ABSTRACT  Isozymes of alcohol and other dehydrogenases convert ethanol and retinol to their corresponding aldehydes in vitro. In addition, new pathways of retinol metabolism have been described in hepatic microsomes that involve, in part, cytochrome P450s, which can also metabolize various drugs. In view of these overlapping metabolic pathways, it is not surprising that multiple interactions between retinol, ethanol, and other drugs occur. Accordingly, prolonged use of alcohol, drugs, or both, results not only in decreased dietary intake of retinoids and carotenoids, but also accelerates the breakdown of retinol through cross-induction of degradative enzymes. There is also competition between ethanol and retinoic acid precursors. Depletion ensues, with associated hepatic and extrahepatic pathology, including carcinogenesis and contribution to fetal defects. Correction of deficiency through vitamin A supplementation has been advocated. It is, however, complicated by the intrinsic hepatotoxicity of retinol, which is potentiated by concomitant alcohol consumption. By contrast, β-carotene, a precursor of vitamin A, was considered innocuous until recently, when it was found to also interact with ethanol, which interferes with its conversion to retinol. Furthermore, the combination of β-carotene with ethanol results in hepatotoxicity. Moreover, in smokers who also consume alcohol, β-carotene supplementation promotes pulmonary cancer and, possibly, cardiovascular complications. Experimentally, β-carotene toxicity was exacerbated when administered as part of beadlets. Thus ethanol, while promoting a deficiency of vitamin A also enhances its toxicity as well as that of β-carotene. This narrowing of the therapeutic window for retinol and β-carotene must be taken into account when formulating treatments aimed at correcting vitamin A deficiency, especially in drinking populations.

KEY WORDS  Ethanol; alcohol; retinol; retinoids; β-carotene; carotenoids; pulmonary cancer; vitamin A; beadlets; liver; cardiovascular complications; carcinogenicity; hepatotoxicity; fetal alcohol syndrome; Carotene and Retinol Efficacy Trial; CARET; Alpha-Tocopherol, Beta-Carotene and Cancer Prevention Study; ATBC; review

INTRODUCTION  Retinol is the main compound with vitamin A function; therefore, these 2 terms will be used interchangeably in this review. Like ethanol (ethyl alcohol), retinol is an alcohol and, in vitro, both can be converted to corresponding aldehydes in reactions catalyzed by several isozymes of cytosolic alcohol dehydrogenase (ADH; EC 1.1.1.1). It is not surprising, therefore, that in vitro, and possibly in vivo, these 2 alcohols can interact significantly by competing with each other for the same or similar enzymatic pathways and by interfering with each other’s reactions. In addition, liver stellate cells are the main storage site of retinol in the body and ethanol, mainly through its acetaldehyde product, interacts with these cells in terms of their proliferation (1) and their capacity to produce fibrous tissue (2, 3). Because of these overlapping biochemical and cellular features, it is not surprising that when ethanol is consumed, it affects the physiologic and pathologic functions of vitamin A—its insufficiency as well as its excess. Such interactions also involve the main retinol precursor, β-carotene.

Interactions between retinoids, carotenoids, and ethanol first attracted attention when it was revealed that alcohol consumption results in a striking depletion of hepatic vitamin A, both in experimental animals (4) and in humans (5). These observations prompted many studies that culminated with the discovery of new pathways of retinol metabolism (6, 7) and the description of a toxic interaction between ethanol and β-carotene in nonhuman primates (8). Such interactions may also play a role in the enhanced carcinogenicity observed in smokers given supplements of β-carotene in the Alpha-Tocopherol, Beta-Carotene and Cancer Prevention Study (ATBC; 9) and in the Carotene and Retinol Efficacy Trial (CARET) (10). As a result of the findings of these studies, recommendations for retinol as well as β-carotene supplementation should now take into account individual drinking habits.

INTERACTIONS OF ETHANOL WITH VITAMIN A

Role of nutritional factors and liver injury  It has been a long-standing observation that alcoholics with cirrhosis may suffer from night blindness (11) related to vitamin A...
deficiency (12). Plasma vitamin A (13) as well as retinol binding protein (RBP) concentrations (14) are decreased in patients with alcoholic cirrhosis. These complications have usually been attributed to malnutrition because poor dietary intake is common in alcoholics with (14, 15) or without (16) cirrhosis. It is also possible that these complications may result from hepatic injury because decreased plasma vitamin A and RBP concentrations have been reported in patients with liver disease without apparent alcohol intake (16). These low plasma concentrations may be due to defective synthesis of RBP in the liver (16). Alcoholic cirrhosis is often associated with zinc deficiency (13, 14), which can decrease plasma RBP through impaired mobilization of RBP from the liver (17). In addition to these classic aspects of vitamin A deficiency due either to poor dietary intake or to severe liver disease, direct effects of alcohol on vitamin A metabolism and resulting alterations in hepatic vitamin A concentrations have been elucidated.

Direct effects of ethanol on hepatic vitamin A

Depletion of hepatic vitamin A by ethanol and its mechanism

Patients with alcoholic liver disease have been found to have very low concentrations of hepatic vitamin A at all stages of their disease (Figure 1). The vitamin A concentration in fatty livers was significantly lower (20% less on average) than in normal livers and significantly lower than in the livers of patients with chronic persistent hepatitis, despite the fact that the degree of liver injury associated with fatty liver was moderate and not more severe than in patients with chronic persistent hepatitis, as judged by liver morphology and liver test results (5). Furthermore, in patients with chronic persistent hepatitis and alcoholic liver disease, blood concentrations of RBP and transthyretin were normal and there was no evidence of deficient vitamin A intake. In patients with alcoholic hepatitis, liver concentrations of vitamin A were even lower (≥10 times below normal). The lowest hepatic vitamin A concentrations were observed in patients with cirrhosis (30 times below normal). Thus, alcoholic liver disease is associated with severely reduced hepatic vitamin A concentrations, even when liver injury is moderate (fatty liver) and when blood concentrations of vitamin A, RBP, and transthyretin are unaffected. Note that serum RBP, which has been considered to be a good index of vitamin A deficiency and a more sensitive index than visual dark adaptation (18), was normal in the patients with alcoholic fatty liver.

Malnutrition, when present, can contribute to hepatic vitamin A depletion, but the patients with low liver vitamin A concentrations in the study of Leo and Lieber (5) appeared well nourished, which suggested a more direct effect of alcohol. In earlier experimental studies, Suschetet (19) found no effect of chronic ethanol consumption on hepatic vitamin A, whereas Baumann et al (20) noted that prolonged ethanol intake decreased hepatic vitamin A. Blomstrand et al (21) observed a similar effect, but in all 3 studies, ethanol was given in drinking water without nutritional controls. Under strictly controlled conditions, chronic ethanol consumption was found to decrease hepatic vitamin A in baboons pair fed a nutritionally adequate liquid diet containing 50% of total energy either as ethanol or isonitrogenous carbohydrate. In these baboons, fatty liver developed after 4 mo of ethanol feeding, with a 59% decrease in hepatic vitamin A concentrations, and fibrosis or cirrhosis appeared after 24–84 mo with a 95% decrease in hepatic vitamin A concentrations (4).

FIGURE 1. Hepatic vitamin A concentrations in subjects with normal livers, chronic persistent hepatitis, and various stages of alcoholic injury. Reprinted with permission from reference 5.
Similarly, hepatic vitamin A concentrations of rats fed ethanol (36% of total energy) decreased after 3 wk (by 42%) and continued to decline up to 9 wk. In contrast, serum vitamin A and RBP concentrations did not change significantly. When dietary vitamin A was increased 5-fold, hepatic vitamin A again decreased in ethanol-fed rats relative to the corresponding controls, and sometimes even compared with the rats given 5 times less vitamin A (without ethanol) (4). To avoid the confounding effect of dietary vitamin A, it was virtually eliminated in some experiments. Under these conditions, the depletion rate of vitamin A from endogenous hepatic storage was observed to be 2.5 times faster in ethanol-fed rats than in controls.

The observations summarized above suggested that the effects of ethanol on vitamin A were not due solely to alcohol-induced severe hepatic damage because retinol depletion was already apparent at the early and relatively benign fatty liver stage. Furthermore, they showed that even when hepatic vitamin A storage is low, plasma vitamin A concentrations are not below normal. Therefore, plasma vitamin A is not necessarily a good marker for hepatic vitamin A storage between ethanol-fed rats and controls was much greater than could be accounted for by the total vitamin A intake. Thus, malabsorption was not the only reason for the depletion of hepatic vitamin A. Two possible mechanisms other than malabsorption were increased mobilization of vitamin A from the liver and enhanced catabolism of vitamin A in the liver or other organs. There is experimental evidence for both. Indeed, vitamin A in the kidneys and testes was increased even when hepatic vitamin A was depleted (4). Furthermore, an acute nonlethal dose of ethanol significantly decreased hepatic vitamin A, whereas serum vitamin A increased as did retinyl esters in serum lipoproteins (22). These changes in blood concentrations were in keeping with earlier observations in animals (23–26) and humans (27, 28). The decrease in hepatic vitamin A after an acute dose of ethanol was preceded by increased serum retinyl esters in serum lipoproteins. Moreover, [14C]vitamin A increased in extrahepatic organs, whereas it decreased in the liver. These results suggest that a shift of vitamin A from the liver to other organs through lipoprotein-bound retinyl esters occurs as a net result of acute ethanol administration. Conceivably, this effect seen after a single dose of ethanol could continue even after a prolonged intake of ethanol and result in a significant depletion of hepatic stores of vitamin A. In addition, accelerated catabolism of retinoids also plays a role in hepatic vitamin A depletion (see below).

**Pathways of hepatic vitamin A metabolism and its interaction with ethanol and other drugs**

The various pathways involved in hepatic vitamin A metabolism are shown schematically in Figure 2 and were reviewed not long ago (29–31). Drugs that induce cytochrome P450 enzymes in liver microsomes were shown to result in a depletion of hepatic vitamin A (32). A similar effect was observed after administration of ethanol (4, 5) and other xenobiotics that are known to interact with liver microsomes, including carcinogens (33). The hepatic depletion was strikingly exacerbated when ethanol and drugs were combined (34), which mimics a common clinical occurrence. Retinoic acid has been shown to be degraded in microsomes of both hamsters (35) and rats (36, 37). In both species, the reported activity was very low compared with the degree of hepatic vitamin A depletion. These observations prompted the search for alternate pathways of retinol metabolism in liver microsomes. Subsequently, a new pathway of retinol metabolism was described: rat liver microsomes, when fortified with NADPH [the reduced form of nicotinamide-adenine dinucleotide (NAD) phosphate (NADP)], converted retinol to polar metabolites, including 4-hydroxyretinol (6). This activity was also shown in a reconstituted monoxygenase system containing purified forms of rat cytochrome P450 enzymes (6), including P450 2B1 (a phenobarbital-inducible isozyme). More recently, it was shown that other cytochromes (eg, P450 CYP1A1) also catalyze the conversion of retinol to retinoic acid (38). Thus, it is now increasingly apparent that microsomal cytochrome P450 plays a role not only in the detoxification of foreign compounds, but also in important physiologic processes, including vitamin A metabolism and maintenance of vitamin A homeostasis (39).

In addition to the cytochrome P450– and NADPH-dependent systems, a new microsomal NAD+-dependent retinol dehydrogenase was described (Figure 2) (7). The classic pathway for the conversion of retinol to retinal in the liver involves an NADP-dependent cytosolic retinol dehydrogenase (CRD), believed to be similar, if not identical, to liver cytosolic ADH (40, 41). The observation that a strain of deer mice lacks this enzyme without apparent adverse effects (42) prompted a search for an alternate pathway for the production of retinal, the precursor of retinoic acid. Evidence was obtained for the existence of an NADP-dependent microsomal retinol dehydrogenase (MRD) (7), which can convert retinol to retinal by using NAD, and retinal to retinol by using NADH as cofactors, whereas with NADP and NADPH it is less active. It is distinct from the cytochrome P450 microsomal system on the one hand (43) and the CRD system on the other hand. It can explain how, in the absence of cytosolic ADH, retinoic acid can be found in ADH−dear mice in which the MRD can provide the retinal shown to be the obligatory precursor of the retinoic acid (44). Subsequently, the genes of 3 MRD isoenzymes were cloned, one of which is expressed only in the liver (45) and may correspond to the MRD referred to above. More recently, a CYP2D1-binding protein was cloned with retinal reductase activity in the presence of NADPH. Addition of
CYP2D1 and NADPH-P450 reductase increased the retinal reductase activity (46). Cloning of a complementary DNA (cDNA) encoding an ADH of rat testes and its expression in Escherichia coli was also reported (47).

Some of the reported enzyme activities are low or may require high substrate concentrations, but such systems could nevertheless be meaningful for the control of hepatic vitamin A concentrations as documented by calculations of the corresponding metabolic rates and their substantiation by comparable increases in urinary polar metabolites (39). This is especially pertinent when hepatic vitamin A concentrations exceed the binding capacity of the cytosolic RBP. Furthermore, the activities of the retinol (7) as well as that of the retinal (48) dehydrogenases are inducible by chronic alcohol consumption, which also contributes to hepatic vitamin A depletion. Finally, metabolism of retinol and retinoic acid was also shown in human liver microsomes and purified cytochrome P450 2C8 (49).

Liver abnormalities associated with low vitamin A concentrations

**Mallory bodies and lysosomes**

In subjects with alcoholic liver disease, very low concentrations of hepatic vitamin A were found to be sometimes associated with the presence of Mallory bodies. However, Mallory bodies were also seen in patients with alcoholic liver disease who had relatively normal concentrations of vitamin A and, conversely, patients with drug-induced liver disease and very low concentrations of vitamin A did not have any Mallory bodies (50). Therefore, the postulated causal relation between low hepatic vitamin A concentrations and the appearance of Mallory bodies (51, 52) was not verified, although the possibility has not been ruled out that a low hepatic vitamin A concentration may potentiate some effect of ethanol that could result in the development of Mallory bodies. Although no obvious correlation was found between the appearance of Mallory bodies and the depletion of vitamin A in the liver, it was noted that in patients with severe as well as moderate depletion of hepatic vitamin A, multivesicular lysosome-like organelles were detected in increased numbers (53; Figure 3). These structures had a single crescent-shaped membrane characteristic of lysosomes (54). Such lysosomal lesions had not been related to low vitamin A concentrations before, but the fact that a low hepatic vitamin A concentration contributes to these lesions was also verified experimentally in rats. Whereas multivesicular lysosomes were not seen in rats fed a control diet, consumption of a diet low in vitamin A resulted in the appearance of such lesions (50, 53). Moreover, addition of ethanol to the low-vitamin A diet increased the number of these structures. Thus, it appears reasonable to conclude that in patients with strikingly lowered hepatic vitamin A concentrations, the lower concentrations might contribute to the appearance of these multivesicular lysosomes. The mechanism whereby a diet low in vitamin A might produce these lesions is not known. These vesicles seem to be filled with numerous lipid-like particles, suggesting that impaired lipoprotein secretion might be involved. Indirect evidence in favor of such a hypothesis was provided by the observation of lower circulating VLDLs in rats fed the diet low in vitamin A than in animals fed the regimen adequate in vitamin A. Because vitamin A is required for glycosylation of export proteins (55), ethanol may also favor the appearance of this lesion through its general effect on lipid retention in the liver and the associated steatosis (56). Whether ethanol has a direct effect on lysosomes has been the subject of controversy; both increases (57) and decreases (58) of lysosomal enzymes after ethanol have been reported. Alterations in lysosomal membranes have been described after feeding rats vitamin A–deficient diets (59, 60) and, thus, it is not surprising that a combination of ethanol and low vitamin A results in striking lysosomal abnormalities.

**Fibrosis and stellate cells**

Hepatic vitamin A depletion plays a key role in hepatic fibrosis, and both hepatocytes and stellate cells are involved. Hepatic stellate cells are the principal storage site of vitamin A. Dietary vitamin A is taken up by parenchymal cells and then transferred to stellate cells for storage. Most likely, RBP synthesized in the hepatocytes is necessary for the intracellular transfer (61). It is unclear whether ethanol directly decreases the vitamin A content in stellate cells or whether it interferes with the uptake of vitamin A by parenchymal cells and its subsequent transfer to stellate cells. The activation of stellate cells into myofibroblast-like cells, which then synthesize collagen, is associated with a decrease in vitamin A storage in these cells (62). Furthermore, retinoic acid, and to a lesser extent retinol, was shown to reduce stellate cell proliferation and collagen production in culture (62–64). It is of interest that retinoic acid was also shown to decrease α(I) collagen transcription and message in cultured human lung fibroblasts (65). Conversely, a lack of retinoids could promote fibrosis in these tissues, especially in the liver, consistent with the associated activation of stellate cells (62). Paradoxically, however, an excess of vitamin A may also promote fibrosis (see below).

**Extrahepatic manifestations of vitamin A deficiency and their potentiation by alcohol**

**Magnitude of the problem**

Worldwide, vitamin A deficiency is a significant public health problem. The most important clinical effects of vitamin A deficiency are found in the eyes and are grouped under the term xerophthalmia; ≈500,000 new cases of xerophthalmia with active corneal involvement occur annually in Southeast Asia, and half of these cases are likely to lead to blindness (66). In West-
ern countries, however, dietary vitamin A deficiency does not appear to be a serious public health problem, except when associated with alcohol abuse. Indeed, although postmortem studies have found apparently reduced liver reserves of vitamin A in many people (67, 68), these low reserves do not necessarily reflect borderline intakes, but could be a consequence of metabolic abnormalities, possibly secondary to a high prevalence of alcohol and drug abuse in the populations examined (see above).

Deleterious effects of hepatic vitamin A depletion on carcinogenesis

A place for vitamin A in both cancer therapy and prevention has been proposed (69). Indeed, increasing evidence for a role of vitamin A in the proliferation and differentiation of a variety of human cells makes it apparent that low hepatic vitamin A concentrations may be associated with the development of tumors. In fact, vitamin A deficiency has been shown to be associated with the formation of various types of tumors (70). Epidemiologic studies in the United Kingdom (71–73) and in the United States (74) revealed an increased risk of bronchogenic carcinoma in association with low serum vitamin A concentrations and, in experimental animals, deficiency of vitamin A is known to lead to the development of squamous metaplasia. Such changes in the respiratory epithelium are frequently found to precede or to be associated with tumor development (75–85). Furthermore, several studies of the natural histories of bronchogenic carcinomas in humans suggest that squamous metaplasia is one of the earliest stages preceding the development of carcinomas at the site of origin (75, 81–84).

Retinol and retinoic acid have key functions not only in terms of cellular differentiation and maintenance of the normal integrity of mucosal tissues, but also in the prevention of carcinogenesis through various other mechanisms, including the inhibition of the microsomal activation of chemical carcinogens (86). Retinoids, however, may also acquire cellular toxicity in the liver and other tissues (see below). Enzymes such as cytochrome P450 2C8, shown to be involved in vitamin A metabolism (49), may thereby participate in maintaining the delicate balance between those retinol concentrations that promote cellular integrity and oppose the development of cancer, and those that cause cellular toxicity. In zebra fish, White et al (87) identified the retinoic acid-inducible all-trans-retinoic acid 4-hydroxylase. They also described the isolation and characterization of a cDNA P450 RAI that encodes a novel member of the cytochrome P450 family and identified 4-oxo-retinoic acid and 4-OH-retinoic acid as major metabolic products of this enzyme.

Interaction with ethanol

Concomitant ethanol consumption and vitamin A deficiency was shown to result in an increased severity of squamous metaplasia of the trachea (88, 89). Conceivably, this potentiation of vitamin A deficiency by alcohol may predispose the tracheal epithelium to neoplastic transformation. Indeed, it has been shown in animal studies that vitamin A deficiency enhances the susceptibility to neoplasm and increases carcinogenesis in the respiratory tract after the administration of carcinogenic polycyclic hydrocarbons (90, 91). Conversely, treatment of animals with vitamin A or its derivatives in high doses protects against the induction of tumors of the respiratory tract (78, 92). A relatively high risk of squamous cell carcinoma of the lung was found in a Norwegian population that drank large amounts of alcohol and had a low dietary intake of vitamin A (93). Further-

more, a positive association between alcohol consumption and lung cancer was reported in Japanese men living in Hawaii (94).

In rats administered alcohol with a diet low in vitamin A, tracheal cells showed a striking loss of cilia, and ciliated cells had an increased number of lysosomes (89). This finding is noteworthy because increased numbers of lysosomes have also been reported in the ciliated cells of the hamster tracheas exposed to carcinogens (95) and in livers of vitamin A–deficient rats or patients (see Figure 3). Another abnormality exhibited by the ciliated cells after ethanol consumption in the vitamin A–deficient rats was the appearance of compound cilia (89). Similar abnormal cilia have been observed in animals exposed to carcinogens or cigarette smoke as well as in humans with bronchial cancer (96, 97). In addition, ethanol-induced vitamin A depletion is associated with decreased detoxification of xenobiotics, including carcinogens such as nitrosodimethylamine (86), thereby playing a role in carcinogenesis (see above). Recent data also suggest that functional down-regulation of retinoic acid receptors, by inhibiting biosynthesis of retinoic acid and up-regulating activator protein 1 (c-Jun and c-Fos) gene expression, may be an important mechanism for causing malignant transformation by ethanol (98).

In addition to promoting vitamin A depletion, ethanol may interfere more directly with retinoic acid synthesis because both were shown in vitro to serve as substrates for the same enzymes (99). Specifically, one of the mechanisms by which ethanol induces gastrointestinal cancer may be an inhibition of ADH-catalyzed gastrointestinal retinoic acid synthesis, which is needed for epithelial differentiation. Indeed, class I ADH (ADH-I) and class IV ADH (ADH-IV), which function as retinol dehydrogenases in vitro, are abundantly distributed along the gastrointestinal tract (100). ADH-IV (now called (ADH) (101) has been purified (102), its full-length cDNA obtained, and the complete amino acid sequence deduced (103, 104). A nearly full-length gene (ADH7) was cloned by Satre et al (105); the full-length gene was cloned by Yokoyama et al (106) and localized to chromosome 4.

Birth defects

Deficiency of retinoic acid can produce birth defects and, as discussed before, ethanol promotes deficiency of retinoids. Duester (99, 107) and Pullarkat (108) implicated competitive inhibition, by ethanol, of the biosynthesis of retinoic acid from retinol because ADH-I can contribute to the biosynthesis of retinoic acid from retinol. Indeed, this group identified one human ADH isozyme that exists in the affected embryonic tissues to act as a retinol dehydrogenase catalyzing the synthesis of retinoic acid. Ethanol did, in fact, reduce retinoic acid concentrations in cultured mouse embryos (109). However, other results (110) failed to verify, in conceptual tissues, that competitive inhibition of the conversion of retinol to retinoic acid is a significant factor in ethanol-induced embryotoxicity. More recently, Kedishvili et al (111) characterized the ADH enzyme ADH-F, which oxidizes all-trans-retinol and steroid alcohols in fetal tissues. In any event, vitamin A deficiency, especially when exacerbated by ethanol, is associated with various extrahepatic complications, but, as for the liver, vitamin A excesses are also detrimental, as discussed below.

Abnormalities associated with excess vitamin A

Magnitude and multiple facets of the problem

The consequences of vitamin A deficiency (see above) have been widely reported and, undoubtedly, they influence many
individuals to use vitamin A supplements. Thus, the medically unsupervised use of retinoids is now becoming increasingly popular, especially because vitamin A preparations containing 7500 retinol equivalents (RE) (25,000 IU) vitamin A are widely available in health food stores. An intake of 1 or 2 capsules daily (7500–15,000 RE, or 25,000–50,000 IU) is not uncommon, but it vastly exceeds the recommended dietary allowance for vitamin A of 1000 RE (3330 IU) for males and 800 RE (2664 IU) for females; in fact, a decrease in the recommended dietary allowance to 690 RE (2300 IU)/d for males and to 600 RE (2000 IU)/d for females has been advocated (112). In addition, vitamin A has been used not only for the treatment of xerophthalmia (113), but also for hypogonadism (14) and abnormal dark adaptation (14, 114). The amounts administered, which range from 3000 RE (10,000 IU)/d for ≤ 5 mo to 15,000 RE (50,000 IU)/d for 1 wk, are considered safe because no adverse effects have been reported in normal individuals with these dosages. Vitamin A therapy has also been advocated for patients with biliary cirrhosis (7500–15,000 RE/d, or 25,000–50,000 IU/d) (115) and chronic ileitis (3000 RE/d, or 100,000 IU/d, for up to 14 mo) (116). Moreover, the vitamin is used therapeutically for a variety of dermatologic conditions (117, 118). Again, this use is not commonly associated with toxicity, but the question must be raised whether, on occasion, such therapeutic doses can become toxic. This concern pertains not only to retinol but also to a variety of other retinoids for which toxicity has been reported (119, 120). The issue is complicated by the fact that optimal amounts of vitamin A may vary from individual to individual, without a definitive threshold of toxicity having been established. 

Carcinogenicity, teratogenicity, and hepatotoxicity

Vitamin A deficiency promotes carcinogenesis (see above), but paradoxically, an excess of vitamin A may have a similar effect: Tuyns et al (121) and DeCarli et al (122) noted that foods providing large amounts of retinol increase the risk of cancer of the esophagus and, in an epidemiologic study, the increased cancer risk associated with the use of cigarettes and alcohol was also enhanced with ingestion of foods containing retinol (123). Other food constituents could also play a role in this regard.

The teratogenic potential of an excessive intake of retinoid was shown clearly in experimental animals, as reviewed by Soprano and Soprano (124), with corresponding data evolving in humans: teratogenicity of 13-cis-retinoic acid, used to treat cystic acne, has been established in epidemiologic studies (125). In addition, among babies born to women who took > 3000 RE (10,000 IU) preformed vitamin A/d as supplements, =1 infant in 57 had a malformation (126). However, caution in the interpretation of these data is still indicated (127). Furthermore, acetaldehyde can cross the placenta (128) and may also contribute to the development of fetal alcohol syndrome, the most prevalent cause of preventable congenital abnormalities (129). Therefore, in addition to potentiating the teratogenicity of vitamin A deficiency, alcohol can be expected to aggravate the adverse effects of an excess of vitamin A; this was verified experimentally (130).

An excess of vitamin A is also known to be hepatotoxic (131, 132). Traditionally, vitamin A toxicity was reported after consumption of doses of ≥ 3000 RE (100,000 IU)/d for periods ranging from weeks to months (133, 134). However, daily supplementation with 2 vitamin A capsules, each providing 7500 RE (25,000 IU) vitamin A, for a couple of years was associated with severe vitamin A toxicity and an increase in plasma vitamin A concentrations to 10 times normal in a 16-y-old (135) and in health food users (136). Minuk et al (137) reported observations that suggest toxicity of vitamin A at even lower intakes (6000–13,500 RE/d, or 20,000–45,000 IU/d). The smallest daily supplement of vitamin A reported to be associated with liver cirrhosis is 7500 RE (25,000 IU) taken for 6 y (138). These supplements fall well within common therapeutic dosages and amounts used prophylactically with over-the-counter preparations by the population at large. Thus, a broadening incidence of toxicity should be expected, and early recognition of such toxicity is needed to prevent the development of severe, irreversible, and potentially lethal complications.

Note, however, that the relation between vitamin A and fibrogenesis is complex: in addition to vitamin A promoting fibrogenesis, the opposite effect has been described under certain circumstances. For instance, retinoids have been shown to down-regulate procollagen gene expression in isolated fibroblasts (139), and vitamin A has been reported to suppress carbon tetrachloride–induced hepatic fibrosis (140).

Alcohol as an aggravating factor of vitamin A hepatotoxicity

The mechanism of retinoid toxicity is poorly understood (141), but in recent years the effect of alcohol abuse, one of the most common aggravating factors of vitamin A toxicity, has been elucidated. Potentiation of vitamin A hepatotoxicity by ethanol was first shown in rats fed 2 mo with either a normal amount of vitamin A or 5 times the normal amount (142). Although ethanol alone produced only modest changes and vitamin A supplementation had no adverse effect under these conditions, the combination resulted in striking lesions, giant mitochondria containing paracrystalline filamentous inclusions, and depression of oxygen consumption with 5 different substrates. The potentiation of vitamin A toxicity by ethanol was also seen in patients treated with 3000 RE (10,000 IU) vitamin A/d for sexual dysfunction attributable to excess alcohol consumption (143). The striking mitochondrial lesion in one of those cases is illustrated in Figure 4. In addition to a giant mitochondrion, which was 1000 times its normal size, striking filamentous or crystalline-like inclusions were also seen. Such inclusions were also described by others (137) in the liver mitochondria of patients with hypervitaminosis A. However, the amount of vitamin A consumed in all of these previous cases was much higher than in the one shown in Figure 4, which illustrates the potentiation of vitamin A toxicity by ethanol. The potentiation of vitamin A toxicity by ethanol was most dramatically documented in another study, in which rats were given a combination of vitamin A and ethanol for up to 9 mo (144). There was striking hepatic inflammation and necrosis accompanied by a rise in serum enzymes (glutamate dehydrogenase and aspartate aminotransferase).

Alcohol may also act indirectly by causing liver disease, which, in turn, might affect the capacity of this organ to export vitamin A, thereby enhancing its local toxicity. Indeed, in alcoholics, the carrying capacity of RBP was at times exceeded even with low serum retinol concentrations (145) and, in such cases, caution in the amount of vitamin A used for therapy was advised. Similarly, diets severely deficient in protein may affect the capacity of the liver to export vitamin A and enhance its hepatotoxicity.

Vitamin A supplementation results in an increased number of stellate cells. By contrast, when vitamin A supplementation was
whether carotenoids might serve as effective (but less toxic) substitutes for retinol, especially in alcoholic liver injury attributed, in part, to oxidative stress, and because β-carotene is an antioxidant. It was not known, however, whether β-carotene can actually offset alcohol-induced lipid peroxidation.

Antioxidant properties of β-carotene

β-Carotene has the potential of acting as a more efficient antioxidant than retinol: carotenoids were found to inhibit free radical–induced lipid peroxidation (150, 151) and β-carotene is one of the most efficient quenchers of singlet oxygen (152). A mechanism whereby β-carotene may act as a lipid antioxidant was provided by Burton (153) and β-carotene was also shown to inhibit arachidonic acid oxidation (154). It may prevent lipid peroxidation by acting through specific enzyme inhibition.

Indeed, β-carotene decreases the activity of lipoxygenase toward linoleate (155). Possible interactions between micronutrients and β-carotene in the membrane’s antioxidant defense system have been reviewed by Machlin and Bendich (156).

β-Carotene can also suppress lipid peroxidation in mouse tissues induced by the injection of carbon tetrachloride (157). A study in guinea pigs noted a protective effect against in vivo lipid peroxidation when animals were pretreated with β-carotene (158). Furthermore, Palozza and Krinsky (159) reported that β-carotene inhibited malondialdehyde production in a concentration-dependent manner and delayed the radical-initiated destruction of endogenous α- and γ-tocopherol in rats, and Kim-Jun (160) described inhibitory effects of β-carotene on lipid peroxidation in mouse epidermis. Favorable effects of β-carotene on lipid peroxidation were also reported after an overload of dietary iron in rats (161) and on HepG2 human liver cells subjected to tert-butyl hydroperoxide (162). Chicks fed β-carotene were less vulnerable to hepatic oxidative stress than were unsupplemented controls (163). Singlet oxygen–mediated oxidation of human plasma LDL was also attenuated with carotenoid supplementation (164). However, in a study in rats, Alam and Alam (165) reported no change in either blood or tissue lipid peroxides after ingestion of 180 mg β-carotene·kg⁻¹·d⁻¹ for 11 wk and carotenoids did not protect against peroxidation in choline-deficient rats (166). Note that β-carotene was shown to be an excellent substrate for free radical attack because of the presence of long and conjugated double bonds (167).

Associations between alcohol and liver disease and β-carotene concentrations

Studies in humans have shown that for a given β-carotene intake, there is a correlation between alcohol consumption and plasma β-carotene concentrations (168). Thus, whereas alcoholics generally have low plasma β-carotene concentrations (168, 169), presumably reflecting a low intake, alcohol per se might in fact increase blood concentrations in humans (168). There was also an increase in women with a dose as low as 2 drinks/d (170) as well as in nonhuman primates (8). Indeed, in baboons fed ethanol chronically, liver β-carotene was increased, in contrast with vitamin A, which was depleted. Similarly, plasma β-carotene concentrations were elevated in ethanol-fed baboons (Figure 5), with a striking delay in the clearance from the blood after a β-carotene load. Furthermore, whereas β-carotene administration increased hepatic vitamin A in control baboons, this effect was much less evident in alcohol-fed animals. The combination of an increase in β-carotene and a relative lack of a...
corresponding rise in vitamin A suggests a blockade in the conversion of β-carotene to vitamin A by ethanol. The nature of this putative block is unclear and, in fact, the normal pathways of the conversion of β-carotene to retinal are still the subject of controversy. Classically, β-carotene is believed to be mainly split into 2 molecules of retinal, which is ultimately converted to retinol (171–174). Others, however, have described significant conversion by side-chain oxidation (175, 176); these studies lend support for an eccentric cleavage mechanism in the metabolism of β-carotene into retinoic acid in vivo. The relative roles of these pathways in various physiologic and pathologic conditions remain to be quantified.

There is also appreciable biliary excretion of β-carotene in humans (177). Theoretically, alcohol could increase blood concentrations by impairing biliary excretion, but the decrease in biliary excretion observed in various liver conditions did not result in an increase in plasma β-carotene concentrations (177).

The relation between liver disease and hepatic carotenoids is complex. In most patients with liver disease, absolute concentrations of hepatic α- and β-carotene and retinoids were found to be very low, even in the presence of normal serum concentrations of lycopene, α- and β-carotene, or both; in patients with cirrhosis, hepatic concentrations were particularly low (178). However, even in these patients with very low liver α- and β-carotene concentrations, more than half had blood concentrations in the normal range, suggesting that liver disease interferes with the uptake, excretion, or, perhaps, metabolism of α- and β-carotene. In only one-third of the subjects were α- and β-carotene serum concentrations low, probably reflecting poor dietary intakes.

β-Carotene, alcohol, oxidative stress, and liver injury

Studies by Mobarhan et al (179) showed that replenishing young men with β-carotene can decrease the concentration of circulating lipid peroxides, but it was not reported whether this could be achieved in individuals who continue to drink while taking doses of β-carotene not shown to be toxic in the presence of alcohol. Indeed, in the studies of Mobarhan et al (179), the subjects had stopped consuming alcohol at the time they received the β-carotene. The question therefore remained whether the combination of alcohol and β-carotene, at the dose used for replenishment, will prevent lipid peroxidation without producing some signs of toxicity.

In baboons, consumption of ethanol together with β-carotene resulted in a more striking hepatic injury than did consumption of either compound alone (Figure 6) (8). This toxic interaction in baboons occurred at a total dose of 7.2–10.8 mg β-carotene/d diet (30–45 mg/1000 kcal diet), which is common in subjects taking supplements and is of the same order of magnitude as the amount given in CARET (10), namely 30 mg supplemental β-carotene/d, and in the study of Rust et al (180) (50 mg β-carotene/d for 12 wk). The dose of alcohol administered to the baboons (50% of dietary energy) was equivalent to that of the average alcoholic (181). In rats, the dose of alcohol was even lower (36% of total dietary energy). Nevertheless, the well-known hepatotoxicity of ethanol was potentiated by large amounts of β-carotene and the concomitant administration of both β-carotene and alcohol resulted in striking liver lesions (182). These lesions were characterized at the biochemical level by increased activity of liver enzymes in the plasma, at the light microscopic level by an inflammatory response, and at the ultrastructural level by striking autophagic vacuoles and alterations of the endoplasmic reticulum and the mitochondria (182). In the latter study, β-carotene was administered in beadlets, which enhanced its bioavailability. To determine whether the beadlet carrier itself contributed to the toxicity, rats were given (for 2 mo) vitamin A, β-carotene (with or without beadlets), or corresponding amounts of beadlets without β-carotene, in diets containing either carbohydrates or equivalent amounts of ethanol. The ethanol-induced oxidative stress assessed by an increase in hepatic 4-hydroxyxnonenal and F₂-isoprostanes (measured by gas chromatography–mass spectrometry) was not improved despite a concomitant rise in hepatic antioxidants (β-carotene and vitamin E). Moreover, beadlets resulted in proliferation of the smooth endoplasmic reticulum and in leakage of the mitochondrial glutamate dehydrogenase into the plasma.

FIGURE 5. Effect of chronic ethanol consumption on β-carotene concentrations in baboons fed liquid diets (with or without ethanol) and a 200-g carrot daily (30 mg β-carotene). The liver and plasma β-carotene concentrations measured by HPLC were significantly higher in ethanol-fed animals than in pair-fed controls. Reprinted with permission from reference 8.
reflecting mitochondrial injury (both documented by electron microscopy) (182). The reason for this toxicity is not clear. The composition of the beadlets is proprietary; of the known ingredients, none have been identified as toxic.

**Extrahepatic side effects**

**Cardiovascular complications**

In the ATBC Study (9) and CARET (10) it was noted that, in smokers, β-carotene supplementation increased death from coronary artery disease. The mechanism involved has not been elucidated. Because alcohol increases β-carotene concentrations (see above) and because cardiovascular complications are apparently associated with elevated β-carotene concentrations, it is possible that β-carotene is cardiotoxic, a possibility that is still largely unexplored. However, because of its antioxidant properties, β-carotene has been postulated to have beneficial effects in terms of cardiovascular diseases, but whether this is indeed the case is not clear. In a study by Greenberg et al (183), there was no evidence of lower mortality after β-carotene supplementation among patients with initial β-carotene concentrations below the median for the study group and there was no support for a strong effect of supplemental β-carotene in reducing mortality from cardiovascular disease or other causes. Similarly, the study of Hennekens et al (184) unequivocally ruled out the possibility that there was even a slight reduction in the incidence of mortality from cardiovascular disease with supplementation of 50 mg β-carotene/d or every other day for an average of 12 y. Recent results even suggest that β-carotene participates as a prooxidant in the oxidative degradation of LDL and that elevated LDL concentrations may cancel the protective qualities of α-tocopherol (185). Supplementation with vitamin A was not associated with lower risks of dying from coronary disease in a study by Kushi et al (186).

**Interaction with cancer**

The toxic effects of β-carotene and their interaction with ethanol are associated with an increased incidence of pulmonary cancer. Whereas a protective effect of β-carotene and retinol on ventilatory function in an asbestos-exposed cohort was reported by Chewers et al (187), 2 epidemiologic investigations—the ATBC (9) and CARET (10)—showed that β-carotene supplementation increased the incidence of pulmonary cancer in smokers. Because heavy smokers are commonly heavy drinkers, we raised the possibility that alcohol abuse was contributory (188) because alcohol is known to act as a carcinogen and to exacerbate the carcinogenicity of other xenobiotics, especially those of tobacco smoke (189). Why this should be aggravated by β-carotene is not clear. However, because pulmonary cells are exposed to relatively high oxygen pressures and because β-carotene loses its antioxidant activity and shows an autocatalytic, prooxidant effect at these higher pressures (152), such an interaction is at least plausible and deserves further study. Indeed, subsequently, the data of the ATBC study and CARET showed that the increased incidence of pulmonary cancer was related to the amount of alcohol consumed by the participants (190–192).

In contrast with the findings of the ATBC study (9) and CARET (10), an investigation by Hennekens et al (184) found no comparable complications. However, Hennekens et al (184) did not focus on smokers and used a different preparation of β-carotene; it was not given in beadlets—the carrier of the β-carotene preparation used in the ATBC study (9), CARET (10), and some nonhuman primate (8) and rat (182) studies. As mentioned previously, beadlets may contribute to the β-carotene toxicity (182).

The observations of the potentiation of carcinogenicity by β-carotene and alcohol in smokers was surprising because up to that point, the prevailing view was that β-carotene was an anticarcinogen. Indeed, experimentally, β-carotene was found to prevent tissue vitamin A depletion produced by the carcinogen benzopyrene (193), to attenuate chemical carcinogenesis (194), and to suppress the progression of spontaneous mammary tumors in mice (195). Because 4,4′-diketo-β-carotene, which has no provitamin A activity, can prevent ultraviolet-induced skin tumors in hairless mice (196), it has been proposed that carotenoids may, in part, exert an antitumor effect per se and not only after conversion to retinoids. Furthermore, carotenoids
affect the proliferation and differentiation of certain cell lines. Induction of cell differentiation by a carotenoid without (lutein) and with (β-carotene) vitamin A activity suggested a vitamin A–independent mode of action for carotenoids in cell differentiation (197). However, in a clinical trial, β-carotene failed to prevent colorectal adenomas (198).

Concentrations of carotenoids, retinoids, and tocopherols were also determined in the homogenate of macroscopically normal appearing oropharyngeal mucosa from chronic alcoholics and from control patients. All the alcoholics except one had oropharyngeal cancer. No significant difference was found in tissue concentrations of carotenoids and tocopherols between alcoholics and control subjects. Furthermore, in 7 of 11 control subjects, retinol was undetectable in the oropharyngeal mucosa, whereas in the alcoholics only 2 of 10 had unmeasurable retinol concentrations (199). These results did not support the concept that ethanol-associated oropharyngeal carcinogenesis is due, at least in part, to local deficiencies in retinoids, carotenoids, or α-tocopherol. In fact, because excess retinol may promote carcinogenesis and because retinol supplementation was also done in CARET (9), one may wonder to what extent this may have contributed to the enhanced carcinogenesis, especially in the alcoholics. Indeed, alcohol promotes the carcinogenesis of other compounds (200) and, as discussed before, exacerbates the hepatotoxicity of vitamin A and increases its content in extrahepatic tissues; thus, the adverse effects of a combination of vitamin A and alcohol on these other tissues or disease processes cannot be excluded. Some antimutagenic or anticarcinogenic compounds act through the modulation of the metabolism of carcinogens by reducing their activation or enhancing their detoxification (201), but neither β-carotene (fed or injected) nor an excess of vitamin A induced any significant variation in such enzyme activities (202), although, as mentioned before, retinol inhibits the activities of certain chemical carcinogens (86).

In contrast with the investigations showing a lack of beneficial effects of β-carotene supplementation (reviewed above), a study of nonmelanocytic skin cancer showed that a high intake of vegetables and other β-carotene–containing foods is protective against nonmelanocytic skin cancers (203). Conversely, there are data showing an association between low concentrations of serum β-carotene and the risk of squamous cell carcinoma of the lung (204). However, the latter observations do not necessarily prove a causal link because the beneficial effects may be associated with active nutrients other than β-carotene. Furthermore, human serum carotenoid concentrations were found to be related to lifestyle factors (205). In addition, 4 y of β-carotene supplementation resulted in only a moderate increase in serum β-carotene concentrations and did not significantly change the serum concentrations of other carotenoids (206).

The various effects observed in conjunction with diets rich or poor in β-carotene could be due to carotenoids other than β-carotene or to other associated nutrients. On the other hand, it remains to be determined whether the undesirable effects of β-carotene in smokers pertain to β-carotene–rich foods or to individuals consuming β-carotene in the absence of beadlets, with or without alcohol. In any event, because diets containing foods rich in β-carotene were found to be beneficial (207), achieving the current year 2000 goal of increasing the frequency of consumption of fruit and vegetables in the United States to 5 servings/d (208) should be maintained because the toxic effects of β-carotene and their potentiality by ethanol (reviewed here) have not altered the weight of the evidence of beneficial effects derived from diets rich in fruit and vegetables.

CONCLUSIONS

Consumption of substantial amounts of alcohol is commonly associated with deleterious effects, some of which are due to vitamin A deficiency, which aggravates alcohol-induced liver injury, fetal-alcohol syndrome, and carcinogenesis. Vitamin A deficiency results not only from a poor dietary intake, but may also derive from direct effects of ethanol on the breakdown of retinol in the liver. Vitamin A supplementation in heavy drinkers may be indicated, but it is complicated by the intrinsic hepatotoxicity of large amounts of vitamin A, which is strikingly potentiated by concomitant alcohol use. β-Carotene is a precursor and a nontoxic substitute for retinol, but ethanol interferes with its conversion to vitamin A and even moderate alcohol intake can result in increased concentrations of β-carotene even when the latter is given in commonly used dosages for supplementation. Side effects observed under these conditions include hepatotoxicity, promotion of pulmonary cancer, and possibly cardiovascular complications. Experimentally, the hepatotoxicity of the combination of alcohol and β-carotene was found to be exacerbated when the latter is given as beadlets. Thus, detrimental effects result from a deficiency as well as from an excess of retinoids and carotenoids and, paradoxically, both have similar adverse effects in terms of fibrosis, carcinogenesis, and possibly embryotoxicity. Treatment efforts, therefore, must carefully respect the resulting narrow therapeutic window, especially in drinkers, in whom alcohol narrows this therapeutic window even further by promoting the depletion of retinoids and by potentiating their toxicity.

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