Low acylation stimulating protein levels are associated with cardiometabolic disorders–secondary to autoimmune activation?

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ABSTRACT

Objective: We investigated the possible association of serum acylation stimulating protein (ASP) with cardiometabolic disorders and the evidence of autoimmune activation.

Methods: Population-based randomly selected 1024 participants were cross-sectionally and prospectively analyzed. ASP concentrations were measured with a validated ELISA kit. Correlations were sought separately in subjects with no cardiometabolic disorders (n=427) designated as "healthy."

Results: ASP was positively correlated with total testosterone and inversely correlated with platelet activating factor (PAF), PAF-acetylhydrolase (AH), in each gender, and positively correlated in "healthy" men with lipoprotein [Lp](a) and apolipoprotein B. Correlations of ASP with PAF values ≥22 nmol/L were abolished, contrasted to a strongly inverse one in subjects with PAF <22 nmol/L. In linear regression analyses in the whole sample, ASP was inversely associated independently with PAF and PAF-AH and, in men, positively with Lp(a) and sex hormone-binding globulin. Prevalent and (at 2.0 years' follow-up) incident metabolic syndrome (MetS, n=393), diabetes (n=154), and coronary heart disease (CHD, n=171) were analyzed by sex-, age-, and Lp(a)-adjusted logistic regression, using tertiles of ASP and PAF. The lower two (<42 nmol/L) ASP tertiles were a risk factor in combined sexes for MetS and diabetes. In women, incident CHD was predicted by either reduced or elevated ASP tertiles.

Conclusion: Findings can be explained by the notion of operation of immune responses against both ASP and oxidized PAF-like lipids of Lp(a) to yield for "reduced" values and increased likelihood of cardiometabolic disorders. *(Anatol J Cardiol 2017; 17: 97-106)*

Keywords: acylation stimulating protein, autoimmunity, type-2 diabetes, lipoprotein(a), metabolic syndrome, platelet activating factor, phospholipids

Introduction

Acylation-stimulating protein (ASP) is an adipokine produced by adipocytes and generated by activation of the alternative complement pathway (1), as well as systemically following pro-inflammatory immune activation (2). ASP stimulates free fatty acid incorporation into adipose tissue by increasing triglyceride synthesis and storage (1), increases glucose uptake through enhanced translocation of glucose transporters, and reduces triglyceride lipolysis in adipocytes via inhibition of hormone sensitive lipase (1). Fasting ASP levels are elevated in subjects with obesity (3, 4), insulin resistance (5), and type-2 diabetes (6, 7). Several metabolic disorders, such as polycystic ovary syndrome (8), renal disease (9), nonalcoholic steatotic hepatitis (5), and dyslipidemia (1), are also associated with increased ASP levels (10), regardless of obesity.

Plasma platelet-activating factor (PAF) is a key lipid mediator and activates cells, including monocytes, through the PAF-receptor. This receptor recognizes a specific acetate residue of PAF and the phospholipids (PAF-like lipids) (11). Oxidized phospholipids are inflammatory compounds and structurally mimic PAF and the PAF-like lipids. Oxidation of low-density lipoproteins (LDL) enhances the ability of certain oxidized phospholipids to interact with and activate the PAF-receptor. PAF-like lipids from oxidized LDLs serve as specific agonists that express the PAF-receptor (12). Target cells are then activated leading to inflammatory and thrombotic responses and aggregation (11).

The PAF intercellular signaling system is regulated by several mechanisms including extra- and intracellular acetylhydrolases. Plasma platelet activating factor acetyl hydrolase (PAF-

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AH) [or lipoprotein-associated phospholipid A2 (Lp-PLA2)] regulates inflammation by terminating signals triggered by PAF and oxidized PAF-like lipids (13). PAF-AH degrades (inactivates by hydrolyzing) not only PAF but also oxidized phospholipids with a specific acetate residue generated in settings of inflammation and oxidant stress (11, 14, 15). The roles of endogenous PAF-AH in atherosclerotic complications have been reviewed by Stafforini (15) and Elisaf (16). Intriguingly, elevated serum levels of Lp-PLA2 are known to significantly predict incident coronary artery disease, independently of and in addition to traditional risk factors (17, 18).

A recent report on a smaller sample of the Turkish Adult Risk Factor (TARF) study showed that correlations of ASP with serum triglycerides, glucose, height and age, among others, diverged in direction across genders (19). The reasons for this divergence remained unclear and required further investigation.

We hypothesized that a dysregulation comprising diminished removal by PAF-AH of the acyl group of oxidized phospholipids [mainly on Lp (a)] and PAF may augment oxidant stress and produce pro-inflammatory immune activation which leads under certain circumstances to aggregation between apoA-I and polypeptide/proteins such as Lp(a), creatinine, or ASP, resulting in reduction in their assayable circulating levels. The present study aims to evaluate in a general population the gender-specific interrelations of the adipokine ASP with PAF, PAF-AH, and Lp(a) and its association with respect to likelihood of metabolic syndrome, type-2 diabetes, and coronary heart disease (CHD). By dissecting the study sample into "healthy" and "non-healthy" groups and using dichotomized PAF values, we sought to uncover specific associations which varied depending on the presence of the pro-inflammatory state.

Methods

The study sample of unselected participants of the TARF study (20, 21) is formed by 1024 middle-aged adults in whom combined measurements of serum ASP with either PAF or PAF-AH were made in the surveys 2011 to 2013. Participants were aged 40 years or over and were residents of all 7 regions of Turkey. The study was approved by the Ethics Committee of the İstanbul University Medical Faculty. Written informed consent was obtained from all participants.

Measurements of risk variables

Waist circumference was measured with the subject standing at the end of gentle expiration at the level midway between the lower rib margin and the iliac crest. Neck circumference was measured at the midway of the neck between mid-cervical spine and mid-anterior neck, if palpable, just below the laryngeal prominence. Status of cigarette smoking was categorized into current smokers, former smokers, and those who had never smoked. Blood pressure (BP) was measured in the seated position on the right arm using an aneroid sphygmomanometer

(Erka, Bad Tölz, Germany) after 5 minutes of rest, and the mean of two recordings was computed.

Concentrations of ASP, PAF, and PAF-AH were determined in sera after an overnight fast with commercially available kits based on the enzyme-linked immunoassay method. ASP was purchased from Biotechist Co. (Beijing, China), PAF from Novatein Biosciences Inc. (MA, USA), and PAF-AH from Cusobio Biotech. Co. (Wuhan, China). Serum concentrations of total cholesterol, fasting triglycerides, glucose, and high-density lipoprotein (HDL)-cholesterol (directly without precipitation) were determined by Cobas C501 chemistry analyzer (Roche Diagnostic GmbH, Mannheim, Germany). Concentrations of insulin, SHBG, and total testosterone were determined by the electrochemiluminescent immunoassay method using Roche kits and Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany). Concentrations of serum Lp(a), apoA-I apoB, CRP, complement C3, and rheumatoid factor were measured by kits and nephelometry of Siemens Healthcare Diagnostic Products (Marburg, Germany).

Definitions

Obesity was categorized by a body mass index (BMI) of 30 kg/m^2. Individuals with diabetes were diagnosed using criteria of the American Diabetes Association (22), namely when plasma fasting glucose was ≥7 mmol/L (or 2-h postprandial glucose >11.1 mmol/L) and/or the current use of diabetes medication. Individuals with metabolic syndrome were identified when 3 out of the 5 criteria of the joint conference (23) were met, modified for male abdominal obesity using as a cut-off point ≥95 cm, as assessed in the TARF study (24).

Diagnosis of CHD was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG (25), or on a history of myocardial revascularization. Typical angina and, in women, age >45 years were prerequisite for a diagnosis when angina was isolated. ECG changes of "ischemic type" of greater than minor degree (Codes 1.1–2, 4.1–2, 5.1–2, 7.1) were considered as myocardial infarct sequelae or myocardial ischemia, respectively. CHD death comprised death from heart failure of coronary origin and fatal coronary event.

Data analysis

Tertiles of ASP concentrations were formed by cut-off of 12.2 and 42.2 nmol/L, and for PAF of 15 and 25 nmol/L. Descriptive parameters were shown as means (±standard deviation [SD]). Due to skewed distribution, geometric means were used uniformly for triglycerides, CRP, insulin, sex hormone-binding globulin (SHBG), testosterone, ASP, PAF, PAF-AH, and Lp(a) values. Two-sided t-tests and Pearson's chi-square tests were used to analyze the differences between means and proportions of groups. Pearson correlations served to analyze bivariate correlations. Any subject with CHD was grouped to CHD, while MetS comprised no subjects with diabetes or CHD. We analyzed correlations of selected pa-

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rameters and linear association of ASP after stratifying to participants with MetS, diabetes, or CHD, and to the remaining subjects herein designated as "healthy" individuals. Multiple linear regression analyses were performed with continuous parameters, expressed in terms of an increment of 1 SD in the independent variable. Sex and age-adjusted associations of the tertiles of the studied variables were assessed in logistic regression analyses for MetS, diabetes and, CHD whereby likelihood estimates (OR) and 95% confidence intervals (CI) were obtained. The gradient across high and low ASP tertiles (11-fold) corresponded to 3.4 SD. A value of p<0.05 on the two-tail test was considered statistically significant. Statistical analyses were performed using SPSS-10 for Windows.

Results

Clinical characteristics of 1024 men and women in the study sample are shown in Table 1, sorted by gender. Sex- and age-adjusted estimated marginal means of non-obese subjects [constituted 57.6% of the sample (=590), of whom 333 males], who had identical age to obese individuals were distinct from obese ones by significantly lower concentrations of systolic BP, fasting glucose, insulin, triglycerides, CRP, and C3 levels, higher PAF-AH and SHBG, and by being more frequently current smokers.

At baseline, 597 subjects were identified with a MetS, diabetes, or CHD, and the remaining 427 individuals were categorized as "healthy." During a follow-up of 2.0 years (total 1140 person-years; 44% of subjects lacked a follow-up) 75 cases of MetS, 28 DM, and 37 CHD developed.

Correlations stratified to gender and presence of cardiometabolic disorders
Correlations of ASP, PAF-AH, and PAF with each other and certain other variables are presented separately in participants without ("Healthy") and with MetS, diabetes, or CHD and strati-

Table 1. Anthropometric and biochemical mean values of the study sample (n=1024), by gender, to be compared with those in non-obese subjects

	Whole sample						Non-obese	
	Men n=494			Women n=530			(57.6%) n=590	
	n	Mean	SD	Mean	SD	P	Mean	SD
Age, years	1024	58.6	10.8	58.8	11	0.73	59.0	11.3
Height, cm	1024	**169.1**	7.2	156.2	7.1	<0.001	**164.6**	9.4
Waist circumference, cm	1024	99.4	11	98.4	13.6	0.20	**92.5**	9.4
Neck circumference, cm	954	39.6	3.1	36.1	3.3	<0.001	36.7	3.1
Body mass index, kg/m²	1024	28.3	4.2	**31.2**	6.4	<0.001	**26**	2.5
Systolic BP, mm Hg	1024	130	19	**133**	21	0.023	**128**	19
Acylation stimulating protein,¶ nmol/L	1024	21.4	3.55	20.2	3.24	0.43	21.2	3.3
Platelet activating factor,¶ nmol/L	811	17.0	2.47	17.5	2.36	0.62	17.4	2.4
PAF-AH,¶ ng/mL	803	213	1.67	203	1.67	0.19	**214.6**	1.67
Total cholesterol, mg/dL	1023	197	42.4	**211**	40.6	<0.001	205.6	44
HDL cholesterol, mg/dL	1023	45	11	**52.4**	13.2	<0.001	**50.3**	13
Fasting triglycerides,¶ mg/dL	1021	142	1.68	141	1.58	0.80	**134.5**	1.63
Fasting glucose, mg/dL	1019	104	40	105.6	48	0.53	**99.8**	41.8
Fasting insulin,¶ mIU/L	992	8.64	2.03	9.15	1.92	0.19	**7.53**	1.95
Apolipoprotein A-I, g/L	999	1.417	0.22	**1.564**	0.26	<0.001	1.493	.258
Apolipoprotein B, g/L	1004	1.084	0.29	1.116	0.29	0.15	1.10	.30
Lipoprotein(a),¶ mg/dL	980	10.63	2.92	**13.05**	2.91	0.003	12.0	2.97
C-reactive protein,¶ mg/L	1015	1.86	2.79	**2.50**	3.04	<0.001	**1.71**	2.92
Complement C3, g/L	471	1.26	0.25	**1.346**	0.30	0.001	**1.239**	0.26
SHBG,¶ nmol/L	955	37.2	1.69	**46**	1.80	<0.001	**44.6**	1.73
Total testosterone,¶ nmol/L	965	**26.2**	4.0	1.23	4.0	<0.001	**6.75**	8.44
Current smokers, n, %	1023	153	**31.1**	65	12.2	<0.001	154	**26.7**
Antihypertensive medication, n, %	1017	162	33.2	270	**51**	<0.001	207	**36**

¶Log-transformed values. Increments of 1 SD in log-transformed values are expressed in terms of a factor of the geometric mean. Significant differences in the non-obese from the obese group are highlighted in bold. HDL - high-density lipoprotein; PAF-AH - platelet-activating factor acetyl-hydrolase; SHBG - sex hormone-binding globulin

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Table 2. Pearson correlations between ASP, PAF-AH, and PAF and certain other variables in men (M) and women (F), stratified to "healthy" and those with MetS, diabetes, or CHD

		ASP n=427		n=597		PAF-AH n=340		n=463		PAF n=342		n=469
		"healthy"		CMet. disease		"healthy"		CMet. disease		"healthy"		CMet.
		r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r
Fasting insulin	M	-0.04	0.54	-0.10	0.12	-0.02	0.82	0.03	0.67	0.07	0.38	*0.13*
	F	-0.03	0.66	-0.02	0.75	0.03	0.67	0.01	0.93	-0.07	0.40	0.00
Neck circumference	M	-0.01	0.88	**-0.11**	0.022	**0.21**	0.007	0.04	0.42	**0.21**	0.011	**0.14**
	F	-0.12	0.12	-0.06	0.17	**0.25**	0.002	**0.13**	0.016	0.10	0.23	*0.10*
Fasting glucose	M	*-0.13*	0.067	-0.05	0.25	-0.02	0.79	0.05	0.35	-0.02	0.84	0.07
	F	0.07	0.33	-0.01	0.89	0.06	0.45	**-0.14**	0.005	-0.00	0.96	-0.01
Lipoprotein(a)¶	M	**0.15**	0.042	0.03	0.51	0.09	0.23	0.01	0.79	0.01	0.94	0.05
	F	-0.08	0.28	-0.05	0.26	0.12	0.16	-0.05	0.20	**0.29**	<0.001	**0.16**
Platelet-activating¶ factor	M	**-0.47**	<0.001	**-0.44**	<0.001	**0.30**	0.001	**0.27**	<0.001			
	F	**-0.40**	<0.001	**-0.36**	<0.001	**0.24**	0.016	**0.29**	<0.001			
PAF-AH¶	M	**-0.22**	0.007	**-0.24**	<0.001							
	F	**-0.27**	0.001	**-0.33**	<0.001							
Apolipoprotein A-I	M	*0.14*	0.06	**0.13**	0.005	-0.07	0.32	**-0.12**	0.023	-0.07	0.37	**-0.11**
	F	0.01	0.90	0.00	0.93	-0.03	0.66	**-0.13**	0.014	-0.01	0.94	*-0.09*
Apolipoprotein B	M	**0.15**	0.033	0.07	0.25	0.03	0.69	0.05	0.51	-0.09	0.24	**-0.14**
	F	-0.01	0.87	**0.22**	<0.001	-0.04	0.60	**-0.15**	0.024	-0.04	0.60	**-0.16**
Testosterone¶	M	**0.38**	<0.001	**0.43**	<0.001	**-0.27**	<0.001	**-0.41**	<0.001	**-0.36**	<0.001	**-.30**
	F	**0.35**	<0.001	**0.35**	<0.001	**-0.24**	<0.001	**-0.31**	<0.001	**-0.40**	<0.001	**-.43**
SHBG¶	F	*-0.15*	0.05	**-0.10**	0.024	-0.05	0.52	-0.05	0.38	0.06	0.50	*0.09*
Complement C3	M	-0.11	0.30	**-0.13**	0.04	-0.03	0.78	0.08	0.29	-0.06	0.62	-0.04
	F	-0.12	0.28	**-0.14**	0.021	0.11	0.22	-0.05	0.49	0.03	0.81	0.05
Fast. triglyceride¶	M	-0.05	0.49	0.03	0.71	*0.14*	0.05	**0.14**	0.008	0.01	0.88	-0.01
	F	-0.09	0.24	**0.23**	0.004	0.10	0.20	-0.04	0.38	0.05	0.51	-0.04

¶Log-transformed values. Significant values are highlighted in bold, borderline significant values in italics. CMet. - Cardiometabolic disease. AH - acetylhydrolase; ASP - acylation stimulating protein; PAF - platelet activating factor; SHBG-sex hormone-binding globulin

fied to gender in Table 2. ASP was inversely correlated with PAF-AH, PAF, C3, and SHBG, and positively with total testosterone and apoA-I in men, irrespective of presence of cardiometabolic disorders. In "healthy" males, ASP was positively correlated with Lp(a) and apoB, while being inversely correlated with neck circumference in men with cardiometabolic disorders. While fasting insulin levels were not correlated with any of the 3 substances in each gender or health status, apoB was correlated with ASP in healthy men, and inversely with PAF in men with cardiometabolic disorders, as well as with any of the 3 substances in women with cardiometabolic disorders.

Figure 1 shows correlations between log-transformed ASP and PAF assays in 811 subjects with or without cardiometabolic disorders, separately in sexes and dichotomized categories of PAF. Correlations were uniformly strongly inverse in subjects having serum PAF <22 nmol/L, in clear contradistinction to those at PAF ≥22 nmol/L, independent of the gender or health status.

Linear regression analysis models for ASP were constructed separately in the sexes (Table 3) using PAF, PAF-AH, Lp(a), and SHBG. These showed Lp(a) to be significantly associated in men with 1.4-fold ASP values, independent of SHBG, PAF-AH, or PAF; such association was strongly mediated by PAF and PAF-AH in women.

Findings in logistic regression using ASP, PAF tertiles, and Lp(a) for the likelihood of prevalent and incident cases at final examination for the combined cardiometabolic disorders are presented in Table 4. Compared with the highest ASP tertile, the lowest tertile disclosed significant 1.6 to 2-fold ORs with MetS and diabetes, respectively. The low ASP tertile and elevated Lp(a) levels in men tended to be associated with CHD likelihood.

Table 5 shows results of logistic regression of categories using circulating ASP, PAF tertiles, and Lp(a) for the incidence of the three cardiometabolic disorders. The two low ASP tertiles combined tended to independently predict MetS in both sexes and, in men, diabetes as well as CHD. Low and high ASP tertiles
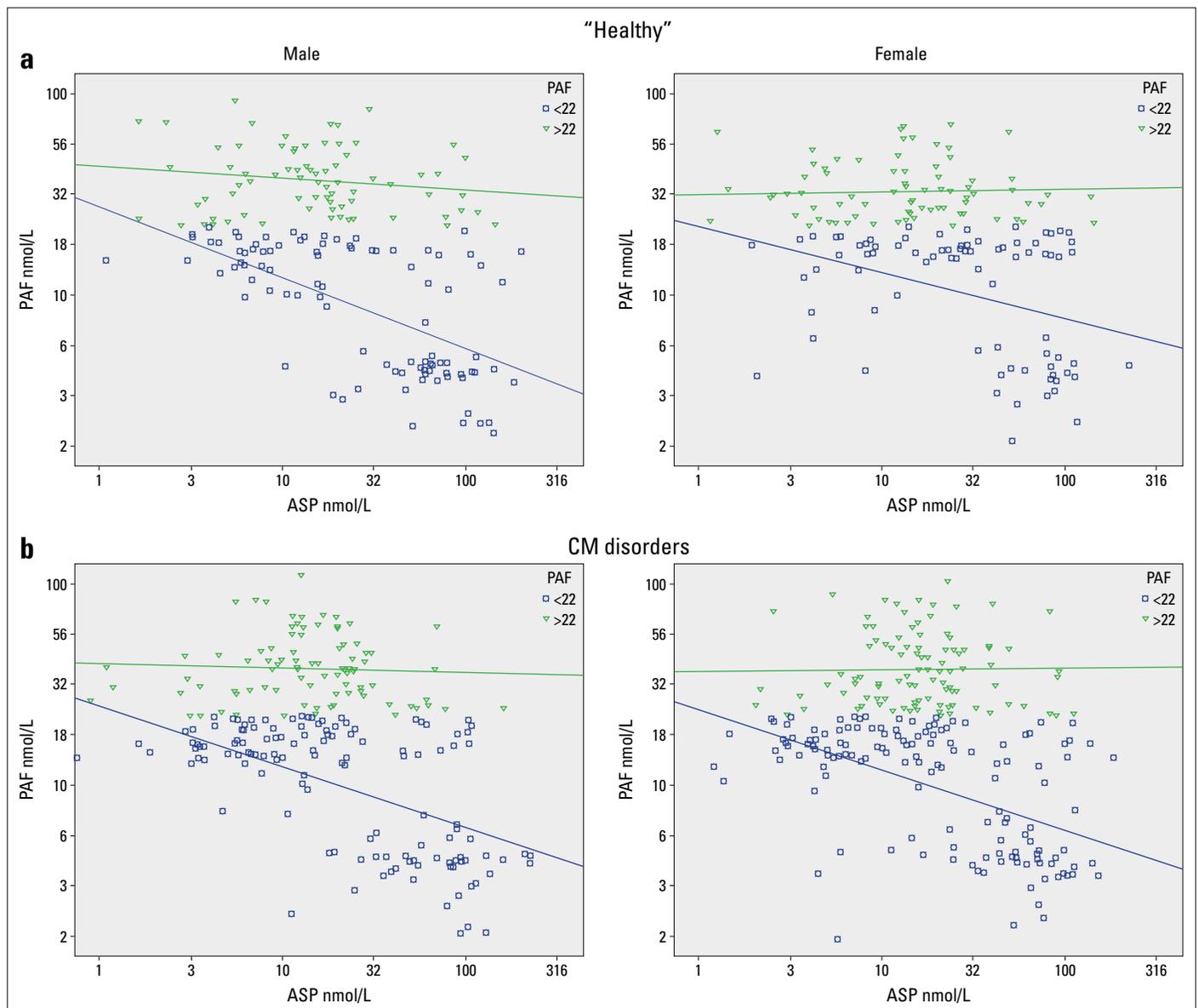
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Figure 1. The plot depicts the correlations between log-transformed acylation stimulating hormone (ASP) and dichotomized platelet activating factor (PAF) concentrations in (a) 177 men (left) and 165 women without cardiometabolic disorders and (b) 221 men (left) and 248 women with cardiometabolic disorders. Individuals with PAF <22 nmol/L (indicated in blue) demonstrate strongly inverse relationship to ASP (ranging from r=-0.52 to -0.64) regardless of gender or health status. This stands in sharp contrast to people with PAF >22 nmol/L (in green), in whom correlations are abolished (ranging from r=-0.18 to +0.05)

combined predicted CHD in women with an OR 6.45 (95% CI 1.35; 30.8) compared to the mid-tertile.

Discussion

In a cross-sectional and brief prospective analysis of a middle-aged population-based sample prone to MetS, we found novel associations of ASP, PAF, and PAF-AH with cardiovascular risk factors as well as cardiometabolic disorders. Regardless of gender and the presence of cardiometabolic disorders, ASP was inversely correlated with PAF-AH, PAF, and C3, and positively with total testosterone and, in men, with apoA-I. ASP was linearly and significantly associated in men with Lp(a), independent of SHBG, PAF-AH, or

PAF. Correlations of ASP with PAF strongly differed in direction depending on dichotomized PAF values. Finally, logistic regression analyses with ASP tertiles indicated that reduced ASP (<42 nmol/L) was associated in both sexes with the risk of MetS and diabetes. In women, incident CHD was predicted by both reduced and elevated, compared to intermediate, ASP tertiles. Findings may be explained by the operation of immune responses against both ASP and oxidized PAF-like lipids of Lp(a), which rendered "reduced" values to be associated with an increase in cardiometabolic disorders.

ASP concentrations in gender and obesity

Though data are scarce on plasma ASP levels, it has been reported that these range in non-obese people from 10 to 58 nmol/L

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Table 3. Multivariable linear regression analysis for acylation stimulating protein in men and women (n=772*)

	Men, n=361			**Women,** n=373		
	ß coeff.	**SE**	*P*	**ß coeff.**	**SE**	*P*
Age, 11 years	*0.93*	1.03	0.066	**0.93**	1.02	<0.001
Lipoprotein(a)¶	1.07-fold	1.14	0.63	1.02-fold	1.13	0.86
PAF¶	**0.26**-fold	1.16	<0.001	**0.32**-fold	1.17	<0.001
SHBG¶	**2.04**-fold	1.36	0.021	0.83-fold	1.26	0.44
constant	**53.7**	1.64	<0.01	**237.7**	1.64	<0.001
r^2	19.5%, *P*<0.001			15%, *P*<0.001		
Model 2	n=361			n=373		
Age, 11 years	0.98	1.03	0.45	0.99	1.02	0.76
Lipoprotein(a)¶	**1.36**-fold	1.14	0.02	*0.81*-fold	1.12	0.059
SHBG¶	0.89-fold	1.35	0.70	**0.67**-fold	1.22	0.039
PAF-AH¶	**0.24**-fold	1.32	<0.001	**0.20**-fold	1.26	<0.001
constant	**783**	2.23	<0.001	**2600**	1.93	<0.001
r^2	7%, *P*<0.001			13%, *P*<0.001		
Model 3	n=377			n=395		
Age, 11 years	0.97	1.03	0.25	0.98	1.02	0.25
Lipoprotein(a)¶	**1.36**-fold	1.14	0.018	*0.82*-fold	1.11	0.07
PAF-AH¶	**0.26**-fold	1.31	<0.001	**0.19**-fold	1.26	<0.001
constant	**606**	2.07	<0.001	**1820**	1.79	<0.001
r^2	7%, *P*<0.001			12.5%, *P*<0.001		
"*Healthy*" participants onlyŤ	n=186			n=186		
Age, 11 years	**0.91**	1.04	0.029	0.93	1.03	0.25
Lipoprotein(a)¶	*1.39*-fold	1.21	0.08	0.82-fold	1.21	0.34
SHBG¶	1.84-fold	1.58	0.39	0.50-fold	1.53	0.10
constant	**25.3**	2.04	<0.001	**146**	2.28	<0.001
r^2	3%, *P*=0.06			2.5%, *P*=0.12		

¶Log-transformed values. *Sample size limited by PAF and PAF-AH values. ß coefficients are expressed for 1SD increment in age and in the log-transformed variables. ŤHaving no MetS, diabetes, or coronary disease. Significant values are highlighted in bold, borderline significant ones in italics. PAF - platelet-activating factor; PAF-AH - platelet-activating factor acetyl-hydrolase; SHBG - sex hormone-binding globulin

and may double in cardiometabolic disorders (26). Geometric mean levels of ASP (21.2 nmoL/L) in the current study were similar in the sexes, which is in agreement with previous reports in the non-obese sample, but importantly did not show any increase in obese individuals. This suggests that obesity is linked to an apparent "reduction" of circulating ASP, possibly indicating that serum ASP partly escapes immuno-assay ability in enhanced low-grade inflammation and parallels a recognized "reduction" of circulating Lp(a) in type-2 diabetes in most ethnicities (27).

Plasma ASP levels were correlated with decreased LDL size in Omani men determined by gradient gel electrophoresis (28). In line with this, age-adjusted ASP levels were positively associated in men with Lp(a) levels, mediated by increments in SHBG.

State in "healthy" subjects

Among "healthy" men, serum ASP was significantly correlated with Lp(a) and apoB, a moiety of Lp(a) and a marker of enhanced systemic inflammation. Positive correlations of ASP with total testosterone point to immune complex-induced "reduction" of ASP accompanying declined testosterone levels. ASP was significantly inversely correlated with its precursor C3 and, in women, was inversely associated independently with SHBG. Correlations in each sex suggested that ASP did not uniformly represent insulin sensitivity.

Low PAF values presumably comprise pro-inflammatory ox-PL and indicate sustenance of oxidative damage to PAF and involvement in immune complex. In subjects having elevated PAF values, the inverse correlation with ASP disappears, irrespective of gender and the presence of cardiometabolic disorders, suggesting that both compounds are trapped in the immune complex, instead of PAF alone.

Increased ASP levels in Turkish women with polycystic ovary syndrome were decreased by metformin therapy (29), and serum ASP was increased by sulfonylurea-mediated improved glycemic control in Turkish obese diabetic women, without correcting

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Table 4. Logistic regression analysis of circulating ASP, PAF, and lipoprotein(a) for prevalent metabolic syndrome, type-2 diabetes, and coronary heart disease (including incident cases at final examination)

	OR	95%CI	OR	95%CI	OR	95%CI
MetS	Total, n=393/765†		Men, n=184/375†		Women, n=209/390†	
Age, 11 years	**1.30**	1.12; 1.51	1.09	0.76; 1.56	**1.49**	1.19; 1.86
ASP mid-tertile 12.2–42.nmol/L	**1.51**	1.02; 2.26	**1.80**	1.02; 3.16	1.23	0.69; 2.18
ASP low tertile <12.2 nmol/L	**1.68**	1.12; 2.52	1.34	0.75; 2.37	**2.05**	1.15; 3.65
Lipoprotein(a)¶, 3-fold	1.04	0.94; 1.14	1.04	0.81; 1.34	1.03	0.90; 1.18
PAF mid-tertile, 15–25 nmol/L	**1.81**	1.22; 2.69	1.43	0.74; 2.06	**2.32**	1.29; 4.17
PAF high tertile, >25 nmol/L	1.23	0.87; 1.76	1.24	0.75; 1.87	1.25	0.76; 2.05
Diabetes	Total, n=154/768†		Men, n=74/376†		Women, n=80/392†	
Age, 11 years	**1.24**	1.05; 1.49	**1.30**	1.00; 1.67	1.17	0.91; 1.51
ASP mid-tertile, avg. 22.nmol/L	**1.96**	1.14; 3.36	1.88	0.88; 3.99	*2.14*	0.97; 4.74
ASP low tertile avg. 6.4 nmol/L	**2.63**	1.54; 4.49	1.85	0.86; 3.98	**3.77**	1.74; 8.17
Lipoprotein(a)¶, 3-fold	0.99	0.88; 1.12	1.12	0.94; 1.33	0.90	0.75; 1.06
PAF mid-tertile, 15–25 nmol/L	1.47	0.90; 2.40	1.22	0.60; 2.81	1.71	0.86; 3.39
PAF high tertile, >25 nmol/L	1.39	0.90; 2.15	1.66	0.89; 3.10	1.19	0.64; 2.20
CHD	Total, n=171/708†		Men, n=72/349†		Women, n=99/359†	
Age, 11 years	**1.75**	1.46; 2.08	**1.56**	1.20; 2.02	**1.94**	1.49; 2.50
ASP mid-tertile 12.2–42.nmol/L	1.27	0.76; 2.10	1.42	0.66; 3.09	1.10	0.56; 2.17
ASP low tertile <12.2 nmol/L	*1.56*	0.94; 2.58	*1.91*	0.88; 4.13	1.25	0.63; 2.47
Lipoprotein(a)¶, 3-fold	*1.11*	0.98; 1.24	*1.16*	0.97; 1.39	1.07	0.92; 1.26
PAF mid-tertile, 15–25 nmol/L	1.23	0.76; 1.98	1.04	0.50; 2.13	1.35	0.72; 1.59
PAF high tertile, >25 nmol/L	0.95	0.16; 1.48	0.97	0.50; 1.87	0.92	0.51; 1.69

¶Log-transformed values. Significant values are highlighted in bold, borderline significant ones in italics. Referent was high ASP tertile (>42.nmol/L). Sex adjustment was made in the models. ASP - acylation stimulating protein; CHD - coronary heart disease; MetS - metabolic syndrome; PAF - platelet-activating factor

lipid abnormalities (6). Both observations are consistent with an amelioration of immune responses by improved insulin resistance/enhanced inflammation.

Inverse association between ASP and metabolic disorders

Regression analyses using ASP tertiles revealed that reduced ASP levels (<42 nmol/L) were a risk factor in combined sexes for MetS and diabetes. Regarding CHD, decrements in ASP tended to be linearly associated in men, while in women, incident CHD risk showed a U-shaped curve. A large prospective study on urban Chinese (n=6209) demonstrated likewise that MetS was independently predicted by plasma fibrinogen levels in females but not in males (30). Furthermore, excess risk of death is additively conferred in Turkish men by the MIF CC-GC genotype and by reduced circulating Lp (a) assays (31). These findings are consistent with the notion that ethnicity-specific sex-dependent prominent autoimmune activation may abolish the linear association of a biomarker and render manifestation of MetS only in one sex.

Hypothesis

An inflammatory compound, OxPL bound to ApoB-100, mediates adverse effects of Lp(a) (32). Oxidation-specific epitopes are thereby generated that are immunogenetic, pro-inflammatory, and pro-atherogenic (32). We have provided epidemiological evidence to the effect that, in middle-aged populations prone to MetS, or in population subsets with impaired glucose tolerance, excess oxidative stress, especially consequent to inadequate hydrolysis of Lp(a) phospholipids, may impair epitopes of endogenous proteins (33) [such as Lp(a), PAF, ASP, and creatinine] which are thereby no longer fully immunoassayable and simultaneously perceived as foreign bodies by protective proteins such as apoA-I to induce immune responses. These immune processes, together with impaired function of apoA-I, are presumably major drivers of MetS and, in women, of diabetes and CHD.

Oxidation of LDL in Lp(a) is recognized to render oxidized phospholipids (ox-PL) to activate the PAF-receptor on the cellular membrane, a receptor that recognizes the acetate residue of the inflammatory compounds PAF and of ox-PL (which are PAF-like lipids) (15). Macrophages, thus activated, induce inflammatory-thrombotic responses and aggregation. Plasma PAF-AH terminates the triggered signals by hydrolyzing PAF and ox-PL generated in settings of inflammation/oxidant stress. This feedback system is reflected by a lack of association between

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Table 5. Logistic regression analysis of circulating ASP, PAF, and lipoprotein(a) for incident metabolic syndrome, type-2 diabetes, and coronary heart disease

	OR	95%CI	OR	95%CI	OR	95%CI
MetS	Total, n=75/278†		Men, n=49/162†		Women, n=26/116†	
Female sex	0.69	0.39; 1.20				
Age, 11 years	0.99	0.74; 1.31	1.09	0.76; 1.56	0.86	0.52; 1.41
ASP tert. 1+2 vs. 3 ≤42.3 nmol/L	*1.78*	0.92; 3.41	1.51	0.67; 3.40	*2.58*	0.82; 8.06
Lipoprotein(a)¶, 3-fold	1.08	0.88; 1.31	1.04	0.81; 1.34	1.17	0.83; 1.65
PAF low-tertile, <15 nmol/L	1.37	0.67; 2.79	1.57	0.64; 3.82	1.28	0.36; 4.54
PAF mid-tertile, 15–25 nmol/L	1.00	0.51; 1.97	1.43	0.59; 3.46	0.62	0.21; 1.79
Diabetes	Total, n=28/479†		Men, n=15/237†		Women, n=13/242†	
Female sex	0. 08	0.37; 1.74				
Age, 11 years	1.23	0.85; 1.80	*1.61*	0.93; 2.77	1.02	0.56; 1.86
ASP tert. 1+2 vs. 3 ≤42.3 nmol/L	2.17	0.79; 5.94	2.15	0.69; 6.75	0.23	0.03; 1.68
Lipoprotein(a)¶, 3-fold	1.00	0.77; 1.31	1.10	0.92; 1.32	0.80	0.53; 1.21
PAF low-tertile, <15 nmol/L	0.97	0.34; 2.76	0.60	0.13; 2.75	0.73	0.20; 2.73
PAF mid-tertile, 15–25 nmol/L	1.12	0.44; 2.82	1.79	0.53; 6.12	0.55	0.12; 2.43
CHD	Total, n=37/423†		Men, n=17/213†		Women, n=20/210†	
Female sex	1.26	0.64; 2.50				
Age, 11 years	1.30	0.91; 1.84	1.14	0.66; 1.94	*1.54*	0.95; 2.50
ASP tert. 1+2 vs. 3 ≤42.3 nmol/L	0.87	0.41; 1.86	1.96	0.62; 6.19	*0.41**	0.14; 1.18
Lipoprotein(a)¶, 3-fold	0.89	0.70; 1.14	0.97	0.67; 1.40	0.80	0.57; 1.14
PAF low-tertile, <15 nmol/L	1.46	0.57; 3.70	3.33	0.77; 14.4	0.66	0.18; 2.36
PAF mid-tertile, 15–25 nmol/L	1.18	0.46; 3.00	2.13	0.48; 9.47	0.70	0.20; 2.39

Annual incidence 127, 28.8, and 42.8 per 1000 persons. ¶Log-transformed values. RRs for the two continuous variables are expressed in 1SD increment. *Low and high ASP tertiles combined vs mid-tertile predicted with an OR 6.45 (95% CI 1.35; 30.8). ASP - acylation stimulating protein; CHD - coronary heart disease; MetS - metabolic syndrome; PAF - platelet-activating factor

ASP and Lp(a) in women compared to a significant association in men. The enhanced inflammation induces aggregation of both Lp(a) and ox-PL with autoimmune components and results in increased insulin resistance.

Implications and future research

We may deduce that, beyond macrophage migration inhibitory factor and creatinine already demonstrated in the TARF, autoimmune activation encompassing ASP and PAF/oxPL on Lp(a) precede the development of MetS. This information may help in the early detection and eventual prevention of MetS relevant to population segments prone to impaired glucose tolerance. Much future research is needed in this area in different ethnicities.

Study limitations and strengths

The essentially cross-sectional design of the study limits the inference of a causal relationship of elicited findings which, nonetheless, are uniformly and consistently novel. The relatively limited sample size precluded the testing of other potential confounders that might have mediated the associations. Measurement of ASP by mass-spectroscopy might contribute

information, and experimental support for the hypothesis with molecular biology is lacking. The contribution to the scarce knowledge regarding the relative impact of the studied parameters in the general population constitutes a strength. The study sample exhibited a high prevalence of MetS, which represents a strength but may limit the applicability of the findings to certain ethnic populations.

Conclusions

ASP is correlated inversely with PAF, assays of which likely comprise ox-PL on Lp(a). The disappearance of the stated correlation at "reduced" PAF levels suggests involvement of both proteins in autoimmune complex. ASP is independently and linearly associated in men with Lp(a). Reduced ASP (<42 nmol/L) is a risk factor in both sexes for MetS and diabetes, and in men tends to CHD. Prediction of incident CHD by lowest and highest ASP tertiles in women is analogous to serum Lp(a) (34). Assumption of operation of immune responses against ASP and PAF-like lipids on Lp(a) can account for escape from assay of damaged PAF and explain the documented increased likelihood of cardiometabolic disorders.

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References

1. Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. Biochim Biophys Acta 2003; 1609: 127-43. **Crossref**

2. Onat A, Can G, Rezvani R, Cianflone K. Complement C3 and cleavage products in cardiometabolic risk. Clin Chim Acta 2011; 412: 1171-9. **Crossref**

3. Maslowska M, Vu H, Phelis S, Sniderman AD, Rhode BM, Blank D, et al. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. Eur J Clin Invest 1999; 29: 679-86

4. Weyer C, Pratley RE. Fasting and postprandial plasma concentrations of acylation-stimulation protein (ASP) in lean and obese Pima Indians compared to Caucasians. Obes Res 1999; 7: 444-52.

5. Yeşilova Z, Özata M, Öktenli C, Bağcı S, Özcan A, Sanisoğlu SY, et al. Increased acylation stimulating protein concentrations in nonalcoholic fatty liver disease are associated with insulin resistance. Am J Gastroenterol 2005; 100: 842-9. **Crossref**

6. Özata M, Güngör D, Turan M, Özışık G, Bingöl N, Özgürtaş T, et al. Improved glycemic control increases fasting plasma acylation-stimulating protein and decreases leptin concentrations in type II diabetic subjects. J Clin Endocrinol Metab 2001; 86: 3659-64.

7. Yang Y, Lu HL, Zhang J, Yu HY, Wang HW, Zhang MX, et al. Relationships among acylation stimulating protein, adiponectin and complement C3 in lean vs. obese type 2 diabetes. Int J Obesity 2006; 30: 439-46. **Crossref**

8. Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K. Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertil Steril 2009; 91: 213-9. **Crossref**

9. Tang JH, Wen Y, Wu F, Zhao XY, Zhang MX, Mi J, et al. Increased plasma acylation-stimulating protein in pediatric proteinuric renal disease. Pediatric Nephrology 2008; 23: 959-64. **Crossref**

10. St-Pierre DH, Cianflone K, Smith J, Coderre L, Karelis AD, Imbeault P, et al. Change in plasma acylation stimulating protein during euglycaemic-hyperinsulinaemic clamp in overweight and obese postmenopausal women: a MONET study. Clin Endocrinol 2009; 70: 539-46. **Crossref**

11. Castro-Faria-Neto HC, Stafforini DM, Prescott SM, Zimmerman GA. Regulating inflammation through the anti-inflammatory enzyme platelet-activating factor-acetyl hydrolase. Mem Inst Oswaldo Cruz 2005; 100(Suppl 1): 83-91. **Crossref**

12. Marathe GK, Zimmerman GA, Prescott SM, McIntyre TM. Activation of vascular cells by PAF-like lipids in oxidized LDL. Vascul Pharmacol 2002; 38: 193-200. **Crossref**

13. Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu Rev Biochem 2000; 69: 419-45. **Crossref**

14. Karasawa K. Clinical aspects of plasma platelet-activating factor-acetylhydrolase. Biochim Biophys Acta 2006; 1761: 1359-72.

15. Stafforini DM. Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2). Cardiovasc Drugs Ther 2009; 23: 73-83. **Crossref**

16. Elisaf M, Tselepis AD. Effect of hypolipidemic drugs on lipoprotein-associated platelet-activating factor-acetylhydrolase. Implication for atherosclerosis. Biochem Pharmacol 2003; 66: 2069-73.

17. Anderson JL. Lipoprotein-associated phospholipase A2: an independent predictor of coronary artery disease events in primary and secondary prevention. Am J Cardiol 2008; 101(suppl): 23F-33F.

18. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol 2006; 26: 2523-9. **Crossref**

19. Rezvani R, Cianflone K, Onat A, Can G. Apparent sex-specific divergence of acylation stimulating protein levels with respect to metabolic parameters of pathogenetic and clinical relevance. J Endocrinol Metab 2012; 2: 1-10. **Crossref**

20. Onat A, Avcı GŞ, Şenocak M, Örnek E, Gözükara Y. Plasma lipids and their interrelation in Turkish adults. J Epidem Commun Health 1992; 46: 470-6. **Crossref**

21. Onat A. Risk factors and cardiovascular disease in Turkey. Atherosclerosis 2001; 156: 1-10. **Crossref**

22. Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003; 26: 3160-7. **Crossref**

23. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109: 433-8.

24. Onat A, Uyarel H, Hergenç G, Karabulut A, Albayrak S, Can G. Determinants and definition of abdominal obesity as related to risk of diabetes, metabolic syndrome and coronary disease in Turkish men: a prospective cohort study. Atherosclerosis 2007; 191: 182-90. **Crossref**

25. Rose G, Blackburn H, Gillum RF, Prineas RJ. Cardiovascular Survey Methods, 2nd Ed. Geneva, Switzerland; WHO: 1982: 124-7.

26. Munkonda NM, Lapointe M, Miegueu P, Roy C, Gauvreau D, Richard D, et al. Recombinant acylation stimulating protein administration to C3+/- mice increases insulin resistance via adipocyte inflammatory mechanisms. PLoS One 2012; 7: e46883. **Crossref**

27. The Emerging Risk Factors Collaboration. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke and nonvascular mortality. JAMA 2009; 302: 412-23. **Crossref**

28. Saleh J, Wahab RA, Farhan H, Al-Amri I, Cianflone K. Plasma levels of acylation stimulating protein are strongly predicted by

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Low serum ASP and cardiometabolic disorders

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waist/hip ratio and correlate with decreased LDL size in men. ISRN Obesity 2013; 2013: 342802. **Crossref**

29. Öktenli C, Özgürtaş T, Dede M, Sanisoğlu YS, Yenen MC, Yeşilova Z, et al. Metformin decreases circulating acylation stimulating protein levels in polycystic ovary syndrome. Gynecol Endocrinol 2007; 23: 710-5. **Crossref**

30. Ding L, Zhang C, Zhang G, Zhang T, Zhao M, et al. A new insight into the role of fibrinogen in the development of metabolic syndrome from a prospective cohort study in urban Han Chinese population. Diabetol Metab Syndr 2015; 7: 110. **Crossref**

31. Onat A, Can G, Çoban N, Dönmez İ, Çakır H, Ademoğlu E, et al. Lipoprotein(a) level and MIF gene variant predict incident meta-

bolic syndrome and mortality. J Invest Med 2016; 64: 392-9.

32. Miller YI, Tsimikas S. Oxidation-specific epitopes as targets for biotheranostic applications in humans: biomarkers, molecular imaging and therapeutics. Curr Opin Lipidol 2013; 24: 426-37. **Crossref**

33. Onat A, Can G. Enhanced proinflammatory state and autoimmune activation: a breakthrough to understanding chronic diseases. Curr Pharm Design 2014; 20: 575-84. **Crossref**

34. Onat A, Çoban N, Can G, Yüksel M, Karagöz A, Ademoğlu E, et al. Low "quotient" Lp(a) concentration mediating autoimmune activation predicts cardiometabolic risk. Exp Clin Endocr Diabetes 2015; 123: 11-8.

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