Subtoxic Hepatic Vitamin A Concentrations in Captive Rhesus Monkeys (*Macaca mulatta*)¹,²

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ABSTRACT Although the rhesus monkey (*Macaca mulatta*) is a widely used experimental animal, its exact vitamin A requirement is unknown. An amount of 430-3600 IU/d [129-1080 retinol equivalents (RE)] is recommended, largely on the basis of depletion studies. Normal hepatic vitamin A appears to be 1 μmol/g liver. Our goal was to determine hepatic vitamin A concentrations of captive monkeys. Liver autopsy samples from rhesus and marmoset (*Callithrix jacchus*) monkeys were obtained from the Wisconsin Regional Primate Research Center. The rhesus monkeys consumed a diet with 40 μ (12 RE) retinyl acetate/g. Male and female monkeys consumed an estimated 250 and 175 g diet/d, respectively. Marmosets were fed a powder-based diet consisting of 20 μ (6 RE) retinyl acetate/g. The marmosets consumed an estimated 25 g of the diet/d. Liver samples were extracted and analyzed by HPLC. The vitamin A concentration of the rhesus monkey livers was very high at 17.0 ± 6.3 μmol/g liver. The hepatic vitamin A of the marmosets was 1.25 ± 0.58 μmol/g liver. Histologic examination of the organs revealed no cellular hypertrophy and hyperplasia in the rhesus monkeys compared with the marmosets. Considering that the natural diet of the rhesus monkey (fruits, seeds, roots and insects) is not high in preformed vitamin A, the vitamin A content of the diet appears excessive, supplying four times the NRC recommendation and resulting in high liver stores.

KEY WORDS: rhesus monkeys marmoset monkeys vitamin A intake liver storage subtoxicity vitamin A status

The rhesus monkey (*Macaca mulatta*) is a widely used experimental animal. Detailed knowledge of its specific nutritional needs is important to ensure that data obtained are not confounded by unintended nutrient deficiency or excess (1). However, the systematic study of the nutrient requirements of animals, particularly of vitamins, has largely passed, predating the extensive use of nonhuman primates for clinical investigation (2). Thus, estimated nutrient requirements for nonhuman primate species are based largely on the experimental production of deficiencies and on nutrient levels that produce adequate health, growth and reproduction (1,3). Additionally, information has been obtained from results of nutrition studies whose primary design was not necessarily to establish nutrient requirements (1). Extensive extrapolation from the nutritional needs of other species, including humans, has also contributed to the estimation of nutrient requirements for nonhuman primates. In formulating the diets for nonhuman primates held in captivity, general practice appears to be to provide some excess of most nutrients to allow for losses during manufacturing and storage (3,4).

The vitamin A requirement for the rhesus monkey has not been adequately determined experimentally (1,2,4–6). Although vitamin A depletion in the rhesus monkey has been studied (7–11), especially regarding ocular and reproductive health, the effects of toxicity other than its teratogenicity and of subtoxicity via chronic ingestion of high physiologic doses have been studied by few investigators. In light of the extensive use of the monkey in research, the recent decrease in dietary vitamin A recommendations for humans (12) and the observation that vitamin A requirements appear to vary with the stages of the life cycle (1), it is of considerable interest to examine the vitamin A status of monkeys used in biomedical research.

Dietary vitamin A is obtained from animal products as preformed retinol (retinyl esters) or as provitamin A carotenoids (e.g., β-carotene, α-carotene, β-cryptoxanthin). Retinyl esters are hydrolyzed to retinol in the intestinal lumen. Retinol is absorbed in the intestine, bound to a specific cellular retinol-binding protein (CRBP II)⁴, and esterified to retinyl esters. The retinyl esters are transported by chylomicrons through the lymph and then as chylomicron remnants to the liver (13). Once in the liver, retinyl esters are hydrolyzed into retinol and either bound to retinol binding protein (RBP) or reesterified for storage, preferentially as palmitate (14). The retinyl esters

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2904
VITAMIN A REQUIREMENT OF RHESUS MONKEYS

2905

TABLE 1
Anthropometric data for rhesus and marmoset monkeys

<table>
<thead>
<tr>
<th>Age</th>
<th>Total body weight</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>kg</td>
</tr>
<tr>
<td>Rhesus</td>
<td>14.1 ± 10.1</td>
<td>8.3 ± 2.4</td>
</tr>
<tr>
<td>Marmoset</td>
<td>5.0 ± 3.9</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± so, n = 10.

Materials and Methods

Animals and diet. Fresh frozen liver autopsy samples from rhesus monkeys (n = 10; 6 male and 4 female; 14.1 ± 10.1 g, range 3.5 to 28.2 g) and from marmoset monkeys (n = 10; 3 male and 7 female; 5.0 ± 3.9 g, range 1.2 to 12.6 g) were obtained from the Wisconsin Regional Primate Research Center (WRPRC). Refer to Table 1 for anthropometric data for the monkeys.

Research and animal care at the WRPRC are regulated by University committees and national agencies to ensure compliance with the Animal Welfare Act. The WRPRC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care-International. Rhesus monkeys were fed Lab Diet #5038 (Purina Mills, St. Louis, MO) consisting of 40 IU vitamin A (as retinyl acetate) per gram dry food and 2.5 mg/kg carotene (Table 2). According to primate center staff, males and females consumed an estimated 250 and 175 g of the diet, accounting for a daily preformed vitamin A intake of 10,000 and 7000 IU for males and females, respectively. Marmoset monkeys were fed Mazuri callitrichid high fiber diet #5M16 (Purina Mills) consisting of 20 IU vitamin A/g, also as retinyl acetate (Table 3). According to staff, the marmosets consumed an estimated 25 g of the diet/d, for a daily preformed vitamin A intake of 500 IU.

Determination of retinyl esters. Rhesus and marmoset liver (100 and 200 mg portions, respectively) were ground with mortar and pestle and dried with sodium sulfate. Purified retinyl acetate (0.5 mg) was added to calculate extraction efficiency. The material was extracted exhaustively and brought to volume (50 mL) with dichloromethane. Aliquots (0.100 mL) were dried down with argon gas and reconstituted with an equal volume of 75:25 methanol/dichloromethane.
and age, liver weight and body weight was performed.gression to determine associations among vitamin A concentration differences between species were performed on the data. Linear re-

Figure 1 [HPLC chromatogram of the retinyl ester profile from the liver of a rhesus monkey. Retinyl acetate was used as an internal standard (added to liver samples before extraction) to calculate extraction efficiency. Identified retinyl esters, in order of elution as shown on the chromatogram are: 14:0, retinyl myristate and 16:1, retinyl palmitoleate (single peak); 15:0, retinyl pentadecanoate; 18:1, retinyl oleate; 16:0 retinyl palmitate; 17:0, retinyl heptadecanoate; and 18:0, retinyl stearate.]

**TABLE 3**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>g/100 g</td>
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</tr>
<tr>
<td>Protein²</td>
<td>22.4</td>
</tr>
<tr>
<td>Fat³</td>
<td>5.7</td>
</tr>
<tr>
<td>Fiber (crude)⁴</td>
<td>5.1</td>
</tr>
<tr>
<td>Nitrogen-free extract (by difference)⁵</td>
<td>58.1</td>
</tr>
<tr>
<td>Vitamins⁶</td>
<td></td>
</tr>
<tr>
<td>Minerals⁷</td>
<td></td>
</tr>
</tbody>
</table>

¹ The nutrient composition is of the dry powder before reconstituting with water.
² Provided primarily as soybean meal, casein, corn gluten meal, gelatin, dried whole egg, dried whey, dehydrated alfalfa meal and fish meal.
³ Provided primarily as soybean oil and animal fat preserved with butylated hydroxyanisole.
⁴ Provided primarily as ground beef pulp, ground whole aspen, solka-floc (nonnutritive fiber) and dehydrated alfalfa meal.
⁵ Provided primarily as glucose, corn flour, wheat middlings, fructose and sucrose.
⁶ Vitamins, as mg/kg: vitamin K as menadione dimethylpyrimidinol bisulfite, 2.7 (menadione); thiamin hydrochloride, 17; riboflavin, 17; niacin, 98; pantothenic acid, 62; choline chloride, 1500; folic acid, 23; pyridoxine, 17; biotin, 0.45; ascorbic acid, 610; vitamin A as retinyl acetate, 6 (retinol equivalents); vitamin D-3 as cholecalciferol, 0.095; vitamin E as d-α-tocopheryl acetate, 250 (α-tocopherol equivalents); vitamin B-12, 0.085.
⁷ Minerals, as mg/kg: calcium, 12,300; phosphorus, 6300; phosphorus (nonphytate), 5200; potassium, 6500; magnesium, 1500; sodium, 3200; chlorine, 4200; sulfur, 1400; iron, 185; zinc, 163; manganese, 130; copper, 27; iodine, 2.4; selenium, 0.28; cobalt, 0.69.

The total vitamin A concentration of the rhesus monkey was 85:15 acetonitrile/dichloroethane plus 0.1% triethylamine. See the total in the rhesus monkeys and 63.7 μmol/g liver for the marmoset monkeys was retinyl palmitate, accounting for 52.2 μmol/g liver of the marmosets (P < 0.0001). The predominant retinyl ester in both monkey livers compared with the marmoset monkey livers. Although irregular, the livers showed no fibrosis or overt pathology as confirmed by the pathologist. Hepatic vitamin A concentration was not correlated with age in this limited sample (P > 0.5). A weak yet nonsignificant inverse relationship existed between hepatic vitamin A concentration and liver weight (P > 0.2).

**RESULTS**

The rhesus monkey livers had very high concentrations of retinyl esters, with a mean of 17.0 ± 6.3 μmol/g (range, 7.2–31; median, 17 μmol/g) or ~16 times more than expected.
on the basis of O'Toole's earlier characterization of normal rhesus monkeys (7). The retinyl ester concentration of the marmoset livers was closer to, yet higher than our expectations at 1.25 ± 0.58 μmol/g (range, 0.44–2.4; median, 1.2 μmol/g). The rhesus monkey diet provided a greater amount of vitamin A than the marmoset diet (see Tables 2 and 3). Specifically, the rhesus monkey diet provided 40 IU/g of food. According to primate center staff, male and female rhesus monkeys consumed an estimated 250 and 175 g diet/day for a daily preformed vitamin A intake of 10,000 and 7000 IU (3000 and 2100 RE), respectively, and an average of 1200 IU (360 RE)/(kg body mass) for the males and 840 IU (250 RE)/(kg body mass) for the females. The marmoset diet provided 20 IU/g of dry powder or half the vitamin A of the rhesus diet. The marmosets, whose daily food intake was estimated 25 g of dry powder, obtained 500 IU (150 RE) of vitamin A/d for a mean of 1600 IU (480 RE)/(kg body mass) for the males and 1500 IU (450 RE)/(kg body mass) for the females. Although the marmosets actually consumed more vitamin A/kg body mass than did the rhesus monkeys, their hepatic vitamin A concentration was 93% less. This is explained in part by the marmosets having nearly double the liver weight of the rhesus monkeys when expressed as a percentage of total body weight (Table 1). Perhaps the vitamin A needs are higher than those of rhesus monkeys when expressed on a body weight basis. The marmoset diet also contains a higher fiber content, which may affect the bioavailability of the vitamin A from the diet. Based upon the 2001 dietary recommendations for adult humans (12), the upper limit of what is considered safe for human intake has been achieved in these rhesus monkeys, i.e., 3000 RE. Considering that the mean body weight of the rhesus monkeys in this study is 8–9 times less than that of the mean healthy human (8.2 vs. 70 kg), this study supports the gradual decrease of vitamin A in the diet and continued monitoring of rhesus monkey vitamin A status.

Bendich and Langseth (23) noted that the livers of various carnivore species may contain >3.4 μmol/g vitamin A and those of herbivores (e.g., chickens, cows, lambs) appear to average 0.17 μmol/g. Schweigert (24) reported similar hepatic vitamin A concentrations for carnivores and herbivores of 0.35–10.5 μmol/g and 0.17–1.4 μmol/g, respectively. In the wild, the rhesus monkeys are neither a strict herbivore nor a carnivore. Its diet is based largely on fruits, seeds, roots, leaves, insects and grubs. Therefore, the hepatic vitamin A concentration of a rhesus monkey in its natural environment could be expected to be closer to that reported for herbivores. However, the rhesus monkeys we analyzed had even higher levels of hepatic vitamin A than most of the carnivorous arctic mammals whose vitamin A concentration is known [Table 5 (23–27)].

The physiology of vitamin A storage in the liver has been well characterized. Much (50–80%) of the total vitamin A (retinol plus retinyl esters) in mammals is stored in the liver (28), mainly in thestellate cells (also known as Ito cells). Stellate cells are specialized lipid-storing cells that also synthesize collagen. With administration of excess vitamin A, the lipid droplets in the stellate cells increase in size (29). Analysis of the lipid droplets in rats by Moriwaki et al. (30) indicates that the major component is retinyl esters. The volume of the lipid droplet can be correlated with the quantity of vitamin A stored in the stellate cell (29). Animals with high vitamin A intakes will thus have a large quantity of vitamin A in stellate cells. Other components of the lipid droplets are triacylglycerol, unesterified retinol, cholesterol esters, cholesterol, free fatty acids and phospholipids, but these appear to be less than half the total in animals with normal vitamin A stores (30). As the stellate cells enlarge with greater lipid and retinyl ester concentration, there is less room for organelles within the cell and compression of nearby cells (31). It is unclear whether there are pathological implications of this compression, at least at subtoxic levels of vitamin A. Zou et al. (32) hypothesized that under a nonpathological (i.e., subtoxic) condition, the enlargement of stellate cells due to high vitamin A stores may protect the liver from fibrogenetic insults by preventing the development of the intracellular organelles that synthesize collagen. This would explain the absence of fibrotic pathology in the rhesus monkey livers we analyzed. We therefore conclude that although the vitamin A liver stores of the rhesus monkeys were high, placing them in a subtoxic-toxic range, their livers showed no fibrosis and no overt pathology. It appears that the natural physiologic defenses against vitamin A toxicity, including the conversion of dietary vitamin A to retinyl esters, the significant storage capacity of the liver and the controlled release of retinol from the liver (33), were sufficient in these monkeys to prevent observable hepatic symptoms of hypervitaminosis A.

Recent research in humans and rats suggests that vitamin A needs may change with age. Specifically, the elderly may have a decreased need for vitamin A due to higher circulating levels. It is unclear whether this is due to increased absorption of vitamin A (34) or decreased clearance of vitamin A from chylomicrons (35,36). Epidemiologic studies have linked excess vitamin A in the elderly with a high incidence of osteoporosis in northern Europe (36). In light of this and other data, the Dietary Reference Intakes for humans for vitamin A were recently lowered to 900 and 700 RE for men and women, respectively (12). Given that monkeys in experimental conditions may live longer than 30 y and that they are used in a variety of research initiatives involving multiple human disease conditions, investigation into their vitamin A needs throughout the life cycle is immediately necessary.

In addition to providing a source for the preformed vitamin A, carotenoids appear to provide other health benefits that are increasingly appreciated. It is widely accepted that β-carotene and other carotenoids are potent antioxidants. Cell culture and human nutrition studies have shown that β-carotene offers protection against some types of cancer and cardiovascular disease and that it stimulates the immune system (13,37,38). Studies among nonhuman primates suggest that diets high in carotenoids may be important not only for protection against cancer but for longevity as well (38). To maintain healthy

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>Hepatic retinyl ester concentration of rhesus and marmoset livers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g total esters</td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:0 + 16:1</td>
<td>12.0 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>15:0</td>
<td>0.60 ± 1.2</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>16:0</td>
<td>52.2 ± 2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>17:0</td>
<td>1.5 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18:0</td>
<td>15.0 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18:1</td>
<td>18.6 ± 2.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin A, μmol/g</td>
<td>17.0 ± 0.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Values are means ± SD, n = 10.*

1 Retinyl esters: 14:0, retinyl palmitate; 16:1, retinyl palmitoleate; 15:0, retinyl pentadecanolate; 16:0, retinyl palmitate; 17:0, retinyl heptadecanolate; 18:0, retinyl stearate; 18:1, retinyl oleate.
colonies of experimental monkeys and to protect against disease processes that may confound research aims, a reformulation of their diets to include more carotenoids may be of value to researchers and monkeys alike.

It is accepted practice for diet manufacturers to include higher micronutrient levels than are deemed necessary to maintain healthy animals. The preformed vitamin A concentration of various commercially available monkey diets ranges from ~20 to 40 IU/g. To allow for losses during manufacturing and storage, manufacturers may actually add even more vitamin A to their products. We conclude that the preformed vitamin A content of the standard monkey diets commercially available today is excessively high for the following reasons: 1) data suggest that the daily vitamin A requirement of the monkeys is in the range of 330-360 IU/d (130–1100 RE) or 10 IU/g diet; 2) the rhesus monkey is physiologically well-suited to obtaining its vitamin A needs from carotenoids; 3) the risk of hypervitaminosis from preformed vitamin A is higher than that from other vitamins; 4) there appear to be age-related changes in vitamin A metabolism, at least in humans, that are currently underappreciated in monkeys and may warrant modification of nutrient levels in the diet; 5) carotenoids may provide benefits to the monkeys (e.g., antioxidant protection, protection against the development of certain diseases) above and beyond supplying vitamin A; and 6) observation of monkeys in natural settings suggests that the majority of the monkey’s vitamin A needs may be met through the ingestion of carotenoids. The subtoxic-toxic hepatic concentrations of vitamin A in the rhesus monkeys we analyzed shows that the diet contains too much preformed vitamin A, substantially more than is required to meet their physiologic needs. A review of the dietary formulations fed to captive monkeys with regard to vitamin A is immediately warranted with a gradual decrease in vitamin A content and continued monitoring of the vitamin A status of the captive monkeys.

ACKNOWLEDGMENTS

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LITERATURE CITED


TABLE 5

Comparison of published hepatic vitamin A concentrations of various mammals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hepatic vitamin A</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halibut</td>
<td>36.1</td>
<td>Bendich (23)</td>
</tr>
<tr>
<td>Polar bear</td>
<td>22.0 ± 7.81</td>
<td>Schweigert (24)</td>
</tr>
<tr>
<td>Arctic fox</td>
<td>12.6</td>
<td>Robbins (26)</td>
</tr>
<tr>
<td>Whale</td>
<td>5.04</td>
<td>Robbins (26)</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>1.08 and 1.07</td>
<td>O’Toole (7)</td>
</tr>
<tr>
<td>Antarctic husky</td>
<td>0.92</td>
<td>Bendich (23)</td>
</tr>
<tr>
<td>Human2</td>
<td>0.008–2.86</td>
<td>Tanumihardjo (19)</td>
</tr>
<tr>
<td></td>
<td>0.049–0.56</td>
<td>Furr (27)</td>
</tr>
</tbody>
</table>

1. Conversion factors: 1 IU = 0.3 retinol equivalent (RE); 1 RE = 1 μg retinol; 1 μg retinol = 0.0035 μmol retinol.
2. Not all humans enrolled in these studies were healthy.

FIGURE 2 Representative liver samples from both a rhesus (top) and marmoset (bottom) monkey were prepared using the hematoxylin and eosin staining technique (Histopathology Laboratory at the UW School of Veterinary Medicine, Madison, WI). Slides were viewed and photographed under magnification (400×). The rhesus monkey liver shows hypertrophy and hyperplasia of the flo cells (appearing as clear white circles), the vitamin A- and lipid-storing cells of the liver. This finding is consistent with the high hepatic concentration of vitamin A in the rhesus monkeys as determined by HPLC.


