ORIGINAL ARTICLE

Positive and negative effects of short-term moderate beer consumption

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Abstract

Alcoholic beverages are an integral part of diets in most countries of Western civilization. Effects of beer on patients with coronary atherosclerosis was studied. Patients were randomly assigned to an experimental (EG) and a control (CG) group, 19 patients each. Only patients of the EG group consumed 330 ml beer daily for a period of 30 days. Before and after completion of the study every patient was examined. A wide range of laboratory tests were performed. In patients of the EG group a tendency to an increase in HDL-cholesterol, a rise in total tocopherol ($P = 0.25$) and α-tocopherol ($P = 0.125$), a decrease in factor VII ($P<0.05$) and a decrease in plasminogen activator inhibitor ($P<0.05$) were seen. In 12 of these 19 patients some qualitative changes in plasma proteins were found: a shift of low-molecular-weight proteins to the basic range and a decrease in their stability. These findings prove that a short period of moderate beer consumption leads to positive changes in lipid metabolism and antioxidant and anticoagulant activity, which are cardioprotective. However, in most patients some qualitative alternations in plasma proteins were detected.

Key words: beer, lipids, tocopherols, proteins

Introduction

Alcoholic beverages have been a food throughout the ages (1) and still are an integral part of diets in most countries of Western civilization (2, 3). At present, on average, alcoholic beverages contribute 4–6% to average energy intake (4). In recent years some epidemiological, experimental and clinical studies have shown that moderate alcohol use has a cardioprotective effect: it induces favourable biochemical changes (5–9). These authors claim that alcoholic beverages protect patients by lowering the levels of plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C), thus improving the plasma LDL-C/HDL-C ratio. It was also found that alcohol consumption enhances antioxidant and thrombolitic activity (3, 5, 7). These positive changes lead to a significant decrease in mortality. Thun *et al.* (9) have compared cause-specific death rates and death from all causes across categories of baseline alcohol consumption. They found that cardiovascular death rates between the ages of 35 and 69 were 30–40% lower among men and women reporting consumption of one drink daily than among teetotallers.

The cardioprotective effect of moderate drinking is known to be connected to improvement of lipid metabolism and anticoagulant and antioxidant activity. Could short-term moderate beer consumption influence these parameters? To answer this question we decided to investigate plasma lipids, tocopherols, factor VIIag, factor VIIc and plasminogen activator inhibitor (PAI) which reflect the anticoagulant status.

It is common knowledge that alcohol consumption has also adverse effects. Alcohol affects negatively protein metabolism (10–12). Some groups have found that even an acute ethanol dose reduces the synthesis rates of intestinal contractile protein (13). It was also shown that ethanol affects the synthesis of protein of skeleton muscle (14). Proteins are oxidatively modified in plasma of alcoholics (15). Could short-term moderate beer consumption also negatively influence plasma proteins? This is very important to know because the plasma proteins comprise a dynamic system with varied biological functions. They are major extracellular components of the circulatory system. Normal conditions of human plasma proteins are very important for pharmaceutics. Binding of new chemical entities to plasma proteins is an issue confronting pharmaceutical companies during development of potential therapeutic agents. Most drugs bind to the most abundant plasma protein, human plasma albumin, at two major binding sites. The structural and functional changes in plasma proteins could affect the effectiveness of the drug treatment. The relationship among physicochemical characteristics of proteins has been extensively investigated on bovine plasma albumin as a model protein. Therefore, these proteins were investigated in this study. In our previous studies, no quantitative changes in plasma proteins after moderate beer consumption were found (16). We have shown that moderate beer consumption is cardioprotective but were concerned about the possible negative effect of alcohol on plasma proteins. We did not find quantitative changes in plasma proteins in patients after moderate beer consumption but could not exclude qualitative changes. Therefore, it was decided to investigate the influence of short-term moderate beer consumption on the structural and functional characteristics of plasma

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proteins in patients with coronary atherosclerosis. To the best of our knowledge, this is the first study to address this issue.

Subjects and methods

Materials. Maccabee beer samples were kindly supplied by Tempo Beer Industries, Natania, Israel. The main components of these samples of beer were proteins (5.2 g/l), total sugars (20.5% of dry matter), alcohol (5.1% v/v) and polyphenols (345 mg/l).

Subjects. The study population was recruited from volunteer patients who had undergone coronary bypass surgery due to three-vessel coronary artery disease (CAD) in the Institute of Cardiology of the University Medical Center, Rehovot, Israel. The subjects gave written, informed consent to a protocol approved by the responsible Institutional Committee on human experimentation based on the Helsinki Declaration of 1975 as revised in 1983. Of the 136 male patients examined, 38 were selected who met the following criteria: (1) clinical manifestation of CAD had appeared at least 2 years before coronary bypass surgery, and after surgery the patients were free of anginal syndrome without additional medication; (2) they were non-drinkers; (3) at least 12 months had passed after the surgery and the results of the laboratory tests were identical to the results of laboratory tests usually performed before coronary bypasses.

Study design. The patients were randomly assigned to an experimental (EG) and a control (CG) group (19 patients each). The patients of the EC group were 47–72 years old and the patients of the CG group were 46–71 years old. All patients completed the trial. Patients of both groups consumed the usual Israeli diet rich in vegetables and fruits and limited quantities of fats as recommended for patients with coronary atherosclerosis. For 30 consecutive days this diet was supplemented once a day with 330 ml Maccabee beer (about 20 g of alcohol) for patients of the EG group while CG patients received 330 ml of Nevitot mineral water instead. Beer and mineral water were consumed during the lunch. A member of the investigation team checked compliance with the diets, life-style and physical activity of the patients daily.

Before and after completion of the experiment all patients were examined. Systolic and diastolic blood pressure, heart rate and weight were registered. A wide range of laboratory tests were performed. During the trial there were no treatment complications.

Laboratory methods. Blood samples a day before and a day after the investigation were collected after an overnight fast. Lipids, tocopherols and quantitative levels of proteins were determined as described (7, 8, 16). To examine possible qualitative changes after beer consumption, the plasma proteins were separated into three groups by ammonium sulfate (albumins, 2 M), ammonium sulfate (globulins, 4 M) and absolute methenol-precipitable proteins (M). (M is the ethanol-precipitable fraction; a high hydrophobicity, i.e. a high M value, is indicative of a low protein stability.) Then, the precipitated fractions were dialysed against water and freeze-dried. Intrinsic fluorescence measurements of proteins were done with a Model FP-770 Jasco spectrofluorometer. Fluorescence emission spectra were taken at excitation wavelengths of 274 and 295 nm and recorded over the frequency range from the excitation wavelength to a wavelength of 500 nm (17, 18). Proteins were dissolved in 0.01 mol/l phosphate buffer pH 7.2. For fluorescence measurements protein solutions were in a concentration of 0.15 mg/ml. The best dissolution was achieved for proteins precipitated with 4 mol/l ammonium sulfate. The protein content was measured by the procedure of Lowry *et al.* (19) and bovine serum albumin (BSA) was used as a standard. Molecular weight was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Two-dimensional gel electrophoresis was done according to Laemmli (20), Otto *et al.* (21) and Hache *et al.* (22). Hydrophobicity (So) was determined by means of 1-anilino-8-naphthalenesulfonate (ANS)-fluorescent probe measurements (23).

Statistical analysis. Values are given as means ± SD of samples analysed 5 times and 95% CI of means. Where appropriate, data were tested by two-way analysis of variance with GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA) followed by Duncan's new multiple range test (Duncan, 1955) to assess differences between groups means. Differences of $P<0.05$ were considered significant.

Results

Clinical data. Means ± SD and 95% CI at baseline in the EG group were: heart rate 64.4±9.0 [45.6-83.2] per minute; systolic blood pressure 145.1±13.1 [117.7-175.2] mmHg; diastolic blood pressure 82.9±7.0 [68.3-97.5] mmHg; and weight 74.7±6.8 [60.5-88.9] kg. In the CG group, corresponding values were and 64.3±9.1 [45.3-83.3] per minute, 145.0±13.2 [117.4-172.6] mmHg, 82.8±7.1 [67.9-97.7] mmHg and 74.8±6.9 [60.4-89.2] kg, respectively.

After completion of the trial, no significant changes in were seen in any of the four parameters in both groups of patients (data not shown).

Laboratory data. Changes in the lipid levels are summarized in Table 1. Statistical evaluation showed no significant changes in levels of plasma lipids in either patient group. Only a tendency to an increase in HDL-C level in patients of the EG group was observed.

Figure 1 summarizes changes in the levels of total tocopherol and α-tocopherol after completion of the trial. An increase in levels of total tocopherol and α-tocopherol was seen in the EG group only ($P<0.025$ and $P<0.0125$, respectively). Changes in the GC group were not significant.

At baseline, the two groups of patients did not differ in factor VIIag, factor VIIc and PAI test results (data not shown). Table 2 summarizes the results after completion of the experiment. A significant decrease in all three parameters was seen in the EG group ($P<0.05$ for all three variables). After the trial, changes in values of factor VIIag, factor VIIc and PAI appeared not to have changed in the CG group. There were no significant differences in plasma pro-

Table 1. Serum lipids (mmol/l) in patients of both groups before and after the experiment.

Experimental group (EG)				Control group (CG)			
TC	LDL-C	HDL-C	TG	TC	LDL-C	HDL-C	TG
Before the experiment							
5.42±0.5	3.71±0.4	0.92±0.1	1.62±0.2	5.44±0.5	3.72±0.4	0.93±0.1	1.62±0.2
(3.83–7.01)	(2.44–4.98)	(0.60–1.24)	(0.98–1.26)	(3.83–7.03)	(2.45–4.99)	(0.61–1.25)	(0.92–1.62)
After the experiment							
5.39±0.5	3.66–0.4	1.01–0.1	1.60±0.2	5.45±0.5	3.73±0.4	0.92±0.1	1.64±0.2
(3.82–7.00)	(2.40–4.91)	(0.69–1.33)	(0.96–2.24)	(3.84–7.04)	(2.46–5.00)	(0.60–1.24)	(1.00–2.28)

Values are means and standard deviations and, in parenthesis, 95% confidence intervals of the means. Abbreviations: TC, total cholesterol; LDL-C, low-density cholesterol; HDL-C, high-density cholesterol; TC, triglycerides.

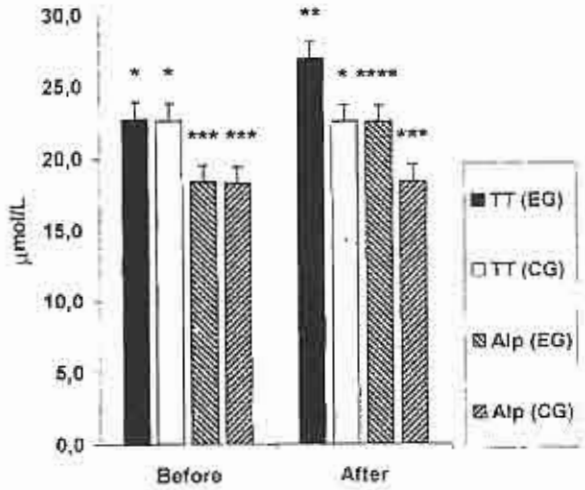

Figure 1. Total (TT) and α- (Alp) tocopherols in experimental (EG) and control (CG) groups. Means ± SD (vertical lines). Bars with a different number of stars are statistically different ($P<0.05$). An increase in the level of total and α-tocopherols is seen in the EG group ($P = 0.025$ and $P = 0.0125$, respectively). In the CG group the changes were not significant.

tein levels in either group before and after completion of the trial (Table 3).

At baseline, the electrophoretic images of plasma total proteins in both groups of patients were similar. These images corresponded to the plasma total protein spots from the data bank. After 30 days of moderate beer consumption, in 12 out of 19 patients of the EG group some qualitative changes in separated plasma proteins were found. The most typical qualitative changes in proteins were seen in patient N. These changes are described in the Figures 2 and 3. Figure 2 shows the changes in the albumin and globulin fractions of patient N. The following changes in the albumins were detected: in the molecular range of 5–20 kDa there were fewer protein spots, and these spots were shifted to the basic region with a pI of ca 7.8. The changes in globulin appeared in the range of 6–20 kDa: the number of spots was higher than before beer consumption.

Changes in methanol-precipitable protein of patient N are presented in Figure 3. The number of spots in the low-molecular-weight range of 6–10 kDa was higher than before beer consumption. It can also be seen that more profound changes after beer consumption appear in methanol-precipitable proteins than in albumins and globulins (Figure 2).

Table 2. Factor VIIag, factor VIIc and plasminogen activator inhibitor (PAI).

Experimental group					Control group				
before experiment		after experiment		P	before experiment		after experiment		P
range	mean ± SD (95% CI)	range	mean ± SD (95% CI)		range	mean ± SD (95% CI)	range	mean ± SD (95% CI)	
Factor VIIag (%)									
91–99	95.9±9.0 (77.1–114.7)	71–89	75.0±8.0 (58.3–91.7)	<0.05	90–99 (77.0–115.0)	96.9±9.1	89–99	96.1±9.0	n.s.
Factor VIIc (%)									
92–99	96.1±9.1 (77.1–115.1)	80–88	74.9±8.0 (58.2–91.6)	<0.05	91–99	96.1±9.0	92–99	96.2±9.2	n.s.
PAI (wl)									
6.9–8.9	7.8±0.7 (6.3–9.3)	5.7–7.5	6.1±0.6 (4.8–7.4)	<0.05	6.8–9.0	7.8±0.7 (6.3–9.3)	6.9–9.0	7.7±0.7 (6.2–9.2)	n.s.

Values are mean ± SD and, in parenthesis, 95% confidence interval (CI).

Table 3. Total protein (g/l), albumin (g/l) and globulin (g/l) before and after the experiment.

	Experimental group			Control group		
	before	after	P	before	after	P
Total protein	52.3–85.5 68.9±9.1 (49.9–87.9)	52.1–85.6 68.8±9.0 (50.0–87.6)	n.s.[a]	52.0–85.4 68.7±9.1 (49.7–87.7)	52.5±85.3 68.8±9.0 (50.0–87.6)	n.s.
Albumin	40.2–62.1 51.1±5.1 (40.4–61.8)	40.3–62.2 51.2±5.0 (40.7–61.7)	n.s.	40.4–62.3 51.2±5.0 (40.7–61.7)	40.5–62.4 51.3±5.1 (40.6–62.0)	n.s.
Globulin	12.7–18.1 15.4±2.7 (9.8–21.0)	12.6–18.0 15.3±2.6 (9.9–20.6)	n.s.	12.5–17.9 15.1±2.6 (9.7–20.5)	12.6–18.2 15.3±2.7 (9.7–20.9)	n.s.

Values, from top to bottom, are range mean ± standard deviation and 95% confidence interval (n = 19).
n.s., non-significant.

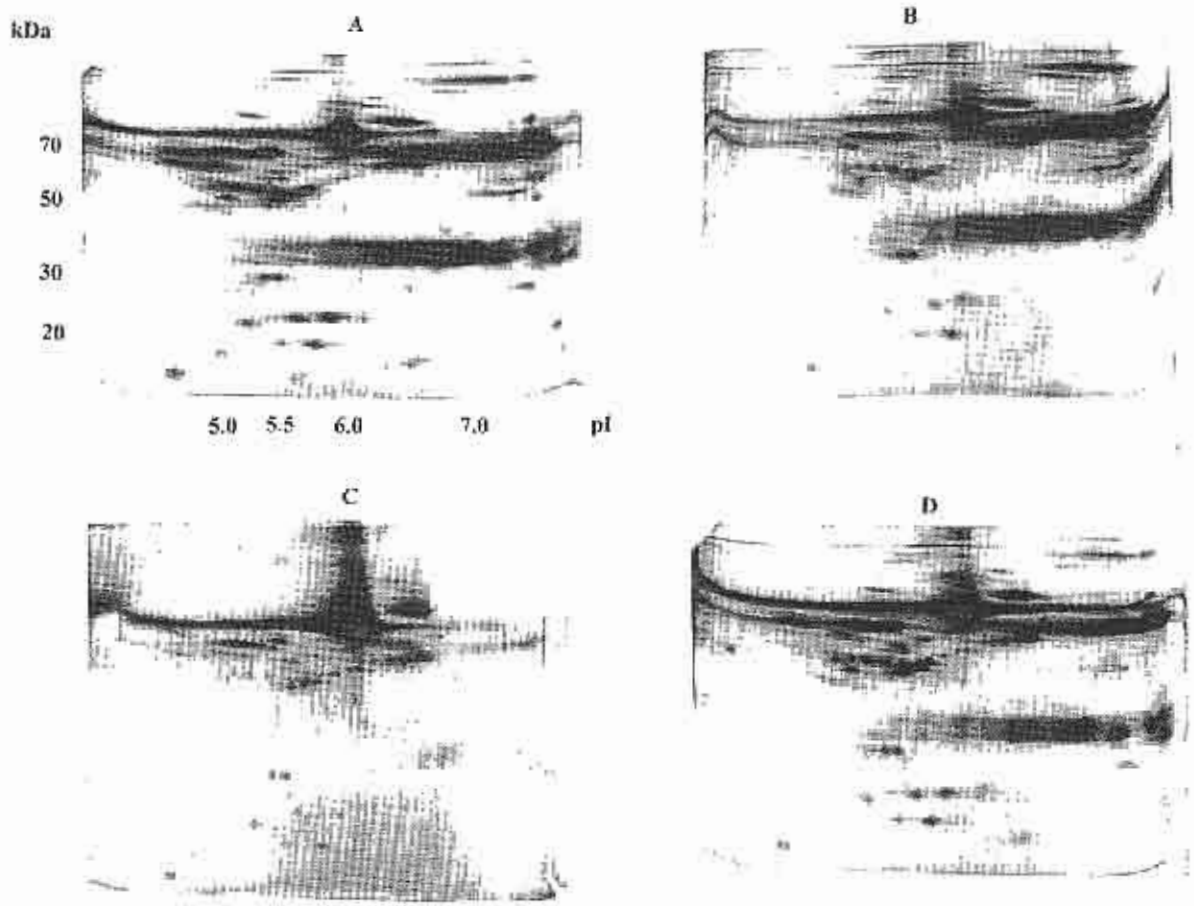

Figure 2. Patterns of plasma proteins in patient N are shown by two-dimensional electrophoresis (2-DE). Albumins before (A), albumins after (B), globulins before (C) and globulins after (D) beer consumption. Albumins and globulins were separated from serum, using different concentrations of ammonium sulfate, then dialysed against water, freeze-dried and dissolved in lysine buffer containing 9 M urea, 70 mM DTT and 2% ampholyte mixture (Servalyte 2-4), 25 mM Tris-/HCl pH 7.1, 50 mM KCl, 3 mM EDTA, 2.9 mM benzamidine, 2.1 µM leupeptin. Then the sample in a concentration of 33 µg/µl was subjected to isoelectrofocusing (IEF) in the first dimension. The IEF gels contained 2% of an ampholyte mixture at pH 2–11. In the second dimension sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used with 15% polyacrylamide gels. The 2-DE gel size was 7–8 cm. The vertical axis shows the molecular mass of proteins in kDa, and the horizontal axis the distribution of proteins by their isoelectric points. Patterns in B show that in the range of 5–20 kDa there are fewer protein spots and the isoelectric points (pI) shift to a pH of ca. 7.8. Patterns in D show that globulin appears between 6 and 20 kDa, the numbers of spots are raised after beer consumption.

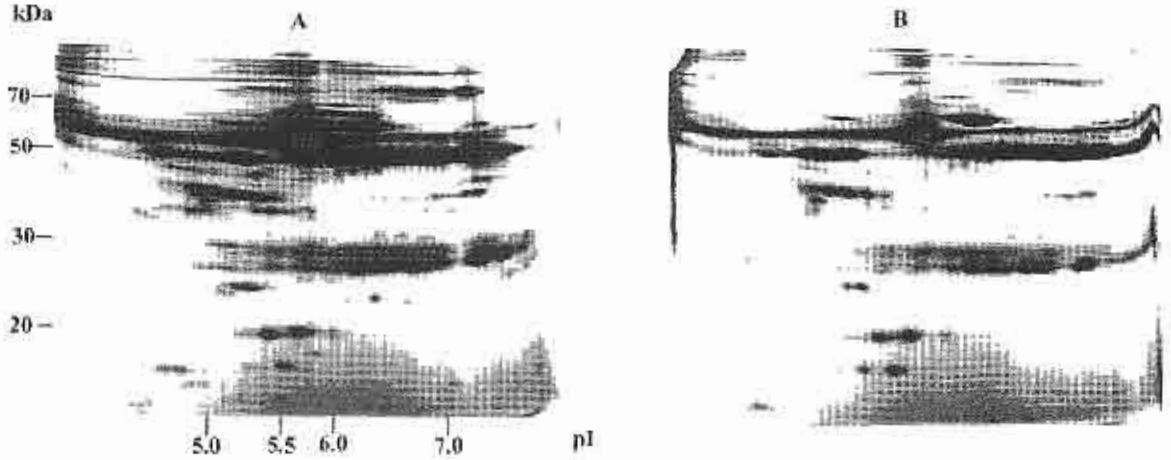

Figure 3. Two-dimensional electrophoresis of methanol-precipitable plasma proteins from patient N before (A) and after (B) beer consumption. These proteins were isolated from serum by methanol, then dialysed against water, freeze-dried and dissolved in the same conditions as for the separation procedure in Figure 2. Spots in B differ from those in A in low-molecular-weight proteins (20–30 kDa).

The methanol-precipitable fraction did not only differ in distribution of protein spots, but also in its functional properties such as hydrophobicity (an indicator of protein stability).

Before the trial the hydrophobicity of albumins (2 M), globulins (4 M) and methanol-precipitable proteins (M) was 9.23±0.8, 23.85±1.8 and 37.4±3.1, respectively (Figure 4). Methanol-precipitable proteins showed a higher hydrophobicity than albumins and globulins. Thus, the data presented in Figure 4 show that the methanol-precipitable fraction is less stable than albumins and globulins in human serum after beer consumption.

No detectable changes in plasma proteins after the trial were found in the CG group.

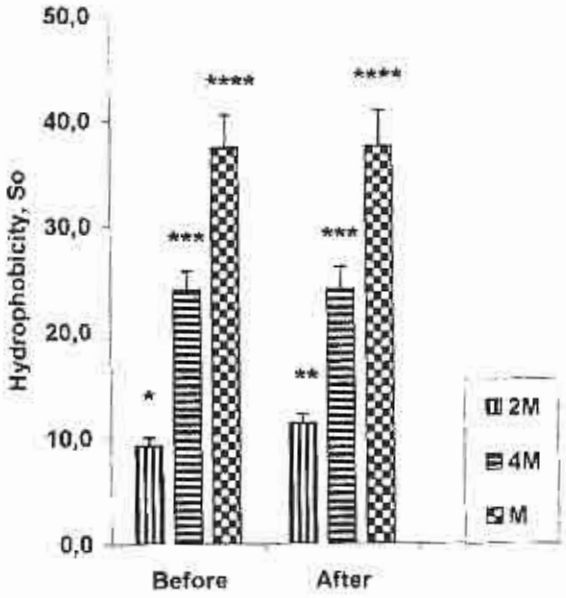

Figure 4. The hydrophobicity of albumins (2 M), globulins (4 M) and methanol-precipitable proteins (M) before and after beer consumption. Mean ± SD (vertical lines). Bars with a different number of stars are statistically different ($P<0.05$). Hydrophobicity (S_0) was determined by 1-anilino-8-naphthalenesulfonate-fluorescent probe measurements. Hydrophobicity reflects the stability of proteins. Thus, the methanol-precipitable fraction is less stable than albumins and globulins in human serum.

Discussion

Epidemiological, experimental and clinical studies convincingly show that moderate drinking can induce favourable biochemical changes and is associated with a decreased mortality from CAD (3, 7–9, 24–27). Alcohol consumption has also adverse effects: it negatively influences protein metabolism (10–12). The aim of this study was to evaluate whether short-term moderate beer consumption could lead to both positive and negative effects.

It is an established fact that elevated levels of total cholesterol, LDL-C, triglycerides, apolipoproteins B and C-III and reduced levels of HDL-C and apolipoprotein A-I are major risk factors for atherosclerosis (28–30). Recent evidence suggests that one of the important mechanisms predisposing to the development of atherosclerosis is oxidation of cholesterol-rich LDL-C particles (31–33). Oxidation of LDL-C enhances its atherogenicity and facilitates penetration of lipids into arterial wall. Thus, prevention of atherosclerosis is a fight against LDL-C oxidation by using antioxidants (34). According to Renaud and Lorgeril (3), the positive effect of alcohol consumption is in prevention of blood coagulation. How were these three parameters affected by short-term moderate beer consumption in our study?

We found a tendency towards an increase in HDL-C, an increase in antioxidant and anticoagulant activity (a significant rise in the contents of both total tocopherol and α-tocopherol and decreases in factor VIIag, factor VIIc and PAI, respectively). Therefore, even short-term moderate beer consumption has a cardioprotective effect. These results support our previous findings and the findings of other groups (3, 7, 8, 24, 26, 27).

Alcohol has not only beneficial but also adverse effects. Some groups have reported that alcoholic consumption negatively influences protein metabo-

lism (10–15). We, who advocate beer consumption (7, 8), have asked ourselves: could moderate consumption of this beverage negatively influence plasma proteins? In our previous study we did not find quantitative changes in plasma proteins in patients with coronary atherosclerosis after short-term moderate beer consumption (16). However, it could not be excluded that there are qualitative changes. Therefore, we decided to investigate the influence of moderate beer consumption on the structural and functional characteristics of plasma proteins. Two-dimensional electrophoresis, intrinsic fluorescence and surface hydrophobicity (So) were used to examine these characteristics of plasma proteins before and after completion of the trial. Like in our previous investigation (16), no quantitative changes in plasma proteins were found. In 12 out of 19 beer consumers a tendency to lower stability and minor structural deviations in plasma proteins were detected. These findings confirm the claims of others that consumption of alcoholic beverages negatively influences protein metabolism (10–15). Thus, what is the answer to the paraphrase of Shakespearean's Hamlet: to drink or not to drink? We cannot tell (1) why only in 12 out of 19 patients the above-mentioned changes in plasma protein were found, (2) whether these changes are transitional and could disappear after termination of beer consumption, (3) if these changes become more profound as beer consumption continues, and (4) if similar changes could appear during consumption of other alcoholic beverages?

In conclusion, in this investigation a tendency towards lower stability and minor structural deviations in plasma protein were detected. However, we also found in most of the beer-consuming patients positive changes in lipid metabolism and increases in anticoagulant and antioxidant activity. These findings allow us to answer positively the paraphrase 'to drink or not to drink'.

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