Published in final edited form as:

Am J Primatol. 2009 October ; 71(10): 813–816. doi:10.1002/ajp.20714.

Hypervitaminosis A in Experimental Nonhuman Primates: Evidence, Causes, and the Road to Recovery

Joseph T. Dever and Sherry A. Tanumihardjo

University of Wisconsin-Madison, Department of Nutritional Sciences, Madison, WI 53706

Abstract

One of the great underlying assumptions made by all scientists utilizing primate models for their research is that the optimal nutritional status and health of the animals in use has been achieved. That is, no nutrient deficiency or excess has compromised their health in any detectable way. To meet this assumption, we rely on the National Research Council's (NRC's) nutritional recommendations for nonhuman primates to provide accurate guidance for proper dietary formulations. We also rely on feed manufacturers to follow these guidelines. With that in mind, the purpose of this commentary is to discuss three related points that we believe have significant ramifications for the health and well-being of captive primates as well as for their effective use in biomedical research. First, our laboratory has shown that most experimental primates are likely in a state of hypervitaminosis A. Second, it is apparent that many primate diets are providing vitamin A at levels higher than the NRC's recommendation. Third, the recommendation itself is based on inadequate information about nutrient needs and is likely too high, especially when compared with human requirements.

Keywords

hypervitaminosis A; marmoset; retinol; rhesus; vervet

INTRODUCTION

Vitamin A (VA) is essential to all primates for vision, growth, and immune function. It is obtained from the diet either from provitamin A carotenoids found in many vegetables (e.g. β -carotene) [Tanumihardjo, 2008] or as preformed VA (retinol and retinyl esters). Commercial lab diets for nonhuman primates as well as most multivitamin supplements for humans contain primarily preformed VA sources, typically as retinyl acetate or palmitate, because they are cheaper to produce, more bioavailable in the gut, and usually more stable than provitamin A carotenoids. However, the overconsumption of preformed VA has recently been identified as a potential public health concern [Penniston & Tanumihardjo, 2003, 2006a]. Ingestion of preformed VA at levels as low as twice the current Dietary Reference Intake has been linked to an increased incidence of hip fractures and bone loss [Melhus et al., 1998; Feskanich et al., 2002; Promislow et al., 2002; Michaelson et al., 2003;

Lim et al., 2004]. Excessive preformed VA may also antagonize the absorption and action of vitamin D (cholecalciferol), a vitamin of which many people may be deficient [Rohde et al., 1999; Rohde & Deluca, 2005; Bjelakovic et al., 2008]. In 2001, the Institute of Medicine lowered the VA Dietary Reference Intakes for humans; however, excessive intake of preformed VA through multivitamin use and food fortification is still a concern in the United States and Europe.

In light of the above findings and the fact that VA status had never been properly measured in nonhuman primates, our laboratory undertook a series of studies designed to assess the VA status of rhesus and marmoset monkeys housed at the Wisconsin National Primate Research Center (WNPRC) and vervet monkeys at the Oregon National Primate Research Center (ONPRC). In our first observational study, published in 2001, captive rhesus monkeys ($n = 10$) were found to have 17-times higher hepatic VA levels ($17.0 \pm 6.3 \mu\text{mol/g}$ liver) than previous values obtained for this species (1.07 and $1.08 \mu\text{mol/g}$ liver). In our study, retinol and retinyl esters were quantified by reversed-phase HPLC [Penniston & Tanumihardjo, 2001]. Hypertrophy and hyperplasia of liver stellate cells, the major cell type responsible for VA storage, were also detected in the rhesus monkeys providing additional evidence that liver VA concentrations were excessively high. At the time of that study, conversations with WNPRC pathologists revealed that this kind of liver pathology had been detected in previous unrelated histological evaluations of rhesus monkey livers. In marmoset monkeys ($n = 10$), hepatic VA concentrations also appeared slightly elevated ($1.25 \pm 0.58 \mu\text{mol/g}$ liver), but to a much lesser degree, and no liver stellate cell hypertrophy was detected. Subsequent studies in rhesus and marmoset monkeys also revealed abundant VA concentrations in the liver ($n = 5/\text{species}$) and kidney ($n = 10/\text{species}$) [Mills et al., 2005]. In fetal livers ($n = 19$) obtained from three Old World monkey species (*i.e.*, rhesus, cynomolgus, and vervet) vitamin A concentrations were high in the second trimester ($0.194 \pm 0.065 \mu\text{mol/g}$) compared with human second trimester reference data (mean of 4 evaluations: $0.056 \mu\text{mol/g}$), although it was not known if these VA concentrations were atypical of monkeys [Mills et al., 2007]. However, despite having high liver VA concentrations, serum concentrations of retinol were found to be within expected ranges in both rhesus and marmoset monkeys consistent with previous findings that retinol concentrations in serum are tightly regulated and not a good indicator of actual VA status [Penniston et al., 2003]. However, retinyl ester concentrations were elevated, which is consistent with hypervitaminosis A. In wild-caught African green vervet monkeys ($n = 13$), high liver VA concentrations ($14.3 \pm 2.3 \mu\text{mol/g}$ liver), stellate cell hypertrophy and hyperplasia, but normal serum retinol concentrations were detected after they had been held in captivity for only two years at the ONPRC [Mills & Tanumihardjo, 2006]. Collectively, we believe that the detection of very high VA concentrations and stellate cell hypertrophy in rhesus and vervet monkeys strongly suggest that hypervitaminosis A is widespread among captive nonhuman primates and that this nutritional anomaly threatens to invalidate any data obtained from their experimental use.

DISCUSSION

Toward the Use of β -Carotene as a Vitamin A Source

In determining the reason for the excessive VA status of experimental primates, one needs to look no further than their diets. In a previous study, we comprehensively evaluated the added VA in seven typical primate diets used at primate centers across the country [Penniston & Tanumihardjo, 2006b] and found that the NRC recommendation for VA intake (10,000 IU/kg feed) was exceeded by an average of 170%. Of those seven diets, only one brand was at the VA recommendation for nonhuman primates (Research Diets Inc., New Brunswick, NJ), while another brand (LabDiets 5037, 5038, 5040, 5045, 5047, and 5050; St. Louis, MO) provided approximately four times that value in their diets (35,000 to 43,000 IU/kg feed). It is unclear why these commercial diets are so high in VA; however, manufacturers routinely add an excess of most nutrients to their products in order to compensate for potential degradation during storage and handling. This practice may be of little consequence for many nutrients, but in the case of VA, our data suggest that the consequences may be exceptionally negative. At the very least, manufacturers of commercial primate diets should lower the amount of preformed VA in their products to the current NRC recommended levels. In conversations with feed manufacturers, they are reluctant to change the formulations because the request needs to be researcher-driven. In conversations with primate researchers, they are reluctant to have the feeds reformulated because they have been using the diets in some cases for decades. Such a change is inconvenient in the short-term due to reformulating and proprietary issues, but manufacturing feed with less VA would be less expensive in the long-term.

Reducing preformed VA levels in primate diets would be the first step in reducing hypervitaminosis A; however, this alone does not comprise the best possible nutritional solution. In the wild, most monkeys are omnivorous with a major part of their diet consisting of fruits and other plant matter rich in provitamin A carotenoids, yet in laboratory diets, these sources of VA are in most cases low or missing. This discrepancy is a major detriment toward the goal of achieving and maintaining normal and optimal nutrition in experimental primates, especially if the natural diet of monkeys is to be considered a model for the proper formulation of their commercialized diets. But more importantly, it is only the consumption of preformed VA sources that can lead to a state of VA toxicity because they are quickly and efficiently absorbed regardless of VA status. VA formation from the carotenoid monooxygenase 1 (CMO1)-mediated cleaving of provitamin A carotenoids is regulated based on current bodily VA demand, perhaps through downregulation of CMO1 via retinoic acid receptor signaling, and thus produces only the amount of VA actually needed for the animal [Bachmann et al., 2002; Lemke et al., 2003]. Therefore, the replacement of preformed VA with β -carotene in primate diets would allow each animal to continually meet their own specific VA needs while preventing any possibility of VA-mediated liver toxicity or bone anomalies. Additionally, β -carotene increases plasma antioxidant capacity in humans [Meydeni et al., 1994] and may help protect against heart disease and cancer [van Poppel, 1996]. These findings further emphasize that β -carotene, besides providing VA at a physiologically-controlled rate, has other benefits that are not endowed by preformed VA. For example, carrot powders not only met the VA needs of Mongolian gerbils, but also

enhanced the antioxidant potential of liver [Mills et al., 2008]. Therefore, to truly optimize the VA status as well as the overall health condition of primates, the addition of β -carotene, perhaps as carrot powder, to commercialized primate diets should be seriously considered.

Toward a Refined Vitamin A Recommended Intake for Nonhuman Primates

β -Carotene may be a more effective VA source for experimental primates; however, its addition may be more expensive. Therefore, a cost/benefit balance must be struck when determining exactly how much β -carotene and preformed VA should be present in primate diets. To find this balance, a significant improvement in our current understanding of the specific VA requirements of nonhuman primate species is necessary. In confirmation of this, the NRC publication on VA requirements for nonhuman primates admits in the opening statement, “Despite relatively extensive studies of the deficiency syndrome, minimal requirements for VA are not well-established” [National Research Council, 2003]. Also not well-established are the “normal” tissue VA concentrations in any species of nonhuman primate except for serum, which is not a good indicator of total VA status. Hepatic VA concentrations are considered to be the best measure of VA status because the liver is the major organ responsible for storage of excess VA. However, a thorough review of the literature, revealed only one publication [O’Toole et al., 1974] where liver VA concentrations in normal (control) rhesus monkeys were reported. The values (1.08 and 1.07 $\mu\text{mol VA/g liver}$), were much lower than the values we detected ($17.0 \pm 6.3 \mu\text{mol VA/g liver}$) [Penniston & Tanumihardjo, 2001].

If experimental primates are in a state of hypervitaminosis A, how are we to determine what their “normal” tissue VA values actually are? One obvious solution would be to measure tissue VA concentrations in wild primates. This would not be a trivial undertaking and would present significant ethical, legal, and logistical problems because the tissue VA measurements would require killing many wild primates. There may, however, be a much more attainable alternative solution that would avoid any use of animals from the wild. In this scenario, a group of captive primates would be given a diet containing high amounts of β -carotene as the sole VA source. Because VA formation from β -carotene is regulated based on bodily VA demand, tissue VA values should plateau at levels that could be interpreted as “optimal” and “normal”. Using this approach, it would also be possible to assess age- and gender-related differences in VA requirements. Furthermore, these studies would simultaneously reveal the amount of dietary β -carotene required to completely meet the VA requirements of primates. Additional experiments could then be undertaken to determine the amount of dietary preformed VA required to attain the optimal tissue VA levels detected in the study using β -carotene. Collectively, this information would provide a much more solid scientific basis for crafting an accurate recommended VA intake and for manufacturing primate diets with an optimal amount of provitamin A carotenoids.

Concluding Remarks

Using primate models to answer sophisticated biological questions requires an equally sophisticated understanding of their basal nutritional needs. We have identified a systemic hypervitaminosis A in at least two different species of experimental primates (*i.e.*, rhesus and vervet). This condition may be causing unknown degrees of data corruption and

erroneous conclusions from any study involving their use, but especially studies aimed at immune function and vaccine development against infectious diseases where VA is a known modulator [Stephensen, 2001]. The excessive concentrations of VA found in these primates are due to high levels of preformed VA in their diet that exceed the current NRC recommendations. We have suggested a series of steps that would help alleviate this problem (Table 1). In the short-term, food manufacturers should be encouraged to lower the levels of preformed VA in primate laboratory diets to the current NRC recommended levels. For a longer-term and more robust solution, it will be necessary to reformulate primate diets to contain a greater proportion of VA content as provitamin A carotenoids. The development of these diets and the establishment of new VA recommendations for nonhuman primates must be based on a modern assessment of VA requirements in each primate species.

Acknowledgments

This commentary refers to previously published research at facilities that adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Nonhuman Primates. The protocols for the care of the animals were approved by the Animal Care and Use Committee of the institution at which they were undertaken and adhere to the legal requirements of the United States. This work was supported partly by NIHNIIDDK 61973, NIH grant T32 DK007665 (JTD), and the Wisconsin National Primate Research Center grant number 5P51 RR 000167 from the NIH National Center for Research Resources (NCRR). Research reviewed in this article was conducted at a facility constructed with support from Research Facilities Improvement Program grant numbers RR15459-01 and RR020141-01. This publication's contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.

References

- Bachmann H, Desbarats A, Pattison P, Sedgewick M, Riss G, Wyss A, Cardinault N, Duszka C, Goralczyk R, Grolier P. Feedback regulation of β , β -carotene 15, 15'-monooxygenase by retinoic acid in rats and chickens. *J Nutr.* 2002; 132:3616–3622. [PubMed: 12468597]
- Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gloud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev.* 2008; 16:CD007176. [PubMed: 18425980]
- Feskanich D, Singh V, Willett WC, Colditz GA. Vitamin A intake and hip fractures among postmenopausal women. *JAMA.* 2002; 287:47–54. [PubMed: 11754708]
- Lemke SL, Dueker SR, Follett JR, Lin Y, Carkeet C, Buchholz BA, Vogel JS, Clifford AJ. Absorption and retinol equivalence of β -carotene in humans is influenced by dietary vitamin A intake. *J Lipid Res.* 2003; 44:1591–1600. [PubMed: 12777466]
- Lim LS, Harnack LJ, Lazovich D, Folsom AR. Vitamin A intake and the risk of hip fracture in postmenopausal women: the Iowa women's health study. *Osteoporos Int.* 2004; 15:552–559. [PubMed: 14760518]
- Melhus H, Michaëlsson K, Kindmark A, Bergström R, Holmberg L, Mallmin H, Wolk A, Ljunghall S. Excessive dietary intake of vitamin A is associated with reduced bone mineral density and increased risk for hip fracture. *Ann Intern Med.* 1998; 129:770–778. [PubMed: 9841582]
- Meydani M, Martin A, Ribaya-Mercado JD, Gong J, Blumberg JB, Russell RM. Beta-carotene supplementation increases antioxidant capacity of plasma in older women. *J Nutr.* 1994; 124:2397–2403. [PubMed: 16856320]
- Michaelsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. *N Engl J Med.* 2003; 348:287–294. [PubMed: 12540641]
- Mills JP, Penniston KL, Tanumihardjo SA. Extra-hepatic vitamin A concentrations in captive rhesus (*Macaca mulatta*) and marmoset (*Callithrix jacchus*) monkeys fed excess vitamin A. *Int J Vit Nutr Res.* 2005; 75:126–132.
- Mills JP, Simon PW, Tanumihardjo SA. Biofortified carrot intake enhances liver antioxidant capacity and vitamin A status in Mongolian gerbils. *J Nutr.* 2008; 138:1692–1698. [PubMed: 18716171]

- Mills JP, Tanumihardjo SA. Vitamin A toxicity in wild-caught African green vervet monkeys (*Chlorocebus aethiops*) after 2 years in captivity. *Comp Med*. 2006; 56:423–427.
- Mills JP, Terasawa E, Tanumihardjo SA. Ingestion of excessive preformed vitamin A by mothers amplifies storage of retinyl esters in early fetal livers of captive old world monkeys. *Comp Med*. 2007; 57:458–462.
- National Research Council. Vitamins. In: Grossblatt, N., editor. *Nutrient requirements of nonhuman primates*. 2nd. Washington, DC: National Academy of Sciences; 2003. p. 113–116.
- O'Toole VA, Fradkin R, Warkany J, Wilson JG, Mann GS. Vitamin A deficiency and reproduction in rhesus monkeys. *J Nutr*. 1974; 104:1513–1524. [PubMed: 4422137]
- Penniston KL, Tanumihardjo SA. Subtoxic hepatic vitamin A concentrations in captive rhesus monkeys (*Macaca mulatta*). *J Nutr*. 2001; 131:2904–2909. [PubMed: 11694616]
- Penniston KL, Tanumihardjo SA. Vitamin A in dietary supplements and fortified foods: Too much of a good thing? *J Am Diet Assoc*. 2003; 103:1185–1187. [PubMed: 12963949]
- Penniston KL, Thayer JC, Tanumihardjo SA. Serum vitamin A esters are high in captive rhesus (*Macaca mulatta*) and marmoset (*Callithrix jacchus*) monkeys. *J Nutr*. 2003; 133:4202–4206. [PubMed: 14652372]
- Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr*. 2006a; 83:191–201. [PubMed: 16469975]
- Penniston KL, Tanumihardjo SA. Vitamin A intake of captive rhesus monkeys exceeds National Research Council recommendations. *Am J Prim*. 2006b; 68:1114–1119.
- Promislow JHE, Goodman-Gruen D, Slymen DJ, Barrett-Connor E. Retinol intake and bone mineral density in the elderly: the Rancho Bernardo study. *J Bone Miner Res*. 2002; 17:1349–1358. [PubMed: 12162487]
- Rohde CM, Deluca HF. All-trans retinoic acid antagonizes the action of calciferol and its active metabolite, 1,25-dihydroxycholecalciferol, in rats. *J Nutr*. 2005; 135:1647–1652. [PubMed: 15987844]
- Rohde CM, Manatt M, Clagett-Dame M, Deluca HF. Vitamin A antagonizes the action of vitamin D in rats. *J Nutr*. 1999; 129:2246–2250. [PubMed: 10573558]
- Stephensen CB. Vitamin A, infection, and immune function. *Annu Rev Nutr*. 2001; 21:167–192. [PubMed: 11375434]
- Tanumihardjo SA. Food-based approaches for ensuring adequate vitamin A nutrition. *Comp Rev Food Sci Food Safety*. 2008; 7:373–381.
- van Poppel G. Epidemiological evidence for beta-carotene in prevention of cancer and cardiovascular disease. *Eur J Clin Nutr*. 1996; 50:S57–61. [PubMed: 8841775]

TABLE 1

A List of Suggested Steps Designed to Alleviate Hypervitaminosis A in Experimental Primates

Timeframe	Action
Short-Term	1) Urge all commercial primate diet manufacturers to reformulate their products to contain preformed VA at NRC recommended levels 2) Urge all primate centers to use only commercial feeds containing preformed VA at NRC recommended levels
Long-Term	1) Reformulate commercial primate diets to include β -carotene as a VA source 2) Determine optimal basal tissue VA levels in nonhuman primates 3) Reassess NRC recommended intake for VA