DOI 10.1002/mnfr.201100687 *Mol. Nutr. Food Res.* 2012, *56*, 866–877

RESEARCH ARTICLE

Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans

Shellen R. Goltz[1], Wayne W. Campbell[2], Chureeporn Chitchumroonchokchai[3], Mark L. Failla[3] and Mario G. Ferruzzi[1]

[1] Department of Food Science, Purdue University, West Lafayette, IN, USA
[2] Department of Nutrition Science, Purdue University, West Lafayette, IN, USA
[3] Department of Human Nutrition, The Ohio State University, Columbus, OH, USA

Received: October 14, 2011
Revised: January 31, 2012
Accepted: February 1, 2012

Scope: Dietary lipids are considered to be primary potentiators of carotenoid absorption, yet the amount and source required to optimize bioavailability has not been systematically evaluated. The objective of this study was to examine the impact of both amount and source of triacylglycerols on postprandial absorption of carotenoids from vegetable salads.

Methods and results: Healthy subjects ($n = 29$) were randomized using a Latin square design (3×3) and consumed three identical salads with 3, 8, or 20 g of canola oil, soybean oil, or butter. Blood was collected from 0–10 h and triacylglycerol-rich fractions (TRLs) were isolated by ultracentrifugation. Carotenoid contents of TRL fractions were analyzed by HPLC-DAD. Considering all lipid sources, 20 g of lipid promoted higher absorption compared to 3 and 8 g for all carotenoid species ($p < 0.05$), except for α-carotene ($p = 0.07$). The source of lipid had less impact on the absorption of carotenoids than amount of lipid. Pooling results from all lipid amounts, monounsaturated fatty acid rich canola oil trended toward enhancing absorption of lutein and α-carotene compared to saturated fatty acid rich butter ($p = 0.06$ and $p = 0.08$, respectively).

Conclusion: While both amount and source of co-consumed lipid affect carotenoid bioavailability from vegetables, amount appears to exert a stronger effect.

Keywords:
Bioavailability / Carotenoids / Clinical trial / Dietary triacylglycerol / Postprandial absorption

1 Introduction

Increased consumption of carotenoid containing foods is associated with a reduced risk of several chronic diseases including cancer [1–5], cardiovascular disease [6–9], and age-related macular degeneration [10–12]. The 2010 Dietary Guidelines encourage consumption of 4.5 cups (approximately 640 g) of carotenoid rich fruits and vegetables per day. However, the average consumption of fruits and vegetables by adults in the USA is only 2.6 cups (approximately 370 g) [13]. This low level of fruit and vegetable consumption is further compounded by the limited absorption of fat soluble, health-promoting, phytochemicals such as carotenoids from foods [14]. The combination of low intake and inefficient absorption of dietary carotenoids diminishes their potential effectiveness as disease preventative compounds.

Carotenoid absorption is a complex process that occurs in several stages including (i) release from the food matrix, (ii) incorporation into bile-salt lipid mixed micelles, (iii) uptake by intestinal epithelia, and (iv) packaging into chylomicrons and secretion into the lymphatic system [14, 15]. Bioaccessibility defines the proportion of carotenoids that have undergone the initial stages, i.e., release and incorporation into mixed micelles [16, 17]. Bioavailability more broadly describes the proportion of carotenoids absorbed from foods and available for utilization, metabolism, or storage by the organism [18]. Because carotenoids are fat soluble, co-consumption of lipids

Correspondence: Dr. Mario G. Ferruzzi, Department of Food Science, Purdue University, 745 Agriculture Mall Dr., West Lafayette, IN 47907, USA
E-mail: mferruzz@purdue.edu
Fax: +1-765-494-7953

Abbreviations: **ANOVA**, analysis of variance; **AUC**, area under the curve; **BHT**, butylated hydroxytoluene; **CHO**, carbohydrate; $\mathbf{C_{MAX}}$, maximum concentration; **FABP**, fatty acid binding protein; **SFA**, saturated fatty acid; **TAG**, triacylglycerol; $\mathbf{T_{MAX}}$, time of maximum concentration; **TRL**, triacylglycerol-rich fraction

in the form of triacylglycerols (TAG) is a critical potentiator of carotenoid bioaccessibility and bioavailability *in vivo*.

While the promoting role of dietary fats and oils on the absorption of carotenoids is well recognized, research addressing the relationship between specific amounts and sources of dietary fat and oil and carotenoid absorption has been limited. Roodenburg et al. demonstrated that 3–5 g of fat was adequate for absorption of α- and β-carotene from supplements in humans [19]. Conversely, Brown et al. found that bioavailability of carotenes and lycopene from vegetable salads in humans was highest when co-consumed with full fat dressing (28 g) compared to reduced fat (6 g) or fat-free salad dressings [20]. Regarding lipid source, Hu et al. demonstrated that saturated fatty acids (SFA) in the form of beef tallow enhanced β-carotene absorption in humans compared to the polyunsaturated fatty acids (PUFA) in sunflower oil [21]. This observation was surprising as monounsaturated fatty acid (MUFA) rich olive oil and PUFA-rich sunflower oil enhance postprandial lipemic response compared to SFA-rich butter [22]. A more detailed understanding of the effect that different amounts and sources of lipid have on carotenoid bioavailability is required to better define recommendations for formulation and dietary strategies to enhance carotenoid absorption from fruits and vegetables.

The objective of this study was to systematically compare how three different amounts of three economically important and commonly consumed dietary TAG sources affect the postprandial absorption of carotenoids from fresh vegetable salads. Canola oil, soybean oil, and butter were used as typical dietary sources rich in MUFA, PUFA, and SFA, respectively. The amounts of lipids in the salad meals were set at 3 g (low fat), 8 g (moderate fat), and 20 g (high fat).

2 Materials and methods

2.1 Materials

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 salad vegetables and butter were purchased at a local market. Canola oil and soybean oil were generously donated by Cargill, Incorporated (Minneapolis, MN).

2.2 Subjects

A total of 37 subjects were enrolled in the study and 34 of these subjects completed all three interventions. Three subjects discontinued participation due to a dislike of the pretesting controlled dietary washout protocol. Of the 34 subjects who completed the study, three had high baseline TAG levels during one or more interventions and their data were excluded due to suspected noncompliance. Data from two additional subjects were excluded due to missed blood draws resulting from veins poorly compatible with phlebotomy. Data from the remaining 29 subjects (14 male, 15 female) are reported. Average age and BMI of the subjects were 27 ± 1 years (range 18–46) and 22.8 ± 0.3 kg/m^2 (range 20–26), respectively.

Informed consent was obtained from all subjects and the study procedures were approved by the Purdue University Committee on the Use of Human Research Subjects. Interested subjects completed health and lifestyle questionnaires and were screened for liver and kidney functions, protein and iron status (anemia), blood glucose, cholesterol, and electrolytes. Exclusion criteria included those who were <18 or >50 years old, had a BMI of <20 or >29 kg/m^2, had a weight change >4.5 kg in the past 3 months, exceeded exercise activities of a recreational level over the past 3 months, had intestinal disorders including lipid malabsorption or lactose intolerance, had abnormal liver or kidney function tests, or had fasting blood glucose >110 mg/dL. Those who smoked, consumed more than two alcoholic drinks per day, used medication affecting lipid profiles or dietary supplements affecting plasma cholesterol (e.g., Benocol or fiber supplements) were also excluded. Additionally, this study excluded menopausal women, those using hormone-based contraceptives, those with abnormal menstrual cycles, and those who were pregnant, lactating or planning to become pregnant.

2.3 Experimental diet

All subjects consumed a low carotenoid diet for 7 days prior to each testing day to sufficiently reduce circulating, endogenous levels of carotenoids and minimize the contribution of carotenoids in VLDL in the triglyceride rich fraction (TRL) as this is known to be elevated from previously consumed meals [23]. During the washout period (days 1–5) subjects were provided with lists of low carotenoid meals, snacks, fruits, vegetables, condiments, and beverages from which to choose. Meals on these days provided no more than 750 μg carotenoid intake per day. On days 6 and 7 (2 days preceding each intervention), subjects consumed a controlled, low carotenoid diet of conventional foods that was provided. The meals on these days were designed to provide 2100, 2500, or 2850 calories per day to best meet the energy needs for each subject. A vegetarian diet (2500 calories) was also provided upon request. Regardless of caloric content, meals were designed to provide less than 500 μg carotenoids daily. The macronutrient and carotenoid contents of these diets are listed in Table 1.

On test day (day 8), subjects consumed the test salad in the morning and were fed a low fat, low carotenoid lunch half way through the test day. The lunch provided 638 kcal, 25 g protein, 130 g carbohydrates (CHO), 1.95 g fat (31%

Table 1. Composition and approximate carotenoid content of controlled diets[a)]

Calorie level	Calories provided	Protein	CHO	Fat	Total carotenoids
	(kcal)	(%)	(%)	(%)	(μg)
2100 (Day 6)	2210	18	58	24	266–326
2100 (Day 7)	2128	19	59	22	321–381
2500 (Day 6)	2555	19	56	25	279–339
2500 (Day 7)	2423	19.5	57	23.5	349–409
2850 (Day 6)	2859	19	55	26	301–361
2850 (Day 7)	2869	20	55	25	394–454
Vegetarian 2500 (Day 6)	2469	20	61	19	279–339
Vegetarian 2500 (Day 7)	2450	15	61	24	293–353

a) Estimated via ProNutra software (Version 3.3.0.10, Viocare, Inc, Princeton, NJ).

MUFA, 40% PUFA, 29% SFA), and 172 μg carotenoids. (The vegetarian lunch provided 645 kcal, 26 g protein, 131 g CHO, 1.9 g fat, and 172 μg carotenoids.) All foods were weighed and prepared by research staff in the Department of Nutrition Science Metabolic Kitchen at Purdue University. The nutrient content of all meals was estimated using ProNutra software (Version 3.3.0.10, Viocare, Inc, Princeton, NJ).

2.4 Test salads

All vegetables for experimental salads were analyzed initially for carotenoid content to ensure provision of an appropriate carotenoid dose. Test salad composition and average carotenoid content was designed based on per capita consumption and serving weights of individual carotenoid rich vegetables [24] with minor modifications to ensure balanced delivery of all major carotenoid species including lutein, zeaxanthin, α-carotene, β-carotene, and lycopene. Representative samples of weekly test salads were homogenized using an immersion blender (KitchenAid, Benton Harbor, MI), flushed with nitrogen, and stored at −20°C until analysis. Salad composition and average carotenoid contents provided throughout the study are shown in Table 2 and Supporting Information Figure 1. Total carotenoid content of test salads during the study period ranged from 16 to 32 mg and provided and average of 25 mg of total carotenoids per serving. *Cis* isomers were present only in low quantities, accounting for <8% of lutein, <3% of β-carotene, and <13% of lycopene. Zeaxanthin, originating from Chinese wolfberries, was primarily present as di- and monoesters (86.2%) with only 13.8% free or nonesterified. Individual and total carotenoid concentrations in TRL fractions of plasma were normalized by salad carotenoid concentrations obtained during the same week of testing (Supporting Information Fig. 1) to offset weekly variability in composition.

Table 2. Composition and average carotenoid content of test salad[a)]

Vegetable composition		Carotenoid content	
Ingredient (Raw)	Weight (g)	Species	Weight (mg)
Beefsteak tomatoes	100	Lutein	4.22 ± 0.15
Julienne carrots	62	Zeaxanthin	6.86 ± 0.14
Baby spinach	70	α-cryptoxanthin	0.03 ± 0.00
Romaine lettuce	25	β-cryptoxanthin	0.28 ± 0.01
Chinese wolfberry	5	α-carotene	3.12 ± 0.19
		β-carotene	6.32 ± 0.22
		Lycopene	4.38 ± 0.24 (4.16 *trans*, 0.22 *cis*)
Total	262		25.21 ± 0.75

a) Values obtained via carotenoid extraction, as described in Section 2, and analysis via HPLC-DAD.

Canola oil, soybean oil, and butter were selected as test oils and fat based on their common use in food preparation and processing, as well as their relative levels of MUFA (65%; mainly as oleic acid in canola oil), PUFA (59%; mainly as linoleic acid in soybean oil), and SFA (61%; mainly as palmitic and myristic acids in butter), respectively (Supporting Information Table 1). The three lipid levels included in meals were chosen to best estimate a low (3 g), moderate (8 g), and high (20 g) lipid load for each source of fat. Subjects were instructed to pour the oil or melted butter onto the salad and consume the test salad within 30 min. After consumption of the salad, subjects were instructed to wipe the salad and oil bowls with a piece of white bread to ensure that all butter or oil was ingested.

2.5 Experimental design

Each subject completed three separate treatments (designated by groups A, B, or C in a 3×3 Latin square design) with the order of consumption randomized and separated by a 3-week interval (Supporting Information Fig. 2). During each treatment, subjects consumed identical vegetable salads, differing only in amount and source of dietary lipids added as designated by treatment group. The 3-week interval between treatments was designed to allow for equilibration and assessment of female subjects in the follicular phase, as phase in the menstrual cycle may affect the concentration and distribution of carotenoids in lipoprotein fractions [25, 26].

On day 1 of each treatment period, subjects arrived at the clinical research center (CRC) following a 12-h fast. A trained phlebotomist collected 5 mL of blood into EDTA-containing tubes, which were immediately placed on ice,

protected from light, and centrifuged to collect plasma (3000 × g, 15 min, 4°C). Aliquots of plasma were flushed with nitrogen and stored at −80°C. Plasma samples were later analyzed for carotenoid content and compared to day 8 (test day) baseline plasma carotenoid levels to assess compliance to the washout and controlled diet periods. Subjects were then instructed on how to follow the washout dietary protocols to be followed for the next 5 days. On day 6 of each study period, subjects were given a cooler of food at the metabolic kitchen containing their controlled meals for days 6 and 7.

On each of three test days (day 8 of each test period), subjects returned to the CRC following a 12-h 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 (20 mL), subjects immediately consumed the experimental salad with the randomly assigned fat or oil. Upon completion of the salad, blood samples (15 mL) were collected hourly for 10 h. Blood samples were collected by syringe, transferred to EDTA-containing tubes and placed on ice, protected from light, and centrifuged (3000 × g, 15 min, 4°C) to separate plasma for immediate isolation of TRL fractions or for future assessment of compliance to the washout and controlled diet periods. Aliquots of plasma used to assess dietary compliance were flushed with nitrogen and stored at −80°C until analysis. Sterile physiological saline (3 mL) was injected into the catheter via the obturator after each draw to prevent clotting. Additionally, an intravenous drip of physiological saline (9 g NaCl/L) was initiated upon catheter placement and maintained at 30 mL/h to keep the line patent. Saline was removed from the line before each collection of blood. The low carotenoid (< 2 mg), low fat (< 2 g) lunch (~650 calories) was given after blood was collected at hour 5. Water was allowed ad libitum throughout the day. A courtesy meal was provided to all subjects following completion of each test day.

2.6 TRL isolation

TRL fractions were isolated via ultracentrifugation as described by Weintraub et al. with minor modifications [27]. Briefly, fresh plasma was transferred to centrifuge tubes (Ultra Clear; Beckman Instruments, Inc, Palo Alto, CA) and preserved with EDTA, sodium azide, aprotinin, and phenylmethyl sulfonyl fluoride as described by Gianturco and Bradley [28]. Preserved samples were overlaid with a density solution of 1.006 g/mL and were centrifuged (L8-M 70; Beckman Instruments Inc) in a swinging bucket rotor (SW41ti) at 152 000 × g for 35 min at 20°C. TRL fractions were isolated (0.6 mL), standardized to 5 mL with saline, flushed with nitrogen, and stored at −80°C until analysis. All isolations were carried out under red light to minimize photo-oxidative reactions.

2.6 Carotenoid analysis in plasma and TRL fraction

Carotenoids were extracted from plasma and from plasma TRL fractions as described by Brown et al. [20]. Samples were initially deproteinated with methanol and subsequently extracted three times with acetone:petroleum ether (1:2 with 0.1% BHT). Ether layers were combined and dried under vacuum using a RapidVap (Labconco Corporation, Kansas City, MO). Dried samples were resolubilized in ethyl acetate:methanol (1:1) and analyzed immediately. Carotenoid analysis was completed by HPLC as described by Kean et al. [29] using a Hewlett Packard model 1090A HPLC pump, model 79880A diode array detector, and a YMC Carotenoid C30 column (2.0 × 150 mm, 3 μm particle size). Lycopene was detected at 470 nm, while all other carotenoid species were detected at 450 nm. Chemstation software and data management system (Rev. A.10.02 [1757], Agilent Technologies, Santa Clara, CA) were used to collect, integrate, and store the chromatographic data.

2.7 Extraction and HPLC analysis of carotenoids in salad vegetables

Five grams of test salad homogenate was combined with ~0.5 and 1 g of sodium bicarbonate and celite, respectively, and carotenoids were extracted with a 1:1 solution of acetone:petroleum ether (0.1% BHT). The salad solvent suspension was vacuum filtered through two no. 1 Whatman filter papers. The filter cake was re-extracted two additional times with acetone/petroleum ether (1:1, 0.1% BHT) and combined acetone/petroleum ether layers were saponified by mixing with 40% KOH in methanol on a magnetic stir plate for 30 min at room temperature in the dark. Following saponification, the mixture was quantitatively transferred to a separatory funnel, where the ether layer was washed with distilled water, collected and subsequently poured through a column of sodium sulfate to remove residual water, and brought to a total volume to 100 mL with petroleum ether. Three mL aliquots were dried by vacuum and stored at −80°C until analysis. Dried extracts were resolubilized in ethyl acetate:methanol (1:1, v/v), and analyzed by HPLC-DAD as described above.

2.8 Analysis of triacylglycerol and cholesterol content of TRL fractions

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

2.9 Statistical analyses

A power analysis was conducted based on carotenoid postprandial response data from Brown et al. [20]. Based on an anticipated 40% differences between groups and approximately 40% standard deviation in carotenoid response, a group size of nine subjects results in a power of 0.81 ($\alpha < 0.05$). Therefore, it was estimated that a total of 27 subjects would provide an adequate sample size to detect differences between treatments in the 3×3 design.

Postprandial TRL TAG and carotenoid concentrations were baseline corrected using fasting values. Baseline corrected 0–10 h area under the curve ($AUC_{0\text{-}10h}$) for TAG and carotenoids were calculated using the PK functions plug-in for Microsoft Excel [30]. Baseline corrected $AUC_{0\text{-}10h}$ were normalized by salad carotenoid concentrations obtained during the same week of testing. Normalization was carried out by dividing $AUC_{0\text{-}10h}$ 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. Normalization was carried out for both individual and total carotenoid content. 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 ± standard error of the mean (SEM). The main effects of lipid source, lipid dose, and the interaction between source and dose on TAG and carotenoid $AUC_{0\text{-}10h}$, C_{MAX}, and T_{MAX}, values, as well as differences in plasma carotenoid concentrations before and after washout periods, were estimated by ANOVA using the mixed procedure in SAS 9.1.4 (Cary, NC), with subject as the random variable. Differences between treatments were determined by Bonferroni's multiple comparison test ($\alpha<0.05$, two tailed). Plots of the data were evaluated to confirm normal distribution and homogeneity of variance prior to ANOVA. The Corr procedure in SAS was used to determine Pearson correlations between carotenoid and TAG AUC values. Data are reported as mean ± SEM.

3 Results

3.1 Adequacy of combined washout and controlled diet period

Significant reductions ($p < 0.05$) were observed in concentrations of each individual carotenoid and for total carotenoids in plasma following the 7-day washout and controlled diet periods, indicating adequacy of the washout protocol (Supporting Information Fig. 3).

3.2 Change in TRL carotenoid content

The effects of co-consumed lipid on carotenoid bioavailability were primarily assessed for the most abundant carotenoids in the test salad including the xanthophylls, lutein and zeaxanthin (as zeaxanthin esters), and the apolar carotenes and lycopene. Although less abundant carotenoids (α-cryptoxanthin, β-cryptoxanthin, and *cis*-lycopene) were not significantly affected by lipid, data for these compounds are included in the calculation for total carotenoid absorption. Additionally, no significant differences were noted in individual or total carotenoids response based on factors such as gender, intervention group (A, B, or C as designated by the Latin square design), and intervention number (1st, 2nd, or 3rd).

3.2.1 Total carotenoids

Analysis of TRL $AUC_{0\text{-}10h}$ values revealed that the main effect of lipid amount significantly affected absorption for combined carotenoids ($p = 0.01$). Considering all sources, presence of 20 g lipid promoted greater absorption of total carotenoids compared to both 3 g (106.5 versus 66.0 nmol L^{-1} h, $p = 0.03$) and 8 g (106.5 versus 60.0 nmol L^{-1} h, $p = 0.02$) (Fig. 1A). There was no significant difference in absorption of carotenoids co-consumed with either 3 or 8 g lipid. The main effect of lipid source was not as strong as lipid amount and did not significantly affect absorption of total carotenoids ($p = 0.13$). Comparison of individual sources revealed that MUFA-rich canola oil promoted the highest absorption of carotenoids (92.5 nmol L^{-1} h) followed by PUFA-rich soybean oil (79.8 nmol L^{-1} h) and SFA-rich butter (60.2 nmol L^{-1} h), however these differences were not statistically significant (Fig. 1A). C_{MAX} values for the main effects reflected trends observed for $AUC_{0\text{-}10h}$ data. The results of these main effects also generally reflected the lipemic responses following the consumption of test meals, and the AUC for total carotenoids was significantly correlated with the AUC for TAG ($p < 0.01$). However, considering all lipid sources, a stronger dose response relationship was observed when evaluating the lipemic response (Fig. 1, Supporting Information Table 2). Also, both canola oil and soybean oil promoted significantly greater amounts of TAG in the TRL fraction compared to butter.

Although the overall interaction between type and amount of lipid did not significantly impact total carotenoid $AUC_{0\text{-}10h}$ values, there was a clear dose–response relationship within soybean oil responses (Fig. 2A, Table 3). This trend became more apparent when evaluating C_{MAX}, as consumption of 20 g of soybean oil significantly enhanced these values compared to 3 g ($p < 0.01$) and 8 g ($p = 0.01$) of soybean oil (Table 3). This dose–response relationship was not observed with either canola oil or butter. The large amount of carotenoid absorption following consumption of 3 g of canola oil may be responsible for the lack of a dose–response relationship from this oil at 8 and 20 g. Similarly, absorption of total carotenoids was doubled when consumed with 3 g of canola oil compared to 3 g of soybean oil or 3 g of butter. This enhancement of

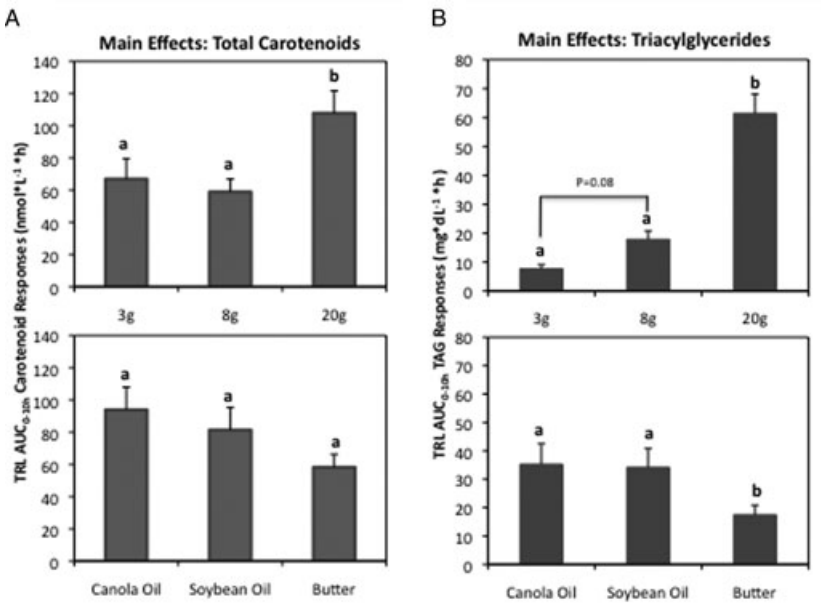

Figure 1. Main effects of lipid source (considering all amounts) and lipid amount (considering all sources) on absorption of carotenoids and TAG. (A) Mean (±SE) TRL $AUC_{0\text{-}10h}$ carotenoid concentrations after consumption of salads with 3, 8, and 20 g of canola oil, soybean oil, and butter. (B) Mean (±SE) TRL $AUC_{0\text{-}10h}$ TAG concentrations after consumption of salads with 3, 8, and 20 g of canola oil, soybean oil, and butter. Significant differences between lipid amounts and lipid types are denoted by different letters ($p < 0.05$).

carotenoid absorption with 3 g of canola oil occurred in the absence of an elevated lipemic response (Fig. 2).

3.2.2 Xanthophylls

For both lutein and zeaxanthin, there was a significant main effect of lipid amount on $AUC_{0\text{-}10h}$ independent of source ($p < 0.01$). As with total carotenoids, 20 g of lipid promoted significantly higher absorption of lutein compared to both 3 g (15.0 versus 7.9 nmol L^{-1} h, $p = 0.01$) and 8 g (15.0 versus 6.1 nmol L^{-1} h, $p < 0.01$). Similarly, 20 g of each lipid promoted significantly greater absorption of zeaxanthin compared to either 3 g (6.5 versus 2.8 nmol L^{-1} h, $p < 0.01$) or 8 g (6.5 versus 2.7 nmol L^{-1} h, $p < 0.01$). There was no significant difference in absorption of lutein or zeaxanthin from salads containing either 3 or 8 g of lipid. Also, there was a significant ($p < 0.05$) main effect of lipid source for lutein, but not zeaxanthin. Considering all lipid amounts, canola oil promoted greater absorption of lutein than butter (11.7 versus 6.6 nmol L^{-1} h, $p = 0.06$). This trend became significant when evaluating differences in C_{MAX} ($p = 0.04$). Otherwise, C_{MAX} values reflected $AUC_{0\text{-}10h}$ values for xanthophyll main effects. As for total carotenoids, the AUC values for lutein and zeaxanthin were significantly correlated to the TAG AUC values ($p < 0.01$).

While the interaction of type and amount of lipid was not significant for absorption of the two xanthophylls overall, individual differences within specific lipid sources were observed (Table 3, Supporting Information Fig. 4). For both lutein and zeaxanthin, 20 g of soybean oil increased $AUC_{0\text{-}10h}$ and C_{MAX} values compared to 3 and 8 g of this oil ($p < 0.05$). Similarly, differences in xanthophyll absorption were observed for same amount of different lipid sources. For example, 20 g of soybean oil appeared to promote higher $AUC_{0\text{-}10h}$ values for lutein compared to 20 g butter ($p = 0.09$). This trend became significant when evaluating C_{MAX} values ($p = 0.04$). Differences in amount and source of co-consumed lipid did not significantly affect T_{MAX} (Table 3).

3.2.3 Carotenes

Absorption of both α- and β-carotene was significantly affected by amount of co-consumed lipid ($p = 0.02$ and $p < 0.01$, respectively). Considering all three lipid sources, 20 g promoted significantly higher absorption of α-carotene compared to 3 g (13.5 versus 8.2 nmol L^{-1} h, $p = 0.03$) and trended toward promoting higher absorption compared to 8 g (13.5 versus 7.5 nmol L^{-1} h, $p = 0.07$). Similarly, 20 g of lipid in any form tested promoted significantly higher absorption of β-carotene compared to both 3 g (33.1 versus 19.5 nmol L^{-1} h, $p < 0.01$) and 8 g (33.1 versus 17.9 nmol/L, $p = 0.01$). There was no significant difference in absorption of α- or β-carotene for salads containing either 3 or 8 g of lipid. The source of lipid had less impact on the absorption of carotenes than on xanthophylls, and this main effect was not significant. However, when compared individually, canola oil trended toward enhancing absorption of α-carotene compared to butter (12.3 versus 6.8 nmol L^{-1} h, $p = 0.08$). C_{MAX} values for the main effects of α- and β-carotene reflected trends observed for $AUC_{0\text{-}10h}$ data. As with the xanthophylls and total carotenoids, AUC values for the carotenes were significantly correlated to the TAG AUC values ($p < 0.05$).

Also similar to the xanthophylls and total carotenoids, the interaction of type and amount of lipid on absorption

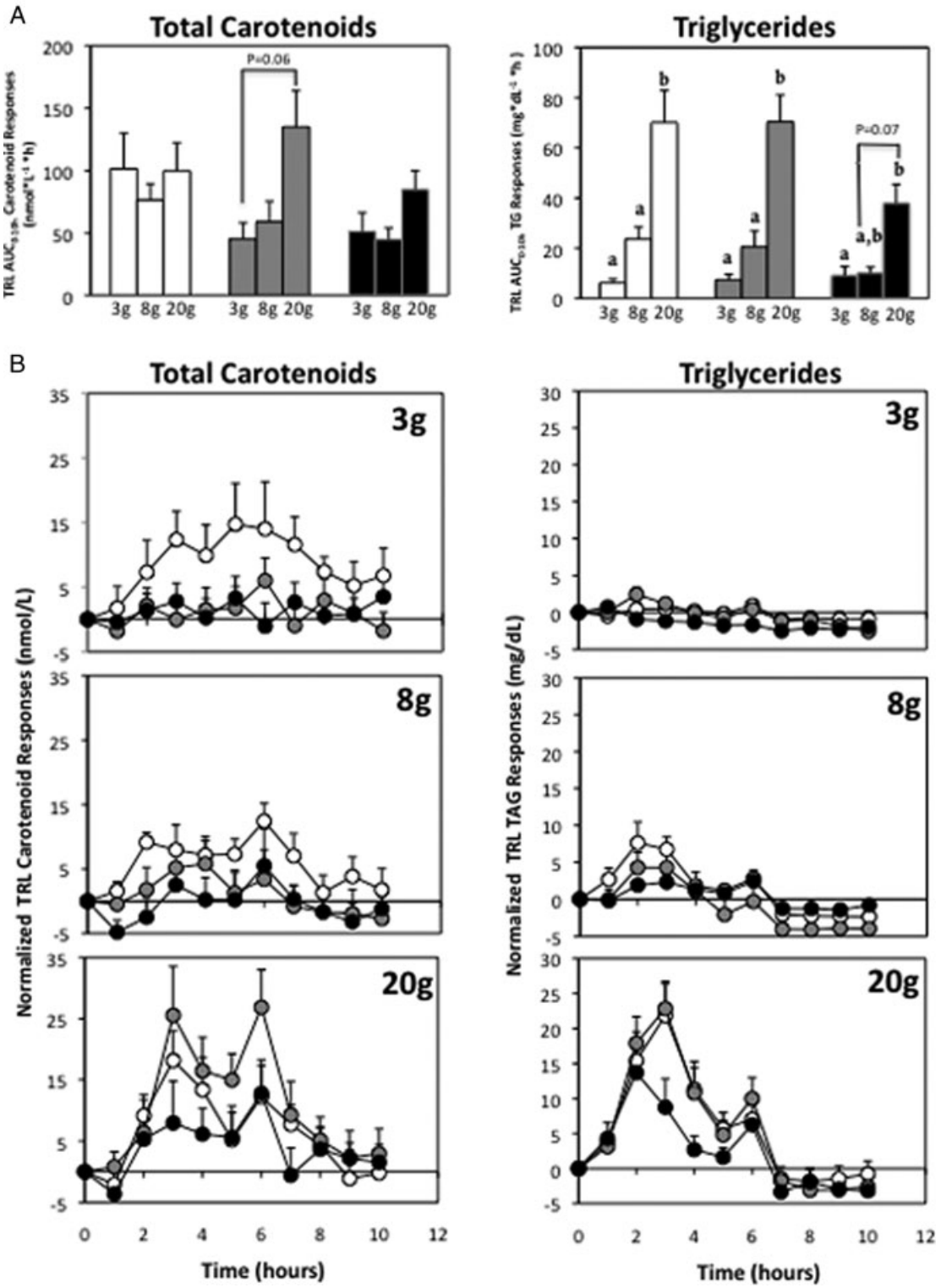

Figure 2. (A) Interaction of lipid source and amount on absorption of carotenoids from salad by human subjects. Mean (±SE) TRL $AUC_{0\text{-}10h}$ carotenoid and TAG concentrations following ingestion of salads with either 3, 8, and 20 g of canola oil (○), soybean oil (◍), and butter (●). Significant differences between amounts of the same dietary lipid are denoted by different letters ($p < 0.05$). (B) Pharmacokinetic response of total carotenoids following consumption of salad meals with different sources and amounts of lipids. Mean (±SE) for TRL total carotenoids and TAG following ingestion of salads with either 3, 8, and 20 g of canola oil (○), soybean oil (◍), and butter (●).

Table 3. Mean (±SE) baseline corrected and normalized TRL carotenoid $AUC_{0\text{-}10h}$, C_{MAX}, and T_{MAX} values[a),b),c)]

		Area under the curve—$AUC_{0\text{-}10h}$ (nmol L^{-1} h)					
Main effects		LUT	ZEA	AC	BC	LYC	Total
3 g		7.9 ± 1.3[a]	2.8 ± 0.7[a]	8.2 ± 2.8[a]	19.5 ± 4.8[a]	6.4 ± 1.0[a]	66.0 ± 12.5[a]
8 g		6.1 ± 0.8[a]	2.7 ± 0.6[a]	7.5 ± 1.1[a,b]	17.9 ± 2.4[a]	8.0 ± 2.0[a]	60.0 ± 7.9[a]
20 g		15.0 ± 2.4[b]	6.5 ± 0.9[b]	13.5 ± 1.7[b]	33.1 ± 4.0[b]	17.9 ± 3.6[b]	106.5 ± 13.9[b]
Canola oil		11.7 ± 1.8	4.5 ± 0.8	12.3 ± 2.7	27.1 ± 4.7	11.8 ± 2.4	92.5 ± 13.3
Soybean oil		10.6 ± 2.2	4.2 ± 0.9	10.0 ± 2.0	24.7 ± 4.1	12.4 ± 3.5	79.8 ± 14.0
Butter		6.6 ± 1.0	3.3 ± 0.6	6.8 ± 0.8	18.7 ± 3.0	8.1 ± 1.5	60.2 ± 8.1
		Area under the curve—$AUC_{0\text{-}10h}$ (nmol L^{-1} h)					
Lipid amount	Lipid source	LUT	ZEA	AC	BC	LYC	Total
3 g	Canola	12.0 ± 2.4	4.7 ± 1.3	15.0 ± 7.7	29.8 ± 12.3	7.8 ± 1.4	101.5 ± 28.6
	Soybean	6.0 ± 2.3[a]	1.3 ± 0.6[a]	5.4 ± 1.1	16.3 ± 4.4	6.3 ± 2.4	45.3 ± 12.9
	Butter	5.6 ± 1.8	2.6 ± 1.1	4.1 ± 0.8	12.5 ± 5.0	5.1 ± 1.6	51.1 ± 15.1
8 g	Canola	8.0 ± 1.7	2.3 ± 0.6	10.3 ± 1.7	25.1 ± 4.5	12.1 ± 6.2	76.5 ± 12.8
	Soybean	4.9 ± 1.1[a]	2.8 ± 1.0[a]	6.4 ± 2.3	17.8 ± 4.5	7.9 ± 2.4	58.9 ± 16.5
	Butter	5.3 ± 1.6	2.9 ± 1.1	5.8 ± 1.1	10.9 ± 2.3	4.1 ± 0.8	44.7 ± 9.1
20 g	Canola	15.0 ± 4.0	6.6 ± 1.5	11.7 ± 2.0	26.4 ± 5.6	15.6 ± 4.1	99.6 ± 22.7
	Soybean	20.9 ± 4.7[b]	8.6 ± 1.5[b]	18.3 ± 4.0	40.0 ± 8.7	23.0 ± 9.1	135.0 ± 29.0
	Butter	9.1 ± 2.1	4.3 ± 1.1	10.5 ± 1.6	32.7 ± 5.4	15.2 ± 4.2	84.9 ± 14.8
		Concentration maximum—C_{MAX} (nmol L^{-1})					
Lipid amount	Lipid source	LUT	ZEA	AC	BC	LYC	Total
3 g	Canola	3.2±0.8	1.1±0.3	3.1±1.2	8.0±3.2 [1]	2.3±0.5	24.3±6.7
	Soybean	1.6±0.6[a]	0.4±0.2[a]	1.5±0.3[a]	4.3±0.7[1,a]	1.4±0.3[a]	9.7±2.4[a]
	Butter	1.4±0.4	0.8±0.2	1.0±0.1	2.9±0.8[2]	1.6±0.3	11.5±2.7
8 g	Canola	2.3±0.7	0.8±0.1	2.5±0.4	6.0±1.1	2.7±1.0	16.3±2.3
	Soybean	1.6±0.3[a]	0.8±0.2[a]	1.9±0.6[a]	5.1±0.9[a]	2.3±0.5[a,b]	13.6±3.1[a]
	Butter	1.7±0.4	0.9±0.2	2.1±0.3	4.5±0.7	2.1±0.5	13.3±2.3
20 g	Canola	3.8±0.6 [1, 2]	1.6±0.2	3.6±0.6	8.3±1.6	4.5±1.2	25.2±4.6
	Soybean	5.4±1.1[1, b]	2.4±0.4[b]	4.8±1.0[b]	13.2±3.4[b]	6.2±2.0[b]	34.1±5.8[b]
	Butter	2.4±0.5[2]	1.3±0.4	3.2±0.7	8.6±1.0	4.6±0.7	19.9±2.0
		Time of maximum concentration—T_{MAX} (h)					
Lipid amount	Lipid source	LUT	ZEA	AC	BC	LYC	Total
3 g	Canola	7.1±0.9	6.3±1.0	5.1±0.4	5.0±0.4	5.1±0.7	4.9±0.3
	Soybean	6.9±0.8	5.4±1.0	5.0±0.5	4.6±0.6	4.1±0.7	5.1±0.7
	Butter	6.2±1.1	4.9±1.1	4.6±0.8	5.0±0.9	4.4±0.9[a,b]	5.4±1.1
8 g	Canola	8.1±0.7	7.6±0.8	5.4±0.4	4.6±0.6	4.6±1.0	6.1±0.2
	Soybean	6.3±0.7	5.8±0.8	5.1±0.6	4.6±0.2	4.2±0.6	4.9±0.6
	Butter	6.3±0.5	5.7±0.6	5.2±0.4	4.9±0.8	6.3±0.7[a]	5.6±0.7
20 g	Canola	5.2±0.5	5.1±0.5	4.6±0.5	4.8±0.5	4.0±0.4	4.9±0.5
	Soybean	5.4±0.1	6.1±0.1	3.6±0.5	3.6±0.5	3.4±0.4	4.1±0.7
	Butter	5.9±1.0	5.8±0.2	5.3±0.5	5.1±0.5	2.6±0.3[b]	4.1±0.6

a) For main effects, for each carotenoid, significant differences between lipid amounts (considering all lipid sources) are denoted by different letters ($p < 0.05$). No significant differences were noted between lipid sources (considering all amounts).
b) For interaction effects, for each carotenoid, significant differences between lipid amounts within the same lipid type are denoted by different letters ($p < 0.05$). Significant differences within the same lipid amount across lipid types are denoted by different numbers ($p < 0.05$).
c) Normalization was carried out by dividing baseline corrected $AUC_{0\text{-}10h}$ 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.

of carotenes was not significant. However, evaluation of individual values reveals that there were several dose–response relationships within specific lipid sources (Table 3, Supporting Information Fig. 5). For example, 20 g of soybean oil promoted greater absorption of α- and β-carotene compared to either 3 or 8 g of this oil. The same trend was noted for absorption of β-carotene from butter as 20 g promoted greater absorption than either 3 or 8 g, although the differences between these amounts and sources were not significant.

As with total carotenoids and xanthophylls, individual differences in C_{MAX} within lipid types accentuated the trends reported in $AUC_{0\text{-}10h}$ data (Table 3). Specifically, consumption of 20 g of soybean oil promoted significantly greater C_{MAX} values compared to 3 g (α-carotene: $p < 0.01$; β-carotene, $p < 0.01$) or 8 g (α-carotene: $p = 0.04$; β-carotene: $p = 0.01$) of soybean oil. Differences in C_{MAX} between equivalent amounts of different lipid sources were not observed. Also, differences in amount and source of co-consumed lipid did not significantly affect T_{MAX} (Table 3).

3.2.4 *All-trans* lycopene

The main effect of lipid amount significantly affected *all-trans* lycopene absorption as observed for total carotenoids, xanthophylls, and carotenes ($p<0.01$). Considering all lipid sources, 20 g promoted greater absorption than either 3 g (17.9 versus 6.4 nmol L^{-1} h, $p < 0.01$) or 8 g (17.9 versus 8.0 nmol L^{-1} h, $p < 0.01$). There was no significant difference in absorption of *all-trans* lycopene when the amount of co-ingested lipid was either 3 or 8 g. As for zeaxanthin and the carotenes, the main effect of lipid source did not significantly affect the overall absorption of *all-trans* lycopene, and there were no differences in absorption when comparing each lipid source individually. As with all carotenoid species, lycopene AUC and TAG AUC values were significantly correlated ($p < 0.01$).

Also similar to the xanthophylls and carotenes, the interaction was not significant. However, increasing doses of canola oil and soybean oil did result in dose-depended increases in lycopene absorption (Table 3, Supporting Information Fig. 6). Changes in C_{MAX} for lycopene were consistent with these trends in $AUC_{0\text{-}10h}$ and accentuated $AUC_{0\text{-}10h}$ soybean oil responses. Consumption of 20 g of soybean oil promoted significantly greater C_{MAX} values compared to 3 g ($p = 0.02$) and trended toward significance compared to 8 g ($p = 0.07$) of soybean oil (Table 3). Also, T_{MAX} for lycopene was significantly affected by lipid amount ($p < 0.01$). Overall, T_{MAX} occurred earlier following consumption of 20 g lipid compared to 8 g lipid (Table 3).

4 Discussion

Previous reports have shown that both amount [19, 20, 31] and source [21] of lipid as TAG can affect the absorption of carotenoids. In this study, the combined effect of both lipid amount (3, 8, and 20 g) and source (MUFA-rich canola oil, PUFA-rich soybean oil, and SFA-rich butter) on carotenoid bioavailability from vegetable salads was evaluated simultaneously in human subjects. Our results suggest that within reasonable dietary levels, the amount of co-consumed lipid appears to be a more significant influence on overall carotenoid absorption than source of lipid.

Considering all three sources, 20 g ingested lipid promoted significantly higher absorption compared to both 3 and 8 g for all carotenoid species and total carotenoids (except for 20 *versus* 8 g for α-carotene, $p = 0.07$) (Fig. 1; Table 3). However, this dose-dependent effect across all lipid sources was primarily driven by the response from soybean oil, as there were no significant differences in absorption between the three amounts of canola oil or butter (Table 3). Overall, these findings are consistent with those from Brown et al. [20], who provided subjects with a salad of similar size and composition compared to the present study and concluded that the threshold for optimal carotenoid absorption from salads exceeds 6 g of fat for a single meal. In their study, Brown et al. observed a significant dose-dependent increase in the absorption of α-carotene, β-carotene, and lycopene when consumed with 0, 6, and 28 g of canola oil. While we observed an overall effect for amount of lipid in the present study, increasing levels of canola oil alone did not significantly increase the absorption of carotenoids to the extent observed by Brown et al. [20]. Differences between the present results and previous reports may be due to several factors. In the current study, we were unable to detect a significant increase between our designated low (3 g) and moderate (8 g) doses of lipid. It is likely that in the absence of a fat-free group in the current study, the 5 g difference in dietary TAG between the 3 and 8 g lipid groups was insufficient to generate a significant increase in carotenoid absorption from raw vegetables. While it was clearly documented that negligible carotenoid absorption takes place in the absence of dietary lipid [20, 31–33], small amounts of co-consumed lipid can significantly improve absorption [33]. Indeed, low levels of co-consumed canola oil (3 g) resulted in appreciable carotenoid absorption in the present study (Table 3). Similarly, modest levels of canola oil (6 g) in the study conducted by Brown et al. [20] provided a significant increase in carotene and lycopene absorption. Brown et al. also observed significantly greater absorption of carotenoids from salad ingested with 28 g canola oil. Considering these previous observations, our use of 20 g as the highest amount of fat may have been below the necessary threshold to induce further increase in absorption from raw vegetables beyond that observed with low to moderate levels (3–8 g) of canola oil. Further, by basing our power calculation from a study that used a range of lipids from 0 to 28 g [20] and then using a range from 3 to 20 g, the potential to detect significant differences in carotenoid absorption between treatment groups may have been reduced. Retrospective power calculations based upon the values obtained from the present study suggest that approximately 14 subjects per group would have allowed for detection of additional significant differences between treatment groups.

Although the effect of lipid source on carotenoid absorption was not as evident as amount, some observations merit further discussion. Specifically, MUFA-rich canola oil enhanced lutein and α-carotene absorption compared to SFA-rich butter (Table 3). Additionally, carotenoid absorption was higher when co-consumed with canola oil compared to

PUFA-rich soybean oil for all carotenoids except lycopene (Table 3). These findings are consistent with previous reports suggesting that absorption of both carotenes [34] and xanthophylls [35] is enhanced when consumed with lipids rich in MUFA compared to PUFA but that these trends are less pronounced for lycopene [36, 37]. Further, from this clinical trial, it appeared that lutein absorption was the most affected by lipid source overall whereas lycopene was the least affected. Zeaxanthin, in apolar mono- and diester forms, was also minimally affected by lipid source. Together, these data may suggest that lipid source may be more influential on the absorption of polar carotenoids such as lutein.

The observed increase in absorption of select carotenoid species with MUFA- compared to PUFA-rich lipid sources may be a result of several factors. First, micelles containing PUFAs are larger in size, resulting in slower diffusion through the unstirred water layer and decreased amounts of absorption [37]. Also, fatty acid binding protein (FABP) is implicated in the intracellular transfer of β-carotene as well as fatty acids [38]. Because PUFA bind more readily to FABP, β-carotene may be affected by competitive binding to FABP when consumed with PUFA-rich soybean oil compared to MUFA-rich canola oil [34]. Therefore, a greater degree of fatty acid unsaturation in co-consumed lipid may decrease incorporation of carotenoids into nascent chylomicrons for secretion into lymph.

SFA-rich lipid sources also promote carotenoid absorption. Hu et al. observed greater absorption of β-carotene from supplements consumed with beef tallow (SFA) compared to sunflower oil (PUFA) [21]. This contrasts with results of the present study where carotenoid absorption was lower when salads were co-consumed with SFA-rich butter compared to MUFA-rich canola or PUFA-rich soybean oil (Fig. 1). However, it is likely that poor responses from SFA-rich butter relative to previously reported results with beef tallow are due to physiochemical differences between the two SFA lipid and carotenoid sources. Hu et al. utilized a supplemental form of β-carotene rather than the raw salad vegetables used in this study. Differences in carotenoid profile and extractability from the matrix may have affected solubilization of carotenoids in mixed micelles. Also, butter, a water-in-oil emulsion, is poorly emulsified in the gut, has limited digestibility, and therefore may have limited ability to aid in carotenoid extraction from the food matrix and micellarization in the gut [22]. Additionally, in comparison to butter, beef tallow contains a high amount of oleic acid, a MUFA, which can enhance carotenoid absorption [39]. Regarding butter, this product contains appreciable amounts of short and medium chain fatty acids (Supporting Information Table 1), resulting in a relatively low lipemic response following ingestion [22]. Huo et al. reported that short chain fatty acids fail to facilitate micellarization of hydrophobic carotenoids during small intestinal digestion [40]. Borel et al. demonstrated that a lower lipemic response decreases carotenoid absorption by reducing chylomicron secretion following medium chain triglyceride (MCT) consumption [40]. Similarly, vanGreevenbroek et al. demonstrated that secretion of chylomicrons from Caco-2 human intestinal cells is stimulated more effectively by MUFA and PUFA than SFA [41]. Collectively, these findings suggest that butter would be a relatively poor selection as a lipid source to enhance carotenoid bioavailability compared to MUFA- or PUFA-rich lipids.

Although there were differential effects of the three lipid sources selected in the present study, TAG source and degree of unsaturation was not found to be as significant a factor as amount of lipid in affecting overall carotenoid absorption. The relatively narrow range of amount of lipid (3–20 g) selected for investigation, while extendable to many common dietary practices, may have tempered the ability to detect possible differences that such sources may have on preabsorptive (digestive release and micellarization) and absorptive (uptake, chylomicron synthesis, and secretion) stages of carotenoid absorption. For example, a dose-dependent response in absorption was not observed when salads were consumed with canola oil, although a robust dose-dependent relationship was observed with soybean oil. The lack of a response with canola oil was surprising considering the report of Brown et al. [20], however, was likely due in large part, to the efficient absorption of carotenoids from salad with only 3 g of canola oil (Fig. 2, Table 3). The inclusion of a fat-free group and higher levels of canola oil, as done by Brown et al. [20] would likely have resulted in observance of a dose response for canola oil as well. However, the low lipemic response with 3 g of canola oil and high extent of carotenoid absorption (Fig. 2) suggest that absorption from the salad meal with low amount of lipid may be significantly impacted by TAG type and specifically by the presence of MUFAs such as oleic acid (Supporting Information Table 1). The impact of oleic acid on chylomicron synthesis and secretion was previously demonstrated [41]. The possibility that a lower dose of a "healthy" oil can be a strong potentiator of carotenoid absorption merits further investigation to elucidate the mechanism behind this observation.

In conclusion, the results of the current study suggest that amount of co-consumed lipid is a primary potentiator of carotenoid absorption from common salad vegetables. Overall, 20 g of lipid promotes greater absorption of carotenoids from a raw vegetable salad than 3 and 8 g for the three different types of lipid tested in this study. However, source of lipid may have distinct impact on carotenoid absorption that merits further study. Compared to MUFA- and PUFA-rich oils, SFA-rich butter appears to be least effective, in the present study, at promoting carotenoid absorption. PUFA-rich soybean oil promotes the greatest dose-dependent response, however, 3 g of MUFA-rich canola oil promotes the same amount of absorption compared to 20 g of the same oil. Overall, considering observed effects on carotenoid absorption from canola oil, these data support the notion that adhering to the 2010 Dietary Guidelines for Americans, which suggest replacing saturated fats with unsaturated fats and consuming oils in smaller quantities, would not negatively impact carotenoid absorption from vegetables. The extent to

which these findings would translate effectively within the context of a balanced and more complex meal containing both protein and carbohydrate ingredients remains to be explored. Additionally, the present study focused specifically on the impact of co-consumed lipid on carotenoid bioavailability. It has been documented that when co-consumed lipid amounts or sources do not facilitate their incorporation into chylomicrons, carotenoids can be stored in epithelial cells until lipids from a subsequent meal are available to promote their absorption [40]. Therefore, it will be important for future research to evaluate not only the impact of co-consumed lipids but also intestinal lipid loading and meal patterns on the bioavailability of carotenoids from common dietary sources.

The authors would like to thank Cargill, Incorporated for generously donating the canola and soybean oils, Lisa Jackman for designing the washout and controlled diet protocols, and the study participants for their time and effort. The authors would also like to recognize Jan Green, Teryn Sapper, Jason Nelson, and Milena Leon for assisting with sample collection, processing, and analysis. This study was funded by USDA-NRI grant no. 2007-02313 and the USDA National Needs Fellowship in Foods for Health.

The authors have declared no conflict of interest.

5 References

[1] Nkondjock, A., Ghadirian, P., Dietary carotenoids and risk of colon cancer: case-control study. *Int. J. Cancer* 2004, *110*, 110–116.

[2] Wu, K., Erdman, J. W., Jr., Schwartz, S. J., Platz, E. A. et al., Plasma and dietary carotenoids, and the risk of prostate cancer: a nested case-control study. *Cancer Epidemiol. Biomarkers Prev.* 2004, *13*, 260–269.

[3] Zheng, W., Blot, W. J., Diamond, E. L., Norkus, E. P. et al., Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. *Cancer Res.* 1993, *53*, 795–798.

[4] Riboli, E., Norat, T., Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am. J. Clin. Nutr.* 2003, *78*, 559S–569S.

[5] Millen, A. E., Tucker, M. A., Hartge, P., Halpern, A. et al., Diet and melanoma in a case-control study. *Cancer Epidemiol. Biomarkers Prev.* 2004, *13*, 1042–1051.

[6] Dwyer, J. H., Paul-Labrador, M. J., Fan, J., Shircore, A. M. et al., Progression of carotid intima-media thickness and plasma antioxidants: the Los Angeles atherosclerosis study. *Arterioscler. Thromb. Vasc. Biol.* 2004, *24*, 313–319.

[7] Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J., et al., The effect of fruit and vegetable intake on risk for coronary heart disease. *Ann. Intern. Med.* 2001, *134*, 1106–1114.

[8] Rissanen, T., Voutilainen, S., Nyyssonen, K., Salonen, J. T., Lycopene, atherosclerosis, and coronary heart disease. *Exp. Biol. Med. (Maywood)* 2002, *227*, 900–907.

[9] Rissanen, T., Voutilainen, S., Nyyssonen, K., Salonen, R., Salonen, J. T., Low plasma lycopene concentration is associated with increased intima-media thickness of the carotid artery wall. *Arterioscler. Thromb. Vasc. Biol.* 2000, *20*, 2677–2681.

[10] Beatty, S., Murray, I. J., Henson, D. B., Carden, D. et al., Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest. Ophthalmol. Vis. Sci.* 2001, *42*, 439–446.

[11] Mares-Perlman, J. A., Fisher, A. I., Klein, R., Palta, M. et al., Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am. J. Epidemiol.* 2001, *153*, 424–432.

[12] Moeller, S. M., Jacques, P. F., Blumberg, J. B., The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *J. Am. Coll. Nutr.* 2000, *19*, 522S–527S.

[13] United States Department of Health and Human Services, U.S.D.o.A., and United States Dietary Guidelines Committee, Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010. 2010.

[14] Yonekura, L., Nagao, A., Intestinal absorption of dietary carotenoids. *Mol. Nutr. Food Res.* 2007, *51*, 107–115.

[15] Yeum, K. J., Russell, R. M., Carotenoid bioavailability and bioconversion. *Annu. Rev. Nutr.* 2002, *22*, 483–504.

[16] Stahl, W., van den Berg, H., Arthur, J., Bast, A. et al., Bioavailability and metabolism. *Mol. Aspects Med.* 2002, *23*, 39–100.

[17] Tyssandier, V., Reboul, E., Dumas, J. F., Bouteloup-Demange, C. et al., Processing of vegetable-borne carotenoids in the human stomach and duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2003, *284*, G913–G923.

[18] Parker, R. S., Swanson, J. E., You, C. S., Edwards, A. J., Huang, T., Bioavailability of carotenoids in human subjects. *Proc. Nutr. Soc.* 1999, *58*, 155–162.

[19] Roodenburg, A. J., Leenen, R., van het Hof, K. H., Weststrate, J. A., Tijburg, L. B., Amount of fat in the diet affects bioavailability of lutein esters but not of alpha-carotene, beta-carotene, and vitamin E in humans. *Am. J. Clin. Nutr.* 2000, *71*, 1187–1193.

[20] Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., et al., Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am. J. Clin. Nutr.* 2004, *80*, 396–403.

[21] Hu, X., Jandacek, R. J., White, W. S., Intestinal absorption of beta-carotene ingested with a meal rich in sunflower oil or beef tallow: postprandial appearance in triacylglycerol-rich lipoproteins in women. *Am. J. Clin. Nutr.* 2000, *71*, 1170–1180.

[22] Mekki, N., Charbonnier, M., Borel, P., Leonardi, J. et al., Butter differs from olive oil and sunflower oil in its effects on postprandial lipemia and triacylglycerol-rich lipoproteins after single mixed meals in healthy young men. *J. Nutr.* 2002, *132*, 3642–3649.

[23] van Vliet, T., Schreurs, W. H., and van den Berg, H., Intestinal beta-carotene absorption and cleavage in men: response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. *Am. J. Clin. Nutr.* 1995, *62*, 110–116.

[24] Service, U.S.D.o.A.E.R., USDA Food Consumption Per Capita Data System. 2004.

[25] Forman, M. R., Beecher, G. R., Muesing, R., Lanza, E. et al., The fluctuation of plasma carotenoid concentrations by phase of the menstrual cycle: a controlled diet study. *Am. J. Clin. Nutr.* 1996, *64*, 559–565.

[26] Forman, M. R., Johnson, E. J., Lanza, E., Graubard, B. I. et al., Effect of menstrual cycle phase on the concentration of individual carotenoids in lipoproteins of premenopausal women: a controlled dietary study. *Am. J. Clin. Nutr.* 1998, *67*, 81–87.

[27] Weintraub, M. S., Eisenberg, S., Breslow, J. L., Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III, and type IV hyperlipoproteinemic individuals. Effects of treatment with cholestyramine and gemfibrozil. *J. Clin. Invest.* 1987, *79*, 1110–1119.

[28] Gianturco, S. H., Bradley, W. A., The role of apolipoprotein processing in receptor recognition of VLDL. *Methods Enzymol.* 1986, *129*, 319–344.

[29] Kean, E. G., Hamaker, B. R., Ferruzzi, M. G., Carotenoid bioaccessibility from whole grain and degermed maize meal products. *J. Agric. Food Chem.* 2008, *56*, 9918–9926.

[30] Usansky, J., Desai, A., Tang-Liu, D., 2011 PK functions for Microsoft Excel. Available from http://www.bo omer.org/pkin/soft.html

[31] Unlu, N. Z., Bohn, T., Clinton, S. K., Schwartz, S. J., Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocado or avocado oil. *J. Nutr.* 2005, *135*, 431–436.

[32] Prince, M. R., Frisoli, J. K., Beta-carotene accumulation in serum and skin. *Am. J. Clin. Nutr.* 1993, *57*, 175–181.

[33] Jayarajan, P., Reddy, V., Mohanram, M., Effect of dietary fat on absorption of beta carotene from green leafy vegetables in children. *Indian J. Med. Res.* 1980, *71*, 53–56.

[34] Hollander, D., Ruble, P. E., Jr., Beta-carotene intestinal absorption: bile, fatty acid, pH, and flow rate effects on transport. *Am. J. Physiol.* 1978, *235*, E686–E691.

[35] Lakshminarayana, R., Raju, M., Krishnakantha, T. P., Baskaran, V., Lutein and zeaxanthin in leafy greens and their bioavailability: olive oil influences the absorption of dietary lutein and its accumulation in adult rats. *J. Agric. Food Chem.* 2007, *55*, 6395–6400.

[36] Lee, A., Thurnham, D. I., Chopra, M., Consumption of tomato products with olive oil but not sunflower oil increases the antioxidant activity of plasma. *Free Radic. Biol. Med.* 2000, *29*, 1051–1055.

[37] Clark, R. M., Yao, L., She, L., Furr, H. C., A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. *Lipids* 2000, *35*, 803–806.

[38] Ockner, R. K., Manning, J. A., Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J. Clin. Invest.* 1974, *54*, 326–338.

[39] Council, N. R. (Eds.), Fat Content and Composition of Animal Products: Proceedings of a Symposium 1976, National Academy of Science, Washington, DC. 1976

[40] Borel, P., Tyssandier, V., Mekki, N., Grolier, P. et al., Chylomicron beta-carotene and retinyl palmitate responses are dramatically diminished when men ingest beta-carotene with medium-chain rather than long-chain triglycerides. *J. Nutr.* 1998, *128*, 1361–1367.

[41] van Greevenbroek, M. M., van Meer, G., Erkelens, D. W., de Bruin, T. W., Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells. *Atherosclerosis* 1996, *121*, 139–150.

