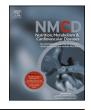
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Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: A randomized feeding trial

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KEYWORDS

Beer; Polyphenols; Alcohol; Adhesion molecules; Cytokines; Cardiovascular disease; Atherosclerosis **Abstract** *Background and aims:* Moderate alcohol consumption exerts a cardioprotective effect, but no studies have evaluated the alcohol-independent cardiovascular effects of the non-alcoholic components of beer. We aimed to evaluate the effects of ethanol and the phenolic compounds of beer on classical and novel cardiovascular risk factors.

Methods and results: Thirty-three high risk male volunteers were included in a randomized, crossover feeding trial. After a washout period, all subjects received beer (30 g alcohol/d, 660 mL), the equivalent amount of polyphenols as non-alcoholic beer (990 mL), and gin (30 g alcohol/d, 100 mL) for 4 weeks. All outcomes were evaluated before and after each intervention period. Moderate alcohol consumption increased serum HDL-cholesterol (~5%), ApoA-I (~6%), ApoA-II (~7%) and adiponectin (~7%), and decreased serum fibrinogen (~8%), and interleukin (IL)-5 (~14%) concentrations, whereas the non-alcoholic fraction of beer (mainly polyphenols) increased the receptor antagonist of IL-1 (~24%), and decreased lymphocyte expression of lymphocyte function-associated antigen-1 (~11%), lymphocyte and monocyte expression of Sialil-Lewis X (~16%) and monocyte expression of CCR2 (~31%), and tumor necrosis factor (TNF)- β (~14%) and IL-15 (~22%) plasma concentrations. No changes were observed in glucose metabolism parameters or in body weight and adiposity parameters.

Conclusion: The phenolic content of beer reduces leukocyte adhesion molecules and inflammatory biomarkers, whereas alcohol mainly improves the lipid profile and reduces some plasma inflammatory biomarkers related to atherosclerosis. Trial registration number: ISRCTN95345245 (http://www.isrctn.org/).

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Introduction

Atherosclerosis, the main cause of coronary heart disease (CHD), is considered a low-grade inflammatory disease mediated by the endothelial secretion of chemokines and adhesion molecules, such as integrins and selectins, which recruit circulating monocytes and T-cells to the endothe-lium and further migrate to the arterial wall triggering atherosclerotic lesions [1].

Moderate alcohol consumption is associated with a decreased cardiovascular risk and mortality independently of the type of alcoholic beverage consumed [2,3]. Nevertheless, red wine, a high polyphenolic fermented beverage, seems to confer greater cardioprotective effects than distilled beverages, which do not contain polyphenols [4], by down-regulating the expression of chemokines and adhesion molecules [5–8]. Recent meta-analyses suggest that beer, a fermented beverage with intermediate polyphenol content, could also confer greater cardioprotection than spirits [9–11], but the results of different trials are controversial, and this question is still under debate [12].

Therefore, we embarked on a randomized, crossover, controlled clinical trial to evaluate and compare the effects of moderate consumption of 30 g alcohol/d of gin, a nonpolyphenolic alcoholic beverage, beer, an alcoholic beverage with a medium polyphenolic content, and the same polyphenolic amount of non-alcoholic beer, a medium polyphenolic non-alcoholic beverage, on several biomarkers related to the early stages of atherosclerosis in subjects at high risk for CHD.

Methods

Subjects

A total of 36 male moderate alcohol consumers between 55 and 75 years of age were recruited for the study in the outpatient clinic of the Internal Medicine Department of our institution. Subjects were at high risk for CVD (family history of premature CVD and/or the presence of diabetes, hypertension, dyslipidemia, and overweight/obesity). Exclusion criteria included documented CVD, human immunodeficiency virus infection, chronic liver disease, malnutrition, neoplastic or acute infectious diseases and customary use of vitamin supplements. Participants were offered free beverages but no monetary compensation. The Institutional Review Board of the hospital approved the study protocol, and all participants gave written consent.

Study design and diet monitoring

The study was an open, randomized, controlled, crossover trial with three intervention periods. Two weeks prior to the study, subjects were asked to maintain their usual diet and to refrain from any alcoholic beverage (run-in period). Baseline data were collected after this run-in period. Following this, participants were individually randomized in a crossover design among six sequences of interventions lasting 4 weeks each, in which the test beverages were provided. Randomization was based on a computergenerated random number table, resulting in six possible intervention sequences. Then, participants were instructed to consume beer (660 mL/day, containing 30 g of ethanol and 1209 mg of total polyphenols), non-alcoholic beer (990 mL/day, containing <1 g of ethanol and 1243 mg of total polyphenols) or gin (100 mL/day, containing 30 g of ethanol and no polyphenols). No washout periods were included in the study. Therefore, the value of the previous intervention or the baseline value (run-in period) in the first intervention was considered as the starting value of each intervention.

The phenolic profile of the beer, non-alcoholic beer and gin used in the trial was determined by SPE-LC-ESI-MS/MS as previously reported [13,14]. No significant differences were observed in the phenolic content of the daily dose of beer and non-alcoholic beer, while gin contained no detectable phenolic compounds (Supplemental Table 1).

Throughout the study the participants were asked to maintain their usual dietary habits, physical activity level and medications, and to abstain from non-alcoholic beer or alcoholic beverages, except those provided by the investigators. Diet monitoring is explained in the Supplemental Material.

Clinical and laboratory measurements

After the run-in period (baseline) and the day after each intervention period, fasting blood, 24-h urine samples and anthropometric measurements were performed with standardized methods, and the blood pressure (BP) and heart rate were measured 3 times at 5-min intervals on the nondominant arm with an oscillometer (Omron 705 CP; Omron Matsusaka Co Ltd, Matsusaka City, Japan) after 15 min resting in a seated position. The mean of the second and the third measures was considered for statistical analysis.

Serum, EDTA-plasma, and urine samples were stored at -80 °C until assayed.

Compliance with the test beers was assessed by measurement of urinary isoxanthohumol (IX), a biomarker of beer and non-alcoholic beer intake. Briefly, the last day of the run-in period and the last day of each intervention subjects were asked to collect 24-h urine. IX was measured in 24-h urine by SPE-LC-MS/MS as previously described (14).

For the measurement of nitric oxide (NO), the release of NO_2^- and NO_3^- , the stable breakdown products of NO in thawed plasma samples, was determined by a chemiluminescence detector in a NO analyzer (Sievers Instruments, Inc., Boulder, CO).

The following parameters were also determined in thawed samples of whole serum or plasma, as appropriate: blood glucose with the glucose oxidase method; cholesterol and triglycerides with enzymatic procedures; HDL cholesterol after precipitation with phosphotungstic acid and magnesium chloride; and homocysteine and vitamin B12 by an automated electrochemiluminescence Download English Version:

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