The relationship between dietary patterns and lipoprotein-associated phospholipase A2 levels in adults with cardiovascular risk factors: Tehran Lipid and Glucose Study

Seyed Hashem Sezavar Seyedi¹, Azadeh Mottaghi¹, Parvin Mirmiran², Mehdi Hedayati³, Fereidoun Azizi⁴

¹Research Center for Prevention of Cardiovascular Diseases, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran, ²Nutrition and Endocrine Research Center, Research Institute of Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, ³Cellular and Molecular Research Center, Research Institute of Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran, ⁴Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

INTRODUCTION

Among several cardiovascular risk factors, hypertension, diabetes, and blood lipid play a crucial role in the plaque formation and cardiovascular disease (CVD) incidence. Atherosclerosis with inflammatory nature was developed by the vessel endothelium dysfunction and oxidative stress.¹ Pathogenesis of CVDs can be detected by the probable involvement of lipoprotein-associated phospholipase A2 (Lp-PLA2), which is known as an inflammatory biomarker. Previous studies have consistently shown a positive relationship between the concentration and activity of Lp-PLA2 in populations with or without coronary artery disease (CAD).²⁻⁴ Some lines of evidence suggest that Lp-PLA2 may be involved in the process of atherogenesis by enhancing the inflammatory processes in the arterial intima.⁵ Lp-PLA2 is an inflammatory biomarker that is involved in the development and progression of cardiovascular diseases. Therefore, understanding the dietary patterns and their impact on Lp-PLA2 levels is crucial for the prevention and management of cardiovascular diseases.

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attached to low-density lipoprotein cholesterol (LDL-C) enters the artery wall, and when LDL-C is oxidized, two pro-inflammatory compounds are produced through the hydrolysis of the ester bonds of oxidized phospholipids in the sn2 positions. These compounds are formed to activate inflammation by recruiting chemokines within the intima of atherosclerotic lesions in humans. In this respect, previous studies have shown that Lp-PLA2 is involved in the progression of atherosclerotic lesions to rupture-prone plaques.

Even though the probable causal role of Lp-PLA2 in atherogenesis and its plausible modification by lipid-lowering drugs have been already assumed, it is not known whether diet and food patterns can reduce the plasma levels of Lp-PLA2. One study conducted on 60 healthy controls showed that a moderate dose (2 g) of n-3 polyunsaturated fatty acid (PUFA) or a high dose (6.6 g) of n-3 PUFA had no effect on the plasma levels of Lp-PLA2. Hatoum et al. conducted a study to determine the dietary factors, lifestyle factors, and clinical measurements associated with Lp-PLA2 activity for 853 women from the Nurse’s Health Study and 878 men from the Health Professionals Follow-up Study who were free of cancer and CVD. Their results showed that substitution of 5% of energy from carbohydrates with protein was associated with reduced activity of Lp-PLA2. Moreover, smoking, taking hormones after menopause, body mass index (BMI), and alcohol consumption are the modifiable risk factors which may influence LP-PLA2 activity.

To the best of our knowledge, no study has investigated the relationship between dietary patterns and LP-PLA2 in Middle Eastern population. The aim of this study is to investigate the relationship between the serum levels of LP-PLA2 and dietary factors among high-risk adults for CVD.

MATERIALS AND METHODS

Study population
This study was conducted within the framework of the Tehran Lipid and Glucose Study (TLGS). As a community-based prospective framework, it was adopted to detect any noncommunicable diseases in a representative sample of ≥3 years old, who resided at District 13 in Tehran, the capital city of Iran. The 1st phase of TLGS was initiated in March 1999 with the ongoing data collection occurring at 3-year intervals.

In the present research, 5605 men and women aged between 40 and 70 years were recruited in the 5th phase of TLGS (2011–2014). We excluded participants if there were no associated data on sex and anthropometric measurements (n = 463), if they were underweight (n = 350), no associated dietary intake information (n = 3695), and if they were under or over reporters of dietary intakes (<800 kcal/day or >4200 kcal/day, respectively) (n = 1097). After exclusions, the final analysis was conducted on the data from 470 participants.

Risk factors for CVDs were total cholesterol (TC) level >200 mg/dl, LDL-C level >100 mg/dl, high-density lipoprotein cholesterol (HDL-C) level <40 mg/dl in men and 50 mg/dl in women, triglyceride (TG) level >150 mg/dl, waist circumference upper than 102 cm in men and 88 cm in women, systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg or on antihypertensive medication, age ≥45 for men and ≥55 for women, and cigarette smoking. The participants in this study had at least five of the eight risk factors mentioned above.

Dietary assessment
A validated semi-quantitative Food-Frequency Questionnaire (FFQ) with 147 food items prepared for TLGS was used to collect the dietary data. Several trained dietitians were employed to question the participants based on their frequencies of each food intake on daily, weekly, and monthly bases during the previous year. The reliability and validity of the FFQ were found to be acceptable in the food groups after the assessment.

Food group data were standardized, and then considering ±4 standard deviation (SD), the data were truncated. The data were normalized using the logarithm. Finally, energy adjustment using the residual methods was performed. We used the factor analysis method to derive the dietary pattern from the dietary information collected from the 22 food groups [Table 1] based on the similarity of their nutrient contents. The factors were rotated by varimax rotation. The number of dietary patterns identified was based on eigenvalues >1, identification of a break point in the scree plot, and interpretability by the use of Horn’s parallel analysis using the software developed by Watkins. Items that had an absolute correlation ≥ 0.2 with a factor were considered to load on that factor and were retained in the calculation of the dietary pattern score. Food items that had absolute correlations <0.2 or cross-loaded on several factors were not included in the calculation of the dietary pattern score. Following data reduction in the factor analysis, three factors were derived. The derived dietary patterns were labeled according to the authors’ data interpretations and those presented in the previous studies. The food groups’ intakes weighed by their factor loadings were summed up to compute the factor score of each pattern; following this, each participant received a factor score for each identified pattern, and the scores were then standardized (mean = 0, SD = 1). Dietary pattern scores were
Table 1: Factor loading of three dietary patterns extracted by the factor analysis

| Food groups          | Healthy | Western | Semi-Mediterranean |
|----------------------|---------|---------|---------------------|
| Fruit and dried fruit| 0.652a  | -       |                     |
| Refined grains       | -0.596  | -       |                     |
| Olives               | 0.541   | -       |                     |
| High- and low-fat dairy products | 0.462 | -       |                     |
| Poultry and fish     | 0.337   | -       |                     |
| Liquid oils          | 0.238   | -       |                     |
| Canned products      | 0.276   | -       |                     |
| Carbonated drinks    | 0.675   | -       |                     |
| Fast foods           | 0.613   | -       |                     |
| Salty snacks         | 0.563   | -       |                     |
| Mayonnaise           | 0.588   | -       |                     |
| Organ meats          | 0.315   | -       |                     |
| Legumes              | 0.638   | -       |                     |
| Potatoes             | 0.649   | -       |                     |
| Egg                  | 0.515   | -       |                     |
| Red meats            | 0.465   | -       |                     |
| Tea and coffee       | 0.242   | -       |                     |
| Percentage of variance explained | 11 | 10 | 7 |

Values are factor loading of dietary patterns (n=470). Factor loading ≤0.2 are not shown; *Eigenvalue >1

Categorized into quartiles. Cutoff points for quartiles of the healthy, semi-Mediterranean dietary pattern, and Western dietary pattern scores were calculated.

Blood collection and laboratory measurements
Participants were asked to take fast for 12 h, and TC, HDL-C, and TG levels were measured using with a Hitachi 911 Analyzer using reagents and calibrators from Roche Diagnostics (Indianapolis, IN, USA); coefficient of variation (CV) were 1.8%. The concentration of LDL-C was measured with a homogeneous direct method from Genzyme (Cambridge, MA, USA); CVs were 3.1%.

The quantitative determination of Lp-PLA2 was measured with a commercial enzyme-linked immunosorbent assay kit (Abcam, USA). Blood pressure was taken on the right arm by a qualified physician after 15-min rest, using a standardized mercury sphygmomanometer, twice in a sitting position; the mean of two measurements was considered as participant blood pressure.

Anthropometrics and lifestyle measurements
A questionnaire was utilized by the trained interviewers to assess the participants’ demographic characteristics of age, educational level, smoking status, and level of physical activity expressed as metabolic Equivalent hours per week. The current smokers and nonsmokers were grouped as those smoking daily or occasionally and those having stopped smoking or never smoking, respectively. Physical activity level was determined using a Modifiable Activity Questionnaire (MAQ) translated in Persian. This questionnaire is based on the times and frequencies of performing light-, moderate-, high-, and very high-intensity common activities during the previous year. The high reliability and relatively moderate validity of the mentioned MAQ were reported among the studied Tehranian adults.[14] The minimally clothed participants with no shoes were weighed to the nearest 100 g using a digital scale. A tape meter was utilized to measure the participants’ heights to the nearest 0.5 cm while keeping them in a standing position with no shoes. The obtained weights (kg) were divided by the squares of the heights (m²) to calculate BMI. Using a soft tape meter, waist circumference measurement at the widest part over light clothing was done to the nearest 0.1 cm without exerting any pressure against the body based on anatomical landmarks.

Statistical analysis
Based on the hypothesis specified for the effects of dietary patterns on Lp-PLA2, we applied linear regressions to the influencing factors for exposure to a dietary pattern. B values with 95% confidence intervals (CI) were estimated for the effects of predicting factors. Separately, Lp-PLA2 was treated as a dependent variable in univariate and multivariate analyses. In multivariate analyses, age, sex, TC, LDL-C, BMI and physical activity, energy intake, hormone therapy for women, and taking blood lipid-lowering drugs were considered as potential confounders. When healthy pattern considered as reference, the association between Western and semi-Mediterranean pattern with Lp-PLA2 levels was compared with the healthy pattern. All statistical analyses were conducted using SPSS (Version 20; Chicago, IL, USA), and P < 0.05 was considered statistically significant.

RESULTS
Following data reduction in the factor analysis, three factors were derived. The derived factors (dietary patterns) were labeled on the basis of the authors’ interpretation of the data and on prior studies.[14] The factor loading of food groups in the three extracted dietary patterns is presented in Table 1. The three dietary patterns were named healthy dietary pattern, Western dietary pattern, and semi-Mediterranean dietary pattern. The healthy dietary pattern was high in fruits and dried fruits, olives, high- and low-fat dairy products, poultry and fish, liquid oils, and canned products. The Western dietary pattern was dominated by carbonated drinks, fast foods, salty snacks, mayonnaise, and organ meats. Finally, the semi-Mediterranean dietary pattern contained legumes, potatoes, eggs, red meats, tea, and coffee. Overall, these dietary patterns explained 27.6% of the total variance.
The characteristics of the study participants across the quartiles of Lp-PLA2 are presented in Table 2. Participants with the highest Lp-PLA2 levels were more likely to be men. Participants in the third quartile of Lp-PLA2 had the highest BMI, although this finding was not significant. For any increase in Lp-PLA2 level, TC and LDL-C levels in the participants also increased. The more active individuals had the lowest levels of Lp-PLA2.

The results of linear regressions on Lp-PLA2 indicated that the Western and semi-Mediterranean dietary patterns had significant effects on changes in Lp-PLA2 levels in univariate analyses [Table 3]. The trend of results showed that when healthy pattern considered as a reference pattern, the Western pattern associated with 0.35 ng/ml increase in Lp-PLA2 levels, whereas 0.12 ng/ml reduction in Lp-PLA2 levels related to semi-Mediterranean pattern.

In multivariate analyses, after adjusting for age, sex, TC, LDL-C, BMI and physical activity, energy intake, hormone therapy for women, and taking blood lipid-lowering drugs as potential confounders, the Western dietary pattern remained a significant factor influencing the Lp-PLA2 level ($\beta$ value: 1.32, 95% CI: 1.05, 1.64; $P=0.035$); in other words, the Western dietary pattern caused a marked increase in Lp-PLA2 levels [Table 4]. Meanwhile, after adjustment for the mentioned confounder factors, the effect of the semi-Mediterranean dietary pattern on Lp-PLA2 disappeared. As seen in univariate analysis, the trend of association between dietary patterns and Lp-PLA2 levels remained unchanged. Although after adjustment for confounders, the association between Western pattern with Lp-PLA2 levels became stronger and 1.32 ng/ml increase in Lp-PLA2 levels compared to reference (healthy pattern) can be seen.

DISCUSSION

The present study was conducted to determine the relationship between dietary patterns and Lp-PLA2 levels in adults with cardiovascular risk factors. A total of 470 adults participated in this study.

The most important finding of this cross-sectional study was the relationship between the Western dietary pattern and increased levels of Lp-PLA2, and even after adjusting for confounding factors, the association remained strong. This is the first study to examine associations between dietary patterns and Lp-PLA2 levels in a large cross-sectional study on adults with cardiovascular risk factors.

Men relative to women had higher levels of Lp-PLA2, a finding that has been consistently observed in other studies. This finding is probably due to the effects of estrogen on reducing the level of Lp-PLA2. Another possible reason for this difference between men and women could be the lower concentrations of LDL-C among the women. There are two ways in which high levels of LDL-C are associated with increased levels of lipase. First, LDL-C is the primary carrier of Lp-PLA2, and approximately 80% of Lp-PLA2 circulates bound to LDL-C, up to an additional 15% circulates with HDL-C, and the rest circulates with very-low-density-lipoprotein. Second, LDL-C is oxidized as a substrate used for Lp-PLA2 activity. This fact can also explain another finding of the present study regarding the relationship between higher levels of LDL-C and Lp-PLA2.

The results of the present investigation showed that Lp-PLA2 levels were significantly lower in more active adults than in other participants. In line with our results,
PUFAs are classified as omega-3 in inflammatory biomarkers, and the incidence of coronary heart disease (CHD) in a European population showed that circulating levels of Lp-PLA2 (women only) were linearly associated with increased waist circumference and decreased physical activity levels. Several studies have shown that physical activity might reduce plasma levels of pro-inflammatory cytokines and upregulate the expression of anti-inflammatory factors in the vascular wall, which may directly inhibit the development of atherosclerosis.[21-23] Our results showed that after adjusting for age, sex, TC, LDL-C, BMI and physical activity, energy intake, fasting blood glucose, hormone therapy for women, and taking blood lipid-lowering drugs were adjusted, BMI=Body mass index; CI=Confidence interval

Table 3: Univariate analysis for the association between dietary patterns and lipoprotein-associated phospholipase A2 (ng/ml)

| Dietary patterns | β  | 95% CI  | P     |
|------------------|----|---------|-------|
| Healthy pattern  | Reference | - | - |
| Western pattern  | 0.35 | 0.11, 0.78 | 0.026* |
| Semi‑Mediterranean pattern | -0.12 | -3.52, -0.16 | 0.043* |

*Statistically significant. CI=Confidence interval

Table 4: Multivariate analysis for the association between the dietary patterns and lipoprotein-associated phospholipase A2 (ng/ml)

| Dietary patterns | β  | 95% CI  | P     |
|------------------|----|---------|-------|
| Healthy pattern  | Reference | - | - |
| Western pattern  | 1.32 | 1.05, 1.64 | 0.035* |
| Semi‑Mediterranean pattern | -0.01 | -0.16, 0.43 | 0.75 |

*Statistically significant. Age, BMI and physical activity, energy intake, fasting blood glucose, hormone therapy for women, and taking blood lipid-lowering drugs were adjusted. BMI=Body mass index; CI=Confidence interval

There are several limitations of this study. First, the analysis was based on cross-sectional data, and thus, causality could not be inferred. Second, the collection of data by the FFQ relies on an individual’s memory, and this method is susceptible to recall bias. Moreover, some individuals cannot accurately estimate the portion size of the food they consume. However, the use of highly trained interviewers in this study reduced this type of error. Third, residual confounding effects could not be avoided. Fourth, there are some limitations of the factor analysis method, namely there are several subjective or arbitrary decisions regarding the use of factor analysis, including consolidation of food items into a food group, number of factors extracted in the rotation method, and interpretability of factors. However, eigenvalues and scree plots are tools that can help extract the best factors. Fifth, questions about physical activity are fully subjective, and their answers are difficult to verify.

Employment of an adequately large population, which was demographically representative of Tehran population, was the greatest strength of this investigation. In this study, we used multivariate analysis to show a relationship between dietary patterns and serum Lp-PLA2 level, and the strength of this method was taking into account the confounding variables.

CONCLUSION

The “Western” dietary pattern was concluded to be associated with higher levels of Lp-PLA2. To counteract their enhanced serum levels, which are directly associated with vascular inflammation and CVDs, we recommend adults to eat more fruits and vegetables.

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Conflicts of interest
There are no conflicts of interest.

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