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Availability and antiperoxidative effects of β-carotene from *Dunaliella bardawil* in alcohol-drinking rats

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The present study demonstrated the high bioavailability and antiperoxidative capacity of the natural β-carotene isomer mixture of Dunaliella bardawil *compared with synthetic β-carotene under alcohol-induced oxidative stress. Weanling rats were adapted to ethanol by increasing ethanol levels in their drinking water to 30% at 5% intervals per week; other rats received water with no added ethanol. One water-drinking group and one alcohol-drinking group with no dietary carotene were used as controls. Two water-drinking groups were supplemented with 1 g/kg diet β-carotene either from* Dunaliella *or a synthetic source, and due to reduced food intake, two ethanol-fed groups received 2 g β-carotene per kilogram of diet from each source. Following 3 months of ethanol consumption, both carotene sources were found to prevent ethanol-induced lipid peroxidation as expressed by the hepatic conjugated oxidized dienes level. However, in the algal-fed rats, hepatic carotene and vitamin A levels were higher. In addition to a lower performance of the group fed ethanol and synthetic β-carotene, there were three deaths in this group.* (J. Nutr. Biochem. 10:449–454, 1999) *© Elsevier Science Inc. 1999. All rights reserved.*

Keywords: *Dunaliella bardawil*; β-carotene; alcohol; lipid peroxidation; vitamin A; rats

Introduction

In recent years the consumption of foods rich in β-carotene has been shown to be associated with reduced risk of several pathologic events. Thus, a large number of epidemiologic and controlled studies have indicated the role of dietary β-carotene in the prevention of certain types of cancer.[1] In addition, Gey et al.[2] demonstrated an inverse relationship between β-carotene intake or plasma level and the risk of cardiovascular disease. The properties of β-carotene as a potent free radical quencher, singlet oxygen scavenger, and antioxidant—not its activity as provitamin A—have been implicated as paramount in this protective role.[3]

Chronic alcohol consumption, which is known to be responsible for the generation of free radicals,[4] results in liver injury accompanied by decreased hepatic vitamin A content in rats, baboons, and humans.[5,6] Therefore, supplementation of β-carotene as a vitamin A precursor as well as an antioxidant was recommended. However, recent studies using alcohol-fed baboons[7] and rats[8] demonstrated that supplementing synthetic β-carotene in the diets of these animals resulted in histologic changes in the liver and potentiation of the hepatotoxicity of alcohol. Furthermore, Ahmed et al.[9] suggested that dietary fortification with synthetic β-carotene during active drinking in humans might lead to hepatotoxic alcohol–β-carotene interactions.

Recently, two human intervention studies noted the possible carcinogenicity of the synthetic *all-trans* β-carotene.[10,11] Therefore, attention was drawn to β-carotene from natural sources, such as that found in fruits and vegetables, which also contain small amounts of other β-carotene isomers.[12]

The unicellular algae *Dunaliella bardawil* has received much attention in recent years as a natural rich source of β-carotene, due to its ability to accumulate large amounts of

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this nutrient, exceeding 12% of its dry weight.[13] The algal β-carotene is composed of *all-trans* and *9-cis* stereoisomers in approximately equal amounts. This unique isomer mixture was shown to accumulate to a higher extent in livers of rats and chicks than synthetic *all-trans* β-carotene.[14,15] The algal β-carotene also demonstrated superior antioxidative properties in human studies under normal nutritional conditions,[16] and was shown to cope more efficiently with dietary oxidative stress caused by feeding oxidized soybean oil to rats.[17]

The present study was undertaken to determine the availability and antiperoxidative efficiency of the natural β-carotene isomer mixture, which was provided to rats in the form of spray dried *Dunaliella bardawil*, compared with the synthetic product. Peroxidation was induced by chronic alcohol consumption.

Methods and materials

Carotene sources

The natural β-carotene source was provided as a spray-dried *Dunaliella bardawil* powder (N.B.T., Eilat, Israel). The synthetic preparation of β-carotene was purchased from Sigma Chemical Co. (St. Louis, MO USA). Analysis by three-dimensional photodiode array high performance liquid chromatography (HPLC)[16] showed that the algal β-carotene was composed of two major isomers: *all-trans* (42%) and *9-cis* (43%). In addition, α-carotene (5%) and several other oxycarotenoids (5%) such as lutein, zeaxanthin, and neoxanthin and a few minor isomers of β-carotene (5%) were present. The preparation of synthetic β-carotene was composed of 97% *all-trans* β-carotene and 3% *15-cis* β-carotene. None of the β-carotene sources showed any significant peaks of other components.

Animals and diets

Weanling Sprague-Dawley female rats (obtained from the animal colony of the Department of Food Engineering and Biotechnology, Technion, Haifa, Israel) were divided into six groups of eight rats each. The animals were housed in wire cages in a room maintained at 23°C with a light–dark cycle of 12 hours, and were fed ad libitum a commercial laboratory animal diet (Koffolk Ltd., Tel-Aviv, Israel). Three groups were gradually adapted to ethanol consumption by increasing ethanol levels in their drinking water from 5 to 30% at 5% intervals per week. The remaining rats continued to receive water and served as age-matched animals. One water-drinking group and one alcohol-drinking group (A and B, respectively) with no added dietary carotene were used as controls. Two of the water-drinking groups were supplemented with β-carotene (1 g/kg diet) either in the form of *Dunaliella bardawil* spray-dried powder (group C) or with synthetic *all-trans* β-carotene (1 g/kg diet; group E). Because preliminary studies indicated that consumption of alcohol reduces food intake to 50 to 60%, two groups of alcohol-consuming animals were supplemented with twice the amount (2 g/kg diet) of β-carotene from each source (groups D and F). The diets were prepared weekly and were kept at 4°C. Appropriate amounts were provided daily to the animals, discarding the residue of the previous rations.

At the end of a 3-month feeding period, the animals were euthanized by carbon dioxide asphyxiation. Following perfusion with cold isotonic saline, the livers and kidneys were removed, blotted, and frozen at −80°C. Blood was collected over ethylenediamine-tetraacetic acid (EDTA) from the abdominal aorta and centrifuged at 1,000 × g for 25 minutes at 4°C, and plasma samples were stored at −80°C.

Carotene and retinol analyses

Livers and kidneys were lyophilized for 36 hours and β-carotene and vitamin A were extracted.[14] Total β-carotene and retinol were determined colorimetrically at 472 nm ($E^{1\%}1_{cm}$ = 2,262) and 325 nm ($E^{1\%}1_{cm}$ = 1,600), respectively. The β-carotene isomeric composition of the dietary mixtures and of the liver extracts was determined by HPLC as previously described.[16] A stainless steel column of 25 cm × 4.6 mm (inner diameter) packed with C18 reversed phase material of 5 μm particle size, and a guard column (Vydac 201 TP 54, The Separation Group, Hesperia, CA USA) were used. Elution was performed with an isocartic solvent, methanol:acetonitrile (9:1, v/v) at a rate of 1 mL/min. The identification of the β-carotene isomers and the retinol and retinyl esters was carried out at 450 nm and 325 nm, respectively, using standards obtained from Sigma Chemical Co.

Conjugated dienes

Conjugated dienes were quantified[16] using the absorption maxima at 232 nm obtained for the liver extracts of the experimental groups in proportion to that of the control, which was arbitrarily assigned the value of 1. HPLC analysis at 232 nm was used to confirm the absorption quantification.

Statistical analysis

Statistical studies were performed using SAS/Stat Version 6.04 software (SAS Insitute, Cary, NC USA). Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test. A probability level of 0.05 was selected as the point at which differences were considered significant. Data are presented as means ± SD.

Results

Consumption of *Dunaliella* powder or synthetic β-carotene without alcohol (groups C and E) had no effect on food intake, body weight, and liver or kidney relative weights (*Table 1*) compared with the control animals (group A). In contrast, alcohol consumption caused a reduction in both food intake and body weight in the control (group B) and in the rats fed β-carotene (groups D and F) from both sources (*Table 1*). There was no significant effect of alcohol intake on liver weight (data not shown). However, alcohol consumption resulted in increased relative liver weight. This is most likely due to the reduced body weight (*Table 1*) observed in the alcohol-consuming rats (groups B, D, and F).

Three of eight animals in the alcohol-consuming, synthetic β-carotene-fed group died during the last month of the feeding period. There were no other deaths in any of the other dietary groups.

Regarding total hepatic β-carotene content (*Table 2*), we demonstrated that regardless of consumption of alcohol, rats fed *Dunaliella bardawil* powder (groups C and D) accumulated significantly higher amounts of hepatic β-carotene than animals fed synthetic β-carotene (groups E and F). Although hepatic β-carotene content in the livers of algal-fed rats (group D) was not affected by alcohol consumption, there was a significant reduction in hepatic β-carotene

Table 1 Body weight (BW), food intake, and relative organ weights in rats consuming alcohol and fed diets supplemented with either *Dunaliella bardawil* powder or synthetic β-carotene for 3 months*

Dietary parameters			Body weight (g)	Food intake (g/day)	Carotene intake (mg/day)	Liver (g/100 g BW)	Kidney (g/100 g BW)
Group	Fluid	Carotene					
A	Water	None	243.6 ± 11.2[a]	18.0 ± 1.2[a]	None	3.68 ± 0.38[b]	0.85 ± 0.04[b]
B	Ethanol	None	216.6 ± 10.8[b,c]	10.5 ± 0.8[b]	None	4.14 ± 0.31[a]	0.99 ± 0.06[a]
C	Water	*Dunaliella*	240.2 ± 14.2[a]	17.9 ± 1.4[a]	179 ± 14	3.69 ± 0.31[b]	0.86 ± 0.08[b]
D	Ethanol	*Dunaliella*	204.2 ± 13.0[c]	9.9 ± 1.1[b]	198 ± 22	4.22 ± 0.46[a]	1.01 ± 0.06[a]
E	Water	Synthetic	239.5 ± 13.8[a]	18.3 ± 0.9[a]	183 ± 9	3.62 ± 0.24[b]	0.86 ± 0.04[b]
F	Ethanol	Synthetic	219.4 ± 12.4[b,c]	10.4 ± 0.4[b]	208 ± 8	4.31 ± 0.42[a]	0.98 ± 0.07[a]

*Values are means ± SD. Values in a column with different superscripts differ significantly ($P < 0.05$).

stores in the counterpart group fed synthetic β-carotene and alcohol (group F). The predominant isomer was the *all-trans* form, which represented 60 to 65% of total β-carotene (*Table 2*). The ratio between *9-cis* to *all trans* β-carotene in the liver was not affected by either ethanol-drinking or the dietary carotene source. Small amounts of α-carotene were detected in the livers of the algal-fed rats only.

β-Carotene content also was enhanced in the kidneys of rats fed both carotene sources. However, renal β-carotene levels were considerably lower in the alcohol-consuming, algal-fed rats (group D), whereas no effect of ethanol was demonstrated in animals fed the synthetic β-carotene (groups E and F; *Table 2*).

Total hepatic vitamin A content was enhanced by the supplementation of carotene from the two sources, both in water- (groups C and E) and in alcohol-consuming (groups D and F) rats (*Table 3*). However, hepatic vitamin A stores were significantly lower in the alcohol-fed rats than in their counterpart controls that consumed water. At this point we should emphasize that in alcohol-fed rats that were not supplemented with dietary carotene (groups A and B), hepatic vitamin A content may have been affected both by reduced food intake, which imposed a lower vitamin A intake, and by a direct alcohol effect on vitamin A metabolism. In fact, although food intake was reduced only by 41.7% (*Table 1*, group B vs. A), hepatic vitamin A content was reduced by as much as 52.8% (*Table 3*). Therefore, the net direct effect of alcohol consumption on vitamin A metabolism was expressed by an 11.1% reduction in hepatic vitamin A content. This effect was reflected by a 16.5% reduction in the main vitamin A storage compound retinyl palmitate (*Table 3*, group B vs. A).

Renal vitamin A content was affected by ethanol in a different way than that in the liver. Ethanol consumption significantly increased vitamin A content in the kidneys of rats fed *Dunaliella* or synthetic β-carotene (*Table 3*).

Ethanol consumption induced oxidative stress (*Figure 1*), as expressed by elevated amounts of hepatic conjugated dienes (group B). The ethanol-induced oxidative stress was counteracted by both carotene sources to the same extent (groups D and F). However, rats fed both β-carotene sources (groups C and E) demonstrated a lower hepatic oxidative state than the water-drinking control animals (group A), with the algal source being more effective than the synthetic one.

Discussion

Peroxidation processes occur constantly in the body throughout life. Peroxidation initiators may be produced in the body as a result of mitochondria, macrophage, or enzyme activity, or may enter the body via food, breath, or skin contact.[18] In the present study, lipid peroxidation was enhanced by alcohol consumption. It was suggested that ethanol or its metabolites alters homeostasis in the liver toward autooxidation, acting as prooxidants or causing a reduction in the body antioxidants level.[19] The effects of synthetic or *Dunaliella bardawil* β-carotenes in counteracting hepatic peroxidation was evaluated using a rat model, either under normal nutritional conditions or alcohol-in-

Table 2 Hepatic and renal β-carotene contents and isomer composition in rats consuming alcohol and fed diets supplemented with either *Dunaliella bardawil* powder or synthetic β-carotene for 3 months*

Dietary parameters			Hepatic β-carotene				Hepatic α-carotene	Renal β-carotene
Group	Fluid	Carotene	Total	*all trans*	*9-cis*	*9-cis/all trans*	Total	Total
A	Water	None	ND	ND	ND	None	ND	1.52 ± 0.38[c]
B	Ethanol	None	ND	ND	ND	None	ND	1.70 ± 0.43[c]
C	Water	*Dunaliella*	107.58 ± 15.57[a]	70.35 ± 13.,81[a]	21.07 ± 11.99[a,b]	0.299	4.41 ± 2.34	9.18 ± 0.73[a]
D	Ethanol	*Dunaliella*	132.30 ± 25.24[a]	78.98 ± 17.95[a]	23.04 ± 9.92[a]	0.292	5.56 ± 1.20	6.48 ± 0.89[b]
E	Water	Synthetic	61.66 ± 10.11[b]	37.04 ± 7.89[b]	10.38 ± 3.64[b,c]	0.280	ND	6.23 ± 1.26[b]
F	Ethanol	Synthetic	38.32 ± 6.44[c]	20.11 ± 3.03[c]	5.73 ± 3.59[c]	0.285	ND	6.48 ± 0.89[b]

*Values are means ± SD. All measurements are in μg/g dry weight. Values in a columm with different superscripts differ significantly ($P < 0.05$). ND, not determined.

Table 3 Hepatic and renal vitamin A and several retinol esters contents in rats consuming alcohol and fed diets supplemented with either *Dunaliella bardawil* powder or synthetic β-carotene for 3 months*

Dietary parameters			Hepatic vitamin A						Renal vitamin A
Group	Fluid	Carotene	Total	Retinol	Retinyl oleate	Retinyl linoleate	Retinyl stearate	Retinyl palmitate	Total
A	Water	None	2,467.5 ± 243.9^{c}	38.9 ± 16.9^{b}	170.8 ± 28.7^{b}	82.1 ± 21.4^{c}	44.8 ± 13.1^{c,d}	1,674.1 ± 129.6^{c}	4.67 ± 1.04^{d}
B	Ethanol	None	1,164.7 ± 198.4^{d}	20.0 ± 14.1^{b}	123.3 ± 21.6^{b}	105.7 ± 12.0^{c}	20.2 ± 10.5^{d}	700.9 ± 159.6^{d}	7.40 ± 0.79^{b}
C	Water	*Dunaliella*	7,011.9 ± 315.0^{a}	98.4 ± 6.6^{a}	379.5 ± 32.7^{a}	226.8 ± 15.1^{b}	117.9 ± 46.2^{a}	4,777.9 ± 428.2^{a}	6.13 ± 1.22^{c}
D	Ethanol	*Dunaliella*	5,464.9 ± 625.6^{b}	37.1 ± 6.8^{b}	393.9 ± 64.3^{a}	362.1 ± 41.5^{a}	63.1 ± 6.4^{b,c}	3,041.6 ± 445.8^{b}	8.72 ± 0.48^{a}
E	Water	Synthetic	7,019.4 ± 569.9^{a}	94.2 ± 17.0^{a}	403.0 ± 9.0^{a}	277.9 ± 11.7^{b}	83.4 ± 10.8^{b}	4,548.6 ± 290.8^{a}	5.53 ± 0.70^{c,d}
F	Ethanol	Synthetic	4,759.7 ± 562.8^{b}	27.5 ± 8.6^{b}	424.1 ± 48.4^{a}	389.2 ± 99.6^{a}	22.9 ± 2.7^{d}	2955.0 ± 369.3^{b}	7.99 ± 0.54^{a,b}

*Values are means ± SD. All measurements are given in μg/g dry weight. Values in a column with different superscripts differ significantly ($P < 0.05$).

duced oxidative stress. Because rats do not accumulate β-carotene in tissues when fed at physiologic levels,[20] high dietary levels of β-carotene were employed. Although very high amounts (1–2 g/kg diet) of synthetic or algal β-carotene were incorporated into the experimental diets, plasma levels of β-carotene were found to be very low (data not shown). In contrast, a marked hepatic accumulation of β-carotene was observed. These results are in agreement with previous rat studies in which synthetic[21] or algal[14] β-carotene were employed. Among the water-drinking animals, the algal-fed rats exhibited a higher hepatic β-carotene content than rats fed diets supplemented with the synthetic β-carotene (*Table 2*), which is in agreement with our previous studies.[14,15] However, although alcohol consumption significantly reduced hepatic β-carotene content in the synthetic β-carotene-fed rats, the enhanced hepatic β-carotene stores in the *Dunaliella*-fed rats were also evident in the alcohol-consuming rats. These results do not agree with a recent study[8] in which ethanol consumption increased hepatic stores of β-carotene in rats fed synthetic β-carotene. The discrepancy between these investigations might stem from the differences in experimental conditions prevailing in the two studies, especially the mode by which ethanol was administered. In the study cited above,[8] the alcohol was given in a liquid diet that contained all the dietary ingredients as well as the carotene source. However, in the present study, alcohol was administered in the drinking water, and the rest of the dietary components, including each of the carotene sources, were supplied in the form of a powdered diet. In addition, the amount of β-carotene consumed by the experimental animals in the present study was approximately 80-fold higher than that administered in the previously mentioned study.[8]

Three of eight rats in the alcohol-drinking, synthetic β-carotene-fed group died during the last month of the feeding period. There were no other deaths in any of the other groups. These results are in agreement with a study that suggested that synthetic β-carotene probably potentiates the toxicity induced by chronic alcohol consumption.[8] The mortality observed in the present study further supports this concept and probably represents an intensified effect caused by the high dietary amounts of the synthetic product, but not by the *Dunaliella* natural isomer mixture. The difference between the two carotene sources might be due in part to the higher availability[14,15] and the superior antioxidative properties of the algal source compared with the synthetic one, as was previously demonstrated.[17]

Several studies demonstrated that chronic alcohol con-

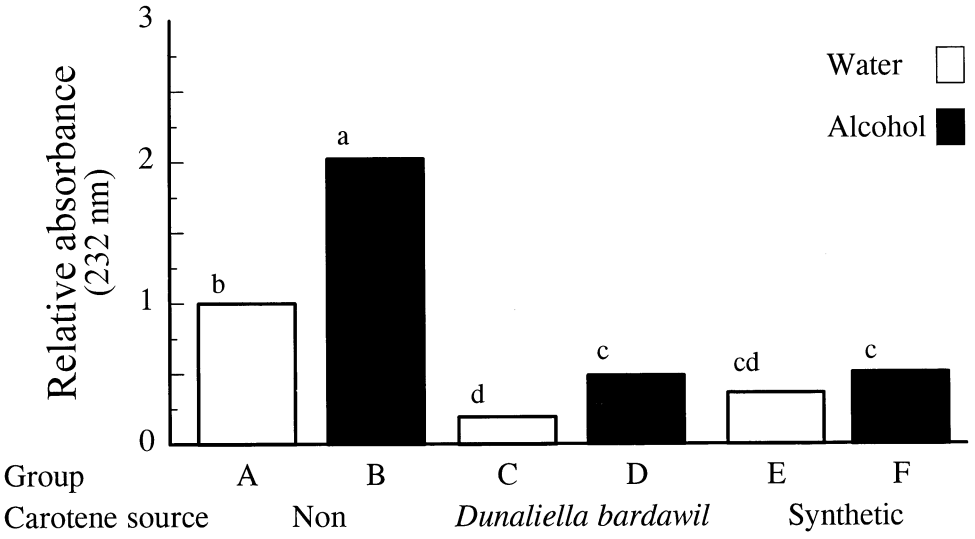

Figure 1 Conjugated dienes expressed as relative absorbance at 232 nm in the livers of rats consuming alcohol and fed for 3 months diets supplemented with either *Dunaliella bardawil* powder or synthetic β-carotene. Values with different letters differ significantly ($P < 0.05$).

sumption results in a decrease in hepatic vitamin A content. It has been suggested[22] that the extent of hepatic vitamin A reduction correlates with the severity of fatty liver, which is one of the earliest pathologic manifestations of alcohol consumption. A liquid alcohol diet with a high fat content (35% of total calories) caused fatty liver that was accompanied by a 42% decrease in hepatic vitamin A content.[5] However, when a liquid diet containing smaller amounts of fat (16.8% of total calories) was used, total hepatic lipid content was not significantly increased with alcohol feeding and only a 35.5% decrease in hepatic vitamin A stores was observed.[22] In the present study, we employed a low-fat diet (approximately 7% of total calories), and when food intake in the ethanol-consuming animals was taken into account, alcohol was found to be responsible for a reduction of only 11.1% in total hepatic vitamin A compared with the control rats (*Table 3*). These results are in agreement with the suggestion that hepatic vitamin A content correlates with dietary fat levels when alcohol is consumed.[22]

Reduced hepatic vitamin A stores may be attributed in part to an alcohol-induced mobilization of vitamin A from the liver to extra hepatic tissues, as has been suggested.[5,22] Our results, which demonstrate a significant increase in renal vitamin A content in the alcohol-fed rats (*Table 3*), support the occurrence of this mechanism. The reduction in hepatic vitamin A also may result from the oxidative stress imposed on the liver by chronic alcohol drinking, as was shown with rats fed oxidized oil.[23]

The direct effect of chronic ethanol consumption on lowering hepatic vitamin A stores was estimated also in rats fed carotene-supplemented diets. Feeding algal β-carotene was found to maintain vitamin A levels to a greater extent than the synthetic β-carotene source. These conclusions were based on the following: In water-consuming rats, supplementation with both carotene sources increased hepatic vitamin A level to the same extent, reaching a level of approximately 7,000 μg/g liver. When the reduced food intake observed in the alcohol-drinking animals is considered, the hepatic vitamin A calculated content should have reached only 3,878 and 3,989 μg/g liver in the algal or the synthetic β-carotene-fed rats, respectively. However, it seems likely that the additional vitamin A amounts of 1,587 μg/g liver found in the algal-fed rats and 771 μg/g liver in the synthetic carotene-fed animals were contributed by each of the carotene sources. Thus, we concluded that under dietary alcohol stress the algal β-carotene can be considered a better dietary source for vitamin A than the synthetic β-carotene. This preferential characteristic also might result from the greater bioavailability of algal β-carotene[14] and its higher efficiency as an antioxidant.[17]

The main purpose of our study was to evaluate the in vivo antiperoxidative potential of *Dunaliella bardawil* β-carotene compared with a synthetic β-carotene source. Therefore, we measured the extent of hepatic lipid peroxidation under oxidative stress induced by alcohol consumption. As already indicated by several investigators,[24] we also failed to observe an increase in the content of hepatic thiobarbituric acid reactive substances following ethanol administration (data not shown). These findings substantiate the criticism regarding the possibility to rely on the determination of thiobarbituric acid reactive substances as the sole index of hepatic lipid peroxidation.[24] Indeed, when the levels of hepatic conjugated dienes were assessed (*Figure 1*), both carotene sources were shown to ameliorate the enhanced ethanol-induced peroxidative stress. Carotene-free, ethanol-fed rats demonstrated significantly higher levels of hepatic conjugated dienes than their counterpart controls. However, when carotene was added to the diets of the alcohol-fed rats, hepatic conjugated dienes were significantly lowered, to the same extent, by both the algal and the synthetic β-carotene sources. Based on the results of the present study, the two β-carotene sources could be considered comparable efficient antioxidants. It is known that the antiperoxidative effect of carotenoids is due to their interaction with free radicals, which leads to the destruction of these molecules.[25,26] Thus, the drastic reduction in the amount of hepatic β-carotene content observed in alcohol-fed rats supplemented with synthetic β-carotene compared with their respective water-drinking counterparts is probably due to this antiperoxidative action. In contrast, no reduction in the level of hepatic β-carotene due to alcohol consumption was demonstrated in *Dunaliella*-fed animals (*Table 2*). The ability to maintain the high level of hepatic β-carotene stores points to the possibility that the algal β-carotene is a more efficient antioxidant than the synthetic β-carotene. Such superior antioxidative beneficial potency was demonstrated previously in rats exposed to high oxygen pressure.[27] The present results are also in agreement with a previous study[17] in which peroxidation was induced by feeding oxidized oil, and a *Dunaliella* extract enriched with *9-cis* β-carotene was supplemented in the rats' diet. In that study the higher ability of the algal β-carotene to cope with oxidative stress compared with the synthetic product was explained by the presence of high levels of dietary *9-cis* β-carotene. It was suggested that due to higher affinity toward free radicals, the *9-cis* isomer possesses a better antioxidative capacity than the *all-trans* β-carotene, and this in turn may result in its enhanced degradation and the reduction in the *9-cis* to *all-trans* isomer ratio. In the present study no reduction in this ratio was observed. It is conceivable that reduction in the *9-cis* to *all-trans* isomer ratio, which should have taken place due to the superior antioxidative properties of the *9-cis* isomer,[17] was masked by other metabolic changes induced by alcohol. Thus, the accumulation of β-carotene isomers in the liver is a net result of several metabolic pathways taking place in vivo. Although *all-trans* β-carotene is known to serve mainly as a vitamin A precursor, the potency of the *9-cis* isomer in this regard is very small.[28,29] Considering other metabolic pathways such as intestinal absorption, tissue uptake, and isomerization, little is known about the impact of these processes on the metabolism and accumulation of each of the two isomers. However, the difference in their extent, in general and especially under various metabolic conditions, including alcohol consumption, might be relevant to the resulting levels of hepatic total β-carotene and to the *9-cis* to *all-trans* β-carotene isomer ratio. Furthermore, the comparison between all the water-drinking rats revealed that *Dunaliella* powder possessed a better antiperoxidative capacity even under normal nutritional conditions (*Figure 1*). This is in agreement with a recent human study in which the prevention of peroxidation was measured in blood samples of

nonsmoking male volunteers who had common dietary habits.[16]

In conclusion, the results reported herein may be of relevance in light of the growing interest in the antiperoxidative and anticancer value of natural β-carotene sources compared with the synthetic product. In addition to the better antioxidative capacity of *Dunaliella* β-carotene compared with the synthetic source, the algal carotene may serve as a more efficient vitamin A precursor under alcohol-induced stress.

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