Absorption and Transit of Lutein and β-Carotene Supplements in the Mongolian Gerbil *(Meriones unguiculatus)*

Anne Laure Escaron and Sherry Ann Tanumihardjo*

Interdepartmental Graduate Program in Nutritional Sciences
University of Wisconsin-Madison, Madison, WI 53706, USA.

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Abstract: Based on a previous carotenoid bioavailability study in Mongolian gerbils *(Meriones unguiculatus),* we hypothesized that gerbils preferentially accumulate ß-carotene over lutein in the liver and lipoproteins. To monitor transit times of these carotenoids through the gastrointestinal tract and concentrations in various tissues and tissue contents, 0.1 μmol each of β-carotene and lutein were given separately as well as in combination to gerbils (n = 30). Contents of stomach, intestines, and ceca were collected at 1.5, 3, and 6 hours following the dose and analyzed for β-carotene and lutein. Mucosal scrapings, liver, and serum were also collected. When β-carotene and lutein were given in combination, 41 ± 11% (mean ± SD) ß-carotene versus 20 ± 4.0% lutein were recovered in total from all tissues and tissue contents. At 3 hours, 45 ± 19% and 55 ± 2.8% of the β-carotene and lutein supplements, respectively, were recovered in the cecum when given separately. When given in combination, 59 ± 32% and 55 ± 25% of the β-carotene and lutein, respectively, were recovered in the cecum. Beta-carotene was up to 45-fold higher than lutein liver concentrations 6 hours after dosing. Gerbils are a useful model for β-carotene bioavailability studies as they absorb and store β-carotene. More studies are needed to determine whether significant extra-hepatic lutein storage occurs in gerbils.

Key words: bioavailability, cecum, carotenoids, tissue distribution

* Corresponding author: Sherry Ann Tanumihardjo, UW-Madison, 1415 Linden Drive, Madison, WI 53706, USA. Fax 608-262-5860. E-mail: sherry@nutrisci.wisc.edu. Presented in part at the 2004 Experimental Biology meeting [Escaron, A.L., Porter Dosti, M.L. and Tanumihardjo, S.A. (2004) Absorption of lutein and β-carotene supplements in the Mongolian gerbil. FASEB J. 18, A, Abstract 131.7].

Abbreviations used: AMD, age-related macular degeneration; β-*apo*-8'CD, β-*apo*-8'carotenyl decanoate; βC, β-carotene; DCE, dichloroethane; EtOH, ethanol; GI, gastrointestinal; LSD, least significant difference; LSI, lower small intestine; L, lutein; MeOH, methanol; USI, upper small intestine.

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Introduction

Many animal studies and clinical trials focus on carotenoids due to their antioxidant properties. Epidemiological studies demonstrate that carotenoids are associated with a decrease in the incidence of malignancies in individuals consuming a diet high in fruits and vegetables [1–3]. Beta-carotene (βC), α-carotene, β-cryptoxanthin, lycopene, lutein (L), and zeaxanthin are the carotenoids most commonly found in human serum [4]. While the location of carotenoids in the human body is fairly well documented, ongoing research continues to elucidate the role that each of these carotenoids plays in human health. βC, along with other carotenoids, is stored mainly in adipose tissue while some βC is stored in liver [4]. βC travels in low-density lipoprotein (LDL) and complements vitamin E in quenching oxidative reactions [5, 6]. L and its isomer zeaxanthin are concentrated in the macula of the human eye [7–10]. This pair of isomers protects the primate retina from photoxidation and reactive oxygen species [11–13]. Of recent clinical interest is the potential role that antioxidants play in delaying age-related macular degeneration (AMD). Controlled clinical trials found that βC supplementation, along with antioxidant vitamins and minerals, protected the elderly from AMD [14, 15]. Furthermore, dietary L and zeaxanthin mitigate increasing density of the ocular lens, a natural part of the aging process, and limit the progression of AMD [13].

Due to interest in understanding carotenoid antioxidant properties and their hypothesized beneficial health effects, various animal models have been explored for their similarity to human carotenoid absorption [16]. Rats efficiently cleave βC to retinol in the intestinal mucosa [17, 19, 20] making them a poor model for βC absorption studies. Ferrets *(Mustela putorius furo)*, preruminant calves, and Mongolian gerbils *(Meriones unguiculatus)*, however, absorb βC intact [16, 19, 21]. In addition to desirable physiologic and disease traits, gerbils were presented as a suitable model for carotenoid absorption research [16]. In a previous study, however, we found that L supplements were not stored in Mongolian gerbil livers [22]. In this paper, we describe the distribution of βC and L supplements in various Mongolian gerbil tissues and tissue contents and further characterize this animal model's absorption of L. Furthermore, we present evidence for interactions during the transit from mouth to cecum of the oxycarotenoid, L, in the context of the hydrocarbon carotenoid, βC.

Materials and Methods

Animals (n = 30) and diet. Upon arrival, 10 male 42-day old Mongolian gerbils (Charles River, Kingston, NY, USA) were randomly assigned to a dose group: 1 gerbil was assigned as control, 3 were assigned to the βC group, 3 were assigned to the L group, and 3 were assigned to receive both βC + L. This process was repeated twice for a total of thirty gerbils. Gerbils were housed singly in solid-bottom plastic cages filled with pine shavings where ambient conditions were maintained between 21.1–23.3°C and 40–55% humidity. An automated dark:light cycle maintained the dark period from 19:00–07:00. Gerbils had free access to water and a pelleted, carotenoid-free diet [22] throughout the week-long study. Gerbils ate 0–11 g feed daily and averaged 5.6 ± 0.5 g/day (mean ± SD). All gerbils were given 105 µL cottonseed oil daily to adapt them to the oral pipetting process. After 6 days of feeding the carotenoid-free diet and administering the oil, gerbils were given the assigned dose. This protocol was approved by the University of Wisconsin-Madison Research Animal Resources Center.

Dose preparation. The L and βC doses were prepared as described before [22] except that both βC and L were dissolved in hexanes instead of ethyl acetate. Cottonseed oil was added, followed by sonification and subsequent evaporation of the solvent under vacuum. The solution concentrations were calculated by measuring the absorbance of a hexanes dilution at 445 and 453 nm for L and βC, respectively, on a UV-visible recording spectrophotometer (Shimadzu UV-2501 PC) using the $E^{1\%}_{1cm}$ for each carotenoid. On day 7 of the experiment, gerbils were given either 101 µL of oil solution to receive 0.1 µmol L (56.9 µg), 105 µL oil solution to provide 0.1 µmol βC (53.7 µg), or 125 µL oil solution for those receiving the combined dose (L + βC) containing 0.1 µmol L and 0.1 µmol βC. The control group was given no cottonseed oil on day 7.

Collection of tissues and tissue contents. For each treatment group (L, βC, or L + βC), 3 gerbils were killed by cardiac exsanguination at 1.5, 3, or 6 hours following the dose. The control group (n = 3) was killed at time 0. Blood, liver, stomach, intestines, and ceca were collected. Blood was processed as previously reported [22].

A 15-cm segment of the small intestine proximal to the stomach was cut. This section was referred to as upper small intestine (USI). The remaining section of the small intestine, approximately a 15-cm segment, was termed the lower small intestine (LSI). The USI and LSI were flushed with 0.9% (w/v) NaCl solution until it contained no particulate matter and the flush was collected. Next, the USI was cut lengthwise, opened, and scraped with a glass slide to collect mucosal cells. USI and LSI flushes, mucosal scrapings, liver, ceca, and stomach samples were immediately placed on dry ice and protected from light. Sam-

ples were then transferred to –80°C for storage until analysis.

Extractions of tissue and tissue contents. Analyses were performed under yellow lighting in order to minimize oxidation and isomerization of carotenoids and retinoids. The USI and LSI flushes were analyzed by nearly identical protocols. Internal standard (IS; 300 µL; 3.4 nmol) β-*apo*-8' carotenyl decanoate (β-*apo*-8'CD) [23]) was added to the entire volume of each flush. Flush volumes were measured and an equal volume of ethanol (EtOH) with 0.1% butylated hydroxytoluene (BHT) (w:v; ICN Biomedicals, Inc.) was added to each sample to denature proteins. Half the sample volume of hexanes was added to the USI flush (range: 3.3–7.3 mL). The sample's volume of hexanes was added to the LSI flush (3.1–4.7 mL). These volumes were used to simplify sample preparation during the extraction process. The solution was mixed by vortex for 30 seconds then centrifuged for 30 seconds (1380 × g), and the hexanes layer was removed. The hexanes extraction was repeated twice and pooled. Solutions were dried under argon and redissolved in 100 µL 50:50 (v:v) methanol:dichloroethane (MeOH:DCE); 25 µL was injected into the high-performance liquid chromatography (HPLC) system.

For the mucosal scrapings, β-*apo*-8'CD (15 µL; 0.2 nmol) was added to each sample after it was weighed (range: 0.4–0.9 g). Proteins were denatured with 1 mL EtOH (0.1% BHT) and samples were extracted with 1 mL hexanes 3 times. Each sample was redissolved in 50 µL 50:50 MeOH:DCE; 35 µL was injected.

Stomach and cecal contents were extracted with the same volumes of solvents as used for the mucosal scrapings. Stomach contents were weighed (0.6–1.7 g); β-*apo*-8'carotenyl decanoate (β-*apo*-8'CD) (160 µL; 1.9 nmol) was added and the extract was redissolved in 200 µL 50:50 MeOH:DCE. Cecal contents were weighed (0.8–1.2 g) and β-*apo*-8'CD (300 µL; 3.6 nmol) was added. Cecal extracts were redissolved in 100 µL 50:50 MeOH:DCE. For both stomach and caecal content extracts, 25 µL was injected.

Serum samples were prepared as reported previously, with minor changes [22]. Briefly, 45 µL β-*apo*-8'CD (0.1 nmol) was added to each sample (660–980 µL). EtOH (0.1% BHT) (1 mL) and hexanes (1 mL) were added. When sample volumes were < 0.7 mL, 0.5 mL of both EtOH (0.1% BHT) and hexanes were added. Samples were redissolved in 50 µL 50:50 MeOH:DCE; 35 µL was injected.

As previously reported for liver analysis [22], sodium sulfate (4.5 g) and IS (200 µL; 9.1 nmol retinyl butyrate) were added to 1.5 g liver. As described by Furr [24], these were ground exhaustively. Alternating washes of dichloromethane and ethyl acetate were added until 50 mL of filtrate (Whatman filter 7 cm No. 1) accumulated. An aliquot of filtrate (10 mL) was evaporated under argon, redissolved in 100 µL 50:50 MeOH:DCE; 50 µL was injected.

Standard curves were compiled from HPLC-purified L and βC to quantify sample carotenoids in all tissues and tissue contents. Retinyl acetate (Sigma) and retinol [24] standard curves were generated to quantify retinoids. The retinol standard curve was used to calculate retinol plasma and liver concentrations whereas the retinyl acetate standard curve was used to quantify retinyl ester peaks in liver. For each liver sample, individual retinol and retinyl ester tissue concentrations were calculated first, then added together to yield total vitamin A.

HPLC system. All extracts described above were injected into a Waters HPLC system (Milford, MA, USA): 717plus autosampler, Resolve™ C18 5-µm 3.9 × 300 mm column, 996 photodiode array detector, and 1525 binary HPLC pump. A gradient system was developed to optimize separation of cis-βC, βC, L, and zeaxanthin [22]. Chromatograms were generated at 325 and 450 nm to characterize retinoids and carotenoids, respectively. The limit of detection for L and βC for this system is 0.7 ng.

Statistical methods. For comparison of gerbil body and liver weights during the three-week experiment, a mixed model was used to represent block (week) as the random effect and treatment (dose administered and time of kill) as the fixed effect. Comparisons of weights by time of kill were made by differences of least squares means. Multiple comparisons were accounted for using the Bonferroni correction. Results were considered significant with adjusted $p < 0.05$. For serum and liver carotenoid concentrations, the general linear model (GLM) procedure was used to compare baseline, 1.5-, 3-, and 6-hour time points for each dose. The least significant difference (LSD) was used to compare group means. The amounts of dose recovered were compared using one-way ANOVA and LSD. Alpha was set at 0.05 to indicate significant differences. All analyses were performed using SAS (Version 8, SAS Institute). Data are presented as means ± SD.

Results

Mongolian gerbil body and liver weights:

Mean body (49 ± 1.4 g) and liver (2.6 ± 0.1 g) weights did not differ among groups due to week of experiment ($p > 0.05$). In addition, there were no differences in body or liver weights between times of kill ($p > 0.05$; data not shown).

Serum and liver βC and L concentrations:

When comparing serum βC and L concentrations at 1.5, 3, and 6 hours following each dose, no significant differences were found ($p > 0.05$) although both carotenoids appear to be cleared from the serum over time (Table I). The highest serum L value was 13 ± 7.7 nmol/L (at 3 hours) when given with βC (Table I). At 6 hours, liver βC concentrations were 7- and 45-fold greater than liver L concentrations when the carotenoids were given separately and together, respectively.

β-*apo*-8'CD (retention time 13.4 min) was used as the internal standard (IS) for all samples except liver. In the intestinal flush, mucosal scraping, and cecal content samples, β-*apo*-8'carotenol (retention time 5.6 minutes) was detected at similar concentrations in control, L, βC, and βC+ L dosed gerbils whereas it was not found in any stomach and serum samples. Hydrolysis of β-*apo*-8'CD is presumed to have occurred in intestinal flush, mucosal scraping, and cecal content samples. Both the β-*apo*-8'CD and the β-*apo*-8'carotenol had λmax at 428 and 453 nm. In calculating extraction efficiency of intestinal flush, mucosal scraping, and cecal content samples, β-*apo*-8'carotenol area under the curves were added to those of β-*apo*-8'CD. βC is converted to β-a*po*-8'-carotenal in rat intestinal extract [17]; however, this conversion does not explain the presence of the carotenol in gerbil tissues and their contents that did not receive βC.

Table I: Serum and liver ß-carotene (βC) and lutein (L) concentrations at various times after supplementation with 0.1 µmol L or βC when given alone and together[1]

Tissue	Dose	Time (h)	L	βC
Serum	Control	0	0	trace[2]
(nmol/L)	L	1.5	8.2 ± 5.1	
		3	7.0 ± 6.1	
		6	2.3 ± 2.8	
	βC	1.5		8.5 ± 9.5
		3		5.8 ± 1.8
		6		3.7 ± 4.1
	L + βC	1.5	6.3 ± 7.2	26 ± 33
		3	13 ± 7.7	6.7 ± 0.97
		6	1.7 ± 1.9	4.0 ± 4.1
Liver	Control	0	0	0.087[3]
(nmol/g)	L	1.5	0.029[4]	
		3	0.013 ± 0.025	
		6	0.026 ± 0.024	
	βC	1.5	0.053 ± 0.066	
		3	0.10 ± 0.071	
		6	0.19 ± 0.12	
	L + βC	1.5	0	0.12 ± 0.069
		3	0.030 ± 0.027	0.15 ± 0.083
		6	0.010[5]	0.11 ± 0.12

[1] Values are means ± SD, n = 3 at each time point. No significant differences between time points (1.5, 3, or 6 hours) were found within a carotenoid dose (L, βC, or L+ βC).
[2] One control gerbil had detectable but not quantifiable serum βC concentrations.
[3] One control gerbil had quantifiable liver βC.
[4,5] n = 1

Dose recovery

When βC and L were given separately, 26 ± 8.5% (mean ± SD) of the 0.1 µmol L and 0.1 µmol βC doses were recovered from all tissues and tissue contents. When βC and L were given in combination, significantly more βC (41 ± 11%) than L (20 ± 4.0%) was recovered in total from all tissues and tissue contents ($p < 0.05$). The total amount of recovered L (from all tissues and tissue contents collected), regardless of whether given alone or in combination, ranged from 24 ± 8.8 nmol recovered at 1.5 hours to 16 ± 6.2 nmol at 6 hours. When given alone, 32 ± 29 nmol of the total βC given was recovered at 1.5 hours versus 23 ± 4.2 nmol at 6 hours. For combined βC, a total of 54 ± 20 nmol was recovered at 1.5 hours while 33 ± 17 nmol was recovered at 6 hours.

Fraction of the dose adjusted for recovery

The fraction of the dose in each tissue or tissue content was adjusted for recovery of the dose. These values sum to 100% for a time point (1.5, 3, or 6 hours) and a carotenoid given (L or βC; whether given separately or in combination). For both single doses at 1.5 hour, 60 ± 26% L and 53 ± 47% βC of the total recovered dose was detected in the stomach (Figure 1A, Figure 2A). For combined doses at 1.5 hours, there were equal amounts of L and βC respectively in the stomach (47 ± 34% and 57 ± 40%) and cecum (40 ± 31% and 39 ± 37%) (Figure 1B, Figure 2B). By 3 hours, significantly more of the total L recovered when given alone was found in the cecum (55 ± 2.8%) compared to the stomach (31 ± 13%). At 3 hours there were equal amounts of βC when given alone in the stomach (47 ± 16%) and the cecum (45 ± 19%). At 6 hours, equal amounts of L and βC respectively were found in the stomach (35 ± 30% and 43 ± 23%) and cecum (60 ± 34% and 53 ± 22%) when given individually (Figure 1A, Figure 2A).

A clearing trend from 1.5 to 6 hours was evident in recovered L percentages of the USI flush (0.63 ± 0.64, 0.44 ± 0.43, and 0.16 ± 0.20% for L alone) and LSI flush (9.0 ± 5.4, 6.6 ± 6.2, and 1.3 ± 0.5% for L alone). A similar trend was observed when βC was administered independently: 22 ± 39, 0.19 ± 0.21, and 0.14 ± 0.25% was re-

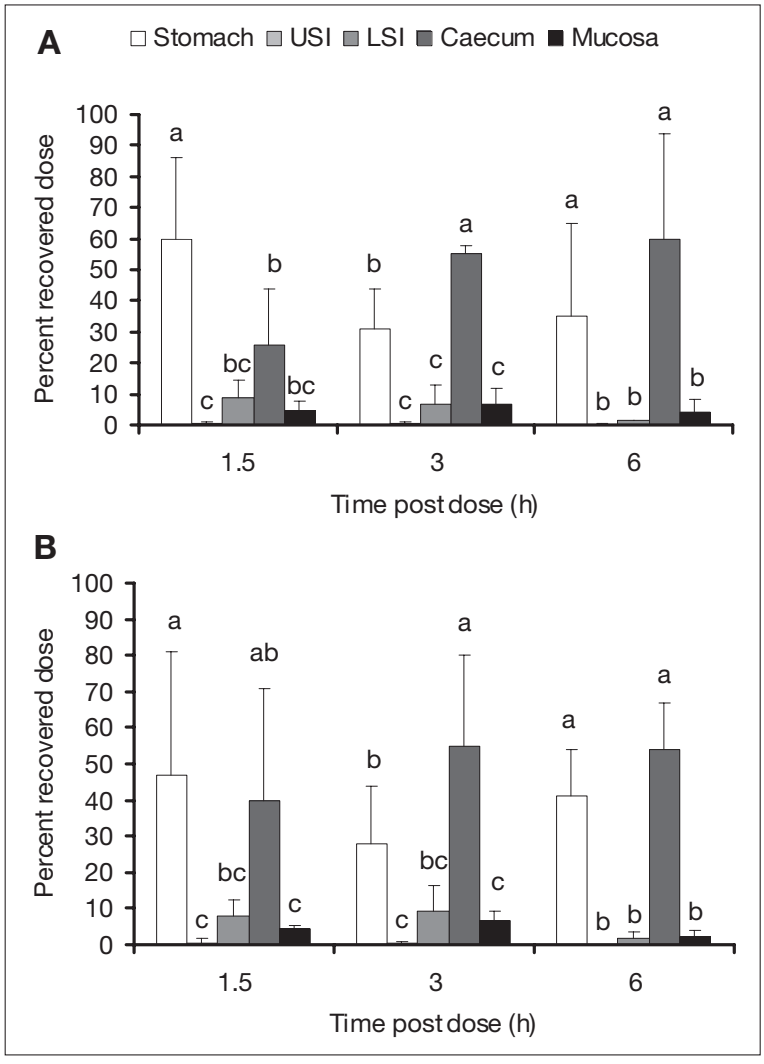

Figure 1: Percent of recovered lutein when given alone *(A)* or in combination with β-carotene *(B)* from contents of stomach, upper small intestine (USI), lower small intestine (LSI), cecum, and mucosal scrapings 1.5, 3, and 6 hours post dose. Values are means ± SD, n = 3. By time point, means without a common letter differ, $\alpha = 0.05$. Control gerbils had undetectable carotenoid concentrations. Serum and liver lutein values are omitted because they represent < 2% of the recovered dose.

covered from the USI flush and 5.8 ± 4.4, 5.4 ± 5.4, and 1.8 ± 1.9% from the LSI flush. When given in combination, βC increased until 3 hours then decreased in both the USI (0.24 ± 0.32, 0.41 ± 0.72, and 0.05 ± 0.01%) and the LSI (3.3 ± 2.3, 5.0 ± 3.8, and 1.4 ± 1.4%). For L given in combination, this same pattern was also found in LSI (7.8 ± 4.8, 9.1 ± 7.1, and 1.6 ± 2.0%). In the USI, when L was given in combination, it was cleared in the same way as when given alone (0.66 ± 0.99, 0.50 ± 0.35, and 0.05 ± 0.04%).

Vitamin A status

All gerbils had sufficient vitamin A status with total liver vitamin A ranging between 0.87–2.1 μmol/liver. These values are from 6 to 15 times the upper cut-off for indicating vitamin A deficiency in humans [18]. Serum retinol concentrations were 1.4 ± 0.2 μmol/L.

Discussion

This experiment describes L and βC transit through the gastrointestinal (GI) tract when given independently or in combination. Most of the carotenoids were found in the upper GI tract (stomach, USI flush, mucosal scrapings) at 1.5 hours post-dose and then moved through the lower intestinal tract (LSI flush and cecum) within 6 hours of the dose.

Previously we reported that Mongolian gerbils given 0.088 μmol L (50 μg) daily for 14 days stored no L in the liver [22]. In that study, gerbils were fasted daily from 08:00–13:00, dosed with L, and then fed a carotenoid-free diet (7% fat) *ad libitum* until the next day at 08:00. While carotenoid absorption may follow a gradual course, the two-week study duration should have been sufficient time for L to accumulate in the liver. Our current findings support this previous result. We found liver L concentrations

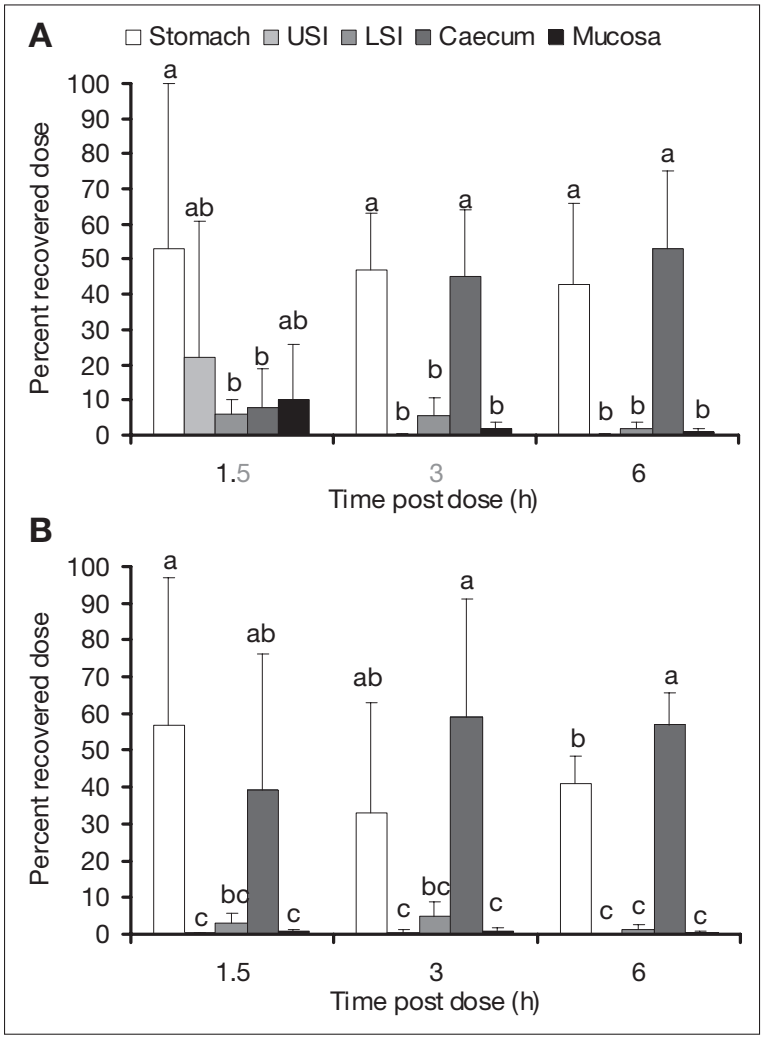

Figure 2: Percent of recovered ß-carotene when given alone *(A)* or in combination with lutein *(B)* from contents of stomach, upper small intestine (USI), lower small intestine (LSI), cecum, and mucosal scrapings 1.5, 3, and 6 hours post dose. Values are means ± SD, n = 3. By time point, means without a common letter differ, $\alpha = 0.05$. Control gerbils had undetectable carotenoid concentrations. Serum and liver β-carotene values are omitted because they represent < 2% of the recovered dose.

were lower than βC both when given alone and in combination. Having anticipated this result, we analyzed 150 mg of liver tissue in this study whereas in the prior study we evaluated 2.5 mg per injection. By 6 hours post-dose, βC accumulated 7- to 45-fold higher than L in the liver when administered alone and in combination, respectively. While both of our studies used L oil supplements, an experiment in which Mongolian gerbils were fed *Arabidopsis* leaf further confirmed that L, in contrast to βC, is not stored in the gerbil liver [25]. These results may indicate liver selective uptake of βC from chylomicron remnants as well as extra-hepatic storage of L in tissues such as adipose, which requires further evaluation. There is evidence for the former possibility in the work of Stahl et al [26] in which they described specific isomeric differences in uptake by humans. All-*trans*-βC preferentially accumulated in chylomicrons and very low-density lipoprotein (VLDL) compared to 9-*cis*-βC after subjects consumed a supplement containing 54% all-*trans* and 37% 9-cis βC. When subjects (n = 4) were given a combined dose of 5.6 and 0.17 μmol/kg body weight of βC and α-carotene, respectively, 50–1000 nmol βC/L increases were found in serum. Serum βC concentrations were highest between 24 and 48 hours after dose administration and returned to baseline between 7 to 9 days [27]. In the gerbils, the peak serum βC concentration occurred in the earliest time points.

When subjects were dosed with single equimolar amounts (0.5 μmol/kg body weight) L, βC, or L + βC, the mean serum L concentration peaked once at 16 hours compared with βC which peaked twice, at 6 and 32 hours. The authors present evidence that these carotenoids interact with one another during absorption and metabolism. When co-administered, L's absorption did not appear to be altered; however, its peak concentration was lower in the presence of βC. The variability in the gerbil serum carotenoid concentrations was large. Keeping this in mind, the peak L serum concentration was higher when administered with βC than when given alone. In contrast, βC's

initial rate of absorption was decreased and the 6 hour serum βC peak was no longer observed. Serum βC concentration did peak at 32 hours and was at a similar concentration as when administered alone [28]. In the gerbils, L may have led to an increase in initial serum βC concentrations.

In the last decade, specific carotenoid binding proteins were identified in various species. In the silkworm larvae *(Bombyx mori)*, a binding protein was isolated that shuttles L between tissues [29,30]. In ferret *(Mustela putorius furo)* livers, a βC-specific protein was purified [31] though its role remains elusive. Perhaps a related protein is present in the Mongolian gerbil acting as a chaperone for increased storage and re-circulation of βC versus L, enhancing liver βC concentrations.

At 3 hours, more L was recovered in the cecum than in the stomach; presumably most of the L was no longer available for absorption at that point. This result explains the low serum L concentration (0.12 ± 0.11 nmol/L) in our previous study [22]. In that study, serum was collected roughly 19 hours after the last L dose. At that point, all supplemental L would have passed through the GI tract, and because absorbed L is not stored in the liver, one would expect to see little L in serum. The current study, using earlier time points, showed that L was minimally absorbed, quickly cleared from the upper GI and serum, and did not accumulate in the liver.

Serum carotenoid concentrations were on the same order of magnitude (nmol) for all treatments; however, they also follow a clearing pattern that could be explained by some uptake into tissues. We previously argued that the Mongolian gerbil is a poor model to test bioavailability of carotenoids by measuring serum carotenoid concentrations [32]. The serum L concentrations we found in the gerbils are three orders of magnitude lower than human serum L concentrations [33], even though the gerbil dose was equivalent to 1 mg L/kg body weight – a level that far exceeds current average daily human carotenoid intake [34]. For some of the gerbils, carotenoids were undetectable in the serum.

While gerbils do absorb and store βC in the liver, they do not seem to re-circulate it like humans. The species-specific distribution of high-density lipoprotein (HDL) versus LDL favors the re-circulation of carotenoids in humans but not Mongolian gerbils. During fasting, about 75% of carotenoids in humans are associated with LDL, the remainder with HDL [35]. In the Mongolian gerbil, the major circulating lipoprotein is HDL, not LDL. Mongolian gerbils had circulating lipoproteins composed of 63% HDL and 17% LDL following a polyunsaturated fat meal [36] similar to the fatty acid composition of our cottonseed oil dose. In comparison, the typical lipoprotein profile in humans is ~30% HDL and ~50% LDL [37–39].

Gerbils are thought to be coprophagous [40] but carotenoid reuptake was most likely very minimal in this experiment due to the short time-course. The unaccounted-for carotenoid may have been excreted, lost during administration, or may have been stored in tissues that were not collected as part of this study. These organs include spleen, kidney, adipose, and lung with the spleen possibly contributing the greatest percentage of unrecovered βC. When a single βC dose (279 nmol) was administered there was no change in those tissues' βC concentrations up to 70 hours following the dose. Pollack *et al* speculate that repeated dosing along with increased monitoring time would lead to extra-hepatic storage of βC [41]. Despite the omission of the tissues cited above, the carotenoid recoveries (28 ± 11%) were higher than previously reported [42], i.e. 0.6–4.1% of supplemental βC recovered 6 hours post-dose. This difference can be partially attributed to carotenoid cecal contents, which were not analyzed previously.

Of note, is the relatively high percentage of the dose that remains in the stomach after 6 hours. We observed this in dosed gerbils whose intensely yellow stomach contents appeared distinct from controls. Gerbils are predominantly grazers. They were not fasted as part of this study. Therefore, complete stomach emptying did not occur as it does in conjunction with meal feeding.

In summary, this study supports our previous finding that Mongolian gerbils are questionable for use in L bioavailability studies though they are a viable and useful choice for α- and β-carotene [32]. Their use as models for interaction studies needs further exploration. Interpreting serum βC data from gerbils, however, suggests that it is an insensitive indicator of βC stores. The lipoprotein profile of gerbils, which is different from that of humans, is an example of species-specific differences that partially explains the low circulating βC and L levels (nmol) seen in this study. The Mongolian gerbil represents a good model for βC bioavailability studies as it absorbs and stores it. Further studies are needed to test for extra-hepatic storage of L in the gerbil.

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Sherry A. Tanumihardjo

University of Wisconsin-Madison
Interdepartmental Graduate Program in Nutritional Sciences
1415 Linden Dr.
Madison, WI 53706 USA