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Coconut Oil Enhances Tomato Carotenoid Tissue Accumulation Compared to Safflower Oil in the Mongolian Gerbil (*Meriones unguiculatus*)

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ABSTRACT: Evidence suggests that monounsaturated and polyunsaturated fats facilitate greater absorption of carotenoids than saturated fats. However, the comparison of consuming a polyunsaturated fat source versus a saturated fat source on tomato carotenoid bioaccumulation has not been examined. The goal of this study was to determine the influence of coconut oil and safflower oil on tomato carotenoid tissue accumulation in Mongolian gerbils (*Meriones unguiculatus*) fed a 20% fat diet. Coconut oil feeding increased carotenoid concentrations among many compartments including total carotenoids in the serum (p = 0.0003), adrenal glandular phytoene (p = 0.04), hepatic phytofluene (p = 0.0001), testicular *all-trans*-lycopene (p = 0.01), and *cis*-lycopene (p = 0.006) in the prostate−seminal vesicle complex compared to safflower oil. Safflower oil-fed gerbils had greater splenic lycopene concentrations (p = 0.006) compared to coconut oil-fed gerbils. Coconut oil feeding increased serum cholesterol (p = 0.0001) and decreased hepatic cholesterol (p = 0.0003) compared to safflower oil. In summary, coconut oil enhanced tissue uptake of tomato carotenoids to a greater degree than safflower oil. These results may have been due to the large proportion of medium-chain fatty acids in coconut oil, which might have caused a shift in cholesterol flux to favor extrahepatic carotenoid tissue deposition.

KEYWORDS: *coconut oil, tomato, bioaccumulation, carotenoids, Mongolian gerbil*

INTRODUCTION

Tomato consumption has been linked to decreased chronic disease risk.[1] In addition to vitamins and minerals, tomatoes contain a variety of carotenoids. Lycopene (LYC) is the major carotenoid found in tomatoes, but tomatoes also contain phytoene (PE), phytofluene (PF), α-carotene (AC), ζ-carotene (ZC), and β-carotene (BC).[1] LYC has garnered much interest because of its ability to quench singlet oxygen, attenuate LDL levels, reduce the risk of cardiovascular disease, and potentially inhibit carcinogenesis.[2,3] However, the other tomato carotenoids may exhibit similar bioactive properties as LYC.[1] Recent studies have suggested that the combination of tomato carotenoids from whole tomato powder may be more effective in disease prevention than LYC alone.[1,4]

Bioavailability describes the degree to which carotenoids may be absorbed and utilized from ingested food sources.[2] Many factors influence carotenoid bioavailability. The species of carotenoid, food matrix, food processing, amount of carotenoid consumed, genetic factors, and nutrient interactions as well as the coconsumption of dietary fat are all contributing factors.[2] The latter point was demonstrated in humans when carotenoids from a vegetable salad were more bioavailable when consumed with a full-fat salad dressing than with a reduced-fat salad dressing.[5] Additionally, tomato carotenoids were more readily absorbed from salsa and salad with the addition of avocado oil or avocado fruit.[6] Dietary fat is believed to facilitate bioavailability at the levels of solubilization, micellarization, and chylomicron packaging of dietary carotenoids.[6−8]

The structural properties of dietary fat also influence carotenoid bioavailability with evidence suggesting fats with differing degrees of saturation differ in their ability to enhance carotenoid absorption. In humans, saturated fat-rich beef tallow increased postprandial BC content in chylomicrons compared to polyunsaturated fat-rich sunflower oil.[9] However, butter, a source of saturated fat, reduced the postprandial chylomicron response in humans when compared with monounsaturated olive oil and polyunsaturated sunflower oil.[10] These studies suggest that monounsaturated and polyunsaturated fats enhance secretion of chylomicrons, whereas saturated fat enhances carotene incorporation into chylomicrons. The specific effects, however, of a saturated versus polyunsaturated fat on tomato carotenoid incorporation into chylomicrons has not been previously reported.

Previous studies have indicated that fatty acid chain length may also influence carotenoid bioavailability and absorption. Borel et al. demonstrated increased solubility of carotenoids as

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Table 1. Gerbil Diet Composition Table

component (g/kg)	18% coconut oil and 2% safflower oil control diet	20% safflower oil control diet	18% coconut oil and 2% safflower oil + 10% tomato powder diet	20% safflower oil + 10% tomato powder diet
sucrose	283	283	242	242
casein[a]	200	200	167	167
cornstarch	119	119	119	119
maltodextrin	94	94	83	83
cellulose	60	60	43.8	43.8
micronutrients				
mineral mix[b]	35	35	35	35
vitamin mix[c]	5	5	5	5
L-cystine	3	3	3	3
choline bitartrate	2.5	2.5	2.5	2.5
$CaHPO_4$	2	2	2	2
MgO	1.75	1.75	1.75	1.75
tomato powder[d]	0	0	100	100
coconut oil	180	0	180	0
safflower oil	20	200	20	200
kcal/g	3.73	3.73	3.73	3.73

[a]Vitamin-free test casein contains 0.9 g protein/g casein. [b]AIN-93G-MX (Teklad) contains calcium carbonate (12.495 g/kg diet); potassium phosphate, monobasic (6.86 g/kg diet); potassium citrate, monohydrate (2.48 g/kg diet); sodium chloride (2.59 g/kg diet); potassium sulfate (1.63 g/kg diet); magnesium oxide (0.85 g/kg diet); ferric citrate (0.21 g/kg diet); zinc carbonate (0.06 g/kg diet); manganese carbonate (0.02 g/kg diet); cupric carbonate (0.01 g/kg diet); potassium iodate (0.35 mg/kg diet); sodium selenate (0.36 mg/kg diet); ammonium paramolybdate, tetrahydrate (0.28 mg/kg diet); sodium metasilicate, nonahydrate (0.0575 g/kg diet); chromium potassium sulfate, doceahydrate (9.6 mg/kg diet); lithium chloride (0.609 mg/kg diet); boric acid (2.85 mg/kg diet); sodium fluoride (2.22 mg/kg diet); nickel carbonate hydroxide, tetrahydrate (0.111 mg/kg diet); ammonium metavanadate (0.231 mg/kg diet); and sucrose (7.73 g/kg diet).37 [c]AIN-93-VX (Teklad) provides niacin (0.015 g/kg diet); calcium pantothenate (0.008 g/kg diet); pyridoxine HCl (3.5×10^{-3} g/kg diet); thiamin HCl (3×10^{-3} g/kg diet); riboflavin (3×10^{-3} g/kg diet); folic acid (1×10^{-3} g/kg diet); biotin (0.1 mg/kg diet); vitamin B12 (0.1% in mannitol) (0.0125 g/kg diet); vitamin E, DL-α-tocopheryl acetate (500 IU/g) (0.075 g/kg diet); vitamin A palmitate (500,000 IU/g) (0.004 g/kg diet); vitamin D3 cholecalciferol (500,000 IU/g) (0.001 g/kg diet); vitamin K1, phylloquinone (0.375 mg/kg diet); sucrose (4.87 g/kg diet).37. [d]Drum-dried tomato powder (Futureceuticals, Momence, IL) contains 3.68 kcal/g, 0.01 g protein/g, 0.03 g fat/g, 0.16 g fiber/g, and 0.52 g carbohydrate/g.

fatty acid chain length decreased.[8] LYC and BC were more soluble in tricaprylin, a source of medium-chain triglycerides, when compared to long-chain triglycerides from fish oil.[8] Incorporation of carotenoids into mixed micelles was enhanced by the addition of long-chain fatty acids in vitro when compared to medium-chain fatty acids.[11] However, carotenoid uptake into Caco-2 cells was not affected by fatty acyl composition of mixed micelles,[11] suggesting triglyceride chain length does not affect carotenoid uptake by enterocytes. Furthermore, absorption of BC was similar when medium- and long-chain triglycerides were compared in the perfused, isolated rat intestine.[12] Borel et al. also observed an increase in BC in chylomicrons when humans ingested a salad with long-chain triglycerides compared to medium-chain triglycerides.[13] In short, whereas medium-chain triglycerides are better at solubilizing LYC and BC than long-chain triglycerides, human and animal trials suggest long-chain fatty acids may facilitate micellarization and incorporation of carotenes into chylomicrons. Intestinal absorption of carotenes does not seem to be significantly affected by fatty acid chain length. Although these studies describe the impact of fatty acid chain length and saturation on specific underlying steps of carotenoid bioavailability, there is little reported on the summative outcome of these altered mechanisms in terms of resultant serum and tissue carotenoid concentrations in response to differing consumed fat types.

Epidemiological evidence suggests regular intake of carotenoid-containing foods, such as fruits and vegetables, may decrease the risk for certain cancers.[3] A consequence of increased bioavailability may be increased bioaccumulation. Tissues with increased carotenoid bioaccumulation may offer some enhanced protection against oxidation and/or other events that lead to cancer.

The Mongolian gerbil (*Meriones unguiculatus*) has been shown to absorb BC and LYC intact.[14] In addition, we have reported that other tomato carotenoids, PE and PF, have marked accumulation in liver, spleen, lungs, testes, adrenal glands, the prostate−seminal vesicle complex, and adipose tissues.[15] The Mongolian gerbil is an excellent model for studies involving carotenoid bioaccumulation because it accumulates tomato carotenoids in levels proportionate to humans.[15]

Previously, we have fed 10% tomato powder diets to mice, rats, and gerbils.[4,15−17] Gerbils fed a 10% tomato powder diet for 26 days, followed by a 2 day wash-out period, had serum LYC levels of 0.007 μmol/dL.[15] Higher serum LYC concentrations have been observed in men ages 19−50 years old with an average serum LYC concentration of 0.05 μmol/dL.[18] Therefore, a 10% tomato powder rodent diet can be utilized on the basis of physiologically relevant serum LYC concentrations similar to those seen in humans.

In addition to carotenoid accumulation, the lipoprotein profiles of the gerbil are more similar to humans than most other rodents, making carotenoid metabolism findings more relevant than those results in other species.[19] A variety of fats have been successfully fed to gerbils to study lipid metabolism. In particular, two very different fats, saturated coconut oil and polyunsaturated safflower oil, were previously fed to gerbils to study lipoprotein metabolism.[20,21] Coconut oil is a good source of medium-chain triglycerides commonly used in enteral feeding solutions for humans.[22] Safflower oil is a common polyunsaturated fat used for cooking and as an ingredient in salad dressings.[23]

How fatty acid chain length affects the absorption and bioaccumulation of the array of tomato carotenoids, including PE, PF, ZC, BC, and LYC, from tomatoes has not been determined. We chose coconut oil and safflower oil to study carotenoid lipid metabolism because of the use of these fats in previous studies[20,21] and the differing fat characteristics that may affect carotenoid accumulation. Therefore, this work compares the effect of medium-chain triglyceride-rich coconut oil versus long-chain triglyceride-rich safflower oil on tissue bioaccumulation of tomato carotenoids from whole tomato powder in Mongolian gerbils.

■ MATERIALS AND METHODS

Chemicals. The HPLC grade solvents hexane, methanol, dichloromethane, methyl *tert*-butyl ether (MTBE), and chloroform were purchased from Fisher Scientific (Pittsburgh, PA, USA). Potassium hydroxide and ammonium acetate were also purchased from Fisher Scientific. Ethanol and polyoxyethylene octylphenyl ether were purchased from Sigma-Aldrich (St. Louis, MO, USA). PE, PF, and ZC standards were purchased from Carotenature (Lupsingen, Switzerland). LYC and BC were extracted from LYC or BC beadlets and obtained from DSM (Heerlen, The Netherlands).

Animal Methods. The University of Illinois Institutional Animal Care and Use Committee (IACUC) approved all procedures with animals. Forty-day-old male Mongolian gerbils were obtained from Charles River Laboratory (Wilmington, MA, USA) (n = 40) and were fed a pelleted chow diet for a 2 day acclimation period. On day 3, gerbils were randomized to either one of two control diets (n = 10/dietary treatment). Diets were modified from previously used high-fat gerbil diets[19] and those used to deliver tomato powder in the diet to rodents [15] (Table1). Control diets consisted of 20% safflower oil (SOC) or 18% coconut oil, with 2% safflower oil (COC) to prevent essential fatty acid deficiency.[24,25] Experimental diets consisted of 20% safflower oil with 10% tomato powder (TPSO) or 18% coconut oil and 2% safflower oil with 10% tomato powder (TPCO). Tomato powder contributed LYC, BC, PE, PF, and ZC to the diet (Table 2). The fatty acid profile for each diet is outlined in Table 3. Coconut oil consisted of 42.5% lauric (C12:0), 15.8% myristic (C14:0), and 8.9% palmitic acids (C16:0). Minor components in coconut oil included oleic acid (C18:1) (7.8%) and linoleic acid (C18:2) (9.3%). The fatty acid profile of safflower oil consisted of 67.8% linoleic acid and 17.2% oleic acid. Minor contributors included 6.4% palmitic acid (C16:0) and 2.6% stearic acid (C18:0) (Table 3). All gerbils were fed 15 g of the respective diet every 2 days for 28 days. Gerbil weights were recorded every 2 days throughout the study to monitor health and growth. On day 30, all four groups were fasted for approximately 4−6 h before being sacrificed. Gerbils were anesthetized with CO_2, blood was collected via cardiac puncture, and gerbils were then euthanized via CO_2 asphyxiation. Following euthanization, animals were partially shaved, and ventral skin samples were taken from each animal. Skin, gonadal adipose, heart, lungs, testes, adrenal glands, and prostate−seminal vesicle complex were removed, weighed, flash frozen in liquid nitrogen, and stored at −80 °C for future analysis. Additionally, livers were perfused with ice-cold 1.15% potassium chloride (KCl) before being frozen and stored.

Table 2. Diet Carotenoid Concentrations[a]

diet	safflower oil + 10% tomato powder (μmol/kg diet, mean ± SEM)	18% coconut oil and 2% safflower oil + 10% tomato powder (μmol/kg diet, mean ± SEM)
phytoene	12.2 ± 1.6	11.5 ± 1.4
phytofluene	5.1 ± 0.6	6 ± 0.3
cis-lycopene	86.2 ± 8.8	81 ± 9.1
all-trans-lycopene	282 ± 47	272 ± 40
total lycopene	368 ± 55	353 ± 49
ζ-carotene	1.5 ± 0.09	1.6 ± 0.07
β-carotene	1.2 ± 0.5	1.5 ± 0.6

[a]Diet carotenoid concentrations for 10% tomato powder + 20% safflower oil diet and 10% tomato powder + 18% coconut oil and 2% safflower oil. Values represent the mean of diet samples analyzed in triplicate ± SEM.

Table 3. Fatty Acid Analysis of Dietary Fats[a]

fatty acid chain length	common name	COC[b] (%)	TPCO[c] (%)	SOC[d] (%)	TPSO[e] (%)
8:0	caprylic	4.7	3.9	0.0	0.0
10:0	capric	5.2	4.9	0.0	0.0
12:0	lauric	42.9	42.2	0.0	0.0
14:0	myristic	15.8	15.9	0.2	0.2
16:0	palmitic	8.9	8.9	6.3	6.5
18:0	stearic	3.0	3.1	2.6	2.6
18:1	oleic	7.7	7.9	17.2	17.1
18:2	linoleic	9.0	9.6	68.0	67.5
18:3	linolenic	0.1	0.1	0.2	0.2
20:0	arachidic	0.1	0.1	0.4	0.3
20:1	gondoic	0.1	0.1	0.2	0.2
22:0	behenic	0.1	0.3	0.0	0.2
total unidentified FA		2.2	2.9	2.7	3.2
total MCFA		52.8	51.0	0.1	0.1
total LCFA		44.6	46.0	95.2	94.9
total fatty acids		99.7	99.9	98.0	98.3

[a]Table 3 represents the percentage of fatty acids based on total fatty acids in the diet run in duplicates. [b]COC, 18% coconut oil and 2% safflower oil. [c]TPCO, 18% coconut oil and 2% safflower oil + 10% tomato powder. [d]SOC, safflower oil. [e]TPSO, safflower oil + 10% tomato powder.

Dietary Carotenoid Extraction. To verify dietary carotenoid content, the entire extraction procedure was performed under yellow light along with other general precautions taken to preserve carotenoids. The extraction method used has been previously described.[16] First, 0.025 g of powdered diet was suspended in 5 mL of ethanol containing 0.1% butylated hydroxytoluene (BHT). Samples were homogenized at level 3 (Power Gen 500, Fisher-Scientific) for 120 s, and 1 mL of saturated potassium hydroxide (KOH) solution was added. Samples were saponified in a 60 °C water bath with intermittent vortexing for 30 min and removed, and 2 mL of deionized water and 6 mL of hexanes were added for biphasic extraction of carotenoids. Samples were vortexed and separated by centrifugation for 10 min, and the organic hexanes phase was removed and reserved. The addition of hexanes, followed by separation and removal, was repeated two more times. Hexanes were pooled and concentrated using a Speedvac concentrator (Speedvac model AS160, Savant, Milford, MA, USA). Samples were stored under argon at −20 °C for l<48 h before HPLC analysis.

Tissue Carotenoid Extraction. Liver carotenoid concentrations were determined by extracting approximately 0.1 g of liver tissue. Whole spleen (∼0.07 g) and prostate−seminal vesicle complex (∼0.4 g) were extracted for each animal. For adrenal glands (∼0.03 g), testes

Table 4. Impact of Dietary Fat on Carotenoid Accumulation in Gerbil Tissues[a]

tissue	dietary treatment group	phytoene (nmol/g)	phytofluene (nmol/g)	*cis*-lycopene (nmol/g)	*all-trans*-lycopene (nmol/g)	ζ-carotene (nmol/g)	β-carotene (nmol/g)	total lycopene (nmol/g)	total carotenoids (nmol/g)
liver	SO[b]	40.4 ± 9.6 a	21.9 ± 4.9 a	47.7 ± 8.7	51.2 ± 13.2	7.7 ± 2.1	3.2 ± 1.6	299.6 ± 113.2	372.7 ± 124.9
	CO[c]	61.8 ± 11.0 b	39.6 ± 4.4 b	59.3 ± 10.7	39.5 ± 8.4	8.8 ± 2.2	1.6 ± 0.6	299.1 ± 96.7	410.8 ± 112.3
spleen	SO	0.001 ± 0.0003	3.8 ± 0.6	17.8 ± 3.4	14.1 ± 2.5 a	0.52 ± 0.15	ND[d]	22.2 ± 8.8 a	36.3 ± 19.3
	CO	0.001 ± 0.0003	5.1 ± 0.8	12.4 ± 2.1	0.73 ± 1.2 b	0.78 ± 0.17	NQ[e]	12.5 ± 4.3 b	25.6 ± 13.2
adrenals	SO	22.5 ± 8.2 a	10.2 ± 2.5	9.4 ± 2.4	5.4 ± 1.4	2.9 ± 7.7	ND	14.8 ± 9.8	50.4 ± 39
	CO	71.4 ± 17.9 b	18.3 ± 3.9	14.2 ± 3.1	6.9 ± 1.5	4.6 ± 1.1	ND	21.1 ± 12	115.5 ± 69.8
lung	SO	0.13 ± 0.01	0.16 ± 0.04 a	0.51 ± 0.17	0.28 ± 0.09	0.06 ± 0.01 a	NQ	0.79 ± 0.8	1.1 ± 1.1
	CO	0.45 ± 0.2	0.42 ± 0.2 b	0.99 ± 0.26	0.44 ± 0.12	0.17 ± 0.03 b	NQ	1.4 ± 1.2	2.4 ± 1.8
testes	SO	ND	0.2 ± 0.04 a	0.09 ± 0.02 a	0.17 ± 0.03 a	0.03 ± 0.006 a	NQ	0.26 ± 0.13 a	0.49 ± 0.23 a
	CO	ND	0.65 ± 0.13 b	0.36 ± 0.08 b	0.57 ± 0.12 b	0.16 ± 0.03 b	NQ	0.9 ± 0.65 b	1.7 ± 1.2 b
prostate seminal vesicle complex	SO	ND	0.026 ± 0.006 a	0.01 ± 0.002 a	0.025 ± 0.01	0.006 ± 0.002 a	ND	0.04 ± 0.04	0.07 ± 0.06 a
	CO	ND	0.086 ± 0.02 b	0.025 ± 0.003 b	0.03 ± 0.004	0.02 ± 0.005 b	ND	0.06 ± 0.02	0.17 ± 0.09 b
serum[f]	SO	ND	ND	0.03 ± 0.002 a	0.003 ± 0.003 a	0.02 ± 0.001 a	ND	0.03 ± 0.005 a	0.05 ± 0.12 a
	CO	0.03 ± 0.006	0.02 ± 0.004	0.04 ± 0.007 b	0.02 ± 0.01 b	0.02 ± 0.004 b	ND	0.06 ± 0.01 b	0.1 ± 0.05 b
skin	SO	NQ	NQ	0.04 ± 0.02	0.04 ± 0.02	ND	ND	0.08 ± 0.04	
	CO	NQ	NQ	0.05 ± 0.02	0.05 ± 0.02	ND	ND	0.1 ± 0.04	

[a]Table 4 values are means ± SEM, $n = 10$. Different letters within rows indicate significant differences within tissues ($P < 0.05$). [b]SO, safflower oil + 10% tomato powder. [c]CO, coconut oil + 10% tomato powder. [d]ND, nondetectable within a tissue. [e]NQ, not quantifiable within a tissue, but detected at very low levels. [f]Units are nmol/dL.

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(~1.1 g), and lungs (~0.4 g), both specimens were pooled and extracted together for each animal. Samples were added to 5 mL of ethanol containing 0.1% BHT followed by manual homogenization. Carotenoids were extracted by hexanes using the above-described protocol.

Skin Carotenoid Extraction. Subcutaneous fat was removed prior to the extraction. The skin extraction procedure was previously described.[26] Briefly, ~0.3 g of skin was flash frozen and ground into a fine powder, and then 1 mL of ethanol with 0.1% BHT, 1 mL of water, and 5 mL of hexane/dichloromethane (5:1, v/v) were added to the samples. The samples were mixed, and the top layer of hexane and dichloromethane was removed. The extraction was repeated, and both hexane and dichloromethane extractions were pooled and dried under argon gas. Samples were stored at −20 °C for <24 h before analysis.

HPLC Analysis. Samples were analyzed using a high-pressure liquid chromatography−photodiode array detector system (HPLC-PDA, Waters, Milford, MA, USA). A C30 reversed phase column (4.6 × 150 mm, 3 μm, YMC, Wilmington, NC, USA) cooled to 18 °C was used with MTBE, methanol, and 1.5% (w/v) aqueous ammonium acetate mobile phases to separate PE, PF, LYC, ZC, and BC. Specific mobile phase compositions and a gradient elution method were used as described previously.[15] Extracts of all tissues, except for the liver, were reconstituted in 40 μL of MTBE. Liver extracts were reconstituted in 300 μL of MTBE. Twenty-seven microliters of the sample reconstituted in MTBE was injected for each tissue or diet for carotenoid analysis. Carotenoids were identified and quantified via UV spectra, retention times, and standard comparison. PE, PF, and ZC standards were purchased, and LYC was extracted from 10% Redivivo beadlets. BC was extracted from 15% beadlets. Samples were analyzed within 48 h of extraction. Samples were kept at 4 °C on an autosampler cooling tray and handled under yellow light to reduce carotenoid degradation.

Hepatic Lipid Extraction. Total liver lipids were extracted following the Folch method.[27] Briefly, 0.5 g of tissue was homogenized in a solution of chloroform/methanol (1:1). A 0.29% sodium chloride (NaCl) solution was added to promote phase separation, followed by centrifugation. Chloroform was added followed by centrifugation and addition of NaCl solution. This step was repeated twice. Supernatant was retained after each of the steps, pooled into preweighed tubes, and evaporated in a fume hood for approximately 48 h, before being stored in a desiccator for 24 h. Samples were then weighed to determine total lipid concentration.

Serum and Hepatic Cholesterol Analysis. Serum total cholesterol concentrations were quantified using a Wako Cholesterol E enzymatic colorimetric assay (Wako Chemicals, Richmond, VA, USA). The protocol was modified to the manufacturer's microtiter procedure, using 3 μL of serum per analysis (Wako Chemicals). Hepatic cholesterol concentrations were determined by reconstitution of extracted hepatic lipids in isopropyl alcohol containing 10% polyoxyethylene octylphenyl ether. All standards were diluted with the same extraction solution, and the remainder of the analysis was performed following the manufacturer's protocol.

Fatty Acid Analysis. Fatty acid analysis was previously described.[28] Briefly, 0.1 g of diet was weighed and 2 mL of a dilute internal standard, tridecanoic acid, was added. Methanolic HCl was added, and the samples were incubated at 100 °C for an hour. Two milliliters of hexanes and 5 mL of potassium carbonate were slowly added to each tube, and the samples were vortexed and centrifuged. The top organic layer was collected, dried under nitrogen gas, and protected from light. The samples were then transferred to gas chromatography vials and analyzed using gas chromatography as previously described.[28]

Statistical Analysis. Tissue carotenoid concentrations in the safflower oil + tomato powder and coconut oil + tomato powder groups were compared using ANOVA, and significant differences were detected using Tukey's Studentized test ($\alpha = 0.05$) when the assumptions of ANOVA were met. When the assumptions of ANOVA were not met, the Wilcoxon test was used to detect differences ($\alpha = 0.05$). Statistical analysis was performed using SAS v. 7.1 (SAS Institute Inc., Cary, NC, USA). Data are reported as the mean ± the standard error of the mean (SEM).

RESULTS AND DISCUSSION

The aim of this study was to compare the effects of an oil high in medium-chain saturated fatty acids (coconut oil) with an oil high in long-chain polyunsaturated fat (safflower oil) on tomato carotenoid tissue accumulation in gerbils. We observed enhanced tissue bioaccumulation of tomato carotenoids in all tissues from animals fed coconut oil except for the spleen and skin as well as differential tissue accumulation between carotenoids unrelated to dietary fat among the tissues measured.

Animal Body and Tissue Weights. The final weight of gerbils at sacrifice did not differ by dietary treatment group ($p = 0.41$) with an average gerbil weight of 69.6 ± 0.8 g. Additionally, the final weight of gerbil livers did not differ by dietary treatment ($p = 0.15$). When compared as percent of body weight, there were some minor organ weight differences between groups. The final weights of gonadal adipose tissue were different between the safflower oil + tomato powder-fed and coconut oil-fed dietary groups ($p = 0.03$), with average weights of 0.90 ± 0.05 and 0.68 ± 0.05 g, respectively. The final weight of the testes was greater ($p = 0.02$) in the safflower oil + tomato powder-fed group, 1.15 ± 0.03 g, compared to the other dietary groups, 1.07 ± 0.02 g. Safflower oil-fed animals had greater ($p = 0.04$) adrenal glandular weights (0.05 ± 0.001 g) compared to coconut oil-fed animals (0.038 ± 0.001 g). In short, gonadal adipose, testes, and adrenal glandular weights were significantly greater in safflower oil-fed animals, which might suggest greater accumulation of fat in these tissues. These fat-specific findings have not been previously reported.

Carotenoid Tissue Accumulation. Carotenoids are bioactive molecules with antioxidant capabilities and may function to reduce the risk for certain chronic diseases and cancers.[3] LYC, PE, and PF have been shown to accumulate in a variety of human and animal tissues including liver, adrenal glands, testes, kidney, pancreas, breast, skin, ovary, spleen, and prostate.[1,3] In the current study, tissue concentrations of PE, PF, *cis*-lycopene (*cis*-LYC), *all-trans*-lycopene (ATLYC), ZC, and BC were quantified in liver, lungs, spleen, adrenal glands, testes, prostate−seminal vesicle complex, serum, and skin (Table 4).

Previously, we have shown that tomato carotenoids differentially accumulate in both male Fisher 344 rat and Mongolian gerbil tissues in levels disproportionate to those fed in the diet.[15,16] In the current study, LYC was the dominant carotenoid in the liver, spleen, testes, lung, serum, and skin. LYC was also the most prevalent carotenoid in the tomato powder, accounting for >90% of the total carotenoid content (Table 2). PE was the most abundant carotenoid in the adrenal glands, accounting for 62% of total carotenoids in the coconut oil-fed and 45% in the safflower oil-fed adrenal glands, despite the fact that PE concentrations in the diet contributed only 2% of the total carotenoids. These results suggest tissue-specific uptake or metabolism of tomato carotenoids consistent with reports from our previous studies.[15,16] Similar to findings by Engelmann, there was no accumulation of PE in the androgen-sensitive tissues, the testes, or prostate−seminal vesicle complex, which suggests a difference in absorption or metabolism of PE in these tissues.[15]

Tissue accumulation of carotenoids was affected by type of fat consumed. Coconut oil feeding increased pulmonary PF (p

= 0.006) and ZC (p = 0.003), adrenal glandular PE (p = 0.05), hepatic PE (p = 0.02) and PF (p = 0.0001), and testicular PF (p = 0.01), *cis*-LYC (p = 0.01), ATLYC (p = 0.010), total LYC (p = 0.01), and ZC (p = 0.02). Coconut oil feeding also increased PF (p = 0.008), *cis*-LYC (p = 0.006), and ZC (p = 0.005) in the prostate−seminal vesicle complex. The prostate−seminal vesicle complex (p = 0.01), testes (p = 0.01), and serum (p = 0.0003) had greater total carotenoid concentrations in animals fed coconut oil (Table 4).

Safflower oil-fed gerbils had greater splenic ATLYC (p = 0.03) and total LYC (p = 0.006) compared to coconut oil-fed animals. Previous studies have observed carotenoid accumulation levels in the spleen to be relatively high compared to other tissues.[15] The spleen is important for immune function because it removes bacteria and debris from the bloodstream.[29] However, carotenoid enrichment of the spleen is poorly understood.

We observed differences in carotenoid accumulation by dietary fat type in the prostate−seminal vesicle complex. PF was the dominant carotenoid in the prostate−seminal vesicle complex of the coconut oil-fed animals, accounting for 50% of the total carotenoids. In contrast, LYC was the dominant carotenoid in the prostate−seminal vesicle complex of the safflower oil-fed animals, accounting for 57% of the total carotenoids. Engelmann observed LYC as the dominant carotenoid in the prostate−seminal vesicle complex when gerbils were fed cottonseed oil, which contains a mixture of saturated, monounsaturated, and polyunsaturated fat.[15] This suggests LYC accumulation in the prostate−seminal vesicle complex is dependent on the type of fat consumed. Polyunsaturated fats may enhance LYC accumulation in the prostate−-seminal vesicle complex, whereas saturated fat from coconut oil enhances total carotenoid accumulation in the prostate−seminal vesicle complex, with PF being the major carotenoid deposited.

The testes of the coconut oil-fed animals had greater total LYC concentrations, but weighed significantly less than those of the safflower oil-fed animals. Studies using carotenoid metabolizing enzyme knockout mice have shown that dietary LYC and tomato powder reduce testicular testosterone, which might explain alterations in growth of the testes.[17]

To our knowledge, this is the first time tomato carotenoids have been reported in skin from Mongolian gerbils (Table 4). ATLYC and *cis*-LYC were consistently detected in skin in quantifiable amounts, but differences between groups were not significant (p = 0.17 and p = 0.38, respectively. PE and PF were detected in skin in fewer than 50% of the animals. PE and PF were considered to be detectable, but unquantifiable, if HPLC signals corresponded with carotenoid concentrations in the injection carrier solvent of <0.2 nM. In the skin, ZC and BC were detected in fewer than 20% of the animals. When detected, ZC and BC were in amounts below the level of quantification (0.9 nM ZC, 0.1 nM BC in the HPLC carrier solvent). BC was detected but unquantifiable in most other tissue extracts. An unidentified compound was found in the skin of animals fed tomato powder diets and in those fed control diets. The compound had a retention time and maximum absorbance (282.8 nm) similar to those of the PE standard (286 nm). However, upon closer inspection, the compound did not have the characteristic spectral fine structure of PE determined by comparison to PE standards.[30] Therefore, this compound was determined not to be PE.

Cholesterol. To evaluate if the change in carotenoid tissue deposition was a result of a change in cholesterol and lipid metabolism, serum and hepatic cholesterol and hepatic lipids were quantified. Animals fed coconut oil diets maintained significantly higher serum cholesterol concentrations (178 ± 8.2 mg/dL) than animals fed safflower oil diets (82 ± 6.7 mg/dL) (p = 0.0001) (Figure 1). Coconut oil is known to increase serum cholesterol in humans due to its high amounts of lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids.[31] The primary fatty acid in coconut oil, lauric acid, accounted for 42% of the fatty acids in the diet of the coconut oil-fed animals (Table 3). The combination of these three cholesterol-raising fatty acids in coconut oil likely contributed to the increased serum cholesterol in the current study.

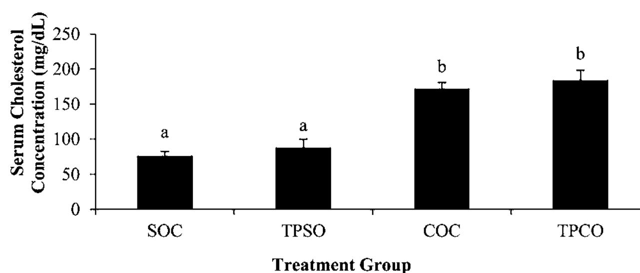

Figure 1. Serum cholesterol concentrations of gerbils fed either a 20% safflower oil diet (SOC; n = 10), a 10% tomato powder + 20% safflower oil diet (TPSO; n = 10), an 18% coconut oil diet + 2% safflower oil (COC; n = 10), or a 10% tomato powder + 18% coconut oil diet + 25% safflower oil (TPCO; n = 10) for 28 days. Significant differences between safflower and coconut oil diets as detected by analysis of variance, Tukey's standardized range test (α = 0.05), are denoted by capital letters. Columns represent the average of 10 analyses performed in triplicate, and error bars represent ± SEM.

In the current study, animals fed safflower oil had significantly higher (p = 0.0003) hepatic cholesterol concentrations (4.0 ± 0.2 mg/g) compared to coconut oil fed groups (2.7 ± 0.14 mg/g) (Figure 2). This finding is consistent with other studies showing that polyunsaturated fats increase hepatic cholesterol uptake.[32,33] In contrast to coconut oil, safflower oil is composed of long-chain polyunsaturated fatty acids, primarily linoleic acid (C18:2) (Table 3).

Saturated fats have long been grouped together for dietary recommendations, but they may not all have the same

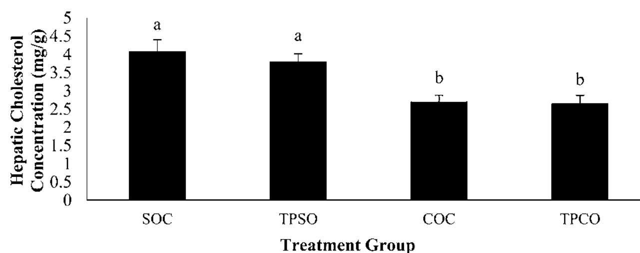

Figure 2. Hepatic cholesterol concentrations of gerbils fed a 20% safflower oil diet (n = 10), a 10% tomato powder + 20% safflower oil diet (n = 10), an 18% coconut oil + 2% safflower oil diet (n = 10), or a 10% tomato powder + 18% coconut oil + 2% safflower oil diet (n = 10) for 28 days. Significant differences between safflower and coconut oil diets as detected by analysis of variance, Tukey's standardized range test (α = 0.05), are denoted by capital letters. Columns represent the average of 10 analyses performed in triplicate, and error bars represent ± SEM.

biological impact. Coconut oil is a unique saturated fat because of its fatty acid profile. The fatty acid profiles of saturated fats can be dramatically different; for instance, butter is primarily composed of short -chain fatty acids, coconut oil is primarily medium-chain fatty acids, and beef tallow consists of long-chain fatty acids.[23] Studies comparing coconut oil to other saturated fats have conflicting results concerning serum cholesterol. When coconut oil and beef tallow were compared in humans, coconut oil increased serum LDL and total cholesterol relative to beef tallow.[34] Similarly, long-chain saturated fatty acids fed to guinea pigs resulted in decreased plasma LDL concentrations when compared to coconut oil.[35] However, butter significantly increased LDL and total cholesterol levels when compared to coconut oil in moderately hypercholesterolemic adults.[36] Differences in saturated fatty acid chain length might account for differences in LDL metabolism and LDL receptor synthesis; therefore, we cannot categorize the effect of all saturated fats on plasma cholesterol on the basis of fatty acid saturation alone.

Tomato powder addition to either safflower or coconut oil diets did not alter hepatic cholesterol concentrations (p = 0.4 and p = 0.37, respectively). Total hepatic lipid concentrations did not differ by dietary treatment group (p = 0.9), with an average concentration of 37 ± 0.8 mg/g for all groups.

Potential Mechanisms for Altered Tissue Carotenoid Accumulation Due to Differential Fatty Acid Chain Length and Saturation and Cholesterol Metabolism by Dietary Fat Treatment. The composition of fatty acids in oils can alter carotenoid solubulization, micellarization, absorption, and chylomicron formation. Whereas in the current study carotenoid absorption was not measured directly, fatty acid chain length does not appear to contribute to differences in carotenoid absorption when different fats are compared.[12] In the current study, carotenoid tissue accumulation was increased in animals fed coconut oil. Previously, Borel et al. observed increased solubility of carotenoids with decreasing fatty acid chain length.[8] Carotenoids are lipophilic compounds and, once released from the food matrix, are solubilized into mixed micelles with bile salts. Medium-chain fatty acids in coconut oil may cause increased carotenoid solubility into mixed micelles compared to the long-chain fatty acids from safflower oil.

Previous studies have indicated increased BC and LYC micellarization with the addition of long-chain triglycerides.[11] However, the current study found that coconut oil, a source of medium-chain fatty acids, increased carotenoid tissue deposition when compared to a long-chain polyunsaturated fat, safflower oil. In contrast to the current study, this previous study used tricaprylin as a source of medium-chain fatty acids, which is high in caprylic acid (C8:0).[11] Caprylic acid is shorter than the primary fatty acids found in coconut oil. Relative to tricaprylin, longer chain medium-chain fatty acids in coconut oil may enhance the carotenoid absorption properties to a greater degree than shorter chain medium-chain fatty acids and to the same extent that long-chain fatty acids do. The comparison of different medium-chain fatty acids' effects on carotenoid metabolism has not been determined; however, the composition of medium-chain triglycerides is important because of the potential impact chain length has on micellarization and incorporation of carotenoids into chylomicrons.

Previous studies have shown increased incorporation of BC and LYC into chylomicrons when long-chain triglycerides were used.[13] However, in the current study, coconut oil facilitated carotenoid tissue accumulation to a greater degree than safflower oil. Because coconut oil is composed of medium-chain fatty acids, these fatty acids can bypass chylomicron formation and be absorbed directly by the portal vein.[13] This results in decreased postprandial chylomicron formation with medium-chain fatty acids.[13] Additionally, it has been described that medium-chain triglycerides can be incorporated into chylomicrons when they are the only source of fat in the diet.[37] In the current study, gerbils were fed a 20% fat diet of 18% coconut oil and 2% safflower oil or 20% safflower oil. The high amount of medium-chain triglycerides in coconut oil may have facilitated incorporation into mixed micelles and absorption by enterocytes; however, the mechanism of carotenoid absorption is unclear.

We observed increased tissue accumulation of tomato carotenoids in every tissue measured except for the spleen and skin in animals fed coconut oil. Chylomicrons are eventually transported to the liver as chylomicron remnants, where hepatic metabolism of carotenoids can occur.[17] In the liver, carotenoids are repackaged into very low-density lipoproteins (VLDL) and released into circulation, where they have access to peripheral tissues. While in circulation, VLDLs lose triglycerides and become denser lipoproteins: intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL). LDLs and IDLs are returned to the liver, where 80% of the LDL is catabolized.[38] Saturated fats can interrupt this cycle by reducing hepatic uptake of LDL.[29,39] The primary fatty acids in coconut oil (lauric, myristic, and palmitic acids) reduce LDL receptor activity,[31,39] thus increasing serum cholesterol and leaving more LDL free to recirculate to peripheral tissues. Nonpolar carotenes, specifically LYC, are carried primarily in the LDL fraction of lipoproteins.[40] In the current study, serum cholesterol in the coconut oil-fed animals was significantly increased and hepatic cholesterol was significantly decreased when compared to safflower oil-fed animals. Perhaps a coconut oil-mediated change in LDL levels altered the flux of carotenoids between the liver and peripheral tissues, resulting in increased tissue-specific accumulation of carotenoids and/or decreased clearance of carotenoids by the liver.

However, with regard to the differential accumulation of tissue carotenoids by dietary fat (Table 4) in tissues enriched with carotenoids by coconut oil feeding, there might be a concurrent shift in tissue fatty acid profile to one more similar to that of coconut oil. Perhaps tissues that accumulated tomato carotenoids preferentially utilize medium-chain fatty acids, and as a result of increased tomato carotenoid solubility in medium-chain fatty acids,[8] carotenoid accumulation was greater. Although tissue fatty acid profiles were not measured in the current study, studies in dairy cattle have shown that dietary fatty acid manipulation can significantly shift the fatty acid profiles of muscle and milk.[41] Further insight into the interaction between dietary fat and tissue carotenoid accumulation may be gained by examining tissue fatty acid profiles.

Carotenoid Stability in the Diet. Polyunsaturated fats are unstable because their double bonds are susceptible to oxidation.[29] To account for possible fat-specific carotenoid degradation, carotenoid stability in the diet was measured. Carotenoid content of the diet was measured prior to being exposed for 2 days in the animal facility and again after exposure. There was no difference in carotenoid content of the diet due to oil type during the 2 day exposure period (data not shown). Thus, the differences in carotenoid accumulation are not the result of carotenoid degradation in the diet.

In summary, differential accumulation of tomato carotenoids in liver, spleen, lungs, adrenal glands, testes, prostate−seminal vesicle complex, serum, and skin was observed independent of fat type in the Mongolian gerbil. In addition, we observed fat type-specific effects on carotenoid accumulation. Coconut oil feeding resulted in increased tomato carotenoid bioaccumulation compared to safflower oil in all tissues measured except the spleen and skin. Increased tissue accumulation may have been a result of increased solubility of tomato carotenoids in the intestinal lumen, portal absorption of medium-chain fatty acids, a cholesterol-mediated change in the flux of carotenoids between the liver and peripheral tissues, facilitated carotenoid cellular uptake by specific fatty acids, or the combination of the four. Research investigating the influence of different saturated fats of differing chain lengths on tomato carotenoid accumulation and cholesterol flux should be undertaken. It is important to understand the differential accumulation patterns of carotenoids in specific tissues and how dietary fats can affect these profiles when animal or human feeding trials with carotenoids are conducted.

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Notes

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ABBREVIATIONS USED

COC, coconut oil; TPCO, coconut oil + 10% tomato powder; SOC, safflower oil; TPSO, safflower oil + 10% tomato powder; LYC, lycopene; ATLYC, *all-trans*-lycopene; *cis*-LYC, *cis*-lycopene; PE, phytoene; PF, phytofluene; BC, beta-carotene; ZC, zeta-carotene; BHT, butylated hydroxytoluene; HPLC-PDA, high-performance liquid chromatography−photodiode array.

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