Dietary patterns are associated with hyperhomocysteinemia in an urban Pakistani population.

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Dietary Patterns Are Associated with Hyperhomocysteinemia in an Urban Pakistani Population

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Abstract

Little attention has been given to the association of dietary patterns with plasma homocysteine. Our objective in this study was to identify major dietary patterns and investigate their association with plasma homocysteine. In a cross-sectional survey, 872 healthy adults (355 males, 517 females; aged 18–60 y) were enrolled from an urban population in Karachi. Dietary intake was assessed by a FFQ. We used factor analysis to define major dietary patterns. Fasting concentrations of plasma or serum homocysteine, folate, pyridoxal-phosphate (PLP; coenzyme form of vitamin B-6), and vitamin B-12 were measured. Three major dietary patterns were identified and labeled as “prudent diet,” “high animal-protein diet,” and “high plant-protein diet.” We observed a protective effect of the prudent dietary pattern for the highest quartile of intake compared with the lowest quartile of hyperhomocysteinemia when the model was adjusted for age, gender, household income, BMI, tobacco chewing, and smoking [OR = 0.52 (95% CI = 0.30–0.90); P = 0.01]. The high plant-protein diet pattern was inversely related to hyperhomocysteinemia, with a higher intake being protective. Compared with the 1st quartile, the adjusted OR was 0.42 (95% CI = 0.25–0.69; P = 0.001) for the 4th quartile. The high animal-protein diet was positively associated with hyperhomocysteinemia, with participants in the highest quartile of intake having the greatest increase in risk [OR = 2.10 (95% CI = 1.22–3.60); P = 0.007]. Plasma homocysteine concentrations appeared to be correlated more with circulating folate (r = –0.25; P < 0.001) than with PLP (r = –0.02; P = 0.663) or vitamin B-12 (r = –0.16; P < 0.001). A diet rich in fruits and uncooked vegetables decreased the risk of hyperhomocysteinemia, whereas diets rich in red meat, chicken, and tea with milk were positively associated with hyperhomocysteinemia. J. Nutr. 140: 1261–1266, 2010.

Introduction

The association between plasma homocysteine concentrations and the development of various chronic diseases is gaining increasing recognition. Results pertaining to the association of homocysteine with cardiovascular disease (CVD) are still controversial. There are published reports that show no association between homocysteine and major cardiac events (1–3). However, several other studies have reported homocysteine as an independent risk factor for CVD (4,5). A meta-analysis revealing homocysteine as a modest predictor of ischemic heart disease adds further support (6). It is recommended that concentrations of homocysteine should be <15 μmol/L to avoid several disease conditions (7).

Hyperhomocysteinemia has been reported to be quite prevalent among South Asians, especially in apparently healthy Pakistani individuals (8–11). There is plenty of evidence to suggest that high concentrations of plasma homocysteine are due to low serum concentrations of folate, vitamin B-12, and vitamin B-6 (12,13). Industrialization and urbanization have led to an emergence of dietary patterns in the population that may lead to the development of chronic diseases. It has been reported that Western diet patterns (high in fried foods, salty snacks, eggs, and meat) are strong predictors of cardiovascular events (14,15). On the other hand, the prudent dietary pattern (known to be rich in grains, legumes, vegetables, fruits, and fish and poor in red meat and animal products) showed protective effects for chronic diseases or their determinants, such as myocardial infarction (15). Despite such protective effects of a prudent dietary pattern that is commonly consumed in South Asia, CVD and other chronic diseases are becoming a serious problem throughout the region, including Pakistan. One particular reason for such findings could be the major modifications in diet (both in...
developed and developing countries like Pakistan), such as the transition from plant-based dietary products to animal-based food products. Therefore, understanding patterns of dietary intake in countries like Pakistan and their association (if any) with some known risk factors for CVD would be helpful in developing prevention programs for overcoming such health issues. It has been demonstrated that the Indo-Mediterranean diet, rich in α-linolenic acid (a constituent of whole grains, fruits, vegetables, walnuts, and almonds), is responsible for significant reduction in coronary artery disease (CAD) morbidity and mortality in this region compared with the step I National Cholesterol Education Program prudent diet (16).

Because of the marked variations in diet between developed and developing countries, identification of dietary patterns that could be related to low homocysteine concentrations and high B vitamin status would be helpful in making dietary recommendations for this region. This approach is in line with the recommendations from the AHA and other study groups that a Mediterranean-style diet has a preventive effect on the development of CVD and, therefore, could be beneficial (17,18). As a result, appropriate selection of diet might provide an economically feasible approach to reducing CAD in regions like South Asia.

A number of studies have been carried out in developed countries to determine the association of hyperhomocysteinemia with dietary patterns (19–21). However, there are very few reports from developing countries and, to the best of our knowledge, there is no South Asian study reporting the relationship of dietary patterns with hyperhomocysteinemia. The determination of dietary patterns would therefore be helpful in identifying any specific patterns in the diet of Pakistan that could be associated with the risk of hyperhomocysteinemia. The purpose of this study was to identify major dietary patterns of food intake in a Pakistani population residing in Sultanaabad, which is a low-income neighborhood in Karachi, and to investigate the association of dietary patterns with plasma homocysteine concentrations.

Materials and Methods

The participants were 872 healthy adults (355 males and 517 females; age range 18–60 y) living in a low-income urban area of Karachi, Pakistan. In this cross-sectional survey, systematic random sampling was used to select houses and 1 individual was selected from each house. Every 4th house was selected from a total pool of 4000 houses available in the target locality.

Healthy individuals who consented to participate in the study were screened using a questionnaire. Anthropometric measurements and blood samples from fasting subjects were obtained to measure study outcome variables and their determinants. Sociodemographic characteristics such as age, gender, household income, education, smoking habits, and tobacco chewing were recorded. We assessed eating habits using a simple 15-item food group frequency questionnaire. Major food sources of B complex vitamins were selected as the food items on the questionnaire. Information on the number of times each food item was consumed per month, per week, or per day was recorded. Frequency of each food item was then converted to per-day consumption. For example, a response of 5 servings/mo was converted to 0.16 servings/d or a response of 2 servings/wk was converted to 0.29 servings/d. The questionnaire used in this study was not validated; however, it has face validity because similar kinds of questionnaires have been used previously (15,22). Moreover, some of the food items used in this questionnaire have been previously studied for their association with CVD, B vitamins, and homocysteine (20,21).

Plasma and serum were separated and kept frozen at −70°C within 4 h after collection of blood. Serum lipids were measured within 48 h of collection of the samples. Colorimetric kits (RANDOX Laboratories) were used to estimate total cholesterol, HDL-cholesterol, triglycerides, and creatinine in serum. LDL-cholesterol was calculated using Friedewald’s formula (23). Serum samples were also analyzed for folate and vitamin B-12 using radioassays (24,25). Plasma homocysteine was measured using an immunaoassay-based kit (Abbott Laboratories). Hyperhomocysteinemia was defined as >15 μmol/L (7). Pyridoxal phosphate (PLP; a coenzyme form of vitamin B-6) was chosen as the standard for defining vitamin B-6 status (26). For determination of PLP in plasma, a modification of the method of Camp et al. (27), as described previously, was used (28). Intra-assay CV for plasma PLP and homocysteine were <9%. Moreover, intra-assay precision for folate and vitamin B-12 in serum was <15%. The minimum limits of detection for serum folate, serum vitamin B-12, plasma PLP, and plasma homocysteine were 1.13 nmol/L, 36.9 pmol/L, 3.2 nmol/L, and 4 μmol/L, respectively.

All individuals who reported using alcohol, oral contraceptives, or antiepileptics, were pregnant, had diabetes mellitus or renal disease (assessed by serum creatinine concentrations above 115 μmol/L), or who took B vitamin supplements during the last 6 mo were excluded from the analysis, because these conditions can affect homocysteine concentrations. Of 872 participants, 57 were not included in the final analysis because of incomplete questionnaires or refusal to give blood for biochemical analysis. Our final sample size was 815 participants. Ethical clearance of the study was obtained from the Ethics Review Committee of the Aga Khan University and all the participants provided informed consent.

Statistical analysis. This analysis had 2 major phases: developing dietary patterns using factor analysis and then logistic regression analysis to predict the association of hyperhomocysteinemia with dietary patterns. Factor analysis was used to identify common underlying dimensions (factors or patterns) of the dietary data. To generate uncorrelated factors, factors were rotated orthogonally. The number of factors to be retained in the model was determined on the basis of Eigenvalue (>1), scree plot, and factor interpretability (29). Initially, we obtained 6 uncorrelated factors from this analysis; however, we decided to use the first 3 factors in our prediction models, because the other 3 factors had high loading on very few food groups. The analyses were conducted with the data reduction procedure in SPSS 16 (version 16 for Windows, Apache Software Foundation).

Continuous variables were expressed as means ± SD and categorical variables as n (%). Categorical variables were analyzed by chi-square to compare the frequencies across the quartiles of each dietary pattern. For continuous variables, ANOVA was used to compare the means across the quartiles of each dietary pattern. All P-values were 2 sided at α = 0.05.

Logistic regression analysis was used to examine the association between the quartiles of the 3 dietary patterns (independent variables) and hyperhomocysteinemia (dependent variable) and each model was also adjusted for age, gender, household income, BMI, tobacco chewing, and smoking status. Values are expressed as OR (95% CI).

Results

Three major dietary patterns were identified by using factor analysis and subjectively labeled as “prudent diet,” “high animal-protein diet,” and “high plant-protein diet.” The prudent diet was characterized by a high intake of eggs, fish, uncooked vegetables, juices, and bananas and other fruits. The high animal-protein diet pattern was characterized by a high intake of meat, chicken, wheat, bananas, and tea with milk, whereas the high plant-protein diet was characterized by a large intake of cooked vegetables and legumes and a small intake of meat (Supplemental Table 1).

Each dietary pattern was classified into quartiles. Descriptive information for each of the dietary patterns by quartiles of intake is presented in Table 1. The age of participants in the highest quartile of the prudent diet pattern was significantly less than for those in the lowest quartile of the same dietary pattern.
The proportion of males was less in the lowest quartile of prudent diet compared with the highest quartile (P = 0.009). Similar findings were obtained for the proportion of males in the highest and lowest quartiles of the high animal-protein diet pattern (P < 0.001). In comparison, women scored higher in the 1st and 2nd quartiles of the prudent and high-animal-protein diet patterns and in the 3rd and 4th quartiles of the high plant-protein diet pattern. A higher percentage of individuals with no education were in the lowest quartile of the prudent diet compared with the highest quartile. Similarly, a significantly higher proportion of individuals with an education level of 12th grade and above were in the highest quartile of the prudent diet compared with the lowest quartile. Such a trend was also observed with education status in the quartiles of the high plant-protein diet pattern. A higher percentage of individuals with no education in the lowest quartile of intake, the adjusted OR was 34% (95% CI = 0.40–0.85) for the 4th quartile.

There was an inverse association between the high plant-protein diet pattern and hyperhomocysteinemia. Compared with the lowest quartile of intake, the adjusted OR was 48% (95% CI = 0.30–0.90) protective effect for hyperhomocysteinemia compared with the lowest quartile of intake.

The highest quartile of the high animal-protein diet pattern was positively associated with hyperhomocysteinemia. Compared with the lowest quartile of intake, the adjusted OR was 2.1 (95% CI = 1.22–3.60) for the 4th quartile.

TABLE 1 Characteristics of study population in the lowest to highest quartiles of different dietary pattern scores

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Prudent diet</th>
<th>High animal-protein diet</th>
<th>High plant-protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
<td>Q2</td>
<td>Q3</td>
</tr>
<tr>
<td>n</td>
<td>204</td>
<td>204</td>
<td>204</td>
</tr>
<tr>
<td>Age, y</td>
<td>35 ± 11</td>
<td>33 ± 11</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>Household income, 2</td>
<td>5000 Rs</td>
<td>88 (43)</td>
<td>93 (46)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>177 (87)</td>
<td>176 (86)</td>
<td>175 (88)</td>
</tr>
<tr>
<td>Only tobacco</td>
<td>2 (11)</td>
<td>7 (4)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Tobacco with betel nut</td>
<td>25 (12)</td>
<td>21 (10)</td>
<td>23 (11)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>185 (91)</td>
<td>183 (90)</td>
<td>181 (89)</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (9)</td>
<td>21 (10)</td>
<td>22 (11)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD or n (%).
2 1 Rupee (Rs) = 0.012 US$.
3 P-trend value was based on ANOVA when row variable was continuous (age) and χ² test when row variable was categorical.

Concentrations of homocysteine and various biomarkers across quartiles of dietary pattern factor scores are shown in Table 2. Plasma homocysteine was higher in the highest quartile of the high animal-protein diet compared with the lowest quartile (P < 0.001). In contrast, plasma homocysteine was significantly lower in the highest quartile of the high plant-protein diet pattern compared with the lowest quartile. Serum folate concentrations were greater in the 4th quartiles of the prudent diet and high plant-protein diet patterns compared with their respective 1st quartiles (P ≤ 0.01). The plasma PLP concentration was significantly higher in the highest quartile of the prudent diet compared with the lowest quartile. Vitamin B-12 concentrations did not differ among the quartiles of the various dietary patterns. Circulating homocysteine concentrations correlated more with those of folate (r = −0.25; P < 0.001) than with PLP (r = −0.02; P = 0.663) or vitamin-B12 (r = −0.16; P < 0.001) among consumers of the prudent and high-plant-protein diet patterns.

HDL cholesterol was significantly higher in individuals in the highest quartile of the prudent diet pattern compared with the lowest quartile. The BMI was greater in the lowest quartile of the prudent dietary pattern compared with the highest quartile even when the model was adjusted for age and gender. The waist:hip ratio (WHR) was greater in the highest quartile of the high animal-protein diet compared with the lowest quartile.

The unadjusted prudent diet pattern and hyperhomocysteinemia were not associated (Table 3). However, when the model was adjusted for age, gender, household income, BMI, tobacco chewing, and smoking, the highest quartile of the prudent diet pattern had a 48% (95% CI = 0.30–0.90) protective effect for hyperhomocysteinemia compared with the lowest quartile of intake.

The highest quartile of the high animal-protein diet pattern was positively associated with hyperhomocysteinemia. Compared with the lowest quartile of intake, the adjusted OR was 2.1 (95% CI = 1.22–3.60) for the 4th quartile.

There was an inverse association between the high plant-protein diet pattern and hyperhomocysteinemia. Compared with the reference group, protection for hyperhomocysteinemia with the high plant-protein diet pattern was 34% (95% CI = 0.40–1.07) for the 2nd quartile, 49% (95% CI = 0.31–0.83) for the...
TABLE 2 Circulating concentrations of biomarkers and anthropometric measures by quartiles of dietary patterns1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Prudent diet</th>
<th>High animal-protein diet</th>
<th>High plant-protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q12</td>
<td>Q4</td>
<td>P2,4</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>15.78 ± 12.5</td>
<td>13.97 ± 7.7</td>
<td>0.26</td>
</tr>
<tr>
<td>Folate, nmol/L</td>
<td>13 ± 6.6</td>
<td>15.95 ± 10.65</td>
<td>0.01</td>
</tr>
<tr>
<td>PLP, nmol/L</td>
<td>30.82 ± 40.1</td>
<td>39.32 ± 43.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin B12, pmol/L</td>
<td>317 ± 162</td>
<td>322 ± 175</td>
<td>0.85</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.14 ± 0.88</td>
<td>4.08 ± 0.91</td>
<td>0.99</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>2.48 ± 0.82</td>
<td>2.3 ± 0.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.47 ± 0.21</td>
<td>1.56 ± 1.08</td>
<td>0.33</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.01 ± 0.21</td>
<td>1.08 ± 0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.50 ± 5.5</td>
<td>23.13 ± 5.4</td>
<td>0.008</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.07</td>
<td>0.83 ± 0.08</td>
<td>0.18</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 Lowest quartile.
3 P-value was based on ANOVA comparing means of quartiles.
4 P-value was based on ANOVA comparing means of quartiles adjusted for age (y) and gender.

3rd quartile, and 58% (95% CI = 0.25–0.69) for the 4th quartile when the model was adjusted for age, gender, household income, BMI, tobacco chewing, and smoking.

Discussion
The association of single food items with different diseases or biomarkers, such as homocysteine, has been reported previously (30–34). Such analyses are important but are not without limitations, because people do not eat individual nutrients and food items alone; rather, these are consumed in combination with other food items. Keeping in view how different foods and nutrients are consumed in combinations, hypotheses have been put forward to investigate the effect of overall diet on the disease of interest (35,36). As a result, dietary pattern analysis has emerged and factor analysis is commonly used to explore dietary patterns (15,37,38).

Using factor analysis, we were able to define 3 dietary patterns in our study population. These dietary patterns were labeled as prudent diet, high animal-protein diet, and high plant-protein diet. The high animal-protein diet pattern as defined by us was quite similar to the pattern defined earlier by Zobairi et al. (39).

South Asian diets are rich in grains, legumes, vegetables, fruit, and fish and low in red meat and animal products, similar to the 3rd pattern that we identified. In other words, these patterns are culturally and ethnically specific; however, they are not much different in the context of food items and patterns from those identified in other parts of the world. The prudent dietary patterns generated from the INTERHEART, Health Professional Follow-Up Study, and the Nurses’ Health Cohort using factor analysis also appear to be similar to the pattern identified in this study (15,20,22). However, the high loading of eggs in the prudent diet identified in this study was different from the above-mentioned studies, because the grouping of eggs was reported mostly as part of Western dietary patterns.

The protective role of the high plant-protein diet pattern and the prudent diet pattern against hyperhomocysteinemia is similar to the findings of some other reports (20,21,40), indicating that such dietary patterns were positively associated with serum folate concentrations and inversely associated with plasma homocysteine. The relationship of increased intake of the high plant-protein diet with serum folate appears to be stronger compared with the association between increased consumption of the prudent diet and folate. This may explain why we observed low levels of homocysteine in individuals consuming a more high plant-protein diet. The high plant-protein diet pattern was bipolar, with negative loading for meat (rich in methionine) and positive loadings for cooked vegetables and legumes. We postulate that the bipolar nature of this pattern may be associated with increased concentrations of folate, thereby providing protection against hyperhomocysteinemia in individuals in the highest quartile. High loading of eggs as part of the prudent diet in the present study merits some discussion. The inverse relationship of the prudent diet with homocysteine may be due in part to the higher consumption of eggs, which contain large amounts of the methyl donor choline (41). Specifically, the choline metabolite betaine can be used as an alternate of folate for the remethylation of homocysteine to methionine. The inverse relationship of high plant-protein diet and prudent diet patterns with plasma homocysteine and the
positive association of these diets with serum folate indicate that adequate consumption of fresh fruits and vegetables could be beneficial in reducing the risk of hyperhomocysteinemia. Some of the components of the high plant-protein diet pattern and the prudent diet pattern are also part of the prudent patterns identified to be protective for coronary heart disease in other studies (14,15,22).

The adverse role of high consumption of the high animal-protein diet with hyperhomocysteinemia could be due to an increased intake of red meat and chicken (rich sources of methionine). In South Asian diets, meat and chicken are not only rich sources of saturated fat, but their cooking is also generally carried out in oil or “vanaspati ghee” (hydrogenated oil). Recently, a positive association between dietary saturated fat intake and plasma homocysteine has been reported (42). This may explain why the high animal-protein dietary pattern with high loadings for meat, chicken, and tea with milk is associated with an increased risk of hyperhomocysteinemia in the present study. The other possible reason could be the frequent use of tea with milk, which is known to be positively associated with hyperhomocysteinemia (40,43,44). Although we think that the effect of tea in terms of polyphenol concentration may not be very significant (45), it could be adding to the homocysteine concentrations by decreasing the bioavailability of folate and, hence, affect the homocysteine remethylation cycle (46).

We observed associations of the prudent diet and high animal-protein dietary patterns with physical measures of obesity (BMI and WHR). After adjusting for age and gender, the protective role of the prudent diet on BMI indicates that a balanced diet such as the prudent diet is less likely to lead to weight gain. The adverse effect of high intake of animal protein on the WHR may be due to the extensive use of cooking oil in the preparation of dishes with red meat and chicken in this part of the world.

This analysis is one of the few large epidemiological studies to focus on this region and, perhaps, the first in South Asia highlighting the impact of dietary patterns on hyperhomocysteinemia. The 3 dietary patterns observed in this study, we contend, represent the most common types of dietary intake patterns of the low-income urban population of Pakistan. The dietetic connotations of these patterns are helpful in that they are commonly explicable and could offer a clear public health direction. Increased consumption of a high plant-protein diet and reduced intake of a high animal-protein diet turn out to be the recommended dietary patterns to keep the concentrations of homocysteine within acceptable limits. Furthermore, the protective effect of vegetables and fruits and some undesirable effects of tea with milk and red meat observed in this study population were in line with the results reported for Western populations (47). As mentioned above, Pakistanis are among those populations having high concentrations of plasma homocysteine and a high rate of CAD (8,9). An association of hyperhomocysteinemia with some of the common dietary patterns suggests that diet may have a major role in the high prevalence of CAD in this population. The maximum level of protection from hyperhomocysteinemia observed in participants in the highest quartile of the high plant-protein diet strengthens the notion that the use of fresh fruit and vegetables would be protective against the development of high concentrations of homocysteine. However, further large scale cohort studies involving both the rural and urban Pakistani populations need to be conducted for validation of our findings, because this was a small, cross-sectional study conducted in a low income community in Karachi. We used a 15-item food group frequency questionnaire. Although lack of validation of the dietary questionnaire could be regarded as a limitation of the study, we still think that we were able to gauge the usual dietary intake of participants. Another point to mention is that we were not able to adjust our models for total energy intake, because we did not have sufficient information for nutrient analysis. However, an alternative adjustment has been done by adjusting the regression model for BMI, as previously recommended by Willet (48). Because older participants and women had higher scores in the 1st quartile of the prudent diet whereas younger participants and men had higher scores in the 4th quartile of this dietary pattern, it is suggestive that menopausal status might have an impact on these associations. We conjecture that, in future studies, the use of a detailed dietary questionnaire, inclusive of sugar and sugar products, milk and dairy food, and information regarding menopausal status and physical activity would be helpful to ascertain the relationship between dietary patterns and homocysteine while adjusting for total energy intake.

In conclusion, of the 3 dietary patterns observed among the Karachi urban population, the prudent diet (with the highest quartile of intake) shows a protective effect toward the development of hyperhomocysteinemia. Moreover, increased intake of a high plant-protein diet decreases the risk of hyperhomocysteinemia by ~50%. Furthermore, we observed that the use of high animal-protein diet in this population could, perhaps, be one of the reasons for hyperhomocysteinemia.

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