

# Increased concentration of plasminogen activator inhibitor-1 and fibrinogen in individuals with metabolic syndrome

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**Abstract.** Metabolic syndrome (MS) is closely linked to a generalized metabolic disorder referred to as insulin resistance. Disturbances in the hemostasis and fibrinolytic systems are a feature of MS. The aim of this study was to determine the concentration levels of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) in a group of patients with MS with respect to a non-MS group, and to evaluate their possible relation with other risk factors in MS. The study was carried out in a total of 186 male and female non-smoking individuals aged 45-64 years, 93 with MS (ATP III criteria) and 93 without MS. Plasmatic levels of PAI-1 were measured by ELISA, and those of fibrinogen by the Claus method. The plasmatic levels of PAI-1 (men  $49.2 \pm 19.8$  vs.  $35.0 \pm 12.2$  ng/ml and women  $42.0 \pm 19.7$  vs.  $31.6 \pm 14.6$  ng/ml;  $p=0.0026$ ) and fibrinogen ( $274.0 \pm 82.1$  vs.  $232.7 \pm 66.6$  ng/ml;  $p=0.0002$ ) were significantly higher in the MS group than in the non-MS group. PAI-1 was significantly associated with diastolic blood pressure, triglycerides and waist circumference. Fibrinogen was negatively associated with HDL-c. High plasmatic levels of PAI-1 and fibrinogen contribute to the cardiovascular risk that characterizes individuals with MS.

## Introduction

The term metabolic syndrome (MS) describes a collection of lipid and non-lipid risk factors for cardiovascular disease (CVD), and is closely related to a generalized metabolic disorder referred to as insulin resistance (1-3). The National Cholesterol Education Program/Adult Treatment Panel (ATP) III has proposed a definition of MS (4). According to these

criteria, 23.7% of North Americans 20 years of age or older presented with MS in 2001 (5). In Chile, the prevalence in adults was 23.0% in 2003 (6). We found that the prevalence of MS in adults in our city (Talca), according to the ATP III and International Diabetes Federation criteria after adjustment for age and gender, was 29.5 and 36.4%, respectively (7).

Fatty tissue is considered to be an endocrine tissue that secretes several adipokines, as well as plasminogen activator inhibitor-1 (PAI-1). These molecules regulate the metabolic aspects of carbohydrates and lipids, the endothelium, inflammatory processes, hemostasis, and other processes (8,9).

Various studies have confirmed that MS is a predictor of an elevated risk of coronary heart disease (10-13). Disturbances in the thrombotic and fibrinolytic systems have been described in MS. Several studies have established that risk factors for CVD include high levels of PAI-1 and fibrinogen (14-17). In order to explore this relationship, we measured the concentrations of PAI-1 and fibrinogen in Chilean subjects with and without MS.

## Patients and methods

**Patients.** The study subjects were randomly selected from individuals enrolled in the 'Cardiovascular Profile of Adults of Talca, 2005' project, the initial results of which were recently published (18). A total of 186 non-smoking individuals 45-64 years of age were selected from two groups: a group of patients with MS (45 men and 48 women) and a group without MS (38 men and 55 women). All subjects included in this study were white Hispanics.

ATP III criteria (4) were used for the diagnosis of MS, including the presence of three or more of the following factors: waist circumference ( $>102$  cm for men and  $>88$  cm for women), high blood pressure ( $\geq 130/85$  mmHg or arterial hypertension in treatment), hyperglycemia ( $>100$  mg/dl or diabetes in treatment; modified according to ADA 2005) (19), hypertriglyceridemia ( $>150$  mg/dl) and low HDL-c ( $<40$  mg/dl for men and  $<50$  for women).

Anthropometric and arterial pressure measurements, as well as blood extractions, were made at the School of Health Sciences, Universidad de Talca, Chile (described by health professionals according to national ministerial and international norms) (20,21).

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Table I. Characteristics of the study population by gender.

	Metabolic syndrome group (n=93)		Non-metabolic syndrome group (n=93)		p-value	
	Men (n=44)	Women (n=49)	Men (n=38)	Women (n=55)	Men	Women
Age (years)	54.6±5.7	54.1±5.7	54.2±5.0	52.9±6.0	0.7211	0.2901
Glycemia (mg/dl)	124.8±44.8	110.1±41.8	96.9±32.3	91.5±24.0	0.0016	0.0076
Total cholesterol (mg/dl)	207.0±49.4	206.4±38.1	192.2±34.5	204.6±32.0	0.1142	0.7865
LDL-c (mg/dl)	117.7±28.3	123.0±31.3	112.1±31.3	116.7±28.0	0.4120	0.2856
HDL-c (mg/dl)	41.8±12.0	48.0±11.9	49.9±13.1	64.7±15.2	0.0048	<0.0001
Triglycerides (mg/dl)	262.0±197.5	176.2±64.1	161.3±99.1	114.3±40.8	0.0041	<0.0001
Waist circumference (cm)	105.0±10.1	98.7±10.1	93.5±8.7	86.3±11.3	<0.0001	<0.0001
Systolic pressure (mmHg)	143.5±15.3	138.5 ±20.0	133.0±17.5	127.9±19.6	0.0046	0.0077
Diastolic pressure (mmHg)	88.2±10.7	83.7±9.7	82.8±11.7	76.1±10.0	0.0322	0.0002
BMI (kg/m <sup>2</sup> )	30.7±3.9	33.5±6.7	27.1±2.9	27.0±5.0	<0.0001	<0.0001

Data are expressed as the mean ± standard deviation. HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; BMI, body mass index.

Table II. Percentage of subjects meeting ATP III criteria in the metabolic syndrome and non-metabolic syndrome groups.

	Metabolic syndrome group (n=93)		Non-metabolic syndrome group (n=93)		p-value	
	Men (n=44)	Women (n=49)	Men (n=38)	Women (n=55)	Men	Women
Hypertension (≥130/85 mmHg)	90.9	83.7	55.3	47.3	<0.001	<0.001
Low HDL-c (men <40 mg/dl, women <50 mg/dl)	56.8	71.4	15.8	9.1	<0.001	<0.0001
High triglyceride (>150 mg/dl)	72.7	63.3	36.8	9.1	0.0020	<0.0001
High glycemia (≥100 mg/ml)	72.7	51.0	21.1	9.1	<0.0001	<0.0001
High waist circumference (men >102 cm, women >88 cm)	54.6	87.8	7.9	34.6	<0.0001	<0.0001

Data are expressed as a percentage. HDL-c, high-density lipoprotein cholesterol.

**Laboratory assays.** To determine fibrinogen plasmatic levels, the Claus method (DG-FIB Kit Grifols) was used with a Clot-1-coagulometer (RAL, Barcelona, Spain). For quantification of PAI-1, the ELISA Kit Zymutest (Hyphen Biomed) was used. The optical densities (OD) were read at 450 nm by a StatFax-2600 microplate reader (Awareness Technology Inc., Palm City, Florida).

**Statistical analysis.** Covariance analysis was performed to evaluate the plasma levels of PAI-1 and fibrinogen in the MS and non-MS groups, controlled by age and gender. In order to identify which MS diagnostic criteria individually affect plasma levels of PAI-1 and fibrinogen, a multivariate regression analysis was performed with adjustment for age and

gender. Linear regression analysis was carried out to evaluate the effect of the number of MS components in plasma levels of PAI-1 and fibrinogen controlled by age and gender. Diagnostic regression measures were used to evaluate normality and homocedasticity assumptions. SAS 9.1.3 and SPSS 14.0 were used for statistical analysis. A 5% level of significance was used.

## Results

The characteristics of the population studied are presented in Table I. The percentages of subjects in the MS and non-MS groups meeting MS criteria according to the ATP III definition are presented in Table II.

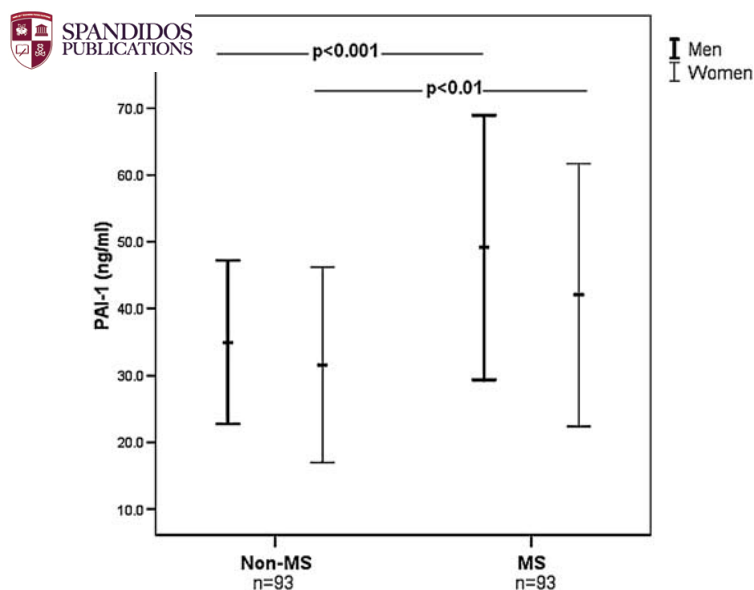


Figure 1. Mean and standard deviations for plasma concentrations of plasminogen activator inhibitor-1 (PAI-1) in the metabolic syndrome (MS) and control (non-MS) groups.

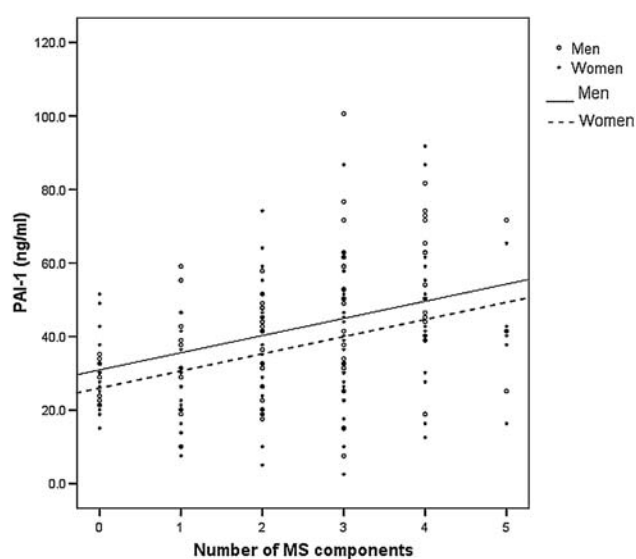


Figure 2. Relationship between PAI-1 concentrations and the number of metabolic syndrome (MS) criteria met (n=186).

Plasma concentrations of PAI-1 were significantly higher in subjects with MS in comparison to the non-MS subjects (men  $49.2 \pm 19.8$  vs.  $35.0 \pm 12.2$  ng/ml and women  $42.0 \pm 19.7$  vs.  $31.6 \pm 14.6$  ng/ml;  $p < 0.0001$ ) (Fig. 1). Significant differences in the plasma concentrations of PAI-1 were found according to gender ( $p < 0.037$ ). PAI-1 levels were associated with diastolic blood pressure ( $p = 0.028$ ), triglyceride ( $p = 0.03$ ) and waist circumference ( $p = 0.01$ ) (controlled by age and gender). Plasma levels of PAI-1 concentration were linearly related to the number of MS criteria, with a different intercept by gender ( $p < 0.001$ ) (Fig. 2). Each additional MS component increased PAI-1 concentrations by  $4.7$  ( $2.9$ - $6.4$ ) ng/ml.

Plasma concentrations of fibrinogen were significantly higher in subjects with MS ( $274.0 \pm 82.1$  mg/dl) than in the non-MS group ( $232.7 \pm 66.6$  mg/dl) ( $p = 0.0002$ ) (Fig. 3). There

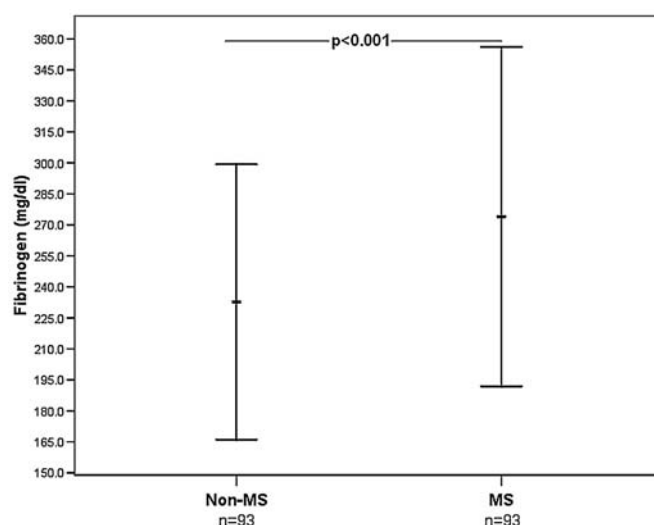


Figure 3. Mean and standard deviations for plasma concentrations of fibrinogen in the metabolic syndrome (MS) and control (non-MS) groups.

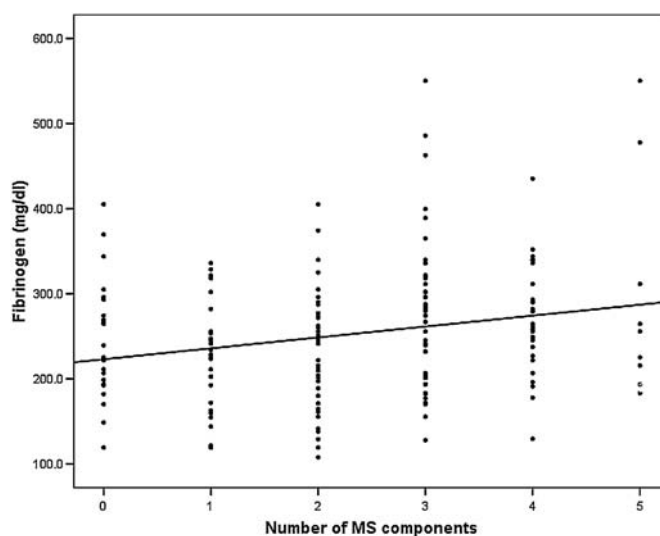


Figure 4. Relationship between fibrinogen concentrations and the number of metabolic syndrome (MS) criteria met (n=186).

were no differences associated with gender or age in the plasma concentrations of fibrinogen, which were only associated with HDL-c ( $p = 0.02$ ) (controlled by age and gender). Fibrinogen concentration was linearly related to the number of MS components, without gender-related differences ( $p < 0.001$ ) (Fig. 4). Each additional MS component increased the fibrinogen level by  $12.8$  ( $4.9$ - $20.7$ ) mg/dl.

## Discussion

Cardiovascular disease is a major cause of mortality worldwide (22). Evidence suggests that metabolic syndrome is associated with CVD (23,24). PAI-1 and fibrinogen, as non-traditional risk factors of CVD, have been associated with an elevated risk of CVD in the general population (25).

Abnormalities in hemostasis represent a well-known link between MS and thrombosis. Patients with MS have higher plasma concentrations of pro-thrombotic factors (fibrinogen,

von Willebrand factor) as compared to controls (26). Similarly, plasma concentrations of PAI-1 have been shown to be higher in obese patients than in non-obese controls (27). It has been proposed that the secretion of IL-6 by adipose tissue, combined with the actions of adipose tissue-expressed TNF- $\alpha$  in obesity, may underlie the association of insulin resistance with endothelial dysfunction, coagulopathy and coronary heart disease (28). Another possibility is that various hormonal abnormalities (androgen, catecholamines) associated with the accumulation of body fat may contribute to the impairment of the coagulative pathway in obesity (28).

Concerning plasminogen activator inhibitor-1, we found elevated plasma levels of PAI-1 in the MS group (men and women) compared with the non-MS group. MS subjects in our study presented similar plasma levels of PAI-1 to those found in other studies: 42-50 ng/ml in subjects 30-75 years of age with MS (29-31). On the other hand, similar to our study, Pankow *et al* (32) found elevated levels of PAI-1 in men ( $33.2 \pm 38.9$  ng/ml) compared to women ( $24.2 \pm 34.8$  ng/ml) with MS, 40-69 years of age.

We found significant linear associations between PAI-1 and diastolic blood pressure, triglycerides, waist circumference and the number of MS criteria. Other studies found statistically significant correlations between PAI-1 and triglycerides (33), waist circumference (34), HDL-c (33,34), BMI (35) and the number of MS criteria met.

Studies have concluded that the most important factor for increased plasma levels of PAI-1 is obesity, but additional variables explaining these levels may exist (31,36,37). In this context, Pankow *et al* (32) proposed the existence of polymorphisms which regulate PAI-1 levels.

High levels of PAI-1 in subjects with MS may be explained by elevated proinflammatory cytokines, such as tumor necrosis factor (38), dyslipidemia (39) platelet hyperactivity (40) and elevated insulin levels (41), as these induce PAI-1 secretion from the endothelium, liver and adipose tissue. Additional studies are needed to identify the genetic factors, environmental factors, and gene environment interactions regulating PAI-1 concentrations.

Concerning fibrinogen, we found that fibrinogen plasmatic levels were significantly elevated in MS subjects with respect to the non-MS group. Other studies found, in individuals of a similar age, values slightly higher than ours, with a mean of approximately 305 mg/dl in an MS group (31,35,42,43).

We found a correlation between plasma levels of fibrinogen and HDL-c and the number of MS criteria met. Other researchers found correlations with HDL-c (34), total cholesterol (44), BMI, systolic and diastolic pressure, HDL-c and triglycerides (43), and the number of diagnostic criteria of MS met (31). Mertens *et al* (36) found no significant differences between subjects with and without MS.

It is possible that the increase in fibrinogen (by favoring coagulation) and PAI-1 (by inhibiting the fibrinolytic system) in MS subjects favors the development of thrombotic arterial events.

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