Avocado Consumption Enhances Human Postprandial Provitamin A Absorption and Conversion from a Novel High–β-Carotene Tomato Sauce and from Carrots

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Abstract

Dietary lipids have been shown to increase bioavailability of provitamin A carotenoids from a single meal, but the effects of dietary lipids on conversion to vitamin A during absorption are essentially unknown. Based on previous animal studies, we hypothesized that the consumption of provitamin A carotenoids with dietary lipid would enhance conversion to vitamin A during absorption compared with the consumption of provitamin A carotenoids alone. Two separate sets of 12 healthy men and women were recruited for 2 randomized, 2-way crossover studies. One meal was served with fresh avocado (Persea americana Mill), cultivated variety Hass (delivering 23 g of lipid), and a second meal was served without avocado.

In study 1, the source of provitamin A carotenoids was a tomato sauce made from a novel, high–β-carotene variety of tomatoes (delivering 33.7 mg of β-carotene). In study 2, the source of provitamin A carotenoids was raw carrots (delivering 27.3 mg of β-carotene and 18.7 mg of α-carotene). Postprandial blood samples were taken over 12 h, and provitamin A carotenoids and vitamin A were quantified in triglyceride-rich lipoprotein fractions to determine baseline-corrected area under the concentration-vs.-time curve. Consumption of lipid-rich avocado enhanced the absorption of β-carotene from study 1 by 2.4-fold ($P < 0.0001$). In study 2, the absorption of β-carotene and α-carotene increased by 6.6- and 4.8-fold, respectively ($P < 0.0001$ for both). Most notably, consumption of avocado enhanced the efficiency of conversion to vitamin A (as measured by retinyl esters) by 4.6-fold in study 1 ($P < 0.0001$) and 12.6-fold in study 2 ($P = 0.0013$). These observations highlight the importance of provitamin A carotenoid consumption with a lipid-rich food such as avocado for maximum absorption and conversion to vitamin A, especially in populations in which vitamin A deficiency is prevalent. This trial was registered at clinicaltrials.gov as NCT01432210.

Introduction

Vitamin A deficiency affects a staggering percentage of the population, especially in the developing world. Globally, it is estimated to affect 0.9% of preschool children and 7.8% of pregnant women (1). It is the leading cause of blindness in children and is also associated with increased burden of infectious disease, xerophthalmia (dry-eye syndrome), night blindness, and increased risk of mortality (2). In contrast, vitamin A sufficiency is associated with growth promotion, cellular differentiation, proper immune function, proper embryonic development, induction of gap junction communication, and light adaptation (3–5).

In the United States, it is estimated that ~30% of the vitamin A consumed is derived from provitamin A carotenoids, found primarily in orange fruits and vegetables and deep-green leafy vegetables (6). In contrast, in developing countries, a majority of the diet consists of grains and staple crops, with fruits and vegetables, as well as animal products, making up a much smaller percentage of overall food intake (7,8). In these countries, fruit and vegetable consumption are responsible for ≥70% of vitamin A intake in the form of provitamin A (9,10). Provitamin A carotenoids, including β-carotene and α-carotene, contain ≥1 unsubstituted β-ionone ring that confers provitamin A activity. These carotenoids must be enzymatically cleaved by
β-carotene oxygenase 1 (BCO1)\(^a\) at the central double bond to produce vitamin A (11). In humans, BCO1 is highly active in the intestinal enterocytes and the liver (12).

Bioavailability of a carotenoid is defined as the amount of the carotenoid (as parent carotenoid or carotenoid metabolite) that is absorbed and packaged with lipids into chylomicrons and released into circulation after the consumption of a carotenoid-containing meal. The bioavailability of provitamin A carotenoids can be measured by monitoring provitamin A carotenoid and retinyl ester concentrations in the postprandial TG-rich lipoprotein (TRL) fraction of plasma that contains chylomicrons (6).

Bioavailability has been shown to be affected by a number of factors. Studies by our group and others have demonstrated that the consumption of a carotenoid-containing meal with lipid or lipid-rich avocado dramatically enhances provitamin A carotenoid bioavailability compared with a meal with no lipid (13–16). Likewise, increasing concentrations of meal lipid leads to increasing amounts of carotenoid absorption, to a certain degree (14–16). However, a definitive understanding of provitamin A carotenoid absorption and metabolism in humans, relative to the provitamin A content in foods, is still lacking.

Various postprandial human studies have assessed the conversion of provitamin A carotenoids to vitamin A when comparing food matrices (17), a food source to a vitamin A reference dose (18,19), or co-consumption with medium- and long-chain FAs (20). In addition, animal studies have revealed that the chronic consumption of provitamin A carotenoids with higher concentrations of lipid leads to both higher intestinal BCO1 activity (21) and higher hepatic vitamin A stores (22,23) compared with animals consuming the same meal with less lipid. However, the impact of the absence and presence of dietary lipid on provitamin A conversion to vitamin A from a single meal has not been well investigated in humans.

Our primary objective was to determine whether adding lipid, in the form of lipid-rich avocado, to a carotenoid-rich meal would promote the absorption of provitamin A carotenoids and enhance intestinal conversion to vitamin A. Participants consumed a meal with or without avocado in combination with a serving of a novel, high–β-carotene tomato sauce (containing nutritionally relevant amounts of β-carotene) for study 1 or carrots (containing β-carotene and α-carotene) for study 2. The immediate postprandial concentrations of parent carotenoids and retinyl esters were measured in the TRL fraction of plasma. The absorption of other carotenoids (i.e., lutein) and vitamins E and K-1 (i.e., α-tocopherol and phylloquinone, respectively) from the avocado fruit were also investigated.

**Participants and Methods**

**Participants.** Two separate sets of healthy adult volunteers (aged 19–37 y) were recruited for each study (study 1, \(n=12\); study 2, \(n=12\)). Previously published data were used to perform power calculations to estimate required sample size to determine statistically significant changes in our primary endpoints of TRL AUC of β-carotene (15), α-carotene (15), and retinyl esters (20). For a significance level \(\alpha=0.05\), a paired \(t\) test indicated that an enrollment of 12 participants would provide >80% power to observe statistically significant differences in all primary analytes of interest in study 1 and study 2.

Inclusion criteria specified that participants be between 18 and 70 y of age, nonpregnant, nonsmoking, normocholesterolemic (<200 mg/dL total cholesterol), and normolipidemic, have a BMI of 17–30 kg/m\(^2\), no history of cancer, and no gastrointestinal diseases or diabetes, and not be using medication affecting lipid uptake or transport. Written informed consent was obtained from all participants before beginning the study, and all clinical procedures were performed at the Clinical Research Center (CRC) of Ohio State University. The study was approved by the Institutional Review Board of Ohio State University (protocol No. 2011H0159) and the CRC of Ohio State University (Center for Clinical and Translation Science No. 987). The study was registered at clinicaltrials.gov as NCT01432210.

**Experimental design.** Individuals who were interested in participating in the study consented at the initial CRC visit. Vitals and a blood sample were taken to check blood lipid and cholesterol concentrations, and the health and lifestyle questionnaire was administered. For each crossover study, an equal number of men and women were randomly assigned to 1 of 2 feeding groups. Participants were asked to abstain from consuming foods rich in provitamin A and vitamin A for 2 wk before daylong clinic visit 1. After an overnight (12 h) fast, participants arrived at the clinic in the morning and had a catheter inserted. Baseline blood (0 h) was drawn, and then participants immediately consumed the test meal. One group consumed the test meal containing avocado on daylong visit 1, and the other group consumed the test meal alone on daylong visit 1. Participants were given 20 min to eat the sauce and 30 min to eat the carrot meal. Participants were allowed to consume water ad libitum throughout the course of their daylong visits. Blood samples were then taken at 2, 3, 4, 5, 6, 8, 10, and 12 h after the meal was consumed. A lunch meal very low in carotenoids, provitamin A, and lipid was served at 4.5 h. Participants returned home, continued the low provitamin A and vitamin A diet for 2 additional weeks, and then again returned to the clinic for daylong visit 2. Participants crossed over to the test meal they had not yet consumed on daylong visit 2.

**Blood lipids.** Blood lipids were tested at all 3 clinic visits using a Dimension Xpand Plus Automated Clinical Chemistry Analyzer (Siemens) and are shown in Table 1.

**Test foods and meals.** For study 1, the test food consisted of a novel variety of tomato (Solanum lycopersicum L.) rich in β-carotene (variety 97L97) that was developed using traditional crossbreeding techniques and grown at Ohio State University North Central Agricultural Research Station near Fremont, Ohio (24). Tomatoes were harvested and processed into tomato juice using a hot-fill process in a pilot plant of the Food Industries Center of Ohio State University. Later, the tomato juice was concentrated in a steam-jacketed kettle to 15° Brix, hot-filled

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\(^a\) Abbreviations used: BCO1, β-carotene oxygenase 1; CRC, Clinical Research Center; TRL, TG-rich lipoprotein.
into No. 300 cans to produce a shelf-stable product. For study 2, the test food consisted of raw petite baby carrots that were purchased from a local grocery store in Columbus, Ohio. Avocados (Persea americana Mill), cultivated variety Hass, were provided by the Hass Avocado Board. The FA profile of Hass avocados consists of predominantly MUFAs (60% oleic, 6% palmitoleic) with some PUFAs (15% linoleic, 2% α-linoleic) and SFAs (16% palmitic, 1% stearic) (25). Avocados were peeled and seeded just before the test meal preparation (further described below).

For both studies 1 and 2, test foods were served with the breakfast meal. For study 1, 300 g of processed sauce was weighed and served at room temperature with or without 150 g of sliced, fresh avocado. For study 2, 300 g of raw petite baby carrots were weighed into a bowl and served at room temperature with or without guacamole consisting of 150 g of freshly mashed avocado, 5 mL (1 teaspoon) of lemon juice, 0.25 g (1/8 teaspoon) of garlic powder, and 0.7 g (1/8 teaspoon) of salt. Participants were also given 1 English muffin (57 g) to completely clean and consume the sauce from the bowl for the sauce study or to clean and consume the guacamole from the bowl for the carrot study. In addition, cooked egg whites (from 2 eggs, 66 g), a medium banana (118 g), and a cup of coffee (237 mL) were served with breakfast. The breakfast with tomato sauce alone provided 406 kcal, with 17 g of protein, 2 g of lipid, and 80 g of carbohydrate. The breakfast with carrot alone provided 275 kcal, with 3 g of protein, 23 g of total carbohydrate, and 80 g of carbohydrate. The salt content in the 150 g of sliced, fresh avocado was 0.7 g. No other condiments were used to prepare the test meal.

### Carotenoid extraction from food
The raw carrots were blended in a food processor yielding a fine pulp. An aliquot of 2 g of carrot pulp, sauce, or mashed avocado was weighed into 12-mL glass tubes. Five milliliters of methanol were added, and the mixture was probe sonicated. The sample was centrifuged at 2000 × g for 10 min. The methanol was decanted into a clean glass vial, and 5 mL of hexane/acetone (1:1) was added to the remaining pellet. The sample was again sonicated and centrifuged at 2000 × g for 10 min, and the hexane/acetone extraction was removed and combined with the methanol. The hexane/acetone extraction was repeated twice more. To the pooled extracts, 10 mL of water and 1 mL of saturated aqueous NaCl solution were added to induce phase separation. The extract was shaken, and the upper phase was separated and made up to 25 mL. An aliquot was removed, dried under nitrogen gas, and stored at −20°C before HPLC analysis the next day, following the method used for the TRL fractions.

### Extraction and analysis of TRL fractions
The blood preparation, TRL isolation, carotenoid extraction, and HPLC-photodiode array-MS/MS quantitation information were detailed previously (26).

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### Table 1

| Gender | Participants | Age | BMI | Plasma total cholesterol | Plasma TG |
|--------|--------------|-----|-----|--------------------------|-----------|
|        | n | y | kg/m² | mg/dL | mg/dL |
| Sauce study (study 1) | | | | | |
| F | 5 | 24.6 ± 4.6 | 22.4 ± 3.3 | 167 ± 24.7 | 79.2 ± 42.9 |
| M | 6 | 26.7 ± 5.0 | 25.8 ± 2.2 | 151 ± 26.9 | 107 ± 65.1 |
| Carrot study (study 2) | | | | | |
| F | 6 | 28.5 ± 5.0 | 23.1 ± 2.7 | 172 ± 14.8 | 59.2 ± 29.8 |
| M | 6 | 27.2 ± 4.0 | 25.3 ± 2.4 | 166 ± 31.2 | 88.3 ± 87.6 |

1 Values are means ± SDs. Characteristics between genders within each study are not statistically different from each other using a 2-tailed unpaired Student’s t test (P < 0.05).

### Conversion efficiency

To estimate the extent of vitamin A formation (Efficiency A1) in the enterocyte from the β-carotene absorbed in study 1, we used a previously published equation (27), Eq. 1:

\[
\text{Efficiency A1} = \left\{ \frac{\text{AUC}_{\text{retinyl esters}}}{2} \right\} \times 100.
\]

Carrots contain 2 sources of provitamin A: 1) β-carotene; and 2) α-carotene. α-Carotene is a nonsymmetric provitamin A carotenoid, and thus cleavage by BCO1 can only produce 1 molecule of vitamin A (in contrast to cleavage of β-carotene, which can produce 2 molecules of vitamin A). Therefore, a different equation must be used to estimate the extent of vitamin A formed in the enterocyte from both β-carotene and α-carotene absorbed in study 2 (Efficiency A2). Previously published equations (28) were used with slight modifications. The contribution X of both carotenos to the TRL vitamin A pool was calculated by taking into account the relative proportion of β-carotene and α-carotene in the test meal in Eq. 2:

\[
X = \left\{ \frac{\text{AUC}_{\text{retinyl esters}} \times \text{mg β-carotene fed}}{2 + \text{mg total carotenes fed}}} \right\} + \left\{ \frac{\text{AUC}_{\text{retinyl esters}}} {\text{mg α-carotene fed/total carotenes fed}}} \right\}.
\]

For example, for the carrot and avocado meal, the equation is as follows:

\[
X = \left\{ \frac{\text{AUC}_{\text{retinyl esters}} \times \text{(27.4 mg × 2/46.2 mg)}}{\text{mg α-carotene fed/total carotenes fed}}} \right\}.
\]

This value was then divided by the sum of the estimated total carotenes (β-carotene + α-carotene) absorbed from the meal, using Eq. 3:

\[
\text{Efficiency A2} = X / (\text{AUC}_{\text{total β-carotene}} + \text{AUC}_{\text{total α-carotene}} + X) \times 100.
\]

### Statistical analysis

Baseline characteristics of the participants for both study 1 and study 2 were compared between genders using a 2-tailed unpaired Student’s t test (Table 1). Bioavailability of each compound is expressed as the baseline-corrected AUC value in the TRL fraction for the 12 h after meal consumption (i.e., measured TRL amounts of the analyte are normalized to the t = 0 blood draw). AUC values were determined using trapezoidal approximation. A mixed-effects regression approach appropriate for the AB/BA crossover design was used to model each of the outcomes (29). Fixed effects for treatment (test meal alone or with avocado) and period and a random effect for participant were included. Raw AUC values for all compounds were right skewed and were log transformed to meet the model assumptions of normality and homoscedasticity. Thus, AUC median values and the 25th and 75th percentiles after each meal are reported. Interactions between treatment and baseline participant characteristics (age, gender, BMI, LDL, HDL,
and total cholesterol, and TGs) were tested and included in the model if significant at a 0.05 level. Because of the log transformation of the outcomes, model coefficients were interpreted in terms of fold changes. All fold changes are multiplicative (e.g., a 2-fold increase indicates a doubling of the initial value). All analyses were conducted in SAS version 9.3 (SAS Institute).

Results

Participants. Table 1 provides the baseline characteristics of study participants at their initial visit to the clinic. Twelve participants completed study 1 (10 Caucasians, 1 of Indian origin, 1 of Chinese origin), and 12 participants completed study 2 (7 Caucasians, 4 African Americans, 1 of Indian origin). After reviewing the data, 1 Caucasian female participant in study 1 appeared to be a “nonresponder” after carotenoid consumption. Nonresponders were reported previously for carotenoid absorption (30,31), although this seems to be a small percentage of the population (20). Although this participant indicated that she typically followed a “Paleo diet” in the health questionnaire (defined as no grains, processed foods, or added sugar; lots of meat, fruits, vegetables, and full-fat dairy products), the data do not suggest that this affected her carotenoid amount. Given this anomalous response, this participant’s data were dropped from the final dataset.

Absorption of carotenoids. Table 2 provides the amount of fat-soluble carotenoids and vitamins of interest provided by each test food. Median AUC values for nutrients of interest and fold differences between the test meal with and without avocado are provided in Table 3 for study 1 and Table 4 for study 2.

Baseline-corrected plasma TRL concentrations of β-carotene (Fig. 1A) and retinyl esters (Fig. 1B) after consumption of the sauce with or without avocado in study 1 are depicted. Nonresponders were reported previously for carotenoid absorption (30,31), although this seems to be a small percentage of the population (20). Although this participant indicated that she typically followed a “Paleo diet” in the health questionnaire (defined as no grains, processed foods, or added sugar; lots of meat, fruits, vegetables, and full-fat dairy products), the data do not suggest that this affected her carotenoid amount. Given this anomalous response, this participant’s data were dropped from the final dataset.

Conversion efficiency. Figure 3 plots the percentage conversion of provitamin A to vitamin A for each participant when the tomato sauce meal was consumed alone compared with the sauce meal with avocado. For study 1, the range of β-carotene conversion to vitamin A for the sauce alone was 5–47%, with a mean of 22%, whereas the sauce and avocado meal was 22–48%, with a mean of 33%. A strong linear relationship between conversion efficiency of the 2 meals was observed. An equal conversion after consumption of both test meals would result in a regression line through the origin with a slope of 1 (Fig. 3, black line). However, all data points fall in the sector above the black line. Thus, conversion was observed to be more efficient after consuming the lipid-rich test meal. Participant with low conversion efficiency when consuming the sauce meal alone had not improved conversion when the meal was consumed with avocado. Participant with high conversion efficiency with sauce alone had less improvement when the meal was consumed with avocado.

Although a similar linear trend for conversion efficiency was observed with the carrot study, there was much wider variation, with approximately half of the data points falling above a slope of 1 and half falling below. Furthermore, the linear relation was weaker (R² = 0.30). The ratio of α-carotene to β-carotene in the carrot meal (~1:1.4 α-carotene:β-carotene) was mostly maintained in the blood plasma of participants when they consumed the carrot meal with avocado, but this ratio was not maintained when participants consumed the carrot alone (data not shown). The range of total carotene (i.e., β-carotene + α-carotene) conversion to vitamin A from the carrot meal alone was 0–64%, with a mean of 27%, and the carrot with avocado meal was 8–69%, with a mean of 34%, demonstrating a very large interindividual variation.

Discussion

The 2 studies presented herein provide some intriguing results that have direct implications relevant to maximizing provitamin

### Table 2 Fat-soluble nutrient and phytochemical profiles of test foods

| Test food       | β-Carotene (mg) | α-Carotene (mg) | Lutein (mg) | Lycopene (mg) | α-Tocopherol (mg) | Phylloquinone (μg) |
|-----------------|-----------------|-----------------|-------------|---------------|------------------|-------------------|
| Sauce alone     | 33.7 ± 0.21     | ND              | ND          | 2.34 ± 0.01   | ND                | ND                |
| Sauce with avocado | 33.7 ± 0.21 | 0.014 ± 0.007  | 0.12 ± 0.03 | 2.34 ± 0.01   | 2.80 ± 0.29      | 26.2 ± 9.8        |
| Carrot alone    | 27.3 ± 0.7      | 18.7 ± 5.5      | 0.40 ± 0.11 | 0.04 ± 0.01   | 0.0008 ± 0.0009  | 19.8 ± 8.6        |
| Carrot with avocado | 27.4 ± 7.9   | 18.8 ± 5.5      | 0.50 ± 0.13 | 0.04 ± 0.01   | 2.80 ± 0.29      | 46.6 ± 19.9       |

1 Limits of detection were detailed previously (26). ND, not detected.
2 Values are reported as means ± SDs of analyte in 300 g of test food (sauce or carrot), n = 3.
3 Values are reported as means ± SDs of analyte in 300 g of test food (sauce or carrot) + 150 g of avocado, n = 3.
A absorption and efficient conversion to vitamin A. In both studies 1 and 2, the bioavailability of provitamin A carotenoids was considerably improved when the test food was consumed with lipid-rich avocado. These results further support previous findings from our group and others (13–16) that increasing amounts of meal lipid increases carotenoid absorption compared with lower amounts of lipid or no lipid. Likewise, a previous study (15) demonstrated that lipid-rich avocado is just as effective as an equivalent amount of pure avocado oil in enhancing carotenoid absorption. When compared with these previous studies (14,15), we observed a smaller magnitude of AUC carotenoid increase when our test meals were consumed with avocado. This difference may be attributed to the larger dose of carotenoid delivered from the sauce in study 1 and from the carrots in study 2 compared with the previous work (11.5 mg dose of carotenoid delivered from the sauce in study 1 and from avocado). This difference may be attributed to the larger dose of carotenoid delivered from the sauce in study 1 and from the carrots in study 2 compared with the previous work (11.5 mg of \( \beta \)-carotene and 6.6 mg of \( \alpha \)-carotene) (15). In addition, at higher doses, transporter-facilitated carotenoid absorption was shown to be saturable (32), and, furthermore, carotenoids may compete for absorption (33,34).

In study 2, the ratio of AUC\( \beta \)-carotene to AUC\( \alpha \)-carotene was almost equal to the ratio of \( \beta \)-carotene to \( \alpha \)-carotene in carrots when the meal was fed with lipid-rich avocado. Thus, under these meal conditions, \( \beta \)-carotene and \( \alpha \)-carotene appear to be absorbed equally. In contrast, this ratio was not maintained when carrots were fed alone, although very little carotenoid was absorbed in general after this test meal. Results from previous human studies are mixed. Some studies reported that carrot \( \beta \)-carotene absorption was approximately double that of carrot \( \alpha \)-carotene when compared on an equimolar basis, as measured by blood response (17) or fecal carotenoid excretion (35). In contrast, other studies reported a greater percentage absorption of \( \alpha \)-carotene relative to \( \beta \)-carotene from carrots after both postprandial (28) and chronic (36) consumption studies. Many factors likely contribute to the disparity between these results.

Strikingly, avocado consumption with the test meals in studies 1 and 2 also led to higher absolute amounts of retinyl esters (i.e., vitamin A) in the TRL fraction. As a consequence of enhanced carotenoid absorption, the presence of more provitamin A to be converted could at least partially explain the increased appearance of retinyl esters. However, co-consumed lipid may also directly affect other variables that affect conversion, as suggested by a few animal studies. In 1 study, Mongolian gerbils were fed a diet containing carrot powder with 10% lipid (\( n = 12 \)) or 30% lipid (\( n = 12 \)) for 2 wk (22). Animals in the 30% lipid group had considerably higher vitamin A concentrations but lower \( \beta \)-carotene concentrations in liver compared with the 10% lipid group, demonstrating higher conversion with a higher amount of dietary lipid (22). A similar study in ferrets compared the effect of 4 wk of consumption of \( \beta \)-carotene with 6%, 13.4%, or 23% lipid (23). A stepwise increase in dietary lipid was correlated with a stepwise increase in hepatic retinyl ester stores, whereas hepatic \( \beta \)-carotene concentrations for 13.4% or 23% lipid were approximately double those of the 6% group (23). Furthermore, higher consumption of unsaturated lipids was shown to enhance the specific activity of BCO1 in rodents, whereas higher consumption of saturated lipids did not substantially increase BCO1 activity (13). Together, these studies suggest that consuming a higher amount of dietary lipid might increase the conversion rate of provitamin A to vitamin A, particularly when unsaturated lipids (like those found in avocado) are consumed. Besides enhanced enzymatic activity, other research has demonstrated that dietary lipids are necessary for chylomicron synthesis in the enterocyte (37). Thus, increased amounts of retinyl esters in the chylomicron fraction may be a product of increased synthesis and release of chylomicrons containing retinyl esters. Regardless of the mechanism(s) involved, increasing vitamin A formation and delivery to the circulatory system by consuming lipid-rich avocados has practical implications for populations in which vitamin A deficiency is prevalent.

Higher mean conversion rates were reported for \( \beta \)-carotene from various preparations of carrots compared with our

### Table 3: Study 1: AUC and fold differences of carotenoids and vitamins after consumption of sauce alone or with avocado in healthy participants

|          | \( \beta \)-Carotene | \( \alpha \)-Carotene | Retinyl esters | Lutein | Lycopene | \( \alpha \)-Tocopherol | Phylloquinone |
|----------|----------------------|-----------------------|---------------|--------|----------|------------------------|--------------|
|          | nmol/L               | nmol/L                | nmol/L        | nmol/L | nmol/L   | nmol/L                 | nmol/L       |
| Sauce alone (AUC) | 202 (111, 273)       | ND                    | 127 (25, 327) | ND     | 110 (19, 256) | ND                     | ND           |
| Sauce with avocado (AUC) | 437 (269, 730)       | ND                    | 367 (237, 802) | 15 (6.5, 74) | 111 (52, 221) | 4.4 (1.0, 7.4)       | 7.9 (7.0, 16) |
| Fold difference\(^2\) | 2.35 (1.89, 2.93)    | N/A                   | 4.63 (2.84, 7.54) | N/A    | 0.84 (0.30, 2.38) | N/A                   | N/A          |
| \( P \) | <0.0001              | —                     | <0.0001       | —      | 0.71     | —                     | —            |

\(^1\) AUCs are presented as medians (25th, 75th percentiles). \( n = 11 \) (5 females, 6 males). N/A, not applicable; ND, not determined.

\(^2\) Fold difference between carrot co-consumed with avocado vs. carrot alone based on log values presented as geometric means (95% CIs).

### Table 4: Study 2: AUC and fold differences of carotenoids and vitamins after consumption of carrots alone or with avocado in healthy participants

|          | \( \beta \)-Carotene | \( \alpha \)-Carotene | Retinyl esters | Lutein | Lycopene | \( \alpha \)-Tocopherol | Phylloquinone |
|----------|----------------------|-----------------------|---------------|--------|----------|------------------------|--------------|
|          | nmol/L               | nmol/L                | nmol/L        | nmol/L | nmol/L   | nmol/L                 | nmol/L       |
| Carrot alone (AUC) | 88 (24, 125)       | 70 (31, 97)          | 51 (22, 97)  | 34 (3.5, 63) | ND     | ND                     | 0.5 (0.0, 0.7) |
| Carrot with avocado (AUC) | 366 (276, 460)     | 260 (170, 313)       | 327 (234, 490) | 39 (7.4, 70) | ND     | 1.6 (1.0, 2.8)       | 4.6 (3.9, 10) |
| Fold difference\(^2\) | 6.63 (4.05, 10.9)\(^3\) | 4.83 (3.17, 7.36)\(^3\) | 12.6 (3.51, 45.4) | 0.77 (0.15, 4.03) | N/A    | N/A                   | 15.0 (7.19, 31.3) |
| \( P \) | <0.0001              | <0.0001               | 0.0013        | 0.73   | —        | —                     | <0.0001      |

\(^1\) AUCs are presented as medians (25th, 75th percentiles). \( n = 12 \) (6 females, 6 males). N/A, not applicable; ND, not determined.

\(^2\) Fold difference between tomato sauce co-consumed with avocado vs. sauce alone based on log values presented as geometric means (95% CIs).

\(^3\) Fold difference at age 28 y based on log values.
A mean conversion efficiency of 44% was observed for carrot puree, 59% for boiled mashed carrots, and 63% for raw chopped carrots (each delivering 18.6 mg of β-carotene co-consumed with 9 g of safflower oil). In contrast, similar conversion rates were reported when 15 mg of β-carotene was fed in pure oil (33–71% conversion) (38) or palm oil (conversion rate of 69–71%) (39). These studies fed lower doses of β-carotene, which may have contributed to the higher efficiency of conversion.

In study 1, those participants with lower conversion efficiency had a more appreciable increase in conversion with lipid-rich avocado than those with higher efficiency of conversion. It is likely that an individual’s vitamin A status will have an impact on provitamin A absorption and subsequent conversion. The liver is the central storage organ of vitamin A and regulator of circulating retinol concentrations (40), whereas blood retinol concentrations remain constant over a wide range of intakes unless an individual is severely deficient or has excessively high consumption (41,42). Thus, the most accurate way to assess vitamin A stores in a healthy population is to estimate liver stores with a deuterated-retinol dilution method (43,44). Because we assumed that all of our participants, as healthy adults living in the United States, had adequate or higher hepatic vitamin A stores, we did not measure this value. We believe it is more likely that differences in percentage conversion between participants could be related to specific polymorphisms in proteins involved in carotenoid uptake, transport, and/or metabolism in the enterocyte, as reviewed recently (45).

In study 2, the weaker relation of conversion efficiency with and without avocado lipid observed in the carrot study (28). A mean conversion efficiency of 44% was observed for carrot puree, 59% for boiled mashed carrots, and 63% for raw chopped carrots (each delivering 18.6 mg of β-carotene co-consumed with 9 g of safflower oil). In contrast, similar conversion rates were reported when 15 mg of β-carotene was fed in pure oil (33–71% conversion) (38) or palm oil (conversion rate of 69–71%) (39). These studies fed lower doses of β-carotene, which may have contributed to the higher efficiency of conversion.
ester as substantial AUC changes for phylloquinone were observed when carrot with avocado meal (Table 2). Studies demonstrating likely due to the significant increase in phylloquinone amounts in the carrot study different for study 2 because of the low and comparable lutein consumed. regions where red tomatoes are traditionally grown and orange tomatoes could be adapted in vitamin A–deficient used to ameliorate this problem. Similarly, this novel variety of dense versions of currently consumed vegetables (55–57), were provitamin A amounts (53,54), and substituting more nutrient–ified rice (52), selective plant breeding of crops with higher fortification (47–49), supplementation (50,51), genetically mod–developing world, and a variety of strategies, including food to determine the mechanism(s) responsible. Future studies are needed matrix effects (cooked tomato sauce vs. raw carrot) may have calculation and not a measured value. Differences in food Mongolian gerbils (46). However, this is a mathematical decision to assume that could be explained by a number of factors, for example, the contribution of α-carotene in carrots to the vitamin A pool. Our the sauce meal alone was much higher. In addition, the uptake of β-carotene from the carrot meal alone was generally very low (Fig. 2). In contrast, the absorption of β-carotene from the sauce meal alone was much higher. In addition, the difference between the results of the sauce and carrot studies could be explained by a number of factors, for example, the contribution of α-carotene in carrots to the vitamin A pool. Our decision to assume that α-carotene provides half as much retinyl ester as β-carotene in Eq. 2 is supported by a previous study with Mongolian gerbils (46). However, this is a mathematical calculation and not a measured value. Differences in food matrix effects (cooked tomato sauce vs. raw carrot) may have also affected the conversion efficiency. Future studies are needed to determine the mechanism(s) responsible.

Vitamin A deficiency represents a real problem in the developing world, and a variety of strategies, including food fortification (47–49), supplementation (50,51), genetically modified rice (52), selective plant breeding of crops with higher provitamin A amounts (53,54), and substituting more nutrient–dense versions of currently consumed vegetables (55–57), were used to ameliorate this problem. Similarly, this novel variety of orange tomatoes could be adapted in vitamin A–deficient regions where red tomatoes are traditionally grown and consumed.

Differences in lutein AUC values were not significantly different for study 2 because of the low and comparable lutein content of the 2 test meals (Table 2). We observed a statistically significant increase in phylloquinone amounts in the carrot study when consumed with avocado compared with carrot alone, likely due to the ~2.4-fold higher dose of phylloquinone in the carrot with avocado meal (Table 2). Studies demonstrating substantial AUC changes for phylloquinone were observed when ~0.4–1 mg of this nutrient was fed to humans (58–60) compared with the sauce study may be attributed to the fact that the uptake of carotenes from the carrot meal alone was generally very low (Fig. 2). In contrast, the absorption of β-carotene from the sauce meal alone was much higher. In addition, the difference between the results of the sauce and carrot studies could be explained by a number of factors, for example, the contribution of α-carotene in carrots to the vitamin A pool. Our decision to assume that α-carotene provides half as much retinyl ester as β-carotene in Eq. 2 is supported by a previous study with Mongolian gerbils (46). However, this is a mathematical calculation and not a measured value. Differences in food matrix effects (cooked tomato sauce vs. raw carrot) may have also affected the conversion efficiency. Future studies are needed to determine the mechanism(s) responsible.

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In conclusion, consuming provitamin A carotenoids with lipid–rich avocado enhances carotenoid absorption in healthy humans. A notably higher concentration of vitamin A was observed in the TRL fraction when the carotene–rich tomato sauce or carrots were fed with lipid–rich avocado compared with no avocado. Furthermore, consuming lipid–rich avocado with provitamin A from a high–β–carotene tomato sauce led to higher conversion efficiency to vitamin A in participants with low conversion efficiency. This observation highlights the importance of consuming provitamin A carotenoids with lipid in the meal, especially in vitamin A–deficient populations in which maximum delivery of active vitamin A is desired.

### Acknowledgments

The authors thank Dr. Dennis Pearl for his advice regarding the statistical analysis of the data and the Hass Avocado Board for providing avocados for the feeding study. R.E.K., D.M.F., E.H.H., S.K.C., and S.J.S. designed the research; R.E.K., J.L.C., and R.M.S. conducted the research; R.E.K., R.M.S., and G.S.Y. analyzed the data; R.E.K. wrote the paper; and S.J.S. had primary responsibility for the final content. All authors read and approved the final manuscript.

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