Research Article

Received: 20 March 2023 Revised: 16 June 2023 Accepted article published: 4 July 2023 Published online in Wiley Online Library: 28 July 2023

(wileyonlinelibrary.com) DOI 10.1002/jsfa.12826

Effect of moderate beer consumption (with and without ethanol) on cardiovascular health in postmenopausal women

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Abstract

BACKGROUND: The main aim of this 2-year non-randomized parallel controlled clinical pilot trial was to evaluate the long-term effect of a moderate daily intake of beer (with and without alcohol) on cardiovascular health in postmenopausal women. A total of 34 participants were grouped into three study arms: 16 were administered alcoholic beer, 6 consumed non-alcoholic beer, and 12 were in the control group. Changes in glucose metabolism, lipid profile, liver enzymes, anthropometric measurements, body composition, and blood pressure variables were monitored. Data on medical history, diet, and exercise were collected, and gustatory capacities were determined.

RESULTS: Moderate consumption of beer, both alcoholic and non-alcoholic, seemed to have positive effects on biochemical indicators of cardiovascular health in postmenopausal women, with 660 mL day^{-1} of non-alcoholic beer reducing low-density lipoprotein cholesterol blood levels, and 330 mL day^{-1} of alcoholic beer increasing high-density lipoprotein cholesterol. The evolution of changes in android and gynoid fat percentage and their ratio differed significantly between study groups, which was attributable to either the interventions or the disparity between groups regarding the time elapsed since menopause onset. Iso-α-acids recognition threshold could be involved in intervention group election, whereas the sensory phenotypes studied were not associated with alcohol drinking frequency.

CONCLUSIONS: Moderate beer consumption was found to improve the lipid profile of postmenopausal women, although their effects in preventing cardiometabolic alterations deserve further research (trial registration number: ISRCTN13825020; https://doi.org/10.1186/ISRCTN13825020).

Supporting information may be found in the online version of this article.

Keywords: phytoestrogens; polyphenols; alcohol drinking; lipid profile; bitter taste; PTC phenotype

INTRODUCTION

Natural menopause is defined as the permanent cessation of spontaneous menses due to loss of ovarian follicular activity, which is accompanied by a drop in steroid and peptide hormone production.[1,2] Cardiovascular (CV) disease (CVD) is the main cause of morbidity and mortality in postmenopausal women worldwide,[3] in whom the rate of CV events is 2.6-fold higher than in premenopausal women of the same age.[4] This can be explained by the metabolic alterations associated with a decline in circulating oestrogen levels after the onset of perimenopause.[5,6] A frequent metabolic disorder associated with menopause is dyslipidaemia, involving a significant increase in serum levels of triglycerides, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), TC/high-density lipoprotein cholesterol (HDL-c) ratio, and apolipoprotein (Apo) B. This condition, together

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with the aging process, accelerates the onset of atherosclerosis[7,8] and is associated with an increase in body weight, accumulation of abdominal fat, and the development of insulin resistance, glucose intolerance, and high blood pressure (BP), resulting in a higher risk of diabetes, coronary heart disease, heart failure, and stroke, among others.[9]

In the absence of established recommendations regarding hormone replacement therapy for the prevention of chronic diseases,[10] a growing number of women are seeking alternative natural treatments to reduce the risk of developing CVD and other postmenopausal symptoms. Therefore, the safety and effectiveness of alternative strategies to hormone replacement therapy have become a relevant subject of research.[11,12] Phytoestrogens are plant compounds similar in structure to endogenous oestrogens, which allows them to act as selective modulators of oestrogen-dependent signals.[13] Prenylflavonoids, a subclass of flavonoid polyphenols, found in hops (*Humulus lupulus* L.), have phytoestrogenic activity.[14] Among them, 8-prenylnaringenin (8-PN) has generated the greatest interest.[15] Although found in beer, the main source of 8-PN for humans is through the conversion of isoxanthohumol (IX) by the intestinal microbiota.[16]

The bitter taste of beer depends to some extent on the content and relative proportions of iso-α-acids (IAAs), also known as isohumulones (i.e. isohumulone, iso-cohumulone, iso-adhumulone).[17] Individual sensitivity to these compounds is mediated by bitter taste receptors (T2Rs), specifically by T2R1, T2R14, and T2R40.[18] The concentration of these compounds depends on the type of beer production and the hop variety. The application of sensory analysis in nutritional and clinical research is shedding light on individual acceptance of dietary interventions and contributing to the design of personalized nutrition aimed at improving health and understanding the mechanisms involved.

Considering all the scientific evidence mentioned herein thus far, the purpose of this research was to evaluate the effect of a moderate daily intake of beer, with or without alcohol, on CV health in postmenopausal women. The study was carried out over 2 years with repeated measurements of various CV health-related parameters. Additionally, the characterization of the sensory perception of subjects was evaluated to study their influence on group intervention election as well as alcohol drinking habits.

MATERIAL AND METHODS

Experimental design, study population, and recruitment

This study was a 2-year non-randomized parallel controlled clinical trial with three study arms: one group was administered the equivalent of 14 g of ethanol per day in the form of alcoholic beer (AB; 330 mL day^{-1}); another consumed non-alcoholic beer (NAB; 660 mL day^{-1}); and the control group refrained from consuming alcoholic beverages, NAB, and hop-derived products. Participants were allocated to a study group after a run-in period of 15 days, and all were requested not to consume any other alcoholic beverage during the study. The eligible participants were 45- to 70-year-old women with a confirmed menopausal status. Also, as one of the main objectives of the study was to assess the effect of beer consumption on bone health,[19] many of the exclusion criteria were related to this issue. The participants were assigned to an intervention group according to their preference. The detailed experimental design can be found in a previous publication.[19]

A total of 37 women were selected and agreed to be part of the study, 34 of whom completed the entire intervention. All participants signed informed consent forms, were required to make four assessment visits during the intervention period (at baseline, and at 6, 12, and 24 months) and were later invited to the sensory analysis. The study was conducted in compliance with the Declaration of Helsinki, and all procedures were approved by the Bioethics Commission of the University of Barcelona (Institutional Review Board: IRB 00003099) in March 2017 for the main study and in July 2022 for the complementary sensory evaluation.

Intervention product characterization and compliance

The detailed characterization of the intervention product can be found in our previous publication,[19] and phytoestrogens (xanthohumol, IX, 6-prenylnaringenin, and 8-PN) content and alcohol in the beer doses supplied to each intervention group are detailed in Supporting Information Table 1. The volume of NAB administered was double that of AB, owing to the detrimental impact of non-alcoholic brewing processes on total prenylflavonoid content.[20]

To standardize the daily dose of phytoestrogens administered to each intervention group (AB and NAB), a specific brand of beer and volume were selected. Participants were encouraged to drink the beer at mealtimes, in accordance with the Mediterranean dietary pattern.[21]

The intervention products were provided monthly throughout the study. The compliance level was assessed from 7-day dietary records reviewed at each visit, as well as objectively by the measurement of urinary IX, a specific biomarker of beer consumption. This analysis was performed in 24-h urine samples collected at the four visits (at baseline and at 6, 12, and 24 months) and stored in aliquots at 80 °C, using solid-phase extraction liquid chromatography–tandem mass spectrometry.[22]

Measurements and outcome assessment

Covariates assessment

Information was collected at baseline and updated at each visit. The interviews were standardized and structured, using a questionnaire with sociodemographic and medical questions with an emphasis on CV health, such as age, baseline diseases, medication history, and current and past smoking and alcohol drinking habits. Physical activity was estimated by the Minnesota leisure-time questionnaire and expressed as the daily metabolic equivalent of tasks (MET-min/day).[23]

Dietary intake was estimated annually through a validated semi-quantitative food frequency questionnaire consisting of 151 items,[24] and as detailed previously.[19] The estimation of micronutrients in the diet was carried out in the same way, and the absolute values of those with a possible influence on CV health were reported.[25-27] Furthermore, the overall diet quality was evaluated using the 14-point Mediterranean diet adherence questionnaire as an index of healthy eating at baseline.[28]

Anthropometric measurements and body composition

Anthropometric data were obtained at each visit; namely, weight, height, body mass index (BMI), waist circumference (WC), and waist/hip ratio (WHR). The measurements were performed by trained technical health personnel following standardized protocols.[29] Diastolic BP (DBP) and systolic BP (SBP) were measured by a validated semi-automatic digital sphygmomanometer (Omron HEM-705CP model)(Madison, WI, USA) in triplicate.

Total and regional body composition were estimated using dual-energy X-ray absorptiometry (GE-LUNAR iDXA Prodigy equipment). Fat mass index (FMI; kg m^{-2}) and lean mass index (kg m^{-2}) were obtained from total fat mass (kg) and total lean

mass (kg) respectively in relation with height. Also, the percentage of android and gynoid fat was calculated, along with the android-to-gynoid fat ratio. All these measurements were evaluated at baseline and then annually by the CETIR medical group (CETIR grup Mèdic, Barcelona, Spain).

Biochemical analyses

Overnight fasting blood and spot urine samples were collected at each visit (between 8 and 9 a.m.). The automated biochemical profiles were analysed at the Biomedical Diagnostic Centre of the Hospital Clinic. The lower detection limit of plasma E2 was 12 pg mL^{-1}; thus, levels below this limit were defined as 11 pg mL^{-1}.

Sensory analysis

Recognition thresholds (RTs) were measured using a same–different task approach, as described elsewhere,[30] with some modifications. Sucrose (sweet), monosodium glutamate (umami), sodium chloride (NaCl; salty), citric acid (sour), phenylthiocarbamide (PTC; bitter), quinine (bitter), and sinigrin (bitter) were supplied by Sigma Aldrich (St Louis, MO, USA), and an IAA-rich extract (bitter) was supplied by Molina for Brewers (Hopalpha ISO 30%, Molina for Brewers, Barcelona, Spain). Distilled water was used as the solvent to prepare the corresponding solutions and as a blank. Sample sets were administered in ascending concentrations (Supporting Information Table 2), placing 0.5 mL of each sample at room temperature on the tongue. For each pair of samples, participants had to indicate whether they detected a difference in taste and to recognize the corresponding basic taste. The assay stopped after correct recognition at a given concentration. RTs were scaled in multiples of one standard deviation. Test solutions were randomized and blinded for participants, who were requested not to smoke, chew gum, or eat/drink any product except for water for 2 h before the test.

The total taste score (TTS), as an overall sensitivity measure, was calculated as the sum of the normalized RT scores divided by the total number of tastants assessed ($n = 8$). Total bitterness score (TBS), as an approximation of overall sensitivity to bitterness, was calculated as the sum of the four normalized RT scores for bitterness divided by four. Cronbach's α was used to evaluate the TTS (α: 0.552) and TBS (α: 0.548) internal reliability. Participants with a PTC RT score of 1 (≤0.7 μmol L^{-1}) were classified as super-tasters; those with a score of 2 or 3 (3.5–14 μmol L^{-1}) as tasters; and those with a score higher than 3 (>14 μmol L^{-1}) were in the non-taster group.[30]

The perceived intensity of sweetness, bitterness, and sourness of the AB and NAB brand used in the study was recorded with the nose covered using a Likert scale (null, light, moderate, high). Hedonist perceptions of AB and NAB were also recorded using the same instrument with the nose uncovered. Liking for AB and NAB in general was assessed through a yes/no question.

Statistical analyses

Differences in baseline characteristics and median RTs between groups were assessed using the Kruskal–Wallis test for continuous variables followed by the *post hoc* Dunn's multiple comparisons test when significant differences were observed. For qualitative variables, the chi-square test was performed and expressed as number (n) and proportion (%). Spearman's correlation was applied to study the relationship between different RTs and the hedonic score and the perceived intensity of beer tastes: sucrose RT and sweetness, citric acid RT and sourness, and IAA RT and bitterness. Differences between AB and NAB three-dimensional intensities were studied with a matched-pair signed-rank test.

The existence of possible intragroup differences in dietary intake and physical activity during the intervention (at baseline and at 12 and 24 months) was analysed with a matched-pair signed-rank test for symmetrically distributed variables, whereas a sign test of matched pairs was used for asymmetric variables. Meanwhile, differences at the intergroup level were analysed with the Kruskal–Wallis test followed by Dunn's *post hoc* test in the case of statistical significance.

The effect of the intervention on biochemical, anthropometric, and BP variables was tested through the generalized estimating equation model, comparing repeated measurements over time (identity link function, first-order autoregressive correlation, and robust standard error parameters were specified). Three adjustment models of increasing complexity were generated to avoid other factors influencing the outcomes. Data were expressed as adjusted mean differences and their 95% confidence intervals. A time–exposure interaction term allowed the evaluation of potential differences between intervention groups in response to changes over time. A test was also performed to measure the trend of the extended response over time as a continuous variable. Additionally, an analysis of variance for repeated measures in order to study the time–exposure interaction among the three study groups was carried out.

The evolution of variables with statistically significant differences between study groups at the end of the intervention according to the generalized estimating equation was graphed. Intra- and inter-group differences for each of these variables were assessed in the same way as for the dietary intake and physical activity variables throughout the intervention.

All statistical analyses were performed with STATA software package 16.0 Special Edition (StataCorp LLC, College Station, TX, USA). Statistical tests were two-sided and *P*-values below 0.05 were considered significant. Figures were prepared using the Prism 9.0.0 software package.

RESULTS

Baseline characteristics of the study participants

Baseline characteristics of each study arm are shown in Supporting Information Tables 3 and 4. Briefly, the total sample of volunteers had a median (Q1, Q3) age of 55 years (53, 58 years) and BMI of 26.3 kg m^{-2} (24.7, 29.0 kg m^{-2}). The only variables detected with a statistical difference were related to alcohol consumption, with members of the AB group reporting a higher intake compared with the other two groups, with a preference for fermented beverages such as beer and wine. Based on the dietary reference values published by the European Food Safety Authority,[31] the diet of the participants was characterized as being low in carbohydrates, very high in dietary fibre, high in fat (especially saturated), and high in sodium (Supporting Information Table 5). The nutritional status of most participants was in the normal or overweight category, with a high WC and a WHR bordering on the limit of abdominal obesity, implying an increased risk of metabolic complications[32] (Supporting Information Table 4).

Regarding biochemical markers, despite significant differences were found in the plasma liver enzymes aspartate aminotransferase and gamma-glutamyl transferase (GGT) at baseline, with higher levels observed in the AB group than the control group, but all were within the reference range, indicating that the differences observed are clinically irrelevant. As expected, follicle-

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

stimulated hormone (FSH) levels were high in the three groups, with the AB group presenting significantly higher levels. Finally, all the remaining laboratory values were within the normal ranges, except for TC, which was slightly above the reference limit (>200 mg dL^{-1}) in the AB and NAB groups (Supporting Information Table 4).

Study compliance

The dietary self-records and interviews indicated that the general level of compliance was 100% for the 34 volunteers who completed the 2-year intervention. Based on the presence or absence of the beer biomarker IX in 24-h urine samples (limit of detection <0.04 ppb), determined at baseline for the run-in period and then throughout the study, compliance was respectively 50% and 97.9% for the AB group, 83.3% and 77.8% for the NAB group, and 100% and 97.2% for the control group.

Controlled covariates

Statistical differences between groups were only observed at the dietary level, specifically in carbohydrate intake, which was significantly higher in the NAB group at 24 months compared with the other two groups. Although NAB consumption was responsible for an average of 5.2% of the daily energy intake in the form of carbohydrates compared with 1.8% provided by AB, these differences could be a consequence of the low carbohydrate intake by the AB and control groups; a significant decrease in consumption of this macronutrient was even observed in the AB group throughout the intervention. A lower consumption of simple sugars, expressed as a percentage of energy intake, was also detected in the AB group compared with both the control and NAB groups at 12 months, being similar to the NAB group only at 24 months. The daily intake of saturated fatty acids in the control group tended to decrease over the course of the intervention (Supporting Information Table 5). As expected, alcohol consumption was significantly higher in the AB group than in the other groups at each assessment visit. However, it should be noted that the intake in the AB group was also significantly higher at the end of the intervention than at baseline.

Glucose control and lipid profile

During the intervention, no significant differences were observed between the study groups regarding changes in glycaemia, glycosylated haemoglobin, triglycerides, and Apo A1 (Table 1). At 12 months, Apo B levels had decreased more in the NAB group than in the control and AB groups. However, these differences lost statistical significance when the whole intervention was considered.

An important finding is that HDL-c levels of AB-consuming postmenopausal women had increased significantly at 24 months compared with the control group, exhibiting a continuous growing trend over time (*P*-trend: 0.006). Consequently, the TC/HDL-c and LDL-c/HDL-c ratios of the AB group decreased significantly compared with the control group in the same period, and linearly with time–exposure (*P*-trends: 0.004 and 0.003 respectively).

Another interesting outcome was that serum LDL-c levels in the NAB consumers had decreased significantly compared with the control group at 12 and 24 months and the AB group at 12 months. Also, the NAB group showed a significant decrease in TC levels at 12 months compared with both the control and AB groups. Nevertheless, these differences lost their significance when the whole intervention was considered. In contrast, no significant differences were found when comparing changes in either LDL-c or TC in AB consumers with those of the control group. Supporting Information Fig. 1 summarizes and illustrates the evolution of the biochemical markers that differed significantly between the study arms after 24 months of intervention.

Liver enzymes

At 24 months, GGT levels had increased significantly in both the AB and NAB groups compared with the control group, whereas no significant differences were observed between groups in aspartate aminotransferase and alanine aminotransferase levels. All the liver enzymes tested remained within the normal range of values throughout the study.

Anthropometric measurements, body composition and BP

No significant differences were observed between groups regarding changes in BMI, WHR, and FMI (Table 2). Although a greater decrease in WC was observed at 12 months in NAB consumers compared with the AB and control groups, and the total fat mass had increased significantly in AB consumers compared with the control group, the differences lost statistical significance at 24 months. Additionally, the two beer-drinking groups had worse values than the control group in terms of android and gynoid fat mass percentages and the android/gynoid ratio, with a slight increase observed at 24 months, compared with a marked decrease in the control group. Changes in the AB group were not statistically different compared with the NAB group.

Finally, significant differences were observed in DBP between the AB and NAB groups at 24 months, with an expected increase of 0.21 mmHg (95% confidence interval (CI): 0.03, 0.40; *P*-trend: 0.023) for every 12 additional months of consuming 330 mL day^{-1} of AB in comparison with the habit of consuming 660 mL day^{-1} of NAB during the 2-year period. No significant differences were found regarding SBP between the study groups.

The progressive changes in the variables that differed significantly between groups at the end of the intervention are illustrated in Supporting Information Fig. 2. Additionally, the FMI, a variable of particular interest, was graphed to visualize the evolution of body composition in each group throughout the intervention.

Sensory analysis

The participants who chose to drink beer (either AB or NAB) had a higher mean TTS (i.e. lower taste sensitivity) than those in the control group (mean TTS for the two beer groups: 0.237 ± 0.142; control group: 0.137 ± 0.103; *P*-value: 0.050). Similarly, the beer consumers had a higher mean RT for IAA and TBS (IAA-rich extract RT score: 4.3 ± 1.8; IAA-rich extract RT: 62.8 ± 52.3 μmol L^{-1}; TBS: 0.45 ± 0.23) than the control group (IAA-rich extract RT score: 3.4 ± 1.5; IAA RT: 31.89 ± 40.5 μmol L^{-1}; TBS: 0.28 ± 0.14).

The IAA RT was higher in NAB consumers than in the control group (IAA RT score for the NAB group: 5.6 ± 0.5; control group: 3.4 ± 1.5; AB group: 3.8 ± 1.8; *P*-value: 0.061). In contrast, the three study arms did not differ in the sucrose RT (*P*-value: 0.738), PTC phenotype distribution (*P*-value: 0.713), or TTS (*P*-value: 0.115).

The beers differed significantly in terms of taste intensity: AB was found to be more bitter (*P*-value: <0.001) and less sweet (*P*-value: 0.041) than NAB. The mean sourness intensity score for AB was the only recorded dimension that correlated well with the corresponding RT for the molecule eliciting this basic taste (*r*: 0.391; *P*-value: 0.048). Additionally, the IAA RT was inversely

Table 1. Effects of intervention on biochemical markers of cardiovascular disease risk and liver enzymes at 12 and 24 months of follow-up

	AB group *versus* CG					NAB group *versus* CG					AB group *versus* NAB group					
	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	*P*-value ANOVA
Glycaemia (mg dL^{-1})																0.843
Model 1	1.44 (−3.61, 6.48)	0.576	1.58 (−2.14, 5.31)	0.405	0.408	3.20 (−3.36, 9.75)	0.339	0.09 (−8.21, 8.40)	0.983	0.998	−1.76 (−8.95, 5.43)	0.632	1.49 (−6.86, 9.85)	0.726	0.712	
Model 2	1.44 (−3.60, 6.48)	0.576	1.58 (−2.14, 5.31)	0.405	0.410	3.20 (−3.36, 9.75)	0.339	0.09 (−8.21, 8.40)	0.983	0.999	−1.76 (−8.95, 5.44)	0.632	1.50 (−6.86, 9.85)	0.726	0.713	
Model 3	1.64 (−3.30, 6.58)	0.515	1.72 (−1.94, 5.38)	0.357	0.363	3.55 (−2.85, 9.95)	0.277	0.25 (−7.71, 8.22)	0.950	0.971	−1.91 (−8.93, 5.11)	0.594	1.46 (−6.54, 9.47)	0.720	0.705	
HbA1c (%)																0.487
Model 1	−0.16 (−0.37, 0.04)	0.119	−0.06 (−0.31, 0. 18)	0.611	0.598	−0.14 (−0. 37, 0.09)	0.238	−0.14 (−0.45, 0.17)	0.370	0.379	−0.02 (−0.23, 0.18)	0.811	0.08 (−0.15, 0.30)	0.498	0.533	
Model 2	−0.16 (−0.37, 0.05)	0.125	−0.06 (−0.31, 0.18)	0.614	0.603	−0.14 (−0.37, −0.09)	0.238	−0.14 (−0.45, 0.17)	0.372	0.381	−0.02 (−0.22, 0.18)	0.836	0.08 (−0.14, 0.30)	0.496	0.529	
Model 3	−0.16 (−0.37, 0.04)	0.119	−0.07 (−0.31, 0.17)	0.568	0.558	−0.14 (−0.38, −0.09)	0.227	−0.14 (−0.45, 0.16)	0.356	0.365	−0.02 (−0.22, 0.18)	0.843	0.07 (−0.15, 0.29)	0.514	0.547	
Triglycerides (mg dL^{-1})																0.669
Model 1	1.94 (−24.82, 28.69)	0.887	−1.10 (−20.74, 18.53)	0.912	0.919	−9.74 (−31.46, 11.98)	0.379	8.98 (−20.17, 38.13)	0.546	0.669	11.68 (−11.54, 34.90)	0.324	−10.08 (−39.13, 18.97)	0.497	0.620	
Model 2	−3.73 (−29.53, 22.06)	0.777	−5.20 (−23.26, 12.88)	0.574	0.537	−11.86 (−36.00, 12.30)	0.336	9.70 (−21.85, 41.26)	0.547	0.683	8.12 (−10.33, 26.57)	0.334	−14.89 (−45.10, 15.31)	0.334	0.397	
Model 3	−11.64 (−45.32, 22.04)	0.498	−8.58 (−25.59, 10.43)	0.376	0.362	−18.38 (−52.20, 15.44)	0.287	8.07 (−25.11, 41.23)	0.634	0.815	6.74 (−10.56, 24.05)	0.445	−16.65 (−46.21, 12.92)	0.270	0.330	
TC (mg dL^{-1})																0.188
Model 1	−1.91 (−19.71, 15.89)	0.833	2.94 (−11.68, 17.56)	0.694	0.694	−25.25 (−47.31, −3.19)	**0.025**	−12.97 (−32.04, 6.10)	0.183	0.212	23.34 (3.33, 43.35)	**0.022**	15.91 (−0.24, 32.05)	0.053	0.069	
Model 2	−1.89 (−19.72, 15.94)	0.835	2.78 (−11.97, 17.53)	0.712	0.713	−25.19 (−47.30, −3.09)	**0.025**	−12.91 (−32.01, 6.19)	0.185	0.215	23.30 (3.31, 43.30)	**0.022**	15.69 (−0.54, 31.91)	0.058	0.075	
Model 3	−2.42 (−20.49, 15.64)	0.792	2.76 (−11.94, 17.46)	0.713	0.715	−24.78 (−46.62, −2.94)	**0.026**	−12.64 (−31.57, 6.28)	0.190	0.220	22.36 (2.49, 42.22)	**0.027**	15.40 (−0.60, 31.40)	0.059	0.076	
LDL-c (mg dL^{-1})																0.138
Model 1	−5.86 (−21.19, 9.46)	0.453	−6.05 (−19.11, 7.00)	0.364	0.361	−30.51 (−49.36, −11.66)	**0.002**	−24.23 (−46.81, −1.66)	**0.035**	0.059	24.65 (5.75, 43.55)	**0.011**	18.18 (−2.65, 39.01)	0.087	0.146	
Model 2	−5.87 (−19.30, 9.52)	0.455	−6.19 (−19.30, 6.91)	0.354	0.352	−30.47 (−49.41, −11.53)	**0.002**	−24.20 (−46.60, −1.81)	**0.034**	0.057	24.59 (5.70, 43.48)	**0.011**	18.01 (−2.63, 38.65)	0.087	0.146	
Model 3	−6.30 (−22.00, 9.41)	0.432	−6.22 (−19.57, 7.14)	0.361	0.361	−29.85 (−48.79, −10.91)	**0.002**	−23.49 (−45.65, −1.33)	**0.038**	0.067	23.55 (4.54, 42.57)	**0.015**	17.27 (−3.03, 37.57)	0.098	0.159	
HDL-c (mg dL^{-1})																0.166
Model 1	4.50 (−2.59, 11.59)	0.213	8.92 (2.22, 15.62)	**0.009**	**0.008**	0.80 (−7.51, 9.11)	0.851	2.78 (−6.48, 12.03)	0.556	0.564	3.70 (−3.38, 10.79)	0.306	6.14 (−3.02, 15.30)	0.189	0.184	
Model 2	4.37 (−2.76, 11.49)	0.235	8.90 (2.30, 15.50)	**0.008**	**0.007**	0.58 (−7.78, 8.94)	0.892	2.58 (−6.62, 11.77)	0.583	0.589	3.79 (−3.38, 10.96)	0.300	6.32 (−2.67, 15.31)	0.168	0.164	
Model 3	3.63 (−3.32, 10.57)	0.306	9.01 (2.47, 15.55)	**0.007**	**0.006**	−0.22 (−8.22, 7.78)	0.956	1.85 (−7.61, 11.32)	0.701	0.702	3.85 (−3.39, 11.09)	0.297	7.16 (−2.21, 16.52)	0.134	0.133	
TC/HDL-c ratio																0.086
Model 1	−0.34 (−0.75, 0.07)	0.107	−0.54 (−0.93, −0.14)	**0.008**	**0.007**	−0.48 (−0.98, 0.02)	0.059	−0.35 (−0.93, 0.24)	0.246	0.337	0.14 (−0.36, 0.65)	0.581	−0.19 (−0.76, 0.38)	0.509	0.364	
Model 2	−0.35 (−0.76, 0.06)	0.099	−0.55 (−0.94, −0.16)	**0.006**	**0.006**	−0.49 (−0.98, 0.01)	**0.047**	−0.36 (−0.94, 0.23)	0.231	0.317	0.15 (−0.35, 0.65)	0.558	−0.19 (−0.77, 0.39)	0.513	0.378	
Model 3	−0.36 (−0.77, 0.04)	0.078	−0.55 (−0.92, −0.18)	**0.004**	**0.004**	−0.52 (−1.02, −0.31)	**0.037**	−0.34 (−1.03, 0.34)	0.325	0.272	0.16 (−0.32, 0.65)	0.515	−0.17 (−0.74, 0.40)	0.554	0.413	
LDL-c/HDL-c ratio																0.081
Model 1	−0.30 (−0.67, 0.07)	0.116	−0.48 (−0.82, −0.14)	**0.006**	**0.005**	−0.49 (−0.90, −0.07)	**0.023**	−0.43 (−0.91, 0.04)	0.076	0.120	0.19 (−0.26, 0.63)	0.415	−0.05 (−0.52, 0.42)	0.836	0.572	
Model 2	−0.31 (−0.68, 0.07)	0.107	−0.49 (−0.83, −0.15)	**0.005**	**0.004**	−0.50 (−0.91, 0.09)	**0.017**	−0.42 (−0.96, 0.11)	0.123	0.105	0.19 (−0.25, 0.63)	0.391	−0.05 (−0.52, 0.42)	0.843	0.590	
Model 3	−0.32 (−0.69, 0.04)	0.084	−0.49 (−0.81, −0.17)	**0.003**	**0.003**	−0.53 (−0.94, −0.12)	**0.011**	−0.47 (−0.92, 0.13)	**0.044**	0.076	0.21 (−0.21, 0.63)	0.329	−0.02 (−0.49, 0.44)	0.928	0.663	
Apo A1 (mg dL^{-1})																0.484
Model 1	−2.69 (−15.12, 9.75)	0.672	8.27 (−7.20, 23.75)	0.295	0.293	−13.38 (−30.42, 3.66)	0.124	−0.54 (−19.21, 18.13)	0.955	0.970	10.69 (−5.02, 26.41)	0.182	8.81 (−6.24, 23.86)	0.251	0.274	
Model 2	−2.70 (−15.13, 9.74)	0.671	8.27 (−7.21, 23.74)	0.295	0.294	−13.37 (−30.42, 3.68)	0.124	−0.55 (−19.22, 18.11)	0.954	0.972	0.67 (−0.505, 26.40)	0.183	8.82 (−6.19, 23.84)	0.249	0.272	
Model 3	−2.55 (−15.09, 10.00)	0.691	8.74 (−6.76, 24.25)	0.270	0.270	−13.58 (−30.32, 3.16)	0.112	−0.89 (−19.55, 17.76)	0.925	0.993	11.03 (−4.20, 26.27)	0.156	9.63 (−5.83, 25.09)	0.222	0.241	
Apo B (mg dL^{-1})																0.367
Model 1	−2.29 (−12.00, 7.42)	0.644	1.69 (−6.82, 10.19)	0.698	0.645	−13.34 (−21.64, −5.03)	**0.002**	−1.93 (−17.00, 13.13)	0.801	0.883	11.05 (0.87, 21.23)	**0.033**	3.62 (−11.30, 18.54)	0.634	0.893	
Model 2	−1.96 (−11.42, 7.51)	0.685	1.83 (−6.75, 10.41)	0.676	0.628	−13.36 (−21.69, −5.03)	**0.002**	−1.42 (−16.43, 13.58)	0.852	0.844	11.40 (1.48, 21.32)	**0.024**	3.26 (−11.48, 17.99)	0.665	0.918	
Model 3	−2.07 (−11.58, 7.43)	0.669	1.62 (−6.76, 9.99)	0.705	0.652	−13.28 (−21.46, −5.10)	**0.001**	−1.30 (−15.84, 13.24)	0.861	0.844	11.20 (1.22, 21.19)	**0.028**	2.92 (−11.40, 17.23)	0.690	0.936	
AST (U L^{-1})																0.542
Model 1	−4.40 (−10.65, 1.86)	0.168	−1.17 (−6.94, 4.60)	0.692	0.662	−1.19 (−4.83, 2.45)	0.521	1.23 (−4.61, 7.06)	0.680	0.710	−3.21 (−9.09, 2.68)	0.285	−2.39 (−9.95, 5.16)	0.535	0.534	
ALT (U L^{-1})																0.678

Table 1. Continued

	AB group *versus* CG				NAB group *versus* CG				AB group *versus* NAB group				
	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	*P*-value ANOVA
Model 1	−2.60 (−8.80, 3.60)	0.412	3.00 (−1.44, 7.45)	0.235	−0.26 (−5.34, 4.82)	0.920	3.23 (−4.14, 10.60)	0.390	0.416	−2.34 (−9.47, 4.80)	0.521	−0.23 (−8.21, 7.74)	0.955	0.922	
GGT, (U L^{-1})															0.186
Model 1	0.13 (−3.95, 4.21)	0.950	4.46 (0.92, 8.01)	**0.011**	0.29 (−2.94, 3.51)	0.861	6.22 (3.77, 8.65)	**<0.001**	**<0.001**	−0.16 (−4.61, 4.30)	0.945	−1.75 (−5.42, 1.92)	0.350	0.412	

Abbreviations: AB, alcoholic beer; NAB, non-alcoholic beer; CG, control group; HbA1c, glycosylated haemoglobin; TC, Total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.
Note: Generalized estimating equation models were used to compare the differences between the study groups throughout the intervention with respect to their own baseline values. Data are expressed as the mean of the differences (95% confidence interval (CI)). Model 1: adjusted by age at baseline. Model 2: adjusted like model 1 plus follicle-stimulating hormone concentration at baseline. Model 3: adjusted like model 2 plus smoking habit, total energy intake, physical activity as MET-min/day, and hypocholesterolaemia (for lipid profile variables) at baseline. *P*-value: group × time interaction; *P*-trend: group × time interaction (continuous). *P*-value analysis of variance (ANOVA) repeated measures: group × time interaction. Values <0.05 are statistically significant and highlighted in bold.

correlated with the hedonic liking score for AB (*r*: −0.506; *P*-value: 0.014), but not with bitterness intensity (*r*: 0.127; *P*-value: 0.535).

In response to a yes/no question about liking AB, 70.0% of the control groups and 80.0% of the NAB group gave a negative answer, whereas all members of the AB group declared they liked AB (*P*-values: 0.001). On the other hand, 70.0%, 50%, and 20% of the control, AB, and NAB groups respectively stated they did not like NAB (*P*-value: 0.186). The PTC phenotype and the sucrose RT were not associated with alcohol drinking frequency.

DISCUSSION

Although consumption of beer has been associated with negative health effects in the general population, such as weight gain and visceral fat accumulation,[33,34] many studies have reported that moderate beer intake can have beneficial effects on the lipid profile, CV risk, and mortality, mainly attributable to the polyphenol content.[35-37] Nevertheless, few studies evaluating the relationship between beer and CVD have stratified their conclusions by sex.[36] The previously published mid-term results of the present clinical trial were encouraging in terms of effects on CV risk factors, with a reduction in serum levels of LDL-c and a slightly decreasing trend in DBP observed after 6 months of NAB consumption.[38]

The nutritional status and anthropometric measurements related to abdominal obesity at baseline indicated that the study population was at CV risk. This was expected, as an increase in fat mass and its redistribution at the abdominal level are well-documented morphological changes associated with menopause.[32] Although significant differences in alcohol consumption were found between the three study arms at baseline, the levels of intake could be considered as low risk, not exceeding 16 g day^{-1}, which might be the amount recommended for women for a cardioprotective effect.[36]

An improvement in HDL-c levels in healthy subjects resulting from moderate and regular alcohol consumption has been repeatedly reported in the literature, although the mechanisms involved are still not entirely clear.[39-41] In addition, studies have described this effect in consumers of AB but not NAB, which seems to support the hypothesis that it is essentially a consequence of alcohol consumption.[35,42] As well as HDL-c, the ratios of TC/HDL-c and LDL-c/HDL-c have been described as CV risk indicators with greater predictive value than the individual lipid parameters used independently.[43] Therefore, their reduction represents a beneficial factor for the health of low to moderate AB consumers.

Similar to the results of the present clinical trial, studies on chronic but moderate beer administration and CV health in humans found that NAB consumers had significantly lower TC levels than AB consumers did, regardless of sex.[35] Moreover, the NAB arm of the present study also showed a significant long-term decrease in serum LDL-c levels compared with the control group, which accounts for the greater reduction in TC in NAB drinkers than in the other groups. It can be hypothesized that this beneficial effect on the lipid profile is due to the bioactive fraction of beer, considering that studies on phytoestrogen supplementation in postmenopausal women for more than 8 weeks also report improvements in serum TCs and LDL-c.[44] However, these improvements were more evident in participants with high baseline cholesterol levels, which is consistent with the characteristics of the present study population. Indeed, a prospective, randomized cross-over trial (RCT) reported that the changes in the lipid profile in moderate consumers of NAB or AB (men and women)

Table 2. Effects of intervention on body composition and blood pressure at 12 and 24 months of follow-up

	AB group *versus* CG					NAB group *versus* CG					AB group *versus* NAB group					
	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	Mean difference 12 months (95% CI)	*P*-value	Mean difference 24 months (95% CI)	*P*-value	*P*-trend	*P*-value ANOVA
BMI (kg m^{-2})																0.738
Model 1	−0.00 (−0.98, 0.97)	0.993	0.58 (−0.36, 1.51)	0.225	0.218	−0.38 (−1.34, 0.59)	0.447	0.17 (−1.06, 1.40)	0.782	0.778	0.37 (−0.77, 1.51)	0.522	0.41 (−0.67, 1.48)	0.462	0.456	
Model 2	−0.10 (−1.10, 0.91)	0.853	0.45 (−0.51, 1.42)	0.359	0.358	−0.42 (−1.37, 0.53)	0.359	0.19 (−1.04, 1.41)	0.767	0.763	0.32 (−0.82, 1.47)	0.578	0.27 (−0.84, 1.38)	0.638	0.639	
Model 3	−0.01 (−1.02, 0.99)	0.981	0.50 (−0.48, 1.48)	0.318	0.315	−0.39 (−1.31, 0.52)	0.400	0.22 (−1.00, 1.45)	0.719	0.714	0.38 (−0.72, 1.49)	0.498	0.28 (−0.80, 1.36)	0.616	0.619	
WC (cm)																0.565
Model 1	−0.62 (−3.34, 2.10)	0.656	1.05 (−3.37, 5.48)	0.641	0.638	−4.04 (−7.25, −0.84)	**0.013**	−0.96 (−6.81, 4.88)	0.747	0.753	3.42 (0.25, 6.60)	**0.035**	2.02 (−3.94, 7.97)	0.507	0.510	
Model 2	−0.68 (−3.47, 2.10)	0.632	0.26 (−4.58, 5.10)	0.918	0.914	−4.02 (−7.34, −0.70)	**0.018**	−0.86 (−6.66, 4.94)	0.771	0.776	3.34 (0.05, 6.62)	**0.046**	1.12 (−5.09, 7.32)	0.724	0.726	
Model 3	−0.60 (−3.40, 2.19)	0.672	0.29 (−4.56, 5.13)	0.908	0.903	−4.00 (−7.25, −0.73)	**0.016**	−0.75 (−6.45, 4.96)	0.798	0.801	3.39 (0.19, 6.58)	**0.038**	1.03 (−5.12, 7.18)	0.742	0.742	
WHR																0.725
Model 1	0.00 (−0.03, 0.04)	0.770	0.02 (−0.03, 0.04)	0.446	0.440	−0.03 (−0.06, 0.01)	0.116	0.00 (−0.04, 0.05)	0.914	0.914	0.03 (−0.00, 0.07)	0.064	0.02 (−0.05, 0.08)	0.583	0.578	
Model 2	0.00 (−0.03, 0.04)	0.774	0.02 (−0.04, 0.07)	0.528	0.522	−0.03 (−0.06, 0.01)	0.125	0.00 (−0.04, 0.05)	0.906	0.906	0.03 (0.00, 0.07)	0.068	0.01 (−0.05, 0.08)	0.662	0.657	
Model 3	0.00 (−0.03, 0.04)	0.766	0.02 (−0.04, 0.07)	0.549	0.542	−0.03 (−0.07, 0.01)	0.130	−0.00 (−0.04, 0.05)	0.899	0.899	0.03 (0.00, 0.07)	0.071	0.01 (−0.05, 0.08)	0.688	0.683	
FMI (kg m^{-2})																0.626
Model 1	0.59 (−0.17, 1.34)	0.128	0.53 (−0.34, 1.34)	0.234	0.229	−0.06 (−1.33, 1.45)	0.932	−0.15 (−1.40, 1.10)	0.817	0.806	0.53 (−0.85, 1.90)	0.453	0.68 (−0.37, 1.72)	0.207	0.198	
Model 2	0.45 (−0.33, 1.23)	0.257	0.39 (−0.49, 1.27)	0.384	0.399	−0.03 (−1.46, 1.39)	0.962	−0.18 (−1.47, 1.10)	0.780	0.773	0.48 (−0.95, 1.92)	0.508	0.58 (−0.54, 1.69)	0.310	0.316	
Model 3	0.76 (−0.00, 1.53)	0.052	0.38 (−0.61, 1.37)	0.454	0.370	−0.04 (−1.17, 1.24)	0.954	−0.12 (−1.36, 1.15)	0.869	0.838	0.60 (−0.42, 1.62)	0.249	0.37 (−0.80, 1.53)	0.533	0.300	
Total fat mass (%)																0.339
Model 1	1.85 (−0.06, 3.64)	**0.043**	0.71 (−0.67, 2.09)	0.314	0.313	−0.24 (−4.21, 3.73)	0.904	0.19 (−3.33, 3.71)	0.916	0.919	2.09 (−1.83, 6.02)	0.296	0.52 (−2.92, 3.95)	0.768	0.764	
Model 2	1.91 (−0.13, 3.69)	**0.036**	0.74 (−0.62, 2.11)	0.286	0.295	−0.20 (−4.13, 3.74)	0.921	0.20 (−3.29, 3.69)	0.912	0.917	2.11 (−1.77, 5.99)	0.286	0.55 (−2.85, 3.94)	0.752	0.754	
Model 3	2.00 (0.20, 3.81)	**0.030**	0.69 (−0.76, 2.13)	0.351	0.347	−0.27 (−4.16, 3.61)	0.890	0.13 (−3.36, 3.62)	0.941	0.943	2.28 (−1.60, 6.15)	0.249	0.56 (−2.82, 3.93)	0.747	0.748	
Android fat mass (%)																**0.001**
Model 1	2.82 (−0.03, 5.68)	0.053	4.69 (1.28, 8.10)	**0.007**	**0.006**	1.81 (−1.03, 4.64)	0.211	7.31 (2.84, 11.79)	**0.001**	**0.001**	1.02 (−2.22, 4.25)	0.539	−2.62 (−6.32, 1.08)	0.165	0.170	
Model 2	2.86 (0.01, 5.71)	**0.049**	4.72 (1.33, 8.11)	**0.006**	**0.006**	1.82 (−1.01, 4.65)	0.209	7.30 (2.83, 11.77)	**0.001**	**0.001**	1.47 (−2.18, 4.27)	0.525	−2.58 (−6.27, 1.11)	0.170	0.170	
Model 3	8.19 (4.69, 11.68)	**<0.001**	3.98 (0.79, 7.17)	**0.014**	**0.008**	1.57 (−1.19, 4.32)	0.265	6.33 (1.64, 11.02)	**0.008**	**0.002**	1.93 (−3.27, 7.12)	0.467	−2.22 (−5.72, 1.27)	0.213	0.180	
Gynoid fat mass (%)																**0.037**
Model 1	0.87 (−0.77, 2.52)	0.299	3.76 (0.64, 6.87)	**0.018**	**0.018**	0.44 (−0.97, 1.86)	0.537	4.01 (0.58, 7.46)	**0.022**	**0.021**	0.43 (−1.21, 2.06)	0.610	−0.26 (−2.35, 1.82)	0.805	0.808	
Model 2	0.89 (−0.75, 2.53)	0.288	3.76 (0.64, 6.87)	**0.017**	**0.017**	0.45 (−0.97, 1.86)	0.536	4.01 (0.57, 7.44)	**0.022**	**0.021**	0.44 (−1.19, 2.07)	0.596	−0.24 (−2.32, 1.83)	0.819	0.825	
Model 3	0.82 (−0.85, 2.49)	0.337	3.73 (0.65, 6.83)	**0.018**	**0.017**	0.36 (−1.06, 1.79)	0.616	4.03 (0.59, 7.46)	**0.022**	**0.021**	0.45 (−1.17, 2.08)	0.585	−0.29 (−2.38, 1.80)	0.785	0.788	
Android/gynoid ratio																0.056
Model 1	0.06 (−0.02, 0.12)	**0.043**	0.02 (−0.03, 0.08)	0.407	0.430	0.05 (−0.02, 0.12)	0.152	0.09 (0.01, 0.18)	**0.033**	**0.039**	0.01 (−0.06, 0.08)	0.780	−0.07 (−0.15, 0.01)	0.095	0.102	
Model 2	0.06 (−0.03, 0.12)	**0.041**	0.02 (−0.03, 0.08)	0.382	0.412	0.05 (−0.02, 0.12)	0.148	0.09 (−0.01, 0.18)	**0.033**	**0.038**	0.01 (−0.06, 0.08)	0.765	−0.07 (−0.15, 0.01)	0.098	0.104	
Model 3	0.09 (0.01, 0.17)	**0.033**	0.02 (−0.03, 0.07)	0.426	0.446	0.05 (−0.02, 0.12)	0.134	0.09 (−0.01, 0.18)	**0.036**	**0.036**	0.04 (−0.06, 0.13)	0.431	−0.07 (−0.15, 0.01)	0.077	0.083	
DBP (mmHg)																0.782
Model 1	−0.54 (−5.40, 4.32)	0.828	−0.13 (−5.93, 5.67)	0.965	0.961	−4.59 (−10.02, 0.85)	0.098	−5.03 (−11.34, 1.27)	0.118	0.109	4.05 (−1.18, 9.28)	0.129	4.90 (0.49, 9.32)	**0.029**	**0.026**	
Model 2	−0.56 (−5.42, 4.30)	0.822	−0.15 (−5.96, 5.65)	0.959	0.952	−4.60 (−10.03, 0.83)	0.097	−5.05 (−11.36, 1.26)	0.116	0.108	4.04 (−1.18, 9.27)	0.127	4.90 (0.49, 9.31)	**0.029**	**0.026**	
Model 3	−0.26 (−5.18, 4.65)	0.916	0.33 (−5.50, 6.16)	0.912	0.917	−4.63 (−10.03, 0.77)	0.093	−4.98 (−11.26, 1.31)	0.121	0.112	4.36 (−0.56, 9.59)	0.101	5.30 (0.97, 9.64)	**0.017**	**0.023**	
SBP (mmHg)																0.448
Model 1	−5.16 (−12.94, 2.61)	0.193	−1.65 (−10.46, 7.16)	0.713	0.693	−4.09 (−14.44, 6.25)	0.438	−3.03 (−12.27, 6.21)	0.521	0.503	−1.07 (−11.36, 9.23)	0.839	1.38 (−7.05, 9.80)	0.749	0.748	
Model 2	−5.23 (−13.03, 2.57)	0.189	−1.74 (−10.59, 7.12)	0.701	0.678	−4.18 (−14.50, 6.13)	0.427	−3.08 (−12.31, 6.16)	0.514	0.495	−1.05 (−12.32, 9.23)	0.842	1.34 (−7.08, 9.75)	0.755	0.756	
Model 3	−5.15 (−12.62, 2.32)	0.177	−1.31 (−10.07, 7.44)	0.769	0.749	−4.39 (−14.41, 5.63)	0.391	−2.34 (−11.62, 6.94)	0.621	0.606	−0.76 (−10.47, 8.95)	0.878	1.02 (−7.61, 9.66)	0.816	0.726	

Abbreviations: AB, alcoholic beer; NAB, non-alcoholic beer; CG, control group; BMI, body mass index; WC, waist circumference; WHR, waist/hip ratio; FMI, fat mass index; DBP, diastolic blood pressure; SPB, systolic blood pressure.

Note: Generalized estimating equation models were used to compare the differences between the study groups throughout the intervention with respect to their own baseline values. Data are expressed as the mean of the differences (95% confidence interval (CI)). Model 1: adjusted by age at baseline. Model 2: adjusted like model 1 plus follicle-stimulating hormone concentration at baseline. Model 3: adjusted like model 2 plus smoking habit, total energy intake, physical activity as MET-min/day, and antihypertensive treatment (for blood pressure variables) at baseline. *P*-value: group × time interaction; *P*-trend: group × time interaction (continuous). *P*-value analysis of variance (ANOVA) repeated measures: group × time interaction. Values <0.05 are statistically significant and highlighted in bold.

followed a different pattern according to whether baseline LDL-c levels were below or above 130 mg dL^{-1}.[45]

Consumption of AB or NAB did not have an impact on glucose-related variables during the 2-year intervention. Similarly, two previous studies found the same result in a 33 high CV risk male cohort[42] and in a 51 non-diabetic postmenopausal women cohort.[46] Considering that these two RCTs involved short interventions of 4 weeks and 8 weeks respectively, our study provides additional relevant information about long-term beer consumption, which had neither positive nor negative effects on blood glucose levels and glycosylated haemoglobin in non-diabetic postmenopausal women.

The analysis of liver enzymes indicated that daily moderate consumption of either AB or NAB had a low risk of hepatic disease for the sample population. Other studies have similarly found that consumption of AB in moderate doses (e.g. one can of beer per day for women) does not modify or induces only small changes in liver enzymes compared with the reference ranges in plasma.[45,47] The cause of changes in GGT after regular NAB consumption remains unknown. In any case, these findings need careful interpretation, as the liver enzymes analysed were at normal basal levels and the participants did not suffer from previously diagnosed hepatic diseases.

The anthropometric and body composition parameters measured in the present study are frequently used as risk factors for CVD and associated mortality.[45,48–50] Thus, the fact that no significant long-term differences were found in BMI, FMI, WHR, and WC between those who drank either type of beer or the control group is a relevant health outcome, which is supported by other studies.[42,45] Although both studies were of short duration, their results are consistent with those obtained in the present clinical trial, as we found that long-term moderate beer intake did not have a significant impact on those CVD risk factors.

Beyond the traditional estimators of abdominal fat, such as WC, with inter- and intra-evaluator variability inherent to the measurement, more sensitive techniques are used to characterize body composition. Accordingly, changes in android and gynoid fat percentage and their ratio were found to differ significantly between the study groups. The effect of low to moderate drinking beer on abdominal fat is a controversial issue, with conflicting evidence in the literature. The consumption of AB has been directly and proportionally correlated with the accumulation of visceral adipose mass,[33,34] but other studies describe the opposite or null effect.[51,52] In the present study, the dietary covariates were controlled and the participants were encouraged to drink beer at mealtimes. No significant differences in daily energy or carbohydrate intake were reported between the AB and control groups, and, paradoxically, the AB group significantly decreased their carbohydrate intake in relation to the baseline. On the other hand, the pronounced reduction in android and gynoid fat observed in the control group was not associated with alcohol consumption, which remained at negligible levels throughout, but this could be related to a decrease in the percentage of saturated fatty acids in the daily energy intake. An accelerated increase in fat mass occurs during the first 4 years after menopause, together with changes in its distribution, after which the process slows down.[53,54] Considering that, at the beginning of the trial, the time since menopause onset was approximately 3.6 years in the control group, compared with 2.3 years in the AB group and 1.9 years in the NAB group, it can be presumed that the variations in body composition associated with menopause had decreased for the control group but not for the other participants.

Although a significant increase in DBP was observed in the AB group compared with the NAB group, this effect was not significantly different compared with the control group's evolution. In a meta-analysis of controlled clinical trials, eight studies reported no significant differences in DBP or SBP between the AB and control groups, whether NAB or placebo.[35] Additionally, a systematic review and meta-analysis of cohort studies found no increased risk of hypertension in women consuming one to two servings of alcohol per day (relative risk: 0.94; 0.88–1.01) compared with abstainers.[55] The mechanism underlying the effect of alcohol on BP has not yet been fully elucidated,[56] although the impact of light to moderate alcohol consumption is expected to be short-term and reversible.[57]

The liking of beer was a significant factor in the participant's choice of study arm. Our findings suggest that the sensory perception of beer could be conditioned at least partially by the IAA RT. Participants in the NAB and control groups reported a lower alcohol intake at baseline, which could indicate that they did not like alcohol and/or the bitterness of beer, as suggested by Guinard *et al.*[58] Although the IAA content of the beers administered in the present study was not quantified, levels of approximately 40–100 μmol L^{-1} have been reported in alcoholic lager and 35 μmol L^{-1} in non-alcoholic lager.[59] Thus, the mean IAA RT for AB and NAB was similar to or above the expected IAA content in lager beers, which could explain the null correlation between this sensory factor and bitterness and indicate a promising biological implication, because some beer matrices may not contain this component at a suprathreshold level for some subjects. Hedonic considerations might also have affected how subjects approached the intensity score procedure.

It has been suggested that drinking behaviour and preferences for specific alcoholic beverages might be influenced by genetic variations in taste receptors. Accordingly, the PTC taster phenotype was found to predict a lower average consumption of standard alcoholic drinks,[58,60,61] although this was not the case in the present study. The IAA together with the low drinking habit might explain why some participants chose to be included in the NAB group, whereas IAA RT itself might explain the beer's arm choices (either AB or NAB). Further research on individual taste sensations (sensitivity, intensity, and hedonism) in large cohorts would be useful to identify individuals that may benefit from moderate beer consumption or those more likely to accept and comply with a moderate beer intervention.

One of the main strengths of this study is its 2-year duration, considerably longer than other similar studies, which made it possible to evaluate the long-term effects of alcohol and bioactive components of beer in postmenopausal women. In addition, the findings of the study expand the limited existing evidence for the effect of beer on CV health in this population and shed light on its protective action against CV alterations associated with menopause. Limitations of this clinical trial are the small sample size, along with the uneven size of the study arms, the lack of power calculation, and the *post hoc* analysis. Additionally, as the study was initially planned to assess the effects of beer intake on bone health, the exclusion criteria did not include a family history of CVD, so certain results may be biased by this parameter. Moreover, the AB and NAB doses differed not only in the presence/absence of ethanol, but also in the prenylflavonoid profile, which may have influenced the results of the intervention. Comparisons with studies on other phytoestrogen food sources (e.g. soya, flaxseed, red clover) are difficult due to differences in components, doses, and duration as well as individual inter-

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variability in compound metabolism. Finally, the sensory evaluation was carried out after the end of the intervention.

CONCLUSION

To sum up, this clinical trial can serve as a pilot study for future research, as the results indicate that beer consumption has positive effects on some CVD risk factors. It remains unclear whether these improvements are due to the antioxidant and anti-inflammatory properties of beer polyphenols or whether phytoestrogenic components (also phenolics) regulate blood lipids by acting as replacements of endogenous oestrogens. Nevertheless, these findings could be of interest for food innovation strategies to boost the healthy properties of beer, considering also that 14 g day^{-1} of alcohol in beer form a significantly increased serum level of HDL-c. Further RCTs about the effect of non-alcoholic and alcoholic beer fractions on CVD risk factors in postmenopausal women should be performed to confirm these results. Also required are more *in vivo* and *in vitro* studies on the mechanisms of action underlying the effects observed in these types of intervention. Furthermore, this study supports the future role of sensory nutrition in the development of food sciences clinical trials and the discussion of findings.

ACKNOWLEDGEMENTS

We thank all the volunteers for their participation and contribution to the trial. We wish to thank the CCiT-UB for the use of the mass spectrometry equipment.

AUTHORS' CONTRIBUTIONS

Conceptualization: AT, JM, RE, and RL; methodology: MT, PM; validation: AT, JM, RE, and RL; formal analysis: MT, PM; data curation: MT, PM; writing – original draft preparation: MT, PM; writing – review and editing: AT, JM, RE, and RL; supervision: JM, RE, and RL; project administration: RL; funding acquisition: RL. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

RL and AT have received funding from The European Foundation for Alcohol Research (ERAB). RL has received lecture fees and travel support from Cerveceros de España and Wine in Moderation. RE reports grants from Fundación Dieta Mediterránea (Spain) and Cerveza y Salud (Spain). He also reports personal fees for giving lectures from Brewers of Europe (Belgium), Fundación Cerveza y Salud (Spain), Lilly Laboratories (Spain), and Wine and Culinary International Forum (Spain); non-financial support to organize a National Congress on Nutrition; and feeding trials with product from Grand Fountain and Uriach Laboratories (Spain). No other conflicts of interest related to the current paper were reported.

FUNDING

This research has been supported by the European Foundation for Alcohol Research (ERAB) (EA 1514, EA 1515, and EA 1517) PID2020-114022RB-I00 and CIBEROBN from the Instituto de Salud Carlos III, ISCIII from the Ministerio de Ciencia, Innovación y Universidades (AEI/FEDER, UE), Generalitat de Catalunya (GC) (2021-SGR-00334). INSA-UB is a Mara de Maeztu Unit of Excellence (grant CEX2021-001234-M funded by MICIN/AEI/FEDER, UE). None of the funders had a role in the study design, implementation, analysis or interpretation of the data, or the writing of the manuscript. MT is grateful for the APIF 2018–2019 fellowship from the University of Barcelona. AT is a Serra-Hunter fellow.

DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are available upon request from RL.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Hoga L, Rodolpho J, Gonçalves B and Quirino B, Women's experience of menopause: a systematic review of qualitative evidence. *JBI Database of Systematic Reviews and Implementation Reports* **13**:250–337 (2015).
2 World Health Organization Scientific Group, Research on the menopause in the 1990s. WHO Technical Report Series (1996).
3 Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA *et al.*, The *Lancet* Women and Cardiovascular Disease Commission: reducing the global burden by 2030. *Lancet* **397**:2385–2438 (2021).
4 Boardman HMP, Hartley L, Eisinga A, Main C, Roqué i Figuls M, Bonfill Cosp X *et al.*, Hormone therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev*: 2015 (2015).
5 Ko S-H and Kim H-S, Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women. *Nutrients* **12**:202 (2020).
6 Karppinen JE, Törmäkangas T, Kujala UM, Sipilä S, Laukkanen J, Aukee P *et al.*, Menopause modulates the circulating metabolome: evidence from a prospective cohort study. *Eur J Prev Cardiol* **29**:1448–1459 (2022).
7 Barańska A, Błaszczuk A, Kanadys W, Baczewska B, Jędrych M, Wawryk-Gawda E *et al.*, Effects of soy protein containing of isoflavones and isoflavones extract on plasma lipid profile in postmenopausal women as a potential prevention factor in cardiovascular diseases: systematic review and meta-analysis of randomized controlled trials. *Nutrients* **13**:2531 (2021).
8 Ambikairajah A, Walsh E and Cherbuin N, Lipid profile differences during menopause: a review with meta-analysis. *Menopause* **26**:1327–1333 (2019).
9 Sánchez-Martínez L, Periago M-J, García-Alonso J, García-Conesa M-T and González-Barrio R, A systematic review of the cardiometabolic benefits of plant products containing mixed phenolics and polyphenols in postmenopausal women: insufficient evidence for recommendations to this specific population. *Nutrients* **13**:4276 (2021).
10 Kim J-E, Chang J-H, Jeong M-J, Choi J, Park J, Baek C *et al.*, A systematic review and meta-analysis of effects of menopausal hormone therapy on cardiovascular diseases. *Sci Rep* **10**:20631 (2020).
11 Glisic M, Kastrati N, Musa J, Milic J, Asllanaj E, Portilla Fernandez E *et al.*, Phytoestrogen supplementation and body composition in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. *Maturitas* **115**:74–83 (2018).
12 Li J, Liu Y, Wang T, Zhao L and Feng W, Does genistein lower plasma lipids and homocysteine levels in postmenopausal women? A meta-analysis. *Climacteric* **19**:440–447 (2016).
13 Sirotkin AV and Harrath AH, Phytoestrogens and their effects. *Eur J Pharmacol* **741**:230–236 (2014).
14 Myasoedova V, Kirichenko T, Melnichenko A, Orekhova V, Ravani A, Poggio P *et al.*, Anti-atherosclerotic effects of a phytoestrogen-rich herbal preparation in postmenopausal women. *Int J Mol Sci* **17**: 1318 (2016).
15 Tronina T, Popłoński J and Bartmańska A, Flavonoids as phytoestrogenic components of hops and beer. *Molecules* **25**:4201 (2020).
16 Pohjanvirta R and Nasri A, The potent phytoestrogen 8-prenylnaringenin: a friend or a foe? *Int J Mol Sci* **23**:3168 (2022).

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17 Parker DK, Beer: production, sensory characteristics and sensory analysis, in *Alcoholic Beverages*, ed by John Piggott. Woodhead Publishing, pp. 133–158 (2012).
18 Intelmann D, Batram C, Kuhn C, Haseleu G, Meyerhof W and Hofmann T, Three TAS2R bitter taste receptors mediate the psychophysical responses to bitter compounds of hops (*Humulus lupulus* L.) and beer. *Chemosens Percept* **2**:118–132 (2009).
19 Trius-Soler M, Tresserra-Rimbau A, Moreno JJ, Peris P, Estruch R and Lamuela-Raventós RM, Effect of moderate beer consumption (with and without ethanol) on osteoporosis in early postmenopausal women: results of a pilot parallel clinical trial. *Front Nutr* **9**:1014140 (2022).
20 Boronat A, Soldevila-Domenech N, Rodríguez-Morató J, Martínez-Huélamo M, Lamuela-Raventós RM and de la Torre R, Beer phenolic composition of simple phenols, prenylated flavonoids and alkylresorcinols. *Molecules* **25**:2582 (2020).
21 Estruch R and Salas-Salvadó J, Towards an even healthier Mediterranean diet. *Nutr Metab Cardiovasc Dis* **23**:1163–1166 (2013).
22 Quifer-Rada P, Martínez-Huélamo M, Chiva-Blanch G, Jáuregui O, Estruch R and Lamuela-Raventós RM, Urinary isoxanthohumol is a specific and accurate biomarker of beer consumption. *J Nutr* **144**: 484–488 (2014).
23 Elosua R, Garcia M, Aguilar A, Molina L, Covas M-I and Marrugat J, Validation of the Minnesota leisure time physical activity questionnaire in Spanish women. *Med Sci Sports Exercise* **32**:1431–1437 (2000).
24 Juton C, Castro-Barquero S, Casas R, Freitas T, Ruiz-León AM, Crovetto F *et al*., Reliability and concurrent and construct validity of a food frequency questionnaire for pregnant women at high risk to develop fetal growth restriction. *Nutrients* **13**:1629 (2021).
25 Wang YJ, Yeh TL, Shih MC, Tu YK and Chien KL, Dietary sodium intake and risk of cardiovascular disease: a systematic review and dose-response meta-analysis. *Nutrients* **12**:2934 (2020).
26 Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC *et al*., Dietary intake and blood concentrations of antioxidants and the risk of cardiovascular disease, total cancer, and all-cause mortality: a systematic review and dose-–response meta-analysis of prospective studies. *Am J Clin Nutr* **108**:1069–1091 (2018).
27 Yin T, Zhu X, Xu D, Lin H, Lu X, Tang Y *et al*., The association between dietary antioxidant micronutrients and cardiovascular disease in adults in the United States: a cross-sectional study. *Front Nutr* **8**: 1256 (2022).
28 Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J *et al*., A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr* **141**: 1140–1145 (2011).
29 World Health Organization, *WHO STEPS Surveillance Manual: The WHO STEPwise Approach to Chronic Disease Risk Factor Surveillance/ Noncommunicable Diseases and Mental Health*. World Health Organization, Geneva, Switzerland (2005) Available: https://apps.who.int/iris/handle/10665/43376.
30 Trius-Soler M, Bersano-Reyes PA, Góngora C, Lamuela-Raventós RM, Nieto G and Moreno JJ, Association of phenylthiocarbamide perception with anthropometric variables and intake and liking for bitter vegetables. *Genes Nutr* **17**:12 (2022).
31 European Food Safety Authority, Dietary reference values for nutrients summary report. *EFSA Supporting Publ* **14**:e15121 (2017).
32 World Health Organization, *Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation*. WHO, Geneva, Switzerland (2011).
33 Larsen BA, Klinedinst BS, Le ST, Pappas C, Wolf T, Meier NF *et al*., Beer, wine, and spirits differentially influence body composition in older white adults – a United Kingdom biobank study. *Obes Sci Pract* **8**:641–656 (2022).
34 Wannamethee SG, Shaper AG and Whincup PH, Alcohol and adiposity: effects of quantity and type of drink and time relation with meals. *Int J Obes* **29**:1436–1444 (2005).
35 Spaggiari G, Cignarelli A, Sansone A, Baldi M and Santi D, To beer or not to beer: a meta-analysis of the effects of beer consumption on cardiovascular health. *PLoS One* **15**:e0233619 (2020).
36 Marcos A, Serra-Majem L, Pérez-Jiménez F, Pascual V, Tinahones FJ and Estruch R, Moderate consumption of beer and its effects on cardiovascular and metabolic health: an updated review of recent scientific evidence. *Nutrients* **13**:879 (2021).
37 Sandoval-Ramírez BA, Lamuela-Raventós RM, Estruch R, Sasot G, Doménech M and Tresserra-Rimbau A, Beer polyphenols and menopause: effects and mechanisms – a review of current knowledge. *Oxid Med Cell Longev* **2017**:4749131 (2017).
38 Trius-Soler M, Marhuenda-Muñoz M, Laveriano-Santos EP, Martínez-Huélamo M, Sasot G, Storniolo CE *et al*., Moderate consumption of beer (with and without ethanol) and menopausal symptoms: results from a parallel clinical trial in postmenopausal women. *Nutrients* **13**:2278 (2021).
39 Arranz S, Chiva-Blanch G, Valderas-Martínez P, Medina-Remón A, Lamuela-Raventós RM and Estruch R, Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. *Nutrients* **4**:759–781 (2012).
40 Rosales C, Gillard BK, Gotto AM and Pownall HJ, The alcohol–high-density lipoprotein athero-protective axis. *Biomolecules* **10**:987 (2020).
41 Koppes LLJ, Twisk JWR, Van Mechelen W, Snel J and Kemper HCG, Cross-sectional and longitudinal relationships between alcohol consumption and lipids, blood pressure and body weight indices. *J Stud Alcohol* **66**:713–721 (2005).
42 Chiva-Blanch G, Magraner E, Condines X, Valderas-Martínez P, Roth I, Arranz S *et al*., Effects of alcohol and polyphenols from beer on atherosclerotic biomarkers in high cardiovascular risk men: a randomized feeding trial. *Nutr Metab Cardiovasc Dis* **25**:36–45 (2015).
43 Millán J, Pintó X, Muñoz A, Zúñiga M, Rubiés-Prat J, Pallardo LF, *et al*., Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag* **5**:757–765 (2009).
44 Wolters M, Dejanovic GM, Asllanaj E, Günther K, Pohlabeln H, Bramer WM *et al*., Effects of phytoestrogen supplementation on intermediate cardiovascular disease risk factors among postmenopausal women: a meta-analysis of randomized controlled trials. *Menopause* **27**:1081–1092 (2020).
45 Padro T, Muñoz-García N, Vilahur G, Chagas P, Deyà A, Antonijoan R *et al*., Moderate beer intake and cardiovascular health in overweight individuals. *Nutrients* **10**:1237 (2018).
46 Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS and Taylor PR, Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women. *JAMA* **287**:2559–2562 (2002).
47 Romeo J, González-Gross M, Wärnberg J, Díaz LE and Marcos A, Effects of moderate beer consumption on blood lipid profile in healthy Spanish adults. *Nutr Metab Cardiovasc Dis* **18**:365–372 (2008).
48 Balkau B, Deanfield JE, Després JP, Bassand JP, Fox KAA, Smith SC, et al. International day for the evaluation of abdominal obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. *Circulation* **116**:1942–1951 (2007).
49 Flegal KM, Kit BK, Orpana H and Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA* **309**:71–82 (2013).
50 Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. *New England Journal of Medicine* **359**:2105–2120 (2008).
51 Dorn JM, Hovey K, Muti P, Freudenheim JL, Russell M, Nochajski TH *et al*., Alcohol drinking patterns differentially affect central adiposity as measured by abdominal height in women and men. *J Nutr* **133**: 2655–2662 (2003).
52 Tolstrup JS, Halkjær J, Heitmann BL, Tjønneland AM, Overvad K, Sørensen TI *et al*., Alcohol drinking frequency in relation to subsequent changes in waist circumference. *Am J Clin Nutr* **87**:957–963 (2008).
53 Palla G, Ramírez-Morán C, Montt-Guevara MM, Salazar-Pousada D, Shortrede J, Simoncini T *et al*., Perimenopause, body fat, metabolism and menopausal symptoms in relation to serum markers of adiposity, inflammation and digestive metabolism. *J Endocrinol Invest* **43**: 809–820 (2020).
54 Juppi H, Sipilä S, Fachada V, Hyvärinen M, Cronin N, Aukee P *et al*., Total and regional body adiposity increases during menopause – evidence from a follow-up study. *Aging Cell* **21**:e13621 (2022).
55 Roerecke M, Tobe SW, Kaczorowski J, Bacon SL, Vafaei A, Hasan OSM *et al*., Sex-specific associations between alcohol consumption and incidence of hypertension: a systematic review and meta-analysis of cohort studies. *J Am Heart Assoc* **7**:e008202 (2018).
56 Puddey IB and Beilin LJ, Alcohol is bad for blood pressure. *Clin Exp Pharmacol Physiol* **33**:847–852 (2006).
57 Cushman WC, Alcohol consumption and hypertension. *J Clin Hypertens* **3**:166–170 (2001).

58 Guinard J-X, Zoumas-Morse C, Dietz J, Goldberg S, Holz M, Heck E *et al.*, Does consumption of beer, alcohol, and bitter substances affect bitterness perception? *Physiol Behav* **59**:625–631 (1996).
59 Česlová L, Holčapek M, Fidler M, Drštičková J and Lísa M, Characterization of prenylflavonoids and hop bitter acids in various classes of Czech beers and hop extracts using high-performance liquid chromatography–mass spectrometry. *J Chromatogr A* **1216**:7249–7257 (2009).
60 Beckett EL, Duesing K, Boyd L, Yates Z, Veysey M and Lucock M, A potential sex dimorphism in the relationship between bitter taste and alcohol consumption. *Food Funct* **8**:1116–1123 (2017).
61 Duffy VB, Davidson AC, Kidd JR, Kidd KK, Speed WC, Pakstis AJ *et al.*, Bitter receptor gene (*TAS2R38*), 6-*n*-propylthiouracil (PROP) bitterness and alcohol intake. *Alcohol Clin Exp Res* **28**:1629–1637 (2004).

