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Beer increases plasma antioxidant capacity in humans

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The positive association of a moderate intake of alcoholic beverages with a low risk for cardiovascular disease, in addition to ethanol itself, may be linked to their polyphenol content. This article describes the effect of acute ingestion of beer, dealcoholized beer, and ethanol (4.5% v/v) on the total plasma antioxidant status of subjects, and the change in the high performance liquid chromatography profile of some selected phenolic acids (caffeic, sinapic, syringic, and vanillic acids) in 14 healthy humans. Plasma was collected at various times: before (T0), 1 hour after (T1), and 2 hours after (T2) drinking. The study is part of a larger research planned to identify both the impact of brewing on minor components potentially present in beer and their metabolic fate in humans. Beer was able to induce a significant ($P < 0.05$) increase in plasma antioxidant capacity at T1 (mean ± SD: T0 1,353 ± 320 μM; T1 1,578 ± 282 μM), returning close to basal values at T2. All phenolic acids measured in plasma tended to increase after beer intake (20% at T1, 40% at T2). Syringic and sinapic acid reached statistical significance ($P < 0.05$ by one-way analysis of variance-Fisher's test) at T1 and T2, respectively. Plasma metabolic parameters (glucose, total cholesterol, triglycerides, and uric acid) and plasma antioxidants (α-tocopherol and glutathione) remained unchanged. Ethanol removal impaired the absorption of phenolic acids, which did not change over the time of the experiment, accounting for the low (and not statistically significant) increase in plasma antioxidant capacity after dealcoholized beer drinking. Ethanol alone did not affect plasma antioxidant capacity or any of the antioxidant and metabolic parameters measured. (J. Nutr. Biochem. 11: 76–80, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

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Introduction

Several epidemiologic studies have indicated the association of moderate ethanol consumption with a reduction in all-cause mortality, particularly with a reduced risk of coronary heart disease (CHD).[1,2] With regard to the form by which ethanol is assumed, Grønbaek et al.[1] reported that beer and wine are associated with a reduced mortality from CHD, whereas spirits lead to an increased risk.

Ethanol is able to increase high density lipoprotein (HDL)-cholesterol plasma level, to decrease platelet aggregation, and to enhance blood fibrinolysis, all events linked to a low risk of CHD.[3–5] However, the protective effects of some alcoholic beverages (wine and beer) may result from (or be implemented by) its nonethanol component, prevalently consisting of nonvitamin phenolics.[6–8]

Once absorbed, phenolic compounds present in beer and wine seem to be able to contribute to the total antioxidant capacity of plasma (TRAP)[7,8] and possibly of other body compartments, thus reinforcing the defenses against the oxidative stress. However, there is little scientific information on their bioavailability, time and site of absorption, influence of ethanol, and metabolic fate in humans.

This study aimed (1) to study the effect of beer drinking on the antioxidant capacity of human plasma, (2) to evaluate the effect of ethanol on the absorption of phenolics, and (3) to study the time course of the absorption of selected phenolics (caffeic, sinapic, syringic, and vanillic acids).

This article is part of a larger program focusing on the nutritional properties of beer. A more in-depth analytical and technological study in close connection with this article is published elsewhere.[9]

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Methods and materials

Experimental protocol

Fourteen healthy, fasting nonsmokers (7 males and 7 females; 25–45 years) received in the morning 500 mL of beer. The beers used in this study were 4.5% ethanol lager types produced in Italy, coming from four representative brands and anonymously provided. Each brand was administered to at least three subjects. Subjects were either nondrinkers or were social drinkers (<28 and <14 g ethanol per day, for males and females, respectively), and they were not taking dietary antioxidant supplements. Because the parameters evaluated in the four beer brands did not differ significantly, data are presented as a single group. Blood was collected before (T0) and 1 (T1) and 2 (T2) hours after beer drinking.

To discriminate the effect of phenolic compounds from that of ethanol, 500 mL of dealcoholized beer or a 4.5% solution of ethanol in tap water were administered to a smaller group of 7 subjects (3 males and 4 females; 25–45 years). Ethanol was removed from beer by lyophilization and samples were reconstituted with pure water. No significant changes in the concentration of phenolic acids were observed after lyophilization (data not shown).

Blood samples were centrifuged and plasma was immediately analyzed for total antioxidant capacity. Plasma samples for metabolic control (glycemia, total cholesterol, triglycerides, and uric acid) and for antioxidants (vitamin E and glutathione) were stored at −80°C until the analysis.

Chemicals and reagents

Methanol and acetone [high performance liquid chromatography (HPLC) grade] were produced by Carlo Erba (Milan, Italy). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) came from Aldrich Chemical Co. (Milwaukee, WI USA). 2,2′-Diazo bis amidine propane dihydrochloride (AAPH) came from Polyscience (Warrington, PA USA). All other chemicals came from Sigma Chemical Co. (St Louis, MO USA).

Blood glycemia, total cholesterol, triglycerides, and urate were measured by commercial kits purchased from Sigma Chemical Co. Vitamin E was analyzed by HPLC as previously described;[10] glutathione was measured in plasma according to Neuschwander-Tetri and Roll.[11]

The total antioxidant capacity of beer samples and plasma was measured as previously described.[12] In brief, a fluorescent probe (R-phycoerithrin, R-PE) loses its fluorescence when exposed to a constant flow of peroxyl radicals, generated by the thermal decomposition of AAPH. Solutions or body fluids containing antioxidants are able to inhibit R-PE oxidation in a dose-dependent manner. Therefore, by standardizing with a known antioxidant (Trolox), it is possible to quantify the antioxidant capacity of the sample. The antioxidant capacity of beer samples is expressed (as conventional for foods) as Trolox equivalents (TE), defined as the antioxidant capacity of 1.0 mM Trolox.[13] The antioxidant capacity of plasma (TRAP) is expressed as μmoles of peroxyl radicals trapped by 1 L of plasma (μM).

Measure of phenolic compounds in plasma

A reliable HPLC-electrochemical method has been set up for measuring selected phenolic acids (caffeic, sinapic, syringic, and vanillic). To evaluate the rate of absorption, plasma phenolic pattern was determined at three different times (before and 1 and 2 hours after drinking beer, dealcoholized beer, or hydroalcoholic solution).

The method is able to evaluate plasma concentrations of phenolic compounds as low as 0.2 nanogram/mL. Samples (500 μL of beer or plasma) were acidified with 1.0 N HCl to pH 1.5 ± 0.1. Three hundred mg of solid NaCl were added with mixing. Then samples were extracted with four 1.0-mL parts of HPLC-grade diethyl ether on a vortex for 2 minutes. The joined organic layers were evaporated to dryness and the residue was dissolved in mobile phase. Mobile phase consisted of two solutions: Solution A was 0.22 M acetic acid and Solution B was methanol. A binary gradient (ranging from 7 to 24% methanol) was applied to a reverse phase ODS-2 (150 × 4.0 mm) analytical column maintained at 30°C.

The eluate was monitored with an ESA (Bedford, MA USA) Coulochem II electrochemical detector equipped with a conditioning cell (Model 5021) followed by the analytical cell (Model 2011). Settings were as follows: the conditioning cell and the first electrode of the analytical cell were set at −100 mV; the second electrode was the analytical one and was set at +600 mV. The output of the detector was registered on a Perkin Elmer Turbochrom Chromatography workstation.

Statistical analysis

Data were expressed as mean and standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA).

Table 1 Plasma TRAP values after administration of 500 mL in bolus of beer, dealcoholized beer, or a 4.5% solution of ethanol in tap water

	Whole beer (μM)	Dealcoholized beer (μM)	Hydroalcoholic solution (μM)
Time 0	1,353 ± 320	1,341 ± 420	1,440 ± 261
Time 1 h	1,578 ± 282*	1,453 ± 483	1,400 ± 289
Time 2 h	1,290 ± 312	1,464 ± 537	—

*$P < 0.05$ from time 0 by one-way analysis of variance (Fisher's test).
Values are expressed as mean ± SD.
TRAP–total antioxidant capacity of plasma.

Results

As previously reported,[14] beer contains an appreciable amount of phenolic compounds that contribute to the overall antioxidant capacity of the product. The four brands tested have an antioxidant capacity of 0.718 ± 0.028 TE (ranging from 0.634 to 0.800), which is much lower than that of red wines (19.8 TE) and of the same order of magnitude as the antioxidant capacity of white wines (0.950 TE).[7]

In our study, the ingestion of 500 mL of beer in bolus produced a statistically significant increase (approximately 17%) in TRAP at T1 (*Table 1*), decreasing close to basal values after 2 hours (T2). Although ethanol removal did not affect the original phenolic content of the beer, its administration failed to induce a significant increase in plasma TRAP. The administration of the hydroalcoholic solution did not influence plasma TRAP. All treatments did not affect any of the metabolic parameters measured (total cholesterol, triglycerides, glycemia, and uricemia) or the antioxidant markers (vitamin E and glutathione) (*Table 2*).

The changes in antioxidant capacity did not reflect in relevant differences in the plasma levels of the selected phenolic acids after the ingestion of 500 mL of either whole beer or dealcoholized beer (*Table 3*). An unknown peak, which is present in plasma even in fasting conditions,

Table 2 Plasma values of some metabolic parameters after administration of 500 mL in bolus of beer, dealcoholized beer, or a 4.5% (v/v) solution of ethanol in tap water

	Whole beer			Dealcoholized beer			Hydroalcoholic solution		
	Time 0	Time 1 h	Time 2 h	Time 0	Time 1 h	Time 2 h	Time 0	Time 1 h	Time 2 h
Total cholesterol, mg/dL	192 ± 28	192 ± 30	191 ± 28	162 ± 26	171 ± 27	175 ± 38	213 ± 33	200 ± 38	—
Triglycerides, mg/dL	107 ± 88	119 ± 73	101 ± 60	70 ± 23	73 ± 29	69 ± 21	154 ± 125	155 ± 112	—
Glycemia, mg/dL	97 ± 16	93 ± 23	86 ± 8	94 ± 18	78 ± 12	99 ± 3	101 ± 29	102 ± 18	—
Uric acid, mg/dL	4.5 ± 1.8	5.0 ± 1.6	5.2 ± 1.5	4.6 ± 1.1	5.1 ± 1.0	5.2 ± 1.9	4.5 ± 1.4	4.6 ± 2.0	—
Vitamin E, mg/dL	10.7 ± 2.5	10.4 ± 2.5	10.0 ± 2.0	9.3 ± 1.4	10.1 ± 1.5	9.6 ± 1.5	12.3 ± 1.4	10.5 ± 2.0	—
Glutathione, μM	5.9 ± 1.5	6.1 ± 0.8	—	5.6 ± 0.6	5.8 ± 1.1	—	4.9 ± 0.7	5.3 ± 1.6	—

There are no statistically significant differences by one-way analysis of variance (Fisher's test) among times in the three experimental groups. Values are expressed as mean ± SD.

increases after ingestion of both whole and dealcoholized beer (approximately 7- and 4-fold, respectively, expressed as area). This compound, which is not present in beer, could be a common metabolite of phenolics, still maintaining a high antioxidant capacity. In fact, the peak coincides with the maximum of the antioxidant capacity (T1); (*Figure 1*).

The sum of the phenolic acids we measured tended to increase in the case of the whole beer (24 μg/mL at baseline, 33 μg/mL at T1, and 42 μg/mL at T2), whereas that of dealcoholized beer seemed to have no effect (34 μg/mL at baseline, 28 μg/mL at T1, and 29 μg/mL at T2); (*Table 3*).

Discussion

Large population studies have shown a U-shaped relation between alcohol and mortality, the mortality rate being lower in people reporting moderate alcohol intake than in either nondrinkers or heavier drinkers (>34 g alcohol/day).[15,16] Moreover, moderate consumption of alcoholic beverages, in particular wine and beer, is reported to be associated to a diminished mortality from cardiovascular diseases.[1,17]

Both ethanol (at low doses) and phenolic compounds play important and different roles in protection against CHD. Platelet aggregation, for example, which is the mechanism underlying myocardial infarction, is reported to be affected by ethanol,[4] but also by some phenolic compounds present in wine and beer.[6,18] Moreover, ethanol, in addition to its direct effect on platelet function, HDL metabolism, and fibrinolysis (all involved in the pathogenesis of cardiovascular diseases), could play an important indirect role in the absorption of phenolic compounds. Phenolics are aromatic compounds that are hardly soluble in water, but easy soluble in ethanol. The increased solubility of these compounds in hydroalcoholic solutions may affect the rate and the amount of their absorption. This is supported by the results of Miyagi et al,[19] who found a significant in vitro inhibition of human low density lipoprotein (LDL) oxidation in the presence of both red wine and grape juice. In vivo ingestion of red wine was able to significantly protect LDL, suggesting that flavonoids in red wine can be absorbed more efficiently than flavonoids in grape juice.

Our results on the plasma antioxidant activity seem to be in agreement with the data reported by Miyagi et al.[19] In fact, although the intake of whole beer significantly increased plasma TRAP, dealcoholized beer showed just a slight tendency to increase the antioxidant capacity, and ethanol (as expected) had no effect.

Our data on phenolic absorption suggest that whole beer is able to transfer its phenolic compounds (at least the

Table 3 Plasma levels of phenolic acids after drinking of whole (W) and dealcoholized (D) beer

Phenolics		Plasma T0 (ng/mL)	Plasma T1 (ng/mL)	Plasma T2 (ng/mL)	*P*
Caffeic acid	W	13.7 ± 11.1	16.8 ± 24.6	22.8 ± 27.0	0.7068
	D	15.6 ± 14.9	7.7 ± 5.1	6.2 ± 4.7	0.4360
Sinapic acid	W	1.5 ± 2.1	1.9 ± 2.5	5.8 ± 5.8*	0.0761
	D	0.4 ± 0.8	0.2 ± 0.4	0	0.0961
Syringic acid	W	0.9 ± 0.9	5.1 ± 5.9*	1.9 ± 1.9	0.7575
	D	7.0 ± 5.5	8.2 ± 6.3	7.9 ± 7.3	0.9507
Vanillic acid	W	7.6 ± 6.5	8.8 ± 2.3	11.4 ± 10.6	0.5760
	D	10.8 ± 6.6	11.5 ± 6.5	14.7 ± 0.9	0.7548
Unknown peak†	W	49 ± 7	349 ± 65	208 ± 36	0.0003
	D	73 ± 13	296 ± 60	221 ± 25	0.0041
Sum	W	23.7 ± 12.1	32.7 ± 25.5	41.9 ± 31.3	0.3466
	D	33.8 ± 20.7	27.6 ± 11.5	28.7 ± 1.6	0.8190

*$P < 0.05$ from time 0 by one-way analysis of variance (Fisher's test).
†The values of the unknown peak are expressed as area *10^6.
Values are expressed as means ± SD of 8 (W) and 5 (D) subjects.

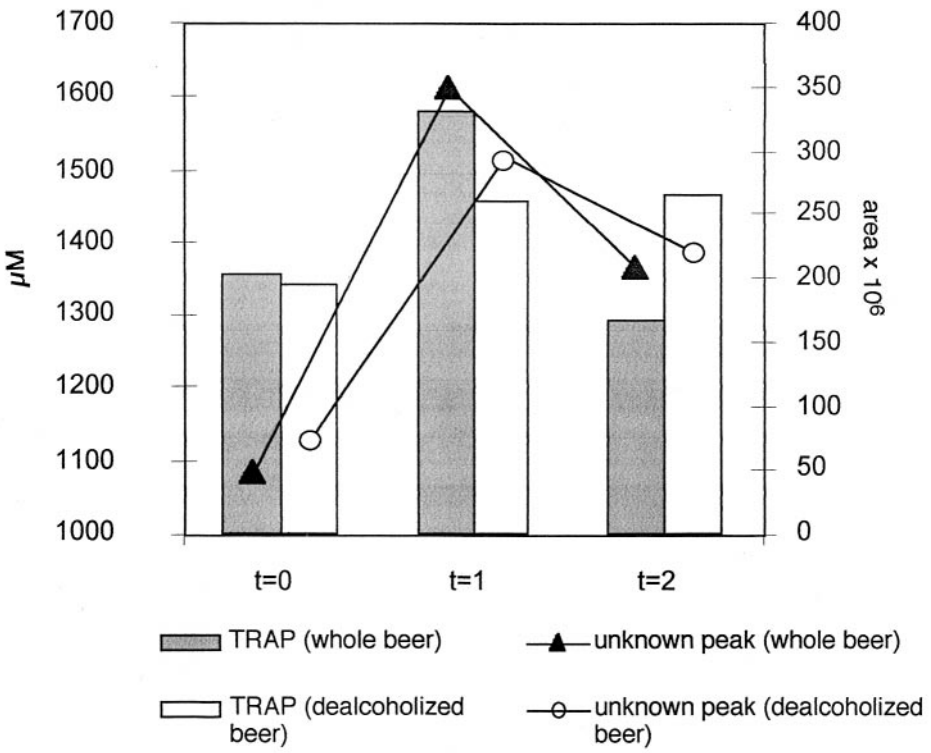

Figure 1 Relationship between the increase of plasma antioxidant capacity (TRAP) and the area of the unknown peak after supplementation of beer and dealcoholized beer.

molecules detectable in our analytical conditions) to body fluids more efficiently than dealcoholized beer (*Table 3*). Moreover, although the behavior of the unknown peak follows that of plasma TRAP, which peaks at T1, plasma phenolics continue to increase (*Table 1*). Although other compounds that are present in beer and not measured in the present study may contribute to the plasma antioxidant capacity, this peak could represent a key molecule in the modulation of plasma antioxidant capacity. Its presence in plasma even in fasting conditions, and its increase after phenol's intake, suggests that the peak can represent either a long-living metabolite of phenolic compounds or an unidentified molecule endowed with antioxidant activity, as suggested by its electrochemical detectability. The unknown peak also increases to a minor extent in the absence of ethanol (dealcoholized beer). In this case, it is also possible to note a parallel, but not significant, increase in plasma TRAP (*Figure 1*).

Gorinstein et al.[20] reported a significant beneficial antioxidant effect in rats supplemented for 4 weeks with both alcohol-containing and alcohol-free beer. The discrepancy with our results in the effect of alcohol-free beer can probably be explained by the modality of intake: acute, as our case, versus chronic.

Similar results on the variation of plasma antioxidant capacity after acute administration have been reported by different authors for white wine.[7,21] Although the methodologies employed were slightly different, the combined results indicate a consistent increase of plasma antioxidant capacity induced by "whole" white wine[21] and a lack of effect by the same amount of "dealcoholized" white wine.[7]

In conclusion, our findings indicated that beer, which has a moderate antioxidant capacity coupled with a low ethanol content, is an alcoholic beverage that is able to improve plasma antioxidant capacity without the negative effects produced by high doses of ethanol. In fact, although the amount of ethanol present in 500 mL of beer (approximately 18 g) did not induce any appreciable change in the markers of metabolic control (triglycerides, uric acid, and glycemia), it is able to facilitate the transfer of the antioxidant capacity from beer to body fluids, probably through the increase of the absorption of phenolic compounds.

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