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The influence of alcohol-containing and alcohol-free beverages on lipid levels and lipid peroxides in serum of rats

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It is an established fact that moderate consumption of alcoholic beverages leads to some positive biochemical changes in blood that are widely regarded as indicators of improved prevention of atherosclerosis. However, at present, there are different opinions regarding the biologically active compounds of alcoholic beverages that bring about these changes. This experiment was conducted on 60 male Wistar rats, which were divided into five groups, each of which contained 12 rats: four experimental groups (EG1, EG2, EG3, EG4) and one control group (CG). During 4 weeks, all groups of rats were fed basal diet (BD) supplemented with dry red wine (EG1), beer (EG2), lyophilized dry red wine (EG3), or lyophilized beer (EG4). The rats of the CG were fed BD only. The rats of EG1 and EG2 were fed BD supplemented daily with 2.0 mL of wine and 6.0 mL of beer, respectively. The rats of EG3 and EG4 were fed BD supplemented daily with lyophilized wine and lyophilized beer at a concentration corresponding to an intake of 2.0 mL of original wine and 6.0 mL of original beer, respectively. Before and after completion of the trial, a wide range of laboratory tests including lipids and lipid peroxides were performed. The results of this investigation reveal that both original and lyophilized wine and beer exercise statistically significant beneficial lipidemic and antioxidant effects by reducing total cholesterol (TC), low density lipoprotein cholesterol, triglycerides, and lipid peroxides ($P < 0.05$ for all) and by elevating the high density lipoprotein cholesterol:TC ratio. There were no statistically significant differences in the results between groups fed BD supplemented with original wine and beer versus groups fed BD supplemented with lyophilized wine and beer. Therefore, it can be concluded that the biologically active compound of these beverages is their dry matter containing inter alia polyphenols in relatively high concentrations. (J. Nutr. Biochem. 9:682–686, 1998)

Keywords: original wine; original beer; lyophilized wine; lyophilized beer; lipids; lipid peroxides; rats

Introduction

Atherosclerosis is the pathanatomic basis of coronary artery disease (CAD), which is the most dangerous disease in Western industrialized countries.[1,2] It has been established that moderate consumption of alcoholic beverages leads to positive biochemical changes in the blood of the consumer, which are widely regarded as indicators of improved prevention of atherosclerosis.[3–6] Our own experiments on rats and clinical investigations show that moderate wine and beer consumption leads to some positive changes in lipid metabolism, antioxidant activity, and blood coagulation.[7–9] However, at present, there are different opinions regarding the biologically active compounds of alcoholic beverages that bring about these changes. Some authors suggest that alcohol per se is responsible for this beneficial effect; other investigators have found that lyophilized wines and beer can positively influence lipid metabolism and antioxidant activity of rats.[9,10] To answer this question, we conducted an

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investigation on rats fed diets supplemented with different alcohol-containing and alcohol-free beverages. There are conflicting reports about the activity of different alcoholic beverages, but most investigators agree that red wine and beer are preferable.[9,11,12] Therefore, in this experiment, alcohol-containing and alcohol-free dry red wine and beer were used as a supplement to basal diet (BD).

Methods and materials

Wines and beer

Dry red wine and Maccabee beer were used. Part of these beverages was freeze-dried to receive alcohol-free examples.

Animals and diets

The experiment was conducted on 60 male Wistar rats with a standard weight of 120 g each. Rats were divided into five groups of 12 animals each: four experimental groups (EG1, EG2, EG3, and EG4) and one control group (CG). The rats were housed individually in stainless steel metabolic cages and fed BD consisting of 70.5% starch, 18% ovalbumin, 5% salt-mix, 5% sunflower oil, 1% cod liver oil, 0.3% choline chloride, and 0.2% vitamins. The salt mixture contained (per kg of diet): $CaHPO_4$, 15 g; K_2HPO_4, 2.5 g; KCl, 5 g; NaCl, 5 g; $MgCl_2$, 2.5 g; Fe_2O_3, 2.5 mg; $MnSO_4$, 125 mg; $CuSO_4 \cdot 7H_2O$, 0.2 mg; $ZnSO_4 \cdot 7H_2O$, 100 mg; and KlO_3, 0.4 mg. The vitamin mixture included (per kg of diet): thiamin, 20 mg; riboflavin, 15 mg; pyrodoxin, 10 mg; nicotinamide, 100 mg; calcium panthotenate, 70 mg; and folic acid, 5 mg. During 4 weeks of the experiment all groups of rats were fed BD supplemented with dry red wine (EG1), beer (EG2), lyophilized dry red wine (EG3), or lyophilized beer (EG4). The rats of the CG were fed BD only. The rats of EG1 and EG2 were fed BD supplemented daily with 2.0 mL of wine and 6.0 mL of beer, respectively. The rats of EG3 and EG4 were fed BD supplemented daily with lyophilized wine and lyophilized beer at a concentration corresponding to an intake of 2.0 mL of original wine and 6.0 mL of original beer, respectively. The diets were served once daily at 10 AM ad libitum; beverages and distilled water were introduced by stomach intubation at the same time. The energy of the BD supplemented with beverages for rats of the EGs (397.3–401.7 kcal/100 g of diet) and the energy of the BD for rats of CG (393.7 kcal/100 g of diet) did not differ significantly.

Assays

We registered the growth of the animals on a weekly basis. Before and after completion of the 4-week feeding period, we drew blood samples from the tail vein. Blood from each rat was placed in a plastic tube containing heparin and centrifuged at 10,000 × g for 15 minutes. After centrifugation plasma was removed and a wide range of laboratory tests was performed immediately. These tests included inter alia total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), and lipid peroxides (LP). We determined TC, HDL-C, and TG enzymatically. TC and TG were measured as described by Trinder and Webster[13] with special Bio Merieux kits (PAP 100, cat. number 6.122.4 and cat. number 6.123.6, respectively; Marcy l'Etoile, France). HDL-C was determined by the same enzymatic methods after the precipitation of LDL-C and very low density lipoprotein cholesterol (VLDL-C) fractions with phosphotungstic acid in the presence of magnesium ions with a kit (cat. number 6.159.1; Bio Merieux). LP were determined colorimetrically (Tateishi et al.[14]) in direct reaction between methylene blue derivative (MCDP, 10-N-Methyscarbamoyl-3,7-dimethylamino-10H-phenothiazine) catalyzed by hemoglobin using kit 9#CC-004 from Kamiya Biomedical Company (Seattle, WA USA). LDL-C was calculated according to the Friedewald formula (Friedewald et al.[15]).

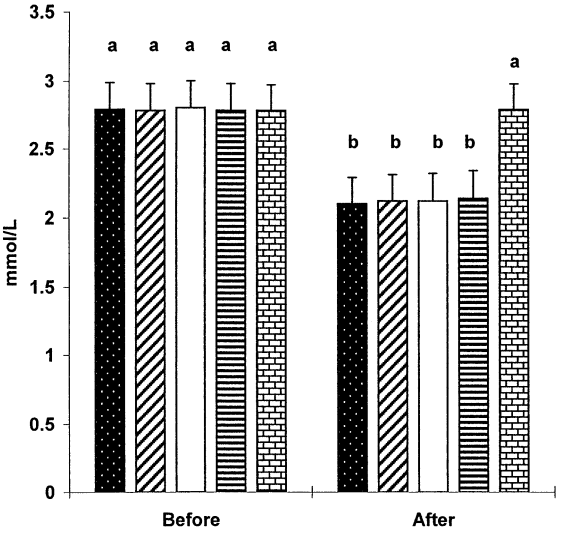

Figure 1 Total cholesterol metabolism before and after the experiment in (▩) experimental group (EG) 1, (▨) EG2, (□) EG3, (▤) EG4, and (▦) the control group (CG). Mean ± standard deviation (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

Statistical analysis

To verify the statistical significance of all parameters, we calculated the values of means and standard deviation (M ± SD) and 95% confidence intervals (CI) of means. To compare several groups, analysis of variance (ANOVA) was used. *P*-values of less than 0.05 were considered as statistically significant.

Results

Food intake was 381.6 ±10.2, 378.6 ±11.1, 371.9 ±9.2, 369.8 ±13.1, and 369.6 ±13.1 g/4 weeks for EG1, EG2, EG3, EG4, and CG, respectively. Body weight gain was 93.6 ±2.6, 92.0 ±2.9, 91.8 ±3.2, 89.5 ±3.9, and 89.1 ±3.8 g/4 weeks for EG1, EG2, EG3, EG4, and CG, respectively. The differences in food intake and the body weight gain were not statistically significant. Therefore, the addition to BD of beverages did not significantly change diet intake and body weight gain of the rats.

The results of the TC, LDL-C, HDL-C, TG, and LP tests before and after completion of the investigation are graphically presented in *Figures 1 through 5*, which are based on the M±SD data. *Figure 1* shows that before the investigation the differences in the TC in all five groups were not statistically significant. After 4 weeks of feeding, we registered a significant decrease in the level of TC in all four EGs (for all groups, $P < 0.01$). ANOVA showed that there were no differences between EGs fed BD supplemented with original or lyophilized beverages.

Before the investigation the differences in the level of LDL-C in all five groups were statistically not significant (*Figure 2*). Similar to TC, after 4 weeks of the feeding period, a statistically significant decrease was found in the level of LDL-C in all four EGs (for all groups, $P < 0.01$). ANOVA

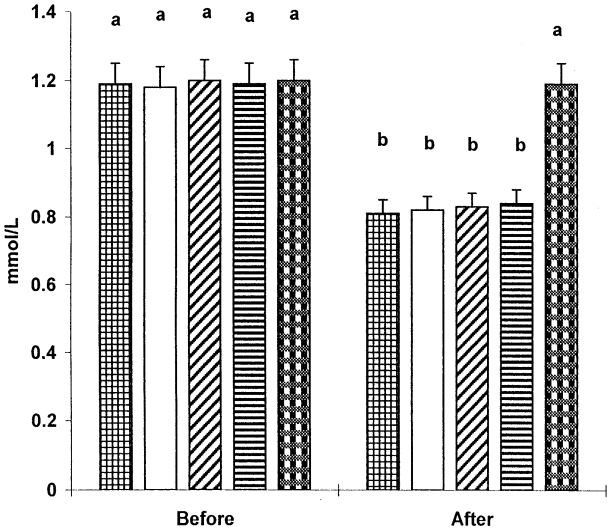

Figure 2 Low density lipoprotein cholesterol (LDL-C) metabolism before and after the experiment in (▦) experimental group (EG) 1, (□) EG2, (▨) EG3, (▤) EG4, and (▩) the control group (CG). Mean ± standard deviation (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

showed that there were no differences between EGs fed BD supplemented with original or lyophilized beverages.

Before the investigation the differences in the level of HDL-C in all five groups were not statistically significant. *Figure 3* reflects the changes in HDL-C metabolism after completion of the experiment. No statistically significant decrease in the level of HDL-C was noted in any of the EGs. The HDL-C:TC ratio was increased in all EGs but this was not statistically significant. ANOVA indicated that there were no differences between EGs fed BD supplemented with original or lyophilized beverages.

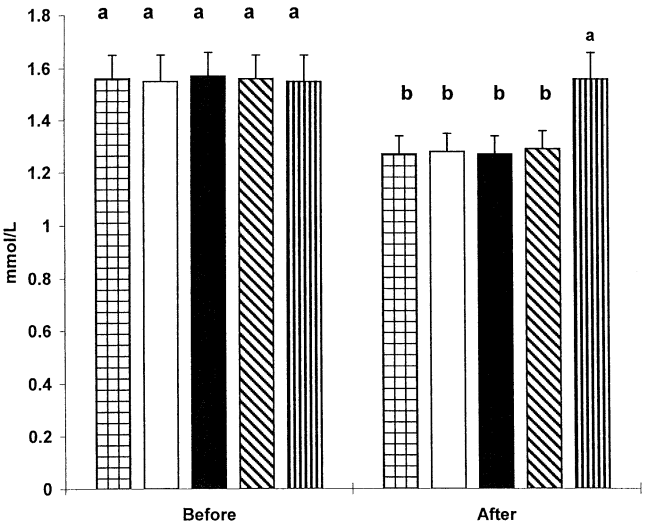

Figure 3 High density lipoprotein cholesterol (HDL-C) metabolism before and after the experiment in (⊞) experimental group (EG) 1, (□) EG2, (■) EG3, (▧) EG4, and (▥) the control group (CG). Mean ± standard deviation (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

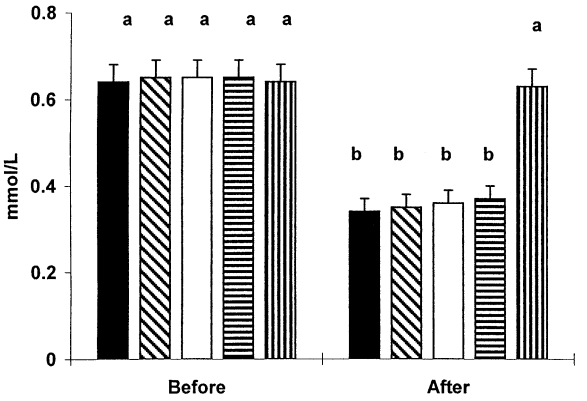

Figure 4 Triglycerides metabolism before and after the experiment in (■) EG1, (▧) EG2, (□) EG3, (▤) EG4, and (▥) EG4, (CG). Mean ± standard deviation (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

Before the investigation the differences in the level of TG in all five groups were not statistically significant. *Figure 4* summarizes the changes in TG metabolism. A statistically significant decrease was found in the level of TG in all EGs (for all groups, $P < 0.01$). ANOVA showed that there were no differences between EGs fed BD supplemented with original or lyophilized beverages.

Before the investigation the differences in the level of LP in all five groups were not statistically significant. *Figure 5* reflects the changes in LP in all EGs and the CG after completion of the investigation. After 4 weeks of feeding, there was a statistically significant decrease in the levels of LP (for all groups, $P < 0.005$). ANOVA showed that there were no differences between EGs fed BD supplemented with original or lyophilized beverages.

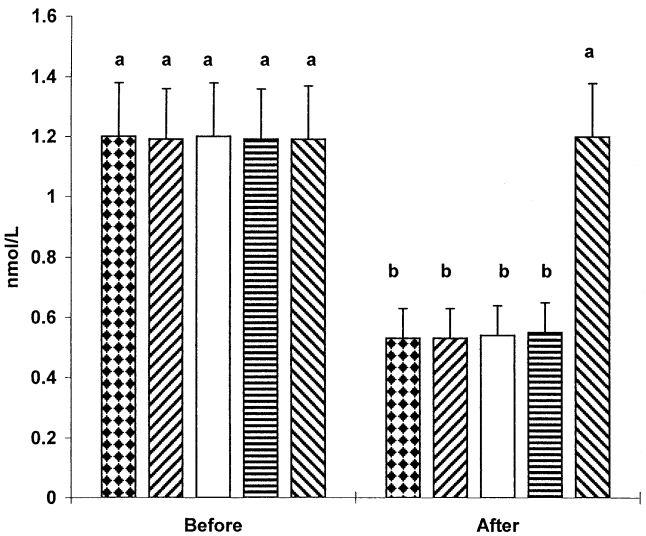

Figure 5 Lipid peroxides before and after the experiment in (⊞) EG 1, (▨) EG2, (□) EG3, (▤) EG4, and (▧) the control group (CG). Mean ± standard deviation (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$).

Discussion

Atherosclerosis is the pathanatomic basis of CAD, which is the most dangerous disease in Western industrialized countries.[1,2] Researchers agree that cholesterol is the "building material" for atherosclerotic plaques, which lead to occlusion of arteries in general and coronary arteries in particular.[16–18] In the last few years, many authors have claimed that proper diet can be very effective in the prevention of atherosclerosis.[19,20–23] Some investigators propose including in diets new kinds of vegetables and fruits to increase its antilipidemic and antioxidant effects.[6,7,24,25] However, the role of one permanent part of diets in Western countries—alcoholic beverages—still needs to be investigated. It is an established fact that moderate consumption of alcoholic beverages leads to a decrease in morbidity of CAD and a decreased mortality from CAD.[11,12,19] Recently, results of the most comprehensive study to date were published.[26] Among 490,000 men and women who used alcohol beverages, 46,000 died during 9 years of follow-up. The investigators compared cause-specific death rates and death from all causes across categories of baseline alcohol consumption. They found that the rates of death from all cardiovascular diseases between the ages of 35 and 69 years were 30 to 40% lower among men and women who reported drinking one alcoholic beverage daily than among nondrinkers. Most authors agree that moderate alcohol consumption leads to positive biochemical changes in the blood of the consumer, which are widely regarded as indicators of improved prevention of atherosclerosis.[3–6]

Our own experiments on rats and our clinical investigations show that even a short term of moderate wine or beer consumption leads to positive changes in lipid metabolism, antioxidant activity, and blood coagulation.[7–9] However, at present, there are different opinions regarding the biologically active compounds of these beverages that bring about the above mentioned changes. Some authors suggest that alcohol per se is responsible for this beneficial effect, but others found that the use of lyophilized wines and beer positively influence consuming persons.[9,10] Others indicate that those studies provide strong evidence that all alcoholic drinks are linked with low coronary risk.[27]

To find out which compounds of alcoholic beverages lead to positive changes in lipid metabolism and antioxidant activity, we conducted the above described experiment on rats. The study reveals that both original and lyophilized wine and beer provide beneficial lipidemic and antioxidant effects by reducing TC, LDL-C, TG, and LP and by elevating the HDL-C:TC ratio. There were no statistically significant differences in the results of the investigation between groups fed BD supplemented with original wine and beer and groups fed BD supplemented with lyophilized wine and beer. Therefore, it can be suggested that the biologically active compound of these beverages is their dry matter, which contains inter alia a high percentage of polyphenols.[9,10] It can be suggested that the relatively low alcoholic content in the alcohol-containing beverages we used (11% and 4% volume for red wine and beer, respectively) is one of the reasons for their minimal influence on the parameters we studied.

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