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The non-alcoholic fraction of beer increases stromal cell derived factor 1 and the number of circulating endothelial progenitor cells in high cardiovascular risk subjects: A randomized clinical trial

Gemma Chiva-Blanch [a,b], Ximena Condines [c], Emma Magraner [c], Irene Roth [a,b], Palmira Valderas-Martínez [a,b], Sara Arranz [a,b], Rosa Casas [a,b], Miriam Martínez-Huélamo [b,d], Anna Vallverdú-Queralt [b,d], Paola Quifer-Rada [b,d], Rosa M. Lamuela-Raventos [b,d], Ramon Estruch [a,b,*]

[a] Department of Internal Medicine, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Villarroel 170, 08036 Barcelona, Spain
[b] CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, (CIBERobn) and RETIC RD06/0045, Spain
[c] Primary Care Research Group, IDIBAPS, Centre d'Assistència Primària ABS Les Corts (AS), GESCLINIC, Barcelona, Spain
[d] Nutrition and Food Science Department, XaRTA, INSA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain

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ABSTRACT

Rationale: Moderate alcohol consumption is associated with a decrease in cardiovascular risk, but fermented beverages seem to confer greater cardiovascular protection due to their polyphenolic content. Circulating endothelial progenitor cells (EPC) are bone-marrow-derived stem cells with the ability to repair and maintain endothelial integrity and function and are considered as a surrogate marker of vascular function and cumulative cardiovascular risk. Nevertheless, no study has been carried out on the effects of moderate beer consumption on the number of circulating EPC in high cardiovascular risk patients.
Objective: To compare the effects of moderate consumption of beer, non-alcoholic beer and gin on the number of circulating EPC and EPC-mobilizing factors.
Methods: In this crossover trial, 33 men at high cardiovascular risk were randomized to receive beer (30 g alcohol/d), the equivalent amount of polyphenols in the form of non-alcoholic beer, or gin (30 g alcohol/d) for 4 weeks. Diet and physical exercise were carefully monitored.
Results: The number of circulating EPC and EPC-mobilizing factors were determined at baseline and after each intervention. After the beer and non-alcoholic beer interventions, the number of circulating EPC significantly increased by 8 and 5 units, respectively, while no significant differences were observed after the gin period. In correlation, stromal cell derived factor 1 increased significantly after the non-alcoholic and the beer interventions.
Conclusions: The non-alcoholic fraction of beer increases the number of circulating EPC in peripheral blood from high cardiovascular risk subjects.
Clinical trial registration: http://www.controlled-trials.com/ISRCTN95345245 ISRCTN95345245

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1. Introduction

Endothelial progenitor cells (EPC) are bone-marrow-derived stem cells with the ability to differentiate into mature endothelial cells [1]. There is growing interest into circulating EPC as a mechanism to repair and maintain endothelial integrity and function by replacing denuded parts of the artery. Endothelial injury may play a role in the pathogenesis of atherosclerosis, arterial thrombosis and hypertension. Indeed, the balance between endothelial injury and recovery is extremely important to reduce cardiovascular events since mature endothelial cells possess limited regenerative capacity and, therefore, EPC are considered as one of the mechanisms to maintain endothelial function (reviewed in Refs. [2,3]). Circulating EPC levels have been proposed as a surrogate marker for vascular function [4] and cumulative cardiovascular

* Corresponding author. Department of Internal Medicine, Hospital Clinic, Villarroel 170, 08036 Barcelona, Spain. Tel.: +34 93 227 54 00x2907; fax: +34 93 227 93 65.
E-mail address: restruch@clinic.ub.es (R. Estruch).

risk because of the strong correlation between the number of circulating EPC and flow-mediated brachial-artery reactivity and a decreased Framingham risk factor score [5].

On the other hand, moderate alcohol consumption is associated with a decreased risk of cardiovascular mortality independently of the type of beverage consumed [6]. Nevertheless, fermented alcoholic beverages (i.e. wine and beer) seem to confer greater cardioprotective effects than distilled beverages probably because of their higher polyphenolic content [7], although the mechanisms involved are not fully understood. *In vitro* or animal studies have observed that red wine polyphenols increase the number and functionality of EPC [8–12], suggesting a possible explanation for this greater cardioprotective effect. To our knowledge, few studies have evaluated the effects of red wine or beer polyphenols on circulating EPC [13,14] in healthy individuals. An enhancement of circulating EPC was observed after red wine consumption [13,14] but not after beer consumption [14]. Nevertheless, no studies have been carried out in high cardiovascular risk patients, who show greater endothelial damage than healthy individuals.

We embarked, therefore, on a study to evaluate the effects of moderate beer consumption and its fractions (alcoholic and non-alcoholic) on the number of circulating EPC and EPC-mobilizing factors in subjects at high risk of cardiovascular disease (CVD), in whom diet and exercise were carefully monitored.

2. Subjects and methods

2.1. Subjects

A total of 36 high-risk male subjects aged between 55 and 75 years were recruited for the study in the outpatient clinic of the Internal Medicine Department at our institution. The subjects included in the trial were moderate alcohol consumers (1–3 drinks/day) and had diabetes mellitus or ≥ 3 of the following cardiovascular disease risk factors: tobacco smoking, hypertension, plasma LDL cholesterol ≥ 160 mg/dL, plasma HDL cholesterol ≥ 35 mg/dL, overweight or obesity (body mass index ≥ 25 kg/m^2), and/or family history of premature coronary heart disease (CHD). Exclusion criteria included documented CHD, stroke or peripheral vascular disease, human immunodeficiency virus infection, alcoholic liver disease, alcoholism or toxic abuse, malnutrition and neoplastic or acute infectious diseases. The Institutional Review Board of the hospital approved the study protocol and all participants gave written consent before participation in the study. The trial was registered in the Current Controlled Trials at London (http://www.controlled-trials.com/), International Standard Randomized Controlled Trial Number at http://www.isrctn.org/, as ISRCTN95345245.

2.2. Study design

The study was an open, randomized, crossover, controlled clinical trial, which included three 4-week intervention periods. Two weeks prior to the study the subjects were asked to maintain their usual diet and to refrain from consuming any alcoholic beverage. Baseline data were collected after this run-in period. Following this, participants were individually randomized in a crossover design among three treatment sequences lasting 4 weeks each, in which the test beverages were provided. Randomization was based on a computer-generated random number table, obtained by a secretary who did not participate in the recording and evaluation of the data, resulting in six possible diet sequences. Participants were assigned to the different interventions and received gin (100 mL–30 g of ethanol/day), beer (660 mL–30 g of ethanol/day), or the same amount of polyphenols as beer in the form of non-alcoholic beer

(990 mL). None of the participants consumed multivitamin or vitamin E supplements or anti-inflammatory drugs (steroids, NSAIDs or aspirin >100 mg/day). The beer and the non-alcoholic beer were of the lager type from the same Spanish commercial brand. The phenolic composition of the beers used in the study is detailed in Supplemental Table 1. The alcoholic degree of the beverages was 38, 5.4 and <0.1%, for gin, beer and non-alcoholic beer, respectively. The phenolic profile of beer and non-alcoholic beer was determined by the SPE-LC-ESI-MS/MS as described previously and adapted to beer samples [15]. The daily intake of polyphenols derived from non-alcoholic and beer interventions showed no significant differences (Supplemental Table 1), while the phenolic content of gin was negligible [16].

2.3. Diet and exercise monitoring

Subjects were asked to exclude alcoholic beverages as well as non-alcoholic beer 15-d before the first intervention (run-in period) and during the study. Subjects were also asked to maintain their lifestyle habits and not to change their dietary pattern during the study. Natural foods rich in antioxidants, especially fruit and vegetables, were especially monitored to ensure that individual diets had a similar antioxidant content throughout the study. After the run-in period and the day after each intervention period, the medical history was reviewed and the Minnesota Leisure Time Physical Activity Questionnaire was administered. In addition, the last week of the run-in period and the last week of each intervention period, the subjects were asked to fill in a validated 7-d food record questionnaire. The food records were used to assess nutrient intake and to monitor adherence to the study protocol. Compliance with the test drinks was also assessed by measures of urinary biomarkers of beer polyphenol intake. Foods were converted into nutrients using the Food Processor Nutrition and Fitness Software (esha Research, Salem, OR), adapted to local foods. At the end of each study intervention, a clinician assessed any adverse effects from the interventions by administering a checklist of symptoms, including bloating, fullness or indigestion, altered bowel habit, dizziness and other symptoms possibly associated with the interventions.

2.4. Methods

Fasting blood samples and 24-h urine were collected at baseline (the last day of the run-in period) and the day after the last day of each intervention (beer, non-alcoholic beer and gin). Serum and urine samples were stored at $-80\,^{\circ}$C until assayed. The clinical investigators and laboratory technicians were blinded to the interventions. To assess the compliance of interventions, isoxanthohumol (IX), a biomarker of beer intake, was determined in 24-h urine by SPE-LC-MS/MS as described previously [17].

2.4.1. EPC determination

A 10 mL aliquot of whole blood after 12 h fasting was used for EPC quantification. Samples were processed within 1 h after collection and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density-gradient centrifugation. Recovered cells were washed twice with phosphate buffered saline (PBS), and the pellet was resuspended at a concentration of 10^7 cells/mL of PBS/2% fetal bovine serum (FBS) after counting in a Neubauer chamber. A volume of 100 μL of the suspension (10^6 cells) was incubated for 1 h in the dark at 4 $^{\circ}$C with 10 μL of the monoclonal antibodies against human KDR (PE-labeled), CD34-FITC and CD133-APC (MAC's Milteny Biotech, all), known to be expressed in EPC [18]. At the end of the incubation, 1 μL of 7-aminoactinomycin D (7AAD) (Sigma-Aldrich) was added, cells were washed with PBS/2% FBS and fixed in