Dietary Serine Intake and Higher Risk of Hypertension: Tehran Lipid and Glucose Study

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A B S T R A C T

Background and Objectives: Recent studies focus on vasoactive properties of protein, which is dependent to its source and amino acid composition. Our aim was to evaluate the association between dietary serine intakes and hypertension incident.

Materials and Methods: We used the data of 4287 subjects aged 20-70 years, who participated in the fourth phase of Tehran Lipid and Glucose Study (2008-2011) and were followed up to the fifth phase (2011-2014). At baseline, the participants were free of hypertension, cardiovascular diseases, and cancer. Serine dietary data were collected through a valid and reliable food frequency questionnaire. Incidence of hypertension was identified after three years of follow up. To investigate the association of serine intake and incidence of hypertension, multivariable adjusted models of logistic regression were used, and odds ratios (ORs) across quartiles of serine were reported.

Results: After three years of follow up, 429 (10%) incident cases of hypertension were ascertained. The OR of the highest quartile of serine intake was 1.43 (95% CI: 1.05-1.95; P for trend: 0.03) compared to the lowest adjusted for age and sex. After further adjustment for body mass index (BMI), diabetes status, physical activity, smoking status, and dietary intake of energy, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, calcium, magnesium, sodium, potassium and fiber, the OR of the highest vs. the lowest quartile of serine intake was 1.70 (95% CI: 1.18-2.44; P for trend=0.005).

Conclusions: These findings suggest that dietary serine intake may be directly associated with the risk of hypertension incident.

Keywords: Serine, Amino acid, Hypertension

Introduction

Hypertension is one of the most common causes of health care problems relating to chronic diseases such as cardiovascular diseases, stroke, and diabetes (1, 2). The prevalence of hypertension was reported to be about 22% in Iran during 1999-2000 (3), and coincidentally, its global prevalence was 26.4%, which is anticipated to reach 29.2% by 2025 (1). Lowering blood pressure (BP) can dramatically reduce the risk of cardiovascular diseases and its mortality rate (2). Previous studies reported that the risk of mortality due to stroke and cardiovascular diseases can be decreased by 6% and 4%, respectively, only by lowering 2 mmHg of systolic BP (4, 5).

Hypertension is a multi-factorial disease influenced by several factors such as environmental
The purpose of the current study was to determine the association between serine intake from diet and BP (7-9). As vasoactive properties of dietary protein are likely dependent on its protein and amino acid composition (10), recent studies address the relation of different amino acids with BP (7, 8, 10-13). Two cross-sectional studies in the framework of the International Collaborative Study of Macronutrients, Micronutrients and Blood Pressure (INTERMAP) indicate that higher dietary intake of glycine is related to 2–3 mmHg higher systolic BP, and higher intake of glutamic acid is associated with 1.5 and 1 mmHg lower systolic BP and diastolic BP, respectively (7, 13). The association of dietary serine and BP investigated in only two studies (11, 13). Serine is a non-essential amino acid that is needed to some of signaling pathways and biosynthetic functions including synthesis of glycine, cysteine, tryptophan, sphingolipids, phosphatidylserine, DNA and RNA components, and remethylation of homocysteine; it further acts as one of the carbon unit donors in the folate cycle (14). Although serine is one of the prominent amino acids in proteins with vegetable source (15) and plant protein has inverse relation with BP (16-18), the findings of previous cross-sectional studies suggest no relation between dietary serine intake and BP (11, 13). However, a recent investigation from the European Prospective Investigation into Cancer (EPIC) study demonstrated that higher serum concentration of serine is a strong predictor of hypertension development (19).

To the best of our knowledge, the association between dietary serine intake and hypertension has not been investigated in previous cohort studies, so the purpose of the current study was to determine the relation between serine intake from diet and incidence of hypertension in the framework of Tehran Lipid and Glucose Study (TLGS).

**Materials and Methods**

**Subjects:** The present study conducted prospectively in the framework of TLGS, which is a prospective study aimed to determine the prevalence and identify the risk factors of chronic diseases, began in 1999–2001 on 15,005 participants, aged 3-75 years, residents of district No. 13 of Tehran, the capital of Iran. The TLGS is an ongoing study, and the participants are followed up every three years for updating their demographics and lifestyle, biochemical, clinical, and dietary information (20). In the fourth phase (2008-2011) of TLGS, the anthropometric, clinical, and dietary measurements of 7956 subjects were collected. For the current study, 6493 participants aged 20-70 years were followed up to the fifth phase (2011-2014) of TLGS (median follow up; 3.1 years). After exclusion of the subjects with over- and under-reporting in the dietary data (energy intake higher than 4200 and lower than 800 kcal/d) (n=317), the subjects with the history of stroke, cardiovascular diseases, and cancer (n=43), those with hypertension diagnose in the fourth phase (n=1057), and pregnant and lactating women (n=106), finally, 5004 subjects remained. Some individuals fell into more than one category. Of 5004 subjects, 4287 were followed up and remained for final analysis (follow up rate, 86%).

The proposal of this study was approved by the Ethics Committee of Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Written informed consent was obtained from all participants.

**Dietary assessment:** Dietary intake data of the participants were collected using a valid and reliable food frequency questionnaire (FFQ) (21). The trained dietetics asked the participants to report the frequency consumption of each food item in the last year in daily, weekly, and monthly basis. The reported frequency of portion sizes for each consumed foods items, which was reported in the household measures, was converted into grams. For calculating the energy and nutrients intakes, we used the United States’ Department of Agriculture (USDA) Food Composition Table (FCT). For some traditional foods that are not listed in USDA FCT, the Iranian FCT was used. The data of dietary serine intake were calculated from USDA (USDA National Nutrient Database for Standard Reference, Release 28) FCT of 2015 (http://www.ars.usda.gov/ba/bhnrc/ndl), which was based on the chemical analysis of the amino acid composition of food items.

**Physical activity assessment:** To obtain measurements of physical activity, the Modifiable Activity Questionnaire (MAQ), which has been modified and validated previously among Iranians population, was used (22). The trained interviewers asked the participants to report and identify the frequency and time spent during the last year on common activities according to a list of activities of...
daily life; physical activity levels were expressed as metabolic equivalent hours per week (MET-h/wk).

**Clinical and biological measurements:** Anthropometric measurements including height, weight, and waist circumference were done by expert interviewers. Weight measurement was conducted using digital scale (Seca 707; Seca Corporation, Hanover, Maryland; range, 0.1-150 kg) with the precision of 100 gram, while the subjects were in light clothing and without shoes. Height was measured using a stadiometer (model 208 portable body meter measuring device; Seca) whereas the participants were in a standing position without shoes and their shoulders were in a normal state. BMI was calculated as weight (kg) divided to height (m²). The measurement of waist circumference (WC) was done with the accuracy of 0.1 cm on the level of the umbilicus using an outstretched tape meter and without pressure on the body surfaces. BP was measured by experienced physicians using a standard mercury sphygmomanometer, and Korotkoff sound technique with an accuracy of 2 mm Hg. Systolic BP was determined with the onset of the first sound heard, and diastolic BP was determined with the disappearance of the sound. After 5-minute resting on a chair, BP was measured twice on the right arm with a minimum interval of 30 seconds. Final BP was considered as the average of two measurements.

For the measurement of serum glucose and lipid profile, the blood samples were obtained after 12-14 hours of overnight fasting at 7:00-9:00 AM from all participants. The samples were immediately centrifuged within 30-45 minutes of collection, and all the blood analyses were done at the TLGS research laboratory.

Fasting plasma glucose (FPG) was measured on the day of sampling with an enzymatic colorimetric method using glucose oxidase. The inter- and intra-assay CV for glucose was 2.2%. Measuring of triglycerides (TGs) was done using enzymatic colorimetric method with glycerol phosphate oxidase. The inter- and intra-assay CVs for TGs were 0.6 and 1.6%, respectively. After precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid, serum high-density lipoprotein-cholesterol (HDL-C) was measured. Enzymatic colorimetric tests were used to assay total cholesterol (TC) with cholesterol esterase and cholesterol oxidase. Inter- and intra-assay CVs for both TC and HDL-C were 0.5 and 2%, respectively. Low-density lipoprotein-cholesterol (LDL-C) was computed from serum TC, TGs, and HDL-C concentrations using Friedewald formula and expressed in mg/dl. Analysis of all samples was conducted using Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands) and commercial kits (Pars Azmoon Inc., Tehran, Iran).

**Definitions:** Hypertension was determined based on the Eighth Joint National Committee (JNC 8), which defined hypertension for subjects ≥ 60 years as systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg, or taking antihypertensive medications, and for subjects < 60 years as systolic BP ≥ 150 mmHg, diastolic BP ≥ 90 mmHg, or taking antihypertensive medications (23). Diabetes was determined according to the American Diabetes Association (ADA), which defined diabetes as FPG ≥ 126 mg/dl, or 2-hour blood glucose ≥ 200 mg/dl, or being on anti-diabetic medication (24).

**Statistical analyses:** Data analysis was performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, IL, USA). Normality of the data was checked using histogram chart and Kolmogorov-Smirnov’s test. Baseline characteristics of the subjects were expressed as mean ± SD or median (25-75 interquartile range) for quantitative variables, and number and percentages for qualitative variables across the quartiles of dietary serine intake (in terms of percentage of total protein). To test for a trend across the quartiles of dietary serine intakes, we used linear regression for continuous variables and Chi-square test for dichotomous variables. Risk of hypertension after three years of follow up across the quartiles of dietary serine was assessed using logistic regression with adjustment for potential confounders including age, sex, BMI, diabetes, physical activity, smoking status, and dietary intake of energy, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), calcium, magnesium, sodium, potassium, and fiber. Odds ratio (OR) and 95 % confidence interval (CI) were reported, and P-values <0.05 were considered as statistically significant.

**Results**

The mean ± SD of age and BMI of participants were 39.2 ± 12.0 and 26.8 ± 4.5, respectively, and
42.1% of them were men. After three years of follow up, 429 incident cases of hypertension (10%) occurred. The mean ± SD of dietary serine intakes in terms of gram per day and percentage of total protein were 4.1 ± 1.4 and 5.0 ± 2.0 %, respectively.

Table 1 presents the baseline characteristics of the study population according to the quartiles of dietary serine intake. There were no significant differences for age, BMI, smoking status, physical activity, systolic and diastolic BP, and lipid profiles across the quartiles of serine intake (P > 0.05). Subjects in the highest vs. the lowest quartiles of serine intake had lower intake of energy, carbohydrate, plant protein and fiber (P < 0.001); however, they had higher intake of animal protein, total fat, SFA, and calcium (P ≤ 0.03).

The ORs (95% CI) of hypertension incidence across the quartiles of serine intake for different adjusted models are shown in Table 2. The OR of the highest compared to the lowest quartiles of dietary serine intake was 1.43 (95% CI: 1.05 – 1.95 P for trend: 0.03) adjusted for age and sex. In model 2, after adjusting for BMI, physical activity, smoking and diabetes status, and energy intake, no much change was observed in the OR of hypertension incidence. The OR of the highest quartile of dietary serine intake in the final adjusted model for potential confounders was 1.70 (95% CI: 1.18 – 2.44 P for trend: 0.005) compared to the lowest quartile (Figure 1).

The positive and significant association of serine-hypertension remained (data not shown) after mutual adjustment of 17 other amino acids (except phenylalanine), which was highly correlated with serine (r=0.84).

Table 1. Baseline characteristics of adult participants of the Tehran Lipid and Glucose Study across quartiles of dietary serine intake

<table>
<thead>
<tr>
<th></th>
<th>Q1 (n=1072)</th>
<th>Q2 (n=1072)</th>
<th>Q3 (n=1072)</th>
<th>Q4 (n=1071)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12.5 ± 39.3</td>
<td>38.8 ± 11.8</td>
<td>39.0 ± 11.9</td>
<td>39.9 ± 11.8</td>
<td>0.212</td>
</tr>
<tr>
<td>Men (%)</td>
<td>41.8</td>
<td>44.7</td>
<td>43.1</td>
<td>38.9</td>
<td>0.050</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>4.6 ± 26.7</td>
<td>26.9 ± 4.7</td>
<td>26.6 ± 4.3</td>
<td>27.1 ± 4.6</td>
<td>0.152</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>11.7</td>
<td>11.2</td>
<td>10.9</td>
<td>10.3</td>
<td>0.746</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>108.4 ± 12.0</td>
<td>109.6 ± 11.6</td>
<td>108.5 ± 12.1</td>
<td>108.7 ± 12.0</td>
<td>0.940</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.1 ± 8.5</td>
<td>73.6 ± 8.0</td>
<td>72.6 ± 8.5</td>
<td>73.2 ± 8.6</td>
<td>0.702</td>
</tr>
<tr>
<td>Physical activity (MET/hour/week)</td>
<td>59.4 (25.6 – 94.2)</td>
<td>63.4 (27.8 – 95.5)</td>
<td>59.5 (26.8 – 92.2)</td>
<td>59.5 (23.5 – 92.2)</td>
<td>0.267</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>113.0 (79.0 – 160.0)</td>
<td>111.0 (79.0 – 160.0)</td>
<td>111.0 (78.0 – 161.0)</td>
<td>114.0 (80.0 – 160.0)</td>
<td>0.137</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>48.1 ± 11.3</td>
<td>47.7 ± 12.1</td>
<td>47.6 ± 11.2</td>
<td>48.6 ± 11.6</td>
<td>0.474</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>109.7 ± 32.6</td>
<td>109.6 ± 33.3</td>
<td>110.1 ± 32.4</td>
<td>111.4 ± 30.5</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Dietary intakes

<table>
<thead>
<tr>
<th></th>
<th>Q1 (n=1072)</th>
<th>Q2 (n=1072)</th>
<th>Q3 (n=1072)</th>
<th>Q4 (n=1071)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2525 ± 769</td>
<td>2495 ± 760</td>
<td>2412 ± 731</td>
<td>2226 ± 713</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>59.3 ± 7.5</td>
<td>59.2 ± 6.5</td>
<td>58.7 ± 6.1</td>
<td>57.0 ± 5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein (% of energy)</td>
<td>3.7 ± 14.0</td>
<td>13.5 ± 2.2</td>
<td>13.5 ± 1.9</td>
<td>14.0 ± 2.0</td>
<td>0.885</td>
</tr>
<tr>
<td>Animal protein (% of energy)</td>
<td>7.2 ± 3.4</td>
<td>6.4 ± 2.2</td>
<td>6.5 ± 2.1</td>
<td>7.4 ± 2.1</td>
<td>0.034</td>
</tr>
<tr>
<td>Plant protein (% of energy)</td>
<td>1.96 ± 7.0</td>
<td>7.0 ± 1.5</td>
<td>7.0 ± 1.4</td>
<td>6.5 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fat (% of energy)</td>
<td>6.4 ± 29.4</td>
<td>29.7 ± 6.2</td>
<td>30.2 ± 6.0</td>
<td>31.0 ± 6.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SFA (% of energy)</td>
<td>2.68 ± 8.8</td>
<td>9.4 ± 2.6</td>
<td>10.0 ± 2.5</td>
<td>11.0 ± 2.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PUFA (% of energy)</td>
<td>6.0 ± 1.9</td>
<td>6.0 ± 2.0</td>
<td>5.9 ± 1.9</td>
<td>5.9 ± 1.9</td>
<td>0.279</td>
</tr>
<tr>
<td>Sodium (mg/1000kcal)</td>
<td>463 ± 1501</td>
<td>1529 ± 439</td>
<td>1539 ± 453</td>
<td>1536 ± 446</td>
<td>0.065</td>
</tr>
<tr>
<td>Potassium (mg/1000kcal)</td>
<td>589 ± 1988</td>
<td>1935 ± 527</td>
<td>1930 ± 526</td>
<td>1956 ± 540</td>
<td>0.188</td>
</tr>
<tr>
<td>Calcium (mg/1000kcal)</td>
<td>186 ± 539</td>
<td>503 ± 187</td>
<td>633 ± 187</td>
<td>721 ± 208</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium (mg/1000kcal)</td>
<td>41.4 ± 194.5</td>
<td>199.3 ± 40.7</td>
<td>197.3 ± 38.7</td>
<td>194.3 ± 38.5</td>
<td>0.622</td>
</tr>
<tr>
<td>Fiber (g/1000kcal)</td>
<td>19.6 ± 6.6</td>
<td>20.2 ± 7.3</td>
<td>19.6 ± 6.0</td>
<td>18.5 ± 7.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1. Multivariable odds ratio and 95% confidence intervals (CI) of incident hypertension according to the quartiles of serine intake adjusted for age, sex, diabetes, body mass index, physical activity, smoking, energy, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, fiber, calcium, magnesium, sodium, and potassium intake among the adults of Tehran Lipid and Glucose Cohort Study (P for trend = 0.005).
Table 2. Odds ratios and 95% confidence intervals of incident hypertension across quartiles of dietary serine intake among 20-70 year adults of the Tehran Lipid and Glucose Study

<table>
<thead>
<tr>
<th>Dietary serine intake</th>
<th>Q1 (n=90/1072)</th>
<th>Q2 (n=111/1072)</th>
<th>Q3 (n=106/1072)</th>
<th>Q4 (n=122/1071)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median intake</td>
<td>4.75</td>
<td>4.96</td>
<td>5.12</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>Model 1†</td>
<td>1.00 (Ref)</td>
<td>1.32 (0.97 – 1.80)</td>
<td>1.33 (0.97 – 1.82)</td>
<td>1.43 (1.05 – 1.95)</td>
<td>0.030</td>
</tr>
<tr>
<td>Model 2‡</td>
<td>1.00 (Ref)</td>
<td>1.31 (0.96 – 1.78)</td>
<td>1.34 (0.98 – 1.83)</td>
<td>1.44 (1.06 – 1.95)</td>
<td>0.027</td>
</tr>
<tr>
<td>Model 3‡</td>
<td>1.00 (Ref)</td>
<td>1.42 (1.03 – 1.96)</td>
<td>1.48 (1.06 – 2.06)</td>
<td>1.70 (1.18 – 2.44)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Adjusted for age and sex.
†Additionally adjusted for diabetes, body mass index, physical activity, smoking (yes or no), and daily energy intake.
‡Additionally adjusted for saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, fiber, calcium, magnesium, sodium, and potassium (all continuous).

Discussion

In the current study, conducted among the adult participants of TLGS, we observed a direct association between higher dietary serine intake and incidence of hypertension after three years of follow-up. This association was independent of age, sex, BMI, diabetes, physical activity, smoking status, and dietary intakes of energy, SFA, MUFA, PUFA, calcium, magnesium, sodium, potassium, and fiber.

To the best of our knowledge, the relation of dietary serine and hypertension incidence has not been investigated in any other cohort study. However, association of serine, glutamic acid, cysteine, proline, and phenylalanine, as predominant amino acids in vegetable proteins, with BP was investigated in the cross-sectional design of the INTERMAP study, and no association between serine and BP was found (13). The INTERMAP study was conducted in four countries (United State, United Kingdom, Japan, and China) with different dietary patterns and food habits, whereas our population had more monotonous distribution of nutritional and lifestyle aspects. In the United State, the United Kingdom, Japan, and China, vegetable sources such as soy, legumes, seeds, and nuts are more rich sources of serine amino acid compared to animal sources such as meat and milk (25). However, in our study, 53.6% of serine was supplied from animal sources; despite the fact that in our study, protein intake from vegetable and animal sources was approximately equal (50.1% vs. 49.9%, respectively). In the INTERMAP study, only among the Chinese participants, vegetable sources provided higher protein intake than animal sources, whereas in other three countries, the consumed protein was more from animal sources (13). Furthermore, Moraes et al. found that higher serine intakes among 12-17 year girls tended to increase BP; however, no statistical significant serine-BP relation was observed (11). It is to be noted that serine intake in our study was higher than that of the above mentioned studies. Therefore, it is possible that serine may have adverse effects on BP, if consumed in higher amounts and predominantly from animal sources.

In the present study, higher serine intake was found to be consistently related to increasing hypertension in all the adjusted models. After adjusting for potential confounders, the highest vs. the lowest quartiles of serine intakes increased the risk of hypertension incident by 70%, and there was a significant tendency to increase hypertension incidence across the quartiles of dietary serine intake. This finding is in line with the results of EPIC study, which indicated that higher serum concentration of serine was a strong predictor for the development of hypertension (19).

Although energy, carbohydrate and plant protein significantly decreased across the quartiles of dietary serine, the intakes of fat and animal protein increased; this makes an explanation for the current observed positive association of serine and hypertension. Vegetable proteins showed an inverse association with BP (16-18), whereas animal protein predominantly did not show any relation with BP (16, 26-29). Furthermore, by increasing the serine intake, the dietary fiber significantly decreased, whereas SFA significantly increased. The results of this study are in accordance with the findings of previous studies that
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indicated a positive relation between lower intake of fiber and higher intake of SFA, and higher BP (30, 31).

As this study is the first of its kind investigating the serine-hypertension association, no definite mechanisms have been reported; however, it seems that our findings may be explained through some physiological functions of serine. Higher intakes of serine in diets with moderate protein (10-15%) compete with threonine, which has an inverse and beneficial association with systolic and diastolic BP (12) for entrance into brain (32). Furthermore, serine competes with glutamic acid to cross the blood-brain barrier, so higher dietary intakes of serine may reduce the entrance of glutamic acid to brain, and consequently, decrease the synthesis of BP-beneficial neurotransmitters. Glutamic acid also showed an inverse relation with BP, which may be explained by its contribution on glutathione formation and removing the additional aldehydes (10, 13).

This study has some noteworthy strengths including prospective design, population-based study with relatively high sample size, which is representative of Iranian population, as well as accurate measurements of dietary data (collected by trained dieticians via face-to-face interview) and BP (measured by experienced physicians).

In addition, the study had some limitations including unavailability to serum amino acids that could help us for better interpretation of the study results, and the possibility of occurring residual confounding despite of adjusting the multivariable models of potential confounder variables.

In summary, our findings showed an independent positive relation between dietary serine intake and incidence of hypertension. This study highlights the need for prospective clinical trials to investigate the effect of amino acids’ intake on BP and other chronic diseases.

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