Safety and toxicity of vitamin A supplements in pregnancy

Michael J. Dibley and David A. Jeacocke

Abstract

Vitamin A plays an essential role during fetal development; however, if consumed at high doses it can produce teratogenic effects. Synthetic retinoids are potent teratogens and are contraindicated during pregnancy. β-Carotene is free of toxic effects. Intakes of vitamin A less than 10,000 IU per day during pregnancy have not been associated with birth defects. However, there are conflicting results for intakes of 10,000 IU to 30,000 IU per day. Intakes of vitamin A greater than 10,000 IU per day are not recommended for well-nourished pregnant women. Intakes of 30,000 IU per day of vitamin A in nonpregnant women produce only minor increases in the primary teratogen of vitamin A embryopathy. In vitamin A–deficient populations, doses of vitamin A less than 10,000 IU per day or 25,000 IU per week are considered beneficial to pregnant women without risk to the fetus. In these populations, the risks of teratogenicity from high vitamin A intake may need to be balanced against those from a deficiency.

Introduction

Safety concerns relating to the use of vitamin A supplementation in pregnancy are twofold. First, there exists the risk to the mother of toxicity from sudden excessive intakes or continued high intakes of vitamin A over prolonged periods. Second, there is the potential for teratogenic abnormalities in the fetus at doses below those required to produce toxicity. The nature and clinical features of vitamin A toxicity are discussed, and a detailed account of the biological basis and evidence relating to birth defects arising from vitamin A exposure is presented.

Michael Dibley is affiliated with the Centre for Clinical Epidemiology and Biostatistics, School of Population Health, Faculty of Medicine and Health Sciences, University of Newcastle, in Callaghan, NSW, Australia. David Jeacocke is affiliated with the Newcastle Institute of Public Health in Newcastle.

Types of toxicity

Retinol may result in toxicity when consumed in excessive quantities in both pregnant and nonpregnant women. Both acute and chronic forms of toxicity have been documented in the medical literature. The dietary intakes and characteristic features are described for each form of toxicity.

Acute toxicity

Acute toxicity may arise from ingestion of large quantities of retinol over short periods. Dosages in the order of 100 times the recommended daily allowance (RDA) are required to produce toxicity in adults, and for this reason acute toxicity is quite uncommon [1]. Symptoms of acute toxicity include gastrointestinal upset and neurological symptoms of headaches, blurred vision, vertigo, and muscular incoordination. In more extreme cases, further progression of these symptoms may occur to include drowsiness, malaise, inactivity, itching, skin exfoliation, and worsening vomiting approximately one week later. Lethal doses result in death from respiratory failure or convulsions [1].

Chronic toxicity

Chronic toxicity usually arises from doses less than 10 times the RDA consumed over extended periods, in some cases years, and is usually reversible following cessation of supplement use with complete recovery from toxic effects [1]. The most serious effects of chronic toxicity are on the liver, bone, and vision, where in some cases permanent damage may occur [1]. Hepatic damage from chronic vitamin A ingestion results in histological findings resembling cirrhosis caused by chronic alcoholism [2]. Chronic muscular and skeletal pain may arise [1], as may

psychiatric side effects, including severe depression and schizophrenia [2].

Teratogenic effects of vitamin A in pregnancy

The question about the safety of vitamin A use in pregnancy remains a complex and unresolved issue, even though it is recognized that vitamin A plays an important role in normal embryonic growth and development. The task of defining safe levels of vitamin A intake during pregnancy has received much attention since the discovery that the pharmaceutical agents etretinate and isotretinoin, both of which are vitamin A analogues, are potent teratogens in humans. Animal studies have shown that vitamin A intake may also result in birth defects similar to those produced by vitamin A analogues in humans. These findings have prompted considerable reassessment of the safety of vitamin A consumption in humans during pregnancy. In this section, an overview of the human and animal evidence for teratogenesis from synthetic retinoids, retinol, and research supporting the safety of the use of β-carotene in pregnancy is presented.

Summary of vitamin A metabolism and pharmacokinetics

Recent understanding of the involvement of retinoic acid in genetic regulatory control mechanisms in embryonic tissues suggests that retinoic acid, rather than retinol and retinol esters, may be responsible for the teratogenic effects of vitamin A [3]. The major teratogenic metabolite of retinol is all-*trans*-retinoic acid [4]. Other teratogenic metabolites include all-*trans*-4-oxo-retinoic acid, 13-*cis*-retinoic acid, and 13-*cis*-4-oxo-retinoic acid. It is believed that the teratogenicity of 13-*cis*-retinoic acid arises because of interconversion to all-*trans*-retinoic acid [4]. The rate of this interconversion may vary among women [5]. The metabolite all-*trans*-4-oxo-retinoic acid is thought to have a similar teratogenic potential to all-*trans*-retinoic acid [4].

Other pharmacokinetic properties also affect the serum levels of vitamin A metabolites, including their volumes of distribution and differing half-lives of elimination [6]. Miller et al. [6] reported on the preliminary results of a multicenter study conducted in six countries in which the serum levels of various teratogenic metabolites were analyzed in 85 pregnant women during the first trimester, providing reference values for safe concentrations. The highest plasma concentrations were found for 13-*cis*-4-oxo-retinoic acid. This metabolite is known to have a longer half-life than retinoic acid, which has a relatively short half-life [6]. The pooled effect of a number of teratogenic metabolites, rather than just the intake of retinoic acid, may be important in determining the potential for the development of birth defects. In considering the effects of the teratogenic metabolites on the fetus, it is also unclear which are important: peak concentrations of metabolites or the total cumulative exposure (area under the plasma concentration–time curve) [4].

Buss et al. [4] examined the serum levels of potentially teratogenic metabolites of vitamin A in 10 nonpregnant women, when consumed as dietary retinyl palmitate, both in the form of liver and as a supplement. Dietary retinol in the form of liver produced a smaller and delayed rise in serum retinol levels than vitamin A consumed as retinyl esters. This suggests that the latter form of vitamin A may have a more marked effect in producing teratogenic metabolites. Therefore, not only must pharmacokinetic properties of vitamin A metabolites be considered, but also the dietary form in which vitamin A is consumed.

In healthy nonpregnant female volunteers, dietary intakes of 10,000 IU of vitamin A resulted in serum retinoic acid and 13-*cis*-retinoic acid levels similar to the physiological range of these metabolites observed in pregnant women [3, 6]. Even at dosages of 30,000 IU, minimal differences in physiological levels were detected with mean serum levels at the upper limit of the physiological range [3]. These findings suggest that, on physiological grounds, the dosages required to produce teratogenic effects might be expected to exceed 30,000 IU.

Physiological basis of vitamin A teratogenicity

A further consideration in determining the potential for teratogenicity is the extent to which changes in maternal serum levels of these vitamin A metabolites result in changes in fetal exposure. The ability of retinol to be transferred across the placenta into the fetal circulation has been discussed previously. Importantly, it has been found that the placenta can transfer teratogenic compounds and continue to produce teratogens through ongoing oxidative metabolism and isomerization [7]. Therefore, in considering the potential teratogenic effects of vitamin A, the placental contribution to production of teratogens needs to be considered, as well as the contribution from maternal serum levels.

Evidence suggests that vitamin A transfer is regulated to maintain steady fetal levels of retinol [8]. The transfer of retinol across the placenta is also thought to be a saturable process at high doses [9, 10]. It is believed that maternal retinoic acid acts as the predominant teratogen in humans [3]. In one study, doses of 30,000 IU retinol did not result in serum

levels of vitamin A metabolites exceeding the usual range observed in women during the first trimester of pregnancy [5].

Transfer of vitamin A to the fetus

Little is known about the mechanisms by which the fetus and placenta regulate the transfer of retinol from mother to fetus. One study in mice has shown that during the embryogenic period there is a sudden reduction in maternal retinol levels corresponding to a steady increase in fetal retinol levels [11]. This depletion in maternal serum levels was followed by a subsequent rise in maternal retinol levels from hepatic stores. Little change was observed in the levels of retinyl esters and retinoic acid. Although it has been observed that maternal serum retinol levels drop during human pregnancies, especially in the third trimester (see earlier section), in vitamin A–replete populations this is due mainly to hemodilution. The mean retinol level in fetal liver is low (<20 μg/g); however the retinol concentration does increase as pregnancy progresses, especially in the third trimester [8]. It is important to note that fetal retinol levels do not increase significantly following maternal supplementation [1].

Both retinol and complexes of retinol and retinol-binding protein (RBP) are taken up by the placenta, and retinol is secreted into the fetal circulation [9]. Retinol is more rapidly transferred across the placenta when it is not in a complex with RBP because of its high lipid solubility in the unbound state [7]. Similarly, free retinoic acid can be taken into the placenta. Maternal β-carotene is also thought to be an important source of retinol precursor for the placenta. Retinol-deficient women are thought to have higher interconversion of β-carotene to retinol in order to maintain adequate vitamin A transfer to the fetus [9]. Maternal β-carotene, which is significantly correlated with cord retinol levels for women with serum retinol levels below 15 μg/dl, may therefore be an important source of retinol in vitamin A–deficient women [12].

Fetal RBP is synthesized initially by the amniochorionic membrane and later in gestation by the fetal liver, but it does not appear to be transferred from the maternal to the fetal circulation [9]. It is thought that retinol binds to RBP secreted into the amniotic fluid. This may provide an important source of retinol to the fetus, which is known to swallow 15 ml of amniotic fluid per day by 20 weeks of gestation [9].

Wallingford and Underwood [10] stated that the ratio of maternal to fetal serum retinol levels is approximately 2:1 in healthy women with adequate vitamin A intakes. In cases of deficiency, these researchers commented that it is possible for fetal levels to exceed maternal levels. This transfer process therefore appears to be homeostatically regulated to ensure adequate fetal vitamin A levels [9, 10]. In a study comparing serum retinol levels in postmortems of fetuses from Swedish women to levels in fetuses from Ethiopian women with low vitamin A reserves, similar although slightly lower serum RBP levels were found in cord blood of Ethiopian fetuses [8]. An exponential increase in fetal hepatic retinol reserves that was detected in fetuses of Swedish women in the second and third trimesters was not observed in the fetuses of similar gestation from the vitamin A–deficient Ethiopian women. This study suggested that the fetus can maintain relatively constant serum retinol levels by ensuring that vitamin A is retained in the fetal circulation in preference to storage in the liver [8]. Other studies have also suggested that the fetus attempts to maintain a steady serum retinol level in this way [12].

Synthetic retinoids

Animal studies

The teratogenic effects of isotretinoin have been examined in a number of species, including the mouse, the rat, and the rabbit [13, 14]. Exposure to synthetic retinoids has been shown to affect almost all organ systems [15]. In rhesus monkeys exposed to tretinoin and etretinate, the resulting abnormalities included microtia and craniofacial defects, nervous system abnormalities, eye abnormalities, and thymic abnormalities [14]. Cranial defects, vertebral defects, and limb reduction defects have been observed in monkeys, although they are not prominent findings in human case studies [14]. Animal studies have shown that the type of birth defect is dependent on the stage of pregnancy at which exposure occurs; abnormalities of the head, sensory organs, and cardiovascular system result from exposure shortly after conception, and limb and urogenital abnormalities result from exposure at a later stage [13].

Despite similarities in the patterns of abnormalities observed, differences across species in the degree of teratogenicity of isotretinoin have been reported [14]. The minimum teratogenic dose on a milligram per kilogram basis over a number of exposures is considerably lower in human than in animal studies. In contrast to other primates, where teratogenic dosages have been reported to range from 7.5 to 40 mg/kg/day [13, 14], the teratogenic dose for humans appears to be much lower (0.4 to 1.5 mg/kg/day). The minimum teratogenic dosage of etretinate in humans is 0.2 mg/kg/day, which is also much lower than that in other primates, rats, mice, hamsters, and rabbits (range, 2 to 5 mg/kg/day) [13].

Human studies

The drugs isotretinoin or 13-*cis*-retinoic acid (Accutane, Roaccutane) and etretinate have been shown to be highly teratogenic in humans [16]. These medications are used for the treatment of severe recalcitrant cystic acne and for psoriasis, respectively [17], both disorders that affect young women. Recently, a newer synthetic retinoid, acetretin (Neotigason), has been released to replace etretinate. Acetretin is an active metabolite of etretinate and, like isotretinoin and etretinate, has been classified as having a high risk of causing birth defects [18].

Birth defects arising from isotretinoin produce a well-described phenotype known as retinoic acid embryopathy [19]. Distinctive features include malformations of the ear (microtia, anotia) associated with heart defects (conotruncal and aortic arch abnormalities); brain defects. including hydrocephalus, microcephaly, and cerebellar abnormalities (absence or hypoplasia of the vermis); facial abnormalities (small mandible, cleft palate); and thymic abnormalities [20]. This combination of findings is suggestive that the impaired migration of cranial neural crest cells may be the underlying cause of these abnormalities [17]. These characteristics are similar to the pattern of fetal abnormalities resulting from exposure of pregnant animals to synthetic retinoids [14].

Lammer et al. [19] reported on 21 malformed infants exposed to isotretinoin whose abnormalities included central nervous system malformations (86%), craniofacial malformations (81%), cardiac defects (57%), and thymic malformations (33%) [21]. Infants exposed to isotretinoin in early pregnancy were 26 times more likely to develop abnormalities of the central nervous system, cardiac system, or ear. In addition to these abnormalities, a 22% miscarriage rate was reported [19].

Similar findings were described in 75 pregnant women who were exposed to the agent etretinate or etretin [22]. Following 14 pregnancy terminations, classic features of retinoic acid embryopathy were present in five cases. Typical malformations were detected in six infants of another 29 women who progressed to delivery. The range of dosages giving rise to malformed infants or fetuses varied from 25 to 75 mg [22]. Defects observed in humans have included craniofacial defects (microtia, micrognathia, and low-set ears), central nervous system abnormalities (meningomyelocele, anophthalmia, and brain defects), and skeletal defects (syndactyly, shortened or absent digits, club foot, and multiple synostoses) [18]. Developmental deficits have been observed in over half the children with exposure *in utero* to this agent [18].

An important consideration in potentially fertile women who have been prescribed synthetic retinoid agents is the safe period for conception. The synthetic retinoids isotretinoin, etretinate, and acetretin have half-lives of 20 hours, 120 days, and 50 hours, respectively. These long half-lives require that women delay conception following the cessation of these drugs. The newer agent acitretin has been released to replace etretinate due to its shorter half-life; however, in some women acetretin is converted to etretinate during therapy [18]. A two-year contraception period has been set for etretinate, representing approximately seven elimination half-lives, which is required for elimination of 99% of the drug from the body [22]. A similar time frame has been set for acetretin because of the potential for interconversion to etretinate [18]. A contraception period of at least one full menstrual period (one month) is required for isotretinoin [17].

The fetus of a 22-year-old woman who underwent a pregnancy termination four months after cessation of etretinate for treatment for Darier's disease displayed abnormalities including aplasia of the tibia and fibula and hypoplasia of the left femur [22]. Chan et al. [18] provided data from a prospective study of 45 subjects in whom pregnancy occurred within two years of cessation, resulting in an abnormality in one case or 2% of subjects examined (95% CI, 0%–12%). The pharmaceutical manufacturer Roche prospectively examined a group of 32 women exposed to Acetretin who became pregnant within two years of cessation; no episodes of abnormalities were recorded [18]. In another prospective case study of 48 women becoming pregnant within one month of completing treatment with isotretinoin, two birth defects were reported (4% of subjects; 95% CI, 1%–14%). These case studies confirm the importance of contraception periods for women of reproductive age undergoing treatment with synthetic retinoids.

In some countries, such as Australia, the prescription of synthetic retinoids has been restricted to specialist dermatologists [18]. Guidelines for the prescription of these agents have been recommended by Roche [18] and include the following:

» The possibility of pregnancy must be ruled out by a pregnancy test two weeks before commencing treatment.
» Treatment should be commenced on the second or third day of the next normal menstrual period.
» An effective form of contraception should be used for at least one month before treatment, during treatment, and for at least one month after cessation of treatment with isotretinoin and for two years after treatment with etretinate and acetretin.
» Women should be effectively counseled about the risks to a fetus, and if pregnancy does occur, they should immediately stop taking the drug and seek medical advice.
» To increase understanding of the implications of treatment, a specially designed consent form

should be signed by the patient before commencing treatment.

» Women should not breastfeed while taking oral retinoids. The drugs should not be given to others and should be kept out of reach of children.

Various methods have been attempted to improve compliance with these guidelines by patients and practitioners. Examples include check lists for practitioners, patient education information including pictures of birth defects, "avoid pregnancy" stickers on medication packets, periodic communications with prescribers and pharmacists, partially reimbursed contraception programs by manufacturers, and the use of two forms of contraception simultaneously [18].

Retinol

Animal studies

Rosa et al. [14] cite Geelen [23], who showed that defects similar to those arising from synthetic retinoids also occur because of excessive retinol consumption during pregnancy. In animal studies, naturally occurring vitamin A given at the same stages of embryogenesis produced similar deformities to those arising from synthetic retinoids. Based on animal studies, the dosage of retinol required to produce birth defects is much higher than those of etretinate and isotretinoin [13].

Defects observed in animals closely parallel abnormalities seen in humans from exposure to high doses of vitamin A. Abnormalities common to both animals and humans have been observed in the following organ systems: central nervous system (anencephaly, spina bifida, hydrocephalus); face (cleft lip and palate, micrognathia); ocular system (microphthalmia); abnormalities of the ear, teeth, salivary glands, and aortic arch; heart defects (ventricular septal defects, conotruncal abnormalities); gastrointestinal system (imperforate anus, omphalocele); liver and gallbladder abnormalities; genitourinary malformations (renal agenesis, polycystic kidney, hydronephrosis, genital malformations); endocrine abnormalities (pituitary, thymus, and thyroid abnormalities); skeletal abnormalities (affecting the skull, vertebrae, ribs, and extremities); and situs inversus [15].

When considering the results of animal studies to determine the teratogenic dose of retinol, it should be remembered that the teratogenic dose might differ across species, with primates possibly being less susceptible to vitamin A in forms other than isotretinoin or etretinate [14]. Geelen, who conducted an overview of the animal studies of hypervitaminosis A, commented that the intakes of retinol required to cause teratogenesis in animals are much higher than likely human exposures [23].

In addition to causing birth defects at high doses in animals, deficiency of vitamin A can result in incomplete pregnancies and birth defects [24]. A spectrum of birth defects has been observed similar to those found from excessive vitamin A consumption in a number of species, including the pig, rat, rabbit, cattle, and sheep [24]. These findings suggest that there may be an optimum level of maternal retinol consumption above and below which abnormalities may occur. A comparison of birth defects observed from excessive vitamin A consumption and vitamin A deficiency is shown in table 1.

Human studies

It is noteworthy that in spite of the widespread use of vitamin A supplements [15], relatively few cases of birth defects due to vitamin A teratogenicity have been documented in the medical literature [16]. Consequently, no minimum teratogenic dose for retinol has yet been established.

One possible explanation for the low rates of reporting of vitamin A teratogenicity is failure to recognize excessive retinol exposure. In spite of the low numbers of reports of vitamin A teratogenicity, one study has suggested that as many as 1 in 57 pregnancies exposed to usual vitamin A intakes greater than 10,000 IU per day may result in birth defects [26]. These results have not been confirmed by other studies, which failed to detect a significant association between vitamin A intake at this level and an increased risk of birth defects. A discussion of the evidence supporting vitamin A teratogenicity in humans arising from case studies and epidemiological studies is presented.

Case studies

A summary of reported cases of birth defects arising from high doses of vitamin A in pregnancy is provided in table 2. Vitamin A intakes reported by mothers of affected infants ranged from 25,000 to 500,000 IU and have been described for both a large single dose and long-term consumption throughout the pregnancy. Prior to 1986, the Food and Drug Administration (FDA) had received only two reports of birth defects associated with vitamin A [14]. These two cases were associated with daily vitamin A dosages of 40,000 IU and 60,000 IU during pregnancy, and both reported the congenital defect of microtia, a feature later found to be characteristic of synthetic retinoid embryopathy [14]. Although there are case reports of vitamin A supplementation possibly associated with birth defects, to date no birth defects have been reported from high intakes of vitamin A from food sources [14].

Epidemiological studies

Two case-control studies [16, 32] and one cohort study [26] have provided some evidence of teratogenicity

TABLE 1. Comparison of birth defects caused by vitamin A excess and deficiency

	Vitamin A excess		Vitamin A deficiency	
Defect	Human	Rat	Human	Rat
Absorption (resorption)		↑		↑
Growth Prenatal Postnatal			↓ ↓	↓ ↓
Craniofacial	Sloping forehead Narrow forehead Micrognathia Mandibular/maxillary hypoplasia	Oculofacial syndrome Mandibular hypoplasia Cleft lip/palate		Cleft palate
Eye	Microphthalmia	Anophthalmia/ microphthalmia	Anophthalmia Ocular deformity	Anophthalmia/ microphthalmia Postlenticular fibroplasia Coloboma
Ear	Tiny/stenotic ear canals Microtia/anotia	Malformations		
Central nervous system	Microcephaly	Microcephaly	Microcephaly	
Cranium		Exencephaly		
Brain		Neural tube defects Hydrocephalus	Mental retardation Neural tube defects	Hydrocephalus Neural tube failure in quail
Heart	Ventricular septal defects Auricular septal defects Conotruncal anomalies	Ventricular septal defects Single midline heart tube in chick embryo	Ventricular septal defects Auricular septal defects	Ventricular septal defects Aorticopulmonary septal defect (conotruncal) Branchial arch anomalies

Source: adapted from ref. 25.

from retinol supplementation in humans. The cohort study, conducted by Rothman et al. [26] suggested that daily retinol intakes as low as 10,000 IU may be teratogenic. Since this report, a number of investigators have attempted to further evaluate the safety of this vitamin A intake threshold and have failed to support the findings of Rothman et al. [33–37].

More recently, it has been accepted that folic acid supplementation is protective against birth defects. Many of the studies supporting this discovery have involved multivitamin supplements, including vitamin A at low doses. A small amount of evidence also suggests that low-dose vitamin A consumption may be protective against birth defects in women with inadequate vitamin A intake [25, 33, 38]. The association between vitamin A intake and risk of birth defects in humans is examined in more detail in the following section. The results of studies examining vitamin A intakes greater than 10,000 IU daily are compared with those of studies examining intakes below 10,000 IU.

Risk of birth defects from retinol consumption greater than 10,000 IU/day during pregnancy

All studies examining the relationship between vitamin A consumption and birth deformities have been observational studies. Five case-control studies and five cohort studies have been described in the scientific literature examining the relationship between retinol consumption and birth defects. Birth defects exam-

TABLE 2. Reported case studies of birth defects arising from excessive vitamin A consumption in humans

Study	Dose	Face and head	Cardiac	Genitourinary	Central nervous system	Musculo-skeletal and other
Pilotti and Scorta 1965 (cited in Rosa et al. 1986) [14]	40,000 IU			Bilateral hydroureter		
Morris and Thomson 1974 [27]	Not stated	Cleft palate	Heart defect			
Mounoud et al. 1975 [28]	Single 500,000-IU dose in 2nd month of gestation	Facial palsy, hemifacial atopy, atresia of ear canals, epibulbar dermoid, micrognathia, bilateral oculomotor palsy, left preauricular appendices				
Stange et al. 1978 [29]	150,000 IU days 19–40	Microcephaly		Hypoplastic kidney and adrenals	Microhydro-cephalus	
Bernhardt and Dorsey 1979 [30]	25,000 IU wk 0–13; 50,000 IU wk 14 to term			Unilateral ureteral duplication with one ureter ending in the vagina, hydroneph-rosis, bilateral hydroureter		
Von Lennep et al. 1985 [31]	150,000 IU days 1–24	Low ears, dys-morphic face, pterygium colli		Absence of external genitalia and urethral openings, polycystic kidneys		Partial sirenomelia, lumbar spine dysmorphism, imperforate anus/distend-ed abdomen
Physician report to FDA[a]	40,000 IU preconception to term	Tiny ear canals, facial dys-morphy, high arched palate				
Physician report to FDA[a]	60,000 IU preconception to term	Absent right ear, cleft palate/lip				
Physician report to FDA[a]	50,000 IU wk 3–9	Bilateral cleft lip				

continued

TABLE 2. Reported case studies of birth defects arising from excessive vitamin A consumption in humans *(continued)*

Study	Dose	Face and head	Cardiac	Genitourinary	Central nervous system	Musculo-skeletal and other
NY State BDR[a]	≤25,000 IU preconception to term	Absent right ear and canal				
NY State BDR[a]	≥25,000 IU preconception to term	Absent left auditory canal, Vater's syndrome				
NY State BDR[a]	≤25,000 IU to term	Hypoplastic left ear				
NY State BDR[a]	≥18,000 IU to term	Low deformed ears, micro-gnathia, micro-phthalmia				
NY State BDR[a]	≥33,000 IU to term		Transposition			
Danish National Health Service	50,000 IU	Cleft lip, palate, cheek, jaw, left eye absent				
Roche[b] (4 cases)	Not specified				Spina bifida Hydrocephalus	Club foot Turner's syndrome

a. Cited by Rosa et al. [14].
b. Based on research by Roche (cited by Rosa et al. [14]).
BDR, Birth defect registry; FDA, US Food and Drug Administration. Source: adapted from refs. 6, 14, 15.

ined mostly included cranial neural crest cell deformities, neural tube defects, and all major abnormalities. Selected controls have included mothers of babies without birth deformities and mothers of babies with other forms of abnormalities. Assessment of vitamin A status also varied across these studies. Only two cohort studies and one case-control study [35] assessed the contribution of dietary retinol to total retinol intake from supplements. All studies were capable of providing an estimate of the effects of daily vitamin A intakes from supplements of greater than 10,000 IU relative to intakes of retinol below this level. An overview of the results of these studies is presented below, and the main findings are summarized in table 3.

Case-control studies

Werler et al. [32] examined exposure to one week or more of vitamin A supplementation (estimated >10,000 IU) during the first three months of pregnancy among mothers of 2,658 children with abnormalities of structures derived at least in part from cranial neural crest cells, relative to mothers of 2,609 infants born with other malformations. Mothers of children with birth defects derived in part from neural crest cells were 3.3 (95% CI, 0.8–14.6) and 3.8 (95% CI, 0.9–16.0) times more likely to have consumed both vitamin A supplements and multivitamins in the first and second lunar months, respectively. By the third trimester, the odds ratio for birth defects among children of women consuming vitamin A–containing supplements had declined to 1.9 (95% CI, 0.5–7.4). Similarly, Martinez Frias and Salvador [16], who examined the risk of birth defects among 11,293 pregnant women relative to 11,193 normal pregnancies, observed a nonsignificant 2.7-fold increase in the odds ratio (95% CI, 0.8–11.7) for women consuming greater than 40,000 IU of retinol daily relative to women consuming less than 40,000 IU. Daily intakes of between 20,000 and 40,000 IU in this study were associated with a nonsignificant reduction in the odds ratio of birth defects to 0.5 (95% CI, 0.01–9.5).

Three case-control studies showed no association between retinol consumption and birth defects. Khoury et al. [33] used data from a population-based case-control study of 4,918 major birth defects conducted by the Centers for Disease Control in the 1980s and assessed vitamin supplement intake of three days or more per week in the first month preconception to the end of the first trimester.

TABLE 3. Studies examining associations between vitamin A exposure during pregnancy and the risk of birth defects

Study	Study design	Sample population	Region	Assessment of vitamin A status	Assessment of abnormalities	Results: PR/OR (95% CI) cases vs controls
Mastroiacovo et al. 1999 [37]	Prospective cohort	423 pregnancies (3 major abnormalities)	Europe (13 regions)	Patients ingesting ≥10,000 IU during wk 1–9 of pregnancy	European Network of the Teratology Information Services	Controls = exposure>wk9: 0.28 (0.06–1.23) Controls = unexposed: 0.50 (0.14–1.76)
Major et al. 1998 [36]	Case-control	11 CDH cases 11 healthy controls 7 matched newborn-mother pairs	Quebec, Canada	Cord blood samples from neonates (11) Maternal blood samples at term (7)	CDH cases and controls clinically diagnosed	Mean cord retinol (CDH vs controls) All 11 newborn: (lower $p < .0002$) 7 newborn-mother pairs: Newborn (lower $p = .003$) Mother (higher $p = .041$)
Mills et al. 1997 [35]	Case-control (population-based)	935 major abnormalities 573 normal controls	California and Illinois, USA 1985–87	Questionnaire recording periconceptional vitamin A intake (diet and supplements) (blind assessment at 1–5 mo antenatal)	Mandatory reporting registries, hospital records, "crippled children's" services, perinatal networks and support groups	Supplement only: <5,000 IU vs >8,000 IU All: 1.05(0.51–2.18); CNC: 1.06 (0.31–3.68) Supplement and cereals: <5,000 IU vs >10,000 IU All: 0.73 (0.27–1.96); CNC: 1.09 (0.24–4.98) Organ meat consumption All: 0.82(0.55–1.23)
Shaw et al. 1995, 1996 [34, 39]	Case-control	552,601 deliveries OFC: 731 cases, 734 normal controls CTH: 207 cases, 481 normal controls	California, USA 1986–89 (population-based)	Recall of supplement intake (average 3.5 yr after delivery)	California Birth Defects Monitoring Program	OFC: 0.55(0.21–1.15) CTH: 0 (0–2.2)
Khoury et al. 1996 [33]	Case-control	4,918 all defects 1,623 CNC 3,295 other 3,029 controls	Atlanta, Ga, USA (1980s)	Multivitamin/vitamin A supplement consumption 3 or more times/wk between 1 mo preconception and 3 mo antenatal	Centers for Disease Control population-based case-control study	MV alone All defects: 0.94 (0.86–1.03) CNC: 0.86 (0.76–0.97) VA alone All defects: 0.85 (0.41–1.78) CNC: 1.36 (0.57–3.19) Both MV and VA All defects: 0.60 (0.28–1.29) CNC: 0.69(0.24–1.91)

Rothman et al. 1995 [26]	Prospective cohort	22,748 pregnancies 339 birth defects (genetic defects excluded)	USA	Intake of dietary supplement assessed at wk 15–20 of pregnancy Mean dietary intake during 4 wk of highest consumption of retinol during trimester 1	Women attending 1 of 100 obstetricians for prenatal screening. Mailed questionnaire to obstetrician/mother	Fitted dose-response curve: apparent threshold for rise in CNC defects near 10,000 IU >10,000 IU vs <5,000 IU (food + supplement): CNC: 4.8 (2.2–10.5) All: 2.4 (1.3–4.4)
Czeizel 1993 [38]	Randomized, controlled trial	4,704 pregnancies Vitamin supplement vs trace element supplement each day for 1 mo preconception	Hungary	Folic acid 0.8 mg Retinol 6,000 IU 1989 4,000 IU 1990–91 + other vitamins and minerals	Deliveries and terminations in Hungarian obstetric outpatient units (validated)	Overall: lower in supplemented group ($\chi^2 = 6.68$ $p = .01$) Excluding NTDs: lower in supplement group ($\chi^2 = 4.69$ $p = 0.03$) RR 1.85 (1.02–3.38)
Mills et al. 1992 [40]	Case-control (population-based)	89 NTD cases 178 other pregnancies	Finland	Serum retinol levels determined at time of antenatal screen Questionnaire reporting supplement use in relation to period of neural tube closure (25–27 days of pregnancy)	Finnish Registry of Congenital Malformations (1983–89)	Mean retinol (NS) OR (adj) 0.99 (0.88–1.10) Differences in maternal vitamin use: Overall: ($\chi 2_{(2)}$ $p = .14$) Before neural tube closure: ($p = 0.62$)
Sandford et al. 1992 [41]	Case-control (population-based)	NTD 44 Normal infants 88 (matched)	South Louisiana, USA	Food-frequency questionnaire	Retrospective screening of obstetric records (17 hospitals) (validated)	β-Carotene fruit/vegetable intake (>1/wk): Overall: protective effect ($\chi^2 = 8.07$, $p = .004$) Spina bifida case-control pairs only: protective effect ($\chi^2 = 9.31$, $p = .002$) RR = 0.25
Martinez-Frias and Salvador 1990 [16]	Case-control (hospital based)	11,293 cases 11,193 controls (chromosomal defects excluded)	Spain	Open-ended question about drug use during pregnancy	Hospital surveillance data. Cases detected by local specialists days 1–3 antenatally	<20,000 IU: 0.5 (0.1–1.9) 20,000–39, 999 IU: 0.5 (0.01–9.5) 40,000 IU: 2.7 (0.8–11.7) Reduced risk if exposed later in pregnancy ($p < .05$)

continued

TABLE 3. Studies examining associations between vitamin A exposure during pregnancy and the risk of birth defects *(continued)*

Study	Study design	Sample population	Region	Assessment of vitamin A status	Assessment of abnormalities	Results: PR/OR (95% CI) cases vs controls
Werler et al. 1990 [32]	Case-control	2,658 CNC cases (84% response rate) (chromosomal/Mendelian disorders excluded) Controls: 2,609 other defects (82% response rate)	Boston, Philadelphia, and Iowa, USA; Toronto, Canada	Vitamin A supplement use (daily users for 1 wk vs nonusers in first trimester) Interviewed within 6 mo of birth	Contact with newborn nurseries from birth hospitals and routine review of discharge diagnoses from hospitals and specialty clinics	Any vitamin A supplementation: Lunar month 1: 2.5 (1.0–6.2) Lunar month 2: 2.3 (0.9–5.8) Lunar month 3: 1.6 (0.6–4.5) Vitamin A supplement + multivitamin: Lunar month 1: 3.3 (NS) Lunar month 2: 3.8 (NS) Lunar month 3: 1.9 (NS)
Smithells et al. 1976 [42]	Case-control	3 NTD cases 971 normal controls	Leeds, England	Serum retinol levels determined during 1st trimester	Not stated	Mean serum retinol: higher in cases ($p < .05$) Affected by high retinol intake in 1 case

CDH, Congenital diaphragmatic hernia; CI, confidence interval; CNC, cranial neural crest defect; CTH, conotruncal heart defect; MS/UG, musculoskeletal/urogenital defect; MV, multivitamin; NS, not significant; NTD, neural tube defect; OFC, orofacial cleft defect; OR, odds ratio; PR, prevalence ratio; RBP, retinol-binding protein; VA, vitamin A.

The odds ratio for cranial neural crest deformities was significantly reduced for subjects exposed to intakes of multivitamin supplements typically containing less than 10,000 IU retinol [33] (OR = 0.86; 95% CI, 0.76–0.97) relative to normal controls matched for period of birth, race, and hospital of birth. However, no significant protective associations were observed for subjects consuming supplements containing only vitamin A with higher retinol concentrations of up to 25,000 IU [43] (retinol and multivitamin supplements: OR = 0.69 and 95% CI, 0.24–1.91; retinol only: OR = 1.36 and 95% CI, 0.57–3.19). A similar population-based birth defect registry study by Shaw et al. [34, 39] showed a reduction in conotruncal heart defects (207 cases) and orofacial defects (731 cases) for women exposed to multivitamins containing folic acid and vitamin A during the periconceptional period relative to mothers with normal births over the same time period (orofacial defects: OR = 0.55 and 95% CI, 0.21–1.5; conotruncal defects: OR = 0 and 95% CI, 0–2.2). All birth defects identified were validated based on diagnostic imaging findings, surgery, or autopsy reports and were classified by a medical geneticist.

A third study by Mills et al. examined the intakes of vitamin supplements by mothers of 548 infants born with neural tube defects, 387 infants born with other major (not purely cosmetic) defects, and 573 normal controls [35]. Cases were identified through mandatory reporting registries, hospital records, services for crippled children, perinatal networks, and parent support groups. Mothers were interviewed about the nature of any birth defects that affected their children and later by an interviewer blinded to the existence of birth defects in offspring. Birth defects were validated by ultrasonographic and amniocentesis records, with 89 subjects meeting set criteria consistent with cranial neural crest defects [44–46]. Only 4 of the 1,508 mothers examined had intakes of above 25,000 IU, one of whom belonged to the group of 573 mothers with normal infants. No increased risk of either cranial neural crest defects (OR = 1.09; 95% CI, 0.24–4.98) or overall defects (OR = 0.73; 95% CI, 0.27–1.96) was observed when subjects with combined dietary and supplement intakes below 5,000 IU were compared with subjects with intakes over 10,000 IU. Organ meat consumption was also not associated with an increased risk of defects overall (OR = 0.82; 95% CI, 0.55–1.23).

Cohort studies

Conway [47] conducted a small, nonrandomized intervention in which mothers of infants with cleft lip, palate, or both were supplemented daily with 12,500 USP units of vitamin A plus other vitamins, including 0.5 mg of folic acid during a subsequent pregnancy. Among 48 mothers who received no vitamin sup-

plementation during pregnancy, there were five cases of congenital abnormalities, four of which included repeat cases of cleft lip, palate, or both. No cases of cleft lip or palate were observed in 39 women given supplementation during their subsequent pregnancy. Following this study, Smithells et al. [42] examined a cohort of 900 women to investigate the relationship between micronutrient consumption at 13 weeks of gestation and neural tube defects. A higher mean serum retinol concentration was observed in mothers of babies with neural tube defects (mean, 75.7 μg/dl; 95% CI, 58.5–92.9) than in mothers of unaffected babies (mean, 68.2 μg/dl; 95% CI, 43–108). However, this conclusion was based on a single high retinol level.

Subsequently, Zuber et al. [48] found no visible fetal malformations in 21 of 27 pregnant women for whom pregnancy outcomes were known and who had been exposed to high intakes of vitamin A during pregnancy. Vitamin A intakes varied from 100,000 IU per week to 250,000 IU in one week during pregnancy, with 18 subjects taking 25,000 IU per day.

Rothman et al. [26] conducted the first substantial cohort study on the teratogenic effects of vitamin A using a sample of 22,748 women undergoing screening for maternal serum alpha-fetoprotein or amniocentesis. Unlike earlier researchers, they evaluated retinol consumption taking into consideration dietary retinol consumption as well as that from supplements. Diet was assessed from telephone interviews by study nurses. Dietary retinol intake was calculated as the mean of the highest four weeks of intake during the first trimester. Birth defects were classified into cranial neural crest defects, neural tube defects, musculoskeletal or urogenital defects, and other defects.

A prevalence ratio of 4.8 (95% CI, 2.2–10.5) was observed for women consuming 10,000 IU or more of vitamin A relative to women with intakes of 5,000 IU or less. A total prevalence of cranial neural crest defects for women consuming greater than 10,000 IU of 3.2% was observed [43]. These researchers smoothed the dose-prevalence curve, which was fitted through individual data points by using quadratic splines [26, 43]. A rise in the prevalence of cranial neural crest defects was observed among women consuming more than 10,000 IU, although the curve for total retinol from diet and supplements rose more gradually. Rothman et al. [26] predicted that 1 in 57 babies born to mothers usually consuming more than 10,000 IU would have an abnormality attributable to this exposure.

Mastroiacovo et al. [37] prospectively evaluated the pregnancy outcomes of 423 women accessing the European Network of Teratology Information Service following exposures to vitamin A in excess of 10,000 IU daily or 50,000 IU weekly during pregnancy. Two groups of control subjects were followed: women with high vitamin A exposure later in pregnancy and women with reported exposures to nonteratogenic agents. Cases of chromosomal abnormalities and minor congenital deformities (e.g., preauricular tag) or problems were excluded from the analysis. A researcher blinded to the knowledge of the research hypothesis performed the identification of abnormalities in the women studied through telephone interviews with doctors or mothers.

The median dosage of retinol among cases was 50,000 IU per day (interquartile range, 25,000–60,000 IU per day). The prevalence rate in the cases relative to the control group exposed to retinol later than the first trimester was 0.28 (95% CI, 0.06–1.23). A prevalence rate of 0.5 (95% CI, 0.14–1.76) was also observed when women exposed to nonteratogenic agents were used as controls. These findings suggest no apparent relationship between total retinol dosage during the first trimester and birth defects. In this study of 120 infants exposed to more than 50,000 IU during embryogenesis, no abnormalities were observed. The results of this study do not support the teratogenicity of doses of retinol supplements of 10,000 IU or greater and contrast with the findings of Rothman et al. [26].

Discussion

Estimation of retinol consumption

Few studies have estimated overall retinol consumption in addition to supplement consumption. Only the prospective cohort study by Rothman et al. [26] and the case-control studies by the research teams headed by Mills [35, 40] and Smithells [42] provided estimates of total retinol consumption, either through assessment of dietary intake or serum retinol levels. It is likely, however, that intakes from regular vitamin A–containing supplements in many cases might far exceed intake from routine dietary sources for most participants. Therefore, although misclassification may have arisen for some participants, subjects consuming both vitamin A supplements and multivitamins containing vitamin A would mostly be correctly classified as having intakes exceeding 10,000 IU. Similarly, the study by Mastroiacovo et al. [37], in which exposure to vitamin A supplements was prospectively recorded, should provide fairly accurate records of high-dose vitamin A exposure.

All except one of the case-control studies examined suffered from potential recall bias, with retinol consumption being measured following births [35]. This bias is likely to be of particular importance for the study by Shaw et al. [34], in which the time delays between estimating nutrient consumption and the periconceptional period averaged 3.5 years. These

findings may have caused some misclassification of exposure status, resulting in the possible attenuation of significant results.

Relationship between timing of exposure and birth defects

The studies by Martinez Frias and Salvador [16] and Werler et al. [32], identified a higher risk of birth defects for exposure within the first two months of pregnancy. Martinez Frias and Salvador showed a decreasing risk of birth defects for women consuming doses higher than 40,000 IU from the first and second months of pregnancy to the fifth month and later. Similarly, Werler et al. showed a higher risk of overall deformities (OR = 2.5; 95% CI, 1.0–6.2) and cranial neural crest abnormalities (OR = 2.6; 95% CI, 1.0–6.9) for women exposed to high vitamin A intake in the first trimester.

The classification of vitamin A exposure into intake categories of 20,000 to 40,000 IU and more than 40,000 IU by Martinez Frias and Salvador showed a nonsignificant reduced risk of birth defects (OR = 0.5; 95% CI, 0.01–9.5) [16]. This is contrary to what might have been expected from the findings of Rothman et al. that 1 in 57 pregnant women with intakes beyond this level would be likely to have a child with a birth defect, although a wide confidence interval exists around this estimate [26]. This study has been criticized because of the possibility of recall bias in assessing vitamin A consumption, heterogeneity in the deformities observed, and the relatively low numbers of abnormalities identified in relation to the number of exposures in early pregnancy [37].

Association between vitamin A exposure and various forms of birth defects

Neural crest cell defects

The results of the study by Rothman et al. have been the source of much debate [37, 49]. It has been suggested that the classification used for cranial neural crest cells may have resulted in the inclusion of some birth defects from structures not derived from this cellular origin. An important criticism relates to the low number of cranial neural crest defects that were observed for subjects with dietary intakes exceeding 10,000 IU, of which at least four of the seven cases observed may have been misclassified [37, 50]. Khoury et al. [33] adopted a classification scheme for cranial neural crest defects similar to that used by Rothman et al. Possible misclassification in case definition may therefore have also arisen in this study, which might have been expected to attenuate the significant protective association that was observed for cranial neural crest defects (OR = 0.86; 95% CI, 0.76–0.97) arising from multivitamin consumption. Martinez Frias and Salvador observed higher relative risks of birth defects for subjects with intakes in excess of 40,000 IU, with reduced relative risks being observed for those with intakes below this level [16].

Variation has also existed between studies in the method of determining retinol consumption. The study by Rothman et al. [43] was unusual in that the four weeks of highest retinol intake were examined rather than the mean intake [6]. In addition, when the date of commencement of periconceptional multivitamins was unknown, the median date of commencement of multivitamins by the women examined was used [6]. However, if women with cranial neural crest defects, women with other abnormalities, and unaffected women were randomly misclassified with respect to retinol consumption, this would only be expected to increase the strength of the associations observed. These researchers have also been criticized for having an open-ended final category of retinol consumption (≥15,000 IU), although the analyses performed by these researchers were through individual data points [43]. Further analysis of the data into two groups according to daily intake of vitamin A–10,001 to 20,000 IU and 20,000 IU or greater—also showed a 4.6-fold higher prevalence ratio in the latter category [43]. These findings suggest that the inexactness of dietary assessment alone is unlikely to account for the findings observed in this study.

Shaw et al. [34] observed no evidence of an increased risk of cranial neural crest defects. Multivitamin intake was assessed by telephone interviews performed on average 3.5 years after delivery, with interviewers blinded until the end of the interview as to the birth outcome. Recall bias may have arisen if mothers of infants with birth defects recorded their exposure to vitamin A–containing supplements to a greater extent than mothers of normal infants. The existence of such bias would only be expected to reduce the relative risk estimates towards a protective effect still further. Similarly, the study by Mills et al. [35] failed to show a significantly increased risk of cranial neural crest defects from vitamin A and multivitamin consumption within six months of birth.

The findings of these studies relating to cranial neural crest defects contradict those of two studies showing increased risks of defects and other studies showing no increased risk of defects. Nevertheless, intakes of vitamin A above 10,000 IU daily should not be routinely recommended.

Neural tube defects

Smithells et al. [42] found that the mothers of babies with neural tube defects had higher mean serum retinol levels in the first trimester than mothers with normal births. An important weakness of this study was that a single high retinol level influenced these results. Mills et al. [35] did not confirm these findings

in a much larger study of 89 mothers of babies with neural tube defects. They observed no suggestion of a relationship between increased serum retinol and risk of neural tube defects, irrespective of the timing of the exposure in relation to neural tube closure. Sandford et al. [41] showed a significant protective effect of regular β-carotene and vegetable intake on neural tube defects; however, this is likely to have been influenced by folic acid consumption.

Congenital diaphragmatic hernia

A small case-control study by Major et al. [36] compared the retinol status of 11 newborns with congenital diaphragmatic hernia and 11 neonates without abnormalities. Controls were matched for gestational age, and no significant differences were observed in either birthweight or gestational age. Cord blood retinol levels and retinol-binding protein levels were significantly lower in cases than controls ($p < .0002$ and $p = .004$, respectively). In contrast, for seven case-control pairs for which maternal blood was collected, maternal serum retinol and retinol-binding protein levels were higher in cases than controls ($p = .41$ and $p = .004$). These authors suggested that rather than the low levels of retinol arising from low maternal levels, they may reflect a deficiency in the synthesis of retinol-binding protein in the fetal liver in the third trimester or a deficiency of a placental receptor.

Birth defects overall

Only the studies by Rothman et al. [26] and by Martinez-Frias and Salvador [16] suggested possible adverse effects from high levels of vitamin A consumption when birth defects were considered overall. Other studies did not confirm these findings and observed relative risks for birth defects near or below unity in all cases [33, 35, 37]. Rothman et al. [26] showed a significant increase in the risk of birth defects overall for women exposed to vitamin A above 10,000 IU relative to women with intakes below 5,000 IU; however, this increase was less marked than that for cranial neural crest defects. Therefore, concerns are still raised by this study about the safety of vitamin A exposure during pregnancy, even allowing for criticisms relating to possible misclassification of cranial neural crest cell-derived birth defects.

Rothman et al. [26] estimated that 1 in 57 babies would be born with a birth defect to women with intakes above 10,000 IU, a proportion that might be expected to increase with increasing dose based on their findings. The absence of birth defects among children of 120 women consuming greater than 50,000 IU [37] is a noteworthy observation, although the lack of cases may have arisen because of chance. The patient population examined by Rothman et al. [26] was unusual because of the relatively low rate of birth defects overall (1.5% total defects) [37] and because it consisted of women visiting a specialist for antenatal screening tests. The latter factor could introduce a specialist referral bias affecting reporting of outcome, exposure, or both [6]. Further birth defect registry studies may be of value in addressing this question.

Risk of birth defects from inadequate retinol consumption during pregnancy

Another concern is that inadequate dietary retinol intake may also result in birth defects. To date most studies on the effects of vitamin A intake on pregnancy have not been conducted in regions where vitamin A deficiency is endemic, so that limited evidence exists to address this question. Smithells et al. [42] commented that an inverse association between socioeconomic status and birth defects has been observed in a number of population-based studies. These researchers also showed that subjects with lower socioeconomic status have lower serum retinol levels. More recently, the finding that periconceptional multivitamin use in developed countries has resulted in reduced rates of fetal abnormalities also raises questions as to whether lower doses of vitamin A may have a protective effect during pregnancy, since many of these multivitamins contained doses of vitamin A below 10,000 IU [38].

Case-control studies

Evidence of a possible protective effect of low-dose vitamin A has been suggested by Khoury et al. [33], who observed a significant protective effect against cranial neural crest defects following consumption of multivitamins containing vitamin A during early pregnancy. It is unclear, however, whether this effect may have been attributable to other micronutrients within these multivitamin preparations, such as folic acid. The details of this study have been discussed previously.

More recently, a Hungarian population-based case-control study showed that the risk of birth defects may be reduced in women consuming vitamin A supplements during pregnancy [51]. This study examined 20,830 mothers of babies with isolated and unidentifiable multiple congenital malformations between 1980 and 1994, excluding congenital dislocation of the hip, congenital inguinal hernia, hemangiomas, and abnormalities of known origin. A total of 35,727 controls drawn from the national birth registry were matched by sex, birth week, and district of parents' residence. The supplement used was determined by questionnaires sent to all participants and through prenatal care logbooks completed by general practitioners.

Overall, a significantly greater number of controls (9.5%) than cases (7.9%, $p < .0001$) were exposed to vitamin A–containing supplements during pregnancy, this being medically validated in 57.4% of cases and 56.0% of controls. No statistically significant differ-

ences were observed in the proportions of cases and controls using vitamin A–containing supplements for each month of gestation. The odds ratio for a congenital malformation arising from exposure to vitamin A in the first trimester was significantly lower for polydactyly or syndactyly (OR = 0.52; 95% CI, 0.3–0.9), clubfoot (OR = 0.55; 95% CI, 0.4–0.9), and total congenital malformations (OR = 0.79; 95% CI, 0.7–0.8). For exposure at any time throughout the pregnancy, reductions in risk were observed for ear abnormalities (OR = 0.52; 95% CI, 0.3–0.9), cleft palate (OR = 0.53; 95% CI, 0.3–0.9), cardiovascular abnormalities (OR = 0.73; 95% CI, 0.6–0.9), undescended testis (OR = 0.72; 95% CI, 0.6–0.9), club foot (OR = 0.72; 95% CI, 0.6–0.9), and total malformations (OR = 0.79; 95% CI, 0.7–0.9).

Randomized, controlled trials

Results from the Nepal Nutrition Intervention Project (NNIP-2) have recently been evaluated. Unpublished data* from this randomized, controlled trial show a nonsignificant reduced risk of all birth defects (RR = 0.76, $p = .38$) for the group in this population treated with vitamin A or β-carotene relative to the placebo group. Reductions in relative risk were observed for cleft lip and palate (RR = 0.46, $p = .20$) and eye defects (RR = 0.21, $p = .05$). A nonsignificant higher risk of other craniofacial defects (RR = 1.86, $p = .37$) and ear or preauricular tags (RR = 1.19, $p = .72$) was observed.

Discussion

The study by Czeizel and Rockenbauer [51] is one of the largest case-control studies on this research question and therefore provides considerable power to examine the association between supplement consumption and birth defects. The weaknesses of this study were the failure to analyze the data according to dosage of vitamin A, the potential for recall bias, the possibility of a confounding effect due to folic acid consumption, and the low response rate among controls. Although dosage was not examined in the analyses, nearly all daily intakes of retinol for exposed subjects in the first trimester were in the range between 5,000 and 10,000 IU. No differences were seen in the proportion of cases and controls consuming vitamin supplements containing folic acid. The findings described could be explained if mothers who offered to act as controls had been more likely to consume supplements during pregnancy than potential controls who did not respond. No attempts were made to compare responders and nonresponders in terms of vitamin A consumption. Recall bias might also explain these findings, although the opposite finding of overreporting of vitamin A intake in cases relative to controls might be considered a more likely outcome to arise from this form of bias.

Despite the differences between the Hungarian population [51] and the Nepalese population [38] in terms of adequacy of maternal vitamin A intake, some similarities may exist in terms of the impact of low-dose vitamin A supplements on birth defects. Both studies showed lower overall rates of abnormalities and a reduced risk of cleft palate deformities. Czeizel and Rockenbauer [51] also reported a reduced risk of eye abnormalities, although this was not statistically significant (OR = 0.50; 95% CI, 0.1–2.0).

Fetal behavioral deficits from excessive vitamin A during pregnancy

Animal studies have shown behavioral deficits in the offspring of animals exposed to high dosages of vitamin A in early pregnancy, including delays in learning time [52]. These deficits have been shown to occur at doses in animals below levels required to produce gross teratogenic effects [15]. Hutchings et al. [53] found that the behavioral deficits did not correlate with brain size. It is possible that damage in hippocampal and cerebellar regions of the brain may be responsible for the behavioral defects [52].

β-Carotene

Absence of toxic effects

Excessive intakes of β-carotene are known to be nontoxic [1]. Dosages equivalent to 30 mg of β-carotene daily or greater result in hypercarotenemia and carotenodermia, which produces a characteristic yellowing of the palms and soles [1, 54]. These clinical features are reversible upon cessation of high β-carotene intake [54].

Evidence supporting safety in pregnancy

Animal studies. To date there have been no studies implicating β-carotene as a teratogen. Animal studies have shown that doses up to 1,000 mg/kg are not carcinogenic [17]. Polifka et al. [55] described one study in which 1 to 3 ml of red palm oil, a rich source of β-carotene and other carotenoids, was administered to pregnant rats that subsequently showed an increased frequency of fetal death, growth retardation, and malformations (anencephaly, ocular abnormalities). They stated that other studies have failed to confirm these findings in rats and rabbits. It was therefore concluded that some component other than β-carotene in the

* West KP. Effects of vitamin A supplementation of women during pregnancy on teratogenicity: a review of current literature and response to the National Technical Review (Bangladesh) 1999 (unpublished).

palm oil might have been responsible for the birth defects described in this animal study.

Human studies. Hathcock et al. [15] reported that infants born with carotenemia to mothers with high β-carotene intakes have been found to be normal. It is believed that the failure of large doses of β-carotene to increase serum levels of retinol may be responsible for the absence of a toxic effect [17]. Bendich and Langseth [52] described a study in which despite high dosages of supplemental β-carotene, sufficient to triple serum carotene levels, no increase in serum retinol was observed. β-Carotene has also been used in humans to treat protoporphyria at doses up to 180 mg per day without any increase in serum retinol levels [15].

Conclusions and recommendations

A number of policy statements have provided recommendations regarding safe levels of consumption and administration of vitamin A during pregnancy in relation to fetal development [3, 13, 56–59]. Recently, the World Health Organization (WHO) conducted a consultation to review this question [3]. In addition, the American College of Obstetricians and Gynecologists (ACOG) released a recent policy statement on vitamin A supplementation during pregnancy [58]. The recommendations from these policy statements are evaluated below in light of the evidence presented.

To whom is it safe to give vitamin A during pregnancy?

Existing recommendation (ACOG [58]):

> Dietary intake of vitamin A in the United States appears adequate to meet the needs of most pregnant women throughout gestation. Therefore, routine supplementation during pregnancy is not recommended.

Vitamin A and its derivatives are known to play an important role during morphogenesis [24]. Evidence in animals suggests that during pregnancy there is a reduction in serum retinol levels during the embryogenic period, which is met by an increase in serum retinol from maternal hepatic retinol stores. This suggests that vitamin A supplementation of pregnant women in areas where vitamin A deficiency is endemic and in whom hepatic stores may be inadequate could be beneficial.

Although many women in the fertile age range have suboptimal vitamin A intake, routine supplementation is not recommended in areas where vitamin A deficiency is not endemic [1, 60]. Given that the RDA is designed to provide a dosage of vitamin A that would conservatively ensure adequate hepatic vitamin A levels for three months across a population, allowing for groups with lower dietary intakes, it is likely that in industrialized countries there is little need for supplementation. This assumes, however, that groups with marginal deficiency do not fall substantially below the RDA. For women meeting the RDA, it is unlikely that any benefit would be obtained from additional vitamin A supplementation during pregnancy.

Further research would provide a clearer picture of the need for supplementation in high-risk population groups in developed countries and in subjects with potentially lower retinol consumption, including strict vegetarians [58], migrants from areas where vitamin A deficiency is endemic [61], and women not consuming liver [60]. The data from Czeizel [38], which suggested that low doses of vitamin A supplementation during pregnancy might be protective against birth defects in developed countries, warrant further examination and confirmation.

What is a safe dosage of vitamin A during pregnancy?

Existing WHO recommendations [3]:

> …independent of vitamin A status, 10,000 IU (3,000 μg retinol equivalents) per day is the maximum daily supplement to be recommended at any time during pregnancy.
>
> **Where vitamin A deficiency is endemic** among children under school age and maternal diets are low in vitamin A, health benefits are expected for the mother and her developing fetus with little risk of detriment to either from either a **daily supplement** not exceeding 10,000 IU of vitamin A (3,000 μg retinol equivalents), or a **weekly supplement** not exceeding 25,000 IU of vitamin A (8,500 μg) at any time during pregnancy.
>
> In this regard a single dose >25,000 IU is not advisable, particularly between day 15 and day 60 following conception (day 0); beyond 60 days after conception, the advisability of providing a single dose of >25,000 IU is uncertain; any risk of non-teratogenic developmental toxicity is likely to diminish as pregnancy advances. In the case of pregnant women who may be reached only once during pregnancy, health workers should balance possible benefits from improved vitamin A status against potential risk of adverse consequences from receiving a supplement.
>
> **Where habitual vitamin A levels exceed at least three times the RDA** (about 8,000 IU or 2,400 μg retinol equivalents) there is no demonstrated benefit from taking a supplement. On the contrary, the potential risk of adverse events increases with higher intakes—above about 10,000 IU—if supplements are routinely ingested.

None of the human studies examined has shown

a significantly greater risk of birth defects from consumption of vitamin A supplements of less than 10,000 IU per day. However, as discussed previously, there is likely to be no additional benefit to pregnant women who consume the RDA of vitamin A from taking additional dietary supplements of vitamin A. Based on one experimental study, dosages of vitamin A below 30,000 IU did not appear to be associated with levels of teratogenic metabolites higher than typically experienced in pregnant women. This again supports the safety of the maximum recommended dosage of 10,000 IU daily during pregnancy. Given the known potential teratogenic nature of vitamin A, its supplementation for women of childbearing age in well-nourished populations is not recommended at this point. At a population level, further studies would be necessary to determine whether benefits may exist from low-dose supplements in industrialized countries. Dosages of vitamin A below 10,000 IU should be adequate to meet the supplementary needs of women with inadequate intakes in industrialized countries and probably in developing countries.

At what period during pregnancy is it safe to provide vitamin A?

The recommendations discussed have provided safe ranges of vitamin A intake at all stages of pregnancy. Animal studies and human epidemiological studies suggested that the first trimester, during which organ development occurs, is the most critical period, particularly the first two months [14, 32]. Animal studies showing behavioral deficits from vitamin A exposure in later pregnancy suggest that it is not advisable to exceed the recommended intakes discussed at any stage throughout the pregnancy, although this risk might be expected to decrease as pregnancy progresses [3].

What form of vitamin A is safe? Dietary sources or supplements?

Buss et al. [4] have shown that dietary retinol produces less marked increases in serum levels of teratogenic metabolites than retinol consumed in the form of dietary supplements. Following consumption of 50 mg of dietary retinol, both the peak levels of retinol and the area under the plasma–concentration time curve showed negligible differences from endogenous levels [6]. In addition, only minimal increases above physiological levels of retinoic acid and 13-*cis*-retinoic acid have been observed following doses of vitamin A supplements as high as 30,000 IU. These findings suggest that both dietary and supplemental forms of retinol are safe at the levels stated in the existing WHO recommendations.

Because liver varies in vitamin A content, and frequent liver consumption could result in intakes above the recommended limit of 10,000 IU, van den Berg et al. [60] suggested restricting liver consumption to no more than one serving per day. They suggested that intakes resulting from consumption at this level would be unlikely to cause problems, although consumption regularly throughout pregnancy might be best avoided.

Given the findings that many women who do not consume liver may have vitamin A intakes below the RDA, these researchers cautioned against campaigns aiming at the avoidance of vitamin A–rich products. Some evidence exists that low-dose supplementation may be protective during pregnancy, particularly in populations with vitamin A deficiency; however, further confirmation of these findings is required.

The preferred form of vitamin A supplementation during pregnancy is as β-carotene. β-Carotene does not produce teratogenic effects during pregnancy in humans or animals. The fact that β-carotene plays an important role as a source of placental vitamin A provides further evidence that, when supplementation is necessary, vitamin A supplementation of pregnant women should be in this form.

Consideration should be given to the development of dietary interventions using food sources rich in β-carotene to provide additional vitamin A when needed rather than vitamin A supplements. This approach may allow pregnant women to achieve other nutritional requirements as well as their vitamin A requirements. In support of this approach is a report from rural Gambia in which vitamin A was increased by the use of sun-dried mangoes [3]. Strategies of this type warrant further evaluation.

Dangers of synthetic retinoids

Extreme caution should be taken to prevent pregnancy from occurring in women of childbearing age requiring synthetic retinoids for medical indications. These agents are known to be potent teratogens and produce a well-described phenotypic pattern of abnormalities. Detailed guidelines that have been proposed to prevent pregnancy have been discussed previously in this document. These pharmaceutical agents should only be prescribed by appropriately qualified medical personnel and with clear counseling about measures to prevent pregnancy, the teratogenic risks of these agents, and the need for a safe contraception period following their cessation.

References

1. Olson JA. Vitamin A. In: Ziegler EE, Filer LJ, eds. Present knowledge in nutrition. Washington, DC: International Life Sciences Institute (ILSI) Press 1996:109–19.
2. Muenter MD. Editorial: Hypervitaminosis A. Ann Intern Med 1974;80:105–6.
3. World Health Organization. Safe vitamin A dosage during pregnancy and lactation. Ottawa: Micronutrient Initiative 1998;98(4):1–34.
4. Buss NE, Tembe EA, Prendergast BD, Renwick AG, George CF. The teratogenic metabolites of vitamin A in women following supplements and liver. Hum Exp Toxicol 1994;13:33–43.
5. Wiegand UW, Hartmann S, Hummler H. Safety of vitamin A: recent results. Int J Vitam Nutr Res 1998;68:411–6.
6. Miller RK, Hendrickx AG, Mills JL, Hummler H, Wiegand UW. Periconceptional vitamin A use: how much is teratogenic? Reprod Toxicol 1998;12:75–88.
7. Miller RK, Faber W, Asai M, D'Gregorio RP, Ng WW, Shah Y, Neth-Jesse L. The role of the human placenta in embryonic nutrition. Impact of environmental and social factors. Ann NY Acad Sci 1993;678:92–107.
8. Gebre-Medhin M, Vahlquist A. Vitamin A nutrition in the human foetus. Acta Paediatr Scand 1984;73: 333–40.
9. Artacho CA, Piandosi R, Blaner WS. Placental transfer of vitamin A. Sight and Life Newsletter 1999;3:23–8.
10. Wallingford JC, Underwood BA. Vitamin A deficiency in pregnancy, lactation, and the nursing child. In: Bauernfeind JC, ed. Vitamin A deficiency and its control. New York: Academic Press, 1986:101–52.
11. Satre MA, Ugen KE, Kochhar DM. Developmental changes in endogenous retinoids during pregnancy and embryogenesis in the mouse. Biol Reprod 1992;46:802–10.
12. Dimenstein R, Trugo NM, Donangelo CM, Trugo LC, Anastacio AS. Effect of subadequate maternal vitamin A status on placental transfer of retinol and beta-carotene to the human fetus. Biol Neonate 1996;69:230–4.
13. Teratology Society. Teratology society position paper: recommendations for vitamin A use during pregnancy. Teratology 1987;35:269–75.
14. Rosa FW, Wilk AL, Kelsey FO. Teratogen update: vitamin A congeners. Teratology 1986;33:355–64.
15. Hathcock JN, Hattan DG, Jenkins MY, McDonald JT, Sundaresan PR, Wilkening VL. Evaluation of vitamin A toxicity. Am J Clin Nutr 1990;52:183–202.
16. Martinez-Frias ML, Salvador J. Epidemiological aspects of prenatal exposure to high doses of vitamin A in Spain. Eur J Epidemiol 1990;6:118–23.
17. Pinnock CB, Alderman CP. The potential for teratogenicity of vitamin A and its congeners [see comments]. Med J Aust 1992;157:804–9.
18. Chan A, Hanna M, Abbott M, Keane RJ. Oral retinoids and pregnancy. Med J Aust 1996;165:164–7.
19. Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW, Lott IT. Retinoic acid embryopathy. N Engl J Med 1985;313: 837–41.
20. Brent RL, Hendrickx AG, Holmes LB, Miller RK. Teratogenicity of high vitamin A intake. N Engl J Med 1996;334:1196.
21. Thomson EJ, Cordero JF. The new teratogens: accutane and other vitamin–A analogs. MCN Am J Matern Child Nurs 1989;14:244–8.
22. Monga M. Vitamin A and its congeners. Semin Perinatol 1997;21:135–42.
23. Geelen JA. Hypervitaminosis A induced teratogenesis. CRC Crit Rev Toxicol 1979;6:351–75.
24. Gerster H. Vitamin A–functions, dietary requirements and safety in humans. Int J Vitam Nutr Res 1997;67:71–90.
25. Zachman RD, Grummer MA. The interaction of ethanol and vitamin A as a potential mechanism for the pathogenesis of fetal alcohol syndrome. Alcohol Clin Exp Res 1998;22:1544–56.
26. Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. Teratogenicity of high vitamin A intake. N Engl J Med 1995;333:1369–73.
27. Morriss GM, Thomson AD. Vitamin A and rat embryos. Lancet 1974;2:899–900.
28. Mounoud RL, Klein D, Weber F. Report of a case of Goldenhar's syndrome: acute intoxication by vitamin A in mother during pregnancy. J Genet Hum 1975;23:135–54.
29. Stange L, Carlstrom K, Eriksson M. Hypervitaminosis A in early human pregnancy and malformations of the central nervous system. Acta Obstet Gynecol Scand 1978;57:289–91.
30. Bernhardt IB, Dorsey DJ. Hypervitaminosis A and congenital renal anomalies in a human infant. Obstet Gynecol 1979;43:750–5.
31. Von-Lennep E, El-Khazen N, De-Pierreux G, Amy JJ, Rodesch F, Van-Regemorter N. A case of partial sirenomelia and possible vitamin A teratogenesis. Prenat Diagn 1985;5:35–40.
32. Werler MM, Lammer EJ, Rosenberg L, Mitchell AA. Maternal vitamin A supplementation in relation to selected birth defects. Teratology 1990;42:497–503.
33. Khoury MJ, Moore CA, Mulinare J. Vitamin A and birth defects [letter]. Lancet 1996;347:322.
34. Shaw GM, Wasserman CR, Block G, Lammer EJ. High maternal vitamin A intake and risk of anomalies of structures with a cranial neural crest cell contribution [letter; comment]. Lancet 1996;347:899–900.
35. Mills JL, Simpson JL, Cunningham GC, Conley MR, Rhoads GG. Vitamin A and birth defects. Am J Obstet Gynecol 1997;177:31–6.
36. Major D, Cadenas M, Fournier L, LeClerc S, Lefebvre M, Cloutier R. Retinol status of newborn infants with congenital diaphragmatic hernia. Pediatr Surg Int 1998;13:547–9.
37. Mastroiacovo P, Mazzone T, Addis A, Elephant E, Carlier P, Vial T, Garbis H, Robert E, Bonati M, Ornoy A, Finardi A, Schaffer C, Caramelli L, Rodgriguez-Pinella E, Clementi M. High vitamin A intake in early pregnancy and major malformations: a multicentre prospective controlled study. Teratology 1999;59:7–11.

38. Czeizel AE. Prevention of congenital abnormalities by periconceptional multivitamin supplementation. BMJ 1993;306:1645–8.
39. Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. Am J Med Genet 1995;59:536–45.
40. Mills JL, Tuomilehto J, Yu KF, Colman N, Blaner WS, Koskala P, Rundle WE, Forman M, Toivanen L, Rhoads GG. Maternal vitamin levels during pregnancies producing infants with neural tube defects. J Pediatr 1992;120:863–71.
41. Sandford MK, Kissling GE, Joubert PE. Neural tube defect etiology: new evidence concerning maternal hyperthermia, health and diet. Dev Med Child Neurol 1992;34:661–75.
42. Smithells RW, Sheppard S, Schorah CJ. Vitamin deficiencies and neural tube defects. Arch Dis Child 1976; 51:944–50.
43. Rothman KJ, Moore LL, Singer MR. Vitamin A and birth defects. Lancet 1996;347:321–2.
44. Johnston MC, Sulik KK. Some abnormal patterns of development in the craniofacial region. Birth Defects Orig Arctic Ser 1979;15:23–42.
45. Kirby ML. Cardiac morphogenesis—recent research advances. Pediatr Res 1987;21:219–24.
46. Bockman DE, Kirby ML. Dependence of thymus development on derivatives of the neural crest. Science 1984; 223:500–2.
47. Conway H. Effect of supplemental vitamin therapy on the limitation of incidence of cleft lip and cleft palate in humans. Plast Reconstr Surg 1958;22:450–3.
48. Zuber C, Librizzi RJ, Vogt BL. Outcomes of pregnancies exposed to high dose vitamin A. Teratology 1987;35:42A.
49. Oakley GP, Erickson JD. Vitamin A and birth defects. Continuing caution is needed. N Engl J Med 1995; 333:1414–5.
50. Watkins M, Moore C, Mulinare J. Teratogenicity of high vitamin A intake. N Engl J Med 1996;334:1196.
51. Czeizel AE, Rockenbauer M. Prevention of congenital abnormalities by vitamin A. Int J Vitam Nutr Res 1998; 68:219–31.
52. Bendich A, Langseth L. Safety of vitamin A. Am J Clin Nutr 1989;49:358–71.
53. Hutchings DE, Gibbon J, Kaufman MA. Maternal vitamin A excess during the early fetal period: effects on learning and development in the offspring. Dev Psychobiol 1972;6:445–57.
54. Bendich A. Safety issues regarding the use of vitamin supplements. Ann NY Acad Sci 1992;669:300–13.
55. Polifka JE, Dolan CR, Donlan MA, Friedman JM. Clinical teratology counselling and consultation report: high dose beta-carotene use during early pregnancy. Teratology 1996;54:103–7.
56. American College of Obstetricians and Gynecologists (ACOG) Committee. Vitamin A supplementation during pregnancy. Int J Gynaecol Obstet 1993;40:175.
57. American College of Obstetricians and Gynecologists (ACOG) Committee. Vitamin A supplementation during pregnancy. Int J Gynaecol Obstet 1995;51:286–7.
58. American College of Obstetricians and Gynecologists (ACOG) Committee. Vitamin A supplementation during pregnancy. Int J Gynaecol Obstet 1998;61:205–6.
59. International Vitamin A Consultative Group (IVACG). Safe doses of vitamin A during pregnancy and lactation. Sight and Life Newsletter 1999;1:34–6.
60. van den Berg H, Hulshof KF, Deslypere JP. Evaluation of the effect of the use of vitamin supplements on vitamin A intake among (potentially) pregnant women in relation to the consumption of liver and liver products. Eur J Obstet Gynecol Reprod Biol 1996;66:17–21.
61. Institute of Medicine. Nutrition in pregnancy. Washington, DC: National Academy Press, 1990.