Serum Vitamin A Esters Are High in Captive Rhesus (*Macaca mulatta*) and Marmoset (*Callithrix jacchus*) Monkeys[1]

Kristina L. Penniston, Julie C. Thayer and Sherry A. Tanumihardjo[2]

Integrated Graduate Program in Nutritional Sciences, University of Wisconsin-Madison, Madison, WI 53706

ABSTRACT We showed previously that hepatic vitamin A concentrations of captive rhesus monkeys (*Macaca mulatta*) are subtoxic to toxic, with livers exhibiting stellate cell hypertrophy and hyperplasia. Although marmoset (*Callithrix jacchus*) livers are also high in vitamin A, no stellate cell irregularities were observed. To further characterize the effects of high dietary vitamin A from preformed sources, stored serum samples were analyzed from monkeys used for biomedical research and housed at the Wisconsin Primate Research Center. The monkeys had been fed commercially available monkey diets, providing vitamin A (as retinyl acetate) at levels exceeding NRC recommendations by a factor of four. The serum from both rhesus and marmoset monkeys had total serum vitamin A (retinol, retinyl esters and metabolites) within the expected range for both species, i.e., 1.44 ± 0.34 and 1.41 ± 0.72 μmol/L serum for rhesus and marmoset monkeys, respectively. However, high serum retinyl ester concentrations as a percentage of total serum vitamin A were present in both species, 12 ± 5.1% (range, 5.5–23%) for rhesus and 27 ± 14% (range, 10–57%) for the marmosets. Serum retinol concentrations were normal, i.e., 1.21 ± 0.28 (rhesus) and 0.92 ± 0.43 μmol/L (marmoset), compared with published values. J. Nutr. 133: 4202–4206, 2003.

KEY WORDS: • *hypervitaminosis A* • *monkeys* • *retinyl esters* • *vitamin A* • *vitamin A metabolites*

We previously analyzed the hepatic vitamin A concentrations of captive rhesus (*Macaca mulatta*) and marmoset (*Callithrix jacchus*) monkeys from the Wisconsin Primate Research Center (WPRC)[3] using HPLC. The total vitamin A concentration in the rhesus monkey livers was, on average, 16 times higher than normal based on previous characterizations of hepatic vitamin A in rhesus monkeys. Moreover, liver histology revealed stellate cell hypertrophy and hyperplasia. Although the hepatic vitamin A concentration of the marmoset monkeys was not as high, it nevertheless exceeded what might be considered normal, although histologic inspection did not reveal hepatic abnormalities. Although the monkeys exhibited no outward symptoms of vitamin A toxicity, according to the primate center staff, we concluded that the vitamin A content of the monkeys' diets was excessive and resulted in subtoxic to toxic liver stores (1).

As a follow-up to this research and a way of further elucidating and characterizing the effect of chronically high dietary vitamin A, we analyzed serum from rhesus and marmoset monkeys for both water- and fat-soluble vitamin A metabolites. Although measurement of hepatic vitamin A provides a highly accurate estimate of whole-body reserves, it is not practical for surveying and monitoring of vitamin A status. On the other hand, high circulating retinyl esters and other vitamin A metabolites in serum, if present, would help to clarify the effects and features of vitamin A subtoxicity and are easier to measure. Given the high hepatic vitamin A stores of both species and the chronic high daily intake of retinyl esters, we hypothesized that their serum would reveal elevated circulating retinyl esters and detoxifying metabolites.

MATERIALS AND METHODS

Animals and diet. Frozen serum from adult rhesus monkeys [n = 16; 3 male and 13 female; 13.0 ± 7.4 y (mean ± SD)] and marmoset monkeys (n = 10; 4 male and 6 female; 7.7 ± 2.5 y) was obtained from the WPRC tissue distribution program from monkeys that were killed between 3/29/01 and 4/01/03. Characteristics of the monkeys were recorded (**Table 1**). The WPRC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care-International. University committees and national agencies ensure that WPRC research and animal care complies with Animal Welfare Act regulations. All serum samples were stored at −80°C until analyzed. Rhesus monkeys were fed Lab Diet #5038 (Purina Mills, St. Louis, MO) providing 42 nmol retinyl acetate/g dry food and 4.7 nmol β-carotene/g food. According to the primate center staff, males and females consumed an estimated 250 and 175 g of the diet each day, accounting for a

[1] Supported by National Institutes of Health NID DK061973; Hatch-Wisconsin Agricultural Experiment station number WIS04533; the University of Wisconsin Graduate School; University of Wisconsin-Madison's College of Agriculture Honors program (J.C.T.); and NIH RR00167, granted to the National Primate Research Center at the University of Wisconsin-Madison. This paper is NPRC publication number 43–007.

[2] To whom correspondence should be addressed.
E-mail: sherry@nutrisci.wisc.edu.

[3] Abbreviations used: DRA, 3,4-didehydroretinyl acetate; ORA, 4-oxoretinoic acid; OROL, 4-oxoretinol; RA, retinoic acid; ROL, retinol; RAE, retinol activity equivalents; RAG, retinoyl β-glucuronide; ROG, retinyl β-glucuronide; WPRC, Wisconsin Primate Research Center.

Manuscript received 7 August 2003. Initial review completed 26 August 2003. Revision accepted 12 September 2003.

TABLE 1

Characteristics of rhesus and marmoset monkeys

	Age[1]	Body weight[1]	Liver weight[2]	
	y	*kg*	*kg*	*g/100 g body*
Rhesus	13.0 ± 7.4	8.04 ± 2.7	0.14 ± 0.042	1.76 ± 0.56
Marmoset	7.7 ± 2.5*	0.40 ± 0.049*	0.025 ± 0.0078*	6.2 ± 1.6*

[1] Values are means ± SD, $n = 16$ for rhesus and 10 for marmoset monkeys.
[2] Values are means ± SD, $n = 12$ for rhesus and 10 for marmoset monkeys.
* Different from rhesus monkeys, $P < 0.05$.

daily preformed vitamin A intake of 10,000 and 7000 IU [3000 and 2100 retinol activity equivalents (RAE)] for males and females, respectively. Marmoset monkeys were fed the Mazuri callitrichid high fiber diet #5M16 (Purina Mills) providing 21 nmol retinyl acetate/g diet. According to the staff, the marmosets, both male and female, consumed an estimated 25 g of the diet/d, for a daily preformed vitamin A intake of 500 IU (150 RAE). Detailed nutrient compositions of both diets were published previously (1).

Determination of serum vitamin A. Serum samples were thawed under yellow lights and extracted using a modification of a published procedure (2). Serum (1 mL) was placed into a test tube and 2 mL of ethanol (0.1% BHT), 2 mL of ethyl acetate and 40 μL of 3,4-didehydroretinyl acetate (0.25 nmol, DRA) were added. DRA was synthesized in our laboratory using published procedures (3,4). We used it as an internal standard to correct for variable recovery. Retinyl acetate was not used because we anticipated that it might be circulating in the serum due to high dietary intake. After being mixed for 30 s on a vortex and centrifuged for 1 min at 1380 × *g*, the supernatant was carefully poured into a separate test tube. The residual pellet was washed twice with 1 mL ethyl acetate each time. The ethyl acetate layers were pooled, mixed on a vortex and centrifuged, and saved for later use. A series of three extractions were then performed on the supernatant. First, 2 mL of purified water and 0.5 mL hexane were added, and the mixture was mixed on a vortex and centrifuged. The top (organic) phase was removed by pipette and reserved. To the remaining aqueous phase was added 50 μL of 10% (v/v) acetic acid and the ethyl acetate from the pellet washes. After mixing on a vortex and centrifugation, the organic phase was removed by pipette and added to the first extraction. The remaining aqueous phase was extracted a third time with 1 mL hexane. The remaining aqueous material was discarded. A final wash was performed on the pooled organic material with 1 mL purified water. After mixing on a vortex and centrifuging, the organic phase was removed by pipette and placed into a clean test tube. This final material was dried under argon, reconstituted with 100 μL 80:20 methanol/dichloroethane, mixed on a vortex, sonicated to ensure complete reconstitution and centrifuged. A 50-μL aliquot was injected onto a gradient HPLC system for analysis.

The reversed-phase gradient HPLC system consisted of a Waters 600E multisolvent delivery system and controller (Milford, MA) set at a flow rate of 1.0 mL/min. A Waters 2487 dual wavelength absorbance detector was set at 335 nm. A linear 20-min gradient of 70:30 methanol/water to 80:20 methanol/dichloroethane (with 0.1% triethylamine and 10 mmol/L ammonium acetate in each solvent) was used followed by 10 min of isocratic elution. After this period, the solvent was returned to baseline conditions, and 10 min was allowed between injections for column equilibration. The stationary phase was a Phenomenex Phenosphere 5-μm, reversed-phase, C-18 ODS column, 150 × 4.6 mm (Torrance, CA). A Shimadzu C-R7A Chromatopac data processor (Kyoto, Japan) recorded and calculated peak areas. A precolumn was used to protect the analytical column from particulate matter. Extraction efficiency, estimated by recovery of internal standard, was 89 ± 9.5%. The CV for this serum extraction method was calculated to be 8.8% for total vitamin A, which includes retinol, retinyl esters and metabolites. Further identification and characterization of the retinyl esters and metabolites was performed on a subset of the monkeys with a second photodiode array HPLC system, which scanned from 210 to 550 nm (Waters 600 solvent delivery system, 717 autosampler, and 996 detector).

Synthesis of retinyl ester standards. Five retinyl esters (laurate, myristate, oleate, palmitate and stearate) were synthesized as standards to determine HPLC retention times and confirm retinoid identity. Esters were synthesized by a condensation reaction of retinol with the individual fatty acid anhydrides (Sigma Chemical, St. Louis, MO) in triethylamine. Standards were purified via TLC before injection onto the HPLC.

Statistical comparisons and vitamin A calculations. Data were entered into a Microsoft Excel spreadsheet for analysis. Single factor ANOVA and appropriate *t* testing were performed on the data to determine differences between species. Linear regression to determine associations among vitamin A concentration and age, liver weight, body weight and other variables was also performed. Total serum vitamin A was calculated as the sum of the following vitamin A compounds and metabolites: 4-oxoretinoic acid (ORA), 4-oxoretinol (OROL), retinoyl β-glucuronide (RAG), retinyl β-glucuronide (ROG, generously donated by A.B. Barua, Iowa State University), retinoic acid (RA), retinol (ROL) and 6 identified retinyl esters. Values in the text are means ± SD. The differences and relationships were considered significant at $P < 0.05$.

RESULTS

Vitamin A compounds eluted in the following order: ORA, OROL, RAG, ROG, RA, ROL, DRA (the internal standard), retinyl acetate, retinyl laurate, retinyl linoleate and/or myristate, retinyl palmitate + oleate (co-eluted) and retinyl stearate. Compounds were identified on the bases of retention times, co-elution of known standards prepared in the laboratory and UV spectra obtained from photodiode array analysis. Although previous studies suggest that retinyl linoleate predominates in primates compared with myristate (5–7), we cannot be certain of the identity of this retinoid because its retention time was nearly the same as that of the retinyl myristate standard.

Similar profiles of vitamin A and metabolites were observed in the serum of rhesus and marmoset monkeys (**Table 2**). The total vitamin A concentration of the rhesus and marmoset monkeys (sum of all metabolites) was 1.44 ± 0.34 and 1.41 ± 0.72 μmol/L, respectively. Compared with the serum of rhesus monkeys, marmoset serum generally contained higher concentrations of all individual retinoids except for ORA and ROL. Serum ROL was 1.21 ± 0.28 μmol/L for rhesus monkeys and 0.92 ± 0.43 μmol/L for marmoset monkeys ($P = 0.079$). For rhesus monkeys, 85 ± 4.5% of the vitamin A existed as ROL and 12 ± 5.1% (range, 5.5–23%) as retinyl esters. This compared with 68 ± 13% as ROL and 27 ± 14% (range, 10–57%) as retinyl esters in marmoset monkeys (**Fig. 1**). The proportions of vitamin A esters differed between species ($P = 0.011$). Retinyl palmitate and retinyl oleate (not resolved) were the major circulating esters in both species. Serum concentrations of RAG and RA tended to be higher in marmoset than in rhesus monkeys ($P = 0.081$ and 0.15, respectively).

Serum ROL was correlated with total serum vitamin A in rhesus ($r = 0.98$, $P < 0.0001$) and marmoset ($r = 0.80$, $P = 0.006$) monkeys. Retinyl esters were correlated with total serum vitamin A in both species ($r = 0.71$ and $P = 0.002$; $r = 0.79$ and $P = 0.007$, respectively). In this small sampling,

TABLE 2

Serum vitamin A and metabolites in rhesus and marmoset monkeys[1,2]

	Rhesus	*n*[3]	Marmoset	*n*[3]
	μmol/L		*μmol/L*	
ORA	0.0048 ± 0.0025	4	0.011 ± 0.014	5
OROL	0.0054 ± 0.0059	10	0.0076 ± 0.0092	7
RAG	0.021 ± 0.014	9	0.046 ± 0.028	6
ROG	0.021 ± 0.015	13	0.028 ± 0.040	8
RA	0.0072 ± 0.0041	13	0.015 ± 0.010	5
ROL	1.21 ± 0.28	16	0.92 ± 0.43	10
RAC[4]	0.033 ± 0.019	16	0.11 ± 0.072	10
RLaur[4]	0.011 ± 0.0095	16	0.032 ± 0.020	10
RLin/RM	0.013 ± 0.0090	16	0.035 ± 0.043	10
RP + RO	0.089 ± 0.051	16	0.16 ± 0.21	10
RS	0.043 ± 0.031	16	0.081 ± 0.12	10
Total[5]	1.44 ± 0.34	16	1.41 ± 0.72	10

[1] Values are means ± SD.

[2] Abbreviations: ORA, 4-oxoretinoic acid; OROL, 4-oxoretinol; RAG, retinoyl β-glucuronide; ROG, retinyl β-glucuronide; RA, retinoic acid; ROL, retinol; RAC, retinyl acetate; RLaur, retinyl laurate; RLin/RM, retinyl linoleate and/or retinyl myristate; RP + RO, retinyl palmitate + retinyl oleate (co-eluted); RS, retinyl stearate; TOTAL, total serum vitamin A.

[3] *n* = number of observations for which a metabolite was observed; others were not detectable.

[4] Significant difference between species, $P < 0.05$.

[5] Sum of ORA, OROL, RAG, ROG, RA, ROL, RAC, RLaur, RLin/RM, RL, RP + RO, and RS.

neither total serum vitamin A concentration nor circulating esters were correlated with age in either species. No association was found between serum vitamin A concentration and liver weight.

DISCUSSION

The present study was conducted to compare total serum vitamin A and the relative contribution of various vitamin A metabolites between two species of monkeys whose hepatic vitamin A stores were high (1). We adapted a published procedure (2) for total serum vitamin A, which includes retinol, retinyl esters and metabolites (CV = 8.8%). This method may prove useful for assessing the risk for vitamin A toxicity because it provides information not only on retinyl palmitate, the ester customarily measured in most clinical situations, but also on other retinyl esters, ROL and vitamin A metabolites.

When total serum vitamin A for the rhesus and marmoset monkeys is compared with published values, i.e., 0.70–2.79 μmol/L in humans (8) and 1.22–1.75 μmol/L in rhesus monkeys (9,10), both species had normal concentrations at 1.44 ± 0.34 and 1.41 ± 0.72 μmol/L for rhesus and marmosets, respectively. Thus, despite previously reported subtoxic hepatic vitamin A concentrations (1), both species have normal circulating vitamin A. This is not surprising, despite the high hepatic stores, because retinol concentrations are homeostatically controlled in plasma and usually vary only slightly depending on body stores, even in toxicity (11,12). A chronic high intake of vitamin A, leading to chronically high circulating vitamin A esters, would presumably reduce endogenous ROL mobilization from liver and maintain serum vitamin A within or near the normal range. In support of this, we did observe normal serum ROL concentrations in both species, i.e., 1.21 ± 0.28 and 0.92 ± 0.43 μmol/L for rhesus and marmoset monkeys, respectively. Published serum ROL concentrations for rhesus and marmoset monkeys are few. Rogers et al. (13) observed a range of 1.12–2.25 μmol/L serum ROL in rhesus monkeys, which are Old World monkeys, and concentrations ranging from 0.32 to 0.69 μmol/L in cebus monkeys, New World relatives of marmosets. The researchers concluded that New World monkeys might normally have lower serum ROL concentrations than Old World monkeys. Although our observation of serum ROL in marmoset monkeys is markedly higher than that of cebus monkeys, it is lower than the serum ROL of rhesus monkeys ($P = 0.079$), which agrees with the earlier conjecture that New World monkeys have lower circulating ROL than Old World monkeys.

If only serum ROL in the rhesus and marmoset monkeys at the WPRC was examined, without regard to total circulating vitamin A, retinyl esters, or hepatic vitamin A, we might assume that the monkeys had normal vitamin A status. This, however, is not the case because we previously reported high hepatic vitamin A for both species, particularly for rhesus monkeys, and hypertrophic and hyperplastic hepatic stellate cells in those monkeys (1). Although not reported here, livers from a subset of monkeys whose serum was analyzed for this study were analyzed for vitamin A. Once again, hepatic vitamin A was extremely high, nearly identical to values we found earlier (1), suggesting that the vitamin A status of these monkeys had not changed recently. We concluded that serum ROL is not, therefore, an appropriate surrogate for vitamin A status in these monkeys whose total body reserves of vitamin A are in the subtoxic to toxic range.

Serum retinyl esters were relatively high, especially in the marmoset monkeys (27 ± 14% of total vitamin A), supporting our hypothesis that monkeys whose hepatic vitamin A stores are high and whose daily diet is high in preformed vitamin A would have high circulating esters. Few studies have characterized serum retinyl ester concentrations. Bankson et al. (5) determined retinyl ester con-

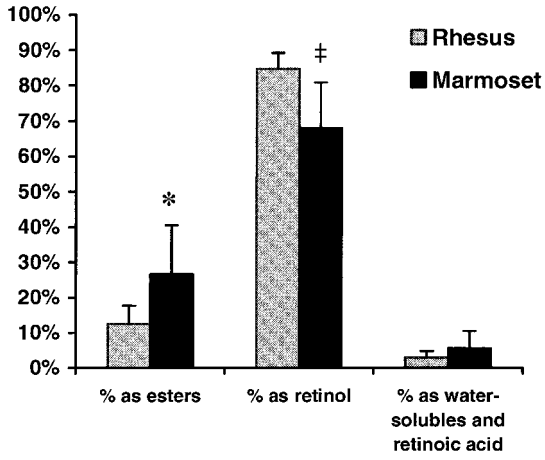

FIGURE 1 The percentage of total serum vitamin A from retinyl esters, retinol and water-soluble metabolites + retinoic acid in rhesus ($n = 16$) and marmoset ($n = 10$) monkeys with a subtoxic to toxic vitamin A status. Values are means ± SD. Symbols indicate different from rhesus monkeys: *$P = 0.011$, ‡$P = 0.0022$.

centrations in fasting humans and found a mean of 6% of total circulating vitamin A as retinyl esters (range reported, 0–18%). Similarly, Krasinski et al. (14) found a range of 2–6% of total circulating vitamin A as retinyl esters in healthy, fasting human adults. Ballew et al. (15) examined data from the third National Health and Nutrition Examination Survey and found that 67% of the adults surveyed had serum retinyl ester concentrations <10% of total serum vitamin A, the level widely accepted as a cut-off indicator of excess vitamin A (14–17), at least in humans. High circulating retinyl esters are normally found only in the chylomicra after the intake of a meal, before they are taken up by the liver as chylomicron remnants. Most of the rhesus and marmoset monkeys in this study had been food deprived and had not ingested a meal immediately before being killed; therefore, the chylomicron vitamin A would have already been cleared by the liver. On the basis of these observations, the proportions of serum vitamin A as retinyl esters in both species were substantially higher than normal, with means of 12 ± 5.1 and 27 ± 14% of the total in rhesus and marmosets, respectively.

There are several possible explanations for the differences between the two species studied, i.e., the higher hepatic vitamin A concentrations in rhesus monkeys and the higher retinyl ester concentrations in marmoset monkeys. Previously, we showed that marmoset monkeys consumed more dietary vitamin A/kg body weight than did the rhesus monkeys (480 vs. 450 RE/kg body weight for males and 360 vs. 250 RE/kg body weight for females), yet they did not have a higher hepatic vitamin A concentration (1.25 ± 0.58 for marmoset vs. 17.0 ± 6.3 μmol/g for rhesus liver) (1). Moreover, the marmoset livers did not show the marked hypertrophy and hyperplasia of stellate cells, as did the rhesus livers. The marmoset livers were larger as a proportion of body weight (6.2 ± 1.6 g/100 g) than those of rhesus monkeys (1.7 ± 0.64 g/100 g). Because retinyl esters are distributed more or less evenly throughout hepatic tissue (18,19), marmoset livers may have an increased capacity to store vitamin A without incurring stellate cell irregularities. Although lower circulating retinol concentrations are normally found in New World monkeys (13), an alternative explanation might be that the marmosets' high daily intake of preformed vitamin A elevates circulating retinyl esters, thus depressing the mobilization of hepatic retinol. The higher retinyl esters in the marmosets compared with rhesus monkeys may reflect a distinction between New and Old World monkeys or may be a result of the higher retinyl ester composition, on a body weight basis, of the marmoset diet.

In conclusion, our findings characterize the vitamin A and metabolites in serum of rhesus and marmoset monkeys whose vitamin A status is subtoxic to toxic (1). We found normal total serum vitamin A concentrations but relatively high percentages of circulating retinyl esters in both species. Our findings are consistent with what is known about vitamin A homeostasis and toxicity. Symptoms of toxicity, such as enlarged hepatic stellate cells or high circulating retinyl esters, may be present even though total serum vitamin A or serum ROL concentrations are within normal limits. Vitamin A toxicity is associated with serum retinyl esters > 10% of total serum vitamin A in humans and rats (8,20). Retinyl ester concentrations in excess of 10% of total vitamin A were found in both species in this study.

Our findings further indicate the need to reformulate the monkey diets to include less preformed vitamin A. Current diets are formulated using incomplete information on the nutrient requirements of monkeys and are based on extrapolation from the nutritional needs of other species (21,22). Furthermore, many diet manufacturers add an excess of some nutrients to allow for losses during processing and storage (22,23), providing as much as four times the NRC recommendation for vitamin A (24). This is a matter of concern because vitamin A accumulates in the liver and is not readily excreted. Increasing evidence suggests that vitamin A may be required in lower amounts than previously recognized, particularly during aging (25,26). More research is required to establish accurate ranges of normal serum vitamin A concentrations and those of ROL, retinyl esters, and other vitamin A metabolites, for rhesus and marmoset monkeys, recognizing that there may be significant differences between species. Future analysis of liver and serum of younger rhesus monkeys at the WPRC is planned in an effort to determine the effect of a change in diet that was implemented in January 2003. The new diet reportedly contains less vitamin A as retinyl acetate (24 nmol/g food) and more as β-carotene (15 nmol/g food) than the previous diet.

LITERATURE CITED

1. Penniston, K. L. & Tanumihardjo, S. A. (2001) Subtoxic hepatic vitamin A concentrations in captive rhesus monkeys (*Macaca mulatta*). J. Nutr. 131: 2904–2909.
2. Barua, A. B. (1990) Analysis of water-soluble compounds: glucuronides. Methods Enzymol. 189: 137–145.
3. Barua, A. B. & Ghosh, M. C. (1972) Preparation and properties of 4-oxoretinoic acid and its methylester. Tetrahedron Lett. 18: 1823–1825.
4. Tanumihardjo, S. A., Barua, A. B. & Olson, J. A. (1987) Use of 3, 4-didehydroretinol to assess vitamin A status in rats. Int. J. Vitam. Nutr. Res. 57: 127–132.
5. Bankson, D. D., Russell, R. M. & Sadowski, J. A. (1986) Determination of retinyl esters and retinol in serum or plasma by normal-phase liquid chromatography: method and applications. Clin. Chem. 32: 35–40.
6. Eckhoff, C., Bailey, J. R., Collins, M. D., Slikker, W., Jr. & Nau, H. (1991) Influence of dose and pharmaceutical formulation of vitamin A on plasma levels of retinyl esters and retinol and metabolic generation of retinoic acid compounds and β-glucuronides in the cynomolgus monkey. Toxicol. Appl. Pharmacol. 111: 116–127.
7. Schmidt, C. K., Brouwer, A. & Nau, H. (2003) Chromatographic analysis of endogenous retinoids in tissues and serum. Anal. Biochem. 315: 36–48.
8. Bendich, A. & Langseth, L. (1989) Safety of vitamin A. Am. J. Clin. Nutr. 49: 358–371.
9. Vahlquist, A. & Nilsson, S. (1979) Mechanisms for vitamin A transfer from blood to milk in rhesus monkeys. J. Nutr. 109: 1456–1463.
10. Vahlquist, A. & Nilsson, S. (1984) Vitamin A transfer to the fetus and to the amniotic fluid in rhesus monkey (*Macaca mulatta*). Ann. Nutr. Metab. 28: 321–333.
11. Smith, R. E. & Goodman, D. S. (1976) Vitamin A transport in human vitamin A and toxicity. N. Engl. J. Med. 294: 805–808.
12. Hathcock, J. N., Hattan, D. G., Jenkins, M. Y., McDonald, J. T., Sundaresan, P. R. & Wilkening, V. L. (1990) Evaluation of vitamin A toxicity. Am. J. Clin. Nutr. 52: 183–202.
13. Rogers, E. J., Sabrah, T., Hebert, J. R., Ausman, L. M., Hegsted, D. M. & Nicolosi, R. J. (1993) A comparison of serum retinol concentration between human and different species of normo and hypercholesterolemic nonhuman primates fed semi-purified diets with defined amounts of vitamin A. Comp. Biochem. Physiol. 106A: 749–754.
14. Krasinski, S. D., Russell, R. M., Otradovec, C. L., Sadowski, J. A., Hartz, S. C., Jacob, R. A. & McGandy, R. B. (1989) Relationship of vitamin A and vitamin E intake to fasting plasma retinol, retinol-binding protein, retinyl esters, carotene, α-tocopherol, and cholesterol among elderly people and young adults: increased plasma retinyl esters among vitamin A-supplement users. Am. J. Clin. Nutr. 49: 112–120.
15. Ballew, C., Galuska, D., and Gillespie, C. (2001) High serum retinyl esters are not associated with reduced bone mineral density in the third National Health and Nutrition Examination Survey, 1988–1994. J. Bone Miner. Res. 16: 2306–2312.
16. Goodman, D. S. (1981) Vitamin A transport and delivery and the mechanism of vitamin A toxicity. In: Retinoids: Advances in Basic Research and Therapy (Orfanos, C. E., Braun-Falco, O., Farber, E. M., Grupper, C., Polano, M. K. & Schuppli, R., eds.), pp. 31–39. Springer-Verlag, New York, NY.

17. Olson, J. A. (1984) Serum levels of vitamin A and carotenoids as reflectors of nutritional status. J. Natl. Cancer Inst. 73: 1439–1444.
18. Underwood, B. A. (1984) Vitamin A in animal and human nutrition. In: The Retinoids, Vol. 1 (Sporn, M. B., Roberts, A. B. & Goodman, D. S., eds.), pp. 281–392. Academic Press, New York, NY.
19. Blomhoff, R. & Wake, K. (1991) Perisinusoidal stellate cells of the liver: important roles in retinol metabolism and fibrosis. FASEB J. 5: 271–277.
20. Gerster, H. (1997) Vitamin A—functions, dietary requirements and safety in humans. Int. J. Vitam. Nutr. Res. 67: 71–90.
21. Knapka, J. J., Barnard, D. E., Bayne, K. A. L., Lewis, S. M., Marriott, B. M. & Oftedal, O. T. (1995) Nutrition. In: Nonhuman Primates in Biomedical Research (Bennett, B. T., Abee, C. R. & Henrickson R., eds.), pp. 211–248. Academic Press, San Diego, CA.
22. Baskerville, M. (1999) Old world monkeys. In: The UFAW Handbook on the Care and Management of Laboratory Animals, Vol. 1 (Poole, T., ed.), pp. 611–628. Blackwell Science, Malden, MA.
23. Oftedal, O. T. (1992) The nutritional consequences of foraging in primates: the relationship of nutrient intakes to nutrient requirements. In: Foraging Strategies and Natural Diet of Monkeys, Apes and Humans (Whiten, A. & Widowson, E. M., eds.), pp. 1–9. Clarendon Press, Oxford, UK.
24. National Research Council (2003) Nutrient Requirements of Nonhuman Primates, 2nd rev. ed., pp. 113–116. National Academy of Sciences, Washington, DC.
25. Hollander, D. & Dadufalza, V. (1990) Influence of aging on vitamin A transport into the lymphatic circulation. Exp. Gerontol. 25: 61–65.
26. Russell, R. M. (2000) The vitamin A spectrum: from deficiency to toxicity. Am. J. Clin. Nutr. 71: 878–884.