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Bioactivity of beer and its influence on human metabolism

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Abstract
Extracted total phenols, flavanols and flavonoids were measured in beer samples and their quality as antioxidants was measured by two modified antioxidant methods: the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS $^{\bullet+}$) and the β-carotene-linoleate model system (β-carotene). It was found that the antioxidant potential of beer was well correlated with flavanols and flavonoids and was slightly lower with total polyphenols (R^2 values from 0.8203 to 0.9393). Forty-two male non drinkers, hypercholesterolaemic volunteers ages 43–71 after coronary bypass surgery, were randomly divided into experimental (EG) and control (CG) groups, each of 21 participants. The antiatherosclerotic diet of the EG group was supplemented for 30 consecutive days with 330 ml beer per day. Could short-term beer consumption affect not only the risk factors of coronary atherosclerosis, but also the markers of this process: plasma albumin and its antioxidant activity? For this goal, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, plasma albumin and fibrinogen, and the antioxidant activity were determined. After the trial a significant improvement in the plasma lipid levels, and an increase in the plasma antioxidant and anticoagulant activities in patients of the EG group was registered. A parallel increase in the plasma albumin concentration and its antioxidant activity was observed. In conclusion, short-term beer consumption on the basis of the bioactivity of the beverage positively affects plasma lipid levels, plasma antioxidant and anticoagulant activities. The increase in the plasma albumin concentration and its antioxidant activity could be the markers of atherosclerosis status.

Keywords: *Beer, bioactivity, plasma albumin, lipids*

Introduction

Beverages are an integral part of diets in most western countries and consist about 4–6% of the average energy intake in spite of the ethanol toxicity (Christiansen et al. 1994). The levels of antioxidant substances, polyphenols, minerals, trace elements,

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and vitamins in malt, hops and beer are important (Paganga et al. 1999; Arnous et al. 2001; Cepicka and Karabin 2002; Szwajgier et al. 2005).

The relationships of the beer levels of some vitamins and blood homocysteine levels that are important markers of health have been reviewed (Kellner et al. 2002; Salonen et al. 2003; Kondo 2004).

One of the reasons for the widespread alcohol consumption is the claim of some investigators that alcoholic beverages have a cardioprotective effect. Moderate consumption of alcoholic beverages leads to improved lipid metabolism and to increased antioxidant and anticoagulant activity (Gorinstein et al. 1997; Buemann et al. 2002) on the basis of beer polyphenols, which act as antioxidants.

Atherosclerosis is still one of the most dangerous diseases in western industrialized countries in spite of the success of preventive measures (Wasserman et al. 2003), and hypercholesterolaemia is still the main risk factor for this disease. However, only oxidized low-density lipoprotein cholesterol (LDL-C) particles are able to penetrate arterial walls and cause their occlusion. The polyphenol action could maintain the integrity of the endothelial function by reducing the formation of superoxide. Moreover, these antioxidants may protect against low-density lipoprotein oxidation and modulate the macrophage attack on the endothelium (Buemann et al. 2002).

Most of the investigators claim that oxidized LDL-C is an important marker for the prevention of atherosclerosis and its monitoring has to be done (Hashimoto et al. 2001; Hendriks et al. 2001). It was shown that plasma albumin is a significant and independent predictor of the number of atherosclerotic plaques, and that epidemiological and clinical data consistently show that a reduced level of plasma albumin is associated with an increased incidence and mortality risk from atherosclerosis (Weijenberg et al. 1997; Malatino et al. 1999; Mukamal et al. 2004). Less is known about the level of plasma albumin as a marker of atherosclerosis. There are many explanations of the connection between atherosclerosis and the level of plasma albumin and beer consumption. The most convincing is that the plasma albumin level is inversely correlated with fibrinogen, and hypoalbuminaemia may contribute to atherosclerosis via increased synthesis of fibrinogen (Kim et al. 1997; Mosesson et al. 2001; Palmieri et al. 2003; Schillinger et al. 2004). Atherosclerosis is associated not only with LDL-C oxidation, but also with oxidative modification of proteins. Some reports explain the link between moderate alcohol consumption and lower cardiovascular disease risk (Brenner et al. 2001; Hashimoto et al. 2001; Sierksma et al. 2002).

Therefore, it was decided to study the influence of short-term moderate beer consumption on plasma lipid levels and plasma antioxidant and anticoagulant activities and to prove whether plasma albumin determination could be a reliable marker of the status of atherosclerosis prevention on the basis of the bioactivity of this beverage. As far as we know there are no previous such publications.

Materials, subjects and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), β-carotene, butylated hydroxyanisole (BHA), p-dimethylaminocinnamaldehyde (DMACA), and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St Louis, MO,

USA), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was obtained from Fluka Chemie (Buchs, Switzerland). All reagents were of analytical grade.

Materials

Maccabee beer samples were used in this study.

Determination of the bioactive substances

Proteins, albumins, glucose, maltose, maltotriose, dextrins and alcohol were determined in beer by conventional analyses.

Total polyphenols. Total polyphenols were measured at 765 nm using Folin–Ciocalteu reagent with gallic acid as a standard, and were expressed as milligrams per gallic acid equivalent (Singleton and Rossi 1965).

Flavonoids. The absorbance of flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$, and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as milligrams per litre of catechin equivalent (Liu et al. 2001).

Flavanols. To beer (0.2 ml), diluted 1:100 with MeOH, 1 ml DMACA solution (0.1% in 1 N HCl in MeOH) was added. The absorbance at 640 nm was then read after 10 min against a blank prepared similarly without DMACA, and the concentration of flavanols was estimated from a calibration curve, constructed by plotting known solutions of catechin (1–16 mg/l) against the absorbance. Results were expressed as milligrams per litre catechin equivalents (Arnous et al. 2001).

Antioxidant activity using the β-carotene linoleate model system. This system comprised β-carotene (0.2 mg) in 0.2 ml chloroform, linoleic acid (20 mg), Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) and 40 ml oxygenated water. Four millilitre aliquots of this emulsion were added to test samples containing 0.2 ml beer and were evaluated in terms of bleaching of the β-carotene, measuring the absorbance at 470 nm: $AA = 100 \ [1 - (A_0 - A_t)/(A_0^o - A_t^o)]$, where A_0 and A_0^o are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A_t and A_t^o are the absorbance measured in the test sample and control, respectively, after incubation for 180 min. The results were expressed as the percentage of inhibition. BHA was used for comparison in both methods (Singh et al. 2002).

The Trolox equivalent antioxidant capacities. Trolox equivalent antioxidant capacities (TEAC) were determined by reaction with $ABTS^{\bullet+}/K_2S_2O_8$ and with $ABTS^{\bullet+}/MnO_2$. This method is sensitive and based on two different reaction mixtures.

1. The 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). The absorbance was monitored exactly 1 and 6 min after addition of 990 μl $ABTS^{\bullet+}$ solution to 10 μl beer or plasma or Trolox (final concentration, 0–20 μM) in phosphate-buffered saline.

2. ABTS$^{\bullet+}$ was also prepared by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide. This solution was then diluted in a 5 mM phosphate-buffered saline, pH 7.4, to an absorbance of 0.70.

The percentage decrease of the absorbance at 734 nm in mixtures 1 and 2 was calculated and plotted as a function of the concentration of the samples and of Trolox for the standard reference data, and expressed as Trolox equivalent (mMTEL^{-1}) (Miller et al. 1996; Onate-jaen et al. 2006).

Subjects

The study population was recruited from patient-volunteers who previously underwent coronary bypass surgery, due to coronary artery disease, at the Institute of Cardiology of the University Medical Center, Rehovot, Israel. As in our previous investigation (Gorinstein et al. 1997), the patient-volunteers gave written, informed consent to a protocol approved by the responsible institutional committee on human experimentations, based on the Helsinki Declaration of 1975 as revised in 1983. One hundred and thirty-six male patients between the ages of 43 and 71 years were examined. All of them underwent bypass surgery due to three-vessel coronary artery disease. Forty-two non-drinkers, suffering also from hypercholesterolaemia, were chosen for further investigation. These 42 hypercholesterolaemic patients were randomly divided into two groups, the experimental (EG) and control (CG) groups, each of 21 participants. During the experiment there were no treatment complications, and all patients completed the trial.

Study design

All patients consumed the usual Israeli diet recommended for patients with coronary atherosclerosis: rich in vegetables and fruits and with limited quantities of fats—about 1800 calories (Gorinstein et al. 1997). This diet was supplemented for 30 consecutive days by 330 ml beer (about 20 g alcohol) once a day for the EG group patients. During this period the CG group patients, instead of beer, drank 330 ml mineral water Netivot, whose content of minerals was similar to those of beer. Both beverages were consumed during lunches. An assigned member of the investigation team checked daily the consumption of diets, the lifestyle and physical activity of the patients.

Laboratory methods

Every patient was examined before and after completion of the study. Systolic and diastolic blood pressures, heart rate and weight were registered. Blood samples 1 day before and 1 day after investigation were collected after an overnight fast. A wide range of laboratory tests were performed: plasma total cholesterol, LDL-C, high-density lipoprotein cholesterol, triglycerides, plasma albumin and fibrinogen, their antioxidant activity, the prothrombin time, factor VII antigen (factor VIIag), factor VII coagulant activity (factor VIIc) and plasminogen activator inhibitor (PAI). All tests were performed as previously described (Gorinstein et al. 1997).

Plasma albumin was precipitated with 2 M ammonium sulfate, fibrinogen with methanol, then purified by sequential DEAE anion-exchange chromatography, dialysed against water for 72 h, and lyophilized (Gorinstein et al. 2003).

Protein assays were performed by the Lowry method (Lowry et al. 1951). Human serum albumin and fibrinogen were used as standards. The TEAC test was used for determination of the serum antioxidant and serum protein activities (Miller et al. 1996).

Statistical analyses

Values are presented as the mean $\pm$ standard deviation of five-times-analysed *in vitro* beer samples. Where appropriate, the data were tested by one-way analysis of variance. $P < 0.05$ was considered significant.

Results

In vitro *results*

Effect of total phenols, flavanols and flavonoids. The results of the major components of the beer samples used (Table I) are in accordance with the data of others (Innes 1998). The antioxidant potential of the beer samples used was 1.83 ± 0.1 and 2.09 ± 0.2 mMTEL^{-1} with $K_2S_2O_8$ or with MnO_2 tests, respectively. The antioxidant activity of the measured standards (mMTEL^{-1}) was placed in the following order: ferulic acid $(1.90) > p$-coumaric $(1.86) >$ BHA $(1.13) >$ caffeic acid (0.93). The antioxidant activity of beer determined with β-carotene (% of inhibition) was about 28.1 ± 2.6. The antioxidant activity of the measured standards by β-carotene (% of inhibition) was placed in the following order: BHA $(94.4) >$ caffeic acid (37.9) $> p$-coumaric $(16.0) >$ ferulic acid (10.2). The two independent antioxidant methods give a slightly different order of measured antioxidants, but the beer samples showed relatively high values and were close to the antioxidant activity of ferulic acid and *p*-coumaric acid, because these antioxidant methods are based on different mechanisms. TEAC/ABTS is based on electron transfer and inhibition of linoleic acid oxidation (β-carotene) involving hydrogen atom transfer reactions; therefore, the reaction of beer extracts would be slightly different. High correlation coefficients were also obtained in other reports on beer samples using ABTS$^{\bullet +}$ (Onate-jaen et al. 2006).

Our results are in correspondence with others (Szwajgier et al. 2005), showing that the antioxidant activity of beer depends mostly on the amount of ferulic acid, which presents in free form, but the main form is the bound form—feruloylated oligosaccharides. Previous research showed that feruloylated oligosaccharides more effectively inhibited lipid and low-density lipoprotein oxidation than free ferulic acid. Plotting the total phenol concentration against antiradical activities (ABTS and β-carotene), the corresponding correlation coefficients (R^2) obtained were 0.8462 and 0.8203, indicating that there is a rather moderate connection between the total phenol content and antioxidant properties of the beers. By contrast, the correlation of total flavanol and flavonoid concentrations with the same antiradical activities gave R^2 values of 0.9324 and 0.9153, and 0.9393 and 0.9173, respectively (Figure 1). This finding suggests that the total flavanol and flavonoid contents may be strongly related to the antioxidant properties of the beers and highly associated with scavenging of free radicals.

In vivo *results*

The heart rate, systolic and diastolic blood pressures and the weight of the patients after the trial were without significant changes (data not shown).

Table I. Protein, carbohydrate and polyphenol profiles of the investigated beer samples.

Proteins (g/l)	Glucose (% on dry substance)	Maltose (% on dry substance)	Maltotriose (% on dry substance)	Dextrins (% on dry substance)	Alcohol (% volume)	Polyphenols (mg gallic acid equivalents/l)	Flavonoids (mg catechin equivalents/l)	Flavanol (mg catechin equivalents/l)
5.5±0.5	0.9±0.1	38.5±3.2	30.5±3.1	30.1±3.1	5.4±0.5	510.0±5.3	45.0±4.7	40.3±4.2

Values are the mean±standard deviation of five measurements.

Blood lipids

The beer-supplemented diet decreased serum lipids levels after completion of the investigation (Figure 2) in the EG group versus the CG group: total cholesterol by 19.3%, LDL-C by 29.6%, and triglycerides by 36.6%. The increase in the high-density lipoprotein cholesterol in the EG group versus the CG group was not significant ($P < 0.05$).
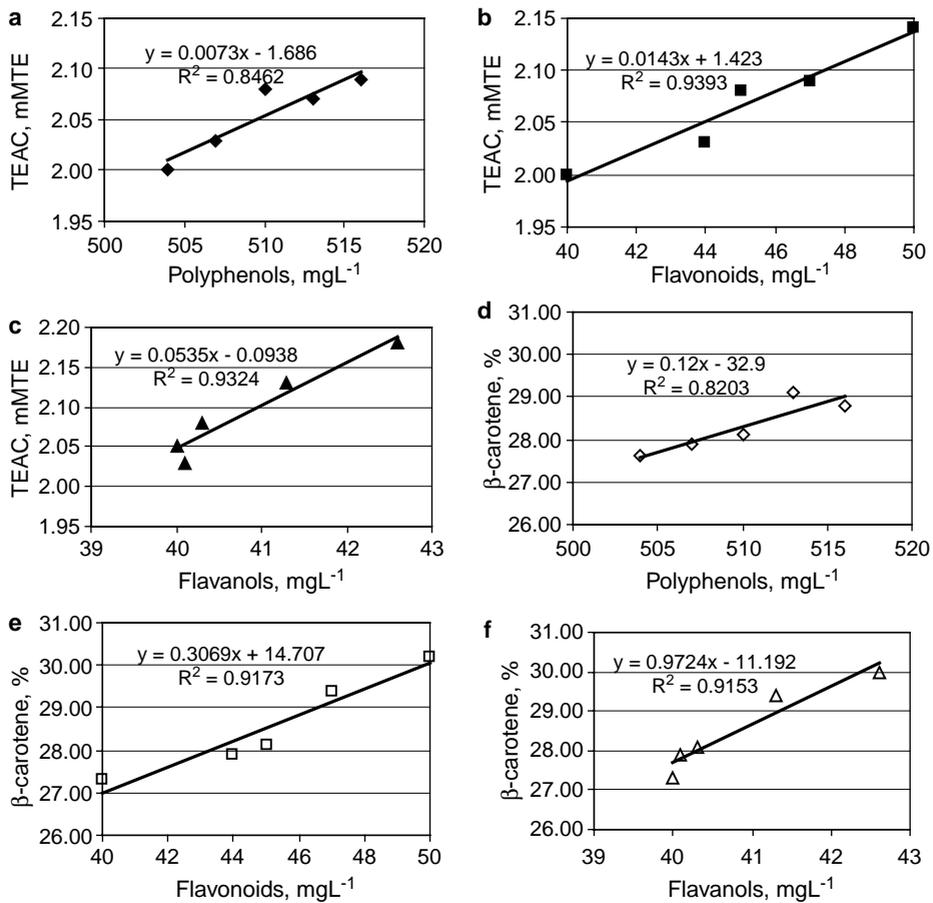

Figure 1. (a) Correlation between the content of polyphenols (mg/l, x axis), (b) flavonoids (mg/l, x axis), (c) flavanols (mg/l, x axis) and TEAC (mMTEL^{-1}, y axis), (d) polyphenols (mg/l, x axis), (e) flavonoids (mg/l, x axis), (f) flavanols (mg/l, x axis) and β-carotene bleaching effect (inhibition%, y axis).

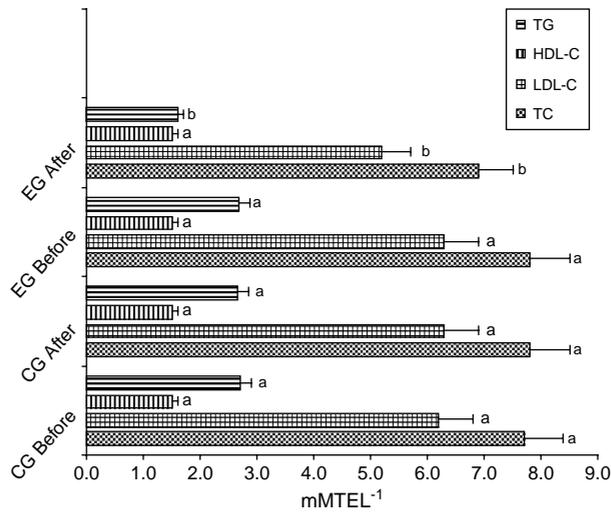Figure 2. Serum lipids before and after completion of the trial. Mean±standard deviation (horizontal lines). $n = 21$. Bars with different letters are significantly different ($P < 0.05$).

Blood coagulation indices

After completion of the investigation (Table II) the values of all studied haemostatic indices (prothrombin time, factor VIIag, factor VIIc, PAI and fibrinogen concentration) were decreased. A significant decrease was only observed for factor VIIag and factor VIIc ($P < 0.05$). The fibrinogen concentration decreased from 11.6 ± 0.9 to 10.3 ± 0.8 mMTEL^{-1}; however, this decrease was not significant ($P > 0.05$).

Blood proteins

The changes in plasma albumin concentration and its antioxidant activity in patients of the EG and CG groups after completion of the investigation are presented in Table III. The increase in plasma albumin concentration in patients of the EG group versus the CG group was not significant ($P > 0.05$): 46.3 versus 44.2 g/l ($+4.7\%$). However, the antioxidant activity in patients of the EG group versus the CG group was significantly increased: 1.59 versus 1.21 mMTEL^{-1} ($+21.0\%$) ($P < 0.05$).

Table II. Changes in some studied haemostatic factors after completion of the investigation in the patients of the experimental and control groups.

Haemostatic factor	EG group		CG group	
	Before	After	Before	After
Prothrombin time (%)	97.2 ± 2.74^a	93.1 ± 2.71^a	97.1 ± 2.73^a	97.3 ± 2.75^a
Factor VIIag (%)	99.3 ± 2.76^a	79.5 ± 2.14^b	99.4 ± 2.77^a	99.2 ± 2.76^a
Factor VIIc (%)	98.2 ± 2.75^a	80.2 ± 2.74^b	98.4 ± 2.78^a	98.3 ± 2.74^a
PAI(IU/ml)⋆	7.1 ± 0.5^a	6.7 ± 0.5^a	7.2 ± 0.5^a	7.1 ± 0.5
Serum fibrinogen (mmol/l)	11.6 ± 0.9^a	10.3 ± 0.8^a	11.5 ± 0.8^a	11.6 ± 0.9^a

Values are the mean±standard deviation. $n = 21$ Means in rows with different superscript letters are significantly different ($P < 0.05$). ⋆IU/ml, international units/ml.

Table III. Changes in the plasma albumin concentration and its antioxidant activity in the experimental and control groups after completion of the investigation.

	Albumin concentration (g/l)	Antioxidant activity (mmol/l)
EG before	44.1 ± 3.2^a	1.21 ± 0.12^a
CG before	44.3 ± 3.3^a	1.22 ± 0.13^a
EG after	46.3 ± 3.4^a	1.59 ± 0.14^b
CG after	44.2 ± 3.1^a	1.21 ± 0.12^a

Means with different subscript letters differ significantly ($P < 0.05$).

Antioxidant activity

The kinetics of the ABTS scavenging effect of human serum is shown in Figure 3a. The antioxidant capacity of patients PT1, PT2 and PT3 varied and can be compared with glutathione of 0.025 mg/ml. The antioxidant activity of human serum as well as of albumin and fibrinogen fractions increased after beer consumption. The antioxidant activity determined by two substances with the same scavenger gave comparable results, but with MnO_2 the determined antioxidant values were about 12% higher than with $K_2S_2O_8$, as expressed by the percentage of inhibition during the reaction time (Figure 3a).

The changes in the antioxidant activities of albumin and fibrinogen fractions after beer consumption are shown in Figure 3b and are expressed as the percentage of inhibition of the scavenging capacity of the ABTS radical during 6 min of reaction time. After beer consumption the antioxidant activities in the two serum protein fractions increased.

After the trial a significant increase of the serum antioxidant activity in patients of the EG group was found (Figure 4): 1.89 versus 1.40 $mMTEL^{-1}$ ($+35.0\%$) ($P < 0.05$). No significant changes in all studied variables were found in patients of the CG group.

Discussion

Recent interest in food phenolics has increased greatly, because of their antioxidant and free radical scavenging abilities. Popular beverages in the world include tea, coffee, cocoa, beer, wine and fruit/vegetable juices. All of these beverages contain phenolic compounds (Lugasi 2003; Lugasi and Hovari 2003).

A series of studies using animal models and human being have shown that beer provides plasma with significant protection from oxidative stress and improves lipid metabolism, and suppresses atherosclerosis (Gorinstein et al. 1997, 2003; Cepicka and Karabin 2002; Vinson et al. 2003; Kondo 2004).

Supplementation of diets with a combination of vitamins, which possess antioxidant properties, slows down atherosclerotic progression in hypercholesterolaemic persons (Salonen et al. 2003). To be effective in prevention of atherosclerosis in general, and of coronary atherosclerosis in particular, a natural product has to possess hypolipidaemic, antioxidant and anticoagulant properties. Our results *in vitro* confirmed other reports (Lugasi 2003) that the average concentration of total polyphenols in lager and dark beers was 376 and 473 mg/l, respectively, and showed slightly higher amounts of polyphenols (Table I). The beer samples used exhibited a strong hydrogen-donating
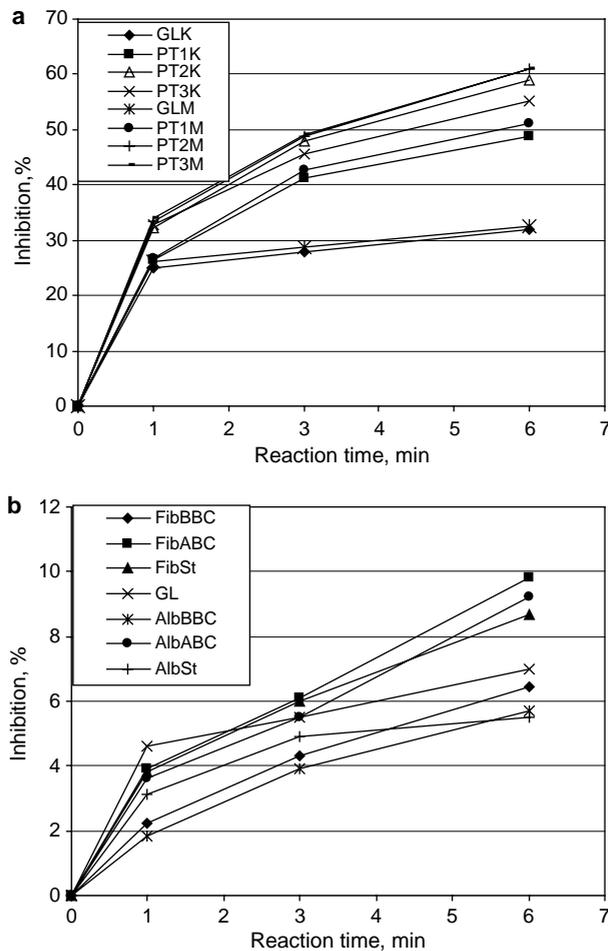
Figure 3. ABTS scavenging effect of: (a) human serum — the ABTS radical cation was produced by reacting with manganese dioxide (M) (GLM, glutathione; PT1M, patient N1; PT2M, patient N2; PT3K, patient N3) and with potassium persulfate (GLK, glutathione; PT1K, patient N1; PT2K, patient N2; PT3K, patient N3), with glutathione at a concentration of 0.025 mg/ml; and (b) serum fibrinogen and albumin before and after beer consumption (FibBBC, fibrinogen before beer consumption; FibABC, after beer consumption; FibSt, fibrinogen standard; AlbBBC, albumin before beer consumption; AlbABC, albumin after beer consumption; AlbSt, albumin standard; GL, glutathione, 0.025 mg/ml).

property and reducing power in two independent antioxidant assays (Figure 1). The antioxidant properties were dependent not only on the polyphenol content, but also on other substances. *In vitro* studies have shown that not only total polyphenols are responsible for the antioxidant activity of beer samples, but also flavonoids and flavanols. These results are supported by other investigations that the beers' lipoprotein antioxidant quality was clearly superior to that of vitamin antioxidants and to that of the phenol ingredients, suggesting synergism among the antioxidants in the mixture (Cepicka and Karabin 2002; Salonen et al. 2003; Vinson et al. 2003).

Our results were supported also by Ghiselli et al. (2000), who showed that the positive association of a moderate intake of alcoholic beverages with a low risk for
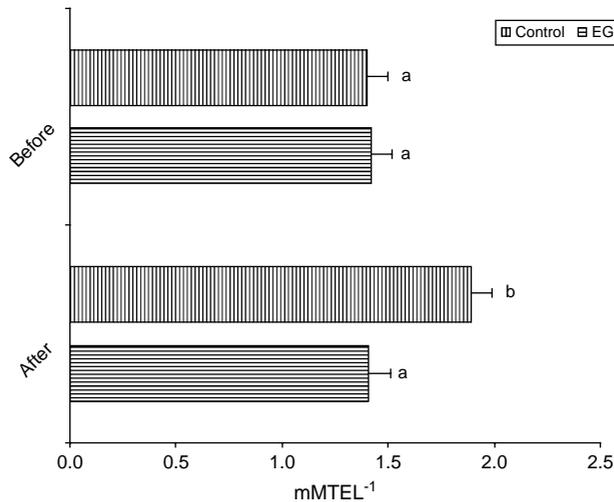
Figure 4. The changes of the serum antioxidant activity after completion of the investigation. Mean $\pm$ standard deviation (horizontal lines). Bars with different letters are significantly different ($P < 0.05$).

cardiovascular disease, in addition to ethanol itself, may be linked to their polyphenol content.

The results obtained in this report using moderate consumption of beer support other reports: while the inverse association between red-wine consumption and cardiovascular risk is globally recognized as the French paradox, many epidemiological studies have concluded that beer and red wine are equally beneficial (Kondo 2004).

Innes (1998) suggests that beer is preferable to other alcoholic beverages. Effectiveness, low cost, acceptable adverse-event profile and single-dose dispensers of beer are the basis for replacing red wine with beer as the cardioprotective beverage. Also, other investigators have indicated that beer is one of the preferable alcoholic beverages for prevention of atherosclerosis (Brenner et al. 2001; Kondo 2004). The concentration of polyphenols and antioxidant properties, except for the chelating ability of beers, measured in the same *in vitro* test system was very similar to those characteristics of white wine, while the extent of chelating ability of beers showed a similarity to that of red wines. Studies evaluating the relative benefits of wine versus beer versus spirits suggest that moderate consumption of any alcoholic beverage is associated with lower rates of cardiovascular disease. Although beers have antioxidant capacity like white wines while having half the alcoholic content of wines, beers should be good sources of antioxidant polyphenols. Moderate consumption, as part of a well-balanced diet, cannot be criticized either medically or socially and may have a beneficial effect on reducing oxidative disorders (Lugasi 2003). The same conclusions were postulated by Lugasi and Hovari (2003)—that lower amounts of phenolics were observed in beer and white wines in comparison with red wine. All beverages exhibited significant antioxidant properties such as the hydrogen-donating ability, reducing power, chelating ability and total antioxidant status value. These antioxidant properties strongly correlated with the total polyphenol content of the beverages.

We have found that short-term moderate beer consumption leads to positive changes in plasma lipid levels: a significant decrease in the plasma total cholesterol and

LDL-C and triglycerides. Our data are contrary to the data of some other authors, who claim that moderate consumption of alcoholic beverages leads first of all to an increase in high-density lipoprotein cholesterol and has little effect on total cholesterol and LDL-C (Sillanaukee et al. 2000; Hashimoto et al. 2001). This discrepancy could be explained by the fact that the decrease in the plasma total cholesterol and LDL-C was reported only in patients with hypercholesterolaemia (Gorinstein et al. 2003). We investigated only patients with such plasma lipid disturbances. It also has to be underlined that the beer consumption in this trial was only of a limited period (30 consecutive days). The reported data are in accordance with additional conclusions (Vinson et al. 2003) that, at the high dose, beer significantly decreased cholesterol and triglycerides acting as *in vivo* antioxidants by decreasing the oxidizability of lower density lipoproteins. The polyphenols in the beers appeared to be responsible for the benefits of beer in the hamster model of atherosclerosis. Lager inhibited atherosclerosis at a human equivalent dose. Similar conclusions were obtained by Cepicka and Karabin (2002) about the health-prevention properties of beer polyphenolics.

After the trial a significant increase of the antioxidant activities of both serum and serum proteins (albumins and fibrinogen) was found. These findings are in accordance with the data of others, who demonstrated that alcohol consumption increases the plasma antioxidant activity (Brenner et al. 2001; Buemann et al. 2002; Kellner et al. 2002).

After the trial a significant increase in the serum antioxidant activity in patients of the EG group was found (Figure 4): 1.89 versus 1.40 $mMTEL^{-1}$. Our results were similar to others showing that beer was able to induce a significant ($P < 0.05$) increase in plasma collected at various times: before (T0), 1 h after (T1), and 2 h after (T2) drinking. The antioxidant capacity at T1 (T0 = 1.353 mM; T1 = 1.578 mM) returned close to basal values at T2 (Ghiselli et al. 2000).

The high content of bioactive compounds in beer is the most possible explanation of its high antioxidant activity. This suggestion is supported by Paganga et al. (1999), who have proved that the antioxidant activities of one glass of red wine (150 ml) is equivalent to 12 glasses of white wine and only to 3.5 glasses of beer. Therefore some authors claim that moderate consumption of alcoholic beverages can be such a remedy (Buemann et al. 2002). Our results correspond with Hendriks et al. (2001), who examined the effects of moderate alcohol consumption (35 g/day) on postprandial lipoprotein metabolism in two groups of healthy middle-aged men who had different plasma total cholesterol, triglyceride concentration, and body mass index, which are three major risk factors for coronary artery disease.

After completion of this trial an increase of the plasma anticoagulant activity was found: a decrease in the values of the prothrombin time, factor VIIag, factor VIIc and PAI tests and fibrinogen concentration. Also, some qualitative changes in fibrinogen structure were noted: fibrinogen as one of the plasma circulating proteins links with coronary atherosclerosis and blood coagulation (Mosesson et al. 2001; Mukamal et al. 2004). It was reported that the fibrinogen level predicts cardiovascular events independent of traditional risk factors (Palmieri et al. 2003). Our results were similar to others showing that 3 weeks of beer consumption significantly decreased (12.4%) fibrinogen levels (Sierksma et al. 2002).

Some authors have demonstrated that moderate drinking leads to a decrease in the plasma circulating fibrinogen concentration (Kim et al. 1997; Mosesson et al. 2001; Gorinstein et al. 2003; Palmieri et al. 2003). The results of the present investigation

are in accordance with the data of the aforementioned authors. We also found that short-term moderate beer consumption leads to a decrease in the content of the serum fibrinogen and to certain qualitative changes in its structure (Gorinstein et al. 2003). In other reports, alcohol intake was associated with lower levels of inflammatory markers (factor VIII coagulant activity), and levels of fibrinogen and albumin) as markers of systemic inflammation in older adults free of cardiovascular disease (Mukamal et al. 2004).

Epidemiological investigations reported that a reduced level of serum albumin is associated with an increased morbidity and mortality from coronary artery disease. A recent study confirms that a low level of serum albumin is associated with increased risk of all-cause and cardiovascular mortality as well as with coronary heart disease and stroke incidence (Shaper et al. 2004). It was shown that low serum albumin is a powerful predictor of cardiovascular adverse events in healthy subjects and patients with subclinical atherosclerosis (Schillinger et al. 2004). The same authors claim that low plasma albumin may be particularly useful for risk prediction in patients with few traditional risk factors of atherosclerosis. In this prospective clinical study we found that short-term moderate beer consumption led to an increase in the concentration of serum albumin and in its antioxidant activity. These findings were observed simultaneously with the improvement in the serum lipid level and antioxidant and anticoagulant activities, and a certain decrease in the concentration of serum fibrinogen. However, the increase in the concentration of serum albumin was not significant ($P > 0.05$). This could be explained by the fact that the consumption of beer in this investigation was only of a limited period (30 consecutive days). There is therefore a need for further investigations, which must be of longer duration, to determine whether the serum albumin concentration as its antioxidant activity could also serve as a reliable marker of the status of the atherosclerosis prevention in beer-consuming patients.

Conclusions

Short-term moderate beer consumption positively influences plasma lipid levels, plasma antioxidant and anticoagulant activities in hypercholesterolaemic patients. The plasma albumin antioxidant activity can be a status marker of atherosclerosis prevention in hypercholesterolaemic consumers of beer.

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