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^{13}C-lutein is differentially distributed in tissues of an adult female rhesus macaque following a single oral administration: a pilot study

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Abstract

Despite the growing awareness regarding lutein's putative roles in eyes and brain, its pharmacokinetics and tissue distribution in primates has been poorly understood. We hypothesized that ^{13}C-lutein will be differentially distributed into tissues of an adult rhesus macaque (*Macaca mulatta*) three days following a single oral dose. After a year of pre-feeding a diet supplemented with unlabeled lutein (1 μmol/kg/d), a 19-year-old female was dosed with 1.92 mg of highly enriched ^{13}C-lutein. Tissues of a non-dosed, lutein-fed monkey was used as a reference for natural abundance of ^{13}C-lutein. On the third day post-dose, plasma and multiple tissues were collected. Lutein was quantified by HPLC-PDA and ^{13}C-lutein tissue enrichment was determined by LC-Q-TOF-MS. In the tissues of a reference monkey, ^{12}C-lutein with natural abundance of ^{13}C-lutein was detectable. In the dosed monkey, highly enriched ^{13}C-lutein was observed in all analyzed tissues except for the macular and peripheral retina, with the highest concentrations in the liver, followed by adrenal gland, and plasma. ^{13}C-lutein accumulated differentially across six brain regions. In adipose depots, ^{13}C-lutein was observed, with the highest concentrations in the axillary brown adipose tissues. In summary, we evaluated ^{13}C-lutein tissue distribution in a non-human primate following a single dose of isotopically labeled lutein. These results show that tissue distribution 3 days following a dose of lutein varied substantially dependent on tissue type.

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Keywords

lutein; biodistribution; rhesus macaque; isotopic tracer; mass spectrometry

1. Introduction

Lutein, a 40-carbon oxygenated carotenoid, cannot be endogenously synthesized in mammals. Thus, the amount of lutein in the body is determined by dietary sources including dark green leafy vegetables, fruits, and yellow and orange egg yolks. Lutein and its isomer zeaxanthin selectively accumulate in the macular region of the primate retina to form yellow coloration (termed macular pigment) and to protect the retina against harmful blue light and oxidative stress [1]. Epidemiological studies show that the consumption of lutein and zeaxanthin is strongly associated with a reduced risk of age-related macular degeneration, a leading cause of irreversible blindness in the United States [2, 3]. Recently, it has been suggested that lutein may also play a role in brain function. Lutein is the predominant carotenoid in neural tissues of both infants and older adults even though it's not the major carotenoid in the US diet [4, 5]. When comparing dietary intake to brain function, several studies have reported associations between lutein and zeaxanthin supplementation and enhanced cognitive function in adults [6–8].

Despite growing interest in lutein, there has been limited research on its pharmacokinetics and tissue distribution in humans or nonhuman primates. Since it is difficult to completely deplete lutein in the body, especially in the retina [9], the use of isotopically labeled lutein is a good strategy to investigate its bioavailability and metabolism *in vivo*. Although a few studies have examined the time course of plasma responses to isotopically labeled lutein in human subjects, it is not possible in humans to obtain detailed information on tissue uptake [10, 11]. Previously, we established a carrot cell suspension culture system to produce enriched ^{13}C-lutein and demonstrated proof-of-principle of tissue uptake through subsequent dosing of an adult female rhesus macaque [12]. Expanding on that work, this pilot study investigated how ^{13}C-lutein was distributed into tissues of the adult rhesus macaque three days following a single oral dose. We hypothesized that ^{13}C-lutein signals would be different across tissues.

2. Methods and Materials

2.1. Animals and Diet

Animal care and *in vivo* dosing were performed at the Oregon National Primate Research Center at Oregon Health and Science University. All procedures in this study were approved by the Institutional Animal Care and Use Committee of Oregon Health and Science University, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A female, 19-year-old rhesus macaque was fed a standard laboratory diet (Monkey Diet Jumbo, LabDiet) (Table 1), supplemented daily with 570μg/kg diet/day of unlabeled lutein (FloraGloR beadlets, 5% lutein, DSM) mixed into various treats such as marshmallow, peanut butter and chocolate for the previous 12 months. The animal was anesthetized with ketamine (10 mg/kg) and dosed with 1.92 mg of ^{13}C-lutein solubilized in

a mixture of monoglyceride and diglyceride oil (Abbott Nutrition) via orogastric intubation. The bioproduction, purification, and analysis of the ^{13}C-lutein dose and details of the dosing procedure were described previously [12]. In the lutein dose, the extent of labelling with ^{13}C was as follows: all 40 carbons were labelled in 64.7 ± 0.9% of lutein molecules, 39 carbons in 27.0 ± 0.4%, and 38 carbons in 8.3 ± 0.9%. Three days after oral dosing and after an overnight fast, the monkey was humanely euthanized by a veterinary pathologist under deep pentobarbital anesthesia. Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and processed to obtain plasma, and tissues were collected, snap-frozen in liquid nitrogen, and stored at −80° C until analysis. Tissues collected included samples of liver, heart, kidney, adrenal gland and quadriceps; adipose tissue from four sites (mesenteric, subcutaneous from abdomen and thigh, and brown axillary adipose tissues); samples of 6 brain regions (occipital, parietal and temporal cortex, medulla, pons, and subcortical white matter); a 4 mm punch of the macular retina, centered on the fovea; and peripheral retina. Tissues of a non-dosed monkey were used as a reference to determine the natural abundance of ^{13}C-lutein. Throughout the text, ^{13}C-lutein stands for the highly enriched ^{13}C-lutein. Reference tissues were obtained from a male, 180-day-old rhesus macaque, who consumed unlabeled lutein via breast milk and supplemental foods for six months. Analytical measurements were conducted in accordance with and approved by University of Illinois at Urbana-Champaign institutional biosafety committee project.

2.2. Purification and quantification of lutein from monkey tissues

Carotenoids were extracted in replicates with methods depending on the tissue type as previously described [13]. Each tissue extract was reconstituted in 40 μL of ethanol/methyl tert-butyl ether mixture (1:1, by vol) and injected onto an Alliance HPLC system (e2695 Separation Module) equipped with a 2998 photodiode array detector (PDA) (Waters). The extract was separated on a reverse-phase C30 column (4.6 × 150 mm, 3 μm; YMC) maintained at 18 °C. A phase gradient method used for carotenoid separation was based on the method of Yeum et al. [14]. Total lutein was identified via absorption spectrum, retention time, and standard comparison, and quantified by an external standard curve method. The lutein fraction was collected, aliquoted, and solvents were evaporated under argon.

2.3. Mass spectrometry

The dried lutein fraction was reconstituted in LC mobile phase (acetonitrile/methanol/10mM ammonium acetate=539:441:20, by vol; pH=4.5) prior to analysis. All samples were analyzed on a system consisting of a Thermo UltiMate 3000 UHPLC, a Bruker atmospheric pressure chemical ionization (APCI) II source, and a Bruker maXis 4G quadrupole time-of-flight mass spectrometer (Q-TOF-MS). The LC separation involved 30 min of isocratic elution at 0.3 mL/min flow rate at 20 °C on a Thermo Acclaim C30 column (3 μm, 2.1 × 150 mm), connected after a Thermo Acclaim C30 guard cartridge (5 μm, 2.1 × 10 mm). The operation parameters for the APCI source included: capillary 3500 V, corona 7000 nA, nebulizer 2 bar, dry gas 4 L/min, dry temperature 200 °C, vaporizer temperature 400 °C. Tandem MS analysis was performed for structure confirmation of target analytes, and high resolution and mass accuracy MS measurements were conducted for determination of ^{12}C-lutein/ ^{13}C-lutein ratios. Mass spectrometer calibration was performed with syringe infusion of the APCI/APPI calibrant solution (Fluka) before each sample run. Due to the low amount

of ^{13}C-lutein in adipose and brain tissues, the lutein fractions from 2–4 tissue extract replicates were combined and used for ^{13}C-lutein detection.

2.4. Statistical analyses

All data were presented as mean from 1–4 replicates. Repeatability of measurements was shown as relative standard deviation (RSD, %) = standard deviation/mean × 100%.

3. Results

3.1. Accumulation of total lutein in rhesus macaque tissues

Total lutein (unlabeled + ^{13}C-labeled) in monkey tissues was extracted and separated by HPLC-PDA. Fig. 1 depicts representative HPLC chromatograms of liver extracts of the monkey dosed with ^{13}C-lutein and the reference monkey obtained at 445 nm as well as the ultraviolet/visible absorbance spectrum of lutein. The retention time and absorbance spectrum of lutein were identical between liver extracts of the dosed and the reference animals. Total lutein was detectable in all tissues examined and was differentially distributed depending on tissue type. Total lutein was most concentrated in the macular retina (5897 pmol/g), followed by adrenal gland and liver (3403 and 2677 pmol/g, respectively; Table 2).

3.2. ^{13}C-lutein detection in rhesus macaque tissues

The total lutein concentrations and ratios of ^{13}C-lutein and ^{12}C-lutein in each tissue allow the determination of ^{13}C-lutein concentrations. We compared our previous method [12], which utilized a triple quadrupole mass spectrometer operating in multiple reaction monitoring mode with a Q-TOF-MS instrument. The Q-TOF-MS was superior and enabled lutein measurement with high accuracy and monoisotopic signal detection (mass error < 5 ppm). The ratio of ^{12}C-lutein and ^{13}C-lutein was calculated from the intensities of corresponding $[M–H_2O+H]^+$ monoisotopic signals observed in the same mass spectrum (^{12}C-lutein – H_2O+H: *m/z* = 551.4253±0.0028 Da; ^{13}C-lutein – H_2O+H: *m/z* = 591.5593±0.0030 Da). Both ^{12}C-lutein and ^{13}C-lutein were observed in the dosed monkey liver, whereas ^{12}C-lutein, but not ^{13}C-lutein, was detectable in the reference monkey liver (Fig. 2). Measurements demonstrated good repeatability, with RSD ≤ 3.9% for triplicate evaluations of the liver (Supplemental Table S1). MS/MS analysis was also performed for structural confirmation of ^{12}C-lutein and ^{13}C-lutein detection. The representative MS/MS spectra are shown in Fig. 3. The matching of fragmentation patterns between unlabeled lutein standard (Fig. 3A) and the dosed monkey liver extract (Fig. 3B) confirmed that the *m/z* 591.5592 ion is a completely labeled ^{13}C-lutein ($^{13}C_{40}H_{56}O$) with the loss of one water molecule.

In the reference monkey, ^{12}C-lutein, but not ^{13}C-lutein, was detectable in all tissues examined. In contrast, ^{13}C-lutein isotopomers were detected in multiple tissues of the ^{13}C-dosed macaque, with the highest concentrations of ^{13}C-lutein found in the liver (257 pmol/g), followed by adrenal gland (114 pmol/g), plasma (27.9 pmol/mL), kidney (5.57 pmol/g), and heart (5.36 pmol/g) (Table 2). As expected, lower signal intensity led to an increase in %RSD (Supplemental Tables S1-S3).

^{13}C-lutein was differentially deposited across brain regions. Among six different brain regions examined (temporal cortex, occipital cortex, parietal cortex, medulla, pons, and subcortical white matter), the occipital cortex exhibited the highest ^{13}C-lutein concentration (2.47 pmol/g), as well as the highest concentration of ^{12}C-lutein. ^{13}C-lutein also accumulated differentially among four different adipose depots, with the highest concentrations of ^{13}C-lutein in axillary brown adipose tissue. ^{13}C-lutein was undetectable in macular and peripheral retina samples, despite high concentrations of ^{12}C-lutein. Except in samples with undetectable ^{13}C-lutein isotopomers, the profile of ^{13}C-lutein isotopomers found in tissues of the dosed monkey were similar to that in the dose, with the highest amount of uniformly labeled lutein, followed by lutein with 39 ^{13}C and 38 ^{13}C atoms, respectively. Information on ^{13}C-lutein concentrations in tissues, organ weights, and estimated blood volume [15, 16] were used to estimate the % recovery of the ^{13}C-lutein dose in selective tissues. The total recovery of the ^{13}C-lutein dose from tissues (plasma, liver, heart, kidney, adrenal gland and brain) was 1.4%, with the majority being in the liver.

4. Discussion

Lutein preferentially accumulates in primate retina and brain, and this biological selectivity suggests critical or essential roles in these tissues. Long-term lutein supplementation leads to dose-dependent increases in serum lutein and macular pigment in human subjects [17]. In addition, we recently showed that the lutein deposition in multiple tissues of infant rhesus macaques, including retina and brain regions, varied in response to 6 months of breastfeeding or formula-feeding [18]. However, lutein pharmacokinetics are poorly understood. This pilot study is the first to demonstrate a determination of tissue bioaccumulation patterns of a single dose of ^{13}C-lutein in an adult nonhuman primate.

We found that 1.4% of the highly enriched ^{13}C-lutein dose was recovered in plasma and the measured tissues of the dosed rhesus monkey, while ^{13}C-lutein was undetectable in any tissues of the reference monkey. Most of the consumed dose is assumed to be excreted via feces and urine, while additional dose accumulated in other unmeasured tissues. De Moura et al. reported that 45% of a ^{14}C-lutein dose was excreted via feces and 10% via urine in an adult woman in the first 2 days following dosing [11]. That study also reported that ^{14}C-lutein was absorbed and appeared in the bloodstream within one hour after consumption, reaching maximum blood levels at 14 hours after ingestion.

It is noteworthy that ^{13}C-lutein was differentially distributed depending on tissue type, being particularly enriched in the liver and adrenal glands. Interestingly, after 12 months of unlabeled dietary lutein exposure, the adrenal glands accumulated higher concentrations of total lutein than the liver, whereas after tracer dosing, the ratio of ^{13}C-lutein/^{12}C-lutein (^{13}C/^{12}C) in the adrenal glands (3.5%) was relatively lower than in the liver (10.6%), heart (7.0%), and kidney (6.6%). It has been reported that the adrenal glands accumulate high concentrations of carotenoids in humans [19], possibly due to highly expressed LDL receptors and scavenger receptor class B type 1 (SR-B1), as well as a high rate of lipoprotein uptake [20–22]. Slow uptake, rapid metabolism, or enhanced export of lutein might explain the relatively low adrenal ^{13}C/^{12}C ratio. In the retina, no ^{13}C-lutein was detected, even though ^{12}C-lutein is highly concentrated, particularly in macular retina. This might be

attributable to slow uptake and turnover of lutein in the retina, as suggested in previous studies in humans [23, 24]. Clearly, a limitation of this study is that only 1 dosed monkey was tested at one time point. Additional studies at other time points, including younger monkeys of both genders, are needed to clarify if these observations indicate differential kinetics of lutein tissue uptake, metabolism, and/or recycling into circulation.

This pilot study demonstrates that a single oral dose of ^{13}C-lutein enabled the tissue distribution to be determined in a rhesus macaque. Our current work was able to detect ^{13}C-lutein isotopomers in tissues of the dosed monkey by using LC-APCI-MS with high sensitivity, allowing determination of its absolute content. We provide evidence to support our hypothesis that ^{13}C-lutein was differentially distributed across various tissues, including multiple brain regions; however, it was undetectable in the retina measured three days after a single dose. This suggests that distribution of lutein in the macaque is substantially dependent on tissue type. Follow-up studies are required to further investigate the pharmacokinetics of lutein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations:

APCI	atmospheric pressure chemical ionization
ASAT	abdominal subcutaneous adipose tissue
BAT	brown adipose tissue
MAT	mesenteric adipose tissue
ND	not detected
PDA	photodiode array detector
Q-TOF-MS	quadrupole time-of-flight mass spectrometer
RSD	relative standard deviation
SWM	subcortical white matter
TSAT	thigh subcutaneous adipose tissue

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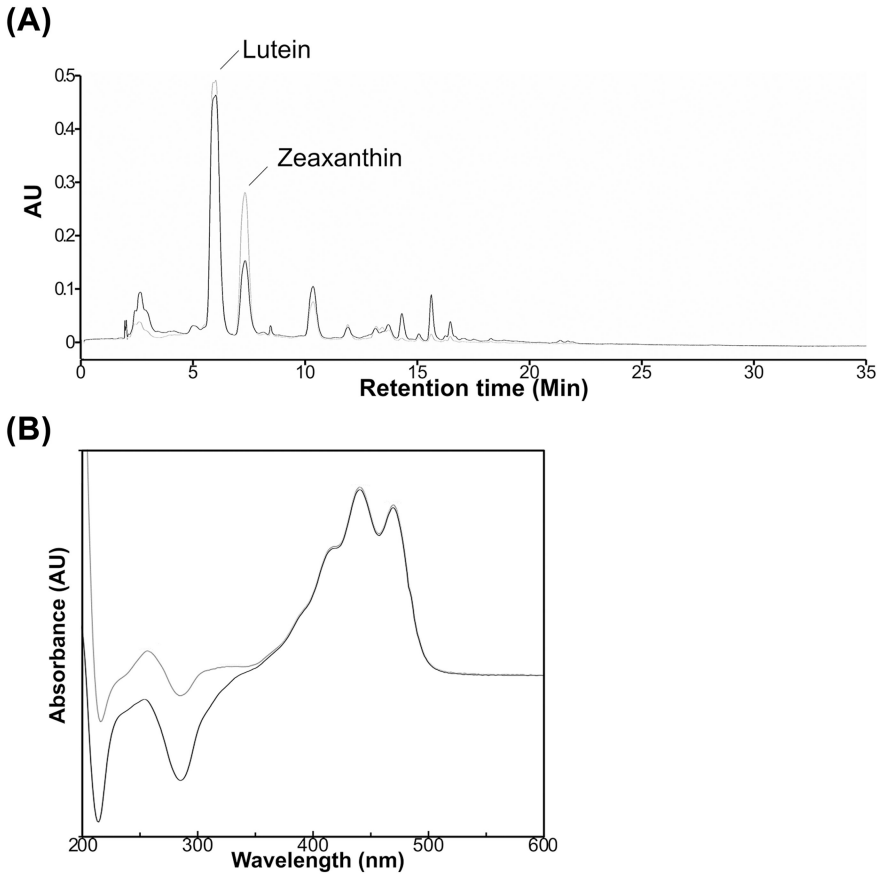

Fig. 1.

HPLC chromatogram (A) and absorbance spectrum (B) of lutein peaks from liver extracts of an adult monkey dosed with ^{13}C-lutein (Black) and a reference monkey (Grey).

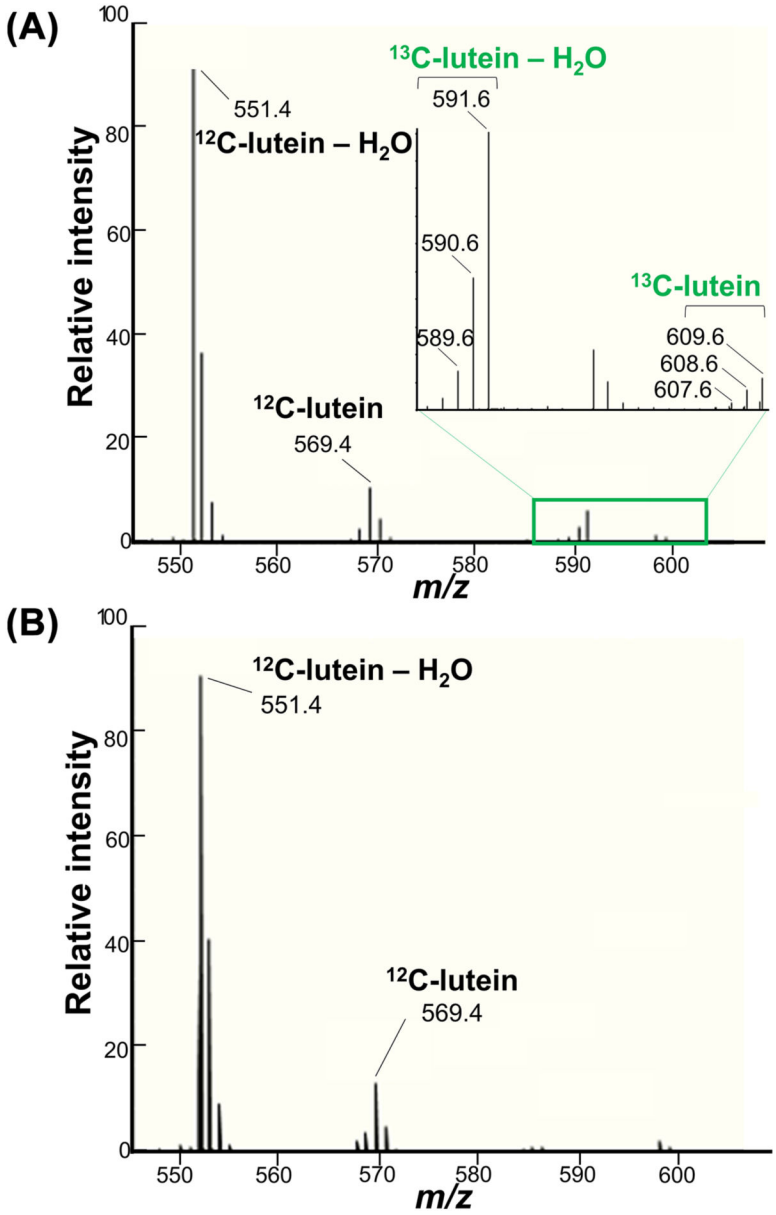

Fig. 2.

Mass spectra of the dosed monkey liver extract (A) and the reference monkey liver extract (B). The mass spectra were acquired during lutein elution from the column. Only the 545 to 610 region is presented.

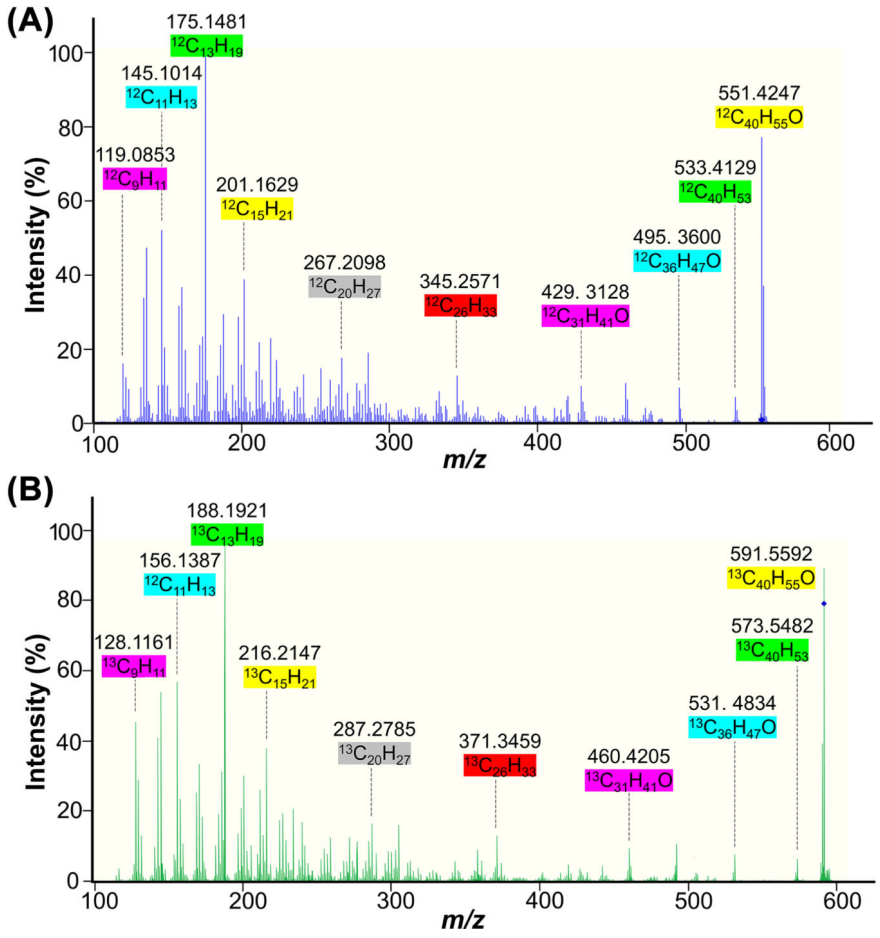

Fig. 3.

Tandem mass spectra of the fragmentation of unlabeled standard of ^{12}C-lutein ($^{12}C_{40}H_{56}O$–H_2O; *m/z* 551.4247) (A), and the ^{13}C-lutein–H_2O ion (*m/z* 591.5592) from the dosed monkey liver extract (B).

Table 1.

Macronutrient composition of the diet[1] (Monkey Diet Jumbo) fed to rhesus macaque monkeys

Standard laboratory diet	
Protein, g/kg	156
Fat (ether extract), g/kg	50
Carbohydrate (nitrogen-free extract), g/kg	600
Fiber (crude), g/kg	42
Minerals[2], g/kg	52
Vitamins[3]	-
Gross energy, kcal/g	4.08

[1]Provided by Labdiet and the moisture content was 100g/kg.

[2]Minerals: calcium, 9.0 g/kg; phosphorus, 9.3 g/kg; potassium, 7.5 g/kg; magnesium, 1.8 g/kg; sulfur, 2.4 g/kg; sodium, 2.5 g/kg; chloride, 3.7 g/kg; fluorine, 19 ppm; iron, 220 ppm; zinc, 110 ppm; manganese, 97 ppm; copper, 21 ppm; cobalt, 0.53 ppm, iodine, 1.3 ppm; chromium, 0.01 ppm, selenium, 0.37 ppm.

[3]Vitamins: carotene, 1.7 ppm; vitamin K, 3.2 ppm; thiamin hydrochloride, 8.3 ppm; riboflavin, 8.6 ppm; niacin, 113 ppm; pantothenic acid, 60 ppm; choline chloride, 1200 ppm; folic acid, 7.9 ppm, pyridoxine, 14 ppm; biotin, 0.1 ppm; B12, 73 ppm; vitamin A, 20 IU/g, vitamin D3, 6.7 IU/g, vitamin E, 110 IU/kg, ascorbic acid, 0.5g/kg.

Table 2.

Lutein isotopomer profiles in the multiple tissues of a monkey dosed with ^{13}C-lutein[1]

	Organ	Total lutein [pmol/g]	Lutein Monoisotopic Peak Intensities Ratios				^{13}C-lutein [pmol/g]
			$^{13}C_{40}/^{12}C_{40}$	$^{13}C_{39}{}^{12}C / {}^{12}_{40}$	$^{13}C_{38}{}^{12}C_2 / ^{12}C_{40}$	$^{13}C / {}^{12}C_{40}$	
	Plasma	276[2]	7.07%	3.23%	0.96%	11.3%	27.9[2]
	Liver	2677	6.56%	3.12%	0.92%	10.6%	257
	Heart	148	4.24%	2.06%	0.61%	6.91%	9.43
	Kidney	160	4.18%	1.84%	0.53%	6.54%	9.79
	Adrenal gland	3403	2.13%	1.04%	0.29%	3.46%	114
	Quadriceps	70.4	1.09%	0.36%	nd[3]	1.45%	0.74
Adipose	**BAT**	250	0.58%	0.15%	0.04%	0.76%	1.94
	MAT	191	0.17%	nd	nd	0.17%	0.32
	ASAT	222	0.26%	0.06%	nd	0.32%	0.74
	TSAT	110	0.10%	nd	nd	0.10%	0.11
Brain	**Temporal cortex**	88.2	1.77%	0.64%	nd	2.41%	2.08
	Occipital cortex	115	1.34%	0.71%	0.14%	2.20%	2.47
	Parietal cortex	71.3	1.29%	0.56%	nd	1.85%	1.58
	Medulla	28.9	1.00%	nd	nd	1.00%	0.29
	Pons	33.6	0.88%	nd	nd	0.88%	0.29
	SWM	48.3	0.64%	nd	nd	0.64%	0.33
Eye	**Macular retina**	5897	nd	nd	nd	nd	nd
	Peripheral retina	9771	nd	nd	nd	nd	nd

[1] Data represent values from 1–4 replicates of tissue samples.

[2] pmol/mL

[3] nd = ^{13}C-lutein not detected.

BAT, brown adipose tissue; MAT, mesenteric adipose tissue; ASAT, abdominal subcutaneous adipose tissue; TSAT, thigh subcutaneous adipose tissue; SWM, subcortical white matter.