Betaine and Choline Intakes are Related to Total Plasma Homocysteine: Health Survey of São Paulo, Brazil

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Abstract

Background: High concentrations of plasma homocysteine (Hcy) have been associated with increased risk of cardiovascular diseases. Hcy can be decreased by remethylation to methionine, which uses folate or betaine as a donor of the methyl group.

Objectives: To evaluate the intake of betaine and choline and its relation to homocysteine in residents of the city of São Paulo.

Methods: Data from 584 individual, of both sexes, from the population-based study ISA-SP 2008. Geometric averages of Hcy were analyzed according to choline and betaine intake tertiles and trend test was applied.

Results: The study analyzed 584 individuals: 222 (38.0%) men and 362 (62.0%) women, mean age 55.0 ± 19.0 years. The prevalence of hyperhomocysteinemia was higher among men (28.0%), the elderly (21.0%) and those with lower household income (21.0%). Approximately 31.0% of individual with hyperhomocysteinemia presented folate deficiency (<7.5 nmol/L) and 26.0% presented vitamin B12 deficiency (<200 pmol/L). There was a decrease in the geometric means of homocysteine according to an increase in betaine tertiles in both sexes, adults, normal and in all categories of education. Choline was related to Hcy in both sexes, higher household income individuals, non-smokers and alcohol consumers.

Conclusions: This study suggests the importance of betaine intake due to its inverse relationship with the concentration of Hcy in adults and elderly in the city of São Paulo. Choline played a protective role in specific subgroups of the population.

Keywords: Betaine; Choline; Homocysteine; Diet; Cardiovascular diseases

Introduction

High plasma homocysteine concentrations have been associated with increased risk for developing cardiovascular diseases, especially atherosclerosis and coronary ischemia events¹⁻⁴.

A biologically plausible mechanism for the atherothrombotic effect of homocysteine refers to its autoxidation with subsequent generation of hydrogen peroxide, damaging the endothelial cells and causing the proliferation of smooth muscle cells of vessels⁵⁻⁶. High concentrations of homocysteine can still activate inflammatory responses involved in the etiology of atherosclerosis⁶.

Reduction in homocysteine concentrations may occur through remethylation to methionine, using folate or betaine as donors of the methyl group. Betaine obtained directly from the diet or from the oxidation of its precursor, choline, can remethylate homocysteine via the enzyme betaine-homocysteine methyltransferase (BHMT). The activity of BHMT becomes crucial when folate availability is reduced due to low intake or low use of the folate pool by organism⁷. Besides the folate, deficiencies of vitamins B6 and B12 may also contribute...
to the elevation of plasma homocysteine⁸, since they are interrelated compounds in the homocysteine metabolic cycle. Regardless of folate and vitamins B6 and B12, higher dietary intakes of choline and betaine were associated with lower concentrations of homocysteine⁹.

The main dietary sources of choline are animal products such as red meat, eggs and poultry; while betaine is obtained mainly from grains¹⁰.

Considering the importance of betaine and choline in homocysteine metabolism and the lack of studies dedicated to investigating its use in humans, this study aims to assess the intake of these nutrients and to investigate its relationship with plasma homocysteine in adults and elderly in São Paulo.

Methods

The data used in this study came from the cross-sectional population-based “ISA - Capital 2008”, with a probabilistic sample of residents from the urban area of the city of São Paulo, Brazil¹¹. Adults (20-59 years) and elderly (≥60 years) of both sexes with full food consumption data were selected for this study (n=584).

The Research Ethics Committee from Faculdade de Saúde Pública da Universidade de São Paulo approved the study under no. 2001 and all participants signed an Informed Consent Form.

Data collection for the study ISA-Capital was conducted from 2008 to 2011. Demographic, socioeconomic and lifestyle variables (dietary intake, physical activity, smoking and intake of alcohol) were obtained at home through questionnaires given by trained interviewers.

In the next stage, blood samples were collected, anthropometric measurements were taken (weight, height and waist circumference) and the blood pressure of the same individuals assessed in the first stage of the study was checked. For this, there was another home visit by a trained nurse, following standardized procedures developed specifically for the study. The second dietary measure was collected one week before the home visit, by telephone interview and in order to investigate the association of dietary variables with anthropometric and biochemical variables.

Diet analysis

We obtained the first measure of food consumption by a 24-hour food recall applied by the Multiple Pass Method developed by the US Department of Agriculture, in which data collection is structured around five steps¹². This method helps the individuals record any food and drink consumed the day before the interview and report them in detail, reducing errors in dietetic measures¹³.

In the second measure of dietary intake, the 24-hour food recall was applied by telephone by Nutrition students, according to the procedures of the Automated Multiple Pass Method (AMPM), also divided into five stages, including direct typing in the program Nutrition Data System for Research. Students were trained to use the program and used an explanatory manual to assist them in collecting data.

The study employed the statistical modeling technique Multiple Source Method (MSM), an online platform to estimate the usual intake of nutrients based on consumption data from two 24-hour recalls. MSM provides estimates of usual intake through the combination of likelihood and amount of consumption¹⁴.

Biomarkers

Blood collection was performed at home by a trained nursing professional after the participant’s 12-hour fasting period. About 10 ml of blood was collected by venipuncture in two dry tubes centrifuged at 1448 rpm for 15 min at room temperature. After centrifugation, plasma samples were stored at -80 °C.

For homocysteine measurement, the high-performance chromatography method (HPLC) was used. Serum vitamin B6 analysis was performed by HPLC¹⁵ while serum folate and vitamin B6 levels were measured by the electrochemiluminescence method¹⁶-¹⁸.

For the classification of individuals with hyperhomocysteinemia, the following cutoff points were adopted: Hcyp ≥12 mmol/l, for individuals aged 15-65 years, and Hcyp ≥16 mmol/l, for those aged >65 years.

Statistical analysis

For each individual, the average intake of choline and betaine was calculated and measured by both 24-hour recalls. The intake of these nutrients has been described as median due to the lack of normal distribution of choline and betaine revealed by the Skewness-Kurtosis test.
The geometric averages of plasma homocysteine were analyzed according to tertiles of choline and betaine intake and nonparametric trend test was conducted. Stata version 12.0 was employed, considering a level of significance of 5%.

The relative contribution (RC) of food to betaine and choline was calculated according to Block\textsuperscript{20}, in which:

\[ RC = \frac{\text{Total Choline or Betaine from the diet } i}{\text{Total Choline or Betaine from all foods}} \times 100 \]

This study describes only the foods that contributed 1% or more betaine and choline in the diet of individuals in the study.

**Results**

The study analyzed 584 individuals: 222 (38.0%) men and 362 (62.0%) women, of which 285 (49.0%) are elderly. The mean age of the study population was 55.0±19.0 years; the average age of the non-elderly group was 39.0±12.0 years, while the average age of the elderly was 71.0±7.0 years.

Table 1 shows the prevalence of hyperhomocysteinemia according to demographic, anthropometric and lifestyle variables. The prevalence of hyperhomocysteinemia was statistically higher in males (28.0%) compared to women (10.0%) (p<0.001). In addition, hyperhomocysteinemia was more prevalent in the elderly (21.0%) and in individuals with household income equal or below the minimum wage (21.0%).

People with hyperhomocysteinemia showed significant differences in the consumption of energy and nutrients compared to individuals without hyperhomocysteinemia, except for carbohydrates, dietary folate equivalent and synthetic folate (Table 2). For biochemical variables, median serum folate, vitamin B6 and vitamin B12 were shown statistically lower in individuals with hyperhomocysteinemia. Furthermore, about 31.0% of individuals hyperhomocysteinemia presented folate deficiency (<7.5 nmol/L) and 26.0% had a vitamin B12 deficiency (<200 pmol/L).

### Table 1

| Variables          | Plasma homocysteine |   |   |   |
|--------------------|---------------------|---|---|---|
|                    | Normal\textsuperscript{a} | High\textsuperscript{a} |   |   |
|                    | n\textsuperscript{c} | % | n\textsuperscript{c} | % |
| Sex                | Male                | 159 | 71.6 | 63 | 28.4 | <0.001 |
|                    | Female              | 325 | 89.8 | 37 | 10.2 |
| Age group          | Non-elderly         | 259 | 86.6 | 40 | 13.4 |
|                    | Elderly             | 225 | 78.9 | 60 | 21.1 |
| Nutritional status | Low weight          | 44 | 84.6 | 8 | 15.4 |
|                    | Eutrophy            | 191 | 83.0 | 39 | 17.0 |
|                    | Overweight          | 106 | 86.9 | 16 | 13.1 |
|                    | Obesity             | 126 | 81.3 | 29 | 18.7 |
| Education          | ≤8 years            | 297 | 82.0 | 65 | 18.0 |
|                    | >9 years            | 184 | 84.0 | 35 | 16.0 |
| Income             | ≤1 minimum wage     | 196 | 79.0 | 52 | 21.0 |
|                    | >1 minimum wage     | 288 | 85.7 | 48 | 14.3 |
| Smoking            | Former smoker       | 116 | 78.9 | 31 | 21.1 |
|                    | Smoker              | 86  | 81.1 | 20 | 18.9 |
|                    | Non smoker          | 282 | 85.2 | 49 | 14.8 |
| Alcoholism         | Yes                 | 212 | 81.5 | 48 | 18.5 |
|                    | No                  | 272 | 84.0 | 52 | 16.0 |

\textsuperscript{a}Average Hcyp: 9.4 µmol/l (n=484)

\textsuperscript{b}Average Hcyp: 20.4 µmol/l (n=100)

\textsuperscript{c}Individuals with incomplete data have been excluded
Table 3 shows the geometric averages of plasma homocysteine according to tertiles of betaine intake for demographic, socioeconomic and lifestyle variables. There was a decrease in the geometric averages of plasma homocysteine according to an increase in consumption tertiles of betaine for all variable categories considered, except for the elderly, non-normal individuals, non-smoking individuals, individuals with household income above one minimum wage and non-alcoholics. As for choline, the relationship with plasma homocysteine levels was observed in both sexes, individuals with household income above one minimum wage, non-smokers and alcohol consumers (Table 4).

Table 5 shows the foods that mostly contribute to the intake of betaine and choline, and average consumption in grams of food and nutrients. About 69.0% of the total dietary betaine was provided by the intake of 10 foods, including white bread (21.0%), cereals and pasta (15.0%) and biscuits (13.0%). As for choline, 60.0% of this nutrient was provided by 10 foods. Of these, beef (20.0%), poultry (11.0%) and eggs (6.0%) had the largest contribution percentages.
Table 3
Geometric averages of plasma homocysteine of the population studied according to tertiles of betaine intake

| Variables                  | betaine (mg) | n   | T1   | T2   | T3   | p-value* |
|----------------------------|--------------|-----|------|------|------|----------|
| Sex                        |              |     |      |      |      |          |
| Male                       |              | 222 | 12.1 | 10.6 | 9.3  | 0.001    |
| Female                     |              | 362 | 8.3  | 8.1  | 7.2  | 0.019    |
| Age group                  |              |     |      |      |      |          |
| Non-elderly                |              | 299 | 9.0  | 8.8  | 7.8  | 0.014    |
| Elderly                    |              | 285 | 12.4 | 11.1 | 10.5 | 0.121    |
| Nutritional status         |              |     |      |      |      |          |
| Low weight                 |              | 52  | 9.4  | 9.5  | 9.1  | 0.328    |
| Eutrophy                   |              | 230 | 10.0 | 9.2  | 8.1  | 0.002    |
| Overweight                 |              | 122 | 8.9  | 8.7  | 7.8  | 0.264    |
| Obesity                    |              | 155 | 9.5  | 9.5  | 8.2  | 0.131    |
| Education                  |              |     |      |      |      |          |
| ≤8 years                   |              | 362 | 10.1 | 8.9  | 8.2  | 0.028    |
| ≥9 years                   |              | 219 | 9.2  | 9.4  | 8.2  | 0.016    |
| Income                     |              |     |      |      |      |          |
| ≤1 minimum wage            |              | 248 | 9.7  | 9.1  | 7.7  | 0.008    |
| >1 minimum wage            |              | 336 | 9.6  | 9.3  | 8.4  | 0.057    |
| Smoking                    |              |     |      |      |      |          |
| Former smoker              |              | 147 | 11.1 | 9.3  | 8.1  | 0.024    |
| Smoker                     |              | 106 | 10.4 | 8.4  | 8.9  | 0.043    |
| Non smoker                 |              | 331 | 8.8  | 9.4  | 7.9  | 0.056    |
| Alcoholism                 |              |     |      |      |      |          |
| Yes                        |              | 260 | 10.8 | 9.2  | 8.6  | 0.001    |
| No                         |              | 324 | 8.8  | 9.3  | 7.7  | 0.132    |

*p-non-parametric trend test

Table 4
Geometric averages of plasma homocysteine of the population studied according to tertiles of choline intake

| Variables                  | choline (mg) | n   | T1   | T2   | T3   | p-value* |
|----------------------------|--------------|-----|------|------|------|----------|
| Sex                        |              |     |      |      |      |          |
| Male                       |              | 222 | 12.7 | 10.5 | 9.6  | 0.005    |
| Female                     |              | 362 | 8.7  | 7.7  | 6.9  | <0.001   |
| Age group                  |              |     |      |      |      |          |
| Non-elderly                |              | 299 | 8.9  | 8.5  | 8.1  | 0.164    |
| Elderly                    |              | 285 | 12.2 | 11.2 | 10.3 | 0.708    |
| Nutritional status         |              |     |      |      |      |          |
| Low weight                 |              | 52  | 9.4  | 9.1  | 9.5  | 0.908    |
| Eutrophy                   |              | 230 | 9.5  | 9.1  | 8.1  | 0.072    |
| Overweight                 |              | 122 | 9.0  | 7.9  | 8.5  | 0.212    |
| Obesity                    |              | 155 | 10.3 | 8.3  | 8.5  | 0.309    |
| Education                  |              |     |      |      |      |          |
| ≤8 years                   |              | 362 | 9.7  | 9.2  | 8.5  | 0.085    |
| ≥9 years                   |              | 219 | 9.8  | 8.8  | 8.3  | 0.061    |
| Income                     |              |     |      |      |      |          |
| ≤1 minimum wage            |              | 248 | 9.2  | 9.3  | 8.1  | 0.186    |
| >1 minimum wage            |              | 336 | 10.2 | 8.7  | 8.5  | 0.024    |
| Smoking                    |              |     |      |      |      |          |
| Former smoker              |              | 147 | 10.1 | 9.8  | 8.5  | 0.327    |
| Smoker                     |              | 106 | 9.9  | 8.6  | 9.4  | 0.935    |
| Non smoker                 |              | 331 | 9.5  | 8.8  | 7.9  | 0.004    |
| Alcoholism                 |              |     |      |      |      |          |
| Yes                        |              | 260 | 10.5 | 9.1  | 8.8  | 0.048    |
| No                         |              | 324 | 9.2  | 8.6  | 7.8  | 0.060    |

*p-non-parametric trend test
Discussion

This study is the first to investigate the relationship between dietary betaine and choline and plasma homocysteine in a population-based sample in individuals from São Paulo.

The higher prevalence of hyperhomocysteinemia in men and in the elderly confirms the results also obtained by Jacques et al.\textsuperscript{21} in the Framingham cohort study. According to Neves et al.\textsuperscript{22}, sex and age are the main physiological factors related to hyperhomocysteinemia. Men have, on average, plasma homocysteine levels 21.0% higher than women. In addition, older individuals tend to have higher circulating levels of homocysteine both resulting from aging\textsuperscript{19} and deficiency of vitamins, especially vitamin B12\textsuperscript{23}.

Most dietary variables presented different intake means according to plasma homocysteine concentrations. Lower median intakes of choline, betaine, natural folate and vitamins B6 and B12 were observed in individuals with hyperhomocysteinemia. Note that these nutrients are interrelated in the metabolic cycle of homocysteine\textsuperscript{4,8,9}.

Table 5

| Food group     | %     | Average consumption (g) | Nutrient (mg) |
|----------------|-------|-------------------------|---------------|
| White breads   | 20.59 | 71                      | 51.02         |
| Cereals and pasta | 15.49 | 202                     | 116.07        |
| Biscuit        | 12.69 | 39                      | 69.97         |
| Whole meal breads | 5.80  | 59                      | 67.69         |
| Mate tea       | 4.80  | 297                     | 220.52        |
| Beer           | 3.11  | 849                     | 68.75         |
| Beef           | 2.34  | 120                     | 14.60         |
| Beetroot       | 1.93  | 51                      | 77.07         |
| Beetroot       | 1.38  | 101                     | 7.04          |
| Pizza          | 1.30  | 176                     | 55.78         |
| Beef           | 19.73 | 120                     | 104.75        |
| Poultry        | 10.52 | 101                     | 81.61         |
| Eggs           | 6.28  | 64                      | 140.21        |
| Milk           | 5.45  | 202                     | 31.24         |
| Ox liver       | 4.08  | 158                     | 695.37        |
| Beans          | 3.24  | 74                      | 27.44         |
| Fish           | 2.39  | 221                     | 142.12        |
| Cakes\textsuperscript{a} | 2.33  | 55                      | 88.26         |
| Sausage        | 2.20  | 84                      | 50.42         |
| Beer           | 1.84  | 849                     | 84.03         |
| French bread roll | 1.71  | 72                      | 10.66         |

\textsuperscript{a}no topping or filling
Individuals with hyper-homocysteinemia also presented lower serum folate and vitamin B6 and B12 concentrations according to the results obtained by Refsum et al. The low availability of folate in the body, indicated by low serum or erythrocyte concentrations suggests that the remethylation pathway of homocysteine to methionine may be dependent on betaine as a donor of the methyl group, which reinforces the importance of adequate intake of betaine and choline for the reduction of homocysteine in individuals from São Paulo.

In this study, individuals in the lowest stratum of per capita household income had a prevalence of hyperhomocysteinemia greater than individuals in the highest income strata. According to the literature, the socioeconomic factor is important and consistent predictor of morbidity and mortality. However, there are no studies in the literature that investigate the influence of socioeconomic variables in plasma homocysteine concentration in individuals, making it difficult to compare and interpret the results.

Plasma concentrations of homocysteine showed a downward trend from the first to the last tertile of intake of betaine for many of the categories studied. Although it is not possible to establish causality due to the cross-sectional design, this study suggests that consumption of betaine exercises a protective role against the increase of plasma homocysteine in individuals from the city of São Paulo. The foods that most contributed to the inclusion of betaine in the diet include breads, cereals and pasta, foods that are also sources of other nutrients involved in homocysteine metabolism, such as the B complex vitamins.

Higher choline consumption was associated with lower plasma homocysteine levels to a smaller number of variables, such as sex (male and female), per capita income (higher than one minimum wage) and alcoholism (alcohol consumers). Thus, the results point to the protective role of choline on plasma homocysteine in specific subgroups of the population. It should be noted that the foods that most contribute for choline intake were animal foods (beef, poultry and eggs), which are also sources of protein, which can increase the endogenous production of homocysteine. Thus, the protective effect of choline can be counterbalanced by the proteins of animal origin in the diet, resulting in no association with plasma homocysteine, as observed in most of the individuals studied.

In relation to smoking, there was no difference in the prevalence of hyperhomocysteinemia. It is known that smoking promotes increased homocysteine concentrations and smokers tend to have lower circulating levels of vitamins B6, B12 and folate, nutrients that are related to the metabolism of homocysteine. The highest intake of betaine was associated with lower homocysteine levels for smokers; for choline, the highest consumption levels were associated with decreased homocysteine only for nonsmokers. Although the reason for these results is not clear, it is possible that differences in food intake between the groups could explain the effects of choline and betaine to homocysteine reduction.

With regard to alcohol consumption, there was no difference in the prevalence of hyperhomocysteinemia among consumers and non-consumers. However, the effects of alcohol on homocysteine plasma concentrations seem to vary according to the degree of consumption. Higher total plasma homocysteine concentrations are directly related to chronic and excessive consumption of alcohol, possibly by interference of alcohol in the cycle of methionine and the antagonistic action in the metabolism of folate. However, moderate consumption of alcohol (≤2 drinks/day) seems to have an effect on lowering total homocysteine. Thus, the findings in the literature suggest an ambiguous effect of alcohol in relation to homocysteine, exercising a protective effect when ingested moderately; and a harmful effect when consumed excessively.

In the present study, plasma homocysteine concentrations among alcohol users revealed a downward trend according to tertiles of choline and betaine intake. In a previous study, Cho et al. observed that even when folate intake is low, as long as choline and betaine intakes are adequate, metabolism of the methyl group can function properly. It is possible that the interference of excessive alcohol consumption in the folate metabolism reinforces the importance of betaine and choline in the remethylation of homocysteine.

Despite the protective role in the reduction of homocysteine levels and thus in the reduction of cardiovascular risk, recent studies show that n-trimethylamine oxide (TMAO), a metabolite formed from the intestinal microbiota of nutrients containing trimethylamine, such as choline, is associated with the pathogenesis of atherosclerosis and the severity of cardiovascular diseases.

The limitations of this study are worth mentioning. Since the data were obtained from a cross-sectional study, one should be cautious in assuming conclusions about the causal effect of exposure of interest on the biochemical factors studied. In addition, there is no data on genetic variations that are known to affect plasma homocysteine concentrations.
Conclusions

This study suggests the importance of food sources of betaine for their protective role in relation to high plasma levels of homocysteine in individuals from the city of São Paulo. As for choline, the results indicate a protective effect in specific subgroups of the population, considering that animal proteins can counteract its effect, resulting in the lack of association with plasma homocysteine.

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Potential Conflicts of Interest

No relevant conflicts of interest.

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Academic Association

This study is not associated to any graduate programs.
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