

## Carotenoid Absorption from Salad and Salsa by Humans Is Enhanced by the Addition of Avocado or Avocado Oil<sup>1,2</sup>

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**ABSTRACT** Dietary lipids are hypothesized to be an important factor for carotenoid bioavailability. However, most carotenoid-rich fruits and vegetables are low in lipids. The objective of this study was to assess whether the addition of avocado fruit as a lipid source enhances carotenoid absorption in humans. Healthy subjects ( $n = 11$ /study) were recruited for 2 crossover, postprandial studies. The effect of avocado addition (150 g) to salsa on lycopene and  $\beta$ -carotene absorption was examined in Study 1, and the absorption of lutein,  $\alpha$ -carotene, and  $\beta$ -carotene from salad in Study 2. Furthermore, the effects of avocado dose (75 vs. 150 g containing 12 vs. 24 g lipid, respectively) and of lipid source (avocado fruit vs. avocado oil) on carotenoid absorption were examined in Study 2. Intact carotenoids were quantified in the plasma triacylglycerol-rich lipoprotein (TRL) fraction during the 9.5 h after consumption of the test meal and expressed as baseline-corrected area under the concentration-vs.-time curve (AUC). The addition of avocado to salsa enhanced lycopene and  $\beta$ -carotene absorption ( $P < 0.003$ ), resulting in 4.4 and 2.6 times the mean AUC after intake of avocado-free salsa, respectively. In Study 2, supplementing 150 g avocado or 24 g avocado oil to salad similarly enhanced  $\alpha$ -carotene,  $\beta$ -carotene, and lutein absorption ( $P < 0.01$ ), resulting in 7.2, 15.3, and 5.1 times the mean AUC after intake of avocado-free salad, respectively (150 g avocado). Neither the avocado dose nor the lipid source affected carotenoid absorption. In conclusion, adding avocado fruit can significantly enhance carotenoid absorption from salad and salsa, which is attributed primarily to the lipids present in avocado. J. Nutr. 135: 431–436, 2005.

**KEY WORDS:** • carotenoids • avocados • postprandial absorption • triacylglycerol-rich lipoproteins • humans

Dietary intake of carotenoid-rich fruits and vegetables has been associated with a reduced risk of a variety of common diseases including multiple types of cancer, cardiovascular diseases, macular degeneration, and cataract formation (1–3). Carotenoids possess antioxidant properties that have been associated with cell protective mechanisms (4), regulation of cell growth, differentiation, and apoptosis (5). A major focus of research has been  $\beta$ -carotene because vitamin A deficiency is a serious health problem in many developing countries. Although carotenoids can be supplemented to the diet, food-based approaches to enhance carotenoid bioavailability deserve attention because they are relatively easy to achieve and are usually more affordable than fortified foods; furthermore,

they can increase the intake of additional health-promoting nutrients and phytochemicals (6,7).

Bioavailability can be defined as the fraction of an ingested nutrient that is absorbed and available for utilization and/or storage (8). Among several methods to assess bioavailability, measuring carotenoids in the triacylglycerol-rich lipoprotein (TRL)<sup>4</sup> fraction of plasma is one of the most commonly used because this fraction represents newly absorbed carotenoids, thus allowing the monitoring of carotenoid absorption from single doses (9).

Because carotenoids are hydrophobic, their absorption depends upon efficient release from the food matrix and subsequent solubilization by bile acids and digestive enzymes, culminating in their incorporation into micelles. Dietary lipids have been considered to be an important factor for stimulation of bile flow into the intestine and micelle formation (10,11). However, limited information exists on the availability of dietary lipids entrapped in the food matrix to enhance carotenoid absorption.

The present investigation addresses the hypothesis that a frequently consumed lipid-rich fruit, avocado, can increase

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<sup>4</sup> Abbreviations used: AUC, area under the concentration-vs.-time curve; OSU, Ohio State University; TRL, triacylglycerol-rich lipoproteins.

carotenoid absorption when consumed together with carotenoid-rich foods and that this increase is comparable to the effect of adding equivalent amounts of fat/oil alone. Avocado (*Persea americana* Mill.) is an oily fruit with an average lipid content of 15%, containing predominantly monounsaturated (18:1) fatty acids (12). Avocados are a rich source of phytochemicals and nutrients such as vitamin E, lutein, glutathione,  $\beta$ -sitosterol, folate, potassium, magnesium, and fiber (13). Furthermore, avocado consumption was reported to be associated with cardiovascular health and cancer prevention (14,15). Per capita annual avocado consumption doubled to 1 kg within the last 30 y in the United States (16).

In this study, absorption of  $\alpha$ -carotene,  $\beta$ -carotene, lutein, and lycopene was investigated after the consumption of a single carotenoid-rich test meal composed of salad or salsa, each with and without avocado or avocado oil as a lipid source. Intestinal absorption was assessed studying the TRL fraction of plasma (9,17), and carotenoids were measured using a highly sensitive electrochemical detector (18) coupled with reversed-phase HPLC.

## SUBJECTS AND METHODS

**Subjects.** Subjects ( $n = 11$ /study) were healthy, nonpregnant, nonsmoking adults, aged 21–42 y (median 28 y). Eligibility was based on a screening test for blood lipids (cholesterol, triacylglycerol) and a health and lifestyle questionnaire. Exclusion criteria included hyperlipidemia, any history of chronic gastrointestinal disease, use of medications affecting lipid metabolism, regular use of carotenoid-containing supplements, and frequent alcohol consumption. The study was approved by the Biomedical Sciences Institutional Review Board of the Ohio State University (OSU) and signed informed consent was obtained.

**Test meals.** Foods frequently consumed with avocados rich in carotenoids and low in fat were chosen as test meals. Test meals were composed of 300 g salsa (Old El Paso-Thick and Chunky Salsa, General Mills) and 3 slices (75 g) of fat-free bread for Study 1 and salad (220 g) containing prepackaged ingredients of 100 g carrots (Premium Fresh Shredded Carrots, Coronet Foods), 40 g lettuce (Precut Dole Hearts of Romaine Lettuce), 80 g baby spinach (Organic Blend Dole Baby Spinach), 2 slices (50 g) of fat-free bread, and 40 g fat-free Italian salad dressing for Study 2; the meals included 240 mL bottled water. The energy and macronutrient content of all test meals were determined by Food Processor™ version 8.1. (ESHA Reach). The salsa meal provided 1.2 MJ energy and was fat-free in the absence of avocado (Mission Produce, Hass California Avocados). When consumed with 150 g avocado, the total energy was 2.44 MJ, with 37% of the energy coming from fat. The energy content of the salad meal was 0.91 MJ with 2.3% from fat. When consumed with 75 and 150 g avocado, the percentage of energy from fat was 31 and 42%, respectively. The fat equivalent of 150 g avocado fruit was 24 g of avocado oil.

**Experimental design.** All subjects were instructed to avoid carotenoid-rich foods for 2-wk as a washout period before each clinical visit. A list of foods to avoid based on USDA-Nutrition Coordinating Center and National Cancer Institute carotenoid database (12) was provided. Study 1 was followed by Study 2.

In Study 1, determination of lycopene absorption from salsa was the primary aim; however, because salsa contains measurable amounts of  $\beta$ -carotene, changes in  $\beta$ -carotene absorption were also reported. Subjects consumed salsa with and without avocado (control) at 2 and 4 wk. In Study 2, the absorption of  $\alpha$ -carotene,  $\beta$ -carotene, and lutein was assessed after the salad consumption alone, with 75 and 150 g avocado, and with 24 g avocado oil (Spectrum Organic Products) at 2, 4, 6, and 8 wk, following a crossover design. In both studies, avocado-containing meals were served in a nonrandomized order to avoid compositional changes in avocados during storage.

Clinical visits (10 h) were conducted at the OSU General Clinical Research Center after overnight fasting (12 h). After baseline blood sampling (0 h), test meals were served at ~0800 h and

consumed under supervision within 20 min. Consecutive blood samples were collected at 2, 3, 4, 5, 6, 8, and 9.5 h after test meal consumption. At 4.5 h, subjects consumed a standardized lunch low in carotenoids and fat, providing 2.67 MJ energy from 127.7 g carbohydrate, 18.5 g protein, and 5.9 g fat. No other foods and beverages except water (consumed ad libitum) were allowed during the 10-h stays.

**Blood sampling and analysis.** Blood samples (12 mL each) were drawn from a forearm vein and immediately centrifuged for plasma separation at  $1250 \times g$  for 10 min at 4°C. Measurements of plasma cholesterol and triacylglycerol levels were based on a spectrophotometric enzymatic method by a Synchron LX 20 system (Beckman Coulter) (19). TRL fractions were isolated from plasma by ultracentrifugation at  $155,000 \times g$  for 30 min at 20°C (Beckman L8-M ultracentrifuge, SW 50.1 swinging bucket rotor) (20).

**Carotenoid extraction and quantification.** All test meal contents were individually analyzed for their carotenoid content by HPLC. Carotenoids were extracted from salsa with acetone:hexane (18), from spinach, lettuce, carrots, and avocado with petroleum ether (21), and from TRL fraction with hexane (22). All procedures were conducted under red light.

Standards of  $\beta$ -carotene, lycopene, and lutein were purchased from Sigma Chemical, and  $\alpha$ -carotene from Fluka Chemie AG. All-*trans* standards were used for the quantitation of both *trans* and *cis* isomers. Identification of *cis* isomers was based on a comparison with previously reported UV-VIS and electrochemical methods (18,23,24).

Carotenoids were analyzed using a Hewlett Packard 1050 HPLC system connected to an electrochemical detector (5600 Coularray™, ESA). A C<sub>30</sub> column (YMC™, 150 mm  $\times$  4.6 mm, 5  $\mu$ m; Waters) was used for the analysis of  $\alpha$ -carotene,  $\beta$ -carotene, lutein (18), and lycopene (24).

**Statistics.** Statistical analyses were performed using SPSS 12.0. Postprandial absorption for each carotenoid in the plasma TRL fraction expressed as baseline-corrected area under the concentration-vs.-time curve (AUC) during the 9.5 h after the test meal consumption was calculated by trapezoidal approximation using Igor Pro 4.06 software (Wave Metrics).

Normal distribution of the differences of AUC (meal with avocado – control) was verified by Q-Q plots and Kolmogorov-Smirnov tests. Homogeneity of variance was verified by Levene's test. In Study 1, nonnormally distributed data were analyzed by Wilcoxon signed-rank tests, and AUC values [(nmol  $\cdot$  h)/L] are expressed as medians with 25th and 75th percentiles. Because conditions of normal distribution and homogeneity of variance were satisfied for Study 2, a general linear multivariate model was developed to investigate the effect of oil type (avocado oil vs. avocado fruit), amount avocado added (75 vs. 150 g), whether any oil/fat addition per se had an effect on absorption, and the influence of gender on carotenoid absorption. Differences in mean AUC after consumption of the test and control meals for individual carotenoids were identified by post hoc tests (Tukey's, Bonferroni). AUC [(nmol  $\cdot$  h)/L], peak concentrations (nmol/L), and carotenoid concentration (mg/g serving) of test meals were expressed as means  $\pm$  SEM. The correlation between age, BMI, and carotenoid absorption was determined by Spearman correlation coefficients. *P*-values < 0.05 were considered significant.

## RESULTS

All subjects ( $n = 11$ , Table 1) completed both studies except for 3 individuals who were unable to schedule another full day at the clinic for the consumption of salad with the 75-g avocado dose ( $n = 8$ ). The total carotenoid content (Table 2) in 300 g salsa was 41 mg with 97% from lycopene and 3% from  $\beta$ -carotene. Salad contained 24 mg carotenoids composed of 48%  $\beta$ -carotene, 25% lutein, and 28%  $\alpha$ -carotene per serving size (220 g). The carotenoid content of each salad ingredient and salsa was comparable to previously reported values (25).

In Study 1, lycopene absorption from salsa with avocado addition was higher than from the control meal ( $P = 0.003$ , Wilcoxon signed-rank test), resulting in 4.4 times the mean

TABLE 1

Subject characteristics at the onset of the study<sup>1</sup>

	Age		BMI	Plasma triacylglycerol	Plasma cholesterol
	<i>n</i>	<i>y</i>	<i>kg/m<sup>2</sup></i>	<i>mmol/L</i>	
Study 1					
Women	5	31.0 ± 3.0	22.5 ± 1.2	0.703 ± 0.127	4.66 ± 0.42
Men	6	28.7 ± 2.1	26.0 ± 1.6	0.809 ± 0.129	4.62 ± 0.41
Study 2					
Women	6	26.2 ± 2.3	21.3 ± 1.0	0.964 ± 0.279	5.18 ± 0.36
Men	5	27.2 ± 2.7	26.0 ± 1.7	0.926 ± 0.151	4.07 ± 0.41

<sup>1</sup> Values are means ± SEM.

AUC after consumption of avocado-free salsa (Table 3).  $\beta$ -Carotene absorption from salsa also was greater when avocado was added ( $P = 0.003$ , Wilcoxon signed-rank test), resulting in 2.6 times the mean AUC after consumption of avocado-free salsa. In Study 2, carotenoid absorption from salad with the addition of either avocado or avocado oil was greater than from salad alone ( $P < 0.0005$ ; Table 3). The high dose of avocado (150 g) and the fat-equivalent avocado oil (24 g) both increased the absorption from salad of all carotenoids studied ( $P < 0.01$ ). Adding 75 g avocado to salad significantly increased absorption of  $\alpha$ -carotene and  $\beta$ -carotene ( $P < 0.003$ ) but not lutein ( $P = 0.07$ ). Adding 75 and 150 g avocado and 24 g avocado oil resulted in 8.3, 7.2 and 8.9 times the mean AUC for  $\alpha$ -carotene, respectively, compared with that after subjects consumed avocado-free salad. Similar results were found for  $\beta$ -carotene (13.6, 15.3, and 17.4 times) and lutein (4.3, 5.1, and 6.7 times), respectively.

The addition of 24 g avocado oil to the salad significantly enhanced the absorption of lutein,  $\alpha$ -carotene, and  $\beta$ -carotene to an extent that was not significantly different from the addition of 150 g avocado fruit. An increase in the avocado dose from 75 to 150 g did not significantly affect the absorption of lutein,  $\alpha$ -carotene, or  $\beta$ -carotene. Men and women did not differ in carotenoid absorption in either study. Age and BMI were not correlated with carotenoid absorption.

In addition to the all-*trans* form, several *cis* isomers of lycopene (23) were detected in the TRL fraction obtained after salsa consumption (results are expressed as total lycopene); 5-*cis* was the predominant isomer. Low amounts of all-*trans*  $\beta$ -carotene and its 9-*cis* isomer were detected and expressed as total  $\beta$ -carotene in salsa. In the TRL fractions obtained from the salad group,  $\alpha$ -carotene,  $\beta$ -carotene including its 9-*cis* isomer, and lutein were quantified.

Peak concentrations (baseline corrected) of 26.5 ± 7.2 and 3.2 ± 1.1 nmol/L plasma for lycopene and 7.9 ± 3.0 and 1.9 ± 0.5 nmol/L plasma for  $\beta$ -carotene in Study 1 were reached in the TRL fraction after salsa consumption with and without 150 g avocado, respectively (Fig. 1). For lutein,  $\alpha$ -carotene and  $\beta$ -carotene, these values were: 2.6 ± 1.0, 0.6 ± 0.3, and 2.2 ± 0.9 nmol/L plasma after salad consumption; 6.9 ± 1.5, 2.8 ± 0.8, and 9.4 ± 2.1 nmol/L plasma for salad consumed with 75 g avocado; 11.6 ± 1.9, 2.7 ± 0.6, and 10.2 ± 1.6 nmol/L plasma for salad with 150 g avocado; 13.5 ± 2.0, 3.5 ± 0.9, and 12.6 ± 2.6 nmol/L plasma for salad with 24 g avocado oil, respectively, in Study 2 (Fig. 2).

No carotenoids were detected in the commercial avocado oil used in the study. However, avocados contributed slightly to the total carotenoid content of the salad (1.3% for 75 g and 2.6% for 150 g avocado addition, Table 2). The moisture and fat content of the avocado fruits were 73.4 ± 0.1 and 16.1 ± 0.1% ( $n = 4$ ), respectively (26).

## DISCUSSION

Previous studies indicated the importance of dietary lipids for effective carotenoid absorption (11,27–29). The present

TABLE 2

Carotenoid contents of the test meals<sup>1</sup>

Ingredient	Weight	$\mu\text{g/serving (edible portion)}$			
		$\alpha$ -Carotene	$\beta$ -Carotene	Lutein	Lycopene
	<i>g</i>	<i>mg</i>			
Salsa	300	ND <sup>2</sup>	1.18 ± 0.03	ND	39.62 ± 0.74
Salad					
Shredded carrot,	100	6.62 ± 0.29	7.51 ± 0.28	0.181 ± 0.02	ND
Romaine lettuce,	40	ND	0.41 ± 0.03	0.44 ± 0.03	ND
Organic baby spinach,	80	ND	3.59 ± 0.03	5.37 ± 0.09	ND
Salad total (serving size)	220	6.62 ± 0.29	11.51 ± 0.24	5.99 ± 0.11	ND
Avocado	75	13.65 ± 0.53	25.50 ± 0.90	268.13 ± 4.05	ND
	150	27.30 ± 1.05	51.00 ± 1.80	536.25 ± 8.10	ND

<sup>1</sup> Values are means ± SEM,  $n = 4$ .<sup>2</sup> ND, not detectable.

TABLE 3

Areas under the curves for carotenoids in the plasma TRL fraction during the 9.5 h after consumption of salsa or salad with or without avocado in humans

	Study 1 (salsa) <sup>1</sup>		Study 2 (salad) <sup>2</sup>			
	Salsa (300 g) (control)	Salsa (300 g) with 150 g avocado	Salad (220 g) (control)	Salad with 75 g avocado	Salad with 150 g avocado	Salad with 24 g avocado oil
<i>n</i>	11	11	11	8	11	11
	(nmol · h)/L					
α-Carotene	ND <sup>3</sup>	ND	-1.32 ± 1.29 <sup>a</sup>	9.58 ± 2.20 <sup>b</sup>	8.12 ± 1.92 <sup>b</sup>	10.45 ± 2.58 <sup>b</sup>
β-Carotene	7.37 <sup>a</sup> (3.82, 20.68)	18.26 <sup>b</sup> (13.75, 26.21)	2.38 ± 5.33 <sup>a</sup>	32.45 ± 6.03 <sup>b</sup>	36.31 ± 5.66 <sup>b</sup>	41.42 ± 8.58 <sup>b</sup>
Lutein	ND	ND	6.45 ± 4.67 <sup>a</sup>	27.78 ± 7.25 <sup>a,b</sup>	33.04 ± 5.17 <sup>b</sup>	43.12 ± 7.03 <sup>b</sup>
Lycopene	18.97 <sup>a</sup> (6.04, 36.28)	68.97 <sup>b</sup> (50.41, 121.84)	ND	ND	ND	ND

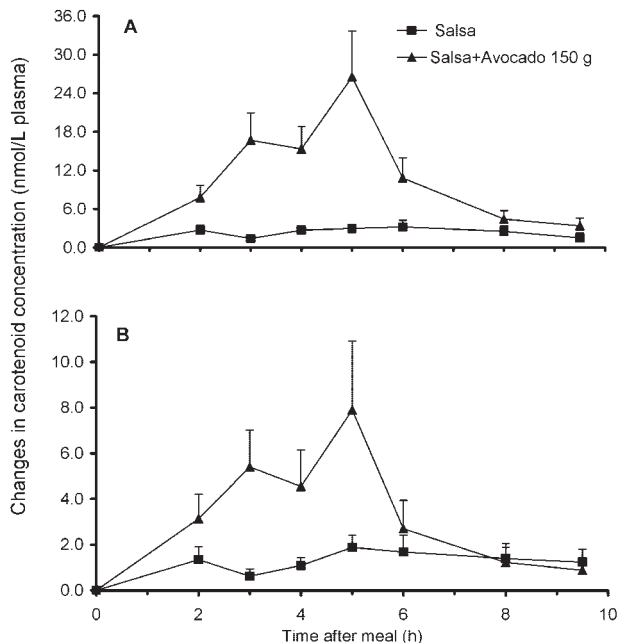
<sup>1</sup> Values are medians (25th and 75th percentiles). Medians in a row without a common letter differ,  $P < 0.003$ .

<sup>2</sup> Values are means ± SEM. Means in a row without a common letter differ,  $P < 0.01$ .

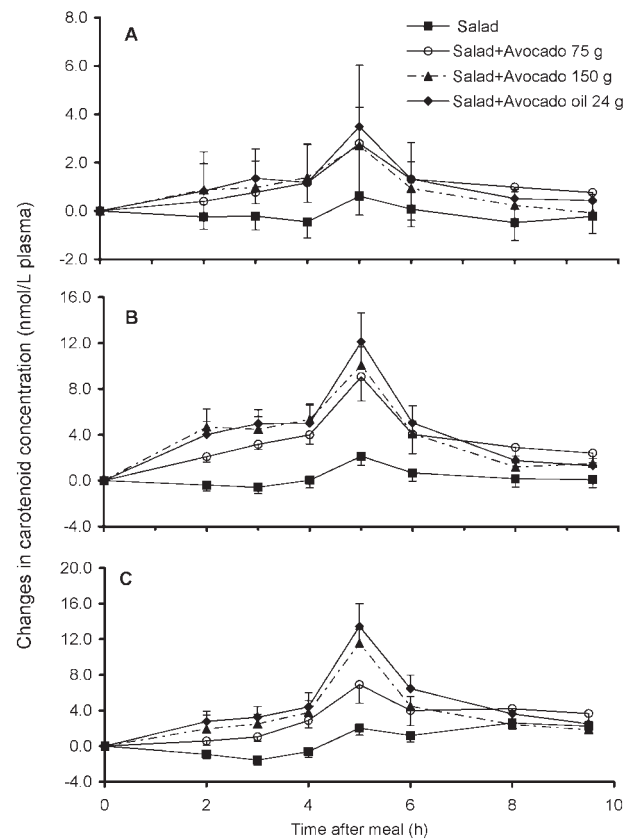
<sup>3</sup> ND, not detectable.

investigation employed commonly consumed carotenoid-rich foods to test the hypothesis that avocado fruit as a lipid source enhances carotenoid absorption to an extent similar to that of an equivalent amount of added oil. The results demonstrate that avocado addition (half or whole fruit, equivalent to 75 and 150 g avocado, respectively) significantly enhances carotenoid absorption from salsa and salad. The comparison of avocado fruit with added avocado oil strongly indicates that the lipid component of the avocado fruit is the critical variable enhancing carotenoid absorption and that the fruit matrix has no negative effect on lipid release. In addition, contrary to a number of previous studies suggesting an inhibitory effect of dietary fiber on carotenoid absorption (17,27,30), our studies

indicate no such effect for dietary fiber present in avocado fruit. These findings add a new perspective to previous studies by demonstrating the possibility of using a lipid-rich fruit instead of added fat/oil for enhancing carotenoid absorption.



**FIGURE 1** Baseline-corrected plasma TRL concentrations of (A) lycopene and (B) β-carotene during the 9.5 h after consumption of salsa with and without avocado in humans. Values are means ± SEM ( $n = 11$ , 5W:6M). Both lycopene and β-carotene AUC were greater after intake of salsa with avocado (150 g) than after consumption of salsa alone (Table 3;  $P = 0.003$ , Wilcoxon signed-rank test).



**FIGURE 2** Baseline-corrected plasma TRL concentrations of (A) α-carotene, (B) β-carotene, and (C) lutein during the 9.5 h after consumption of salad alone ( $n = 11$ ), with 75 g avocado ( $n = 8$ ), with 150 g avocado ( $n = 11$ ), or with 24 g avocado oil ( $n = 11$ ) in humans. Values are means ± SEM. Lutein, α-carotene, and β-carotene AUC were greater after salad consumption with 150 g avocado and 24 g avocado oil than after salad alone (Table 3;  $P < 0.01$ , Tukey's test). The AUCs for salad with 75 g avocado were significantly higher for α-carotene and β-carotene ( $P < 0.003$ , Tukey's test).



Absorption of carotenoids especially from salad was very low when consumed alone (Table 3). Similarly, Brown et al. (28), demonstrated a significantly increased absorption of carotenoids from salads when changing from fat-free to reduced-fat (6 g) dressing, and from reduced-fat to full-fat (28 g) dressing. However, the amount of dietary fat required for optimum carotenoid absorption is still controversial (17,29,31). A minimum intake of 5 g lipids/d was suggested for sufficient absorption of  $\beta$ -carotene (27), whereas other studies recommended the presence of 3–5 g fat/meal for significant  $\beta$ -carotene absorption in adults (10,28). In our study, the low dose avocado (containing 12 g fat) was nearly as effective as the high dose (24 g fat) in increasing carotenoid absorption, indicating a nonlinear relation between the amount of lipid ingested and absorption of carotenoids, at least with a moderate fat intake. It remains to be elucidated, however, whether there is a minimum amount of lipid required for optimal carotenoid absorption that can be applied to various food sources.

The findings of the present investigation also support the hypothesis that specific carotenoids may require different amounts of lipids for optimal absorption (10,32,33). Even though lutein absorption from salad with 75 g avocado was 3.3-fold that of salad alone, this difference was not significant ( $P = 0.07$ ). Roodenburg et al. (10) reported that 3 g fat were sufficient for optimum absorption of  $\alpha$ - and  $\beta$ -carotene, whereas higher amounts of fat (36 g) were required for optimum absorption of lutein. In the salad study, 75 g avocado provided 12 g lipids, which might have been marginal for optimum lutein absorption. Serving unprocessed vegetables in the salad mix might be another possible reason for the relatively low increase in lutein absorption. As shown in previous studies, carotenoids from processed foods are more bioavailable compared with their unprocessed form (34–36). However, it cannot be completely ruled out that the nonsignificant difference in lutein absorption after the addition of 75 g avocado is based on the lower number of subjects ( $n = 8$ ) compared with the number of subjects for whom the comparison between 150 g avocado and 24 g avocado oil was made ( $n = 11$ ).

Some studies have suggested that differences in lipid metabolism (37), body weight, body composition (38), and hormonal status between men and women (27,30) can influence carotenoid metabolism, whereas other studies suggested no effect (39,40). In this study, no significant difference in carotenoid absorption between men and women was observed; however, investigating the effect of sex was not the primary focus of our study. Fairly homogenous plasma lipid profiles in this cohort of men and women may have contributed to this observation because variation in lipid metabolism may be a crucial factor influencing carotenoid absorption (27). In addition, the between-subject variation in AUC data for all carotenoids studied was quite high, most likely due to differences in the kinetics of chylomicron secretion and cleavage or vitamin A status of the subjects (9,17,27). No adjustment for AUC carotenoid response according to triacylglycerol concentrations was made in our studies because Erdman et al. (41) showed that the time course of the appearance and half-life of triacylglycerols and carotenoids in chylomicrons are nearly identical. In addition, the aim of our study was to determine carotenoid absorption per se, without regard to any potential vitamin A activity. Therefore, no effort was made to quantify retinyl ester cleavage products.

The postprandial curves shown in Figures 1 and 2 indicated 2 predominant periods for carotenoid incorporation into the TRL fraction. The first moderate increase (more pronounced in Study 1) occurred ~2–3 h after test meal consumption,

followed by the major response observed at 5 h (after the lunch meal). This was observed in all treatments for all carotenoids studied. This 2-peak absorption pattern is in line with previous findings (34,39). It was postulated that carotenoids are kept in the enterocyte and not released until long-chain fatty acids (12:0–18:0) from a subsequent meal enable carotene packaging into chylomicrons. No secretion of chylomicrons was observed after consumption of a medium-chain fatty acid-containing meal (42). Unsaturated fatty acids, especially oleic acid, were shown to be efficiently incorporated into lipoproteins (43,44). Because California avocados are rich in monounsaturated fatty acids, with oleic acid predominating (~66% of fatty acids) (12), we assume that the fatty acid distribution of avocados facilitates the formation of chylomicrons. However, consumption of the second meal (lunch) containing <6 g fat seemed to further facilitate chylomicron formation and carotenoid absorption, indicating that repeated test meals, even with a relatively low fat content, contribute substantially to carotenoid absorption.

Absorption of  $\alpha$ -carotene,  $\beta$ -carotene, and lutein was remarkably similar for avocado fruit and avocado oil. Despite the food matrix, the lipids present within avocado fruit seemed readily available to facilitate carotenoid absorption. Avocados are considered to be a good source of dietary fiber (6.8 g fiber/100 g edible portion) (12); 72% of these are insoluble fibers (i.e., cellulose, pectins, and hemicellulose) (14). Previous studies reported that dietary fiber might decrease the absorption of carotenoids by entrapping them and increasing fecal excretion of lipid-soluble substances (17,45,46). In contrast to these findings, the present study suggests that the fiber content of avocados did not affect postprandial carotenoid absorption. However, it is difficult to compare different studies when various dietary fibers are consumed. In addition, dietary fibers might have a more pronounced negative effect on carotenoid absorption as measured in plasma during long-term absorption studies compared with postprandial TRL responses because of potentially increased fecal excretion of carotenoids (17).

In conclusion, we observed a notable increase in the absorption of carotenoids from vegetable-based foods consumed with avocados. To our knowledge, this is the first intervention study showing that consumption of a fruit as a lipid source with carotenoid-rich foods enhances carotenoid absorption in humans. The present study also highlights the importance of taking nutritional interactions into account when giving dietary recommendations. Considering that avocados contain a large variety of nutrients such as vitamins, minerals, and monounsaturated fatty acids, adding avocado fruit to carotenoid-containing meals as a lipid source can facilitate carotenoid absorption while offering additional nutritional benefits.

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