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Phenol Antioxidant Quantity and Quality in Foods: Beers and the Effect of Two Types of Beer on an Animal Model of Atherosclerosis

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The free phenols have been measured in 15 lagers, 6 porters and ales, and 11 light and nonalcoholic beers. Phenols were measured colorimetrically using an oxidation−reduction reaction with Folin−Ciocalteu reagent and catechin as the standard. The order of phenol concentration was ales > lagers > low calorie > nonalcoholic. The quality of antioxidants of the major phenols in beers and the quality of beer antioxidants were measured by (1) dose−response inhibition of lower density lipoprotein oxidation and (2) concentration of phenols in the beers at which 50% of the peroxide was destroyed in a luminescent assay for antioxidant activity. 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. The average per capita consumption of beer in the United States in 2000 was 225 mL/day, equivalent to 42 mg/day of catechin equivalents. Beer provides more antioxidants per day than wine in the U.S. diet. A dark beer and a lager beer were given at two concentrations to cholesterol-fed hamsters, an animal model of atherosclerosis. At the high dose ($^1/_2$-diluted beer) both lager and dark beer significantly inhibited atherosclerosis compared to a control of 2% alcohol. At the high dose, lager significantly decreased cholesterol and triglycerides, and both beers acted as in vivo antioxidants by decreasing the oxidizability of lower density lipoproteins. At the low dose ($^1/_{10}$-diluted beer) only the lager beer significantly decreased atherosclerosis compared to the 0.4% alcohol control. The polyphenols in the beers appear to be responsible for the benefits of beer in this model. Lager beer inhibited atherosclerosis at a human equivalent dose in this hamster model of atherosclerosis.

KEYWORDS: Phenols; antioxidants; beer; lipoprotein oxidation; atherosclerosis

INTRODUCTION

Epidemiological evidence from many studies overwhelmingly supports the hypothesis that moderate alcohol consumption is significantly associated with a reduction in coronary heart disease (CHD) mortality (*1*, *2*). Alcoholic beverages that were especially beneficial were beer and red wine (*3*). Ethanol is able to increase the level of high-density lipoprotein (HDL; the "good" cholesterol), to decrease platelet aggregation, and to enhance blood fibrinolysis. All of these mechanisms are associated with a low risk of CHD (*4*−*6*). However, the protective effects of some alcoholic beverages may be the result of the nonalcoholic components such as the phenolics present. There is ample epidemiological evidence that consumption of these phenol compounds reduces the risk of CHD (*7*, *8*).

It has been hypothesized that oxidation of low-density (LDL) and very low (VLDL) density lipoproteins is a crucial step in atherosclerotic lesion formation (*9*). Ethanol is normally assumed to be a pro-oxidant in vivo. However, we have recently demonstrated in a hamster model of atherosclerosis that 6% ethanol consumption produces a decreased susceptibility of LDL + VLDL to oxidation (*10*). Also, beer supplementation to rats resulted in lipoproteins that were more resistant to oxidation (*11*). In a human study the ingestion of 500 mL of beer produced a significant temporal increase in plasma antioxidant activity (*12*).

Beer is a very popular alcoholic beverage and consumed in large amounts in almost all countries, with a yearly intake for 68 reporting countries of 123.4 billion liters in 1998 (*13*). Beer has been produced for over 4000 years by a simple process. Barley and water are combined in the malting process that

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produces fermentable sugars. The sprouted barley grain is then roasted, which creates beer's color and flavor. The roasted barley kernels are then ground into a grain mixture and then mixed with hot water to form a mash. This process allows the enzymes contained in the grain to convert the starches of the mashed grains into sugar. The sweet liquid solution created by the germinated grain water is called wort. Hops, dried flowers from the spice-like hops plant, are then added to the wort and the mixture is boiled in a kettle. The fermentation process is then begun by adding either of two kinds of yeasts, *Saccharomyces cerevisiae*, a top fermenting yeast that produces ales, porters, and stouts, and *Saccharomyces uvarum*, a bottom fermenting yeast that produces lagers (*14*). Barley and hops are the source of phenols in beer, with barley providing the most (*15*). Common beer phenols are flavonols, phenolic acids, catechins, procyanidins, and tannins.

A Belgian team has found that hop extracts were 30 times more effective than malt in inhibiting the peroxy radical oxidation of linoleic acid (*16*). Recently Shibamoto has demonstrated that a volatile extract of beer had antioxidant activity comparable to that of vitamin E (*17*). Also, xanthohumol, the major prenylchalcone in hops and beer, was a much better antioxidant than vitamin E in cupric ion catalyzed LDL oxidation (*18*). We have shown that many phenols are strong antioxidants compared to the vitamin antioxidants, using as a model the oxidation of the lower density lipoproteins (LDL + VLDL) (*19*). Phenols have also been found to enrich these lipoproteins (*20*) after spiking in plasma. Thus, they can provide protection when the lipoproteins penetrate the endothelium of the aorta, where they are subsequently oxidized (*9*). We will therefore investigate the quantity and quality of the phenols present in beers and the pure phenolic constituents. By analogy with wine, it is possible that beer consumption may protect LDL and VLDL from oxidation in vivo and therefore be beneficial with respect to heart disease. We thus undertook an investigation of beer and heart disease using an animal model of atherosclerosis.

MATERIALS AND METHODS

Samples. Beer samples were obtained from commercial sources in both the United States and Canada. Pure phenols found in beer were obtained from Sigma Chemical Co., St. Louis, MO. Beers were refrigerated and analyzed immediately upon opening to prevent loss of phenols by oxidation.

Analysis. Oxidizable phenols were measured in duplicate samples of each beer at 750 nm using the Folin−Cocialteu reagent diluted 5-fold before use (Sigma), with catechin as the standard. Any interference by sulfite was removed by pretreating the beers with acetaldehyde (*21*). To calculate the per capita consumption of phenols from beers, the most recent consumption data from 2000 was used in the calculations (*22*).

The quality of the phenol antioxidants was measured by determining the IC_{50} (the concentration required to inhibit oxidation by 50%) of the pooled phenol extracts for each beer (*23*). Phenols and beers (catechin equivalents) were added to the LDL + VLDL at concentrations ranging from 0.2 to 10 μM followed by a standard oxidation with cupric ions. The oxidation mixture was reacted with thiobarbituric acid, and the products were measured by fluorometry in butanol. A native sample without cupric ions and a blank sample without an antioxidant were also analyzed. All samples were done in duplicate.

The quality of the Canadian beer was determined using a published method (*24*). Briefly, the experiments used luminol adsorbed to bovine albumin as a chemiluminescent detecting agent for reactive oxygen species, modeled using peroxide (hydrogen peroxide). Luminescent counts were detected by a Lumac 2010 luminometer (Celsis, Chicago, IL). Alcohols were added at different concentrations to the assay mixture, and the counts per minute plotted against the beer concentration. Beers were diluted serially 10-fold to a dilution of 10^{-5}. The Folin catechin equivalent concentration of each beer, at which the diluted beer destroyed 50% of the peroxide, is recorded as the inhibitory concentration for 50% inhibition (PIC_{50}).

The ability of polyphenols from beer to enrich LDL + VLDL in plasma and protect them from subsequent oxidation was measured in one lager and one ale sample. Plasma was spiked with the beer to produce phenol concentrations of 50, 100, and 200 μM along with a control, and equilibrated for 1 h at 37 °C. The LDL + VLDL was isolated and oxidized with cupric ions under standard conditions. The kinetics of conjugated dienes formation was determined at 234 nm and the lag time (where the initial slow oxidation line converges with the rapid oxidation line) measured (*23*, *25*). An increase in lag time versus the control indicates antioxidant activity.

Atherosclerosis Study. The hamster was used as a model of atherosclerosis due to the fact that when they are given a high-fat diet, they have a lipid profile very similar to that of humans. Hamsters develop foam cells, the earliest sign of atherosclerosis, after 10 weeks of feeding. Male, weanling, Syrian Golden hamsters were received from Charles River Breeding Laboratories (Wilmington, MA) and given commercial nonpurified rodent chow (Ralston Purina, St. Louis, MO) for 4 weeks. They were then separated into groups, each with comparable average weights. The animals were housed in plastic cages, three or four animals per cage with a bedding of wood chips, in a temperature-controlled room (20 °C) and a 12-h light/dark cycle. They were allowed free access to food and water. Animals were maintained following the guidelines of the University of Scranton Institutional Animal Care and Use Committee. The basal diet consisted of 0.2% cholesterol and 10% coconut oil (*26*). Two Czech beers were investigated: Urquell Pilsner, a lager beer (196 kJ/100 mL, 4.4% ethanol), and Gambrinus Dark (188 kJ/100 mL, 4.2% ethanol), supplied by Plzeñsky Prazdroj, a.s., Plzeň, Czech Republic. The beers were diluted 1/2 and 1/10 with distilled water and mixed with Sweet N'Low (15 g/L). Ethanol at 2.2 and 0.4% with Sweet N'Low was used for the corresponding control groups. Animal weights and food and beverage consumption were measured every 2 weeks during the study.

After 10 weeks of feeding, and after an overnight fast with the beverages replaced by water, the animals were anesthetized with pentobarbital (Sigma Chemical Co.), and a cardiac puncture was performed. Aortas were processed as previously described (*24*, *25*). In brief, the animals were perfused with 10% formaldehyde in phosphate-buffered saline and the aorta was isolated and prepared for histology and staining with Oil Red O. The percent of aortal surface covered with foam cells was then determined. The blood was put in an EDTA microtainer and the plasma isolated and stored at −90 °C until assay. Total cholesterol and triglycerides were measured with a Sigma enzyme assay. The HDL was measured similarly after precipitation of LDL + VLDL by a Sigma phosphotungstate reagent. The LDL + VLDL fraction was separated and isolated with heparin−agarose affinity columns (Sigma) using 200 μL of pooled plasma from each group and eluting with 2.7% NaCl. The fraction was oxidized under standard conditions with 25 μM Cu^{2+} and monitoring of conjugated dienes at 234 nm to determine the lag time (*23*).

RESULTS AND DISCUSSION

In Vitro and ex Vivo Studies. The quantity and quality of the phenol antioxidants in beers and the quality of the pure phenolic antioxidant components of beer are shown in **Table 1**. The average concentration and standard deviations of the groups are shown in **Figure 1** and are the following: ale/porter/stout, 1804 ± 453 μM; lager, 677 ± 278 μM; low calorie, 400 ± 138 μM; nonalcoholic, 346 ± 96 μM. The ales/porter/stouts were significantly higher in phenols than the other beers, $p < 0.001$. There was no significant difference between the lagers and the low calorie or the nonalcoholic beers with respect to phenols. Nonphenolics in beers such as carbohydrates and hydroxymethylfurfural (a Maillard product) did not have a response with the Folin reagent.

Table 1. Phenol Concentration in Beers, Quality of Antioxidants in Beers, and Quality of Phenol Antioxidants in Beers

sample/source	Folin phenols (μM as catechin)	LDL + VLDL oxidation IC_{50} (μM)	peroxides oxidation IC_{50} (μM)
lagers			
domestic	699	0.53	
domestic	754		
domestic	732	0.14	
domestic	165	0.47	
domestic	182	0.64	
Canada	787	0.25	69
Canada	953		12
Canada Ice	867		
Canada Ice	807		39
Canada Ice	1038		79
Canada	682		82
Canada	713		30
Czech Republic	1013	0.30	55
Mexico	461		
The Netherlands	310		
ales/porters/stouts			
domestic	2400	0.15	
domestic	1590	0.61	
domestic	2210	0.38	
Ireland	1134	0.36	59
Czech Republic	1782	0.22	
Canada	1710		6
low calorie			
domestic light	483	0.39	
domestic light	516		246
domestic light	343	0.50	
Canada light	522	0.41	136
Mexico light	371		
domestic light	165	0.36	
nonalcoholic			
Canada	391		
domestic	342	0.50	
domestic	187		
domestic	371		
domestic	440	0.42	
polyphenols found in beer			
syringic acid		2.29	
coumaric acid		1.28	
ferulic acid		0.54	
protocatechuic acid		1.07	
vanillic acid		>10	
caffeic acid		0.24	
p-hydroxybenzoic acid		>10	
tannic acid		0.15[a]	
epicatechin		0.19[a]	
quercetin		0.22[a]	

[a] Reference 19.

According to USDA statistics for 2000 (*22*) the average person in the United States consumes 225 mL of beer/day and 20.7 mL of wine/day. Assuming lager beer is consumed, then the average phenol intake as catechin equivalents is 42 mg/day. The same calculation using an average of our red wine and white wine data of 3397 μM (*28*, *29*) equals 21 mg/day from wines. Thus, beer provides ~2 times more polyphenols than wine on a per capita basis.

The quality of the antioxidants in the beers is a function of the chemical structure of the compounds in beer and was determined by calculating the IC_{50} and PIC_{50} (**Table 1**), with the lower number indicating the higher quality of antioxidants in the beers or the phenolic constituents. There was no significant difference among the types of beers with respect to the quality (IC_{50}) of phenolic antioxidants in the beers. The PIC_{50} values, although at concentrations of catechin equivalents per liter higher than for the lipoprotein oxidation, showed similar trends. The U.S. light beers contained less powerful antioxidants than the corresponding regular lagers when expressed in catechin equivalents. Canadian lagers and stout were also more potent than Canadian lights. The quality of beers' antioxidants (IC_{50}) is much higher than that of vitamin C or vitamin E, 1.45 and 2.40 μM, respectively (*19*). Many of the phenols found in beer were tested individually as shown in **Table 1**. Phenolic acids such as ferulic acid and procatechuic acid that are ubiquitous in beer were poorer antioxidants than the beers. This indicates a possible synergism among the phenols in beer. A low IC_{50} value is especially important for the phenols to act as antioxidants in vivo, because their maximal concentrations in plasma are <1 μM.

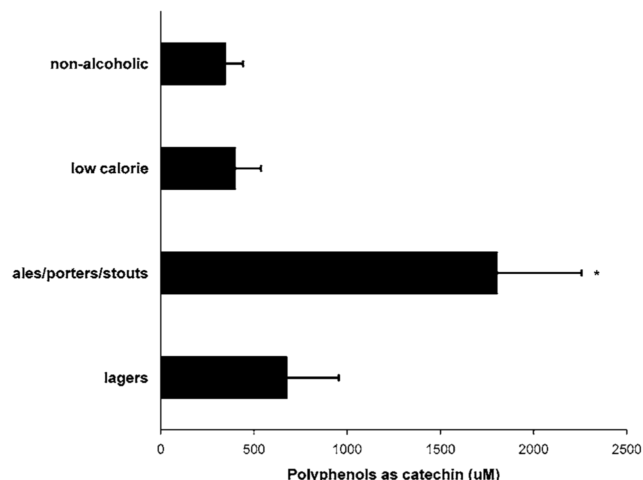

Figure 1. Beer phenols measured as catechin equivalents (average and standard deviation). *, $p < 0.001$ versus other beers.

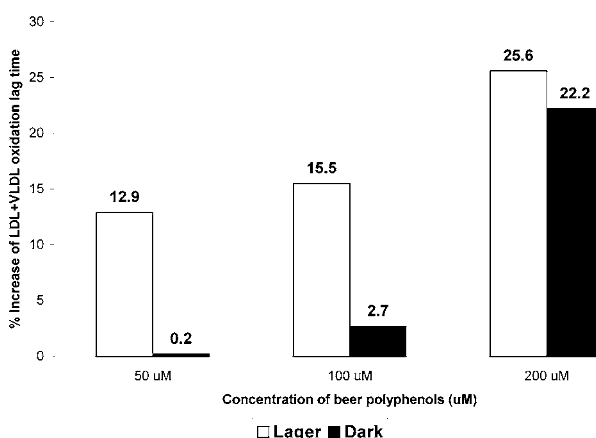

Figure 2. Results of ex vivo spiking of diluted lager (□) and dark beer (■) expressed as catechin equivalents in plasma and compared to a control plasma with no added antioxidants. After equilibration, the LDL + VLDL was isolated and oxidized under standard conditions with cupric ion. Conjugated dienes were measured versus time at 234 nm, and the lag time was calculated. An increase in lag time indicates the presence of lipoprotein-bound antioxidants.

Ex vivo lipoprotein-bound antioxidant activity was measured for two beers, a lager and a dark. The results are shown in **Figure 2**. Lager was superior to dark at the lower concentrations. We have also measured this antioxidant activity for many pure polyphenols, vitamins, and beverages (*20*, *25*). A number of substances such as vitamin E, tea, red wine, and grape juice have also been found to enrich the lipoproteins and protect them from oxidation. Vitamin E, cocoa, and the above beverages were also able to produce an in vivo antioxidant improvement after

Table 2. Effect of Lager and Dark Beer on Beverage Consumption, Lipids, Lipid Peroxidation, and Atherosclerosis in a Hamster Model[a]

group	beverage consumption (mL/animal/day)	plasma cholesterol (mg/dL)	plasma DL cholesterol (mg/dL)	plasma triglycerides (mg/dL)	aortal coverage with foam cells (%)	pooled LDL + VLDL lag time (min)
2% ethanol control	21 ± 4	310 ± 44	75 ± 31	1200 ± 598**	8.29 ± 3.46	50
1/2-diluted lager beer (2% ethanol)	39 ± 8*	132 ± 94*	88 ± 26	288 ± 224***	3.62 ± 3.14†	110
1/2-diluted dark beer (2% ethanol)	41 ± 10*	91 ± 22*	71 ± 23	581 ± 297***	2.73 ± 1.84†,‡	130
0.4% ethanol control	16 ± 5	330 ± 136	86 ± 25	216 ± 131	10.73 ± 2.54	55
1/10-diluted lager beer (0.4% ethanol)	34 ± 9*	297 ± 109	96 ± 24	223 ± 115	8.45 ± 9.73†,‡	75
1/10-diluted dark beer (0.4% ethanol)	34 ± 10*	305 ± 133	86 ± 22	144 ± 83	13.47 ± 4.78	90

[a] Significance: *, $p < 0.001$ vs corresponding control; **, $p < 0.01$ vs 0.4% ethanol; ***, $p < 0.05$ vs control; †, $p < 0.01$ vs control; ‡, $p < 0.001$ vs 1/10-diluted dark beer.

supplementation in humans (*30–34*). We believe this same benefit may occur to humans after drinking beer.

In Vivo Animal Study. The composition of the hamster groups, weights, and food and beverage consumption are shown in **Table 2**. There was no significant difference among any of the groups with respect to weight gain or food consumption, indicating any biochemical difference was not due to different amounts of the atherogenic diet consumed or weight differences. However, there were differences in the amounts of liquid the animals consumed. The animals drank significantly more of the 2% ethanol than of the 0.4% ethanol, $p < 0.05$. Hamsters are known to prefer alcohol to water (*35*), and their blood ethanol concentrations after drinking are similar to those of humans (*36*). Also, the hamsters drank significantly more beer than ethanol at both doses, $p < 0.001$. There were no differences between consumption of the lager and dark beers.

Table 2 also lists the biochemical values for the hamster study. Cholesterol levels were not different between the two doses of alcohol. Lager and dark beer at the high dose significantly diminished cholesterol versus the 2% ethanol control group, 57 and 70% reductions, respectively, $p < 0.001$. The low doses of the beers did not change plasma cholesterol significantly. Thus, there was a dose–response effect of the polyphenols in the beers on cholesterol. A previous study with rats using a nonalcoholic beer also found a significant decrease in serum cholesterol relative to the control group (*37*). There was no effect of alcohol or beers in the present study on plasma HDL. Ethanol at a higher dose of 6% did not change HDL in a previous hamster study (*27*). One of the benefits of moderate alcohol consumption in humans is the resulting increase in HDL that is beneficial for reducing the risk of heart disease (*38*).

There were significantly greater plasma triglycerides with the high dose of ethanol than with the low dose, indicating that ethanol is hypertriglyceridemic ($p < 0.05$). This was also confirmed in a human supplementation study with beer (*39*). Triglycerides, although subject to considerable variation, were significantly decreased by the high dose of lager and dark beer, $p < 0.05$. The lager beer group at the high dose had 50% lower triglycerides than the corresponding dark beer group, which was almost significant, $p = 0.07$. Thus, the beers moderated the hypertriglyceridemic effect of the alcohol at the high dose. There was no effect of the beers on triglycerides at the lower dose. The previous rat study also demonstrated that beer was hypotriglyceridemic (*37*).

Because the ex vivo spiking of beer to plasma resulted in a decrease in LDL + VLDL oxidizability as evidenced by an increase in lag time, LDL + VLDL from the supplementation study was tested on pooled samples from each group. Although no statistics could be done, it appears that ethanol itself has no effect because the lag times were similar for both the low and high ethanol doses. There was a dose–response effect of the phenols from both the lager and dark beers, with the high dose providing a greater increase in lag time. Dark beer appeared to be more effective than lager beer at the same dose.

The most important biochemical parameter, the extent of atherosclerosis, was significantly decreased by both beers at the high dose, 62 and 71% by lager and dark beer, respectively ($p < 0.01$). There was a dose–response effect on atherosclerosis for both beers. At the low dose only the lager beer significantly inhibited atherosclerosis, 21%, $p < 0.01$, and it was significantly better than the low-dose dark beer. The greater effect of the low dose of lager may be due to the greater bioavailability of the polyphenols in lager as compared to dark beer. This low dose of lager beer extrapolated to a 70 kg human dose is equivalent to 1.25 L/day or 3.4 12-oz bottles/day. This is approximately equal to the average male consumption of 1.33 L of beer in Germany (*40*). In this epidemiology study, the lowest relative mortality for beer drinkers compared to non-drinkers was 0.49 and occurred at this intake.

This 62% atherosclerosis reduction with 1/2-diluted lager beer is comparable to that of 1/2-diluted red wine (53%) in our previous hamster study (*27*). The equal effectiveness of beer to red wine occurred despite the much greater polyphenols in the red wine (9280 μM) versus the lager beer (1013 μM) used in the atherosclerosis studies. Thus, beer is equally as effective as red wine, which is hypothesized to be responsible for the "French paradox" of heart disease reduction (*41*). In fact, a U.S. epidemiological study found for men and women drinking 1–2 servings/day, beer consumption provided a lower relative risk of coronary artery disease than wine (*42*). Although it is difficult to draw many inferences from animal studies to human health, our interventional animal model confirms this epidemiological finding. Beer has another advantage over red wine; beer does not raise the level of homocysteine, a risk factor for heart disease, as does wine after moderate human consumption (*43*).

Beers with fewer calories and with less alcohol have considerably lower levels of phenols than regular lagers and dark beers. Beer contains high-quality antioxidants as measured by the oxidation of lower density lipoproteins, which is the initiating step in atherosclerosis. Both lager and dark beer have shown dose–response efficacy in the hamster model as anti-atherosclerotic agents. This study provides evidence of an in vivo antioxidant mechanism for beer that should be investigated in humans. Our animal study and human epidemiological studies indicate the equivalent cardiovascular benefits of beer and red wine.

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LITERATURE CITED

(1) Rimm, E. B.; Giovannucci, E. L.; Willett, W. C.; Colditz, G. A.; Ascherio, A.; Rosner, B.; Stampfer, M. J. Prospective study of alcohol consumption and risk of coronary disease in men. *Lancet* **1991**, *35*, 464–468.

(2) Klatsky, A. L.; Armstrong, M. A.; Friedman, G. D. Alcohol and mortality. *Ann. Intern. Med.* **1992**, *117*, 646–654.

(3) Gronbaek, M.; Deis, A.; Sorensen, T. I.; Becker, U.; Schnohr, P.; Jensen, G. Mortality associated with moderate intake of wine, beer or spirits. *Br. Med. J.* **1995**, *310*, 1165–1169.

(4) Riemens, S. C.; van Tol, A.; Hoogenberg, K.; van Gent, T.; Scheek, L. M.; Sluiter, W. J.; Dullart, R. P. Higher higher density lipoprotein cholesterol associated with moderate alcohol consumption is not related to altered plasma lecithin: cholesterol acyltransferase and lipid protein activity levels. *Clin. Chim. Acta* **1997**, *258*, 105–115.

(5) Renaud, S. C.; Ruf, J. C. Effects of alcohol on platelet functions. *Clin. Chim. Acta* **1996**, *246*, 77–89.

(6) Dimmit, S. B.; Rakic, V.; Puddey, I. B.; Baker, R.; Oostryck, R.; Admas, M. J.; Chesterman, C. N.; Burke, V.; Beilin, L. J. The effects of alcohol on coagulation and fibrinolytic factors: a controlled trial. *Blood Coagul. Fibrinolysis* **1998**, *9*, 39–45.

(7) Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **1995**, *155*, 381–386.

(8) Knekt, P.; Jarvinen R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland; a cohort study. *Br. Med. J*. **1996**, *312*, 478–481.

(9) Steinberg, D.; Parathasarathy, S.; Carew, T. E.; Khoo, J. C.; Witzum, J. L. Beyond cholesterol; modification of low-density lipoprotein that increases its atherogenicity. *New Engl. J. Med.* **1989**, *320*, 915–924.

(10) Trevithick, C. C.; Vinson, J. A.; Caulfeild, J.; Rahman, R.; Derksen, T.; Bocksch, L.; Hong, S.; Stefan, A.; Teufel, K.; Wu, N.; Hirst, M.; Trevithick, J. R. Is ethanol an important antioxidant in alcoholic beverages with risk reduction of cataract and atherosclerosis? *Redox Rep*. **1999**, *4*, 89–93.

(11) Gasbarrini, A.; Addolorato, G.; Simoncini, M.; Gasbarrini, G.; Fantozzi, P.; Mancini, F.; Montanari, L.; Nardini, M.; Ghiselli, A.; Scaccini, C.; Montanari, L.; Nardini, M.; Ghiselli, A.; Scaccini, C. Beer affects oxidative stress due to ethanol in rats. *Dig. Dis. Sci.* **1998**, *43*, 1332–1338.

(12) Ghiselli, A.; Natella, F.; Guidi, A.; Montanari, L.; Fantozzi, P.; Scaccini, C. Beer increases plasma antioxidant capacity in humans. *J. Nutr. Biochem.* **2000**, *11*, 76–80.

(13) ERC Statistics International. *World Beer Market Survey*; London, U.K., 1998.

(14) Bamforth, C. Brewing a better beer. *Chem. Br.* **1997**, *33*, 37–39.

(15) Boivin, P.; Malanda, M.; Maillard, M. N.; Berset, C.; Richard, H.; Hugues, M.; Richard-Forget, F.; Nicolas, J. Role of the natural antioxidants of barley and malt by various methods. *Proc. Congr. Eur. Brew. Conv.* **1993**, *24*, 397–404.

(16) Liégeois, C.; Lermuieau, G.; Collin, S. Measuring antioxidant efficiency of wort, malt, and hops against the 2,2′-azobis(2-amindinopropane) dihydrocholoride-induced oxidation of an aqueous dispersion of linoleic acid. *J. Agric. Food Chem.* **2000**, *48*, 1129–1134.

(17) Wei, A.; Mura, K.; Shibamoto, T. Antioxidant activity of volatile chemicals extracted from beer. *J. Agric. Food Chem.* **2001**, *49*, 4097–4101.

(18) Miranda, C. L.; Stevens, J. F.; Ivanov, V.; McCall, M.; Frei, B.; Deinzer, M. L.; Buhler, D. R. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *J. Agric. Food Chem.* **2000**, *48*, 3876–3884.

(19) Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an *in vitro* oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800–2802.

(20) Vinson, J. A.; Jang, J.; Dabbagh, Y. A.; Serry, M. M.; Cai, S. Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an *in vitro* model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2798–2799.

(21) Singleton, V. L.; Orthoefer, R.; Lamuela-Raventós, R. M. Analysis of total phenols and other oxidation substrates and antixoidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 153–178.

(22) U.S. Department of Agriculture, Economic Research Service. *Statistical Bulletin 965*; Washington, DC, 1999; updated on the web at www.ers.usda/gov/data/foodconsumption/.

(23) Vinson, J. A.; Proch, J.; Bose, P. Determination of quantity and quality of polyphenol antioxidants in foods and beverages. *Methods Enzymol.* **2001**, *335*, 103–114.

(24) Trevithick, J. R.; Dzialoszynski, T. A new technique for enhancing luminol luminescent detection of free radicals and reactive oxygen species. *Biochem. Mol. Biol. Int.* **1994**, *33*, 1179–1190.

(25) Vinson, J. A.; Jang, J.; Yang, J.; Dabbagh, Y.; Liang, X.; Serry, M.; Proch, J.; Cai, S. Vitamins and especially flavonoids in common beverages are powerful in vitro antioxidants which enrich lower density lipoproteins and increase their oxidative resistance after ex vivo spiking in human plasma. *J. Agric. Food Chem.* **1999**, *47*, 2502–2504.

(26) Vinson, J. A.; Hu, S.-J.; Jung, S.; Stanski, A. M. A citrus extract plus ascorbic acid decreases lipids, lipid peroxides, lipoprotein oxidative susceptibility, and atherosclerosis in hypercholesterolemic hamsters. *J. Agric. Food Chem.* **1998**, *46*, 1453–1459.

(27) Vinson, J. A.; Teufel, K.; Wu, N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* **2001**, *156*, 67–72.

(28) Vinson, J. A. Flavonoids in foods as in vitro and in vivo antioxidants. *Adv. Exp. Med. Biol.* **1998**, *439*, 151–164.

(29) Vinson, J. A.; Hontz, B. A. Phenol antioxidant index: comparative antioxidant effectiveness of red and white wines. *J. Agric. Food Chem.* **1995**, *43*, 401–403.

(30) Jialal, I.; Fuller, C. J.; Huet, B. A. The effect of α-tocopherol supplementation on LDL oxidation. A dose–response study. *Arterioscler. Thromb. Vasc. Biol*. **1995**, *15*, 190–198.

(31) Ishikawa, T.; Suzukawa, M.; Ito, T.; Yoshida, H.; Ayaori, M.; Nishiwaki, M.; Yonemura, A.; Hara, Y.; Nakamura, H. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. *Am. J. Clin. Nutr.* **1997**, *66*, 261–266.

(32) Fuhrman, B.; Lavy, A.; Aviram, M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549–554.

(33) Stein, J. H.; Keevil, J. G.; Wiebe, D. A.; Aeschlimann, S.; Folts, J. D. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **1999**, *100*, 1050–1055.

(34) Kondo, K.; Hirano, R.; Matsumoto, A.; Igarashi, O.; Itakura, H. Inhibition of LDL oxidation by cocoa. *Lancet* **1996**, *348*, 1514.

(35) Arvola, A.; Forstander, O. A. Hamster in experiments of free choice between alcohol and water. *Q. J. Stud. Alc*. **1963**, *24*, 591–597.

(36) Keung, W. M.; Lazo, O.; Kunze, L.; Vallee, B. L. Daidzin suppresses ethanol consumption by Syrian golden hamsters without blocking acetaldehyde metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8990–8993.

(37) Gorenstein, S.; Zemser, M.; Weisz, M.; Haruenkit, R.; Trakhtenberg, S. The influence of dry matter of different alcoholic beverages on lipids, proteins, and antioxidant activity in serum of rats. *J. Nutr. Biochem.* **1998**, *9*, 131–135.

(38) Gaziano, J. M.; Buring, J. E.; Breslow, J. L.; Golhaber, S. Z.; Rosner, B.; Van Denburgh, M.; Willett, W.; Hennekens, C. H. Moderate alcohol intake, increased levels of high-density lipoprotein and its sub-fractions and decreased risk of myocardial infarction. *N. Engl. J. Med.* **1993**, *329*, 1829−1834.

(39) Rakic, V.; Puddey, I. B.; Dimmitt, S. B.; Burke, V.; Beilin, L. J. A controlled trial of the effects of pattern of alcohol intake on serum lipid levels in regular drinkers. *Atherosclerosis* **1998**, *137*, 243−252.

(40) Keil, U.; Chambless, L. E.; Doring, A.; Filipiak, B.; Stieber, J. The relation of alcohol intake to coronary heart disease and all-cause mortality in a beer-drinking population. *Epidemiology* **1997**, *6*, 687−688.

(41) Ulbright, T. L. V.; Southgate, D. A. T. Coronary heart disease: seven dietary factors. *Lancet* **1991**, *338*, 985−992.

(42) Klatsky, A. L.; Armstrong, M. A.; Friedman, G. D. Red wine, white wine, liquor, beer, and risk for coronary artery disease hospitalization. *Am. J. Cardiol*. **1997**, *80*, 416−420.

(43) van der Gaag, M. S.; Ubbink, J. B.; Sillanaukee, P.; Nikkari, S.; Hendriks, H. F. Effect of consumption of red wine, spirits, and beer on serum homocysteine. *Lancet* **2000**, *355*, 1522.

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