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EFFECT OF NON-ALCOHOLIC BEER ON OLDER ADULTS´ HOMOCYSTEINE LEVELS

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Abstract: *Objective:* To evaluate the effectiveness of non-alcoholic beer (NAB) and non alcoholic beer with a high content of folic acid (NAB-FA) in reducing hyperhomocysteinemia amongst older adults. *Design:* Quasi-experimental study. *Setting:* Care homes for older adults. *Participants:* Forty older adults aged 72 to 96 years (mean=83), with high serum homocysteine (Hcy) levels, >11μmol/L in women and >12μmol/L in men. *Intervention:* Those who reported liking NAB were randomly assigned to drinking 500 ml/day of NAB (n=8) or NAB-FA (43.7μg of folic acid/100 mL) (n=9). Those who reported not liking NAB were allocated to the control group (n=23), 500ml/day of water. *Measurements:* Serum levels of Hcy, folic acid and cobalamin evaluated at baseline and after the intervention. *Results:* A higher reduction in Hcy levels was observer after beer (NAB and NAB-FA) than after water ingestion (3.2μmol/L, interquartile range (IQR)=3.2μmol/L, p<0.001 compared with 0.9μmol/L, IQR=1.5μmol/L, p<0.001). Compared to the water ingestion, this reduction in Hcy levels was higher after NAB-FA intake (3.55μmol/L, IQR=2.1μmol/L, p=0.011) than after NAB (2.45μmol/L, IQR=6.3μmol/L; p=0.132). *Conclusion:* Three week intake of non-alcoholic beer significantly decreased Hcy levels in older adults with advanced age. When folic acid was integrated into NAB, a considerably lower dose of folic acid was necessary in order to reduce Hcy plasma levels than previously described for that purpose.

Key words: Homocysteine, folic acid, cobalamin, non-alcoholic beer, older adults.

Introduction

Total homocysteine (Hcy) is an independent and significant risk factor for vascular diseases, as it is related to genetic, nutritional and pathological factors (1, 2). In fact, nutritional deficiencies of folic acid, pyridoxine and cobalamin are connected to high Hcy levels, since they act as coenzymes or cofactors in enzymatic metabolism of Hcy (3-5). Normal levels of plasma Hcy are considered to be between 5 and 12μmol/L (5) but there is an increase in these levels with age, which may be related to decreased concentration of vitamins required for the synthesis of Hcy (1, 6). Gender, smoking and lack of exercise also influence Hcy levels, as well as high consumption of alcohol and coffee (1, 6-8).

There is some evidence that consumption of beer, unlike wine and spirits, is associated with lower levels of Hcy (6, 9-11). This possible protective effect has been attributed to folic acid content in beer and the absence of this vitamin in other alcoholic beverages (6, 9). Other beer components can also contribute to the low levels of Hcy associated with beer consumption. Besides having high water content, beer is a source of minerals, soluble fibre and B vitamins such as niacin, pyridoxine, riboflavin and folic acid (12). The bioavailability of folic acid in beer is high, since it is not subjected to any heat treatment or oxidation (6). Its richness in phenolic compounds, in particular, xanthohumol, isoxanthohumol and naringinine, which are known to have a high antioxidant potential, should also be emphasized (13). Non-alcoholic beer (NAB) may provide further benefits in Hcy metabolism, as high consumption of alcohol has been related to elevated levels of Hcy (1, 6-8).

Due to the absence of experimental data in animals and the scarcity in humans regarding the possible effectiveness of beer in reducing Hcy levels, this study aims to determine whether supplementation with NAB and non-alcoholic beer with high content of folic acid (NAB-FA) decreases Hcy levels in older adults.

Subjects and methods

A quasi-experimental study was conducted in older adults residing in care homes. A convenience sample of

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older adults with high serum Hcy levels (>11μmol/L in women and >12μmol/L in men) (14) were previously identified in a descriptive study (15). The existence of cognitive ability which was assessed by the Abbreviated Mental Test Score (16) and the informed consent were also inclusion criteria.

The sample size was calculated assuming a significance level of 0.05, a statistical power of 80%, a minimal detectable difference in the outcome variable (Hcy difference) of 3 μmol/L and a standard deviation (SD) of the outcome variable (Hcy difference) of 1.5 μmol/L. The two latter assumptions were made on basis of previous reports of differences in levels of Hcy between beer drinkers and non-drinkers. A total sample of 12 patients in each treatment group was defined. Because of the expected dropouts, a larger sample size was recruited.

Fifty three older adults who met the inclusion criteria and showed interest in participating in the study were invited to take part. Thirteen older adults did not complete the study for various reasons: death (n=1), hospitalization (n=2), refusal to draw blood at the end of the study (n=6), moving to another care home (n=3) and very high variation of the Hcy levels between the beginning and end of the study (39.7μmol/L, n=1). These thirteen older adults who did not complete the study were compared with participants and there were no differences between them regarding the baseline levels of Hcy, folic acid and cobalamin.

The older adults who reported liking NAB and wished to consume the study doses of NAB during the study were randomly assigned to the beer intervention groups, NAB (n=8) and NAB-FA (n=9). Those who reported not enjoying NAB were allocated to the control group (n=23). During a period of three weeks and on a daily basis, one group consumed 500 mL of NAB, a second group had 500 mL of NAB-FA supplemented with 40μg folic acid/100mL and the control group consumed 500 mL of water. The choice of the 3 week period was based on a previous experimental study (21). The dose of 40μg folic acid/100mL of NAB was selected in order to provide half of the daily Recommended Dietary Allowance for an adult (400μg folic acid) (17). The total amount of folic acid present in 500mL of NAB was 18.3μg in and 218.3μg in NAB-FA. The NAB and NAB-FA were provided by UNICER (Unicer – Bebidas, SA, Matosinhos, Portugal). This company has been responsible for its production and assays of folic acid as well as for tests of stability.

Beer and water were distributed and consumed during lunch and dinner. Subjects but not investigators were blinded to distribution of NAB or NAB-FA. Adherence and tolerance to supplementation was recorded by the researchers. The majority of the participants consumed the entire study doses while a minority of them left <50mL.

Assessment of plasma levels of Hcy, folic acid and cobalamin was conducted at baseline and at the end of the study. The blood collection was conducted at the beginning of the morning under the same conditions for all the participants. A sample of 4 mL of venous blood was drawn from the cubital vein. The samples were centrifuged and the packaging and transportation were held in a refrigerated environment. Measurement of levels of folic acid, cobalamin and Hcy was performed using chemiluminescence (Immulite ® 2000, Siemens).

Information was collected by two previously trained interviewers, using a structured questionnaire regarding characteristics of the groups on demographic and social data, general health, physical activity, smoking and drinking habits and anthropometric parameters. Nutritional status was evaluated by the Mini Nutritional Assessment, (18) the degree of functional dependence was determined by the Barthel Index (19) and the presence of depressive symptoms and behaviours was evaluated by the Centre for Epidemiologic Studies Depression Scale (20).

This protocol was approved by the Ethics Committee for Health of the Hospital of São João, Porto, Portugal and by the care home administration boards. The participants gave their informed consent, according to the principles laid down in the Declaration of Helsinki. Older adults who showed low levels of folic acid and/or cobalamin were referred to their doctor in charge. All participants were advised to practice healthy lifestyles both at the beginning and at the end of the study as some had high levels of Hcy.

The intention to treat data analysis technique was used, i.e., data from participants who missed study doses was considered as they had completed the full treatment. Differences in baseline characteristics of the groups were calculated by comparing proportions (chi-square or Fisher's exact test) and for continuous variables using ANOVA test. The Kolmogorov Smirnov test was used to assess the normality of distribution. The intervention and control groups were compared before and after supplementation by Wilcoxon test. The differences before and after supplementation of levels of folic acid, cobalamin and Hcy, between each of the intervention groups and control group, were evaluated with Mann-Whitney test. Difference between baseline and intervention was adjusted for baseline values with ANCOVA. A p value <0.05 was considered significant. All analyses were carried out using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

A total of 40 older adults aged from 72 to 96 years with a mean (SD) of 83 (5) years participated in the study. No differences were found between baseline characteristics of control and intervention groups for gender, physical activity, drinking habits, the degree of functional dependence, the presence of signs and/or symptoms of

depressive behaviour, nutritional status and serum levels of Hcy, folic acid and cobalamin (Table 1). NAB and NAB- FA groups were older than the control group, both presenting a mean age of 85 years, with respectively a SD=6 years and SD=3 years, while the control group had a mean age of 81 years (SD=5 years) (p=0.041). When NAB and NAB-FA groups were compared together with the control group, a significant reduction in Hcy levels of 3.2μmol/L (interquartile range (IQR)=3.2μmol/L, p<0.001) was seen (Table 2).

Table 1
Baseline characteristics of the study subjects

	Control Group (n = 23)	NAB Group (n = 8)	NAB-FA Group (n = 9)	p [b]
Sex [a]				n.s.
Female	17 (73.9)	5 (62.5)	6 (66.7)	
Male	6 (26.1)	3 (37.5)	3 (33.3)	
Physical activity [a]				n.s.
Yes	4 (17.4)	0 (0)	2 (22.2)	
No	19 (82.6)	8 (100)	7 (77.8)	
Alcohol consumption [a]				n.s.
Drinker	8 (34.8)	4 (50.0)	3 (33.3)	
No-drinker	10 (43.5)	2 (25.0)	2 (22.2)	
Ex-drinker	5 (21.7)	2 (25.0)	4 (44.5)	
Degree of functional dependence [a,c]				n.s.
Light	11 (47.8)	6 (25.0)	4 (44.4)	
Independent	12 (52.2)	2(75.0)	5 (55.6)	
CES-D [a,c]				n.s.
Without depression	11 (47.8)	5 (62.5)	2 (22.2)	
Mild to moderate depression	12 (52.2)	3 (37.5)	7 (77.8)	
Mini Nutritional Assessment [a,c]				n.s.
Normal nutritional status	17 (73.9)	7 (87.5)	6 (66.7)	
At risk of undernutrition	6 (26.1)	1 (12.5)	1 (33.3)	
BMI (Mean (standard-deviation)) [c,d]				.
Men	25.7 (2.3)	29.6 (8.0)	24.3(3.3.)	n.s
Women	29.7 (4.9)	27.4 (6.2)	27.4 (2.9)	n.s

a. n (%); Qui square or Fisher exact test; b. p >0.05; n.s, non-significant; c. No participants within the classes of serious or moderate functional dependence, having possibility of major depression or undernourished; d. Anova test; NAB, Non-alcoholic beer; NAB-FA, Non-alcoholic beer with a high content of folic acid; CES–D, Centre for Epidemiologic Studies Depression Scale; BMI, body mass index.

When compared separately with control group, the NAB-FA group showed a reduction in Hcy levels about four times higher (3.6μmol/L, IRQ=2.1μmol/L) compared to the control group (0.9μmol/L, IRQ=1.5μmol/L) (p=0.011). NAB supplemented older adults showed a slight but non-significant decrease in Hcy levels when compared to the control group. The intervention resulted in a decrease of Hcy levels for the total sample from a mean value of 15.4μmol/L (SD=5.09μmol/L) to 13.6μmol/L (SD=3.82μmol/L), p<0.001. This decrease occurred in 77.5% (n=31) of the sample (mean=-2.76μmol/L, SD=2.52μmol/L). Participants who showed an increase in these levels (n=9) belonged to the control group (n=7) and to the NAB group (n=2). The intervention had no effect on folic acid and cobalamin levels (Table 2).

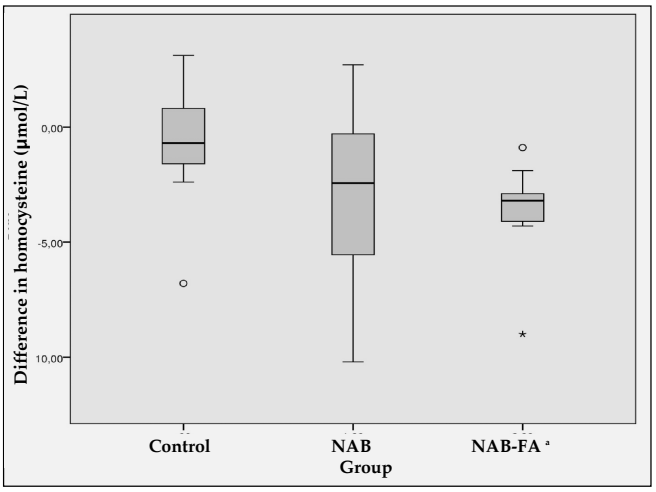
Table 2
Levels of homocysteine, folic acid and cobalamin at baseline and after the intervention and their differences

	Control group n = 23	NAB group n = 8	NAB-FA group n = 9
Homocysteine (μmol/l) [a]			
Baseline	13.2 (4.3)	15.6 (6.0)	14.5 (8.0)
After intervention	13.3 (4.1)	14.2 (7.2)	11.3 (6.1)
p [b]	0.273	0.069	0.008
Folic acid (ng/ml) [a]			
Baseline	6.0 (3,3)	5.8 (4.4)	3.9 (2.1)
After intervention	6.1 (5.9)	6.5 (6.5)	5.1 (5.1)
p [b]	0.360	0.139	0.086
Cobalamin (pg/ml) [a]			
Baseline	454.0 (240.5)	382.5 (310.3)	323.5 (97.3)
After intervention	479.0 (238.0)	441.0 (462.8)	310.0 (111.3)
p [b]	0.193	0.225	0.575
Dif. homocysteine (μmol/L) [a]	-0.90 (1.50)	-2.45 (6.30)	-3.55 (2.10) [c,d]
Dif. folic acid (ng/ml) [a]	0.00 (1.35)	0.85 (2.73)	1.05 (1.62)
Dif. cobalamin (pg/ml) [a]	39.0 (154.5)	63.0 (143.8)	-40.5 (117.5)

a. Results expressed as median (IRQ); b. Wilcoxon test test; p<0.05, level of statistical significance; c. Mann-Whitney for NAB-FA vs control group, p=0.008; d. Ancova test for NAB-FA vs control group, p=0.011; NAB, non-alcoholic beer; NAB-FA – non-alcoholic beer with a high content of folic acid; Dif., Difference between after the intervention and baseline.

During the three weeks of intervention, older adults supplemented with NAB and NAB-FA did not show any signs of displeasure nevertheless three participants mentioned having slight gastrointestinal discomfort. Of those, two were from NAB and one was from NAB-FA group.

Figure 1
Variation in levels of homocysteine in control and intervention groups



Discussion

A higher reduction in Hcy levels was observed after beer consumption than after water intake. This reduction was more pronounced in the NAB-FA group. These results are comparable to previous studies that showed a reduction of Hcy levels with beer intake (6, 9, 12, 21). In

an experimental study, involving eleven individuals, Van der Gang et al., 2000, evaluated the effect of consumption of red wine, spirits and beer in plasma levels of Hcy. The results showed an increase in Hcy levels of between 8% and 9% after moderate consumption of red wine and spirits, respectively, as compared to water consumption, whereas no increase was recorded after beer consumption (21).

The absence of ethanol in beer in the present study would suggest a relevant reduction of Hcy levels in the group supplemented with NAB, but this decrease did not reach statistical significance. It is unclear whether the effect would have increased with stronger statistical power, but present results give support to include a larger sample size. The limited number of participants was certainly a limitation of our study, as it may not allow us to identify statistically significant differences and therefore increasing the possibility of type II errors. The quasi experimental design, with lack of randomization of control and experimental groups, could be regarded as a weakness in this study. Otherwise, we can suppose that there is no link between the reason for assigning older adults to study groups (liking beer) and the outcome of this study (Hcy levels).

Although in the present study there were no significant differences in the levels of folic acid before and after supplementation in the different groups, we found a significant reduction in Hcy levels of the NAB-FA group. In addition, the intervention resulted in decreased levels of Hcy in the group who consumed NAB-FA compared to those who drank NAB. This difference may be explained by the different intake of folic acid content of beers, 18.3μg/500mL from NAB and 218.3μg/500mL from NAB-FA. This is considered an important finding from this study, showing that a considerably lower dose of folic acid than previously described as necessary for reducing levels of plasma Hcy, was effective when integrated into a NAB.

According to current knowledge, a daily intake of at least 350μg of folic acid is needed to maintain normal or safe plasma concentration of Hcy and daily supplementation with at least 650μg to reduce high concentrations of this amino acid (6). These amounts of folic acid are very difficult to achieve by diet alone and may require the use of supplements or fortified foods. The prevalence of hyperhomocysteinemia in the general population varies between 5-10%, however an increase has been observed in 30-40% of our sample. If population studies are correct, hyperhomocysteinemia seems to be responsible for more than 10% of CVD and therefore represents an important modifiable risk factor for such diseases (22). Wald et al., 2002, showed that an increase Hcy levels of 5μmol/L, increases the risk of ischemic heart disease in 20 to 30% (23).

The determination of serum Hcy is usually restricted to investigations and is not routinely performed, due in part to its high cost. Although evidence on the diagnosis and treatment of hyperhomocysteinemia is insufficient, the recommendation of the food sources of folic acid, vitamin B12 and vitamin B6 should be encouraged (22). In the present study this has been taken into consideration after the first biochemical analysis, which may help explain the slight decrease in the levels of Hcy in the control group and part of the observed reduction in the intervention groups.

The reduction of 3.55μmol/L Hcy levels in the NAB-FA group is relevant since it was estimated that a 25% reduction in the Hcy levels (about 3μmol/L) results in 11% decrease in risk of ischemic heart disease and 19% risk of stroke (24).

Research carried out within the study PREDIMED,(25) showed that moderate beer consumption may have beneficial effects on CVD. Beer consumers had a higher intake of folic acid, vitamins B, D and E, calcium and iron and also a lower body mass index than those who not drink beer. Moreover, participants who consumed beer moderately had a lower incidence of diabetes mellitus and hypertension, known risk factors for CVD, as well as higher levels of HDL cholesterol (25). Another study performed among cloistered nuns, showed that consumption of NAB was reflected in positive results in blood markers of oxidative and inflammatory processes, with an 8% reduction in LDL oxidation (26). These authors suggested that NAB may have a protective effect on lipid oxidation, which turns out to be of great interest since high levels of this lipoprotein are a risk factor for CVD (26). The NAB, with properties similar to traditional beer, may be an alternative for ill and/or medicated older adults. Its pleasant taste and freshness allied to its high water content may contribute, in addition to the advantages already described, to a better state of hydration.

The present study showed a significant reduction of Hcy levels with intake of NAB-FA in older adults. This reduction was achieved with a considerably lower dose of folic acid than previously described as being necessary for this purpose. It would be important to develop further experimental studies with larger sample sizes that may corroborate the results, given the impact of high levels of Hcy in cardiovascular health.

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