Adipocytokines in primary antiphospholipid syndrome: potential markers of low-grade inflammation, insulin resistance and metabolic syndrome

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Abstract

Objective

This study was undertaken to evaluate a possible association of adipocytokines with metabolic syndrome (MetS), inflammation and other cardiovascular risk factors in primary antiphospholipid syndrome (PAPS).

Methods

Fifty-six PAPS patients and 72 controls were included. Adiponectin, leptin, visfatin, resistin, plasminogen activator inhibitor-1 (PAI-1), lipoprotein (a), glucose, ESR, CRP, uric acid and lipid profiles were measured. The presence of MetS was determined as defined by the International Diabetes Federation (IDF), and insulin resistance was rated using the homeostasis model assessment (HOMA) index.

Results

Concentrations of leptin were higher [21.5 (12.9-45.7) ng/mL] in PAPS patients than in the controls [12.1 (6.9-26.8) ng/mL), p=0.001]. In PAPS patients, leptin and PAI-1 levels were positively correlated with BMI (r=0.61 and 0.29), HOMA-IR (r=0.71 and 0.28) and CRP (r=0.32 and 0.36). Adiponectin levels were negatively correlated with BMI (r=-0.28), triglycerides (r=-0.43) and HOMA-IR (r=-0.36) and positively correlated with HDL-c (r=0.37) and anti-β2GPI IgG (r=0.31). The presence of MetS in PAPS patients was associated with higher levels of leptin (p=0.002) and PAI-1 (p=0.03) levels and lower levels of adiponectin (p=0.042). Variables that independently influenced the adiponectin concentration were the triglyceride levels (p<0.001), VLDL-c (P=0.002) and anti-β2GPI IgG (p=0.042); the leptin levels were BMI (p<0.001), glucose (p=0.046), HOMA-IR (p<0.001) and ESR (p=0.006); and the PAI-1 levels were CRP (p=0.013) and MetS (p=0.048).

Conclusion

This study provides evidence that adipocytokines may be involved in low-grade inflammation, insulin resistance and MetS in PAPS patients.

Key words

adipocytokines, inflammation, insulin resistance, metabolic syndrome, primary antiphospholipid syndrome

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Introduction

Antiphospholipid syndrome (APS) is an autoimmune thrombophilic condition characterised by venous and/or arterial thromboses that may or may not be associated with pregnancy complications and are accompanied by the presence of antiphospholipid antibodies (aPL) (1). APS is associated with higher rates of cardiovascular disease (CVD), in part due to accelerated atherosclerosis (2). This phenomenon is the result of traditional cardiovascular risk factors, but the contributions of autoimmune and inflammatory processes have been increasingly recognised (3, 4). In this regard, Sacré *et al*. (5) showed that the prevalence of occult myocardial ischaemia was more than 7 times higher in the patients with APS compared to the controls, despite the low cardiovascular risk, as calculated using the Framingham risk equation.

One of the emerging factors that regulates CVD in APS patients is adipose tissue, which has endocrine functions and is the main source of several mediators known as adipocytokines. These substances have been recognised as key regulators of insulin sensitivity and are powerful predictors for the development of metabolic syndrome (MetS) (6, 7) due to their active participation in the regulation of physiologic and pathologic processes, including immunity and inflammation (8). Although adipocytokines play a key role in the interplay between obesity, inflammation, insulin resistance and atherosclerosis (9), the exact nature and the relative contribution of adipocytokines as potential markers warrant investigation in primary antiphospholipid syndrome (PAPS). In fact, leptin, resistin, visfatin and plasminogen activator inhibitor-1 (PAI-1) have pro-inflammatory and atherogenic properties and are associated with insulin resistance (IR) (10, 11). On the other hand, adiponectin has anti-diabetic, anti-inflammatory and anti-atherogenic effects (12, 13).

Recently, a study observed that patients with systemic lupus erythematosus have increased levels of adiponectin, leptin and visfatin; additionally, lower concentrations of adiponectin and higher concentrations of leptin are associated with IR, body mass index (BMI) and C-reactive protein (CRP) levels (14). In patients with rheumatoid arthritis (RA), leptin is associated with insulin resistance but paradoxically attenuates the effects of insulin resistance on coronary calcification (15).

No data are currently available regarding the association between adipocytokines in patients with PAPS despite the recent report describing a high prevalence of MetS in these patients (2).

The objective of this study, therefore, was to evaluate in PAPS patients a possible association of adipocytokines with inflammation, MetS, PAPS characteristics and other cardiovascular risk factors.

Patients and methods

Patients

A cross-sectional study was conducted that included fifty-six adult patients with PAPS (16) who were followed up at the Antiphospholipid Outpatient Clinic of the Rheumatology Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo from August 2009 to July 2010. Seventy-two age- and sex-matched individuals from the community composed the control group (Fig. 1). The controls did not meet the criteria for APS or any other other autoimmune disease. All participants underwent clinical evaluation with standardised interviews, and all medical charts were extensively reviewed. A blood sample after a 12-hour fasting period was also collected at the time of the study. Exclusion criteria included the presence of secondary APS, an age of less than 18 years, inherited thrombophilias, pregnancy and/or the use of drugs that may interfere with adipocytokine levels, such as statins, methotrexate, hydroxycloroquine, insulin and glitazones. The following clinical parameters were evaluated: venous thrombosis (documented deep vein thrombosis and/or pulmonary embolism), arterial thrombosis (clinically documented stroke, transient ischaemic attacks or acute myocardial infarction), livedo reticularis, thrombocytopenia (<100,000 platelets on at least in two distinct occasions), recurrent spontaneous abortions and in utero foetal loss.

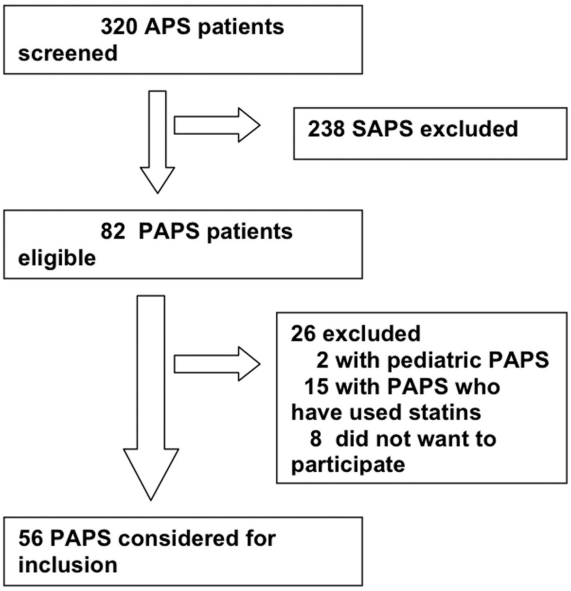

Fig. 1. Trial profile of the study patients screened for inclusion according to the Sapporo criteria for antiphospholipid syndrome (APS).

BMI was calculated based on the following formula: weight/height^2 (kg/m^2); waist circumference was also measured. Obesity was defined as BMI ≥30 kg/m^2 (17). Blood pressure was determined as the average of two measurements that were recorded 5 min apart after the subjects had rested in a supine position for 10 min. Family history of premature coronary artery disease (CAD) was defined as having a first-degree relative with a history of myocardial infarction before the age of 55 years in men or 65 years in women (17). The Framingham risk score was applied to estimate the 10-year risk for CAD and was expressed as a percentage (17).

Dyslipidemia was defined as plasma high-density lipoprotein cholesterol <40 mg/dL, total cholesterol >200 mg/dL, low-density cholesterol >130 mg/dL, triglycerides >150 mg/dL or drug treatment for elevated LDL or TG (17). This study was approved by the Local Ethics Committee, and all subjects signed an informed consent form.

Laboratory evaluation

Immunological and biochemical analyses were performed using the same serum samples. Glucose, uric acid, TSH, free T4 and insulin levels were also measured. Insulin levels were measured using an immunofluorometric assay and reported as μU/mL.

Lipoprotein (a). Lp (a) was measured using an immunoturbidimetric technique with a commercial kit (DiaSorin, Sallugia, Italy). The instrument calibration was performed using calibrators supplied by the kit. The levels of change were defined as those greater than 30 mg/dL.

Adipocytokines. Adipocytokine levels were measured with a personalised panel in which four analytes (leptin, adiponectin, resistin and plasminogen activator inhibitor-1) were assayed using a multiplexed biomarker immunoassay technology (Luminex® Multi-Analyte Profiling x MAP®, Massachusetts, USA). Visfatin was analysed separately by enzyme immunoassay, using the Visfatin C-terminal (Human) EIA kit (Phoenix Pharmaceuticals, Inc. USA) according to the manufacturer's instructions. Intra-assay variation was <10%, and inter-assay variation was <15%.

Serum immunologic analysis. Anticardiolipin antibodies were detected using a commercially available ELISA kit (Enzyme Immunoassay Kit, BIND-AZYME™, Birmingham, UK). The normal cutoffs (based on an assay of 102 samples) have been determined to be 11 GPL U/mL and 11 MGL U/mL. The cutoff values were as follows: <11 GPL/MPL U/mL, negative; ≥11 and <20 GPL/MPL U/mL, indeterminate; and ≥20 GPL/MPL U/mL, positive (18). Lupus anticoagulant (LAC) levels were detected using an activated partial thromboplastin time test (APTT-Diagnostica Stago, France) and a diluted Russel's viper venom time test (dRV-VT-Trinity Biotech, Wicklow, Ireland, UK) according to international guidelines (19). Serum IgG and IgM anti-beta-2-glycoprotein I were detected using an ELISA technique (ORG 521 Anti-beta-2-Glycoprotein I IgG/IgM Mainz,Germany) with cutoff values of 8 U/mL for IgM and for IgG with intra-assay variations of 2.1-5.0% and 2.1-3.8% and inter-assay variations of 2.6-7.95 and 4.1-6.3 for IgG and IgM, respectively.

Lipid profiles. Total cholesterol and triglycerides in serum samples were measured enzymatically (Boehringer Mannheim, Buenos Aires, Argentina, and Merck, Hohenbrunn, Germany, respectively) on a Technicon RA 1000 Analyzer (Technicon Instruments, Tarrytown, NY) (20, 21). HDL cholesterol was obtained after the precipitation of VLDL cholesterol from the serum, LDL cholesterol was isolated using phosphotungstic acid and magnesium chloride (22) and serum levels were determined using the colorimetric method (Roche Diagnostics, Mannheim, Germany). Levels of VLDL cholesterol and LDL cholesterol were estimated because all samples had a triglyceride level of <400 mg/dl (23). VLDL cholesterol levels were determined using the triglyceride level/5 ratio (TG/5) (23), and LDL cholesterol levels were estimated using the following equation: total cholesterol = HDL + TG/5 + LDL (23).

Inflammation markers. High-sensitive CRP levels of all patients were determined by nephelometry, and the results were expressed in mg/L. Erythrocyte sedimentation rates (ESR) were evaluated using the modified Westergren method, and the results expressed as mm/1st hour.

Metabolic syndrome definition. Patients with PAPS and controls were classified as having metabolic syndrome based on the criteria of the International Diabetes Federation (IDF) (24). In 2005, the IDF defined MetS as follows: central obesity, defined as a waist circumference with ethnicity-specific values (waist circumference >80 cm in women and >90 cm in men) plus any two of the following four factors: (1) fasting glucose >100 mg/dL or previously diagnosed

type 2 diabetes; (2) fasting TG >150 mg/d or specific treatment for this dyslipidemia; (3) HDL <40 mg/dL in men and <50 mg/dL in women or the use of a specific treatment for dyslipidemia; or (4) systolic blood pressure (BP) ≥130 mm Hg and/or diastolic BP ≥85 mm Hg or the use of treatment for previously diagnosed hypertension (24).

Insulin resistance. Based on the Inherited Risk of Coronary Atherosclerosis (SIRCA) data (25), the presence of insulin resistance was defined by the homeostasis model assessment (HOMA) index >2.114 that is representative of the top quartile of a non-diabetic population.

Statistical analysis

Demographics and clinical characteristics are expressed as mean ± standard deviation (SD) for continuous variables or as frequencies and percentages for categorical variables. The median (interquartile range) was calculated for continuous variables that were not normally distributed. Comparisons between the patients and the controls as well as between the patients with and without MetS were made using Student's *t*-test or the Mann-Whitney test for continuous variables. Pearson's chi-squared test or Fisher's exact test was used for categorical variables.

We used Spearman correlations to examine the association between adipocytokines, cardiovascular risk factors and markers of inflammation. To determine which factors were independently associated with each adipocytokine, the variables that were significant in the univariate analysis were included in the multivariate linear regression model. It was performed to check the adjustments of the models using the residual analysis. *P*-values less than 0.05 were considered significant. All analyses were performed using statistics software (SPSS 15.0, Chicago, USA).

Table I. Demographics, cardiovascular risk factors, lipid profiles and adipocytokine levels in PAPS patients and controls.

Characteristics	Patients with PAPS (n=56)	Control subjects (n=72)	*p* value
Demographic characteristics			
Age, years	39.8 ± 10.9	38.4 (11.6)	0.50
Sex, n. (% female)	46 (82.1)	59 (82)	0.97
Caucasian race, n. (%)	49 (87.5)	59 (86.3)	0.82
PAPS duration (months)	87.41 ± 67.03		
Cardiovascular risk factors			
Family history of CAD (%)	17 (30.4)	2 (2.8)	<0.001
Framingham score (%)*	3 (1-5.8)	1 (1-3)	0.007
History of diabetes n. (%)	4 (7.1)	1 (1.4)	0.1673
History of hypertension n. (%)	18 (32.1)	5 (1.4)	<0.001
Obesity n. (%)	16 (28.6)	3 (5.3)	<0.001
Dyslipidemia n. (%)	25 (44.6)	33 (45.8)	0.89
Tobacco use n. (%)	7 (12.5)	9 (12.5)	1.0
BMI (kg/m2)	28.2 ± 6.9	23.9 ± 3.18	<0.001
Waist circumference	91.4 ± 16.3	80.9 ± 8.6	<0.001
HOMA-IR index	1.6 (0.8-2.7)	1.0 (0.7-1.5)	0.01
Insulin (uU/mL)	10.2 (9.9)	6.8 (4.3)	0.02
Glucose (mg/dL)	82.1 (19.4)	75.0 (10.8)	0.02
Uric acid (mg/dL)	4.6 (1.7)	4.4 (1.1)	0.28
MetS (IDF) n. (%)	15 (26.8)	5 (6.8)	0.002
Lipid profile			
Triglycerides (mg/dL)	122.8 (67.2)	103.2 (58.8)	0.06
Total cholesterol (mg/dL)	188.3 (40.7)	195.4 (44.9)	0.39
HDL cholesterol (mg/dL)	50.6 (14.3)	60.30	<0.001
LDL colesterol (mg/dL)	114.6 (36.9)	112.9 (41.0)	0.79
VLDL colesterol (mg/dL)	23.7 (12.4)	19.4 (9.2)	0.05
Lipoprotein A (mg/dL)	25 (10-47)	14 (6.8-42.8)	0.28
Adipocytokines			
Adiponectin (ug/mL)	17.6 (14.3)	13.5 (9.3)	0.10
Resistin (ng/mL)	16.6 (6.3)	15.3 (6.5)	0.23
Leptin (ng/mL)	35.5 (32.9)	17.0 (12.5)	0.001
Visfatin (ng/mL)	6.5 (5.5-7.4)	6.0 (5.0-8.5)	0.689
Total PAI-1 (ng/mL)	17.6 (6.2)	17.4 (6.8)	0.77
Inflammation markers			
CRP (mg/L)	3.5 (0.9-7.4)	2.5 (1.0-3.1)	0.03
ESR (mm/1st hour)	12.0 (11.9)	6.2 (4.3)	0.002

PAPS: primary antiphospholipid syndrome; BMI: body mass index; CAD: coronary artery disease; *Within the next 10 years, calculated using the Framingham risk equation; HOMA-IR: homeostasis model assessment index; MetS: metabolic syndrome; IDF: International Federation Diabetes; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: Very low density lipoprotein; CRP: C-reactiveprotein; ESR: erythrocyte sedimentation rate. Data are presented as mean (standart desviations) or percentages or median (interquartile range).

Results

Comparison between the patients and the controls

Patients with PAPS and control subjects were comparable in terms of age (39.8±10.9 *vs*. 38.4±11.6 years, *p*=0.50), sex (82.1 *vs*. 82.0% female, *p*=0.97) and race (87.5 *vs*. 86.3% Caucasian, *p*=0.82), respectively. Arterial thrombosis was observed in 48.2% of the patients, stroke in 33.9%, venous thrombosis in 67.9%, deep venous thrombosis in 53.6%, pulmonary thromboembolism in 26.8%, obstetric events in 39.3%, livedo reticularis in 39.3%, thrombocytopenia in 16.1% and angina in 7.1% of the cases. LAC positivity was observed in 78.6% of the patients, IgG anticardiolipin in 40.7%, IgM anticardiolipin in 16.7%, IgG anti-β2-GPI in 33.9% and IgM anti-β2-GPI in 16.1% of the patients.

The following cardiovascular risk factors were detected more often or were higher in the patients compared to the controls: family history of CAD (30.4 *vs*. 2.8%, *p*<0.001), Framingham score [3 (1-5.8) *vs*. 1 (1–3)%, *p*=0.007], diabetes (7.1 *vs*. 1.4%, *p*=0.16), systemic arterial hypertension (32.1 *vs*. 11.9%, *p*<0.001), BMI (28.2±6.9 *vs*. 23.9±3.18) kg/m^2, *p*<0.001] and lower HDL-c levels [48 (40-61) *vs*. 61 (49–69), *p*<0.001]. Waist circumference was higher in PAPS patients than in controls (91.4±16.3 *vs*. 80.9±8.6), *p*<0.001) as were the HOMA index [1.6 (0.8–2.7)

vs. 1.0 (0.7–1.5), p=0.01] and glucose levels [82.0 (69.3–91.8) *vs*. 75.5 (67.0-82.8) mg/dL, p=0.02]. TSH and free T4 levels did not differ between the patients and the controls (p>0.05). The prevalence of MetS (IDF) was significantly higher in the PAPS group than in the control group (26.8 *vs*. 6.8%, p=0.002). The levels of inflammatory markers, such as CRP [3.5 (0.9–7.4) *vs*. 2.5 (1.0–3.1) mg/L), p=0.03] and ESR [8.0 (5.0–14.0) *vs*. 6.0 (3.0–8.0) mm/1st hour), p=0.002], were significantly higher in the PAPS group relative to the control group (Table I).

Adipocytokines in patients with PAPS and controls

Concentrations of leptin [21.5 (12.9–45.7) *vs*. 12.1 (6.9–26.8) ng/mL), p=0.001] were higher in PAPS relative to the controls. No significant differences were found in the concentrations of adiponectin (p=0.10), resistin (p=0.23), visfatin (p=0.68) or PAI-1 (p=0.77) in both groups.

Correlation between adipocytokines and cardiovascular risk factors and inflammation in PAPS

Adiponectin levels were inversely correlated with BMI (-0.28, p=0.041), VLDL-c (r=-0.41, p=0.003), triglyceride levels (r=-0.43, p=0.001) and HOMA-IR (r=-0.36, p=0.010) but positively correlated with HDL-c (r= 0.37, p=0.006), aCL IgG (r=0.41, p=0.02) and anti-β2GPI IgM (r=0.38, p=0.005). Leptin levels were positively correlated with BMI (r=0.61, p<0.001), waist circumference (r=0.53, p<0.001), glucose (r=0.50, p<0.001), HOMA-IR (r=O.71, p<0.001), PAI-1 (r=0.38, p=0.004), CRP (r=0.32, p=0.020) and ESR (r=0.28, p=0.041). PAI-1 levels were positively correlated with CRP (r=0.32, p=0.0020) and tended to be positively associated with HOMA-IR (r=0.28, p=0.050) but were inversely correlated with aCL IgG (r=-0.53, p<0.001) and anti-β2GPI IgG levels (r=-0.45, p=0.001). The resistin levels were inversely correlated with lipoprotein (a) (r=-0.44, p=0.001), and visfatin levels were not significantly correlated with any metabolic components (Table II, Fig. 2).

Table II. Correlations among adipocytokines, cardiovascular risk factors and inflammation markers in patients with PAPS.

	Adiponectin	Resistin	Leptin	Visfatin	PAI-1
Age	-0.08	0.72	0.07	0.06	-0.03
Duration of PAPS (months)	0.10	0.14	0.09	0.06	-0.16
Body mass index	-0.28‡	0.08	0.61†	-0.05	0.29‡
Abdominal circunference	-0.22	0.16	0.53†	0.12	0.17
Systolic blood pressure	-0.15	0.07	0.17	0.001	-0.07
Diastolic blood pressure	-0.04	0.11	0.00	0.18	-0.08
Glucose (mg/dL)	-0.19	0.10	0.50‡	0.17	0.10
Total cholesterol (mg/dL)	-0.17	-0.20	0.20	0.01	0.10
High-density lipoprotein (mg/dL)	0.37‡	-0.02	-0.08	-0.10	-0.16
Low-density lipoprotein (mg/dL)	-0.22	-0.21	0.16	-0.01	0.10
Very low-density lipoprotein (mg/dL)	-0.41‡	-0.07	0.20	0.06	0.25
Lipoprotein (a) (mg/dL)	-0.08	-0.44‡	0.00	-0.11	-0.05
Triglycerides (mg/dL)	-0.43‡	-0.13	0.21	0.10	0.26
Uric acid (mg/dL)	-0.16	-0.03	0.17	0.01	-0.11
Framingham score	-0.18	-0.20	-0.03	0.11	-0.02
ESR (mm/1st hour)	0.10	0.10	0.28‡	0.12	0.08
CRP (mg/L)	-0.17	0.14	0.32‡	0.05	0.36‡
aCL IgG, GPL (U/mL)	0.41‡	0.19	-0.13	0.25	-0.53†
aCL IgM, MPL (U/mL)	0.16	0.13	-0.05	0.03	-0.13
Anti-β2GPI IgG (U/mL)	0.31‡	0.24	-0.19	0.14	-0.45†
Anti-β2GPI IgM (U/mL)	0.38‡	0.01	-0.15	-0.09	-0.08

aCL: anticardiolipin antibodies; anti-β2GPI: anti-β2-glyprotein I.
†p<0.001; ‡p<0.05.

Metabolic syndrome and adipocytokines in PAPS

Lower concentrations of adiponectin were significantly associated with the frequency of MetS [16.0 (10.2–14.0) *vs*. 25.1 (14.1–39.0), p<0.042], and higher concentrations of leptin and PAI-1 were significantly associated with MetS [45.1 (29.7–76.6) *vs*. 17.0 (10.0-40.3), p=0.002 and 20.5 (16.2–23.8) *vs*. 16.5 (11.4–20.5), p=0.030, respectively] (Table III).

Association between arterial events and adipocytokines in PAPS

No significant differences were found regarding adiponectin (p=0.09), leptin (p=0.11), resistin (p=0.23), visfatin (p=0.60) and PAI-1 (p=0.51) concentrations in patients with and without arterial events (Table IV).

Insulin resistance, inflammation, the metabolic syndrome, antiphospholipid antibodies, dyslipidemia and adipocytokines in PAPS

In the multivariate linear regression model, the variables that were independently associated with adiponectin levels were triglyceride levels (coefficient = 2.12, standard error = 0.64, t-value = 3.33, p=0.002), VLDL-c (coefficient = -10.87, standard error = 3.16, t-value = 0.001, p<0.001) and anti-β2GPI IgG levels (coefficien t = 0.14, standard error = 0.07, t-value = 2.09, p=0.042). The variables that were associated with the leptin concentration were BMI (coefficient = 2.93, standard error = 0.58, t-value=5.05, p<0.001), glucose level (coefficien t = -0.40, standard error = 0.19, t-value = -2.05, p=0.046), HOMA-IR index (coefficient = 7.43, standard error = 1.79, t-value = 4.16, p<0.001) and ESR (coefficient = 0.78, standard error = 0.27, t-value = 2.89, p=0.006). The variables that were associated with the PAI-1 level were CRP (coefficient = 0.39, standard error = 0.15, t-value = 2.59, p=0.013) and MetS (coefficient = 3.46, standard error=1.74, t-value = 1.99, p=0.048).

Discussion

This study is the first to highlight the possible relationship between adipocytokines and MetS in patients with PAPS and its association with insulin resistance and low-grade inflammation.

The inclusion of an age- and gender-matched control group was a necessary condition for the accurate analysis of

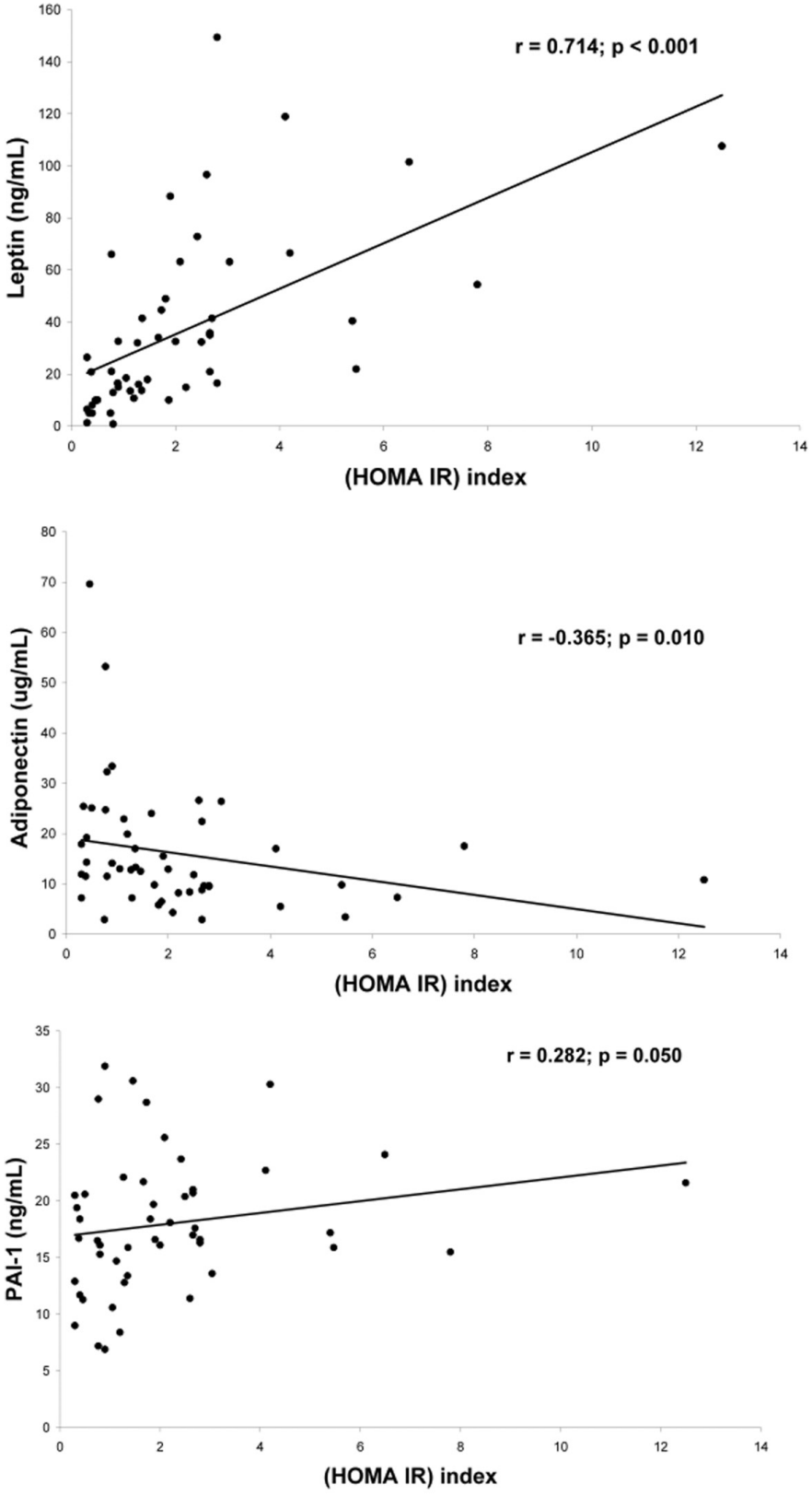

Fig. 2. Correlation between adipocytokine levels and the HOMA-IR index in patients with PAPS.

adipocytokine levels, given that the normal range for these hormones is not well established (9) and that their levels may to be influenced by gender and age (26). In addition, our study exclusively included primary APS because lupus *per se* may be associated with adipocytokine alterations that might hamper data interpretation (14, 27) and pregnancy was also an exclusion criterion since it alters the adipocytokin levels (28, 29). The overall assessment of several traditional and non-traditional cardiovascular risk factors concomitantly with the determination of the levels of five adipocytokines allowed, for the first time, a more precise distinction of the role of each these hormones in metabolic complications associated with PAPS.

Importantly, statins have been reported to improve endothelial function by decreasing PAI-1 and leptin levels (30-32), and glitazone and methotrexate are known to increase adiponectin levels in type 2 diabetic patients and in drug native rheumatoid arthritis, respectively; (33, 34) therefore, the exclusion of patients undergoing these therapies was necessary for the adequate evaluation of these bioactive peptides.

This study confirmed that PAPS patients have increased risk factors for CVD and yielded the novel observation that leptin levels are elevated in PAPS patients compared to controls. Given that this hormone may be directly atherogenic and that leptin levels are linked with cardiovascular events (35), our finding supports the notion that this adipocytokine may be an additional marker for CVD in PAPS. The observed association of leptin with BMI and the abdominal circumference is likely explained by leptin resistance, a condition that impairs satiation, increases the ability to store fat and reduces its oxidation (35). Leptin may be prothrombotic, promoting platelet aggregation and consequently arterial thrombosis (35), although the higher leptin levels observed in our patients with arterial events did not reach statistical significance. A likely explanation for this discrepancy was the fact that these arterial events may have occurred prior to the dosage of this hormone. However, we demonstrated a positive correlation between leptin and PAI-1 levels, which may constitute the mechanism underlying leptin-mediated thrombosis (35). Moreover, leptin levels are positively correlated with the levels of PAI-1 (36), fibrinogen (37) and von Willebrand factor (37) and

Table III. Association between adipocytocine levels and the presence of metabolic syndrome in PAPS patients.

	Metabolic syndrome		
	Present (n=15)	Absent (n=41)	*p*-value
Adiponectin (ug/mL)	10.2 (6.8-16.0)	14.1 (9.8-25.1)	0.042
Resistin (ng/mL)	13.9 (12.6-23.3)	16.0 (12.4-19.3)	0.928
Leptin (ng/mL)	45.1 (29.7-76.7)	17.0 (10.0-40.3)	0.002
Visfatin (ng/mL)	6.5 (5.5-7.5)	6.5 (5.5-7.2)	0.682
PAI-1 (ng/mL)	20.5 /16.5-23.8)	16.5 (11.4-20.5)	0.030

Mann-Whitney test.
Data are presented as median (interquartile range).

negatively correlated with the levels of tissue plasminogen activator (38).

Of note, inflammation in PAPS patients was uniformly low-grade, as also reported by Ames *et al*. (39), reinforcing previous observation that the atherosclerotic process in this syndrome is characterised by elevated triglycerides and low HDL and may involve the interaction with oxidised LDL, beta2-glycoprotein I and inflammatory markers, suggesting that these features resemble the typical changes of acute and chronic inflammatory states (40).

The evaluation of PAPS patients with and without MetS revealed increased leptin levels in the former group. Glucose, BMI, and HOMA-IR were independent factors associated with this cytokine, justifying the observation that these risk factors for CAD may raise the specific thrombotic risk associated with APS and this suggests that patients with PAPS and MetS have additional cardiovascular morbidity, which is greater than the risk associated with each individual component (41). Reinforcing this finding, there is strong evidence that aPL disturb endothelium both *in vitro* (42) and in experimental animal models, by inducing vasculopathy and an endothelial pro-inflammatory/coagulant phenotype (43-44) and in this regard, two studies suggested that LAC is a major risk factor for arterial thrombotic events in young women and aPL carriers (45, 46) and other study showed that average carotid intima-media thickness was greater in PAPS than control patients, suggesting that premature atherosclerosis ia a clinical feature of thrombotic PAPS patients (47).

In our study, we observed positive correlation between anti-β2GPI antibodies and adipocytokines. This finding seems to contrast with previous concept that low concentrations of adiponectin enhance the risk of diabetes mellitus, hypertension and dyslipidemia, ultimately leading to atherosclerosis (10). Nevertheless, recent evidences have suggested that adiponectin is in fact unique, since it may have a pro-inflammatory effect (9) that was reported RA systemically and in synovium (34, 48) as well as, in chondrocytes, lymphocytes and endothelial cells (34).

Alternatively, increased adiponectin levels may reflect a partial compensatory response to increased thrombosis mediated by aPL. Moreover, the overall effect of adiponectin in atherosclerosis may be mediated through interactions with other cardiovascular risk factors rather than the an independent action of aPL in PAPS (10, 14), suggesting that additional factors drive autoantibody-mediated vascular involvement and/or alter the relationship between adipocytokines and aPL in PAPS. Addition to that, in systemic lupus erythematosus and in rheumatoid arthritis, adiponectin levels are increased and show inverse relationship between adiponectin and BMI, insulin resistance and dyslipidemia (14, 15).

In our study, we found higher PAI-1 levels in patients with MetS, and we observed positive correlations between PAI-1 and BMI, inflammation and insulin resistance. Obesity is the central motor of MetS, which also includes impaired fibrinolysis (49). Therefore, increased levels of PAI-1, the main serine protease inhibitor that inhibits fibrinolysis, may be a component of MetS. Reinforcing this idea, MetS was independently associated with PAI-1. Interestingly, recent *in vitro* and *in vivo* studies have shown that in addition to its role in atherothrombosis, PAI-1 is also implicated in adipose tissue development and in the control of insulin signaling in adipocytes (49). These findings suggest that PAI-1 inhibitors may be a surrogate biomarker for cardiometabolic disease (50).

Our novel finding that the altered adipocytokines levels observed in PAPS are associated with MetS, obesity and insulin resistance provides an additional link, for understanding the autoimmune-mediated atherothrombotic vascular disease. Future studies are necessary to evaluate whether these cytokines will be useful markers for these metabolic complications in clinical practice.

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