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Orally Ingested $^{13}C_2$-Retinol is Incorporated into Hepatic Retinyl Esters in a Nonhuman Primate (*Macaca mulatta*) Model of Hypervitaminosis A

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The mechanism responsible for the metabolism of vitamin A during hypervitaminosis is largely unknown. This study investigated hepatic ^{13}C-retinol uptake in hypervitaminotic A rhesus monkeys. We hypothesized that individual retinyl esters would be enriched in ^{13}C after a physiologic dose of $^{13}C_2$-retinyl acetate, thus suggesting de novo in vivo hepatic retinol esterification. Male rhesus macaques (n = 16; 11.8 ± 2.9 y) each received 3.5 µmol 14, 15-$^{13}C_2$-retinyl acetate. Blood was drawn at baseline and 5 h and 2, 4, 7, 14, 21, and 28 d after administration. Liver biopsies were collected 7 d before and 2 d after dose administration (n = 4) and at 7, 14, and 28 d after dose administration (n = 4 per time point). ^{13}C enrichments of retinol and retinyl esters HPLC-purified from liver samples were measured by using gas chromatography–combustion–isotope ratio mass spectrometry. ^{13}C enrichment of total vitamin A and individual retinyl esters were significantly greater 2 d after dose administration compared with baseline levels. In contrast, the concentration of isolated retinyl esters did not always increase 2 d after treatment. Given that the liver biopsy site differed between monkeys, these data suggest that the accumulation of hepatic retinyl esters is a dynamic process that is better represented by combining analytical techniques. This sensitive methodology can be used to characterize vitamin A trafficking after physiologic doses of ^{13}C-retinol. In this nonhuman primate model of hypervitaminosis A, hepatic retinyl esters continued to accumulate with high liver stores.

Abbreviations: GCCIRMS, gas chromatography–combustion–isotope ratio mass spectrometry; IRMS, isotope ratio mass spectrometry; PS/LO, ratio of retinyl palmitate plus stearate to retinyl linoleate plus oleate.

Vitamin A is critical for vision, reproduction, and cellular differentiation.[18] All tissue vitamin A originates as dietary vitamin A, which is predominantly available as preformed vitamin A (that is, retinyl esters)[3] and the provitamin A carotenoids. Retinyl esters are cleaved to retinol in the intestinal lumen, and unesterified retinol is absorbed into the enterocyte.[25] Retinol is esterified within the intestinal mucosa[23,31] and then packaged into chylomicrons. These particles are exocytosed and transported into the general circulation,[4] where they are degraded to chylomicron remnants and taken up by the liver.[5,9] Once in hepatic parenchymal cells, retinyl esters are rapidly hydrolyzed to retinol and, depending on the animals' vitamin A status, retinol is secreted back into plasma bound to retinol-binding protein or transferred to stellate cells, reesterified, and stored.[6] Thus, vitamin A in the liver occurs in 2 forms: as retinol and esterified to various fatty acids. Analysis of hepatic retinyl esters within 30 min of intravenous injection of labeled chylomicron retinyl ester in vitamin-A–sufficient rats recovered 80% to 90% of the dose. During vitamin A sufficiency, a majority of labeled chylomicron retinyl esters are taken up by the liver before subsequent hydrolysis.[5]

Little is known about the storage and metabolism of vitamin A during hypervitaminosis A,[32,49] despite the wide use of retinoids pharmaceutically.[48] An early study involving hypervitaminotic A rats characterized increased concentrations of retinyl esters as percentages of total vitamin A in the plasma profile during excessive consumption of vitamin A.[32] This study further identified that retinyl esters were transported in the serum by means of lipoproteins, thus mediating the vitamin's nonspecific delivery to body tissues.[32] Increased plasma levels of retinyl esters as a percentage of total vitamin A also occurred in human patients[10,28,49] and rhesus monkeys[41] from the same colony as those used in the current study. Numerous case studies in humans document various symptoms of hypervitaminosis A, including dermatologic, hepatic, and neurologic pathologies.[27,30,35,47,48] Hepatic pathologies include abnormal liver function tests consistent with accumulation of lipid-storing droplets. Similar accumulation of lipid droplets has been reported to occur in rhesus monkeys from the same colony as those in the current study.[39]

Previously, liver vitamin A concentrations in captive rhesus monkeys were reported to range from 11.9 ± 5.4 to 18.8 ± 6.4 µmol retinol/g liver,[8,33,39] which are several fold higher than the concentrations considered excessive (that is, 0.70 to 1.05 µmol/g liver) and toxic (that is, 1.05 µmol/g liver) in humans.[36] Hepatic vitamin A concentrations in 2 wild-caught control rhesus monkeys in a vitamin A deficiency study were 1.07 and 1.08 µmol retinol/g liver.[38] Systematic inquiry to uncover the source of the high liver vitamin A concentrations found that the dietary vitamin A intake of captive rhesus monkeys exceeds National Research Council recommendations.[40] Consistent with these excessive dietary vita-

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min A levels, clinical markers of hypervitaminosis A were present.[8] To monitor the trafficking of a vitamin A dose during chronic hypervitaminosis A, we treated rhesus monkeys from the same colony as those cited earlier with $^{13}C_2$-retinyl acetate and collected liver biopsies as part of a study reported elsewhere.[8] The main goal of the previous report was to validate a vitamin A assessment technique using the heavy stable isotope of carbon.

In the current study, we hypothesized that isotope ratio mass spectrometry (IRMS) could be used to detect accumulation of individual hepatic retinyl esters and provide evidence of de novo in vivo hepatic retinol esterification after treatment of rhesus macaques with a physiologic dose of $^{13}C_2$-retinyl acetate. The ^{13}C abundance of HPLC-purified hepatic retinyl esters collected at baseline was compared with that of posttreatment samples. Here we show that hepatic retinyl esters continue to accumulate in a nonhuman primate model by using state-of-the-art analytical methods and ^{13}C-labeled retinol as a tracer.

Materials and Methods

Animals and experimental design. The complete study design is reported elsewhere.[8] Briefly, male rhesus monkeys (*Macaca mulatta*, $n = 16$; 11.8 ± 2.9 y; 7.5 to 15.9 y) were housed at the Wisconsin National Primate Research Center since birth. Overnight-fasted monkeys were dosed orally with 3.5 µmol 14, 15-$^{13}C_2$-retinyl acetate by injecting the oil-based supplement into banana and sealing it with peanut butter. Center staff drew fasting blood samples from all monkeys at baseline and then at 5 h and 2, 4, 7, 14, 21, and 28 d after dose administration. Clinical chemistry results from baseline serum samples have been reported previously.[8] The monkeys' customary vitamin-A–containing feed (providing approximately 6 µmol/day; Teklad Global 20% Protein Primate 2050; Harlan–Teklad, Madison, WI) was returned to each monkey's cage after the 5-h blood draw. Fasting (since the afternoon prior to surgery) liver biopsies (smaller than 1 cm^3) were collected under anesthesia in the same monkeys 7 d before and 2 d after ($n = 4$) the dose, allowing for recovery between procedures; liver biopsies were collected 7, 14, and 28 d ($n = 4$ per time point) after treatment in the remaining monkeys. Buprenorphine (0.01 mg/kg IM) was administered before and after surgery to minimize any discomfort or pain from the procedure. All procedures were approved by the University of Wisconsin-Madison's Research Animal Resources Center. The Wisconsin National Primate Research Center is fully AAALAC-accredited, and research and animal care at this center are regulated by University committees and national agencies to ensure compliance with the Animal Welfare Act.

Materials and instrumentation. 14, 15-$^{13}C_2$-retinyl acetate was synthesized as previously reported.[52] The synthesis used commercially available β-ionone as the starting material, and the source of ^{13}C was ^{13}C-labeled triethylphosphonoacetate (both from Aldrich Chemical, Milwaukee, WI), which adds 2 atoms of ^{13}C to the moiety's backbone. Synthetic $^{13}C_2$-retinyl acetate was purified on 8%-water-deactivated alumina by using highly volatile, organic solvents (that is, hexanes and diethyl ether). The resulting preparation was characterized by using UV–visible spectroscopy, thin-layer chromatography, and HPLC. After 2 rounds of HPLC purification, $^{13}C_2$-retinol had the same retention time (711 s) by gas chromatography–combustion–IRMS (GCCIRMS; Figure 1 A) as the unlabeled retinol.

IRMS (Delta Plus Advantage, ThermoFinnigan [now Thermo Fisher Scientific], Waltham, MA) was used in conjunction with

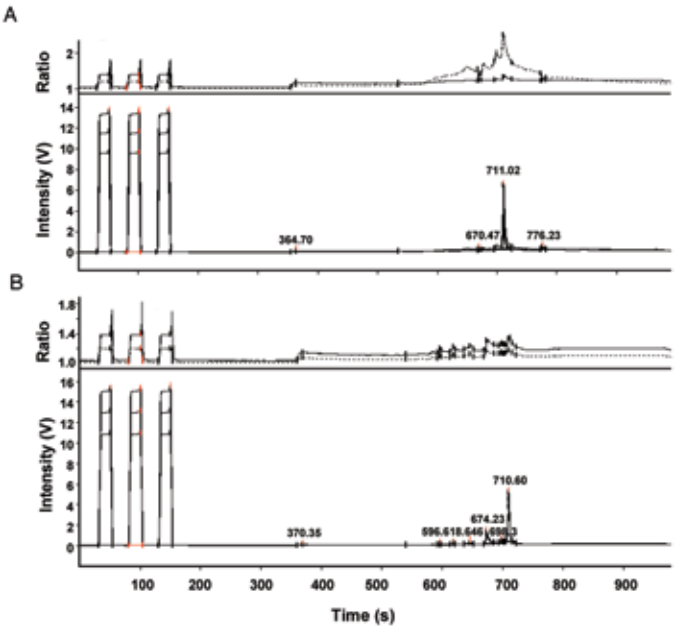

Figure 1. (A) Mass chromatogram for labeled $^{13}C_2$-retinol standard (50 ng/µL; 3% $^{13}C_2$-retinol). (B) Mass chromatogram of retinol from saponified retinyl oleate and palmitate isolated from baseline rhesus monkey liver, indicating that the retinol saponified from hepatic retinyl esters at baseline has the same retention time as the labeled retinol standard. For both panels A and B, the top image indicates the ratio of CO_2 molecules, whereas the bottom image indicates the signal strength (intensity of the peaks in volts). The dotted line in the top images represents the mass 45/44 trace—the variation of mass 45 (predominantly $^{13}CO_2$) to mass 44 ($^{12}CO_2$). The solid line in the top panels represent the mass 46/44 trace —the variation of mass 46 (predominantly $^{12}C^{18}O^{16}O$) to mass 44 ($^{12}CO_2$). The first 3 peaks in both the top and bottom panel are the reference gas (CO_2) pulses.

a gas chromatograph (TraceGC, ThermoFinnigan) for ^{13}C-retinol analysis of serum and liver samples. This instrument measures the ^{13}C accumulation in samples by quantifying the isotope ratio in excess of natural abundance (the amount of background isotope).[15] IRMS quantifies the isotopic ratio of the heavier, rarer isotope (^{13}C) to the lighter, more abundant isotope (^{12}C) with high sensitivity.[19] In the present study, a programmable temperature vaporizing injector introduced the sample onto a guard column (length, 1 m; inner diameter, 0.53 mm; Nonpolar Fused Silica, Supelco, Bellefonte, PA) connected to the analytical column (length, 15 m; inner diameter, 0.25 mm; film thickness, 0.25 µm; J and W Scientific, Wilmington, DE). Samples were oxidized (GC Combustion III, ThermoFinnigan) to CO_2 and a capillary column transported the combusted sample to the IRMS. For the analysis of the monkeys' feed, an elemental analyzer (Elemental Combustion System; Costech Instruments, Valencia, CA) was connected to a Conflo III interface (ThermoFinnigan) to transfer the sample to the IRMS. Data were generated as atom % ^{13}C (At% ^{13}C).

Analysis of liver retinyl ester concentrations and ^{13}C accumulation. After being ground with anhydrous sodium sulfate, liver (50 mg) was extracted into 25 mL dichloromethane for analysis of retinyl ester concentration and enrichment of the ^{13}C isotope. Liver extract (5 mL) was concentrated and injected onto an HPLC column. The retinyl ester fractions corresponding to retinyl linoleate, oleate, palmitate, and stearate were identified and collected

by comparison of retention times with those of standards. Retinyl oleate and palmitate, which eluted close to each other (respectively, 20.9 and 21.9 min), were pooled for IRMS analysis (Figure 1 B). Each retinyl ester fraction was saponified with methanolic potassium hydroxide, extracted, and concentrated. The residue was redissolved in 10 to 45 µL hexanes, and 1.5 µL was injected into the gas chromatograph. Use of a guard column preceding the analytical column preserved retinol for analysis by GCCIRMS without the need for derivatization.

On the same day as analysis of ^{13}C enrichment of liver hepatic retinyl linoleate, retinyl oleate and palmitate, and retinyl stearate, a second aliquot of liver extract (5 mL) was saponified and then analyzed as described for total liver vitamin A (retinol and retinyl esters) ^{13}C enrichment. Analysis of hepatic unesterified retinol (less than 1% of total vitamin A) was attempted, but the mass recovered from HPLC purification was below the instrument's limit of detection. Serum analysis of ^{13}C-retinol by GCCIRMS is reported elsewhere.[26]

Analysis of natural abundance of ^{13}C in rhesus diet. Rhesus feed was ground in a mortar to a fine powder and stored at –80 °C until analysis. Aliquots of powdered feed (1.4 to 2.1 mg; $n = 10$) were weighed into tin cups and introduced into the elemental analyzer-IRMS by means of a zero-blank autosampler. Vitamin A was not isolated from the feed prior to analysis.

Statistical analysis and calculations. Data are shown as mean ± 1 SD. Differences by sampling day in ^{13}C accumulation and the concentrations of liver total vitamin A and individual retinyl esters were analyzed by using the PROC MIXED command for baseline versus 2 d and by using PROC ANOVA for comparisons between days 2, 7, 14, and 28 (SAS Institute, Cary, NC). The ratio of hepatic retinyl palmitate plus stearate to retinyl linoleate plus oleate (PS:LO ratio)[57] was calculated by normalizing each retinyl ester to the estimated liver weight,[39] resulting in the total moles of each of the 4 retinyl esters per liver (µmol/liver). A P value less than 0.05 was considered statistically significant.

Results

Total hepatic vitamin A content in rhesus monkeys. Hepatic vitamin A (retinol + retinyl esters) ranged from 4.2 to 16.8 µmol/g at baseline among the monkeys ($n = 4$) tested, a 3-fold difference overall.[8] Differences in total vitamin A concentration in individual monkeys ranged from –2 to +1 µmol/g liver between baseline and day 2, but mean values (baseline, 11.9 ± 5.4 µmol/g; day 2, 11.9 ± 5.9 µmol/g) did not differ.

Analysis of ^{13}C content in standards, serum, and liver. IRMS measures CO_2 and produces the mass 45/44 trace (indicated by the dotted line in Figure 1) as a way to measure ratios of ^{13}C to ^{12}C. As expected, saponified $^{13}C_2$-retinyl acetate diluted with unlabeled retinol (3% $^{13}C_2$-retinol; 0.26 nmol) was highly enriched for ^{13}C (Figure 1; 2.420 At% ^{13}C). Analysis of unlabeled retinol confirmed that $^{13}C_2$-retinol had the same retention time as the unlabeled compound. The chromatograms of the retinol hydrolyzed from retinyl palmitate and oleate from a baseline liver sample indicated that the elution times of the saponified material were similar to those of the standards. Baseline traces did not reveal peaks in the prepared $^{13}C_2$-retinol standard 45/44 ratio trace because these liver samples were collected at baseline, before administration of the $^{13}C_2$-retinyl acetate dose. These chromatograms represent the range in the amount of ^{13}C evaluated in the present study. GCCIRMS analysis of retinol saponified from livers of rhesus after the $^{13}C_2$-dose showed similar retention times to the prepared standards.

The shape of the serum ^{13}C accumulation curve between 5 h and 28 d after treatment indicates rapid absorption, secretion from liver into plasma, and extrahepatic clearance of plasma $^{13}C_2$-retinol from the $^{13}C_2$-retinyl acetate dose (Figure 2). Total hepatic vitamin A was significantly enriched in ^{13}C at day 2 after treatment compared with baseline (Figure 3). When total vitamin A was examined as individual esters, this relationship was conserved (Figure 3).

Retinyl ester composition of liver. HPLC measurement indicated that concentrations (µmol/g liver) of individual retinyl esters were not always increased at 2 d after dose administration compared with baseline (Table 1). When normalized to reported liver size (µmol/liver),[39] concentrations of retinyl linoleate, oleate, stearate, and palmitate did not differ significantly between baseline and day 2. Because an increase in the PS:LO ratio has been proposed to indicate increased intestinal lecithin:retinol acyltransferase activity compared with acyl-CoA:retinol acyltransferase activity, [57] we calculated this ratio to gain insight into the major pathways potentially responsible for the tremendous retinyl esterification in rhesus liver. However, the ratio did not differ between baseline (2.66 ± 0.38 µmol/liver) and 2 d (2.72 ± 0.33 µmol/liver).

Natural isotopic abundance of feed. The natural isotopic abundance of ^{13}C relative to ^{12}C of the animal feed was 1.085 ± 0.0007 At% ^{13}C. This value is midway between the baseline and postdose abundances of the individual retinyl esters (Figure 3).

Discussion

This study assessed the hepatic uptake of a physiologic dose of retinol in a hypervitaminotic A rhesus macaque model by using sophisticated ^{13}C-tracer methodology. Because they lack the deleterious effects of radioisotopes on human health, stable isotopes are an attractive alternative for a broad range of metabolic studies. The current study demonstrates that the heavy isotope of carbon can be used to trace orally ingested vitamin A. The statistically significant enrichment of total hepatic vitamin A and individual retinyl esters with tracer provides evidence for the incorporation into the liver of the $^{13}C_2$-retinol from the $^{13}C_2$-retinyl acetate dose. Given the observation in hypervitaminotic A laboratory rats that the liver has a finite storage capacity for vitamin A,[32] the accumulation of labeled retinyl esters in the current study suggests that the rhesus monkeys had not reached saturation despite their increased liver vitamin A reserves. In rats with moderate liver vitamin A reserves, hepatic recovery of injected ^{3}H-retinyl ester chylomicrons resulted in 23% to 30% from 2 h to 15 d after the dose.[17] Therefore, the origin of the increased amounts of hepatic retinyl esters in our rhesus monkeys might represent compounds of intestinal origin as well as de novo in vivo hepatic esterification.[5]

The pre- and postdose differences in total liver vitamin A of individual rhesus monkeys may indicate factors related directly to hypervitaminosis A or to the variability of hepatic vitamin A HPLC analysis from different biopsy sites. This variability in hepatic distribution of vitamin A occurs in humans,[1,37] swine,[56,59] arctic animals,[24] lamb,[37] and rats.[24,37] In humans, the right lobe tended to have higher mean vitamin A than the left or caudal lobes, whereas the vitamin A content of the midcentral portion of the right lobe agreed within 15% of that of the liver as a whole.[37]

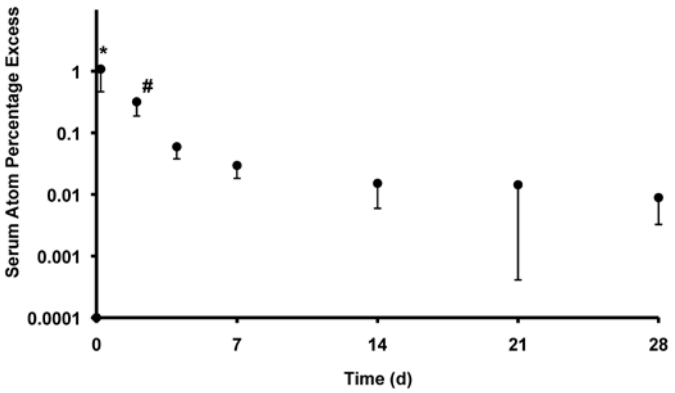

Figure 2. Semilog plot of serum retinol enrichment over time in captive rhesus monkeys given an oral dose of 3.5 µmol $^{13}C_2$-retinyl acetate. Serum retinol enrichment is shown as serum atom percentage excess (adjusted for baseline). Repeated measures using Tukey adjustment for multiple comparisons demonstrate that serum retinol enrichments at 5 h (*) and 2 d (#) after dose administration differ significantly ($P < 0.05$) from those at all other times. Values are presented as mean ± 1 SD (n = 16 per time point, except for 5 h and 28 d, for which n = 15).

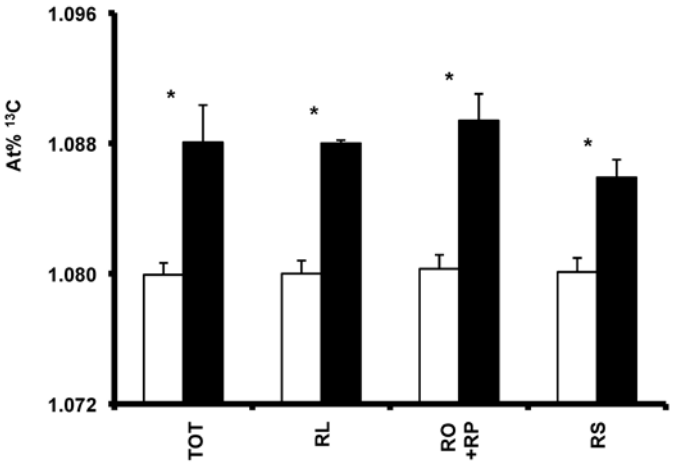

Figure 3. Pre- and postdose At%^{13}C for hepatic retinyl esters that were HPLC-purified and saponified to retinol: total retinyl esters (TOT), retinyl linoleate (RL), retinyl oleate and palmitate (RO + RP), and retinyl stearate (RS). Baseline (white bars) and postdose (black bars) values are given as mean ± 1 SD; values for baseline and day 2 after dose administration reflect the same 4 monkeys at each point. *, 1-way ANOVA by day revealed significant difference relative to baseline value (TOT, P = 0.0065; RL, P = 0.0003; RO + RP, P = 0.0022; and RS, P = 0.0027).

Neither retinol-binding protein nor retinyl palmitate hydrolase were shown to have any specific anatomic location within rat liver, regardless of vitamin A status.[2] In the current study, the hepatic vitamin A concentration of 1 rhesus monkey decreased between baseline and 2 d posttreatment with a coefficient of variation of 13%, which exceeds the reported between-day coefficient of variation for hepatic retinyl ester analysis in rats.[11,13] However, this variation is within that reported for rhesus liver vitamin A concentration.[33,39] In the present study, liver biopsy sites were random.

The relative distribution of retinyl esters reflects that reported for several species, that is, retinyl palmitate is greatest in amount, followed by stearate, oleate, and linoleate.[12] The increase in hepatic retinyl ester quantities 2 d after dose administration suggests that the hepatic enzymatic machinery of hypervitaminotic A rhesus monkeys remains capable of esterifying incoming dietary retinol, indicating that this process is not completely downregulated. The relationship of the rhesus mechanism to that underlying vitamin A trafficking during hypervitaminosis A in humans is unknown. In addition, the calculated increase in concentration coupled with the greater ^{13}C enrichment of the retinyl palmitate fraction on day 2 should be further investigated in an animal model in which the entire liver is accessible. Unlike the total hepatic retinol concentration over the same time span, total liver ^{13}C enrichment of vitamin A increased after ^{13}C-retinyl acetate administration to rhesus macaques.

Table 1. Concentrations of major hepatic retinyl esters in captive rhesus monkeys before (baseline) and 2 d after oral treatment with 3.5 µmol $^{13}C_2$-retinyl acetate

Retinyl ester	Baseline	2 d after dose
Linoleate, 18:2 (µmol/g)	1.37 ± 0.58[a]	1.32 ± 0.63
Oleate, 18:1 (µmol/g)	1.82 ± 0.76	1.83 ± 0.84
Palmitate, 16:0 (µmol/g)	6.83 ± 3.34	6.95 ± 3.68
Stearate, 18:0 (µmol/g)	1.93 ± 0.74	1.87 ± 0.78
Linoleate (µmol/liver)	442 ± 187	427 ± 199
Oleate (µmol/liver)	593 ± 246	591 ± 266
Palmitate (µmol/liver)	2220 ± 1080	2250 ± 1170
Stearate (µmol/liver)	629 ± 243	606 ± 250.

[a]Values (mean ± 1 SD; n = 4) are normalized to the estimated rhesus liver weight[39] at baseline and 2 d postdose. For each retinyl ester, baseline and day 2 values did not differ (paired t-test, $P > 0.05$).

All sources of food would influence the natural abundance of rhesus monkey body tissues. Fruits and vegetables complement the rhesus monkeys' feed. Some of these foods contain provitamin A carotenoids,[26] which are metabolized to vitamin A, confounding the relationship between feed and ^{13}C liver retinyl ester enrichment. These relationships would explain feed natural abundance falling mid-way between the baseline ^{13}C abundance and postdose ^{13}C enrichment of the individual hepatic retinyl esters. Serum enrichment of ^{13}C in the isolated vitamin A peaked by 12 h, and the enrichment-over-time curve in our rhesus monkeys is consistent with patterns seen in humans for recirculation of a ^{13}C-vitamin A dose.[54,55] Given that hypervitaminotic A rats transport a greater percentage of plasma vitamin A as retinyl esters compared with vitamin A-sufficient control rats,[32] subsequent experiments should include analysis of serum for labeled retinyl esters[7] by saponifying the serum retinyl esters and assessing the retinol recovered from this reaction by GCCIRMS.

Studies with deuterated vitamin A rely on GCMS.[14] This technique, which operates across a broad mass range, reaches an isotope ratio relative standard deviation of 1%.[15,16] This precision requires a relatively high dose. Commercial IRMS instruments typically yield isotope ratios of 0.001% or less[15] for synthetic hydrocarbons at natural abundance. Studies in children (whose body weights are similar to those of rhesus monkeys) in whom deuterated vitamin A was measured by GCMS and either electron capture negative chemical ionization or electron ionization was chosen for the detection method, the lowest doses used were 0.21[50] and 1.49[22] µmol $^2H_4$- or $^2H_8$-retinyl acetate/kg body weight, respectively. In adults, the lowest doses for use with electron capture and electron ionization were 0.28[45] and 0.75[21] µmol $^2H_4$-retinyl

acetate/kg body weight, respectively. Therefore, the per-kilogram ^{13}C dose administered to the rhesus monkeys in the present study is similar to that for electron capture analysis. However, the estimated total body reserves in children (that is, less than 0.3 mmol)[50] were much less than those of our hypervitaminotic A monkeys (approximately 5 mmol), and the number of labeled atoms in the ^{13}C tracer was at least half that of ^{2}H. Furthermore, the dose administered to these monkeys (3.5 µmol) was much less than the estimated daily intake from feed (approximately 6 µmol). To our knowledge, GCMS has not been used to measure accumulation of ^{13}C in individual liver retinyl esters after administration of a physiologic dose of a stable isotope, thereby further highlighting the sensitivity of IRMS.

In midgestation fetuses of Old World primates with a maternal history of excessive vitamin A intake, retinyl esters accumulated at levels higher than those observed in prior measurements of fetal human and monkey livers from later stages of development.[34] Retinyl ester concentrations continue to climb throughout life in captive rhesus monkeys that are fed standard laboratory chow; the highest value recorded is 18.8 ± 6.4 µmol retinol/g liver.[33] Therefore, the conservation of de novo retinol esterification may be a protective mechanism during a hypervitaminotic state to mitigate overt signs of chronic toxicity. Whether humans have this adaptive feature of metabolism during hypervitaminosis is unknown, although very high retinyl ester levels have been reported in case studies of excessive vitamin A intake.[30] Baseline serum chemistry profiles for rhesus monkeys in the current study revealed that some of them had abnormal serum triglycerides and circulating liver enzymes (31% and 50%, respectively),[8] both of which are consistent with the liver dysfunction reported in hypervitaminosis A.[47,48]

Hepatic liver vitamin A concentrations in captive rhesus monkeys are at least 4 times that considered sufficient in humans. The effect of this markedly increased liver vitamin A concentration merits further research, given that rhesus monkeys are so widely used for biomedical research,[53] including studies addressing long-term caloric restriction,[43,44] heart disease,[51] diabetes,[20,29] and AIDS.[58] Because rhesus monkeys are phylogenetically close to humans,[42,46] the results of these experiments can be extrapolated to understanding human health and treating human illness. As a result, understanding whether and how hypervitaminosis A (and the associated secondary liver dysfunction) in rhesus monkeys confounds these studies is crucial.

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