Comparative Medicine
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Vol 57, No 5
October 2007
Pages 505–511

Ingestion of Excessive Preformed Vitamin A by Mothers Amplifies Storage of Retinyl Esters in Early Fetal Livers of Captive Old World Monkeys

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Excessive preformed vitamin A (VA) intake is contraindicated during pregnancy because of teratogenic concerns. Recent studies have provided biochemical and histologic evidence of chronic hypervitaminosis A in captive Old World monkeys consuming laboratory diets containing high concentrations of retinyl acetate. To investigate the effects of maternal chronic overconsumption of preformed VA on VA storage in early fetal liver, we analyzed monkey fetal livers ranging from 35 to 93 d gestational age (comparable with mid-first to late second trimester in humans) for VA (n = 19) and retinoic acid (n = 9). Retinyl esters were identified in all fetal livers, and retinol, on a percentage basis, was more abundant in younger fetuses. Liver VA concentration increased with gestational age, ranging from 0.0011 to 0.26 μmol/g in the youngest (35 d) and oldest fetuses (93 d), respectively. Liver VA concentrations (mean ± 1 standard deviation) were 0.023 ± 0.008 μmol/g in early gestation and 0.19 ± 0.06 μmol/g in midgestation fetuses. All-*trans* retinoic acid concentrations were higher in early gestation (99.2 ± 57.0 pmol/g, n = 6) than in midgestation (18.2 ± 6.1 pmol/g, n = 3) but were variable. Liver VA concentrations from midgestation fetuses were higher than those in fetal human and monkey livers from later stages of development, when growth and VA accumulation rates are assumed to be highest. Therefore, excessive intake of preformed VA by the mothers results in amplified early fetal liver retinyl ester storage.

Abbreviations: VA, vitamin A; WNPRC, Wisconsin National Primate Research Center

Vitamin A (VA) is essential for fetal development. Retinoic acid, the active retinoid involved in gene transcription directing tissue growth and differentiation,[14,32] is derived from retinol (Figure 1). All-*trans*-, 13-*cis*-, and 13-*cis*-4-oxo-retinoic acid are teratogenic metabolites of preformed VA.[21] The embryo relies on placental transport of retinoic acid, until it develops the capacity to oxidize retinol endogenously, which occurs early in midgestation for the mouse.[2] Retinoic acid concentration is tightly controlled, and any imbalance resulting from too little or too much maternal VA can be detrimental to the fetus. Deficient VA status during pregnancy results in prolonged gestation, increased fetal resorption, compromised fetal growth, and teratogenesis.[31] Congenital malformations occur when excess VA is consumed, with the severity and specificity dependent on retinoid teratogenicity, dosage, stage of development, and species.[14,32,37]

Retinol that is bound to retinol binding protein from maternal circulation is the predominant source of fetal VA, but retinyl esters carried by lipoproteins provide enough VA to support normal growth when the maternal capacity to transport retinol is ablated in mice in which retinol binding protein has been knocked out.[29] This knockout model has demonstrated that retinol is the primary retinoid responsible for fetal development, whereas retinyl esters are largely responsible for the accumulation of fetal retinoid stores.[30] In light of these observations and the elevated circulating retinyl ester concentrations seen in VA toxicity,[15] we consider the effects of excessive dietary intake of VA by the mothers on placental transmission of VA and fetal retinoid accumulation.

The relationship between maternal VA intake during pregnancy and fetal liver VA accumulation depends in part on maternal intake. Studies in VA-restricted and -nonrestricted mammals demonstrate that VA intake during pregnancy exerts little influence on fetal liver storage, compared with that of maternal VA intake during lactation.[1,5,20] However, evidence suggests that maternal VA restriction during pregnancy can decrease fetal liver VA accumulation and impair growth.[36] The extent to which placental transport regulation is able to attenuate VA transfer to the fetus during times of maternal toxicity or excessive dietary intake has not been delineated.

Fetal and neonatal VA storage patterns vary widely across species, due to unique developmental dynamics, gestation period, animal size, and physiology.[21,32] Throughout the gestation, whole-fetus and organ growth rates increase with gestational age, as do nutrient stores. Fetal growth accelerates during mid- to late gestation[13,31] and is accompanied by an increase in liver VA storage in rats[11] and humans,[7,8] especially when maternal VA reserves are high.[35] Other data from human fetuses suggest that liver VA concentrations increase until sometime in the third trimester, when they decrease until birth,[19] at which time breast milk consumption causes resurgence.[5]

Although data are available for late-stage fetal and neonatal liver VA storage in humans and rodents, few data exist regarding early-stage fetal tissues of all species, including primates. In addition to sharing approximately 95% genetic homology, human

Received: 2 Feb 2007. Revision requested: 12 Mar 2007. Accepted: 15 May 2007.
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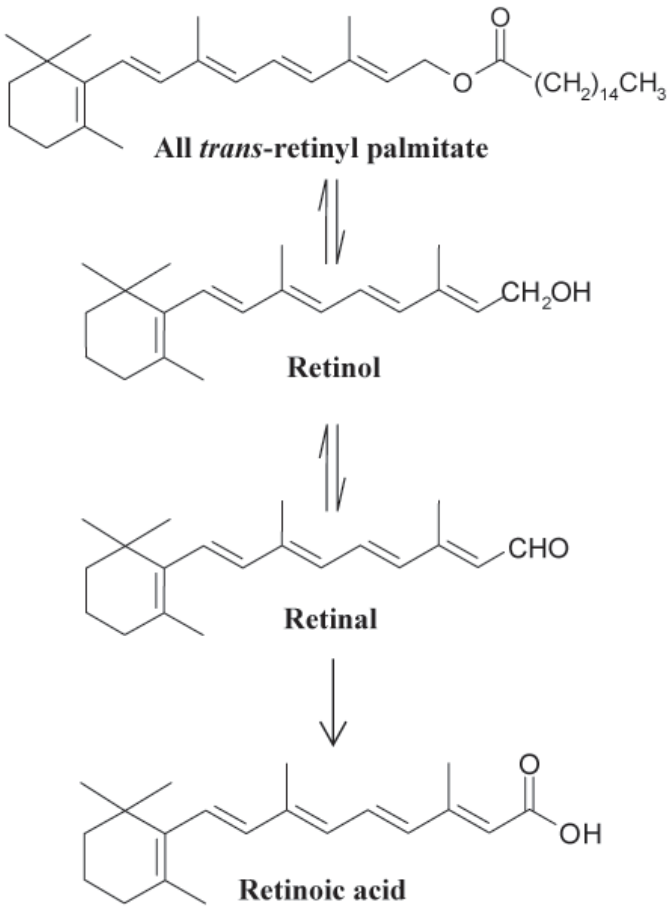

Figure 1. Structures of common forms of vitamin A found in mammals. Retinyl palmitate is the dietary and major storage form and can be hydrolyzed to retinol in the gastrointestinal tract and liver. Retinol can be oxidized to retinal, which is the major form in the visual cycle. Retinal can be oxidized further to retinoic acid, which mediates gene transcription directing tissue growth and differentiation.

and Old World monkey fetal organ growth rates and sizes are comparable throughout development.[3,13] These species also share commonalities in reproductive cycle, gestation period (252 d for humans and 165 d for Old World monkeys), placental structure, and postnatal development, making the fetal monkey a useful model for early human development.[3]

Daily intake of primate laboratory diets has been linked to elevated liver[17,22] and serum retinyl esters,[27] stellate cell hypertrophy and hyperplasia,[18,22] and extrahepatic VA storage[17] in lifetime-captive monkeys and wild-caught monkeys held in captivity for 2 y. These observations could indicate vitamin A toxicity due to excessive dietary intake of preformed VA.[26] However, without clinical or pathologic diagnosis of overt hepatic disease, hypervitaminosis may be more accurate. Regardless, consequences of chronic excessive VA intake before or during pregnancy on fetal development have not been examined.

Materials and Methods

Experimental animals and diet. Livers (n = 19) were obtained from monkey fetuses that were delivered by cesarean section and used for other research purposes at the Wisconsin National Primate Research Center (WNPRC; Madison, WI). Animal protocols were reviewed and approved by the Animal Care and Use Committee at the University of Wisconsin-Madison. The WNPRC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, International. Research and animal care at the WNPRC are regulated by University committees and national agencies to ensure compliance with the Animal Welfare Act.

Table 1. Composition of Teklad Global Primate Diet 2050

Component	g/kg
Protein (crude)[a]	209
Fat (crude)[b]	50
Fiber (crude)[c]	89
Nitrogen-free extract (by difference)[d]	484
Vitamins and minerals[e]	

[a]Provided primarily as soybean meal, corn gluten meal, and corn gluten feed.
[b]Added fat provided primarily as soybean oil and animal fat preserved with butylated hydroxyanisole.
[c]Provided primarily as soybean hulls.
[d]Provided primarily as ground corn, wheat middlings, ground wheat, and sucrose.
[e]Vitamins (mg/kg): vitamin A as retinyl acetate, 6.84; vitamin D3 (cholecalciferol), 0.2; vitamin E as dl-α tocopheryl acetate, 97.46; vitamin K as menadione dimethylpyrimidinol bisulfite complex, 13.4 (menadione); thiamin mononitrate, 18.09; riboflavin, 0.012; niacin, 86.64; pyridoxine hydrochloride, 19.1; pantothenic acid, 29.54; vitamin B12, 0.02; biotin, 0.61; folic acid, 16.77; ascorbic acid as L-ascorbyl-2-polyphosphate, 800; choline chloride, 1962.67; β carotene, 8.18; and inositol, 526. Minerals (g/100 g): calcium, 0.93; phosphorus, 0.75; sodium, 0.37; potassium, 0.7; chloride, 0.60; and magnesium, 0.18. Trace minerals (mg/kg): zinc, 71.47; manganese, 83.78; copper, 17.98; iodine (ethylenediamine dihydriodide), 7.36; iron, 306.83; selenium, 0.19; and cobalt, 2.31.

Three Old World monkey species comprised the sample: rhesus (*Macaca mulatta*; n = 11), cynomolgus (*Macaca facicularis*; n = 4), and vervet (*Chlorocebus aethiops*; n = 4). These species are closely matched in size and have similar lifespans, reproductive cycles, and gestation periods.[33] For reporting and comparison purposes, fetal livers were categorized as early (1 to 55 d) or midgestation (56 to 110 d), according to gestational age. The early gestation group contained 1 vervet and all of the rhesus (n = 11) and cynomologus (n = 4) fetal livers. The remaining vervet fetal livers (n = 3) comprised the midgestation group.

The mothers of the fetal monkeys were adult lifetime captives. Only 12.5% of the dams from the early gestation group were specific pathogen-free, and 31% had given birth previously. The mean age of the early-gestation dams was 7.25 ± 5.21 y. None of the midgestation group dams were specific pathogen-free or had previous parities. Vervet monkeys are not bred at the WNPRC, and dams of these species used in the current study were purchased from other colonies; therefore vervet birth dates were unknown, and age could not be determined. All dams were housed at the WNPRC during pregnancy and fed a 20% protein commercial diet (Teklad Global Primate Diet 2050, Harlan Teklad, Madison, WI; Table 1). The diet contains 22.58 IU (0.024 μmol) preformed VA (as retinyl acetate)/g feed and 0.015 μmol β carotene/g feed. According to primate center staff, rhesus and cynomolgus monkeys consume an average of 100 g feed daily during pregnancy, whereas the slightly smaller vervet monkeys consume 83 g feed daily.[6] These intakes fall into the generally accepted range of 2% to

4% body weight published by diet manufacturers for Old World monkeys.[18] Therefore, pregnant rhesus and cynomolgus mothers consumed approximately 2240 IU (2.4 µmol) VA daily, whereas pregnant vervets consumed approximately 1860 IU (2.0 µmol/d) VA daily. Produce and other enrichments are not included in this estimate.

Determination of vitamin A and retinoic acid concentrations. Liver VA concentrations were determined as previously described[18] with alterations in the amount of tissue used, because of low endogenous concentrations. Briefly, liver (20 to 220 mg) was ground with mortar and pestle and dried with sodium sulfate. Purified retinyl butyrate (0.1 to 0.25 ml, 3.7 to 9.3 nmol) was added to determine the extraction efficiency. Extraction was performed with dichloromethane by using 10 to 25 ml per liver. Aliquots (2 to 10 ml) were dried under argon and solubilized in 50:50 methanol:dichloromethane (100 µl). After being mixed with a vortexer (approximately 30 s) and centrifugation (approximately 1 min at 1380 × *g*), 50 to 80 µl of the supernatant was injected into the high-performance liquid chromotography system. A mobile phase of acetonitrile:dichloroethane (85:15, v:v) with 0.05% triethylamine was pumped through the column at 1.5 ml/min for a run time of 46 min. The chromotography system (Waters, Milford, MA) consisted of a precolumn and C18 column (Sunfire, 5 µm, 4.6 × 250 mm) as the stationary phase, a binary pump (model 1525), an autosampler (model 717), and a 996-photodiode array detector. Chromatograms were generated at 325 nm to quantify retinol and retinyl esters.

Liver retinoic acid concentrations were determined in a subset of livers (n = 9) by using a modified published procedure.[4] Liver (14 to 360 mg) was ground in a tube with a glass stir rod and dried with sodium sulfate. Purified 3, 4-didehydroretinyl acetate (0.04 ml, 1.5 nmol) was added to determine extraction efficiency. Liver was treated with 2 ml ethanol (containing 0.1% butylated hydroxytoluene) and 2 ml ethyl acetate. After being mixed with a vortexer (30 s) and centrifugation (1 min, 1380 × *g*), the supernatant (aqueous phase) was poured into a clean test tube. The residual tissue was washed twice with 1 ml ethyl acetate. The ethyl acetate layers were pooled, mixed, and centrifuged. The residual tissue was washed twice with 1 ml hexanes. The hexane layers were pooled, mixed, and centrifuged.

Three extractions were performed on the aqueous phase. First, 2 ml water and 0.5 ml hexanes were added, and the mixture was mixed and centrifuged; the organic phase was removed and reserved. Second, 100 µl 10% acetic acid (v/v) and the pooled hexanes and ethyl acetate layers from the tissue washes were added to the remaining aqueous phase, and the contents were mixed and centrifuged. The organic phase was removed and reserved; the remaining aqueous phase was extracted once more with 1 ml hexanes, the organic phase was removed and reserved, and the aqueous phase was discarded. The organic material was pooled, washed with 1 ml water, dried under argon, and reconstituted with 200 µl 50:50 methanol:dichloroethane. An aliquot (175 µl) was injected into a high-performance liquid chromotography system by using a published gradient method.[27] A dual-wavelength absorbance detector (SPD-10A, Shimadzu, Kyoto, Japan) was set at 325 and 350 nm for maxima of retinol and retinoic acid, respectively. Peak retention times from standards were used in conjunction with photodiode array absorbance spectra to confirm tissue retinoic acid, retinol, and retinyl esters.

Statistical methods. Values are presented as mean ± 1 standard deviation. Linear regression was used to find correlations between liver VA concentration and gestational age (R, version 2.2.0, R Foundation for Statistical Computing, Vienna, Austria). Main effects were evaluated by using 1-way analysis of variance, and differences between groups were determined by using contrasts if the F test was significant (SAS Software, version 8.2, SAS Institute, Cary, NC). *P* values less than 0.05 were considered significant.

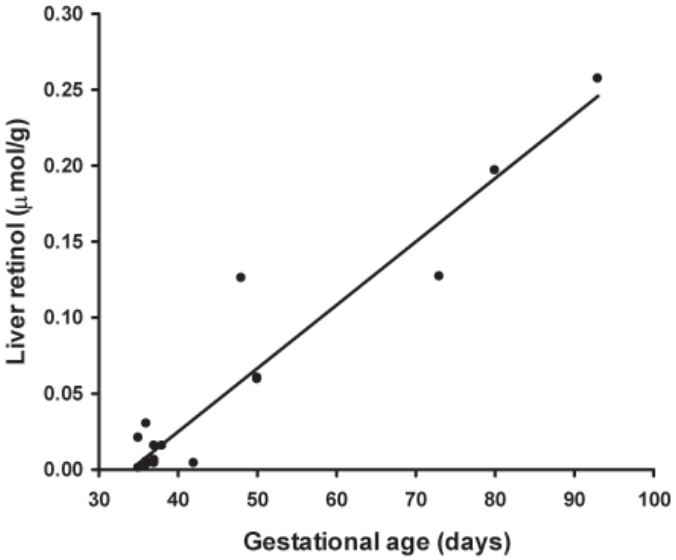

Figure 2. Liver vitamin A (retinol plus retinyl esters) in Old World monkey fetuses according to gestational age. Liver vitamin A increased with gestational age ($r = 0.98$, $P < 0.0001$).

Results

Total liver vitamin A. Total liver VA concentration, represented by retinol and retinyl esters, ranged from 0.0011 µmol/g in the youngest fetus (35 d) to 0.26 µmol/g in the oldest fetus (93 d). Liver VA concentrations (mean ± 1 standard deviation) were 0.023 ± 0.008 and 0.19 ± 0.06 µmol/g for the early and midgestation groups, respectively, and liver weights were 34.8 ± 26.8 and 1360 ± 612 mg, respectively. The difference between group means was highly significant ($P < 0.0001$). Liver VA (retinol plus retinyl esters) was highly correlated with gestational age ($r = 0.98$, $P < 0.0001$; Figure 2). Comparison of regression lines by group showed a significantly ($P < 0.0001$) greater rate of VA accumulation in midgestation livers than early-gestation livers. Midgestation values obtained in the current study are much higher than those reported for other mammals during the mid- and even late-gestation periods (Table 2).

Liver retinol, retinyl esters, and retinoic acid. Retinyl esters accounted for the majority of VA in all fetal livers, and the relative contribution from retinol decreased with increasing gestational age (Figure 3). Retinol concentrations in early-gestation livers (4.0 ± 5.3 nmol/g) were lower than those in midgestation livers (8.0 ± 7.1 nmol/g). Retinyl palmitate was the most prevalent retinyl ester, making up 46% ± 15% and 73% ± 2.4% of the total VA in early and mid-gestation age groups, respectively and 50% ± 17% for all livers. Retinyl oleate (14% ± 2.1%), linoleate (10% ± 3.7%), stearate (6.9% ± 3.6%), and other unidentified esters accounted for the remaining VA. Mean all-*trans* retinoic acid concentrations were 99.2 ± 57.0 pmol/g for the early-gestation group (n = 6) and

Table 2. Comparison of published fetal liver vitamin A concentrations (μg/g) in Old World monkeys, humans, and rodents

Species	Gestation period (trimester)			Reference
	Early (1st)	Middle (2nd)	Late (3rd)	
Old World monkeys				
	6.6 ± 2.4 (n = 16)	55.4 ± 18.6 (n = 3)	not available	Current study
	not available	not available	27.2[a,b]	41
Human				
	6.8 ± 1.30 (n = 5)	12.0 ± 1.29 (n = 11)	18.6 ± 1.24 (n = 4)	35
	9.0 ± 1.9 (n = 8)	8.7 ± 2.3 (n = 11)	14.0 ± 2.1 to 30.4 ± 0.49 (n = 6)	7
	not available	16.9 ± 2.8 to 26.6 ± 6.2 (n = 18)	11.9 ± 3.7 to 29.5 ± 7.3 (n = 18)	19
	not available	not available	16.2 ± 6.2 to 30.6 ± 12.5 (n = 22)	12
Rodents				
Fisher–Wistar rat	not available	not available	5.1 ± 1.4 to 6.9 ± 2.1 (n = 29)	11
Sprague–Dawley rat	not available	not available	9 to 15[b] (n = 10)	5
Mouse	not available	0.2 to 1[b,c] (n ≥ 5 litters)	2 to 14[b,c] (n ≥ 5 litters)	34

Vitamin A concentrations are reported as either mean ± 1 standard deviation or mean ± standard error; 1 μmol retinol = 286 μg.
[a]Liver vitamin A (retinol and retinyl esters) was reported as μg/liver in reference 41. Vitamin A concentration (μg/g) was calculated from μg/liver and published liver weight (g) from age-matched rhesus monkeys from reference 13.
[b]Mean values reported without standard deviation.
[c]Values taken from graphs and should be interpreted as approximations. Within a gestation period, lower and upper range values are at early and late time points, respectively.

18.2 ± 6.1 pmol/g for the midgestation group (n = 3). Retinoic acid concentration marginally decreased with increasing gestational age ($r = 0.61$, $P < 0.079$).

Discussion

This study uniquely measured liver VA concentrations in early and midgestation Old World monkey fetuses. Without species- and age-specific reference values for direct comparison, interpretation is confined to a small number of human studies and 1 monkey study involving late-gestation fetuses. Rhesus monkey fetuses of 121 to 165 d gestational age had retinyl ester and retinol reserves of 1.36 and 0.035 μmol/liver, respectively.[41] Because these values involve late gestation fetuses while our results include fetuses from early and midgestation, liver VA concentrations rather than entire liver reserves are needed for comparison between studies. In light of established fetal organ growth patterns in rhesus monkeys,[13] the estimated VA concentration for late gestation livers is 0.09 and 0.002 μmol/g liver for retinyl esters and retinol, respectively. This estimated retinyl ester concentration is half of the midgestation fetal value (0.19 ± 0.06 μmol/g) in the current study. This difference is likely an indication of elevated fetal liver VA storage in these monkeys, especially considering that late gestation is associated with rapid organ growth and a dramatic increase in liver VA accumulation.[7,11] The estimated percentage of retinol relative to total VA during this period is slightly lower than the values from the current study. This finding is in agreement with the falling retinol to retinyl ester ratio that occurs during fetal development and in postnatal life.[31]

Comparisons of midgestation findings from Old World monkeys with published fetal liver VA values in humans support the conclusion that these fetal monkey livers contain elevated retinyl ester concentrations. The VA concentration in midgestation monkey livers (0.19 ± 0.06 μmol/g) was 3 to 6 times that of second-trimester human livers (0.03 to 0.06 μmol/g) in studies involving mothers with presumably adequate nutritional status.[7,19,35] Midgestation values for our animals also exceeded human third-trimester (late gestation) values in every study examined.[7,12,19,35] In Table 2, published fetal liver VA reference values from data in humans, monkeys, and rodents are juxtaposed with results from the current study.

The gestational period is 252 d for humans and 165 d for Old World monkeys.[3] Several studies have reported progressive accumulation of liver VA throughout gestation,[7,8,35] yet 1 study found that liver VA concentrations increase until 28 wk in humans.[19] This time point would be equivalent to 128 d in Old World monkeys. Therefore, based on the regression developed from the data, the extrapolated liver VA concentration at birth for Old World monkeys is estimated to be between 0.40 μmol/g (at 128 d) and 0.55 μmol/g (for a full-term infant at 165 d), assuming continued

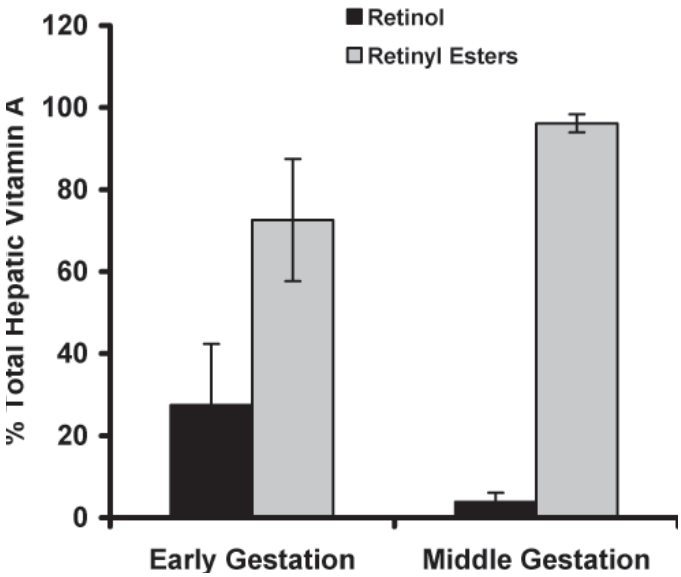

Figure 3. Relative contributions of retinol and retinyl esters to total liver vitamin A in Old World monkey fetuses during early and middle gestation.

accretion.

Primates and rodents share various aspects of fetal development, and genesis of VA-storage capacity may be among them. Liver stellate cells accumulate VA as retinyl esters in lipid droplets.[38] Anatomical analysis has demonstrated that these VA-storing cells first become visible in the fetal mouse liver at day 15 of gestation, the beginning of the late-gestation period.[16] Stellate cell genesis is accompanied by the appearance of measurable liver VA storage, which occurs as early as day 16 of gestation in rats,[39] when synthesis of retinol binding protein begins.[31] If the developmental stages of rat are comparable to those in Old World monkeys, quantifiable liver retinyl ester accumulation likely does not occur during early gestation. Nonetheless, appreciable concentrations of esters were identified, and these levels may be unique to Old World monkeys or may indicate excess VA accumulation due to excessive maternal VA intake. Early VA overload may stimulate premature development of liver stellate cells to accommodate excess VA not used for normal growth and development. Whether this response is mediated by altered gene expression, cellular retinol and retinoic acid binding protein activity, or enzymatic activity involved in retinoid metabolism in the developing fetus is unknown.

Minimal daily teratogenic preformed VA dietary levels have been established for the mouse, rat, hamster, and rabbit and range from 17.5 (rabbit) to 315 (rat) µmol retinol/kg feed.[21] Captive female Old World monkeys at the WNPRC and the Oregon National Primate Research Center (Portland, OR) consumed 3.5 to 7.3 µmol preformed VA as retinyl ester (42 to 45 µmol retinol/kg feed) daily.[18,22] No congenital malformations have been attributed to excessive VA intake in monkeys at either primate center. Although this level of dietary intake causes maternal chronic hypervitaminosis A, it has not led to identifiable birth defects in offspring. Furthermore, the reported range of daily VA intake for rhesus monkeys at 5 primate centers in the United States is 4.2 to 11.2 µmol (36 to 45 µmol retinol/kg feed).[26] At these dietary levels, serum analysis from rhesus monkeys has demonstrated that despite elevated concentrations of retinyl esters, those of retinoic acid and other teratogenic retinoids that could potentially cross the placental barrier are only modestly elevated.[27] Considering that many women in the United States are either prescribed or begin taking over-the-counter vitamin–mineral supplements during the first trimester of pregnancy,[43] it is important to know the degree at which high levels of retinol and retinyl esters are teratogenic to the human fetus. In general, the high intake of preformed VA worldwide has caused concern.[23,25]

Although excessive retinoic acid and analog transmission from mother to fetus does not seem likely in these monkeys, compelling evidence of amplified transfer of preformed VA as retinol or retinyl esters exists. Instead of being converted to teratogenic retinoids, the excessive, maternally derived VA is being stored primarily as retinyl esters in the liver. This effect is accomplished either through fetal conversion of retinol to retinyl esters (from retinol bound to retinol binding protein) or direct transmission of retinyl esters from mother to fetus via chylomicra or lipoproteins or as a combination of these routes. This preferential shunting of maternally derived VA to storage as retinyl esters may be a mechanism to protect against VA toxicity in the fetus.[16] In fact, retinol is sequestered as retinyl esters in zebrafish embryos, presumably to decrease retinoic acid production and act as a store for later larval vision.[10] In addition, retinol is 20 times less teratogenic than is retinoic acid,[32] and monkeys are less sensitive to the teratogenic effects of 13-*cis*-retinoic acid than are humans and rodents.[32] Together, these observations may explain why there have been no reports of VA-induced congenital malformations in primate center neonates, despite evidence of hypervitaminosis A in their mothers.

Liver VA concentrations from midgestation fetuses were higher than those in fetal human and monkey livers from equivalent and later stages of development. Fetal growth velocity, including of the liver, is highest during late gestation (around 125 d gestational age).[13] Furthermore, fetal VA accumulates more rapidly than growth dictates during the third trimester,[7,11] which these fetuses had not yet reached. In addition, retinyl esters were present in all of the early-gestation monkey fetal livers, despite the fact that VA-storing cells do not develop until the onset of the late-gestation period in rats. Amplified fetal liver VA storage in the current sample of Old World monkeys suggests that preformed VA intake levels before or during pregnancy were high enough to overcome the highly regulated mechanisms of placental VA transfer which normally function to keep fetal VA levels low relative to those postnatally. Because these monkeys were lifetime captive for several generations, this mechanism may be an adaptation to constant exposure to a diet high in preformed VA. Further investigation should include assessment of fetal and neonate liver VA storage in wild-caught monkeys.

Limitations of the current study, such as the lack of late-term fetal livers and separate analyses by species, should be addressed in future studies. Our sample consisted of 1 African (that is, vervet) and 2 Asian (that is, rhesus and cynomolgus) species whose ancestors occupied unique ecologic niches. Subtle differences in vitamin A metabolism may have occurred with these different environmental conditions. However, in captive animals, excessive dietary intake of VA is a universal finding across species and is more pronounced in Old World monkeys at multiple primate centers. We have demonstrated this situation by using dietary survey data[26] and biochemical and histologic examinations of monkey

tissue[17,18,22] and serum.[27] Therefore, the basis for our conclusion that maternal excessive intake results in amplified fetal storage is sound.

Maternal VA supplementation is an important issue worldwide, because it is assumed that infants are born without significant liver reserves that are somewhat independent of the mother's VA status. The understanding of the mechanism by which VA is transferred to the fetus and its limitations are paramount as countries decide which programs to implement. Daily administration of low-dose supplements during pregnancy[42] may mimic transfer to the fetus as seen in the monkeys if delivery through the placenta occurs via chylomicra at absorption.[29] However, if elevated concentrations of liver-derived lipoprotein retinyl esters, which are found during hypervitaminosis A, cause the high liver reserves that we noted in these monkey fetuses, then maternal supplementation during pregnancy of mothers with a normal or deficient VA status would benefit the mother more than the fetus. Moreover, large-dose supplementation of lactating mothers does not notably elevate breast milk concentrations for an extended period of time, as modeled in lactating sows,[28] although significant increases in toxic metabolites occur.[24] Perhaps low-dose daily supplementation to lactating women[40] or regular maternal intake of foods rich in VA or provitamin A carotenoids[9] to elevate chylomicron-delivered VA levels in breast milk would benefit neonates most.

Acknowledgments

The authors thank Kim Keen for coordinating the procurement of fetal livers after cesarean section. This research was supported by grant NIH-NIDDK 061973 and the Wisconsin National Primate Research Center grant number 5P51 RR 000167 from the National Center for Research Resources. This research 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 the National Center for Research Resources or National Institutes of Health. The authors do not have any conflicts of interest with the funding agencies of this study.

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