as control (n = 6/group). The dosage of lutein was 1.24 mg/kg BW. Rats were euthanized 14 days after lutein administration and blood was collected. It was shown that plasma lutein was 128.3% higher in the nanoparticle group than in the control group ($p < 0.01$). A dose-dependent experiment was also conducted with 0.1, 1, 10, and 100 mg/kg BW of lutein encapsulated by CHI-OA-ALG (n = 6/group) for 28 days. The plasma lutein concentration was noted to gradually increase with dosage. There was a 1.7-, 3.2-, and 3.9-fold higher plasma lutein concentration with the dosage of 1, 10, 100 mg/kg, respectively, as compared to the 0.1 mg/kg BW dosage ($p < 0.01$). Liver, kidney, eye, brain, spleen, and intestine were collected in the 1 and 10 mg/kg BW dosing groups after 28 days. The 10 mg/kg BW dosing group showed a significantly 1.4-, 3.5-, 2.1-, and 3.0 -fold higher lutein concentration in the liver, kidney, eye, and spleen ($p < 0.01$), respectively, than that of the 1 mg/kg BW dosing group. The lutein concentration in brain and intestine was also increased by 1.3- and 2.5-fold in the 10 mg/kg BW group ($p < 0.05$).

PLGA was utilized along with PEG and phospholipid (PL) in the other two studies published by the same research group (Arunkumar et al. 2015; Ranganathan et al. 2019) with similar experimental designs. The lutein-loaded nanoparticles were prepared by co-precipitation and particles sizes were between 80 to 500 nm (mean = 200 nm) (Arunkumar et al. 2015) and 140 ± 6 nm (Ranganathan et al. 2019), respectively. The EE achieved was 88% ± 2% and 90% ± 2%, respectively. In both studies, 60 male mice consumed lutein-deficit diets for six weeks to induce lutein deficiency. After a single dose of nanoencapsulated lutein by PLGA-PEG (Arunkumar et al. 2015) or by PLGA-PL (Ranganathan et al. 2019) and lutein micelle as control,

blood was collected from 5 mice/group at 2, 4, 8, 12, 24, and 48 h. The dosage of lutein was 0.91 mg/kg BW in both studies. The plasma C_{max} of lutein was reported to be 1.8- and 3.6-fold higher in the PLGA-PEG and PLGA-PL group, respectively, as compared to the control group ($p < 0.05$). The plasma AUC of lutein was 5.4- and 3.9- fold greater in the PLGA-PEG and PLGA-PL group than the control group ($p < 0.05$), respectively. T_{max} (4 hours) was not changed in the treatment groups. In addition, in the PLGA-PL study, 5 Swiss albino mice/group/time point were euthanized at 2, 4, 8, 12, 24, and 48 h after dosing with the liver and eyes collected. Results indicated that lutein C_{max} and AUC increased by 2.5- and 2.9-fold in the liver, and 3.4- and 3.1-fold in the eyes in the treatment group, respectively, as compared to the control group ($p < 0.05$).

PLGA was used by another group of researchers to fabricate nanoparticles (124 ± 4 nm) as the lutein carrier (EE of 52% ± 3%) (Kamil et al. 2016). Ninety-six male Fischer rats consumed lutein deficient diet for two weeks and were randomly divided into two groups to receive either a single oral dose of free lutein or lutein-loaded PLGA nanoparticles at the dosage of 10 mg/kg BW. Blood, liver, mesenteric fat depots, spleen, and lung were collected at 0.17, 0.5, 0.83, 1.17, 2.17, and 4.17 h after lutein administration (n = 8/group/time point). Compared with the free lutein group, 52.1- and 77.6-fold greater plasma C_{max} and AUC were found in the PLGA group ($p \leq 0.05$). It should also be emphasized that lutein was only detected in the plasma in five of the eight rats in the free lutein group at $T_{max,}$ which was 2.17 h. In contrast, all the rats in the PLGA group showed detectable lutein in their plasma after 1.17 h. In addition, the PLGA group showed significantly 3.6- and 19.1-fold higher lutein C_{max} in liver and in spleen at 4.17 h ($p \leq 0.05$). The PLGA group also showed 1.2- and 3.9-fold higher C_{max} in lung and fat depot, although the differences did not reach statistical significance.

PVP with an emulsifier, i.e., Tween 80, was used to encapsulate lutein by vacuum drying (Sato et al. 2018). The size of the freshly made lutein-loaded PVP nanoparticles was 154.3 ± 3.9 nm. Male Wistar rats were divided into the PVP nanoparticles and powder lutein (control) group with 10 rats in each. After a single dose of 2.5 mg/kg BW of lutein, blood was collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h. Liver, kidney, spleen, and intestinal mucosa were collected from three to five rats at 4 h and 24 h. The plasma lutein concentration in the control group was very low (below 5 ng/mL) at all time points, while that in the PVP group was temporarily high at 2, 5, and 10 h (ranging from 150 to 300 ng/mL). At 4 h, the lutein concentration was significantly 4.5-fold higher in the liver in the PVP group than in the control group ($p < 0.05$). A 17-fold higher lutein concentration was detected in the upper intestinal mucosa in the PVP group as compared to the control group ($p < 0.05$). No significant difference in the lutein concentration in kidney or in spleen was noted between groups at 4 h. At 24 h, the PVP group showed a significantly 20-fold higher lutein concentration in the upper intestinal mucosa ($p < 0.05$); there was no significant difference in lutein concentration noted in other tissues between the two groups. In addition, given that newly absorbed lutein is carried by chylomicrons and circulates in lymph before entering blood streams, a thoracic lymph cannulation experiment was also conducted to measure the lymph lutein concentrations after oral dosing. Specifically, lymph was collected every 30 min from 0 to 9 h, and every 60 min from 9 h to 12 h (n = 3–5/group/time point). Results indicated a 20-fold higher lutein accumulation in lymph than that in serum even in the control group at 9 h ($p < 0.05$). A significantly 2.3-fold higher lymph lutein concentration was noted in the PVP group as compared to the control group at 9 h ($p < 0.05$). The PVP group also showed a significantly 4.2-fold higher lutein mass in lymph ($p < 0.05$).

Emulsions

Two studies utilized emulsions to improve the bioavailability of lutein (Table 2). Emulsion systems can be formed by mixing the lipophilic compound (e.g., lutein) with oil and emulsifier into small droplets in an aqueous phase by mechanical agitation or high pressure homogenization (Anton, Benoit, and Saulnier 2008). Besides using the PVP nanoparticles as presented above, Sato et al. (2018) also formulated lutein into an emulsion system consisting of soybean oil, Tween 20, and egg yolk lysophosphatidylcholine. The particle size of the freshly prepared emulsion was 336.9 ± 93.6 nm, but the emulsion underwent phase separation after 6 days of storage. Ten rats were assigned to emulsion group and received the treatment with 2.5 mg/kg BW of lutein. Blood was collected at 1, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 10, 12, and 24 h. Liver, kidney, spleen, upper and lower small intestine were collected after 4 and 24 hours. Different from the PVP group, which showed a dramatically increased plasma (about 300 ng/mL) lutein concentration at 2, 5, and 10 h, the emulsion group showed a slightly 10 to 30 ng/mL higher lutein concentration than the control group in 3-6 h ($p < 0.05$). At 4 h, the liver lutein concentration in the emulsion group was 1.5-fold increased as compared to the control ($p < 0.05$). The concentration of lutein in the lower small intestine also showed a 15-fold increase in the emulsion group ($p < 0.05$). There was no statistically significant difference in the lutein concentration in kidney or in spleen between the control and the emulsion group. Comparing the two treatment groups—the emulsion and PVP groups, the plasma lutein concentration was approximately 100-, 80-, and 75- fold higher in the PVP group at 2, 5, and 10 h (P value not reported), respectively. In addition, at 4 h, the PVP group showed a significantly 3-fold higher liver lutein concentration ($p < 0.05$). Results of the lymph experiment showed that same as the PVP group, the emulsion group exhibited an approximately 2.5-fold and 4.2-fold increased lutein concentration and mass in lymph compared with the control at 9 h after dosing ($p < 0.05$). The lymph lutein concentration and mass at 9 h in the two treatment groups were close. The lymph lutein concentration gradually increased from 4 h in the emulsion group and from 2 h in the PVP group.

In a study by Murillo et al. (2016), the effects of a lutein-loaded nanoemulsion on the bioavailability of lutein, as well as on lipoprotein metabolism and progress of hepatic steatosis were studied. The lutein-loaded nanoemulsion was formulated using a medium-chain triglyceride oil and the d-α-tocopheryl polyethylene glycol succinate as a polymeric emulsifier. The particle size was determined to be 254.2 nm. Twenty-four male Hartley guinea pigs were given a hypercholesterolemic diet to induce hepatic steatosis. The guinea pigs were then randomly assigned into the blank group (receiving no lutein), the control group (receiving power lutein), and the lutein emulsion group (n = 8/group). For the control group and the emulsion group, lutein was incorporated into the diet and administered for 6 weeks, and the dosage of lutein was equivalent to ~14 mg/kg BW per day. Blood, liver, adipose tissue, and eyes were collected at the end of the 6-week intervention. Lutein was not detected in the plasma, eyes, and liver of the blank group, while a significantly 10- to 13-fold lower concentration was detected in the adipose tissue compared with the other two groups ($p < 0.05$). A significantly 2.4- and 1.5- fold increased lutein concentration in plasma and liver was noted in the emulsion group than the control group ($p < 0.001$). No significant difference in lutein concentration in the eyes or in the adipose tissue was noted between the control and the emulsion group. Results on lipoprotein metabolism and hepatic steatosis will be discussed in Discussion session.

Lutein nanoparticles

Four studies used lutein nanoparticles/nanocrystals with or without carriers or excipients (Table 3). Zhang et al. (2015) spray dried lutein into nanoparticles coated with corn starch and the particle size was about 214.7 nm, calculated by the mean of three measurements. Twelve Sprague–Dawley rats were randomly assigned to two groups (n = 6/group) and received a single dose of lutein nanoparticles or powder lutein (control group) both providing 100 mg/kg BW of lutein. Blood was collected at 1, 2, 4, 6, 8, 10, 12, 26, and 32 h after oral dosing. Results showed that plasma C_{max} and AUC of lutein in the nanoparticle group was 1.9- and 1.4-fold higher, respectively, than the control group, with no p value reported. The T_{max} in the control group was 6.0 ± 2.2 h, which was 1.3 h later than that of the nanoparticle group (4.7 ± 3.0 h).

Lutein nanocrystals (particle size: 377.9 ± 32.1 nm) were prepared by anti-solvent precipitation and incorporated into a hydroxypropyl methylcellulose-PEG composite film in a study by Liu et al. (2017). The film was claimed to be fast-dissolving on the tongue and thus releasing the bioactive compound (Khan et al. 2015). As a comparison, raw lutein powder without size reduction (size unknown) was also loaded into the film. A total of 27 Sprague–Dawley rats were randomized into three groups and received lutein solution (control group), the raw lutein film, and the lutein nanocrystal film containing 10 mg/kg BW of lutein, respectively. Blood was collected at 0.083, 0.25, 0.5, 0.75, 1, 3, 5, 8, 12, and 24 h. Plasma T_{max} of lutein was significantly decreased to 0.79 and 0.75 h in the raw lutein film and lutein nanocrystal film groups, respectively, as compared to 3 h in the control group. The raw lutein film and lutein nanocrystal film groups showed an increase in plasma lutein C_{max} by 1.9- and 5.5-fold, respectively, compared with the control group ($p < 0.05$), as well as 1.3- and 2.1- fold higher plasma AUC ($p < 0.05$), respectively. Comparing the two film groups, rats in the lutein nanocrystal film group showed 2.8- and 1.6- fold higher plasma C_{max} and AUC than the raw lutein film group ($p < 0.05$).

Chang et al. (2018) and Wu et al. (2019) both used the anti-solvent precipitation method to prepare lutein nanoparticles and the sizes obtained were 159.5 nm and 164.1 ± 4.3 nm, respectively. In the study by Chang et al. (2018), 12 Sprague–Dawley rats were randomized into two groups and received raw lutein and lutein nanoparticles containing 50 mg/kg BW of lutein, respectively. Blood was collected at 5, 15, 30, 45 min, and 1, 3, 5, 8, 12, and 24 h. The nanoparticle group exhibited 3.2- and 2.3-fold increase in plasma lutein C_{max} and AUC compared to the control group ($p < 0.05$). Although there was no significant difference in T_{max}, the $T_{1/2}$ was shortened by 6.3-fold in the lutein nanoparticle group ($p < 0.05$). Similarly, Wu et al. (2019) used antis-solvent precipitation to produce lutein ester nanoparticles. Lutein ester is the natural form of lutein and has the same spectral characteristics as lutein (Jiang, Chen, and Zhou 2005). Two experiments were conducted using Sprague–Dawley rats and Kunming strain mice, respectively. In experiment 1, 12 female Sprague–Dawley rats were given either a single oral dose of lutein ester nanoparticle or a free lutein ester dose (control) containing 50 mg/kg BW of lutein ester. Blood was collected at 5, 15, 30, and 45 min, and 1, 23, 46, 81, 224 h. Compared with the control group, the plasma lutein C_{max} and AUC were increased by 2.5 and 1.4 times ($p < 0.001$ and $p < 0.05$), respectively, in the treatment group. Plasma lutein T_{max} in the nanoparticle group was significantly shorter than that of the control group (0.25 h vs. 2 h). In experiment 2, Kunming strain mice were given either the lutein ester nanoparticle or free lutein ester and three mice per group were euthanized at each time point (0.25, 0.5, 1, 2, 6, and 24 h) after dosing. Heart, liver, spleen, lung, kidney, brain, and eyes were collected. In the nanoparticle group, the lutein C_{max} in the heart, liver, spleen, lung, kidney, brain, and eyes was increased by 2.1-, 1.6-, 2.0-, 1.4-, 2.3-, 1.5-, and 2.3-fold, respectively, and the AUC increased by 1.7-, 1.9-, 1.9-, 1.3-, 1.3-, 1.7-, and 2.3-fold, respectively, compared to the control group ($p < 0.05$). In addition, the T_{max} for lutein to reach heart, liver, spleen, lung, kidney, brain, and eyes was 1 to 5 h shorter in the treatment group than that in the control group.

Quality assessment

The rating results of each article were assessed using SYRCLE's RoB (Table S1 in Supplemental material). Overall, all studies had "neutral" scores, which were about 4 to 6 "yes" responses received. All the studies clearly stated the research questions and baseline characteristics of the rodent

models, reported the complete outcome data, and had no animal losses in the experiment. Four of the studies did not apply allocation sequence, i.e., the animal model was not randomly assigned to a control group or an experimental group (Arunkumar, Prashanth, and Baskaran 2013; Arunkumar et al. 2015; Ranganathan et al. 2019; Sato et al. 2018), increasing the risk of selection bias. None of the 11 studies mentioned whether the animals were randomly housed during the experiments, and whether the caregivers and investigators were blind of the intervention that each animal received. In addition, none of the studies addressed randomized selection and blindness during the outcome assessment, which increased the possibility of outcome bias.

Discussion

Overall results

The present systematic review aims to evaluate and compare the effectiveness of nanoscale delivery systems on improving lutein bioavailability in rodent models. All studies included in the review reported significant enhancement in the in vivo bioavailability of lutein rendered by various nanoscale delivery methods, as evidenced by increased plasma lutein concentrations and/or higher C_{max} and AUC, compared to their respective control group (Toragall, Jayapala, and Vallikannan 2020; Zhang et al. 2015; Wu et al. 2019; Sato et al. 2018; Chang et al. 2018; Murillo et al. 2016; Kamil et al. 2016; Arunkumar, Prashanth, and Baskaran 2013; Arunkumar et al. 2015; Ranganathan et al. 2019; Liu et al. 2017). The enhancement in lutein bioavailability might have been achieved through various mechanisms. The use of hydrophilic polymeric carriers and emulsions could improve the water dispersibility of lutein and thus lutein may become more accessible in the digestive tract. The polymers and emulsions could also protect the lutein from oxidation and prevent the conversion of carotenoids to epoxides in the gastric juice (Soukoulis and Bohn 2018). In addition, in most cases, lutein was reduced to tiny sizes in the order of hundreds of nm, which could facilitate their penetration through the apical membrane of the small intestine for absorption.

Seven of the 11 studies measured lutein concentration in organs, including the liver, eyes, small intestine, spleen, fat depot, lymph, heart, lung, kidneys, and brain (Arunkumar, Prashanth, and Baskaran 2013; Ranganathan et al. 2019; Kamil et al. 2016; Sato et al. 2018; Murillo et al. 2016; Wu et al. 2019; Toragall, Jayapala, and Vallikannan 2020). The accumulation of lutein in the liver was significantly increased by various nanoscale delivery systems (Arunkumar, Prashanth, and Baskaran 2013; Ranganathan et al. 2019; Kamil et al. 2016; Sato et al. 2018; Murillo et al. 2016; Wu et al. 2019; Toragall, Jayapala, and Vallikannan 2020). It reaffirmed that liver is the primarily organ that responds to increased blood lutein concentration upon oral administration (Itagaki et al. 2006). Nanoscale delivery systems were also shown to more effectively deliver lutein to the eyes (Wu et al. 2019; Kamil et al. 2016; Toragall, Jayapala, and Vallikannan 2020; Ranganathan, Hindupur, and Vallikannan 2016; Arunkumar, Prashanth, and Baskaran 2013). In addition, Wu et al. (2019) noticed significantly higher lutein concentrations in the spleen and the kidney in the treatment group, whereas the lutein concentrations in the heart and the brain were also higher but not significant. However, the two studies that examined lutein concentration in the adipose tissue did not show a significant increase by the nanoscale delivery, although both studies noted a significantly higher lutein concentration in the eyes (Kamil et al. 2016; Murillo et al. 2016). This result is similar to a previous study, where although the macular pigment was significantly increased in the high-lutein diet group, the lutein concentration in adipose tissue did not show a significant increase until 8 weeks (Johnson et al. 2000). It is plausible that lutein in adipose tissue takes a longer time to accumulate and may interact dynamically with the retina to instantly replenish lutein needed by the macula to maintain normal visual function (Johnson et al. 2000).

Comparison among nanoscale delivery systems

While the reported nanoscale delivery systems showed promising performance on improving the bioavailability of lutein, the effectiveness may vary with different techniques and carriers. Direct comparison among those delivery systems is beyond our capability, since there are too many variables in the studies, including particle size, encapsulation efficiency, lutein dosage, study duration, measured outcomes, etc. Specific comparisons are to be attempted by avoiding as many confounders as possible and used for drawing general conclusions.

The size of nanostructures created by the nanoscale delivery techniques varied with specific process, but all ranged from tens to hundreds of nanometers. The micelles in emulsions appeared to be larger than most of the nanoparticles, probably owing to their multilayer architecture and the thermodynamically unstable nature of nanoemulsions, i.e., tendency to coalesce. The larger size might not have affected the efficacy of the delivery system, as they resulted in comparable improvements in plasma response and liver storage of lutein. Emulsion-based systems should have been more appropriate for encapsulating lutein than polymer-based systems, because lutein is a highly lipophilic molecule and thus more compatible with oil than the relatively hydrophilic polymers. Surprisingly, the use of polymers exhibited promising results in stabilizing lutein as well as improving lutein bioavailability.

Among the six studies fabricating lutein-loaded polymer nanoparticles, two used chitosan in the formulation. Chitosan is a polysaccharide produced by the deacetylation of chitin and composed of acetylated and deacetylated β-(1→4)-linked D-glucosamine. Chitosan is hydrophilic although it is not water soluble or dispersible. Arunkumar, Prashanth, and Baskaran (2013) suggested that lutein interacts with chitosan on the molecular level through hydrogen bonding and van der Waals force, and is entrapped by crosslinked chitosan chains. It is likely, but this assembly should be thermodynamically unfavorable, because it is

formed at the expense of more favorable intramolecular interactions, including more hydrogen bonds formed between chitosan molecules and greater van der Waals interaction among the non-polar hydrocarbon chains of lutein. Lutein might exist as tiny lipophilic phases in chitosan nanoparticles immobilized by the cross-linker. In contrast, the construction of CHI-OA-ALG nanoparticle carrier should be more effective in encapsulating lutein (Toragall, Jayapala, and Vallikannan 2020). The use of oleic acid could increase the compatibility of lutein in the polymer nanoparticles. In fact, lutein in oleic acid formed the core of the nanoparticles in an architecture similar to emulsions except that it has a solid wall. Chitosan and alginate are oppositely charged polysaccharides and will form a self-assembled polyelectrolyte complex gel entrapping the lutein-oleic acid core without the use of a cross-linker. A decrease in particle size was also noted, i.e., 40–160 nm compared to 80–600 nm of chitosan nanoparticles (Arunkumar, Prashanth, and Baskaran 2013). Both studies showed a significantly increase in lutein bioavailability by the nanocarriers than that of the control group. Furthermore, the later CHI-OA-ALG nanoparticles also increased the intestinal uptake and the accumulation of lutein in the liver and eyes. Smaller nanoparticles were reported to exhibit higher permeability through the intestinal epithelial cells, therefore increasing the bioavailability of bioactive compounds in circulation (Ranganathan et al. 2019). A previous study showed that an emulsion system with micelle size of 30 nm had a faster drug release rate than that of an emulsion with size of 150 nm (Niamprem, Rujivipat, and Tiyaboonchai 2014).

Three studies used PLGA nanoparticles as lutein carrier and showed significantly higher lutein concentrations in plasma (Ranganathan et al. 2019; Arunkumar et al. 2015; Kamil et al. 2016), liver (Ranganathan et al. 2019; Kamil et al. 2016), and eyes (Ranganathan et al. 2019) than their respective control. PLGA is a popular option as a drug carrier via oral administration due to its safety and stability in the digestive tract (Murugeshu et al. 2011). PLGA is a linear copolymer of lactic and glycolic acids and its relative hydrophobicity increases with the monomer ratio of lactic acid to glycolic acid (Gentile et al. 2014). All three studies used PLGA of copolymer ratio of 50:50 and similar molecular weight. Two of the studies added a second polymer, i.e., PEG and PVA, possibly to facilitate the precipitation of PLGA nanoparticles, and 1 study added PL to increase the compatibility of lutein in the polymer nanoparticle. The PLGA nanoparticles fabricated with PL showed a higher EE ($90 \pm 2\%$ vs. $78 \pm 3\%$) and a smaller particle size (227 ± 12 nm vs. 140 ± 6 nm) as compared to the PLGA-PEG polymer nanoparticles. As an emulsifier, PL could have facilitated the loading and release of lutein and thus lead to higher increase in C_{max}. For instance, Kamil et al. (2016) reported a 52.1-fold higher C_{max} in PLGA nanoparticles group compared to the free lutein group. The increased bioavailability of lutein carried by the polymers may be primarily due to weak intermolecular forces between lutein and the polymer (Arunkumar et al. 2015). Additionally, PLGA and chitosan can stimulate the paracellular absorption of lutein by weakening the force of tight junction (Arunkumar, Prashanth, and Baskaran 2013; Thanou, Verhoef, and Junginger 2001).

A direct comparison between a polymer nanocarrier (i.e., PVP) and an emulsion nanocarrier was made by Sato et al. (2018). Compared with the same control group with the same dosage, the PVP nanoparticle group significantly increased the plasma lutein concentration to 100-, 80-, and 75- fold higher at 2, 5, 10 h, which were much higher than the 1.6-, 2.5-, and 2-fold increase in the emulsion group at the same time points, although p values were not reported. The difference might be explained by the smaller particle size of the polymer nanoparticles (181.8 ± 14 nm) than that of the micelles in emulsion (349.6 ± 109.9 nm) or the difference in their release behavior. Though the release mechanisms of the two nanoscale delivery systems are different, the diffusion and transit of lutein from the emulsion carriers to the absorption site might require a longer time and a higher energy expenditure. The PVP nanoparticle can be directly dispersed in bile and form micelles in the intestine lumen, while lutein emulsion requires emulsification by bile before forming micelles (Sato et al. 2018).

Without being carried by polymers or emulsions, lutein nanoparticles can form a colloidal dispersion in the lumen (Patravale, Date, and Kulkarni 2004) and thus increase the permeability of lutein through the epithelial cells of the small intestine (Arunkumar et al. 2015). Lutein nanoparticles all enhanced the C_{max} and AUC of plasma lutein compared with their respective controls, which were lutein powders of larger size. It is also worth noting that in three of the four studies that used lutein nanoparticles (Zhang et al. 2015; Wu et al. 2019; Liu et al. 2017), the plasma T_{max} of lutein nanoparticle group was between 1.3 h and 2.25 h earlier than that of the control groups. In Wu et al. (2019), T_{max} in different organs was also 1 to 5 h shorter in the treatment group. This may be explained by the faster dissolution of nanosized lutein in the intestine (Chang et al. 2018), which leads to an accelerated absorption and distribution. The changes of T_{max} were not reported with the other two nanoscale delivery systems. This result is consistent with a previous study showing that T_{max} of an isradipine nanosuspension formulation was 1.5 h earlier than the plain drug microsuspension (Shelar, Pawar, and Vavia 2013). Soukoulis and Bohn (2018) stated that lutein nanoparticles may alter the release rate of lutein in small intestines, and minimize the influence of food matrix (e.g., dietary fiber) on the absorption of lutein. The physiological impact of T_{max} alteration on lutein functionality warrants further investigation.

Nanoscale delivery systems on the functionality of lutein

Lutein plays a vital role in maintaining the visual function, cognitive function, and antioxidant capacity. Studies have shown that the increase in serum lutein concentration is positively correlated with the enhanced macular pigment optical density (MOPD) in the retina (Bone et al. 2003;

Connolly et al. 2010; Johnson et al. 2000). Given that nanoscale delivery techniques can effectively increase the serum lutein concentration, they could be promising for enhancing lutein accumulation in the eyes for preventing or ameliorating AMD, the leading cause of blindness in the elderly population. In fact, of the five studies that measured lutein concentration in the eyes, four of them showed a significantly increased concentration within 0.25 h to 24 h (Wu et al. 2019; Toragall, Jayapala, and Vallikannan 2020; Ranganathan et al. 2019; Arunkumar, Prashanth, and Baskaran 2013). Three studies included in the present review investigated the functionalities of lutein in cell lines. Arunkumar et al. (2015) observed a 2-fold greater inhibitory effect of lutein-loaded PLGA nanoparticles on the viability of Hep G2 cells, which belong to a human liver cancer cell line. The incorporation of PL in the nanoparticles showed a more effective anti-tumor effect (Ranganathan et al. 2019). Wu et al. (2019) investigated the antioxidant capacity of lutein nanoparticles, showing a significantly 3.9-fold higher free-radical scavenging capacity by the lutein nanoparticles than bulk lutein. In one of the eleven studies included in this review, the effect of lutein nanoemulsion incorporated into the daily diet on the metabolic conditions of guinea pigs with hepatic steatosis was evaluated (Murillo et al. 2016). Results revealed a 55% reduction in oxidative stress in the lutein emulsion group compared to the control group after the 6-week intervention ($p < 0.05$). The hepatic steatosis score, hepatic triglycerides, and interleukin-6 were also lowered in the treatment group ($p < 0.05$), while the plasma triglycerides, LDL-cholesterol, and HDL-cholesterol were increased ($p < 0.05$). The negative effect of the emulsion on plasma lipids profile may be due to the use of a large amount of median-chain triglycerides in the emulsion.

Nanoscale delivery systems have been shown to enhance the functionalities of lutein in other animal studies. A previous study compared the effects of daily dosing of pure lutein (100 mg/kg BW), lutein-loaded nanoparticles prepared by nanoprecipitation technique (25 and 50 mg/kg BW), and metformin (100 mg/kg BW) on blood glucose using alloxan-induced diabetic rats (Mishra, Malaviya, and Mukerjee 2015). The particle size of nanoparticles was 152.38 ± 6.82 nm. The results indicated significantly 1.8-, 2.1-, 2.2-, and 2.4-fold decreased blood glucose levels ($p < 0.001$) in pure lutein, 25 mg/kg nanoparticle, 50 mg/kg nanoparticle, and metformin groups, respectively (Mishra, Malaviya, and Mukerjee 2015). In another animal study, the effect of nanoencapsulated lutein on the declarative memory of mice was investigated (do Prado Silva et al. 2017). After 14 days of daily dosing, the results showed that feeding the mice 1.5 and 10 mg/kg BW of lutein carried by PVP had the same effect on their recognition index as feeding the mice 100 mg/kg of free lutein. The findings suggest that certain nanoencapsulation techniques can significantly improve the functionality of lutein. As such, the application of nanoscale delivery systems on lutein has enormous potential clinical applications for preventing certain eye diseases and cognitive dysfunctions. These two studies mentioned above were not included in the present systematic review, because lutein bioavailability was not directly evaluated.

Concerns with nanoscale delivery systems

Food safety is the most important benchmark for the selection of nanoscale delivery systems. The natural biopolymers, i.e., chitosan and alginate, and synthetic polymers, including PLGA, PEG, PVA, and PVP, are all biodegradable and biocompatible. As a safety concern in nanotechnology, by reducing the polymers to nano size, they gain much greater ability to penetrate into the intestinal epithelial cells and accumulate in various organs than their bulk counterparts, which simply pass through the gastrointestinal tract. Semete et al. (2010) found that no in vitro cytotoxicity and in vivo morphological alteration was caused by PLGA nanoparticles, even though they were detectable in various organs with liver containing the most abundant PLGA residues. Ranganathan, Hindupur, and Vallikannan (2016) tested the acute and subacute toxicity of lutein-loaded PLGA-based nanoparticles in mice and did not observe any acute or subacute toxicity or adverse reactions as assessed by hematological parameters. The polymer nanoparticles may not cause immediate harm to the organs, nevertheless, it is wise to increase the hydrophilicity of the nanoparticle surface to increase the circulation time of polymer nanoparticles in the blood. Although polymer nanoparticles have been widely used for nanoencapsulation and no side effects were reported (Watkins et al. 2015; Tan et al. 2011; Sanna et al. 2012; Casettari and Illum 2014), they still need to undergo formal regulatory evaluation and approval (Bergin and Witzmann 2013).

The construction of food emulsions is based on food grade ingredients, including oils and emulsifiers, and therefore, safety is usually not a concern. However, the use of oil or fat in emulsions may lead to health concerns, since over consumption of fats could increase the risk of chronic diseases, especially cardiovascular diseases. As mentioned, Murillo et al. (2016) used medium chain triglyceride as the lutein carrier and found elevated plasma LDL and HDL-cholesterol concentrations, and accumulated cholesterol in adipose tissue in Guinea pigs with cholesterol-induced liver damage. Such elevation could increase the risks of hyperlipidemia and inflammation. On the contrary, in a crossover study supplementing lutein for 1 week, healthy adults in the lutein nanoemulsion group did not show any difference in total cholesterol and LDL-cholesterol as compared to the control group that was not provided with the extra fat (Vishwanathan, Wilson, and Nicolosi 2009). However, the length of intervention might be too short to develop any differential effect. Given the health concerns with fat use in emulsions, especially for people with hyperlipidemia, in fact, research efforts have been devoted to develop high quality food emulsions with reduced fat contents (Chung et al. 2016).

In addition, due to the thermodynamic instability of nanoemulsions, they may undergo coalescence, flocculation, stratification, or phase separation during long-term storage.

For instance, in the lutein-loaded nanoemulsion developed by Sato et al. (2018), the particle size increased slightly during the first 3 days, but experienced phase separation after 6 days. A number of factors could induce the instability and deterioration of emulsions and nanoemulsions, such as temperature fluctuation, the presence of light, and the addition of a solvent (e.g., solvents that are miscible with oil or water phase) (Jaiswal, Dudhe, and Sharma 2015). The instability of nanoemulsions affects its applicability and may restrict its application in clinical settings.

In recent years, with the wide application of nanotechnology in the fields of medicine and nutrition, the research and development investment in this field has been continuously focused (Patra et al. 2018). However, the practical application of nanotechnology still faces many uncertainties, such as the safety and toxicity of nanomaterials. In addition, the clinical application of nanotechnology lacks adequate supervision. Although the FDA has approved some nanomaterials, there are no specific regulatory guidelines for the development and characterization of nanomaterials (Wacker, Proykova, and Santos 2016). In addition, more research is needed on the structure and function of various nanomaterials and their interactions with biological systems (Patra et al. 2018). Depending on the formula, single or composite nanomaterials can form aggregates in organisms that can have toxic effects (Wacker, Proykova, and Santos 2016). It is also worth noting that the health status and age of the people who consume these nanoparticles may vary widely. Given that a high dosage of pure lutein up to 1000 mg/kg/day is not toxic for rodent models (Ranganathan, Hindupur, and Vallikannan 2016; Nidhi and Baskaran 2013), research should pay more attention to the potential side effects of carriers used in the nanoscale delivery systems. More animal and human microdosing studies (Henderson and Pan 2010) are needed to promote the development of nanotechnology in the clinical environment.

Strengths and limitations

To the authors' knowledge, this is the first systematic review that evaluates the effectiveness of various nanoscale delivery systems on improving the in vivo bioavailability of lutein. The use of nanoscale delivery systems to enhance the stability and bioaccessibility of carotenoids, including β-carotene, crocin, fucoxanthin, lycopene, lutein, and astaxanthin, has been examined in several previous narrative reviews (Soukoulis and Bohn 2018; Rehman et al. 2020). This review focuses on the in vivo bioavailability of lutein by analyzing plasma lutein concentrations, pharmacokinetic parameters, and lutein accumulation in organs. The comparison among various nanoscale delivery techniques provides valuable information and guidance for developing practical applications using these techniques.

This systematic review also involves some limitations. Only studies using rodent models were included. Rodent models, including mice, rats, and gerbils, are commonly used to study carotenoid absorption and metabolism (Lee et al. 1999; Kamil et al. 2016). Previous studies have shown that the absorption rate and tissue distribution of β-carotene in Mongolian gerbils are similar to those in humans (House, Apgar, and Smith 1997; Lee et al. 1998). However, the digestion process in human is affected by the food matrix and the lutein dosages given to rodents model are usually higher than that could be administered to human (Xue et al. 2015). Previous studies also suggested that the physiological levels of retinal lutein deposition in rodent models may be different from those in humans (Li et al. 2017). As such, animal experiments cannot fully predict the bioavailability and accumulation of lutein in human. To date, only one study was conducted to evaluate the effect of nano technique on lutein bioavailability in human, which showed that lutein nanoemulsions had significantly greater bioavailability than lutein supplement pill (Vishwanathan, Wilson, and Nicolosi 2009). The application of nanoscale delivery systems in more clinical trials is needed. Moreover, most of the reviewed studies relied on the plasma lutein concentration to determine its bioavailability. Sato et al. (2018) showed a significantly higher lutein concentration in lymph stream than that in plasma in both lutein powder and treatment groups. After oral administration, lutein in the small intestine can be passively diffused or transported via two cholesterol transporters, i.e., Niemann-Pick C1 like-1 (NPC1L1) and scavenger receptor class B type 1 (SR-B1), to lymph instead of blood stream (Sato et al. 2012). As such, measuring the lymph lutein concentration is suggested to be included in future studies.

Conclusion

In summary, articles included in this systematic review unanimously revealed the effectiveness of nanoscale delivery systems on improving lutein bioavailability, as evidenced by increased plasma lutein concentrations. In addition, the majority of the studies observed enhanced accumulation of lutein in the liver and the eyes. Different nanoscale delivery systems may have their advantages and drawbacks as discussed, but the current literature supported that polymer nanoparticles, emulsions (including nanoemulsion), and lutein nanoparticles are all promising techniques for enhancing lutein bioavailability and may be applicable for other lipophilic bioactive compounds.

Further studies are suggested to explore the effect of increased lutein bioavailability on its functionality. Several rodent models, such as diabetic rats, high-fat diet induced obese rodent model, AMD mice, and β-carotene oxygenases 1 (BCO1), and β-carotene oxygenases 1 (BCO2) knockout mice (Li et al. 2017) are appropriate animal models for such investigation. BCO1 and BCO2 are the only two enzymes involved in the cleavage of carotenoids in human. A previous study showed that mice deficient in BCO1 and BCO2 gene can cause lutein accumulation in retina and other tissues, which makes this mice model appropriate for determining the accumulation and macular functionality of lutein (Li et al. 2017). Given that all the studies included in this review were conducted in relatively short-term period ranging from 8 h to 6 weeks, longer study duration is

recommended to determine the long-term impact of nanoscale delivery systems on the bioavailability and functionality of lutein, as well as the potential toxicity and side effects of the delivery systems. Once the safe and optimal doses are established, clinical trials are warranted to evaluate the effects of nanoscale delivery systems on improving lutein bioavailability and functionality to benefit human health, especially visual and cognitive functions.

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Declarations of interest

No potential competing interest was reported by the authors.

Abbreviations

AMD	age-related macular degeneration
AUC	area under the concentration-time curve
BW	body weight
EE	encapsulation efficiency
PL	phospholipid
LDL	low-density lipoprotein
HDL	high-density lipoprotein
SR-B1	scavenger receptor class B type 1
BCO1	β-carotene oxygenases 1
BCO2	β-carotene oxygenases 2

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