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High Dose Lycopene Supplementation Increases Hepatic Cytochrome P4502E1 Protein and Inflammation in Alcohol-Fed Rats[1,2]

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Abstract

Recent in vitro evidence suggests that the antioxidant lycopene can prevent alcohol-induced oxidative stress and inflammation. However, knowledge of possible interactions in vivo between escalating doses of lycopene and chronic alcohol ingestion are lacking. In this study, we investigated potential interactions between alcohol ingestion and lycopene supplementation and their effect on hepatic lycopene concentration, cytochrome P4502E1 (CYP2E1) induction, and inflammation. Fischer 344 rats (6 groups, $n = 10$ per group) were fed either a liquid ethanol Lieber-DeCarli diet or a control diet (isocaloric maltodextrin substituted for ethanol) with or without lycopene supplementation at 2 doses (1.1 or 3.3 mg·kg body weight^{-1}·d^{-1}) for 11 wk. Plasma and hepatic concentrations of lycopene isomers were assessed by HPLC analysis. We examined expressions of hepatic CYP2E1 and tumor necrosis factor-α (TNFα) and the incidence of hepatic inflammatory foci. Both plasma and hepatic lycopene concentrations were greater in alcohol-fed rats than in control rats supplemented with identical doses of lycopene. In contrast, alcohol-fed rats had a lower percentage of lycopene *cis* isomers in the plasma and the liver compared with control rats fed the same dose of lycopene. Notably, lycopene supplementation at the higher dose significantly induced hepatic CYP2E1 protein, TNFα mRNA, and the incidence of inflammatory foci in the alcohol-fed rats but not in the control rats. These data indicate an interaction between chronic alcohol ingestion and lycopene supplementation and suggest a need for caution among individuals consuming high amounts of both alcohol and lycopene.

Introduction

Chronic and excessive alcohol intake is associated with increased oxidative stress (1), a decrease in antioxidant enzymes (2), and induction of the cytochrome P4502E1 (CYP2E1)[3] enzyme (3,4). The absence of antioxidants has been shown to further promote alcohol-induced oxidative stress, which could lead to an inflammatory state (5). Chronic alcohol intake also induces bacterial endotoxin (6), which is associated with activation of the nuclear factor-κB inflammatory pathway, leading to the activation of several proinflammatory cytokines,

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[3]Abbreviations used: BW, body weight; CYP2E1, cytochrome P4502E1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TNFα, tumor necrosis factor α.

including tumor necrosis factor-α (TNFα), and increased hepatic infiltration by inflammatory cells. Whereas some antioxidants such as tea polyphenols are protective against alcohol-induced changes in the liver (7,8), others such as vitamin E have no beneficial effect (9).

Whereas the antioxidant properties of lycopene have been demonstrated both in vivo and in vitro (10), recent evidence suggests that it also functions as an antiinflammatory agent (11, 12). It has been demonstrated that lycopene can inhibit the expression of inflammatory cytokines and reverse the loss of antioxidant enzymes induced by inflammation caused by either injecting with lipopolysaccharide or by exposure to iron (11,13,14). Although the data for lycopene's role both as an antioxidant and an antiinflammatory agent in various models are compelling, such effects have not been demonstrated in an in vivo model of alcohol-induced oxidative stress and inflammation. One human study has shown that lycopene was inversely associated with concentrations of alcohol-induced γ-glutamyl-transferase, an indicator of liver damage, in moderate and heavy drinkers (15). An in vitro study demonstrated that in addition to decreasing alcohol-induced oxidative stress, lycopene could also ameliorate alcohol-modified hepatic biomarkers (16). In that study, incubating hepatic HepG2 cells that overexpressed CYP2E1 with lycopene resulted in decreased alcohol-induced apoptosis and hydrogen peroxide formation and restoration of mitochondrial glutathione levels (16). However, no in vivo studies have examined the effect of lycopene on alcohol-induced oxidative stress and inflammation, particularly with regard to dose responsiveness.

The goal of this study was to investigate potential interactions between alcohol feeding and lycopene supplementation in vivo and to determine the effects of escalating doses of lycopene on alcohol-induced hepatic biomarkers.

Materials and Methods

Animal study

Sixty male Fischer 344 rats (2 mo old, weighing 130–140 g) were obtained from Charles River Laboratories and were housed individually in cages in an American Association of Accreditation of Laboratory Animal Care accredited animal facility at the Human Nutrition Research Center on Aging at Tufts University. The rats were housed in a room with controlled temperature (68–72°F), humidity (45–55%), and light (12-h-light/-dark cycles). They were acclimatized over 1 wk on a nonpurified diet (Harlan Teklad LM-485 diet), the composition of which has been previously described (17). The rats were then randomly divided into 6 groups: control + placebo, control + low dose lycopene, control + high dose lycopene, alcohol + placebo, alcohol + low dose lycopene, and alcohol + high dose lycopene and were adapted to the nutritionally adequate Lieber-DeCarli liquid control and alcohol diets (Dyets) over a 2-wk period. The composition of the diets used has been previously published (18); both diets provided 18% of energy from protein and 35% from fat. The control diet provided 47% of energy from carbohydrates. In the ethanol diet, 36% of energy was provided by ethanol and the remaining 11% was provided by carbohydrates. The amount of alcohol ingested in rats fed the ethanol diet is approximately equal to an intake of 100 g/d of alcohol in a human consuming a 2000-kcal (8.4 MJ) diet (varies from 80 to 150 g/d of alcohol depending on energy intake). Neither the control nor the alcohol placebo diets contained lycopene. For dietary lycopene supplementation, water soluble lycopene beadlets, containing 10% wt:wt lycopene (BASF) were blended into the liquid diets [1.1 mg·kg body weight $(BW)^{-1}\cdot d^{-1}$ or 3.3 mg·kg $BW^{-1}\cdot d^{-1}$] and were fed to the rats. The placebo beadlets (BASF) contained the same constituents as the lycopene beadlets except the lycopene. The lower dose (1.1 mg·kg $BW^{-1}\cdot d^{-1}$) is equivalent to a 15 mg/d intake in a 70-kg man based on our previous studies in ferrets (19), and the observed similarity between rats and ferrets in terms of lycopene absorption (20). This low dose is above the average intake of lycopene (9.4 ± 0.3 mg/d) in humans (21). The higher dose (3.3 mg·kg $BW^{-1}\cdot d^{-1}$) is equivalent to a 45 mg/d intake in a 70-kg man and

is achievable by using supplements. For the period of the study, the rats were group pair-fed to the alcohol + placebo group with their respective diets and were given ~70 mL/d of the diet. The diets were prepared twice per week and were stored at 4°C in opaque bottles to prevent degradation of the lycopene. BW were recorded once per week for the period of the study. After 11 wk of treatment, the rats were killed by terminal exsanguination under deep anesthesia. Blood and livers were collected for future analyses. This study protocol was reviewed and approved of by the Animal Care and Use Committee at the Human Nutrition Research Center on Aging at Tufts University.

HPLC analyses

Liver and plasma concentrations of all-*trans* and *cis* isomers (5-*cis*, 13-*cis*, and 9-*cis*) of lycopene were determined by HPLC. A Water's separation system fitted with a C30 column was used to separate lycopene and its isomers. The HPLC system, the mobile phase, and the gradient procedures used have been previously described (22). Liver tissue (100 mg) or plasma (1 mL) was homogenized in 3 mL of saline and ethanol (1:2 ratio). Lycopene was then extracted from the samples using 5 mL of hexane and ether (1:1 ratio) by vortexing for 3 min, centrifuging at 2000 × *g* for 10 min at 4°C, and collecting the upper layer. Samples were extracted 3 times and were evaporated under nitrogen gas, after which they were reconstituted with 100 μL of ethanol and ether (2:1). A 50-μL sample of the final extract was injected into the system to measure lycopene isomer concentrations. In this HPLC system, 13-*cis* lycopene, 9-*cis* lycopene, all-*trans* lycopene, and 5-*cis* lycopene were eluted at 28.4, 32.0, 35.0, and 35.3 min, respectively. Retinyl acetate and echinenone were used as internal controls to determine the efficiency of extraction. Extraction efficiency > 80% was considered adequate to calculate concentrations of all-*trans*, 13-*cis*, 9-*cis*, and 5-*cis* lycopene. All procedures were conducted under red light.

Western blotting

Tissue homogenates were prepared from frozen liver tissue by homogenizing with ice-cold, whole-cell lysate buffer containing inhibitors. Protein concentrations were determined by spectrophotometry using Coomassie Blue and proteins in the tissue homogenates were resolved on a 10% SDS polyacrylamide gel, after which they were transferred onto nitrocellulose membranes. The membranes were blocked using 5% milk and were incubated with the CYP2E1 primary antibody (Chemicon) for 2 h at room temperature followed by the secondary antibody for 1 h at room temperature. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon) was used as a loading control. Results were quantified using a densitometer and are expressed as fold of the control group.

Histopathology

Liver sections were fixed in buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for examination. The number of clusters of infiltrating mononuclear cells (foci of inflammation) on each slide (2 liver sections) was examined at both 10× and 40× magnification and counted by 2 independent investigators who were unaware of the treatment. The mean number of clusters in each group was compared.

Real-time PCR

mRNA levels of TNFα were determined by real-time quantitative PCR. Liver RNA was extracted using TriPure reagent (Roche Applied Science) according to the manufacturer's instructions and cDNA was synthesized using random primer Moloney murine leukemia virus reverse transcriptase (Invitrogen). Primers for rat TNFα and GAPDH were designed using the Primer Express version 2.0 (Applied Biosystems) software. The sequences for TNFα (accession no. NM-012675.2) were as follows: sense, 5′-CCAGACCCTCACACTCA-

GATCA-3′ and antisense, 5′-TCCGCTTGGTGGTTTGCTA-3′. The sequences for GAPDH (accession no. NM-017008.2) were as follows: sense, 5′-AGTGCCAGCCTCGTCTCATAG-3′ and antisense, 5′-CCTTG-ACTGTGCCGTTGAACT-3′. Real-time PCR was performed using the SYBR Green qPCR kit (Invitrogen) according to the manufacturer's instructions on an Applied Biosystems 7000 sequence detection system. Gene expression was quantified relative to the values of the control group after adjusting for GAPDH by the $2^{-\Delta\Delta CT}$ method as described previously (23). Results were expressed as fold of the control group.

Statistical analyses

SPSS (version 14.0) and Graph Pad PRISM (version 3.0) software were used for statistical analyses. Samples identified as outliers by the SPSS statistical software were excluded from the analyses. Two-way ANOVA and trend analyses were used to analyze the data. After determining the presence of significant interactions between alcohol feeding and lycopene supplementation for hepatic lycopene concentrations and all the biomarkers assessed, we used 1-way ANOVA followed by Tukey's honestly significant difference test to test for differences between means of multiple groups for results discussed in this study. Differences between means were considered significant at $P < 0.05$. Results are expressed as means ± SEM.

Results

Body and liver weights

After 11 wk of treatment, the alcohol-fed rats had significantly lower BW and liver weights than the control groups (Fig. 1) as previously documented (24). Lycopene supplementation at the 2 doses did not affect either BW or liver weight. The liver weight:BW ratio was constant between the groups (ranging from 0.029 to 0.031), indicating that decreased liver weight was consistent with the decreased BW in the alcohol-fed rats.

Plasma and liver lycopene concentrations

Lycopene isomers were detected in both the plasma and liver of rats supplemented with lycopene but not in the rats supplemented with the placebo. Although we detected only the all-*trans* and 5-*cis* isomers in the plasma, the liver contained the all-*trans*, 5-*cis*, 9-*cis*, and 13-*cis* isomers of lycopene. In the plasma, we observed a significant dose-dependent increase in lycopene isomer concentrations in alcohol-fed rats (Table 1). In the liver, however, a similar increase was observed in both the control and the alcohol-fed rats (Table 1). Plasma lycopene concentrations increased 48 and 127% (low dose and high dose, respectively) in rats that were fed alcohol vs. rats fed the control diet with the same doses of lycopene. Similarly, total hepatic lycopene concentrations increased 74 and 157% (low dose and high dose, respectively) in rats that were fed alcohol vs. rats fed the control diet with the same doses of lycopene. Both plasma and hepatic lycopene isomer concentrations analyzed by 2-way ANOVA analyses showed that the interaction between alcohol feeding and lycopene supplementation was significant for all the lycopene isomers detected ($P < 0.05$; Table 1). This difference was supported by significant differences between means using 1-way ANOVA analyses and by adjusting for multiple comparisons using Tukey's honestly significant difference test (Table 1).

Based on our HPLC analyses, the lycopene beadlet supplement contained 75 ± 3% of lycopene as the all-*trans* isomer and the remaining 25 ± 3% was present as *cis* isomers. In the livers of rats supplemented with lycopene, however, we detected a higher proportion of lycopene *cis* isomers (between 38 and 61%) compared with what was provided in the diet (Table 1). In the lycopene-supplemented control groups, the percentage of *cis* isomers of lycopene was greater than or almost equal to that of the all-*trans* isomers. In the alcohol-fed rats, however, the percentage of *cis* isomers was less than the all-*trans* isomers of lycopene and was lower than

that detected in the control rats supplemented with the same dose of lycopene. In both the control and alcohol-fed groups, the percentage of lycopene *cis* isomers decreased with increasing lycopene dose in the liver but not in the plasma (Table 1). Apo-8′- and 12′-lycopenals, metabolites of lycopene, were previously detected in the livers of lycopene-supplemented rats (25). In our study, we did not detect apo-8′-, 10′-, or 12′-lycopenals in the liver tissue of rats after lycopene supplementation, although some unidentified lycopene metabolites were detected during HPLC analyses (retention time between 11.6 and 12.2 min).

Hepatic CYP2E1 protein

We assessed the effect of alcohol and lycopene supplementation on hepatic protein levels of CYP2E1. Alcohol feeding significantly increased expression of CYP2E1 compared with control rats (Fig. 2) as previously documented (26). Lycopene supplementation alone did not affect CYP2E1 expression. However, in the presence of alcohol, we observed a trend of increasing CYP2E1 protein expression with an increasing dose of lycopene (*P*-trend = 0.003). High dose lycopene with alcohol feeding increased hepatic CYP2E1 expression compared with rats fed alcohol alone without lycopene (*P* of interaction between alcohol feeding and lycopene supplementation = 0.005).

Hepatic inflammation

Because chronic alcohol intake is associated with significantly increased hepatic TNFα expression and inflammation (27), we assessed hepatic levels of TNFα mRNA (Fig. 3). We did not detect any significant increase of hepatic TNFα mRNA in alcohol-fed rats supplemented with placebo or low dose lycopene vs. control rats treated similarly. However, lycopene supplementation at the high dose induced the expression of TNFα in the liver of alcohol-fed rats compared with the other groups (*P* of interaction between alcohol feeding and lycopene supplementation = 0.032). Lycopene supplementation alone, in the absence of alcohol, at either dose did not affect TNFα expression. We further investigated the effects of lycopene supplementation on hepatic infiltration by mononuclear inflammatory cells (Fig. 4). Alcohol feeding or lycopene supplementation alone did not affect the incidence of hepatic inflammatory foci. However, in the presence of alcohol, high dose lycopene supplementation was associated with an increased incidence of inflammatory foci in the liver (*P* of interaction between alcohol feeding and lycopene supplementation = 0.036).

Discussion

This study clearly shows an interaction between in vivo lycopene supplementation and alcohol intake in a rat model and indicates the need for caution among alcohol drinkers who also consume high amounts of supplementary lycopene. In the present study, we tested 2 doses of lycopene (1.1 and 3.3 mg·kg $BW^{-1}\cdot d^{-1}$), which are approximately equivalent to 15 mg and 45 mg/d lycopene in a 70-kg adult man. The average American diet provides ~9.4 mg/d of lycopene (21); therefore, both the experimental doses were higher than the usual intake. However, these doses are currently present in dietary supplements and are being tested in prostate cancer clinical trials (28–30), emphasizing the importance of our observations from the current animal study. Furthermore, the hepatic lycopene concentrations that we detected (1.07–7.30 nmol/g) in the rats are within the range normally seen in humans (0.1–20.7 nmol/g) (31,32), indicating that the interaction between chronic alcohol intake and supplementary lycopene and their combined effect on hepatic biomarkers takes place at physiologically relevant tissue concentrations of lycopene. Plasma concentrations in the rats (3.82–12.22 nmol/L), however, were much lower than those observed in humans (260–900 nmol/L) (33).

Alcohol-fed rats accumulated more lycopene than control animals supplemented with the same dose of lycopene. Interestingly, chronic alcohol intake is also associated with increased plasma

(34) and hepatic concentrations (35) of another carotenoid, *β*-carotene. The accumulation of *β*-carotene in the liver has been attributed to the inhibition of its catabolism to retinoids by chronic alcohol intake (36). In the case of lycopene, however, the mechanism(s) involved is unknown at present. Multiple mechanisms could be involved; e.g. alcohol intake could increase the solubility and/or bioavailability of lycopene for absorption, or it could decrease lycopene catabolism. Recently, we have shown that the carotene 9′,10′-oxygenase can cleave *cis* isomers of lycopene into apo-10′-lycopenoid metabolites (37) and other investigators have reported apo-8′-and 12′-lycopenals in rat livers after lycopene supplementation (25). However, we did not detect any apolycopenoids in the livers of rats with lycopene supplementation and observed no changes in hepatic carotene 9′,10′-oxygenase protein levels or in the formation of the unidentified polar metabolites in alcohol-fed rats vs. the control rats (S. Veeramachaneni and X-D. Wang, unpublished data). It is, therefore, unlikely that alcohol altered lycopene degradation, suggesting that the increased concentrations of lycopene in vivo might result from enhanced absorption in the gut due to alcohol ingestion. However, this hypothesis needs to be addressed in future studies using intestinal perfusion models as previously described (38).

Though the lycopene beadlet predominantly contained all-*tran*s lycopene, the rat livers had a higher accumulation of *cis* lycopene compared with that in the diet. This observation is similar to what is normally seen in humans, where higher concentrations of lycopene *cis* isomers are detected in the body although dietary lycopene is predominantly in the all-*trans* form (39). In the current study, the percentage of *cis* isomers of lycopene decreased in the livers, but not in the plasma, with increasing lycopene dose (Table 1) in both the control and the alcohol-fed groups. This suggests that the ratio of *cis*/*trans* lycopene isomers in the circulation may not be reflective of isomerization of the all-*trans* isomer to *cis* isomers in the liver. Interestingly, the alcohol-fed rats had a lower percentage of *cis* lycopene accumulation than the control rats fed the same dose of lycopene. It is unknown whether this decrease in *cis* lycopene accumulation is a result of either decreased absorption of *cis* isomers in the gut or decreased isomerization of the all-*trans* isomer to *cis* isomers by chronic alcohol ingestion.

Chronic alcohol ingestion can induce the levels of CYP2E1 (26), which plays an important role in metabolizing excess amounts of alcohol in the liver via the microsomal ethanol oxidizing system (26). During this process, CYP2E1 generates several reactive oxygen species, including hydrogen peroxide and superoxide, leading to increased oxidative stress (1,3,40). Reactive oxygen species generated by CYP2E1 induce mitochondrial damage and induce cell membrane damage by increasing lipid peroxidation and lipid aldehyde formation. Alcohol regulates CYP2E1 levels both by inducing its transcription (41) and by stabilizing the protein against degradation (42,43). In the present study, in addition to the expected induction of CYP2E1 in response to alcohol treatment, we observed a trend of increased CYP2E1 protein with increasing lycopene dose in the alcohol-fed rats. This increase was significantly greater when lycopene was supplemented at the high dose in rats compared with the rats that were supplemented with the placebo. Although the mechanisms for these results are currently unknown, our observation suggests that lycopene supplementation at a high dose may potentiate the harmful effects of excessive alcohol intake. This notion is further supported by the following observations. First, it has been previously documented that chronic alcohol intake was associated with increased incidence of inflammatory foci in the livers of alcohol-fed rats compared with control rats (44,45). Although we observed no significant increase in inflammatory foci with alcohol feeding, we found that rats fed both alcohol and high dose lycopene had a significantly higher incidence of hepatic inflammatory foci compared with the other groups. Second, previous evidence has shown that alcohol induces the expression of TNF*α* in rats (27), and patients with alcoholic liver disease have increased levels of several proinflammatory cytokines, including TNF*α* (46). In our model, hepatic TNF*α* mRNA did not differ between the control and alcohol-fed groups supplemented with placebo. This is in agreement with earlier in vivo data showing no significant differences in TNF*α* expression in

the livers of mice fed control or alcohol Lieber-DeCarli liquid diets (47). However, our observations in Fischer rats are contradictory to findings from another study using alcohol-fed Sprague-Dawley rats (27). Although the exact reasons for the differences in response to alcohol feeding are not known, it is possible that different strains of rats react differently to chronic alcohol feeding. Rats fed both alcohol and high dose lycopene had significantly higher expression of TNFα compared with all other groups. Interestingly, rats fed alcohol and low dose lycopene (equal to one-third of the dose supplemented to the high dose groups) had no significant induction of TNFα expression. Lycopene has been shown to act as a prooxidant or an antioxidant depending on the dose at which it is administered (48,49). In this study, although neither dose was protective, lycopene supplementation at the higher dose had a more pronounced effect on alcohol-related changes compared with the lower dose. Further studies are needed to understand both the interaction between alcohol and lycopene and the mechanisms by which high doses of lycopene, in the presence of alcohol, modulate hepatic inflammation and CYP2E1 expression.

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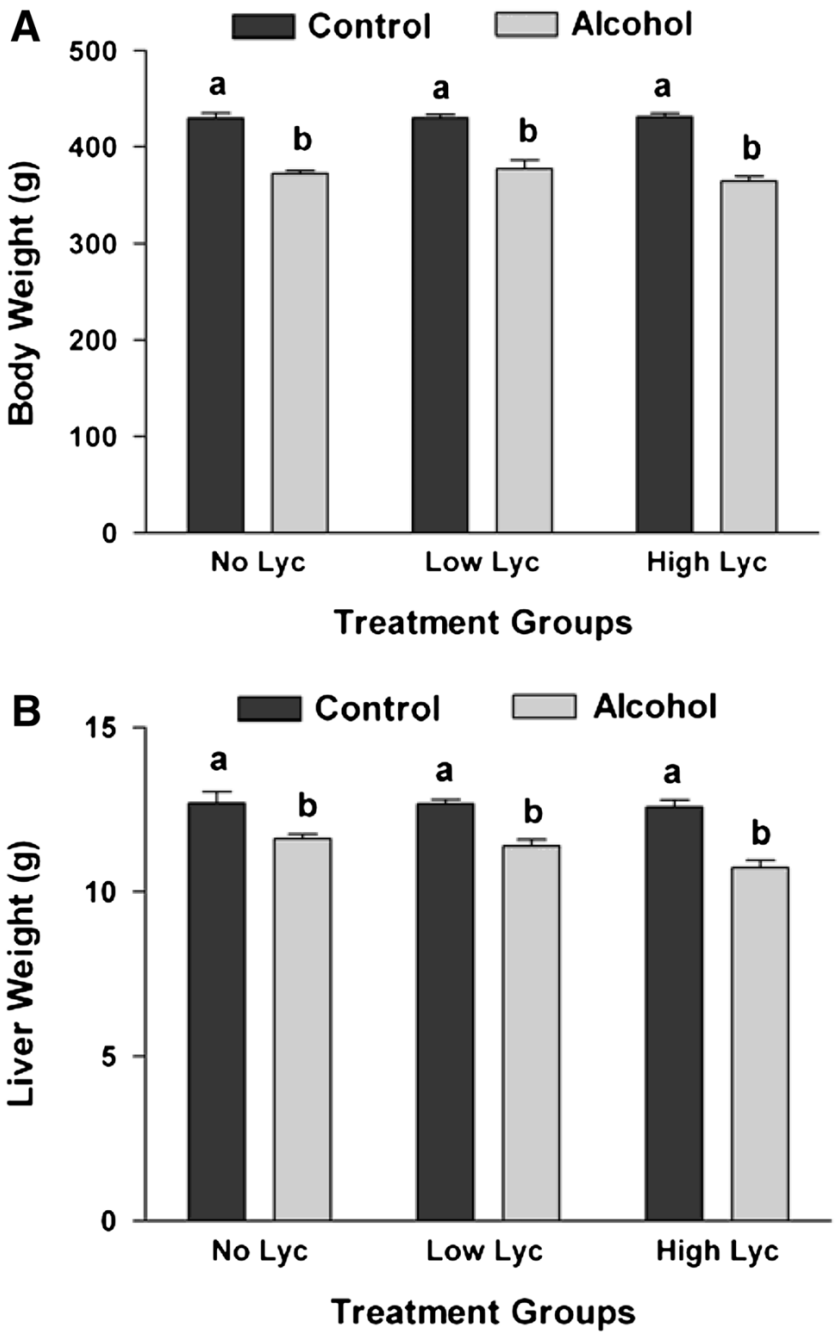

Figure 1.
BW (*A*) and liver weight (B) of rats after 11 wk of feeding either control or alcohol diets with or without lycopene (Lyc) supplementation. Values are means ± SEM, $n = 10$. Bars without a common letter differ, $P < 0.05$.

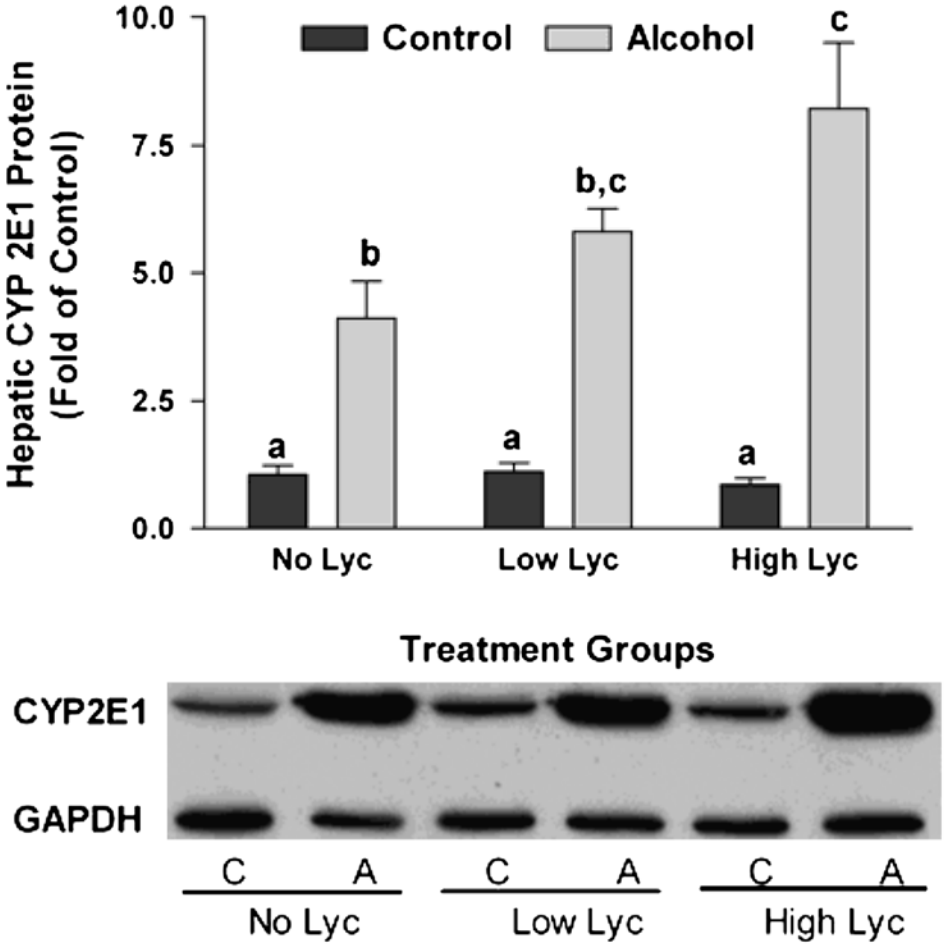

Figure 2.
Induction of hepatic CYP2E1 protein in rats after 11 wk of feeding either control (C) or alcohol (A) diets with or without lycopene (Lyc) supplementation. Representative western blot of CYP2E1 and GAPDH protein expression (inset). Values are means ± SEM, $n = 10$. Bars without a common letter differ, $P < 0.05$.

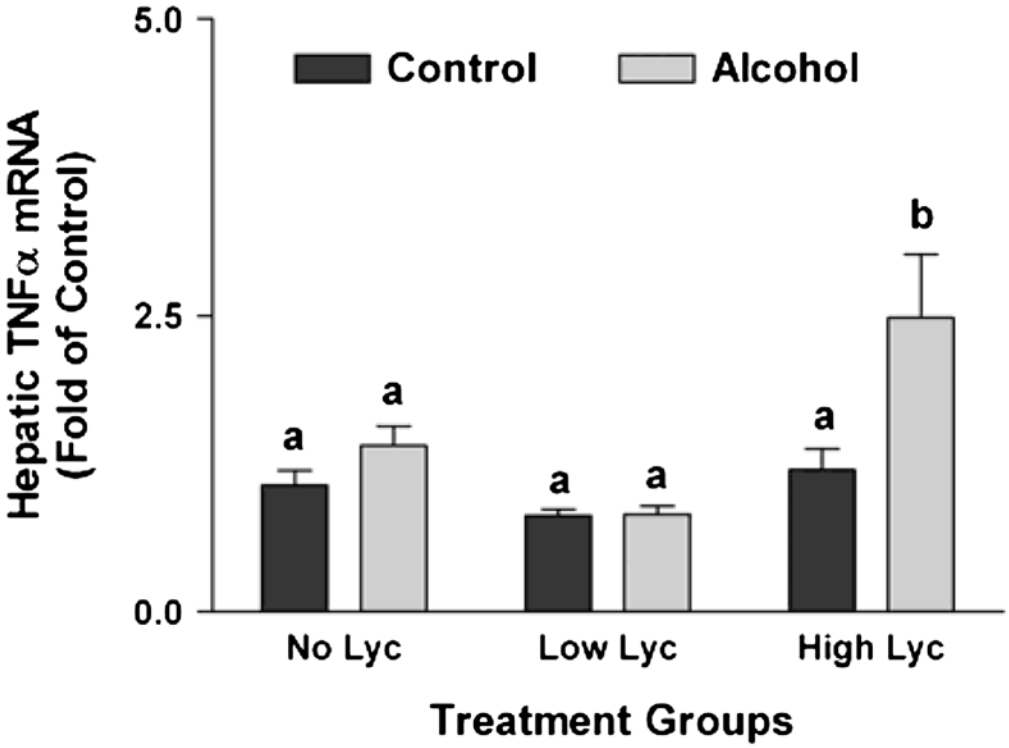

Figure 3.
Expression of TNF*α* mRNA in the livers of rats after 11 wk of feeding either control or alcohol diets with or without lycopene (Lyc) supplementation. Values are means ± SEM, $n = 10$. Bars without a common letter differ, $P < 0.05$.

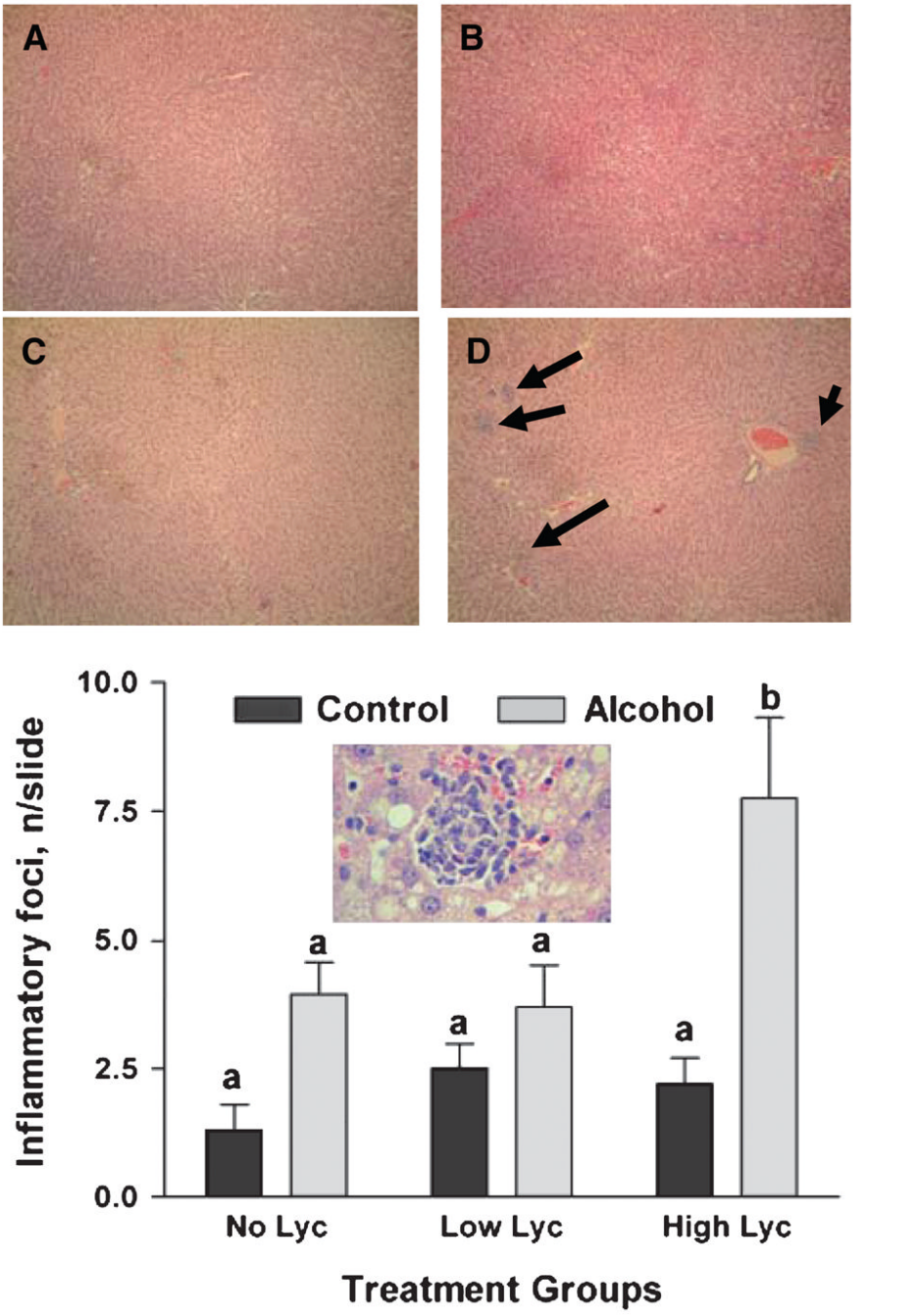

Figure 4.
Incidence of inflammatory foci in the livers of rats after 11 wk of feeding either control or alcohol diets with or without lycopene (Lyc) supplementation. Values are means ± SEM, n = 10. Bars without a common letter differ, $P < 0.05$. Upper panel: Representative image of hepatic inflammatory foci (arrows) from the control (*A*), control + high lycopene (*B*), alcohol (*C*), and alcohol + high dose lycopene group (*D*) at 10× magnification and 40× magnification (inset).

TABLE 1

Plasma and hepatic lycopene isomer concentrations in rats fed diets with or without alcohol and lycopene for 11 wk[1–4]

	Control		Alcohol		*P* of main effects		
	Low Lyc	High Lyc	Low Lyc	High Lyc	Alcohol	Lyc	*P* of interaction
Plasma, nmol/L							
Total lycopene	3.82 ± 0.67[a]	5.37 ± 0.61[a]	5.67 ± 0.66[a]	12.22 ± 1.36[b]	<0.0001	<0.0001	0.0068
All-*trans*	1.75 ± 0.36[a]	2.27 ± 0.29[a]	3.12 ± 0.48[a]	6.45 ± 0.63[b]	<0.0001	0.0001	0.0038
All-*trans*, %	46	42	55	53			
5-*Cis*	2.07 ± 0.33[a]	3.10 ± 0.34[a]	2.55 ± 0.28[a]	5.77 ± 0.79[b]	0.0022	<0.0001	0.0276
5-*Cis*, %	54	58	45	47			
Liver, *nmol/g*							
Total lycopene	1.07 ± 0.10[a]	2.84 ± 0.24[b]	1.87 ± 0.18[a]	7.30 ± 0.31[c]	<0.0001	<0.0001	<0.0001
All-*trans*	0.42 ± 0.05[a]	1.47 ± 0.16[b]	1.00 ± 0.10[b]	4.50 ± 0.21[c]	<0.0001	<0.0001	<0.0001
All-*trans*, %	39	52	54	62			
5-*Cis*	0.34 ± 0.03[a]	0.90 ± 0.08[b]	0.51 ± 0.05[a]	1.98 ± 0.09[c]	<0.0001	<0.0001	<0.0001
9-*Cis*	0.15 ± 0.01[a]	0.23 ± 0.01[b]	0.15 ± 0.01[a]	0.34 ± 0.01[c]	0.0009	<0.0001	0.0016
13-*Cis*	0.16 ± 0.01[a]	0.25 ± 0.01[b]	0.20 ± 0.01[a,b]	0.46 ± 0.02[c]	<0.0001	<0.0001	<0.0001
Cis isomers, %	61	49	46	38			

[1] Values are means ± SEM, n = 9–10. Means in a row without a common superscript differ, $P < 0.05$ (1-way ANOVA followed by Tukey's test for differences between means).

[2] Lycopene (Lyc) was not detected in placebo-fed rats with or without alcohol.

[3] *P*-values of main effects and the interaction were determined by 2-way ANOVA.

[4] One outlier from each of the alcohol + low Lyc and alcohol + high Lyc groups was excluded from final analyses.