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ORIGINAL CONTRIBUTION

Olive oil improves the intestinal absorption and bioavailability of lutein in lutein-deficient mice

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Abstract
Purpose To investigate the influence of olive (OO), groundnut (GNO), soybean (SBO), sunflower (SFO), rice bran (RBO), corn (CO), palm (PO) oil or mixed micelle (control) on absorption kinetics and bioavailability of lutein in lutein-deficient mice. Additional aim was to correlate the activity of intestinal triacylglycerol lipase with intestinal and plasma lutein levels.
Methods After induction of lutein deficiency, mice ($n = 165$) were divided into eight groups (OO, SFO, GNO, RBO, PO, CO, SBO and control; $n = 20$/group) and the remaining ($n = 5$) were used as baseline (0 h). Groups were further divided into four subgroups ($n = 5$/subgroup) and were intubated with lutein (200 μM) dispersed in different vegetable oils. Plasma and tissue (intestine, liver and eyes), lutein, triglycerides, intestinal triacylglycerol lipases and fatty acid profile of plasma and tissues were measured at different time intervals.
Results The percentage area under the curve value for plasma lutein in OO and GNO was higher by 41.8 and 5.1 %, while it was lower in other groups (18.2–53.3 %), when compared to control. Similarly, the percentage area under the curve for eye lutein in OO and GNO groups was higher by 35.2 and 4.8 %, whereas in other groups it was lower (5.4–69 %) than in control. Results show that olive oil facilitates the lutein absorption more compared to other vegetable oils, which may be due to the difference in fatty acid composition and higher activity of intestinal triacylglycerol lipase.
Conclusions Dietary olive oil rich in oleic acid improves the bioavailability and accumulation of lutein in lutein-deficient mice by modifying the intestinal triacylglycerol lipase activity.

Keywords Absorption kinetics · Bioavailability · Lutein deficiency · Triacylglycerol lipase · Vegetable oils

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Introduction

Lutein and its isomer zeaxanthin are the only dietary carotenoids which specifically accumulate in the macula of retina and lens and are known to protect the eyes from UV-light-mediated pro-oxidants [1]. Dietary intake of lutein and zeaxanthin and their concentration in the serum and macula are inversely correlated with age-related macular degeneration [2]. They render antioxidant properties that have been coupled with cell protective mechanism [3], regulation of cell growth, differentiation and apoptosis [4]. Bioavailability of lutein is poorer than that of pro-vitamin A carotenoids owing to its lipophilic nature. Dietary lipids, in general, are reported to facilitate the intestinal absorption of carotenoids [5]. Hence, it is essential to find out a suitable lipid vehicle to achieve an improved bioavailability of lutein, in particular, under a condition like lutein deficiency.

The process of carotenoid absorption is partly identical to that of fats [6], which includes release of carotenoids from the food matrix and conversion into a potentially absorbable mixed micelle and further absorption through enterocytes [7]. Apart from bile salts, biliary phospholipids and cholesterol, mixed micelles also contain free fatty acids and monoglycerides from hydrolysis of triglycerides by lipases [8]. Therefore, ingestion of lutein along with a

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suitable fat is crucial for improved absorption [5]. Other concern that affects the intestinal accessibility of lutein is the transfer of it from food matrix where they cannot be absorbed to the micelles. This is probably a very important step in lutein absorption because apparently the efficiency of lutein transfer directly affects its plasma/serum response. Fat-free or reduced-fat salad dressings limit the absorption of α-carotene, β-carotene and lycopene in humans [9]. Lycopene and astaxanthin showed higher absorption with olive oil than with corn oil emulsion in duodenum of rats [10]. Although we have demonstrated that specific fatty acid such as oleic acid micelles significantly improved the plasma response of lutein in lutein-sufficient rats [11], none of these studies shows how specific vegetable fat influences the intestinal absorption of lutein in lutein-deficient condition and role of intestinal triacylglycerol lipase in it. The present study hypothesized that vegetable oils with varying fatty acid composition undergo intestinal processing differently particularly micellarization of lutein and stimulation of intestinal triacylglycerol lipase activity and thereby augment the bioavailability of lutein. To improve our understanding of vegetable oils on lutein absorption via modulation of lipase activity, the objective of this study was to investigate the effect of orally administered lutein dispersed in vegetable oils on absorption kinetics (plasma response) and tissue distribution in lutein-deficient mice. This study also investigated the usefulness of plasma triacylglycerol and fatty acid profile as a measure of carotenoid absorption from vegetable oils. This study provides a new insight into the selection of suitable lipid vesicle for improved lutein absorption in its deficient condition.

Materials and methods

Standard lutein (99 %), mono-oleo-glycerol, sodium taurocholate, cholesterol and oleic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile and dichloromethane were of high-performance liquid chromatography (HPLC) grade. Acetone, hexane, ethanol, ethyl acetate, ammonium acetate, butylated hydroxyl toluene (BHT), silica gel (60–120 mesh size) and sodium sulfate of analytical reagent grade were obtained from Sisco Research Laboratories (Mumbai, India). Fresh marigold flowers, refined olive (OO), groundnut (GNO), sunflower (SFO), soybean (SBO), rice bran (RBO), corn (CO) and palm (PO) oils were obtained from a local supermarket.

Extraction and purification of lutein

Marigold flower (*Calendula officinalis*) petals were ground in a blender with sodium sulfate (5 g) and 0.1 % BHT in ethanol, and total carotenoids were extracted [12] with ice-cold acetone. The pooled extract (250 ml) was dried over anhydrous sodium sulfate (20 g) and saponified with methanolic KOH (30 %) at room temperature (28 ± 2 °C) for 3 h in dark. After saponification, carotenoids were extracted with 50 ml of hexane and washed with deionized water, and the hexane extract was evaporated to dryness using a rotary evaporator (Buchi, Switzerland), re-dissolved in hexane and purified on an activated silica column (60–120 mesh) eluting with hexane for β-carotene and methanol/dichloromethane (1:1, v/v) for lutein and zeaxanthin fraction. The purity of lutein was ascertained by HPLC (λ_{max} 445.6; purity 96 ± 2 %) and the lutein used for intubation studies [13].

Animals

Animal experiment was conducted after due clearance from the Institutional Animal Ethical Committee. Weanling male albino mice [OUTB/Swiss Albino IND-CFT (2c)], each weighing 22 ± 2 g, were housed in cages in the institute's animal house facility at room temperature (28 ± 2 °C). A 12-h light–dark cycle was maintained, and the mice had free access to feed and water ad libitum.

Induction of lutein deficiency and gavage of lutein

Lutein deficiency was induced in mice ($n = 165$) by feeding a semisynthetic diet [14] devoid of lutein. The composition of diet (g/kg) was as follows: casein (200), methionine (3), cellulose (50), sucrose (600), mineral mix (35), vitamin mix (10), choline bicarbonate (2) and peanut oil (100). On feeding lutein-deficient diet (8 weeks), its deficiency (3.57 ± 0.88 pmol/ml) was confirmed by measuring its plasma level (henceforth referred as lutein-deficient mice) and baseline data on various biochemical parameters were analyzed. Deficient mice ($n = 165$) were divided into eight groups ($n = 20$/group), and the remaining ($n = 5$) were used as baseline (0 h), which was common for all the experimental groups. Lutein-deficient mice were deprived of food for 12 h and were orally administered a pharmacological dose of lutein (200 μM) dispersed in 200 μl of vegetable oil, either OO, GNO, SBO, SFO, CO, RBO or PO separately. In brief, lutein dissolved in solvent (methanol/dichloromethane, 1:1, v/v) was evaporated to dryness under nitrogen, dispersed in different vegetable oils and sonicated for 5 min. The group received lutein (200 μM) dispersed in mixed micelles with no added vegetable oil was considered as control. Each group was further divided into four subgroups ($n = 5$/subgroup) to study time-course plasma kinetics and its tissue levels after 2, 4, 6 and 8 h of intubation. These vegetable oils were selected because of their distinct

differences in fatty acid composition. Mixed micelles in phosphate-buffered saline (200 µl) contained mono-oleoglycerol (2.5 mM), oleic acid (7.5 mM), sodium taurocholate (12 mM) and cholesterol (0.5 mM), with lutein (200 µM) [15]. Lutein concentration in control and vegetable oils was confirmed by HPLC before being fed to mice [13]. At the completion of each time interval, mice were anesthetized with diethyl ether and killed by exsanguination. Blood was collected in heparinized tubes, and plasma was separated by centrifugation at 1,000×*g* for 15 min at 4 °C. Liver, intestine and eyes were excised and washed with ice-cold isotonic saline and homogenized (Potter–Elvehjem homogenizer, Remi Instruments, Ltd., Mumbai, India) separately with nine parts of ice-cold isotonic saline.

Extraction of lutein from plasma and tissues

Lutein and zeaxanthin were extracted from plasma, liver, intestine and eye homogenates as described by Lakshminarayana et al. [13] with slight modification. In brief, 3 ml of dichloromethane/methanol (2:1, v/v) containing 0.1 % BHT in ethanol was added to the plasma and vortexed for 1 min, followed by addition of hexane (1.5 ml) to the mixture, mixed well and centrifuged at 1,000×*g* for 15 min, and the resulting upper hexane/dichloromethane layer was collected. Extraction was repeated two more times for the lower phase with dichloromethane (1 ml) and hexane (1.5 ml); pooled extracts were evaporated to dryness and analyzed by HPLC. In case of liver homogenate, samples were saponified before lutein extraction with 2 ml of 10 M KOH at 60 °C for 45 min. For intestine, upper portion (jejunum) was cut open and the mucosal layer was gently scraped off using cover glass and homogenized separately with ice-cold isotonic saline. Eye samples ($n = 5$) were pooled and homogenized separately with ice-cold saline. The liver, intestine and eye homogenates were used for lutein extraction according to the procedure described for plasma. Handling, homogenization and extraction procedures were carried out at 4 °C under dim yellow light to minimize photo-isomerization.

HPLC analysis of lutein

Lutein extracts of plasma, liver, intestine and eyes were analyzed by a HPLC system (LC-10A; Shimadzu, Kyoto, Japan), equipped with photodiode array detector (SPD-M20A, Shimadzu) [13]. They were separated on a Princeton SPHER C-30 (ODS) column (250 mm × 4.6 mm; 5 µm) isocratically eluting with acetonitrile/methanol/dichloromethane (60:20:20, v/v/v) containing 0.1 % ammonium acetate (mobile phase) at 1 ml/min flow rate, monitored at 445 nm (Shimadzu Class-VP version 6.14 SP1 software). The peak identity of lutein was confirmed by its characteristic spectrum and λ_{max} and quantified by external calibration curve of authentic lutein standard. Results are presented as lutein + zeaxanthin since zeaxanthin is an isomer of lutein (hereafter referred as lutein).

Absorption kinetics of lutein

Absorption kinetics (plasma response) parameters of lutein were calculated as per non-compartmental analysis section of Winnonlin version 3.1 (Pharsight Corporation, CA, USA). The area under the concentration–time curve (AUC) was calculated using the trapezoidal rule with linear interpolation up to the last measured concentration (C_{last}). The mean residence time (MRT_{last}) was computed as $MRT_{last} = AUMC/AUC$, where AUC is area under the curve and AUMC is the area under the moment time. The observed peak concentration (C_{max}) and the time to peak concentration (T_{max}) were also calculated.

Assay for intestinal triacylglycerol lipase

Intestinal triacylglycerol lipase activity was assayed by turbidimetric method using kit (Randox Laboratories Ltd, Antrim, UK), where decrease in turbidity was measured at 340 nm. In brief, intestinal mucosal homogenate was centrifuged at 10,000×*g* for 10 min at 4 °C to remove nuclei and cell debris. The supernatant was used for lipase assays. Protein in the supernatant was measured as per the methods suggested by Lowry et al. [16].

Analysis of triacylglycerides and fatty acids

Plasma lipids were extracted by the method of Folch et al. [17]. Aliquots of lipid extract (0.2 ml) were used to determine triacylglycerides by spectrophotometer (model 1800, Shimadzu) as per the methods suggested by Fletcher [18]. Fatty acid profile of plasma and tissues was analyzed by boron trifluoride in methanol to obtain fatty acid methyl ester (FAME) as per the methods suggested by Morrison and Smith [19].

Statistical analysis

The experimental data obtained for different parameters were subjected to analysis of variance (ANOVA). In case of significant difference, mean separation was accomplished by Tukey's highest significant difference test using ORIGIN 6.0 professional software.

Results

Absorption kinetics of lutein

The time-dependent changes in the postprandial plasma lutein response after a pharmacological dose of lutein dispersed in micelles (control) and vegetable oils (GNO, OO, SFO, SBO, RBO, CO and PO) and HPLC chromatograms at T_{max} are shown in Table 1 and Fig. 1, respectively. The mean ± SD of time-course (0–8 h) plasma lutein concentration is shown in Fig. 2a, b. After gavage, lutein levels reached maximum at 2 h in control, OO and SBO, and at 4 h in SFO, GNO, RBO, CO and PO groups (Fig. 2a, b; Table 1). The mean peak concentration (C_{max}) and AUC values ranged between 29.1 and 86.2 pmol/ml and between 130.8 and 397.3 pmol·h/ml, respectively. The MRT_{last} was lowest for OO group (3.7 h) than for other groups. The mean lutein level at C_{max} was significantly higher ($p < 0.05$) in OO, SFO, SBO, CO and PO groups compared to control. The mean AUC values for plasma lutein (pmol·h/ml) were found to be in the order of OO (397.3) > GNO (296.6) > control (279.1) > SBO (249.9) > RBO (229.1) > SFO (184.3) > CO (147.0) > PO (130.8). The percentage area under the curve value for plasma lutein in OO and GNO groups was higher by 41.8 and 5.1 %, while it was lower by 34.2, 10.8, 18.2, 47.5 and 53.3 % in SFO, SBO, RBO, CO and PO groups, respectively, compared to control.

Tissue accumulation of lutein

The lutein levels in intestine reached maximum at 6 h after administration of lutein (Fig. 2c, d). Further, the AUC value (Table 2) of intestinal lutein was 6.2 % higher in OO than in control group, whereas it was lower by 26.6 % (SFO), 15.9 % (GNO), 68.5 % (SBO), 47.5 % (RBO), 30.2 % (CO) and 88.2 % (PO). Deprivation of lutein did not affect the liver weight and showed no morphological signs during the experimental run. In the liver, lutein level (pmol/g) reached maximum at 4 h in the OO, SBO and RBO groups and at 6 h in control, SFO, GNO, RBO and PO groups (Fig. 3a, b). The liver AUC value of OO was 31.2 % higher than that of the control, while it was lower in other groups (17.3–83.5 %) (Table 2). Lutein levels in the eyes after a dose of lutein (pmol/g) reached maximum at 8 h in all the groups and were significantly different ($p < 0.05$) (Fig. 3c, d). The mean AUC values (Table 2) of lutein in eye (pmol h/g) were in the order of OO (240.4) > GNO (186.4) > control (177.8) > RBO (168.2) > SFO (162.9) > CO (133.3) > SBO (114.6) > PO (55.04), and it was higher in OO (35.2 %) and GNO (4.8 %) groups than in the control group, whereas it was lower in SFO (8.3 %), RBO (5.4 %), SBO (35.5 %), CO (25 %) and PO (69 %) than in the control.

Intestinal triacylglycerol lipase response

Triacylglycerol lipase is an exocrine enzyme essential for the digestion and hydrolysis of glycerol esters of fatty acids, thereby assists the carotenoid uptake. Intestinal triacylglycerol lipase (U/mg protein) activity reached maximum at 2 h in the OO (2.8) group, at 4 h in control (1.2), SFO (1.8), GNO (2.1) and RBO (2.1) groups and at 6 h in SBO (2.1), CO (1.6) and PO (1.0) groups (Fig. 4a, b). Percentage difference at maximum activity of triacylglycerol lipase was higher in OO (132 %), RBO (76 %), SBO (75 %), GNO (72 %), SFO (52 %) and CO (37 %) groups

Table 1 Absorption kinetic parameters (plasma response) of lutein after a single dose of lutein dispersed in vegetable oils in deficient mice

Groups[†]	Parameters					
	T_{max} (h)	C_{max} (pmol/ml)*	C_{last} (pmol/ml)*	AUC (pmol h/ml)	AUMC (pmol h h/ml)	MRT_{last} (h)
Control	2	52.6 ± 13.6[a]	10.4 ± 1.2[a]	279.1 ± 12.6[a]	1092.7 ± 19.6[a]	3.9 ± 0.5
OO	2	86.2 ± 8.7[b]	14.9 ± 5.5[a]	397.3 ± 23.3[b]	1480.1 ± 11.5[b]	3.7 ± 0.2
SFO	4	41.3 ± 8.1[c]	12.6 ± 2.2[a]	184.3 ± 9.7[c]	797.7 ± 11.3[c]	4.3 ± 1.0
GNO	4	59.9 ± 11.6[a]	19.5 ± 11.1[b]	296.6 ± 34.6[a]	1224.1 ± 42.4[b]	4.1 ± 0.6
SBO	2	40.1 ± 5.1[c]	22.9 ± 4.6[b]	249.9 ± 8.5[d]	1075.2 ± 15.7[a]	4.3 ± 0.5
RBO	4	57.7 ± 16.2[a]	10.3 ± 1.1[a]	229.1 ± 17.8[d]	919.8 ± 26.4[a]	4.0 ± 0.2
CO	4	42.9 ± 13.6[c]	7.8 ± 2.2[a]	147.0 ± 21.3[e]	681.4 ± 27.9[d]	4.6 ± 1.1
PO	4	29.1 ± 8.6[d]	13.4 ± 4.2[a]	130.8 ± 9.4[e]	638.5 ± 10.9[d]	4.8 ± 0.9

Values are mean ± SD ($n = 5$). Values not sharing a similar superscript within the column among groups are significantly different ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's test. *C_{max}* maximum concentration, *T_{max}* time at maximum concentration, *C_{last}* concentration of last time point, *AUC* area under curve, *AUMC* area under moment time, *MRT_{last}* mean residence time. * Since lutein and zeaxanthin did not resolve clearly in plasma, results are presented as lutein + zeaxanthin. [†] *OO* olive, *GNO* groundnut, *SFO* sunflower, *SBO* soybean, *RBO* rice bran, *CO* corn and *PO* palm oils

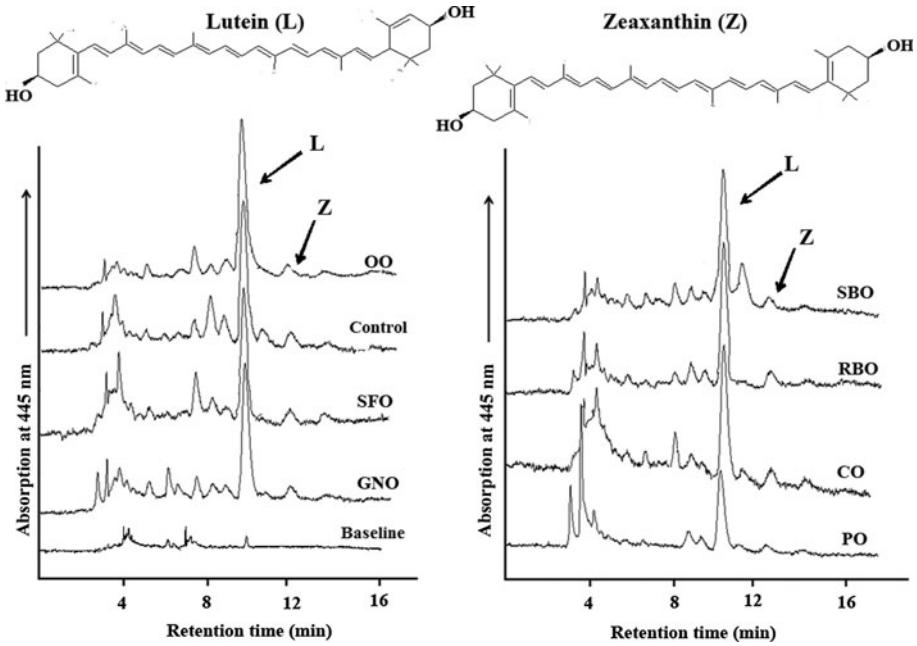

Fig. 1 High-performance liquid chromatographic profile of lutein and zeaxanthin in plasma at baseline and maximum absorption (2 h in control, OO and SBO; 4 h in SFO, GNO, RBO, CO and PO groups) of mice after a single dose of lutein (200 μM) solubilized in micelle (*control*) and various vegetable oils. Refer to Table 1 for abbreviations

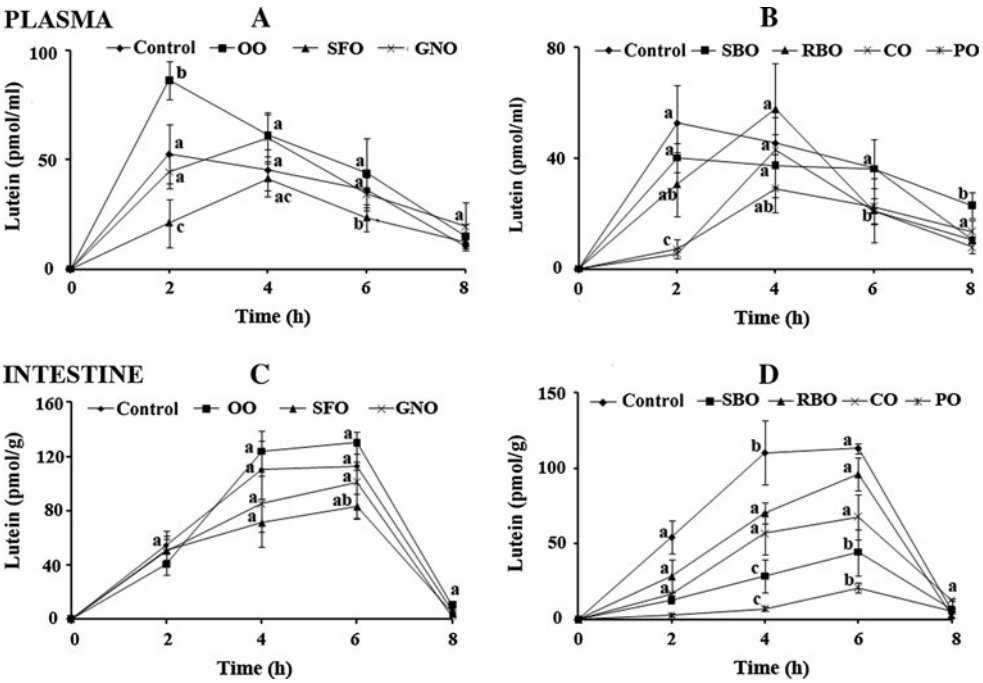

Fig. 2 Postprandial responses of lutein + zeaxanthin in plasma (**a**, **b**) and intestine (**c**, **d**) of mice after a single dose of lutein (200 μM) solubilized in micelle (*control*) and various vegetable oils. Values are mean ± SD ($n = 5$). Values at each time point not sharing a common letter are significantly different ($p < 0.05$) between groups as determined by one-way analysis of variance and Tukey's test. Lutein concentrations are baseline corrected. Refer to Table 1 for abbreviations

when compared to control, whereas it was lower in PO by 17.9 %.

Plasma and tissues fatty acid profile

Plasma fatty acids showed higher oleic acid level in OO (36.4 %) and GNO (27.2 %) groups, while linoleic acid was higher in SFO (39.6 %), SBO (30.6 %) and CO (31.4 %) groups. PO group showed almost equal proportion of 16:0 and 18:1 as 29.3 and 23.4 %, respectively. The level of oleic acid in plasma rose to 18.1 % (control), 36.4 % (OO), 20.7 % (SFO), 27.2 % (GNO), 20.6 % (RBO), 22.2 % (CO) and 23.4 % (PO) from baseline value of 16.7 % (Table 3). Similarly, linoleic acid level altered from 15.1 % (baseline group) to 15.5 % (control), 14.3 % (OO), 39.6 % (SFO), 20.4 % (GNO), 30.6 % (SBO), 25.8 % (RBO), 31.4 % (CO) and 19.9 % (PO). As in the case of plasma, the level of oleic acid was significantly higher ($p < 0.05$) in OO (69.1 %) and GNO (48.1 %), whereas it was lower ($p < 0.05$) in control (17.6 %), SFO

Table 2 AUC values for tissue lutein level after a single dose of lutein dispersed in vegetable oils

Groups[†]	Intestine (pmol h/g)*	Liver (pmol h/g)*	Eye (pmol h/g)*[#]
Control	563.9 ± 24.3[a]	630.9 ± 32.2[a]	177.8[a]
OO	598.8 ± 12.7[a]	827.4 ± 44.5[b]	240.4[b]
SFO	413.8 ± 18.6[b]	187.5 ± 17.3[c]	162.9[a]
GNO	473.9 ± 9.4[b]	520.8 ± 21.2[a]	186.4[a]
SBO	177.3 ± 11.4[c]	241.6 ± 41.5[c]	114.6[c]
RBO	295.8 ± 26.1[d]	395.2 ± 29.6[d]	168.2[a]
CO	393.1 ± 8.5[b]	202.4 ± 18.6[c]	133.3[c]
PO	66.6 ± 6.9[e]	103.5 ± 11.5[e]	55.1[d]

Values are mean ± SD ($n = 5$). Values not sharing a similar superscript within the column among group are significantly different ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's test. * Since lutein and zeaxanthin did not resolve clearly in tissues, results are presented as lutein + zeaxanthin. # Eyes samples were pooled ($n = 5$) for lutein extraction

† Refer to Table 1 for abbreviations

(27.1 %), SBO (28.3 %), RBO (24.8 %), CO (28.9 %) and PO (21.2 %) groups, when compared with baseline value (45.7 %). Likewise, linoleic acid increased significantly ($p < 0.05$) in SFO (48.3 %) and CO (31.0 %), while no difference was observed in other groups, when compared with baseline values (19.2 %) (Table 4). Oleic acid level in the liver (Table 5) increased ($p < 0.05$) in OO (44.2 %) and GNO (40.1 %) groups, whereas it decreased significantly ($p < 0.05$) in SBO (27.4 %) group, though other experimental groups were not significantly different, when compared with baseline values (35.5 %). Linoleic acid level, on the other hand, increased ($p < 0.05$) in SFO (30.9 %) and SBO (27.4 %) groups, when compared with baseline value (22.3 %).

Plasma triacylglyceride response

Carotenoids are transported in association with triacylglyceride-rich chylomicrons and lipoproteins [20]. Hence, the plasma TG level was measured after gavage of lutein with various vegetable oils. As in the case of plasma lutein, the TG levels (mg/ml) reached maximum at 2 h in the control (1.1), OO (1.9) and SBO (0.5) groups, but it was maximum at 4 h in SFO (0.8), GNO (1.5), RBO (0.7), CO (0.3) and PO (0.2) groups (Fig. 4c, d). Further, the TG levels decreased from the maximum absorption to 8 h in all the groups.

Discussion

Dietary lipids reported to affect the carotenoid bioavailability [8]. Fatty acids hydrolyzed from lipids modify the physiological characteristics of micelles [21], which possibly enhance the repartitioning of carotenoids into micelles. Studies show a marked inhibition of lutein, lycopene and astaxanthin bioaccessibility/absorption suspended with palm, fish, corn and coconut oils, whereas enhanced absorption was observed with olive oil, beef tallow and sunflower oil in lutein-sufficient rat model or in vitro [10–12, 22]. However, the mechanism underlying this phenomenon remains largely unknown, especially in

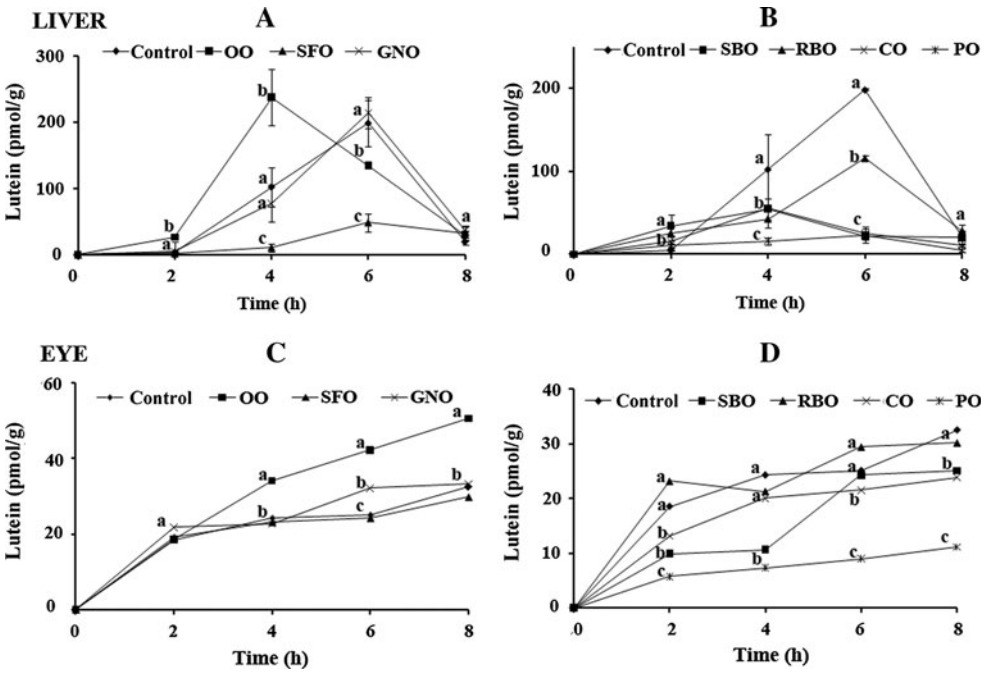

Fig. 3 Postprandial responses of lutein + zeaxanthin in liver (**a**, **b**) and eye (**c**, **d**) of mice after a single dose of lutein (200 μM) solubilized in micelle (*control*) and various vegetable oils. Values are mean ± SD ($n = 5$). Values at each time point not sharing a common letter are significantly different ($p < 0.05$) between groups as determined by one-way analysis of variance and Tukey's test. Lutein concentrations are baseline corrected. Refer to Table 1 for abbreviations

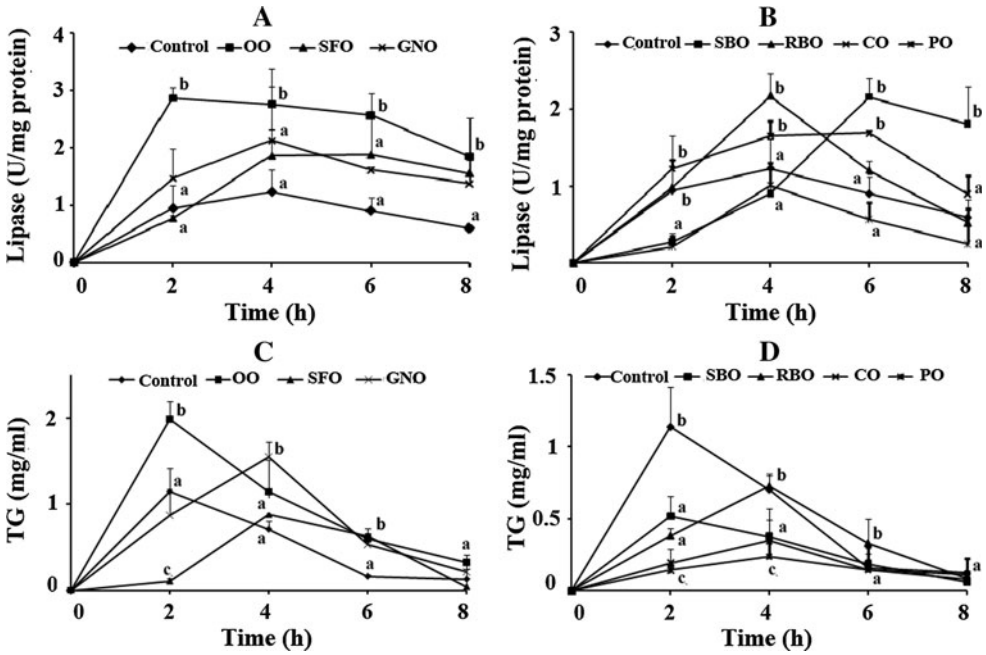

Fig. 4 Postprandial response of triacylglycerol lipase in intestinal mucosa (**a**, **b**) and triacylglycerides (TG) in plasma (**c**, **d**) of mice after a single dose of lutein (200 μM) solubilized in micelle (*control*) and various vegetable oils. Values are mean ± SD ($n = 5$). Values at each time point not sharing a common letter are significantly different ($p < 0.05$) between groups as determined by one-way analysis of variance and Tukey's test. Values are baseline corrected. Refer to Table 1 for abbreviations

Table 3 Plasma (8 h) fatty acid profile of lutein-deficient mice after oral dose of lutein dispersed in vegetable oils

Fatty acid (%)	Groups[†]								
	Baseline (0 h)	Control	OO	SFO	GNO	SBO	RBO	CO	PO
8:0	0.3 ± 0.1^{a}	3.9 ± 0.9^{b}	4.0 ± 1.3^{b}	5.3 ± 0.9^{b}	4.8 ± 0.7^{b}	0.2 ± 0.1^{a}	1.4 ± 0.1^{a}	0.6 ± 0.3^{a}	1.5 ± 1.2^{a}
12:0	4.8 ± 3.4^{a}	5.2 ± 2.5^{b}	2.9 ± 0.3^{ab}	4.4 ± 0.8^{ab}	4.3 ± 1.0^{b}	0.8 ± 0.1^{a}	1.7 ± 0.4^{ab}	2.0 ± 0.1^{a}	2.6 ± 0.6^{b}
14:0	2.1 ± 1.9^{a}	0.5 ± 0.1^{a}	0.3 ± 0.2^{a}	3.1 ± 0.9^{b}	0.8 ± 0.5^{a}	0.2 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}
16:0	22.9 ± 4.3^{a}	23.3 ± 1.4^{a}	25.7 ± 10.3^{a}	16.8 ± 2.2^{a}	18.9 ± 1.2^{a}	19.7 ± 0.2^{a}	23.5 ± 1.7^{a}	23.6 ± 2.8^{a}	29.3 ± 2.6^{ab}
16:1	2.2 ± 0.5^{a}	2.1 ± 0.5^{a}	1.1 ± 0.7^{a}	1.4 ± 0.3^{a}	1.3 ± 0.3^{a}	1.3 ± 0.1^{a}	1.5 ± 0.2^{a}	1.2 ± 0.2^{a}	1.2 ± 0.4^{a}
18:0	9.9 ± 1.5^{a}	10.2 ± 0.5^{a}	26.3 ± 11.8^{b}	8.1 ± 3.1^{a}	8.5 ± 0.3^{a}	9.8 ± 0.9^{a}	10.2 ± 0.8^{a}	9.6 ± 0.7^{a}	10.5 ± 1.2^{a}
18:1	16.7 ± 2.0^{a}	15.8 ± 0.7^{a}	36.4 ± 4.8^{b}	20.2 ± 2.6^{a}	27.2 ± 2.9^{ac}	12.2 ± 8.8^{a}	20.6 ± 0.4^{a}	22.2 ± 1.6^{a}	23.4 ± 4.2^{a}
18:2 (n-6)	15.1 ± 0.5^{a}	15.5 ± 0.5^{a}	14.3 ± 2.2^{a}	39.6 ± 2.6^{b}	20.4 ± 2.1^{a}	30.6 ± 1.4^{ab}	25.8 ± 1.1^{a}	31.4 ± 2.1^{ab}	19.9 ± 1.6^{a}
18:3 (n-3)	3.6 ± 1.3^{a}	1.3 ± 0.2^{a}	ND	ND	ND	0.5^{a}	ND	ND	ND
20:0	12.6 ± 1.5^{a}	19.8 ± 1.9^{a}	ND	ND	ND	0.6 ± 0.3^{b}	ND	ND	ND
20:4 (n-6)	17.8 ± 2.2^{a}	5.4 ± 0.7^{b}	6.2 ± 2.3^{b}	4.5 ± 3.1^{b}	10.1 ± 2.2^{ab}	17.8 ± 2.8^{a}	15.1 ± 2.2^{a}	14.4 ± 1.5^{a}	10.8 ± 2.9^{ab}

Data represent mean ± SD ($n = 5$/time point). Values not sharing a similar superscript within the row among group are significantly different ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's test. *ND* not detected

[†] Refer to Table 1 for abbreviations

lutein-deficient condition. The present study investigated the effect of dietary lipids on absorption kinetics and bioavailability of lutein in lutein-deficient mice. Results revealed that the plasma response (AUC 397.3 pmol h/ml) and tissue accumulation (liver 827.4; eye 240.4 pmol h/g) of lutein were higher in OO and rich in monounsaturated fatty acid (MUFA) in lutein-deficient mice, demonstrating that OO improves the bioavailability of lutein. This is consistent with the previous studies showing that avocado oil and olive oil rich in oleic acid (66 and 74 %) enhance β-carotene and lutein absorption in humans [23] and rats [11]. It is interesting to note that the influential effect of OO on lutein in lutein-deficient mice is significantly higher (397.3 pmol h/ml) than in lutein-sufficient rats (4.6 pmol/ml) [11], indicating that under lutein deficiency, efficiency of lutein absorption is higher. T_{max} of lutein in plasma of OO, control and SBO groups was 2 h, whereas in other groups it was 4 h, showing faster absorption of lutein. The

Table 4 Intestine (8 h) fatty acid profile of lutein-deficient mice after oral dose of lutein dispersed in vegetable oils

Fatty acid (%)	Groups[†]								
	Baseline (0 h)	Control	OO	SFO	GNO	SBO	RBO	CO	PO
8:0	0.3 ± 0.2[a]	0.4 ± 0.2[a]	0.1 ± 0.1[a]	0.3 ± 0.2[a]	0.2 ± 0.1[a]	0.4 ± 0.1[a]	0.2 ± 0.1[a]	0.1 ± 0.0[a]	0.2 ± 0.0[a]
12:0	1.3 ± 0.1[a]	0.9 ± 0.4[b]	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.1[a]	0.5 ± 0.1[ab]	0.1 ± 0.0[a]	0.7 ± 0.0[ab]
14:0	1.2 ± 0.4[a]	1.0 ± 0.9[a]	0.4 ± 0.2[a]	0.4 ± 0.2[a]	0.3 ± 0.2[a]	0.5 ± 0.2[a]	0.2 ± 0.1[a]	0.6 ± 0.1[a]	0.2 ± 0.1[a]
16:0	21.6 ± 8.4[a]	25.8 ± 4.9[a]	16.8 ± 1.1[ab]	11.9 ± 1.7[ab]	13.7 ± 0.3[ab]	19.8 ± 0.4[a]	21.3 ± 1.3[a]	18.5 ± 1.3[a]	21.9 ± 3.5[a]
16:1	2.8 ± 1.4[a]	0.7 ± 0.1[b]	0.3 ± 0.2[b]	2.1 ± 1.8[ab]	0.6 ± 0.3[b]	2.5 ± 0.7[b]	1.8 ± 0.2[ab]	1.4 ± 0.4[ab]	1.2 ± 0.4[ab]
18:0	3.5 ± 1.4[a]	17.9 ± 1.7[a]	1.9 ± 0.6[b]	10.6 ± 0.8[ab]	5.1 ± 0.6[ab]	15.4 ± 6.8[a]	17.4 ± 0.5[a]	11.8 ± 1.5[ab]	9.3 ± 0.5[ab]
18:1	45.7 ± 6.0[a]	17.6 ± 4.6[a]	69.1 ± 2.6[b]	27.1 ± 6.8[a]	48.1 ± 0.5[ac]	28.3 ± 9.2[a]	24.8 ± 0.9[a]	28.9 ± 2.7[a]	21.2 ± 0.1[a]
18:2 (n-6)	19.2 ± 1.1[a]	23.5 ± 4.0[a]	9.33 ± 1.0[b]	48.3 ± 10.6[c]	25.7 ± 0.4[a]	26.5 ± 2.1[a]	22.8 ± 0.3[a]	31.0 ± 0.7[ac]	34.2 ± 1.7[ac]
18:3 (n-3)	1.3 ± 0.2[a]	ND	0.8 ± 0.1[b]	ND	ND	1.1 ± 0.2[a]	0.7 ± 0.1[b]	0.6 ± 0.1[b]	1.7 ± 0.8[a]
20:0	0.3 ± 0.1[a]	ND	1.1 ± 0.1[a]	ND	2.3 ± 1.0[a]	ND	1. 1 ± 0.4[a]	1.0 ± 0.1[a]	0.4 ± 0.1[b]
20:4 (n-6)	6.9 ± 2.9[a]	25.5 ± 1.8[b]	3.4 ± 0.7[a]	5.1 ± 2.1[a]	2.2 ± 0.4[a]	4.2 ± 1.8[a]	6.6 ± 0.1[a]	5.2 ± 0.2[a]	8.5 ± 1.5[a]

Data represent mean ± SD ($n = 5$/time point). Values not sharing a similar superscript within the row among group are significantly different ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's test. *ND* not detected

[†] Refer to Table 1 for abbreviations

Table 5 Liver (8 h) fatty acid profile of lutein-deficient mice after oral dose of lutein dispersed in vegetable oils

Fatty acid (%)	Groups[†]								
	Baseline (0 h)	Control	OO	SFO	GNO	SBO	RBO	CO	PO
8:0	ND	ND	ND	ND	ND	ND	0.1 ± 0.0[a]	0.1 ± 0.0[a]	ND
12:0	0.8 ± 0.1[a]	0.6 ± 0.2[a]	0.4 ± 0.1[a]	0.5 ± 0.3[a]	0.5 ± 0.0[a]	0.5 ± 0.0[a]	0.6 ± 0.1[a]	0.5 ± 0.0[a]	0.7 ± 0.2[a]
14:0	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.1 ± 0.0[a]	0.2 ± 0.1[a]	± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.1[a]	0.3 ± 0.3[a]	0.1 ± 0.0[a]
16:0	23.8 ± 1.3[a]	21.9 ± 1.3[a]	20.7 ± 1.2[a]	19.8 ± 1.9[a]	20.7 ± 1.0[a]	20.9 ± 1.1[a]	24.2 ± 1.0[a]	22.8 ± 2.8[a]	25.1 ± 2.1[ab]
16:1	3.8 ± 0.4[a]	3.8 ± 0.4[a]	3.1 ± 0.5[a]	2.8 ± 0.1[a]	3.0 ± 0.5[a]	3.5 ± 0.2[a]	3.7 ± 1.1[a]	3.7 ± 0.9[a]	2.4 ± 0.7[a]
18:0	4.4 ± 0.9[a]	5.0 ± 0.1[a]	4.1 ± 0.6[a]	3.8 ± 0.4[ab]	3.7 ± 0.9[ab]	6.0 ± 0.4[a]	4.6 ± 1.4[a]	5.8 ± 1.0[a]	5.0 ± 0.8[a]
18:1	35.5 ± 2.1[a]	39.3 ± 0.6[a]	44.2 ± 1.7[ab]	35.1 ± 3.3[a]	40.1 ± 2.1[a]	27.4 ± 3.5[ac]	35.2 ± 4.6[a]	31.5 ± 3.2[a]	34.8 ± 5.7[a]
18:2 (n-6)	22.3 ± 1.2[a]	25.9 ± 0.8[a]	20.1 ± 0.2[a]	30.9 ± 5.6[ab]	24.5 ± 0.5[a]	27.4 ± 1.3[ab]	24.5 ± 0.9[a]	26.2 ± 0.7[a]	17.3 ± 3.1[a]
18:3 (n-3)	2.0 ± 0.3[a]	2.3 ± 0.8[b]	1.1 ± 0.2[a]	ND	ND	1.2 ± 0.1[a]	± 0.1[a]	1.2 ± 0.3[a]	1.2 ± 0.1[a]
20:0	0.2 ± 0.1[a]	0.7 ± 0.3[a]	0.7 ± 0.3[a]	0.8 ± 0.4[a]	0.5 ± 0.1[a]	0.6 ± 0.1[a]	0.6 ± 0.1[a]	0.4 ± 0.3[a]	0.6 ± 0.1[a]
20:4 (n-6)	7.1 ± 1.0[a]	0.3 ± 0.1[b]	5.8 ± 0.7[a]	4.7 ± 1.0[a]	5.5 ± 0.5[a]	8.1 ± 1.0[a]	0.7 ± 0.5[b]	7.4 ± 2.6[a]	6.5 ± 2.9[a]

Data represent mean ± SD ($n = 5$/time point). Values not sharing a similar superscript within the row among group are significantly different ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's test. *ND* not detected

[†] Refer to Table 1 for abbreviations

lower mean residence time of lutein in case of OO (3.7 h) also supports the above results.

Primarily, lipase is secreted from pancreas; however, small intestinal mucosa is also a site for pancreatic triacylglycerol lipase synthesis [24]. Activity of lipase in the intestine lumen can be influenced by the amount and type of dietary lipid [24, 25]. Dietary lipids were hydrolyzed to free fatty acids by intestinal triacylglycerol lipase, which was higher in OO group at T_{max} compared to other vegetable oil groups. Results are in agreement with Birk and Brannon [26], who also demonstrated that medium-chain triglycerides in the diet elevated pancreatic lipase activity in weanling rats. This study also proposes that the oleic acid released upon the action of intestinal triacylglycerol lipase of OO may facilitate micelle formation in intestinal lumen. This may be the reason for higher intestinal triacylglycerol lipase activity in OO, which may be due to susceptibility of OO to lipase [25]. Higher lutein

levels in the liver and eyes of the OO group than in other groups suggest that oleic acid not only influences the intestinal absorption but also facilitates the transport of newly absorbed lutein to its target tissues in the form of lutein esters via lipoprotein, which merits further studies. An elevated level of oleic acid in plasma and tissues further supports the above hypothesis. But it is not clear why intestinal lutein C_{max} was 6 h in all the groups. The potential explanation for this could be a controlled diffusion of lutein across the intestinal lumen. However, the permeation of lutein micelles with oleic acid may be higher in OO group. The difference in plasma TG indicates that the ingested dietary lipid readily altered the plasma TG. Interestingly, the time-course plasma response of lutein is proportional to TG response in plasma of all the groups, indicating that TG is a carrier vehicle for lutein. This is consistent with Lakshminarayana [11] and Mamatha and Baskaran [27] who also reported higher levels of triacylglycerides in plasma after single or repeated doses of lutein in rats. An elevated plasma TG in OO may be due to reconstitution of TG via monoacylglycerol pathway in intestinal epithelium, which is the integral part of chylomicrons [28], and this could be the reason for higher plasma and tissue lutein level. Jackson et al. [29] also reported that short-term ingestion of oleic acid-rich olive oil resulted in higher chylomicrons than that of palmitic acid- and linoleic acid-rich palm and safflower oils in humans and suggested that olive oil may influence the activity of microsomal triglycerides transfer protein (MTP) at enterocyte level, thereby helps in transferring lutein to chylomicrons [30].

The degree of unsaturation in dietary lipid is reported to affect the absorption of carotenoids [31] and the size of micelles [5]. Hollander and Ruble [32] found that the rate of β-carotene disappearance from micellar perfusates in rats intestinal loops was higher when oleic acid was added to perfusates than linoleic or linolenic acids and stated that the rate of transport depends upon the hydrophobicity of fatty acid. The present results reveal that lutein absorption in lutein-deficient mice can be altered by the degree of unsaturation of C-18 fatty acids or the positions of the double bond. This supports the likelihood that the degree of unsaturation of fatty acyl group may influence the post-micellarization processes, which are essential for absorption [10] or incorporation and secretion of lutein in triacylglycerol-rich lipoprotein [31]. Apart from the nature of fatty acids in the lipids, the particle size of the micelles is also one of the factors that can influence the bioavailability of carotenoids [5], which needs further investigation to confirm the hypothesis. The higher level of β-carotene in palm oil can also be a reason for lower lutein bioavailability that may be due to competitive absorption of carotenoids [27].

Conclusion

The choice of carrier lipid and its fatty acid profile is critical to achieve an enhanced bioavailability of lutein in lutein deficiency. The ability of facilitating lutein absorption under its deficiency by olive oil may help in ameliorating the biochemical changes in age-related macular degeneration. However, further research is needed to identify the mechanism responsible for the decreased absorption of lutein with other vegetable oils.

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