

5-2012

Impact of Meal Patterns on Carotenoid Absorption From Vegetables

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IMPACT OF MEAL PATTERNS ON CAROTENOID ABSORPTION FROM
VEGETABLES

By

Teryn Sapper

A Thesis Submitted in Partial Fulfillment
Of the Requirements for a Degree with Honors
(Dietetics)

The College of Nutrition Science

Purdue University

May 2012

West Lafayette, Indiana

Approved by

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ACKNOWLEDGEMENTS

The author of this thesis would like to thank Shellen Goltz for her equal collaboration throughout the clinical trial and composition of this thesis and Dr. Mario Ferruzzi for his guidance throughout the honors process.

***Adapted with permission from; Goltz, Shellen R. Impact of Dietary Lipids and Meal Patterns on Carotenoid Bioavailability from Raw Vegetables. Purdue University, 2012.

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ABSTRACT

Sapper, Teryn N. Bachelors of Science, Purdue University, Spring 2012. Impact of meal patterns on carotenoid absorption from vegetables. Major Professor: Mario Ferruuzi.

While the impact of food composition and processing on carotenoid bioavailability has been the subject of several investigations, the effect of meal patterning remains unknown. The aim of this pilot study was to assess the impact of select consumption patterns on the bioavailability of carotenoids from vegetables. On three randomized testing days, subjects consumed equal amounts of fat-free “chef’s salad” ingredients at two meals representing lunch and dinner, along with raw salad vegetables and 8g canola oil split between meals in the following patterns: 100 and 0%; 75 and 25%; and 50 and 50%. Blood was collected from 0-12h and triacylglycerol rich lipoprotein fractions (TRL) were isolated by ultracentrifugation. TRL carotenoid concentrations were analyzed by HPLC-PDA. Considering all carotenoids, absorption was greatest when $\geq 75\%$ of vegetables were consumed in the first meal ($P < 0.05$). Absorption of apolar carotenes also followed this trend ($P < 0.05$ for α - and β -carotene). For more polar xanthophylls, although not significant, consumption of all vegetables in the first meal promoted greater absorption compared to 75% or less at one time. These data suggest that, within the context of typical meal patterns, daily carotenoid absorption is greatest when consumed in one meal compared to smaller doses over multiple meals.

INTRODUCTION

Carotenoids are the most widely dispersed group of pigments, with approximately 700 different species identified to date (Maiani et al, 2008). Coupled with their antioxidant activity, increased intake of carotenoid-rich vegetables has been associated with reduced risk of several chronic diseases including cancer, macular degeneration, and coronary heart disease (Steinmetz and Potter, 1996; Rissanen et al, 2002; Giovannucci, 2005; Carpentier et al, 2009). Despite these associations, Americans consume only 1.6 cups of the 2.5 cups of vegetables recommended by the 2010 Dietary Guidelines each day (USDA, 2010). Yet, increases in the consumption of several salad vegetables including leafy greens, carrots, bell peppers, and fresh tomatoes are becoming apparent (Wells and Buzby, 2008). Therefore, strategies to optimize absorption of carotenoids from these commonly consumed vegetables are needed.

Absorption of carotenoids occurs primarily in the small intestine, in several steps including: 1) Release from the food matrix, 2) Incorporation into bile-salt mixed micelles, 3) Uptake by epithelial cells, and 4) Chylomicron packaging and secretion into the lymphatic system (Yeum and Russell, 2002; Yonekura and Nagao, 2007). Several factors have been reported to impact carotenoid bioavailability in humans, summarized by the mnemonic “SLAMENGHI”: *S*pecies of carotenoids, *L*inkages at molecular level,

Amount of carotenoid, *Matrix*, *Effectors*, *Nutrient status*, *Genetics*, *Host related factors* and *Interactions* among these variables (Castenmiller and West, 1998).

While many of these factors have been studied extensively, the impact of meal patterning, or the division of carotenoid-rich foods among daily meals, has remained largely unexplored. Suggested USDA meal patterns for a 2,000 calorie diet split recommended vegetables between lunch (1 cup), snack (1/2 cup) and dinner (1 cup) meals (CNNP, 2010). Similarly, sample meal plans developed by the USDA's Center for Nutrition Public Policy split recommended vegetable intake between lunch and dinner meals alone, likely due to American preferences for consuming vegetables at these meals (CNNP, 2011b). Interestingly, one previous report demonstrated that the bioavailability of purified β -carotene was increased when taken in three small daily doses (17 mg each) in place of one large dose (51 mg) (Prince and Frisoli, 1993). However, whether the greatest daily carotenoid absorption will also result from dividing one large dose of commonly consumed vegetables between meals, as suggested by USDA meal patterns, remains to be evaluated.

The objective of this pilot study was to assess the impact of select consumption patterns on the bioavailability of carotenoids from vegetables. This salad provided a total of 2.5 cups of vegetables, the amount recommended daily by the 2010 Dietary Guidelines.

Subjects were instructed to consume these vegetables with additional fat-free, “chef’s salad” ingredients over a two-meal period in three different meal patterns. A moderate amount of canola oil was added to salads as dressing to promote carotenoid absorption while meeting recommendations for added lipids by the 2010 Dietary Guidelines (USDA, 2010).

METHODS AND MATERIALS

Chemicals, Standards, and Test Meal Ingredients

Lutein, zeaxanthin, β -cryptoxanthin, β -carotene, and lycopene standards, as well as aprotinin, phenylmethanesulfonylfluoride (PMSF), sodium azide, ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), sodium bicarbonate, sodium chloride, and anhydrous sodium sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). α -carotene and α -cryptoxanthin standards were purchased from CaroteNature (Lupsingen, Switzerland). All solvents and celite were obtained from J.T. Baker (Phillipsburg, NJ). All chef’s salad ingredients were purchased at a local market. Canola oil was generously donated by Cargill, Incorporated (Minneapolis, MN).

Subjects

Subjects were recruited and screened as outlined in Goltz et al. (2012). However, due to the small sample size of this pilot study, only men were recruited in order to reduce variability of the data collected. It is expected that a full-scale trial with both men and women will be completed in the future to evaluate whether changes in carotenoid bioavailability based upon meal patterns is affected by sex. A total of six subjects were enrolled and all six subjects completed the study. Average age and BMI of the subjects were 28 ± 2 y (range 23-36) and 23.4 ± 0.4 kg/m² (range 21.6-25.0), respectively. Informed consent was obtained from all subjects and the Purdue University Committee on the Use of Human Research Subjects approved study procedures.

Experimental Design

Each subject completed three separate treatments (A, B, and C) in random order (**Table 1**). During each treatment, subjects consumed identical vegetable salads and “chef’s salad” ingredients over a two-meal period, representing lunch and dinner, differing only in the amount of salad vegetables and oil consumed at each meal. In treatment A, subjects consumed 100% of salad vegetables and oil in the first meal to represent a large salad consumed as a full meal. In treatment B, subjects consumed 50% of salad vegetables and oil at each meal, representing two small side salads at each meal. Finally, treatment C represented a scenario in which a large and small salad was consumed at

lunch and dinner meals respectively. A moderate amount (8g total) of canola oil was added to salads each testing day to promote carotenoid absorption as it has been documented that negligible carotenoid absorption takes place in the absences of co-consumed dietary lipid (Jayarajan et al, 1980; Prince and Frisoli, 1993; Brown et al, 2004; Unlu et al, 2005). One slice of bread was provided with each meal and was used to wipe any residual oil from salad bowls to ensure that all oil was consumed.

The experimental time course for each subject lasted for approximately 5 weeks (**Figure 1**). Each testing day was directly preceded by a 5-day washout and 2-day controlled diet period and followed by a 1-week free-living period.

Table 1 Meal Patterns by Treatment

		Treatment		
		A	B	C
<u>Meal #1:</u>	Salad Vegetables	All	1/2	3/4
	Chef's Salad Ingredients	1/2	1/2	1/2
	Canola Oil	8g	4g	6g
	White Bread	1 slice	1 slice	1 slice
<u>Meal #2:</u>	Salad Vegetables	None	1/2	1/4
	Chef's Salad Ingredients	1/2	1/2	1/2
	Canola Oil	0g	4g	2g
	White Bread	1 slice	1 slice	1 slice

On each of the three test days (day 8 of each test period), subjects reported to the CRC following a 12-hour fast, and a catheter equipped with a disposable obturator was inserted into an antecubital vein by a trained phlebotomist. After collection of a baseline blood sample (15mL), subjects immediately consumed test meal #1 (**Table 1**) within 30 minutes. Upon completion of the meal, blood samples (12 mL) were collected at hours 2-10, and 12. Test meal #2 (**Table 1**) was provided at hour 4 and consumed within 30 minutes. Protocols for drawing blood and processing to plasma were identical to those outlined in Goltz et al. (2012). Water was allowed ad libitum throughout the day. A courtesy meal was provided to all subjects following completion of each test day.

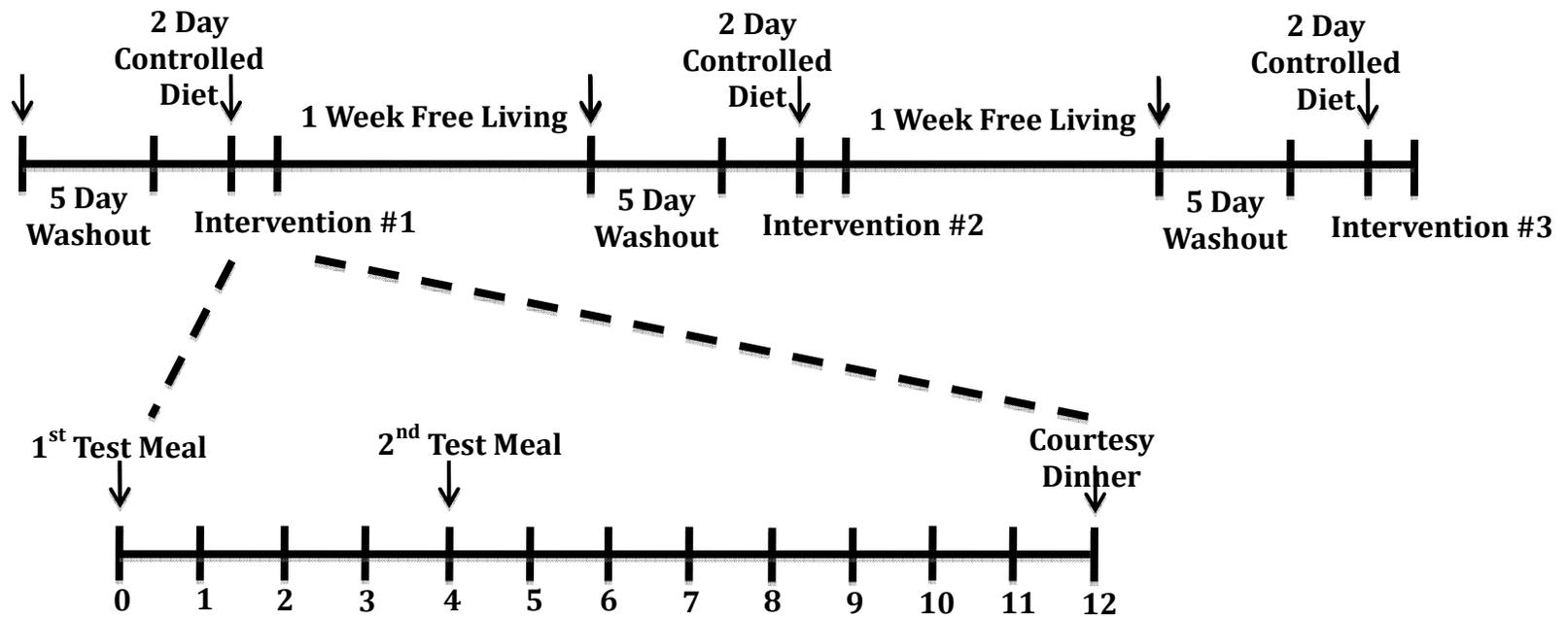


Figure 1 Experimental time course for each subject. Each subject consumed vegetable salads, oil, and additional “chef’s salad” ingredients over a two-meal period (hours 0 and 4) in three different, randomly assigned dosing patterns. Blood was collected at hours 0, 2-10, and 12 for TRL isolation on each intervention day. Collection of blood (□) for plasma carotenoid analysis was conducted at the beginning of each washout period and prior to consumption of each test salad to evaluate compliance to the washout and controlled diets.

Chef's Salad Test Meals

The test meals consisted of salad vegetables and additional, “chef’s salad” protein-based ingredients. As previously noted, canola oil and white bread were also provided with the test meals (**Table 1**). Composition and approximate carotenoid contents of “chef’s salad” ingredients are shown in **Table 2**. Vegetable composition of test salads provided the 2.5 cups of vegetables recommended daily by the 2010 Dietary Guidelines (USDA, 2010). Representative samples of weekly salad vegetables were homogenized using an immersion blender (KitchenAid, Benton Harbor, MI), flushed with nitrogen, and stored at -20°C until analysis. Composition and average carotenoid contents provided by salad vegetables throughout the study are shown in **Table 3**. Total carotenoid content of the test salads during the study period ranged from 20-30 mg and provided an average of 23 mg of total carotenoids per serving (**Figure 3**). Individual and total carotenoid concentrations in TRL fractions of plasma were normalized by salad carotenoid concentrations obtained during the same week of testing to offset weekly variability in composition.

Table 2 Composition and approximate carotenoid content of “chef’s salad” ingredients. ¹

Ingredient	Amount	Approximate Carotenoid Content
Fat Free Turkey	2 oz	0 mg
Fat Free Ham	2 oz	0 mg
Egg Whites	2 oz	0 mg

¹Estimated from USDA National Nutrient Database for Standard Reference (U.S.Department of Agriculture, 2004).

Table 3 Composition and average carotenoid content of salad vegetables. ¹

Vegetable Composition		Carotenoid Content	
Ingredient (Raw)	Weight (g)	Species	Weight (mg)
Beefsteak Tomatoes	100	Lutein	3.17 ± 0.03
Julienne Carrots	62	Zeaxanthin	7.07 ± 0.33
Baby Spinach	70	α-cryptoxanthin	0.05 ± 0.00
Romaine Lettuce	25	β-cryptoxanthin	0.36 ± 0.01
Chinese Wolfberry	5	α-carotene	2.91 ± 0.26
		β-carotene	5.77 ± 0.69
		Lycopene	3.97 ± 0.39
Total	262		23.29 ± 1.54

¹Values obtained via carotenoid extraction, as described in materials and methods, and analysis via HPLC-DAD.

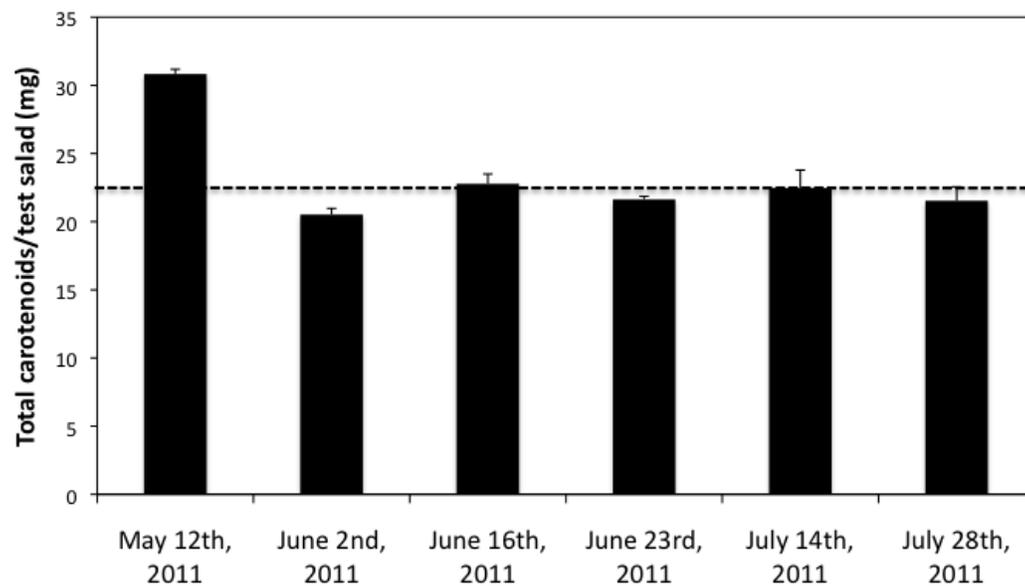


Figure 2 Total carotenoid content of test salads provided throughout the study. Total carotenoid content of the test salads during the study period ranged from 20-30 mg and provided an average of 23 mg per serving (- - -).

TRL Isolation

TRL fractions were isolated via ultracentrifugation based upon the procedures described by Weintraub (Weintraub et al, 1987) and Gianturco and Bradley (Gianturco and Bradley, 1986) as outlined in Goltz et al. (2012).

Extraction and HPLC Analysis of Carotenoids in Plasma and TRL Fractions

Carotenoids were extracted from plasma and from plasma TRL fractions based upon the procedures described by Brown et al (2004) with minor modifications as outlined in Goltz et al. (2012). Carotenoid analysis was completed by HPLC-DAD based upon the methods of Kean et al. (2008) as outlined in Goltz et al. (2012).

Extraction and HPLC Analysis of Carotenoids in Salad Vegetables

Carotenoids were extracted from salad vegetables and analyzed using HPLC-DAD as outlined in Goltz et al. (2012).

Analysis of Triacylglycerol and Cholesterol Content of TRL Fractions

As described in Goltz et al. (2012), TRL fraction total cholesterol and TAG were analyzed in duplicate by a Cobas MIRAS Plus chemistry analyzer (Roche Analytical Instruments, Nutley, NJ).

Data Analysis

Post-prandial TRL TAG and carotenoid concentrations were baseline corrected using fasting values and baseline corrected 0-12 hour area-under-the-curve (AUC_{0-12h}) for TAG and carotenoids were calculated using the PK functions plug-in for Microsoft Excel (Joel I. Usansky). Baseline corrected AUC_{0-12h} were normalized by salad carotenoid

concentrations obtained during the same week of testing in the same manner described in Goltz et al. (2012). The maximum plasma concentration (C_{MAX}), and time at which the maximum plasma concentration was observed (T_{MAX}) were determined from individual plasma pharmacokinetic curves and expressed as mean \pm standard error of the mean (SEM). The impact of meal patterns on TAG and carotenoid AUC_{0-12h} , C_{MAX} , and T_{MAX} , values were estimated by ANOVA using the MIXED procedure in SAS 9.1.4 (Cary, NC). Differences between treatments were determined by Bonferroni's multiple comparison test ($\alpha < 0.05$, two-tailed). Differences in plasma carotenoid concentrations before and after washout periods were determined using a student's T-test (two-tailed). Data are reported as mean \pm SEM.

RESULTS

Adequacy of combined washout and controlled diet period

Significant reductions ($P < 0.05$) were observed in concentrations of each individual carotenoid and for total plasma carotenoids following the 7-day washout and controlled diet periods, indicating adequacy of the washout protocol. Average reductions in carotenoid content following washout protocols were 45.9% for lutein, 43.7% for zeaxanthin, 33.0% for α -cryptoxanthin, 40.7% for β -cryptoxanthin, 38.1% for α -carotene,

44.8% for β -carotene, 36.4% for *cis* lycopene, 57.1% for all-*trans* lycopene, 41.4% for 5-*cis* lycopene, and 42.7% for total carotenoids (**Figure 3**).

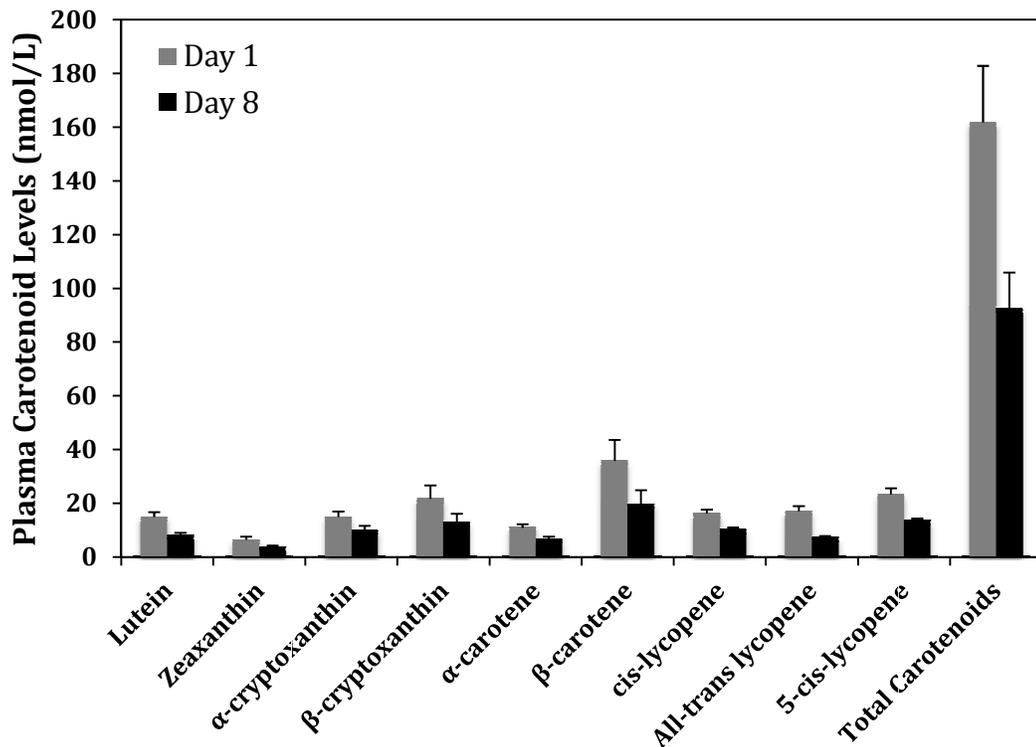


Figure 3. Mean (\pm SE) plasma carotenoid levels before (day 1) and after (day 8) washout and controlled diet periods as analyzed by HPLC-DAD. Significant reductions ($P < 0.05$) were observed in concentrations of each individual carotenoid and for total carotenoids, indicating adequacy of washout protocols.

Change in TRL TAG and carotenoid content

The impact of meal patterns on carotenoid bioavailability was primarily assessed for the most abundant carotenoids in the test salad including the xanthophylls, lutein and zeaxanthin, and the apolar carotenes and lycopene. Although less abundant carotenoids (α -cryptoxanthin, β -cryptoxanthin and *cis*-lycopene) are not shown individually, data for these compounds are included in the calculation for total carotenoid absorption.

When compared by individual treatment group, no significant differences were noted in individual or total carotenoids response based on treatment order. Retrospective power calculations indicated that a total of 14 subjects would have been required to detect differences between individual groups. In an effort to highlight trends observed in the data, results from two treatment groups were combined and compared to the third group for each carotenoid species and for combined carotenoids. More specifically, differences in carotenoid absorption were compared when subjects consumed 100% of salad vegetables and oil in the first meal (treatment A) versus when salads were split into two meals (treatments B+C). Absorption was also compared when subjects consumed the majority of salad vegetables and oil in the first meal (treatments A+C) versus when subjects ate 50% of the vegetables and oil at each meal (treatment B).

Total carotenoid and TAG

Analysis of TRL AUC_{0-12h} values revealed that, although treatments A and C promoted greater absorption of combined carotenoids than treatment B, differences between the three main treatment groups were not significant (**Table 4, Figure 4a**). Following combination of data from treatments A and C, reanalysis suggested that consumption of 75% or more of salad vegetables in the first meal promoted significantly greater absorption compared to consumption of 50% in each meal ($P < 0.05$) (**Table 4, Figure 4a**). Differences between treatments B+C and treatment A were not significant, as the relatively low amount of absorption resulting from treatment B was increased when combined with treatment C. Overall, these results did not reflect the lipemic responses following the consumption of test meals, as TAG AUC_{0-12h} did not differ between groups (**Figure 4a**).

Analysis of PK curves indicated that C_{MAX} values reflected trends observed for AUC_{0-12h} for combined carotenoids with greater values resulting from consuming 75% or more of the test salad in the first meal compared to consumption of 50% at each meal ($P = 0.06$) (**Table 4, Figure 4b**). T_{MAX} values were not significantly different between individual or combined treatments, however the PK response curves did appear quite different, especially between treatments A and B (**Figure 4b**). When 100% of salad vegetables and oil were consumed in the first meal, a large increase in absorption occurred 2 hours after

ingestion of the first meal. TRL concentrations remained elevated through intake of the second meal (hour 4) and up to hour 6 before declining. When 50% of vegetables and oils were consumed at each meal, a relatively small increase in absorption occurred at hour 2 and dropped quickly. The second meal caused an additional peak response in TRL carotenoid concentrations that remained elevated until hour 9. PK response curves for carotenoids and TAG were similar. However, difference in TAG PK response resulted in similar TRL AUC_{0-12h} values between treatments, but differences in carotenoid PK response did not (**Figure 4**).

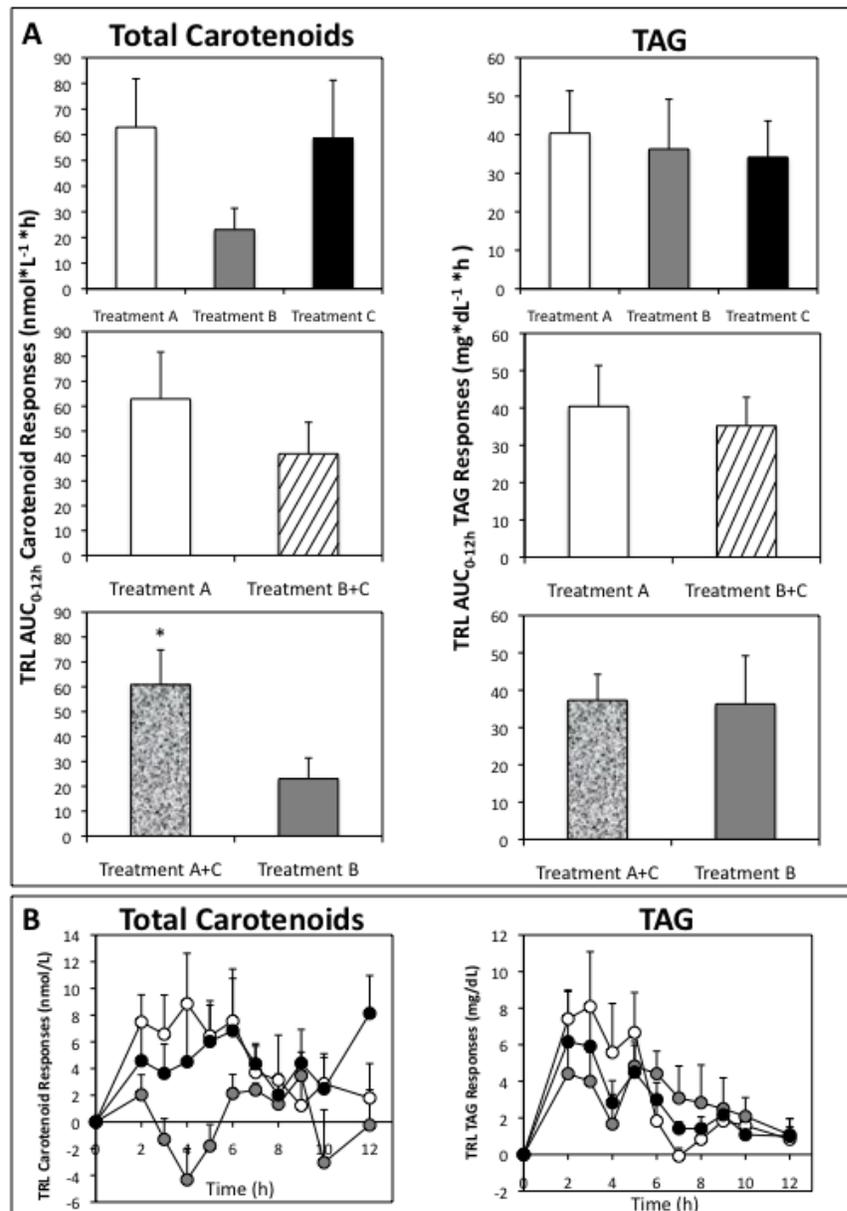


Figure 4 A) Impact of meal patterns on absorption of carotenoids and TAG from salad by human subjects. Mean (\pm SE) TRL AUC_{0-12h} for total carotenoid and TAG concentrations following ingestion of salads when 100% (○), 75% (●), 50% (●), 50%+75% (●), or 75% + 100% (●) of salad vegetables and oil were consumed in the first meal. Significant differences between treatments are noted by an asterisk. **B) Pharmacokinetic response of total carotenoids and TAG following consumption of salads.** Mean (\pm SE) for TRL total carotenoids and TAG following ingestion of salads when 100% (○), 75% (●), or 50% (●) of salad vegetables and oil were consumed in the first meal.

Table 4 Mean (\pm SE) baseline corrected and normalized TRL carotenoid AUC_{0-12h}, C_{MAX}, and T_{MAX} values.^{1,2}

Area Under the Curve – AUC _{0-12h} (nmol*L ⁻¹ *h)						
Treatment	LUT	ZEA	AC	BC	LYC	Total CART
A	8.7 \pm 2.6	3.1 \pm 1.0	5.1 \pm 1.3	22.2 \pm 7.6	6.6 \pm 3.5	62.9 \pm 18.8
B	4.0 \pm 1.3	1.2 \pm 0.3	2.3 \pm 0.8	7.9 \pm 2.5	3.7 \pm 1.3	23.0 \pm 8.3
C	5.1 \pm 1.3	1.5 \pm 0.6	6.1 \pm 2.3	25.5 \pm 11.5	8.0 \pm 2.3	55.2 \pm 22.6
A+C	6.9 \pm 1.5	2.3 \pm 0.6	5.6 \pm 1.3 *	23.8 \pm 6.6 *	7.3 \pm 2.0	60.8 \pm 14.0 *
B+C	4.5 \pm 0.9	1.3 \pm 0.3	4.2 \pm 1.3	16.7 \pm 6.2	5.9 \pm 1.4	40.9 \pm 12.7
Concentration Maximum – Cmax (nmol*L ⁻¹)						
Treatment	LUT	ZEA	AC	BC	LYC	Total CART
A	1.7 \pm 0.4	0.6 \pm 0.2	0.5 \pm 0.2	4.0 \pm 1.4	1.1 \pm 0.4	11.1 \pm 2.9
B	1.0 \pm 0.3	0.3 \pm 0.1	0.6 \pm 0.1	1.9 \pm 0.4	1.2 \pm 0.3	6.0 \pm 1.2
C	0.7 \pm 0.3	0.4 \pm 0.1	1.1 \pm 0.4	3.9 \pm 1.5	1.5 \pm 0.3	10.8 \pm 3.5
A+C	1.4 \pm 0.3	0.5 \pm 0.1	1.0 \pm 0.2	4.0 \pm 1.0	1.3 \pm 0.3	11.0 \pm 2.2
B+C	1.1 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.2	2.9 \pm 0.8	1.3 \pm 0.2	8.4 \pm 1.9
Time of Maximum Concentration – Tmax (h)						
Treatment	LUT	ZEA	AC	BC	LYC	Total CART
A	5.5 \pm 0.8	6.9 \pm 1.0	5.2 \pm 1.1	4.7 \pm 0.7	5.8 \pm 0.9	5.2 \pm 0.6
B	8.0 \pm 1.0	9.1 \pm 1.1	5.6 \pm 1.4	5.5 \pm 1.1	5.8 \pm 1.3	5.2 \pm 1.4
C	8.4 \pm 1.8	8.8 \pm 1.2	6.4 \pm 1.5	5.7 \pm 1.4	6.6 \pm 1.5	7.8 \pm 1.9
A+C	6.8 \pm 1.1	7.8 \pm 0.8	5.7 \pm 0.9	5.2 \pm 0.8	6.2 \pm 1.0	6.5 \pm 1.1
B+C	8.0 \pm 1.4	9.1 \pm 1.1	5.6 \pm 1.4	5.6 \pm 0.8	5.8 \pm 1.4	5.2 \pm 1.7

¹For each carotenoid, significant differences ($P < 0.05$) between groups A + C and B are denoted by an asterisk. No other statistically significant differences are present.

²Normalization was carried out by dividing baseline corrected AUC_{0-10h} values by the percent of carotenoids consumed on the week of testing compared to the average amount of carotenoids provided in all salads throughout the study.

Xanthophylls

Similar to trends observed for total carotenoids, no significant differences were noted between the three main treatment groups. In contrast to total carotenoids, treatments B and C resulted in the similar absorption patterns for both lutein and zeaxanthin, and promoted less absorption compared to treatment A. Thus, although not significant, consumption of 100% of salad vegetables and oil in the first meal promoted seemingly greater absorption of xanthophylls compared to splitting the test meal into two smaller doses (**Table 4, Figure 5a**). Comparing treatments A+C versus treatment B also did not result in significant differences as the relatively low absorption resulting from treatment C dampened the response of treatment A. Neither differences in C_{MAX} nor T_{MAX} values were significantly different between individual or combined treatments (**Table 4, Figure 5b**).

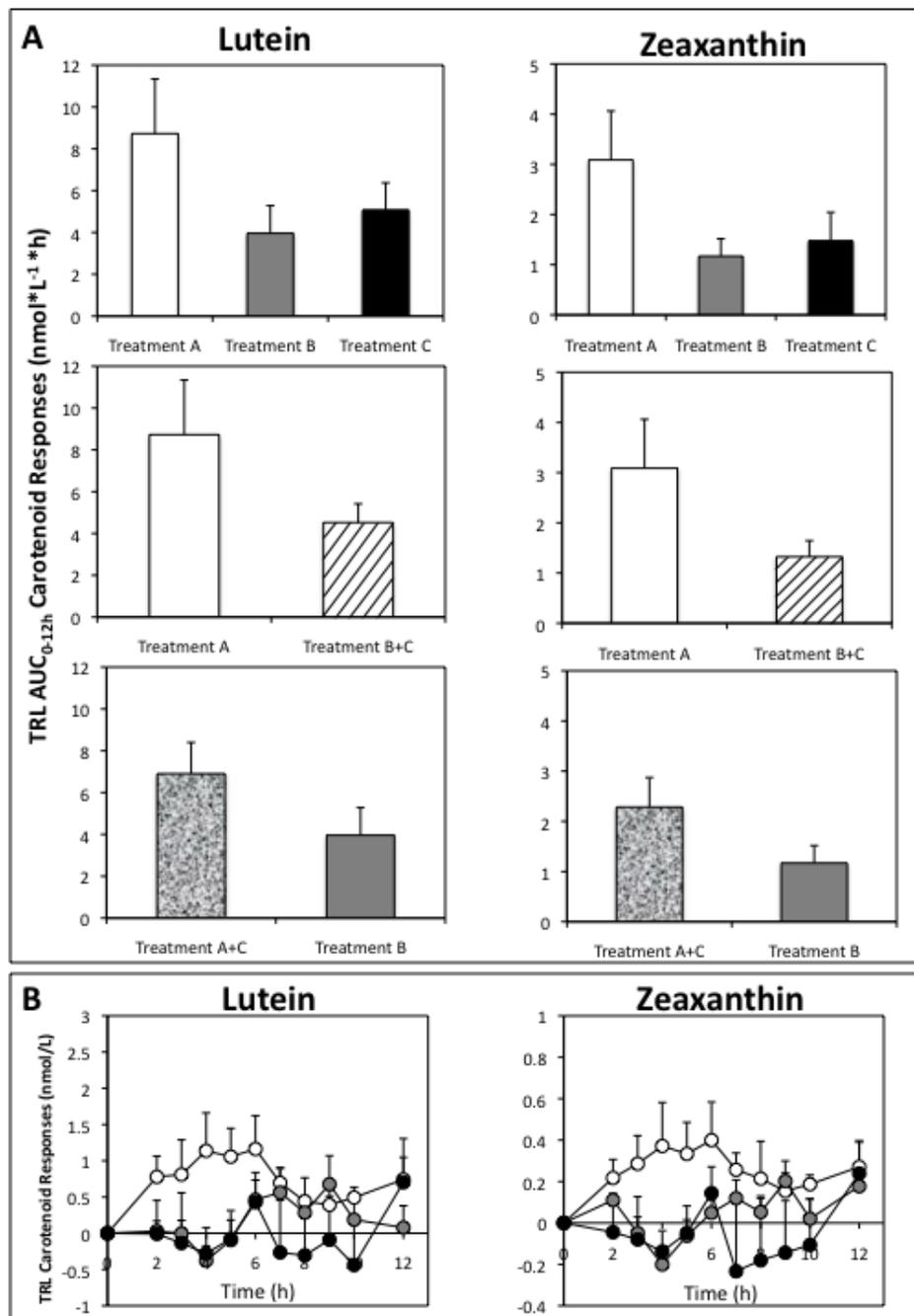


Figure 5 A) Impact of meal patterns on absorption of xanthophylls from salad by human subjects. Mean (\pm SE) TRL AUC_{0-12h} for lutein and zeaxanthin concentrations following ingestion of salads when 100% (○), 75% (◐), 50% (◑), 50%+75% (◒), or 75% + 100% (◔) of salad vegetables and oil were consumed in the first meal. Differences between treatments were not significant. **B) Pharmacokinetic response of xanthophylls following consumption of salads.** Mean (\pm SE) for TRL lutein and zeaxanthin following ingestion of salads when 100% (○), 75% (◐), or 50% (◑) of salad vegetables and oil were consumed in the first meal.

Carotenes

As with total carotenoids, although differences in absorption between the three main treatment groups were not significant, consumption of 75% or more of salad vegetables in the first meal (treatments A+C) promoted significantly greater absorption of both α - and β -carotene compared to consumption of 50% in each meal (treatment B) ($P < 0.05$) (**Table 4, Figure 6a**). Analysis of PK response curves indicated that C_{MAX} values reflected trends observed for AUC_{0-12h} for both carotenes, as higher values resulted from consumption of 75% or more of the test salad in the first meal (α -carotene: $P = 0.09$; β -carotene: $P = 0.08$) (**Table 4, Figure 6b**). T_{MAX} values were not significantly different between treatments, and PK responses exhibited similar trends compared to the curve for total carotenoids (**Figure 4b and Figure 6b**).

All-trans lycopene

As with total carotenoids, xanthophylls, and carotenes, no significant differences were noted between the three main treatment groups. Similar to the carotenes and total carotenoids, absorption of all-*trans* lycopene was promoted when 75% or more of the test salad was consumed in the first meal compared to 50% in each meal, however these differences were not significant (**Table 4, Figure 7a**). C_{MAX} and T_{MAX} values were also unaffected by treatment (**Table 4, Figure 7b**).

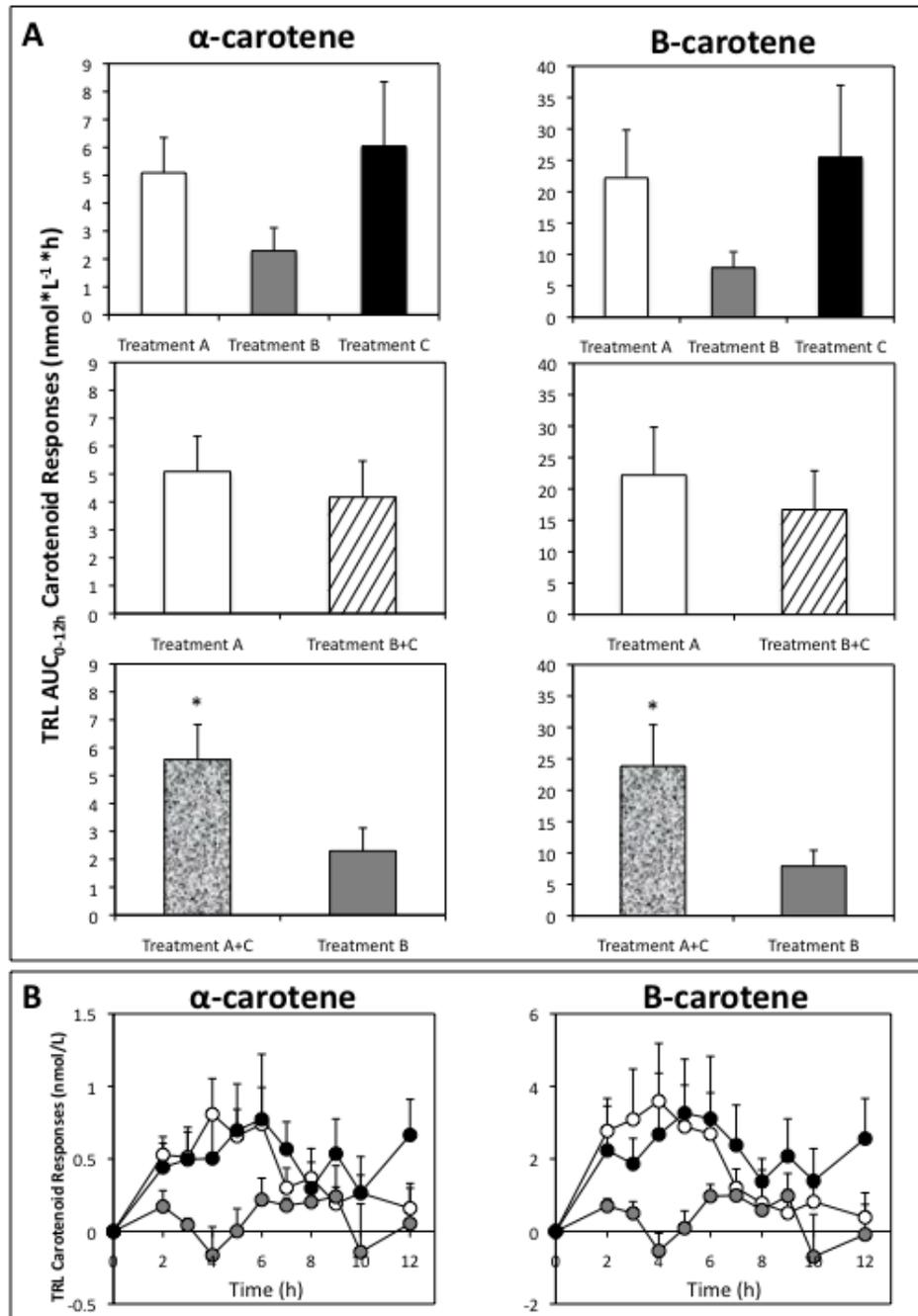


Figure 6 A) Impact of meal patterns on absorption of carotenes from salad by human subjects. Mean (\pm SE) TRL AUC_{0-12h} for α - and β -carotene following ingestion of salads when 100% (○), 75% (●), 50% (●), 50%+75% (●), or 75% + 100% (●) of salad vegetables and oil were consumed in the first meal. Significant differences between treatments are noted by an asterisk. **B) Pharmacokinetic response of carotenes following consumption of salads.** Mean (\pm SE) for α - and β -carotene following ingestion of salads when 100% (○), 75% (●), or 50% (●) of salad vegetables and oil were consumed in the first meal.

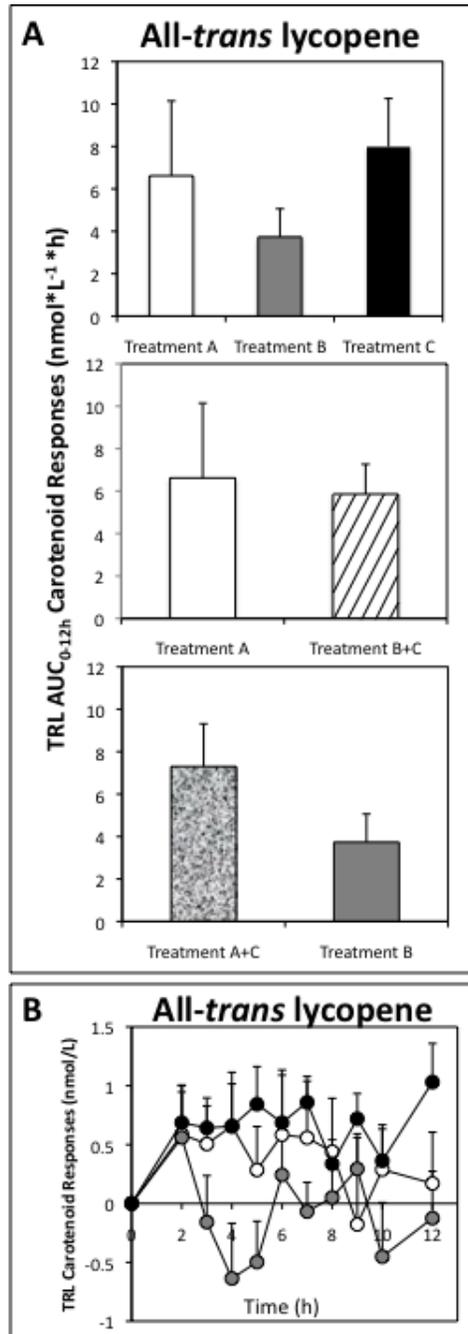


Figure 7 A) Impact of meal patterns on absorption of lycopene from salad by human subjects. Mean (\pm SE) TRL AUC_{0-12h} for all-*trans*-lycopene following ingestion of salads when 100% (○), 75% (●), 50% (●), 50%+75% (●), or 75% + 100% (●) of salad vegetables and oil were consumed in the first meal. Significant differences between treatments are noted by an asterisk. **B) Pharmacokinetic response of carotenes following consumption of salads.** Mean (\pm SE) for all-*trans*-lycopene following ingestion of salads when 100% (○), 75% (●), or 50% (●) of salad vegetables and oil were consumed in the first meal.

DISCUSSION

Several reports have indicated that carotenoid absorption is a saturable process (O'Neill and Thurnham, 1998; O'Sullivan et al, 2007) and that overall bioavailability from supplements can be improved by taking multiple small doses in place of one large dose (Prince and Frisoli, 1993). The present study was designed to evaluate whether a similar paradigm of meal patterning, implemented by dividing one large meal into two smaller meals, would also increase carotenoid absorption from commonly consumed vegetables. More specifically, this study design investigated whether carotenoid absorption was greater when 100% of recommended vegetables were consumed in a large salad as a full lunch meal or when vegetables were split into smaller side salads with simulated lunch and dinner meals. Based upon the results from Goltz et al (2012), a total of 8g of canola oil was provided with the test meals to promote adequate carotenoid absorption. Oil was given in proportion to the amount of salad vegetables in each meal to maintain an equal carotenoid to TAG mass ratio between meals and reduce the influence of dietary lipid on carotenoid bioavailability (Borel et al, 1996).

Results from this study demonstrated that carotenoid absorption from raw vegetable salads was greatest when the majority of the vegetables were consumed together (75% or 100% at once) compared to splitting the dose into two small meals (50% in each meal).

These results were significant for both the carotenes and combined carotenoids. Absorption of xanthophylls and lycopene also followed this trend, although differences in absorption between meal patterns did not reach significance (**Table 4**). Additionally, the meal patterns that promoted the greatest absorption were slightly different between the apolar carotenes and lycopene and the relatively polar xanthophylls. For the xanthophylls, it was clear that consumption of one large dose promoted the greatest absorption, and that splitting the dose in any manner reduced overall bioavailability (**Figure 5**). In contrast, as long as the majority of salad vegetables were consumed together (75-100%), the bioavailability of carotenes and lycopene were higher than when half of the vegetables were consumed at each meal (**Figures 6 and 7**). These minor differences in absorption patterns may have been influenced by the differences in polarity between the carotenoid species. Whereas the relatively polar xanthophylls predominate in the phospholipid-rich emulsion surface of lipid droplets in gastric chyme and readily transfer to mixed micelles in the duodenum during digestion, apolar carotenes are incorporated into the TAG-rich core, and cannot be transferred until after TAG hydrolysis (Borel et al, 1996; Furr and Clark, 1997; Tyssandier et al, 2001). Whether these mechanisms are responsible for the slight differences in absorption observed in this study requires further evaluation.

Overall, these results are in contrast to the findings of Prince and Frisoli (1993) who demonstrated that dividing one large dose of purified β -carotene (51mg) into three smaller doses (17mg each) throughout the day increased bioavailability 3-fold. While differences in the form (supplement versus raw food) and dose may have affected these outcomes, the amount of dietary lipids provided with each carotenoid dose likely played a larger role. Subjects who completed the study by Prince and Frisoli (1993) were instructed to take assigned β -carotene doses with meals that reflected their habitual dietary patterns. Analysis of dietary records indicated that lipid intake averaged 6.5g at breakfast, 28.75g lunch, and 57.25g at dinner. Interestingly, the one large β -carotene dose was given at breakfast, the meal containing the least amount of fat. Therefore, when subjects consumed the divided doses, not only were intestinal carotenoid loads reduced, but also two of these smaller carotenoid doses were consumed with much larger amounts of fat (in the second and third meals). Several reports have indicated that carotenoid absorption from both supplements and food is promoted when taken with increasing amounts of fat (Dimitrov et al, 1988; Prince and Frisoli, 1993; Brown et al, 2004; Unlu et al, 2005). When considering these variables, it becomes unclear whether carotenoid absorption in the study by Prince and Frisoli (1993) was purely impacted by carotenoid dose or also the change in carotenoid to TAG mass ratio (Borel et al, 1996).

Similarly, the relatively low amount of fat provided in the present study may have influenced the outcome and favored absorption when all vegetables and oil were consumed in one meal (Treatment A). It was shown in Goltz et al. (2012) that carotenoid bioavailability of vegetable salads was similar when provided with either 8g or 20g of canola oil. Consequently, this lipid dose was chosen for the present study not only because it was adequate to promote absorption, but also to determine if splitting the salad and oil over two meals could further promote carotenoid bioavailability from this relatively low dose. Increasing bioavailability in this way could have been an alternative method to consuming greater than 20g of dietary TAG with each carotenoid-rich meal, a method that has previously shown to significantly enhance carotenoid bioavailability (Dimitrov et al, 1988; Prince and Frisoli, 1993; Brown et al, 2004; Unlu et al, 2005) but one that makes it difficult to adhere to the USDA's recent recommendations for added oils (maximum of 5-7 tsp or 23-33 g daily) (CNP, 2011a). However, results from this study demonstrated that dividing the salad and oil into two meals reduced carotenoid bioavailability overall. That the TAG to carotenoid ratio did not change between meals but overall absorption was reduced when lipid doses were split may suggest that the absolute amounts provided in the divided doses (2g, 4g, or 6g) were inadequate to promote carotenoid absorption beyond that observed with 8g in a single meal. Taken together, the potential limiting effect of low amounts of dietary lipid consumed with the divided meals in the present study and the large β -carotene dose in the study by Prince

and Frisoli (1993) may account for the difference in results between studies. Therefore, when minimizing added lipids, carotenoid absorption may be the greatest when all vegetables are consumed together in one large meal. However, different outcomes may result if the upper limit of recommended lipid amounts (~30g instead of 8g) is consumed with carotenoid-rich foods over the course of daily meals.

In addition to investigating the impact of meal patterns, this study has allowed for the evaluation of carotenoid absorption within the context of a more balanced and complex meal. Comparing TRL AUC values from subjects consuming vegetable salads with 8g canola oil in Goltz et al. (2012) with subjects completing Treatment A in the present study illustrates that absorption is relatively unaffected by co-consumption of protein and carbohydrate ingredients (**Table 5**). With the exception of α -carotene ($p=0.03$), absorption of individual carotenoids is similar between the studies. Absorption of combined carotenoids is also slightly but not significantly reduced in the present study compared to Goltz et al. (2012), which may be a result of the lower average concentration of carotenoids provided in the test salads (23.3 versus 25.2mg, mainly from differences in α - and β -carotene) as well as the relatively high variability noted in the present study (**Table 5**).

Table 5 Comparison of carotenoid absorption following consumption of salad vegetables with 8g canola oil in the lipids study in Goltz et al (2012) and from treatment A in the present study.

Study	Area under the Curve (nmol*L⁻¹*h)					
	LUT	ZEA	AC	BC	LYC	Total
Goltz et al. (2012) (AUC _{0-10h})	8.0±1.7	2.3±0.6	10.3±1.7	25.1±4.5	12.1±6.2	76.5±12.8
Current Study (AUC _{0-12h})	8.7±2.6	3.1±1.0	5.1±1.3	22.2±7.6	13.0±5.3	62.9±18.8

CONCLUSIONS

Results from this study suggest that carotenoid absorption from raw vegetable salads with a moderate amount of dietary fat is greatest when consumed in one sitting compared to smaller doses over multiple meals. Additionally co-consumption of protein and carbohydrate ingredients does not appear to reduce the bioavailability of carotenoids from these salads. Therefore, daily intake of a large, well-balanced salad containing protein and carbohydrate ingredients and a moderate amount of fat may promote the greatest plasma carotenoid concentration under the current Dietary Guidelines. Although the general population consumes only around half of the recommended 2.5 cups of vegetables each day, intake of salad vegetables is increasing, which suggests that these dietary recommendations may be adopted in an effort to promote health. A larger-scale, longer-term study providing the upper limit of allowable lipids should be completed to better evaluate how meal patterns following the 2010 Dietary Guidelines impact carotenoid bioavailability over time.

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