Comparison of Serum LP-PLA2 Level and some Nutritional Factors between Well-Controlled and Poorly-Controlled Diabetic Patients

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Abstract- Lipoprotein-associated phospholipase A2 (Lp-PLA2) is produced by inflammatory cells, bound to LDL and other lipoproteins, and hydrolyzes oxidized phospholipids in LDL. Type 2 diabetes is the most common form of diabetes some investigations show the association of lipoprotein-associated phospholipase A2 mass and activity with the incidence of cardiovascular disease (CVD) in populations with high prevalences of insulin resistance and diabetes. This study is a cross-sectional descriptive and analytic study on 80 individuals with diabetes referring to the Tehran Diabetes Association. Patients divided into two groups (well-controlled and poorly controlled) based on their HbA1C. Personal information, anthropometric assessments (including height, weight, waist circumference and hip circumference) and semi-quantitative 147 items FFQ was used and vein blood samples were taken. After plasma separation, blood sample used for FBS, HbA1c and LP-PLA2 measurement. The independent sample T test was used for comparing means. Data analyses showed a significant difference between weight and WHR (waist to hip ratio) means in two studied groups, also there was a statistically significant difference in food intake (Energy, carbohydrate, protein, micronutrients percent and some of the micronutrients). FBS, HbA1C and LP-PLA2 means showed statistically significant difference (P<0.001) between two groups. This study showed LP-PLA2 is elevated in poorly-controlled patients compared to well-controlled diabetic patients, which may suggest some nutritional factors contributing to the regulation of this enzyme.

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Keywords: LP-PLA2; Well-controlled diabetes; Poorly-controlled diabetes; Nutrition

Introduction

Diabetes mellitus (DM) in adults is a major worldwide health problem that its prevalence is on the rise in many parts of the developing countries in response to increasing prosperity and sedentary lifestyles. It was reported by American Diabetes Association research that 3.8 % of Americans and 7.7% of the Iranian population are suffering from diabetes in 2011 (1).

Lipoprotein-associated phospholipase A2 (Lp-PLA2), a 45.4-kDa protein, is a calcium-independent member of the phospholipase A2 family that hydrolyzes phospholipids. Monocytes, macrophages, T-lymphocytes and liver cells are mainly responsible for the production of this enzyme. In the bloodstream, two-thirds of the Lp-PLA2 plasma isoform circulates primarily bound to LDL (associated mainly with apolipoprotein B), the other third is distributed between high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL). Lp-PLA2 remains unexposed until LDL is oxidized, once this occurs Lp-PLA2 cleaves the oxidized phosphatidylcholine into two bioactive compounds, i.e. lysophosphatidylcholine (LysPC) and oxidized non-esterified free fatty acids (oxNEFA). These compounds contribute significantly to the inflammation associated with atherosclerosis creating a

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positive feedback loop whereby lsyoPC and oxNEFA activate monocytes/macrophage to secrete cytokines which promote Lp-PLA2 release from macrophages (2,3). Both Lp-PLA2 mass and activity have been shown to be independent predictors of coronary heart disease and ischemic stroke in the general population as well as clinical populations and higher levels of circulating Lp-PLA2 appear to be associated with the atherosclerotic burden as assessed by the extension of coronary calcification, endothelial dysfunction and plaque instability (4,5).

There has been always a continuous seek of new risk factors that predict CVD, especially in those groups that are at a higher risk. A suitable factor should be easy to measure, using inexpensive and standardized commercial assays that have low variability, relatively stable and exhibit minimal circadian variation. Regarding these factors Lp-PLA2 provides an attractive target for assessing cardiovascular disease. In a past decade, many cross-sectional and longitudinal (prospective) epidemiologic studies have shown a relationship of Lp-PLA2 with cardiovascular diseases, however, there have been always some conflicts. These studies that assessed the Lp-PLA2 measurements usually funded or performed by the commercial manufacturers. In 2005, US Food and Drug Administration established an assay to measure Lp-PLA2 mass (PLAC®; diaDexus) for the purpose of predicting CVD risk. More recently, some US consensus groups, such as the National Lipid Association, have recommended measurement of Lp-PLA2 in certain clinical circumstances to assist in identifying patients with risk factors that may not clearly indicate the need for drug therapy (6).

Many inflammatory factors and oxidative stresses were investigated in diabetic subjects and their relationship with blood glucose and glycemic control was reported, but as far as we know, there is a paucity of data regarding the Lp-PLA2 mass and activity in diabetic subjects based on glycemic control. We conducted the present study to evaluate relations between Lp-PLA2 mass between glycemic control and nutritional factors in diabetic patients.

Materials and Methods

Subjects and design

80 diabetic patients (men and women) were selected from patients that admitted to the Iran Diabetes Association (Tehran). Patients were excluded if they had a cardiovascular disease history or myocardial infarction, inflammatory disorders, steroid use, pregnancy and insulin therapy. Volunteered patients were selected by the simple random sampling method and were divided into two groups based on their recent HbA1c and fasting blood sugar (well-controlled diabetes, poorly–controlled diabetes). Finally, they were called for filling questionnaires and taking blood samples.

Anthropometry

Body weight (BW) was determined to the nearest 0.1 kg and standing height to the nearest 0.5 cm. BMI was calculated as weight (kg) divided by height squared (m2). Waist circumference was measured using a cloth tape midway between the lowest rib and the iliac crest and hip circumference as the maximum circumference over the buttocks. Waist-to-hip ratio (WHR) was calculated.

Food intake

The food intake was evaluated by semi-quantitative food frequency questionnaire (147 items FFQ). Energy (kcal), carbohydrates (g), lipids (g), proteins (g), cholesterol mg/dl, fiber (g) and micronutrients were measured through the software N4. All anthropometric and food intake assessment was conducted by one expert in order to minimize the possible errors.

Blood sampling

After 10-12 hour fasting, blood was obtained from the antecubital vein and collected in tubes containing citrate. To obtain plasma, blood was centrifuged for 10 minutes at 4000g. Plasma was frozen in -80˚C till they were assessed.

Analytical methods

Lp-PLA2 was quantified by Elisa kit (diaDexus, Inc. South San Francisco, CA, USA) according to its manual. FBS was assessed with a spectrophotometer and ion-exchange chromatography was used for measurement of HbA1c.

Statistical analysis

Preliminary analyses were performed to ensure no violation of the assumptions of normality and linearity. The Kolmogorov - Smirnoff test was used to evaluate the normality of each variable. For basic comparisons, the two group characteristics were primarily examined by independent t-tests. ANOVA analysis used for Lp-PLA2 comparison, with considering all confounders. All analyses were carried out using SPSS 18.0. Statistical significance was established for P-value <0.05 and P-value < 0.001. Data were shown as mean ± SD.

Results

Table 1 shows descriptive statistics on these subjects. There were 40 subjects in the well-controlled diabetes group and 40 subjects in poorly-controlled diabetes group. For eliminating the co-founder effect of sex, we have included 17 men and 23 women in the groups. The range of subject's age was 44 to 68. The mean of Age did not show the significant difference after analysis (*P*= 0.071). Those in the poorly-controlled group had a high cholesterol and hypertension prevalence (*P*<0.001) in spite of high-dose statins consumption. In the anthropometric assessment two groups were different in weight (*P*=0.018) and waist to hip ratio (*P*<0.001), but other parameters (height, BMI, waist circumference and hip circumference) were not statistically different.

Table 2 shows the dietary intake of two groups that was assessed by the FFQ questionnaire.

Table 1. Descriptive statistics for well-controlled diabetes and poorly-controlled diabetes groups

	Well-controlled diabetes (n=40)	Poorly-controlled diabetes (n=40)	*P*-value
Gender	M:17, F:23	M:17, F:23	-
Age (year)	53.1 ± 7.04	55.7 ± 5.4	0.071
Diabetes in years	4.9 ± 2.92	6.4 ± 3.7	0.039*
Hypercholestrolemia	27.5 %	45 %	<0.001**
Statin therapy	17.5 %	40 %	<0.001**
Hypertension	25 %	40 %	<0.001**
Cigarette	5 %	25 %	<0.001**
Weight (kg)	81.87 ± 6.65	86.55 ± 10.2	0.018*
Height (cm)	168.62 ± 7.73	169.77 ± 6.34	0.469
BMI (kg/cm2)	28.94 ± 2.02	29.88 ± 3.33	0.131
Waist (cm)	100.35 ± 22.61	101.32 ± 9.12	0.801
Hip (cm)	109.96 ± 21.42	105.5 ± 11.24	0.252
WHR	0.90 ± 0.07	0.96 ± 0.08	<0.001**

M= male, F= female, BMI= body mass index, WHR= Waist to Hip Ratio

Table 2. Food intake of well-controlled diabetes and poorly-controlled diabetes groups CHO, carbohydrate, Pro, protein.

	Well-controlled diabetes(n=40)	Poorly-controlle diabetes(n=40)	*P*-value
Energy (kCal/d)	2411.9 ± 283.4	2642.5 ± 231.02	<0.001**
CHO (gr/d)	213.5 ± 34.3	236.72 ± 31.7	<0.003*
Protein gr/d)	103.4 ± 20.6	93.6 ± 16.8	<0.023*
Fat (gr/d)	77.9 ± 19.1	75.8 ± 15.9	0.595
Cholesterol (mg/d)	413.2 ± 93.09	453.2 ± 85.2	0.048*
CHO percent	52.2 ± 3.39	58.9 ± 3.84	<0.001**
Pro percent	24.4 ± 4.08	20.8 ± 3.2	<0.001**
Fat percent	23.9 ± 4.68	21.1 ± 4.08	<0.006*
Fiber (gr/d)	18.3 ± 7.4	12.9 ± 2.35	<0.001**
Sodium (mg/d)	6308 ± 1301	6351 ± 1089	0.871
Iron (mg/d)	8.86 ± 3.2	7.97 ± 3.16	0.217
Calcium (mg/d)	751.4 ± 249	731.3 ± 131.4	0.654
Magnesium mg/d)	297.5 ± 93.9	209.3 ± 45.8	<0.001**
Zinc (µg/d)	10.5 ± 4.1	9.5 ± 3.43	0.232
Selenium (µg/d)	34.2 ± 9.05	30.52 ± 9.02	0.073
Vitamin A (µg/d)	963.7 ± 236.1	944.2 ± 169.7	0.672
Vitamin E (mg/d)	8.14 ± 3.09	7.63 ± 3	0.454
Vitamin C (mg/d)	76.46 ± 21.7	54.78 ± 18.39	<0.001**
Vitamin D (µg/d)	4.98 ± 2.4	4.71 ± 2.36	0.622
Vitamin B1 (mg/d)	1.16 ± 0.37	1.07 ± 0.29	0.254
Vitamin B6 (mg/d)	1.51 ± 0.53	1.26 ± 0.4	0.019*
Folat (µg/d)	407.3 ± 163.8	328.5 ± 89.8	0.01*

There was a significant increment in energy, carbohydrate, cholesterol intake and the portion of carbohydrate in the total daily energy intake in poorly-controlled diabetes group. Along with that, a significant increment was observed in well-controlled diabetes group in fiber, magnesium, vitamin C, vitamin B6 and folate intake and the portion of protein and fat intake in total daily energy intake.

FBS, HbA1C and LP-PLA2. As shown in figure 1, like the FBS (P<0.001), HbA1c had a lower concentration in well-controlled group than poorly-controlled group (P<0.001). LP-PLA2 level in Well-controlled diabetes group was 204.5 ng/ml and in the poorly-controlled diabetes group was 236.1 ng/ml which was statistically minor (P<0.002). In well-control patients FBS, HbA1c and LP-PLA2 had a minimum and maximum range of 76 and 206; 5.7 and 6.9; and 139 and 189, respectively. However, in poorly-controlled patients minimum and maximum range of FBS, HbA1c and LP-PLA2 was 150 and 273; 7.8 and 12.6; and 189 and 311, respectively.

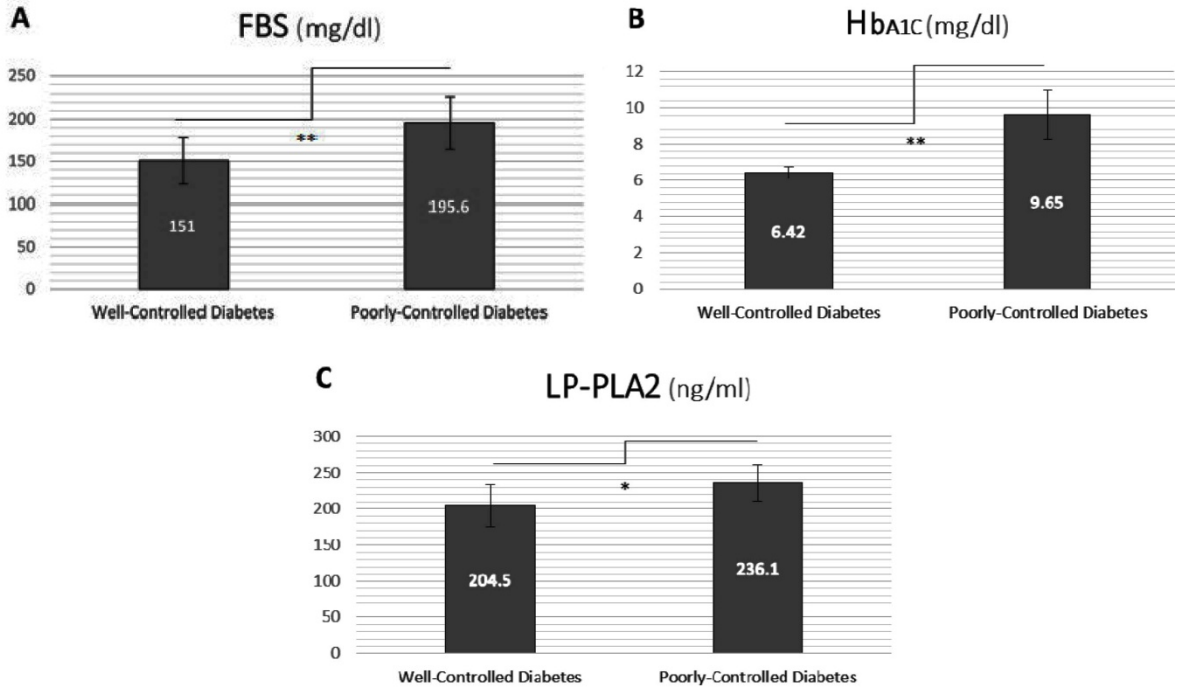

Figure 1. Comparison of FBS(A), HbAC1(B) and LP-PLA2 (C) between Well-controlled diabetes and poorly-controlled diabetes groups (n=40).Data are shown as a mean ± SD. *P < 0.05. ** P < 0.001.

Discussion

Our main goal was to investigate the relationship between some nutritional factors and LP-PLA2 in well and poorly-controlled patients. As we expected, there were so many differences between both groups. We have shown there are significant differences between well-controlled and poorly controlled diabetic patients in factors such as total blood cholesterol, hypertension, smoking and WHR (Table 1). We also demonstrated that LP-PLA2, FBS and HbA1c had a lower concentration in a well-controlled group than poorly-controlled group (Figure 1). In confirmation of our results, previous studies have shown cholesterol level are increased in poorly-controlled diabetic patients (7) and the same is true for hypertension (8), WHR (9) (10) and smoking (11).

By the mean of the semiquantitative FFQ we have clarified that there are significant differences in daily intake of energy and nutrients such as protein, carbohydrate, cholesterol, magnesium, selenium, fiber, vitamin C, vitamin B6 and folate (Table 2). In line with our experiment, some studies suggested that carbohydrate consumption as a percentage of the total energy and low fiber intake are associated with poorly-controlled diabetes, however, in contrast to our findings,

they are mentioned a higher protein intake that it could be due to economic conditions between our studied population (i.e. Developed versus Developing countries) (12). Some experiments have found a lower plasma concentration of vitamin C because of the disease nature itself, not by the dietary intake (13), but our results have shown another possibility. It is not just because of low intake of ascorbic acid in poorly-controlled patients, but may be due to improved intake of well-controlled patients. Like the vitamin C, our results of vitamin B6 consumption have the same reason to be different from other studies (14). Another noteworthy reason that must be mentioned is that the differences between our group selection and design is maybe the cause of the discrepancy compared to other studies, i.e. In our study, we have compared well-controlled diabetic patients to poorly-controlled ones, in contrast to other studies that have chosen healthy subjects to be compared with diabetic patients. Among the micronutrients, it seems that magnesium intake and plasma level had a great association with insulin resistance and type 2 diabetes (15-17). Complementing these results, we have revealed magnesium intake is significantly lower in poorly-controlled patients compared to well-controlled counterparts. There is a decreased plasma level of folic acid in diabetic patients because of the metformin consumption (18), however, some have mentioned a normal plasma status (19). In this study, we have demonstrated a decreased intake of folic acid in poorly-controlled patients. A plausible reason is, probably, the existence of common sources of food between both folic acid and magnesium (20).

FBS and HbA1c both are a reliable marker for monitoring and predicting of type 2 diabetes (21,22). Along with them, LP-LPA2 has shown to be a predictive factor for type 2 diabetes and is associated with insulin resistance (23,24). Furthermore, in this study, we have demonstrated an improvement of these three independent factors in well-controlled diabetic patients. Previous studies have represented that a reduction in LP-PLA2 is achievable by replacing carbohydrates with proteins (25,26). Also, total cholesterol is directly correlated with the LP-PLA2 level (27), however, in animal studies it is shown high cholesterol high fat diet can induce PL-PLA2 activity (28). In our experiment, the well-controlled patient was shown to have a higher fiber intake than poorly-controlled patients. By far as we know, LP-PLA2 is transferred with LDL-cholesterol in the blood (29). On the other hand, both soluble and insoluble fibers can reduce LDL-cholesterol (30,31) so it may be conferred that fibers can decrease LP-PLA2 level by the indirect impact on LDL.

LP-PLA2 have indicated an association with Homocysteine in atherosclerotic patients (32,33). The roles of folic acid, vitamin B6 (34,35) and vitamin C (36) in reducing of homocysteine has been established. So it seems, the different intake of these vitamins in our groups could impact the LP-PLA2 levels. Finally, vegetarians have shown to have a lower LP-PLA2 compared to omnivores (34). Although, magnesium is found abundantly in vegetables (37) but it seems Irrational to attribute the low plasma level of LP-PLA2 to magnesium intake regarding that vegetables are a rich source of either folic acid and vitamin C. Nevertheless, the role of magnesium is ambiguous in modulating of LP-PLA2 because of a shortage of research in this area.

In this study, we assumed that the level of LP-PLA2 is lower in plasma of well-controlled diabetic patients compared to poorly-controlled diabetic patients. As we have shown, in addition to LP-PLA2, HbA1c and FBS had a decreased level in well-controlled patients. Nutritional factors such as carbohydrates, proteins, cholesterol, fiber, magnesium, ascorbic acid, folate and Vitamin B6 have shown to be associated with LP-PLA2. It is acceptable to investigating more nutrients in the aspect of a modulator of LP-PLA2.

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