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The influence of dry matter of different alcoholic beverages on lipids, proteins, and antioxidant activity in serum of rats

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In many western industrialized countries alcoholic beverages are an invariable component of different diets. In the last couple years evidence that moderate consumption of alcoholic beverages leads to some beneficial changes in lipid metabolism and in this way reduces the morbidity and mortality from coronary artery disease (CAD) has been found. In this study we examined the influence of diets supplemented with different lyophilized wines and beer on lipids, proteins, and antioxidant activity in serum of rats. The investigation was conducted on 60 male Wistar rats, divided into three experimental (EG) and one control (CG) groups, each comprised of 15 animals. The rats of the three EGs were fed basic diet (BD) supplemented with South African (SA) dry red wine (EG1), SA dry white wine (EG2), and Israeli Maccabee beer (EG3). The rats of the CG were fed BD only. During 4 weeks of our experiment the animals of EG3 were fed BD supplemented with lyophilized beer at a concentration corresponding to an intake of 6.0 mL of original beer and the rats of EG1 and EG2 were fed BD supplemented with lyophilized wine at a concentration 2.0 mL of original wine daily. Before and after completion of the trial we performed a wide range of laboratory tests including lipids, proteins, and lipid peroxides. The results of our investigation reveal that the dry matter of red wine and beer are the most effective beverages: they exercise beneficial lipidemic and antioxidant effects by reducing total cholesterol (TC), triglycerides, and lipid peroxides and elevating high-density lipoprotein (HDL-C)/TC ratio. All used beverages do not effect the level of proteins in serum of the rats. (J. Nutr. Biochem. 9:131–135, 1998) © *Elsevier Science Inc. 1998*

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Introduction

As early as 1913, Anitschkow and Chalatow[1] have shown on rabbits that diet rich in cholesterol develops atherosclerotic changes in arteries—the pathanatomic basis of coronary artery disease (CAD), which is the most dangerous disease in the western industrialized countries.[2,3] It was established that a proper diet that includes vegetables and fruits can prevent atherosclerosis.[4–7] In most western countries alcoholic beverages are part of different diets[8–11] and consist about 4 to 6% of the average energy intake.[12] There are some authors who claim that alcoholic beverages positively influence lipid metabolism of moderate drinkers.[13–17] Our previous work[18] found positive biochemical changes in

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patients with CAD even after a short-term moderate beer consumption. There are conflicting reports about preference of different types of alcoholic beverages. According to Friedman and Kimball[19] and Klatsky et al.[20] wine and beer more than other alcoholic beverages positively influence the lipid metabolism in alcohol consumers. But Choudhury et al.[21] claim that the type of alcoholic beverages have no significant relationship with serum low-density lipoprotein (LDL) and high-density lipoprotein (HDL-C). In the last years it was demonstrated in vitro and in epidemiological and clinical studies that antioxidants of wine prevent the oxidation of LDL-C—the "building material" for atherosclerotic changes.[7,22–24] The most used alcoholic beverages are different kinds of wine and beer. But what is preferable, wine or beer? And if it is wine, then what kind of wine? If lyophilized beverages can positively influence lipids, proteins, and antioxidant activity in serum of rats fed with different diets supplemented by their dry matter? To answer these questions we decided to conduct a proper investigation.

Methods and materials

Wines and beer

Wines dry red and dry white and beer were freeze-dried and analysis of the necessary components was done. Total phenol content was determined by the Folin-Ciocalteu assay and expressed as gallic acid equivalents.[25] Epicatechin and quercetin were determined by fluorometric and spectroscopic analyses. Quercetin was also determined as total phenol and expressed in molar equivalents of quercetin.[26,27]

Animals and diets

We experimented on 60 male Wistar rats with standard weight of 120 g each. All rats were divided into four equal groups: three experimental (EG) and one control (CG), each of 15 animals. The rats were housed individually in stainless steel metabolic cages and fed a basic diet (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 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. The BD was supplemented with lyophilized beverages only for the rats of the three EGs. During a period of 4 weeks the rats of EG1 were fed BD and 2 mL of South African dry red wine every day, the rats of the EG2 were fed BD and 2 mL of South African dry white wine, and the rats of the EG3 were fed BD and 6 mL of Maccabee beer daily. The rats of the CG received BD only. The diets were served once a day at 10 a.m. ad libitum, together with lyophilized beverages and distilled water introduced by stomach intubation. The energy of the BD supplemented with lyophilized beverages for rats of the EGs (397.3 to 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 recorded 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 and performed a wide range of laboratory tests. These tests included inter alia total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), total protein (TP), albumin (AL), globulin (GL), and lipid peroxides (LP). TC, LDL-C and HDL-C were determined according to Epstein[28] and Onongbu and Lewis.[29] Total protein was determined by Lowry[30] and LP- by MODP method using Kamiya Biomedical kits.

Table 1 The most relevant components of the used wines and beer

Indices	Dry white	Dry red	Beer
Lyophilized weight (g/100 mL)	1.90 ± 0.12	2.80 ± 0.21	3.70 ± 0.29
Alcohol (% volume)	11.34 ± 0.57	11.85 ± 0.58	4.05 ± 0.31
Total polyphenolics (mg/L)	436.2 ± 21.2	2741 ± 198.1	304 ± 15.1
Epicatechin (mg/L)	56.1 ± 2.82	195.1 ± 9.67	65.5 ± 3.15
Quercetin (mg/L)	1.29 ± 0.05	8.11 ± 0.36	0.95 ± 0.04
Caloric content (Kcal/L)	45.65 ± 2.1	48.1 ± 2.1	23.9 ± 1.2

All results are mean values of triplicates ± standard deviation.

Statistical analysis

To verify the statistical significance of all parameters we calculated the values of means, standard deviation (M ± m) and confidence intervals (CI) of means. To compare several groups we used analysis of variance (ANOVA). The P values of <0.05 were adopted as statistically significant.

Results

The composition of the used wines and Maccabee beer before lyophilization is presented in *Table 1*. The wine and beer samples can make a substantial contribution to dietary requirements. The highest amount of total polyphenols, and especially epicatechin and quercetin, were in dry red wine and then in beer, showing the highest antioxidant activity of these beverages.

The results of the growth of the rats of all four groups are summarized in *Table 2*. According to *Table 2*, the addition to the BD of lyophilized beverages did not cause a statistically significant change in diet intake, the body gains of the animals or the efficiency of diets. The results of the TC, LDL-C, HDL-C, and TG tests before and after completion of the investigation are summarized in *Table 3*. Statistical analysis of these data did not find significant differences in the TC, LDL-C, HDL-C, and TG values in all three EGs and CG before the investigation. After 4 weeks of feeding changes in the level of TC in all EGs were found. But statistically significant decrease in the level of TC ($P < 0.01$ and 0.0125) was registered only in EG1- and EG3-fed BD supplemented by dry red wine and beer, respectively. In the EG2-fed BD supplemented with dry white wine these

Table 2 The diet intake, body gains, and diet efficiency ratio

Groups	Av. intake of diet (g/4 weeks)	Av. body gain (g/4 weeks)	Diet efficiency ratio
EG1	381.6 ± 54.1	92.0 ± 29.2	0.241 ± 0.051
EG2	378.6 ± 49.6	93.6 ± 29.9	0.247 ± 0.045
EG3	371.9 ± 54.2	91.8 ± 28.8	0.247 ± 0.047
CG	369.8 ± 49.1	89.5 ± 28.8	0.242 ± 0.048

Table 3 The serum levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in mmol/L (mean values, standard deviations and confidence intervals of means)

Indices	EG1	EG2	EG3	CG
Total cholesterol				
Before	2.79 ± 0.2 [2.36–3.22]	2.78 ± 0.21 [2.33–3.23]	2.80 ± 0.2 [2.33–3.23]	2.78 ± 0.19 [2.38–3.18]
After	2.10 ± 0.16 [1.76–2.44]	2.80 ± 0.2 [2.37–3.23]	2.15 ± 0.15 [1.83–2.47]	2.79 ± 0.2 [2.36–3.22]
LDL				
Before	1.18 ± 0.06 [1.05–1.31]	1.19 ± 0.06 [1.16–1.32]	1.18 ± 0.05 [1.16–1.32]	1.20 ± 0.06 [1.07–1.33]
After	0.82 ± 0.04 [0.74–0.9]	1.2 ± 0.06 [1.07–1.33]	0.84 ± 0.04 [0.76–0.92]	1.19 ± 0.05 [1.08–1.30]
HDL				
Before	1.56 ± 0.09 [1.37–1.75]	1.56 ± 0.1 [1.35–1.77]	1.56 ± 0.09 [1.37–1.75]	1.57 ± 0.1 [1.36–1.78]
After	1.26 ± 0.07 [1.11–1.41]	1.57 ± 0.09 [1.38–1.76]	1.27 ± 0.07 [1.12–1.42]	1.58 ± 0.1 [1.37–1.79]
Triglycerides				
Before	0.64 ± 0.04 [0.56–0.72]	0.65 ± 0.04 [0.57–0.73]	0.64 ± 0.04 [0.56–0.72]	0.65 ± 0.04 [0.57–0.72]
After	0.36 ± 0.03 [0.33–0.39]	0.67 ± 0.04 [0.59–0.75]	0.37 ± 0.03 [0.34–0.40]	0.66 ± 0.04 [0.58–0.74]

changes in the level of TC were not statistically significant ($P < 0.4$). According to *Table 3*, after 4 weeks feeding period the changes in the level of TG in all EGs completely repeat the TC changes. Statistically significant decrease in the level of TG in the EG1 and EG3 (P for both <0.01) and not significant decrease in EG2 ($P < 0.45$) were registered. The changes in the level of LDL-C after completion of the experiment basically repeat the changes in TC, but were not statistically significant in all three EGs. After completion of the trial we found that the changes in the level of HDL-C in all EGs were not statistically significant. But it is important to emphasize that in rats of the EG1 and EG3 the HDL-C/TC ratio has increased from 0.56 and 0.56 to 0.60 and 0.59, respectively.

Table 4 summarized the protein and lipid peroxides levels in all EGs and CG before and after completion of the investigation. Differences in the levels of total proteins, albumin, globulin, and lipid peroxides in all three EGs and CG before investigation were not statistically significant. After 4 weeks of feeding, changes in the levels of total proteins, albumin, and globulin in three EG3 and CG were nonsignificant. However, level of lipid peroxides decreased

Table 4 The serum levels of total protein, albumin, globulin,[1] and lipid peroxides[2] (mean values, standard deviations, and confidence intervals of means)

Indices	EG1	EG2	EG3	CG
Total protein				
Before	66.1 ± 4.4 [56.7–75.5]	65.9 ± 4.3 [56.7–75.1]	66.0 ± 4.5 [56.4–75.6]	66.2 ± 4.4 [56.8–75.6]
After	66.0 ± 4.4 [56.6–75.4]	66.0 ± 4.4 [56.6–75.4]	66.1 ± 4.5 [56.5–75.7]	66.1 ± 4.4 [56.7–75.5]
Albumin				
Before	45.2 ± 4.1 [36.5–53.9]	45.1 ± 4.0 [36.6–53.6]	45.0 ± 4.1 [36.3–53.7]	45.1 ± 4.0 [36.6–3.6]
After	45.1 ± 4.0 [36.6–53.6]	45.2 ± 4.1 [36.5–53.9]	45.1 ± 4.0 [36.6–53.6]	45.2 ± 4.1 [36.5–53.9]
Globulin				
Before	12.3 ± 3.1 [5.7–18.9]	12.2 ± 3.2 [5.4–19.0]	12.3 ± 3.3 [5.3–19.3]	12.1 ± 3.1 [5.5–18.7]
After	12.2 ± 3.2 [5.4–19.0]	12.3 ± 3.1 [5.7–18.9]	12.2 ± 3.2 [5.4–19.0]	12.2 ± 3.1 [5.5–18.8]
Lipid peroxides				
Before	1.20 ± 0.17 [0.84–1.56]	1.21 ± 0.18 [0.83–1.59]	1.19 ± 0.17 [0.83–1.53]	1.21 ± 0.18 [0.83–1.59]
After	0.54 ± 0.09 [0.35–0.73]	0.70 ± 0.10 [0.49–0.91]	0.55 ± 0.09 [0.36–0.74]	1.20 ± 0.17 [0.84–1.56]

[1] Total protein, albumin and globulin in g/L.
[2] Lipid peroxides in μmol/L.

in all three EGs after the trial period. This decrease was statistically significant for EG1 and EG3 ($P < 0.001$ and $P < 0.005$, respectively).

Discussion

In the last few years many authors claim that proper diets can be very effective in prevention of atherosclerosis—the pathanatomic basis of the CAD, which is the most dangerous disease in the western industrialized countries.[5–7] Some investigators propose to include in diets new kinds of vegetables and fruits in order to increase the antilipidemic and antioxidant effects of diets.[31–33] But the role of the old permanent part of diets in the western countries—alcoholic beverages, still needs to be investigated. There are still conflicting reports of the influence on the lipid metabolism and peroxidation of different kinds of wine and beer.[19,21,23] Therefore, we decided to evaluate the influence of diets supplemented with dry red and dry white wines and beer on lipids, proteins, and antioxidant activity of rats. In our previous investigations first on animals[34] and after receiving positive results on patients with CAD[18,35] we studied the influence of alcohol-containing beverages. However, according to some authors[36,37] relatively high alcoholic consumption leads to a prooxidant effect. Therefore, investigation was conducted with different lyophilized alcoholic beverages.

Using alcohol-free beverages we wanted to investigate the influence of their dry matter on lipids, proteins, and antioxidant activity in serum of rats. And in the case of positive influence to continue studies on patients with CAD. An investigation with different alcohol-free beverages would allow us to include patients who do not use alcohol according to their religious believes (about 20% of our population). We used 60 male Wistar rats. They were divided into four equal groups—three EGs and one CG, each of 15 animals. During 4 weeks these groups of animals were fed different diets: EG1—BD and dry red wine; EG2—BD and dry white wine; EG3—BD and beer; and CG—BD only. After completion of the experiment we found that the most marked beneficial effect on lipids exercise dry red wine and beer: these two beverages decrease the level of TC, LDL-C and TG and increase the HDL-C/TC ratio. These results confirm reports on different effect of various alcoholic beverages on lipid metabolism.[17,19,20] It is well known today that the lipids of the atherosclerotic plaques are derived from plasma oxidised LDL-C.[38] Therefore, it was important to examine the antioxidant properties of all three beverages. After completion of the investigation we noted that all beverages exercise antioxidant effect by decreasing the level of lipid peroxides. But the antioxidant action of dry red wine and beer was statistically significantly higher than that of the dry white wine. How it can be explained? It is true that the content of total polyphenolics in dry white wine is statistically significantly higher than in beer. However, the content of epicatechin in beer is statistically significantly higher than in white wine. Epicatechin is one of the unique group of phenolic metabolites of relatively high molecular weight that are named tannins.[39]

It was shown that tannins are 20 times more active than vitamin E,[32] the main and the possibly only role of which is to act as an antioxidant.[40] It is known that ethanol is oxidized to an extremely reactive metabolite, acetaldehyde, which in turn converts to other products that may be responsible for some adverse effects.[41] Even a short-term ethanol consumption is responsible for reduction in cardiac protein synthesis.[42,43] Can a short-term consumption of lyophilized beverages negatively influence the serum proteins? As we have supposed, after 4 weeks of investigation we did not find any quantitative changes in the serum total protein, albumin and globulin in all three EGs. In conclusion, our investigation demonstrates that among used beverages lyophilized dry red wine and beer more positively effect lipids and the antioxidant activity in serum of rats and do not lead to quantitative changes in serum proteins. Therefore, these two beverages can be used for investigation in patients with CAD to decide if lyophilized alcoholic beverages could be a valuable part of atherosclerosis prevention diet.

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