# Carotenoids and alcoholic liver disease

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**Abstract:** Chronic and excessive consumption of alcohol leads to the development of alcoholic liver disease. The depletion of vitamin A is a well-known consequence of alcohol consumption, and may be associated with the observed alcohol-induced hepatic injury. The provitamin A carotenoid β-carotene has been demonstrated to increase alcohol-induced hepatic injury when given in high doses, while low dose supplementation provides protection against hepatic injury. However, it is unknown if the hepatoprotective effects of low dose β-carotene are due to the protective actions of β-carotene itself or if the alterations are due to restored vitamin A levels. Future studies are needed to provide further insight into the specific mechanisms by which β-carotene exerts its protective effect. Further, supplementation studies utilizing high doses of β-carotene in the presence of alcohol must be done with caution.

**Keywords:** Carotenoids; β-carotene; vitamin A; alcoholic liver disease; hepatic injury

Excess consumption of alcohol is commonplace in the United States and worldwide and is known to cause serious organ damage. Specifically, alcohol consumption is one of the most prominent factors contributing to liver disease—a major cause of morbidity and mortality worldwide (1). Alcoholic liver disease (ALD) includes a broad spectrum of disease stages beginning with steatosis (fatty liver) and followed by alcoholic steatohepatitis. These lay the groundwork for progression to the more damaging and irreversible stages of fibrosis and cirrhosis, which increases the risk of hepatocellular carcinoma development (2).

β-Carotene is a provitamin A carotenoid found in many fruits and vegetables that is known to possess potent antioxidant functions (3). In a recent issue of *Hepatobiliary Surgery and Nutrition*, Peng *et al.* explored the effects of β-carotene supplementation on antioxidant capacity and hepatic apoptosis in a chronic ethanol-fed rat model (4). The study supplemented with low dose (0.52 mg/kg BW/day) and high dose (2.6 mg/kg BW/day) β-carotene in rats fed either Lieber-DeCarli control or 35% ethanol diet for a treatment period of 12 weeks. Analysis revealed

that consumption of the ethanol diet resulted in hepatic injury including elevated plasma AST and ALT activities, fatty liver, plasma and hepatic TNFα concentrations, lipid peroxidation, cytochrome P450 2E1 (CYP2E1) protein expression, and apoptosis. Interestingly, β-carotene supplementation provided a hepatoprotective effect against the ethanol-induced hepatic injury seen in the rat model. Both supplementation doses prevented ethanol-induced liver damage and were associated with a significant reduction in liver injury markers AST and ALT. An important finding in this paper was that the two doses of β-carotene supplementation appeared to work via different mechanistic targets. Specifically, the hepatoprotective actions of low dose (0.52 mg/kg BW/day) β-carotene supplementation were associated with the inhibition of mitochondrial-mediated apoptosis via increased Bcl-xL and decreased caspase-3 and -9 hepatic protein expressions. Low dose β-carotene supplementation was also associated with lowered oxidative stress as shown by decreased lipid peroxidation and CYP2E1 protein expression levels. On the other hand, the hepatoprotective actions of high dose

(2.6 mg/kg BW/day) β-carotene supplementation were associated with decreased plasma and hepatic TNFα concentrations and lipid peroxidation. Additionally, high dose β-carotene supplementation resulted in decreased cytochrome c and increased Bcl-2 protein expression (4). In summary, these data provide evidence that supplementation with β-carotene exerts protective effects against alcohol-induced hepatic injury.

Low dose β-carotene supplementation studies have previously demonstrated a beneficial effect against alcohol-induced liver injury via antioxidant activity (5,6). In contrast, high dose β-carotene was shown to have no beneficial antioxidant effect, and was even proposed to act as a prooxidant in the presence of alcohol (7). Indeed, high dose β-carotene supplementation intensified liver damage in an alcohol model (8). Supplementation with β-carotene has also been shown to potentiate alcohol-induced CYP2E1 levels in rat livers, which was confirmed by the corresponding increase in the hydroxylation of p-nitrophenol, a specific substrate for the CYP2E1 enzyme (9). As mentioned, Peng *et al.* demonstrated a decrease in lipid peroxidation with both low (0.52 mg/kg BW/day) and high (2.6 mg/kg BW/day) doses of β-carotene supplementation and a significant decrease in CYP2E1 protein expression with low dose β-carotene supplementation only (4). It is important to note that although this study utilized both low and high supplementation doses, both are considerably lower than the doses found to have detrimental effects in the presence of alcohol. These findings emphasize the importance of low dose β-carotene supplementation with alcohol consumption.
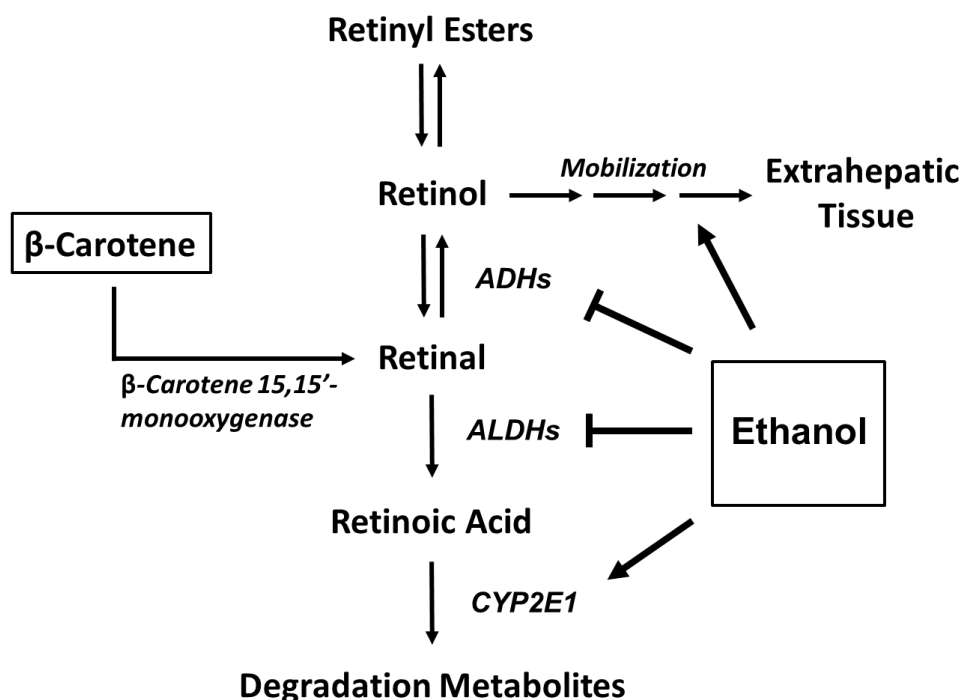
An interesting addition to this study would be to address the possibility that the hepatoprotective effects in the study are due to the conversion of the β-carotene supplement into vitamin A and retinoic acid, the bioactive form of vitamin A. Retinoic acid functions as a nuclear transcription factor ligand and controls the expression of hundreds of genes (10). Vitamin A can be consumed directly from the diet, usually in the form of retinol or retinyl esters from a variety of meat and dairy products. In addition, it can also be produced via enzymatic cleavage from dietary provitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin). Specifically, β-carotene 15,15'-monooxygenase (*Figure 1*) has a high affinity for the cleavage of β-carotene to produce retinal (11). Our lab has demonstrated that alcohol consumption results in the up-regulation of β-carotene 15,15'-monooxygenase (12), which supports the previous observation that heavy consumption of alcohol substantially reduces plasma levels of β-carotene in humans (13,14).

Further, a previous study providing β-carotene as the major source of vitamin A demonstrated that ablation of β-carotene 15,15'-monooxygenase resulted in a dramatic decrease in vitamin A as well as the development of fatty liver (15). Therefore, it is possible that altered vitamin A levels could be associated with the alcohol-induced liver injury observed by Peng *et al.* as well (4).

Chronic and excessive alcohol intake is known to interfere with vitamin A homeostasis and metabolism in several different ways (*Figure 1*) (10,16,17). Ethanol is metabolized primarily in the liver to acetaldehyde via oxidative metabolism, and then converted to acetic acid. The metabolism of vitamin A undergoes a very similar oxidative process including the conversion of retinol to retinal, followed by conversion to the bioactive form, retinoic acid (10). Ethanol and retinol share similar chemical properties, and ethanol has been shown to act as a competitive inhibitor of both ethanol and acetaldehyde dehydrogenases, thus inhibiting vitamin A oxidation to bioactive retinoic acid (16,17). Additionally, induction of the CYP2E1 enzyme by ethanol has been shown to enhance retinol and retinoic acid catabolism (16). Specifically, utilizing the CYP2E1 inhibitor chlormethiazole, we have demonstrated that chlormethiazole normalizes hepatic retinoic acid, retinol, and retinyl ester levels and prevents the appearance of retinoid degradation metabolites in a dose dependent manner (18). Lastly, ethanol has been shown to alter retinoid homeostasis by increasing the mobilization of vitamin A to extrahepatic tissues (10,16).

Alcoholics are reported to have significantly reduced levels of plasma retinol as well as hepatic retinyl ester stores (19-21). Specifically, ALD is associated with depleted levels of hepatic vitamin A (17) and the decrease in hepatic retinoid content is correlated with disease severity (22). Further, animal models have demonstrated that alcohol depletes retinoid stores independent of vitamin A intake and absorption (23). Studies in our laboratory have demonstrated that hepatic concentrations of retinoic acid, retinol, and retinyl palmitate are significantly reduced upon high consumption of alcohol (12,24). Interestingly, multiple studies have shown that supplementation with retinoic acid alleviates alcohol-induced liver injury (25-27). Therefore, it is important to point out that if the β-carotene supplementation restored the vitamin A status to within normal range, this may explain the protective effects of β-carotene against the alcohol-related liver injury seen in the study.

In conclusion, examination of the role of β-carotene

**Retinyl Esters**

**Retinol** → *Mobilization* → **Extrahepatic Tissue**

**β-Carotene**

*β-Carotene 15,15'-monooxygenase*

**Retinal**

*ADHs*

*ALDHs* ⊣ **Ethanol**

**Retinoic Acid**

*CYP2E1*

**Degradation Metabolites**

**Figure 1** Potential mechanisms of how excessive alcohol interferes with retinoid metabolism. Specifically: (I) both retinol and ethanol have similar metabolic pathways, and ethanol can act as a competitive inhibitor of vitamin A oxidation to retinoic acid involving alcohol dehydrogenases (ADHs) and aldehyde dehydrogenases (ALDHs); (II) Ethanol enhances catabolism of vitamin A and retinoic acid by inducing cytochrome P450 enzymes, particularly cytochrome P450 2E1 (CYP2E1) which contributes greatly to alcohol-related liver injury; and (III) ethano alters retinoid homeostasis via increased vitamin A mobilization from liver to extrahepatic tissues. Supplementation with the provitamin A carotenoid, β-carotene, may restore the vitamin A status to within a normal range, thereby providing protection against alcohol-related liver injury.

15, 15'-monooxygenase activity and vitamin A status in β-carotene supplementation studies will help to provide a more detailed explanation of the involvement of retinoid levels in the protection against alcohol-induced hepatic injury. Further, we suggest a need for caution among individuals consuming high amounts of both alcohol and β-carotene supplements.

## Acknowledgements

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