PAI-1 levels are related to insulin resistance and carotid atherosclerosis in subjects with familial combined hyperlipidemia

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ABSTRACT

Familial combined hyperlipidemia (FCH) is a primary atherogenic dyslipidemia with insulin resistance and increased cardiovascular risk. Plasminogen activator inhibitor type 1 (PAI-1) and myeloperoxidase (MPO) activity are associated with proinflammatory and atherothrombotic risk. Our aim was to study the role played by PAI-1 and MPO activity in the carotid atherosclerosis prevalence in FCH subjects. 36 FCH unrelated subjects (17 women) were matched by age and body weight with 36 healthy normolipidemic subjects (19 female). Blood lipids, glucose, insulin, insulin resistance (homeostasis model assessment (HOMA)), MPO, and PAI-1 were determined in both groups. Carotid intima media thickness (IMT) was measured by the same investigator by standardized protocol. No differences in age, body mass index (BMI) or waist circumference were observed between the two groups. HOMA and PAI-1 values were higher in the FCH group, reaching statistical significance in those subjects with insulin resistance. In addition, PAI-1 values correlated significantly with metabolic syndrome components and carotid IMT. It is known that the elevated cardiovascular risk that characterizes FCH is frequently associated with insulin resistance. We have detected that two known proinflammatory and proatherothrombotic factors (MPO and PAI-1) are significantly elevated in FCH subjects with insulin resistance. These results could partly explain the high cardiovascular risk present in FCH subjects.

Significance of this study

What is already known about this subject?
- Familial combined hyperlipidemia (FCH) is associated with high cardiovascular risk.
- The cardiovascular risk of FCH subjects is incremented by insulin resistance; however, there are more unknown risk factors.
- Despite the treatment of known risk factors, there is an unacceptable high rate of cardiovascular disease in these patients.

What are the new findings?
- Prothrombotic factor, plasminogen activator inhibitor type 1 (PAI-1), is elevated in FCH subjects with insulin resistance.
- PAI-1 levels were correlated with carotid intima media thickness.

How might these results change the focus of research or clinical practice?
- These findings help to explain the high cardiovascular risk of FCH subjects.
- Our results may open the door to new therapeutic approaches in these patients.

INTRODUCTION

Familial combined hyperlipidemia (FCH) is a genetic model of primary dyslipidemia and insulin resistance. It is characterized by high risk of premature cardiovascular disease, related to a procoagulant and chronic inflammatory state.

One of the main characteristics of FCH is insulin resistance. It is independent of the grade of obesity or lipoprotein phenotype.[1] However, obesity aggravates the insulin resistance state and the cardiovascular risk in these patients.[2] Furthermore, FCH has been associated with oxidative stress and inflammation, especially in the presence of insulin resistance.[3 4]

Initiation and progression of the atherosclerotic plaque is characterized by a chronic inflammatory state.[5 6] Different studies have shown that acute-phase reactant proteins and many interleukins are predictive factors for the progression and severity of coronary atherosclerosis.[7]

In its evolution, the atherosclerotic plaque may rupture and cause an acute coronary syndrome. Although the mechanisms involved in the plaque erosion and rupture are still incompletely known, plaque instability plays a central role. It is well known that proinflammatory and prothrombotic mediators, such as plasminogen activator inhibitor type 1 (PAI-1) and myeloperoxidase (MPO), are involved in the process of plaque's rupture.

Our goal was to evaluate whether proinflammatory and prothrombotic markers such as MPO and PAI-1 are altered in FCH subjects

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and verify if both are modified by insulin resistance and are related with carotid atherosclerosis.

SUBJECTS AND METHODS

Subjects

Subjects were recruited at the Lipid Unit of our Center by convenience sample. We included 36 subjects with FCH (17 women) and 36 (19 women) age-matched and body mass index (BMI)-matched control subjects. Subjects in the two groups were non-hypertensive, non-diabetic, non-smokers, had no clinical manifestations of cardiovascular disease and were off medical treatment. Their BMI was below 35 kg/m^2 and their age range was between 18 and 65 years.

The diagnosis of FCH was based on the international criteria as previously published by Castro Cabezas *et al*[8]: the presence of mixed hyperlipidemia (total cholesterol (TC) and/or triglyceride (TG) levels exceeding the 90th percentile for our population by age and gender), elevated plasma levels of apolipoprotein B (apoB) (>120 mg/dL) and first-degree relatives with variable lipid phenotypes (IIa, IIb or IV). Family history of premature arteriosclerosis and the absence of xanthomas in the patient and first-degree family members were also considered.

Subjects included in the control group were non-diabetic and normolipidemic (TC concentration <200 mg/dL, TG <150 mg/dL and apoB<100 mg/dL) with no family history of dyslipidemia, cardiovascular disease or diabetes.

Exclusion criteria were similar to previously published.[4 9 10]

The institutional ethics committee approved the study protocol. Written informed consent was obtained from all the subjects included in the study.

Methods

Blood pressure was measured in the sitting position after a 10 min resting period, with two separate measurements. Abdominal circumference was measured in centimeters (point between the low costal rim and the iliac crest) by the same investigator.

Venous samples were obtained after 12 hours overnight fasting. Serum concentrations of TC, TG, high density lipoprotein cholesterol (HDL-C), apolipoprotein B, glucose and insulin were measured by standard methods as previously described.[4] The low density lipoprotein cholestrol (LDL-C) concentration was calculated according to the Friedwald equation. Insulin resistance (IR) was calculated by homeostasis model assessment (HOMA), considering IR if HOMA 3.20.[11] MPO and PAI-1 were determined by high sensitivity multiplex (LINCO*plex* High Sensitivity Human Cytokine Panel Milliplex, Millipore, Darmstadt, Germany).

Human carotid artery ultrasound evaluation was performed as follows. B-mode ultrasound imaging of the right and left carotid arteries was performed using a Siemenes Sonoline G40 instrument (Siemens Medical Solutions, Erlangen, Germany) equipped with 7–10 MHz broadband linear array transducers. Patients were examined in the supine position with the head turned 45° contralateral to the side of scanning. Before obtaining images for intima media thickness (IMT) measurements, B-mode and color Doppler sonographic examinations were done in longitudinal and transverse planes to identify vascular stenoses. A standardized imaging protocol was used for the IMT measurements in agreement with the Mannheim consensus.[12] The primary variable was mean common carotid IMT (CCIMT), defined as the average of distances between the far wall lumen-intima and media-adventitia ultrasound interfaces taken bilaterally, in three different projections (right common carotid artery: 90°, 120° and 150°; left common carotid artery: 210°, 240° and 270°). An experienced sonographer (SMH) performed all examinations. Intraobserver variability was examined in 20 subjects. The coefficient of variability of mean CCIMT was 5.2%.

Table 1 Characteristics of anthropometric measurements and biochemical values in the control and FCH groups

	Control	FCH	p Value
N (M/F)	36 (19/17)	36 (17/19)	0.427
Age (year)	36.8±14.3	46.2±12.2	0.092
BMI	26.9±5.4	27.4±3.7	0.337
Waist (cm)	89.7±14.3	92.4±11.4	0.282
TC (mg/dL)	197.1±24.4	267.5±29.5	
TG (mg/dL)	96.7±43.8	190.8±140.9	<0.001
HDL-C (mg/dL)	55.8±12.9	56.8±13.8	0.714
LDL-C (mg/dL)	122.2±24.5	177.2±25.2	<0.001
ApoB (mg/dL)	88.1±17.1	126.6±17.1	<0.001
Glucose (mg/dL)	88.1±10.5	99.0±14.1	0.001
Insulin (µU/mL)	9.4±6.1	12.5±6.0	0.014
HOMA index	2.08±1.40	3.15±1.87	0.006
MPO (ng/mL)	38.8±158.2	92.8±234.3	0.664
PAI-1 (ng/mL)	41.4±33.1	73.1±67.7	0.005
IMT (mm)	0.47±0.12	0.55±0.11	0.013

Values expressed as mean±SD.
ApoB, apolipoprotein B; BMI, body mass index; FCH, familial combined hyperlipidemia; HDL-C, high density lipoprotein cholesterol; HOMA, homeostasis model assessment; IMT, intima media thickness; LDL-C, low density lipoprotein cholesterol; MPO, myeloperoxidase; N, number of subjects (M, males, F, females); PAI-1, plasminogen activator inhibitor type-1; TC, total cholesterol, TG, triglycerides; waist, abdominal circumference.

Statistical analysis

Statistical analyses were performed using SPSS software, V.15.0. Results are expressed as mean±SD. Due to the sample size and the measurement of variables that do not fulfill the criteria of normality, non-parametric tests were used. The differences between groups were calculated using the Mann-Whitney U-test to compare two variables or the Kruskal-Wallis test for three or more variables. For the comparison of proportions, Fisher's exact test was used. The degree of relationship between two quantitative variables was analyzed by the Spearman correlation coefficient. Two-tailed p values of less than 0.05 were considered as statistically significant.

RESULTS

Anthropometric parameters, laboratory findings, and IMT measurements from both groups of subjects are shown in table 1. No significant differences were found in age, gender, or other anthropometric parameters. Due to the selection criteria, significant differences were observed in lipid values. Blood levels of insulin, glucose, HOMA, and PAI-1 were significantly higher in the FCH subjects than in

Table 2 Comparison between controls and FCH subjects with and without insulin resistance

	Control (n=36)	FCH non-IR (n=19)	FCH IR (n=17)	p Value
MPO (ng/mL)	38.9±158.2	61.8±215.6	127.5±255.5	0.184*, 0.03†, 0.009‡
PAI-1 (ng/mL)	41.4±33.1	49.4±33.6	99.5±55.3	0.284*, <0.001†, 0.016‡
IMT (mm)	0.47±0.12	0.53±0.11	0.57±0.1	0.115*, 0.017†, 0.0519‡

Values expressed as mean±SD.
FCH, familial combined hyperlipidemia; IMT, intima media thickness; IR, insulin resistance (homeostasis model assessment ≥3.2); MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor type-1.
*Control versus FCH without IR.
†Control versus FCH with IR.
‡FCH without IR versus FHC with IR.

controls. Carotid IMT was significantly higher in the FCH subjects.

The values for the two proinflammatory and proatherothrombotic factors studied (MPO and PAI-1) and the carotid IMT measurements are shown in table 2. Subjects were divided into three groups: controls, FCH without and FCH with insulin resistance. Significant differences were found between controls and insulin resistance FCH subjects for any of the three parameters measured. The MPO and PAI-1 values in the insulin resistance FCH group were significantly higher than in non-insulin resistance FCH, but no differences were observed in the IMT measurements.

Figure 1 shows the in-between group differences in PAI-1 and MPO. Significant differences (p<0.001) were observed in the PAI-1 and MPO values between FCH subjects with insulin resistance and the other two groups. No differences were observed in MPO and PAI-1 values between the non-insulin resistance FCH subjects and controls, although there was a progressive increment in PAI-1 values from controls to FCH with insulin resistance.

The correlations between MPO and PAI-1 with different parameters are shown in table 3. A positive correlation with components of the metabolic syndrome was observed for both PAI-1 and MPO values. However, only PAI-1 correlated with IMT values.

DISCUSSION

FCH is characterized by high risk of premature cardiovascular disease affecting both genders, with a higher incidence in subjects with insulin resistance. This could be partly explained by the chronic proinflammatory state and increased oxidative stress that has been observed in FCH subjects, particularly those with insulin resistance.[4 9 10] In the present study, we have observed that, compared with controls, FCH subjects have increased MPO and PAI-1 blood values. The elevation of these two proinflammatory and proatherothrombotic biomarkers was significantly higher in the presence of insulin resistance. These findings may be relevant in view of the evidence correlating MPO plasma values with risk of major cardiovascular events.[13]

Different potential mechanisms could explain MPO contribution to the formation and evolution of the atherosclerotic plaque. MPO facilitates LDL oxidation and foam

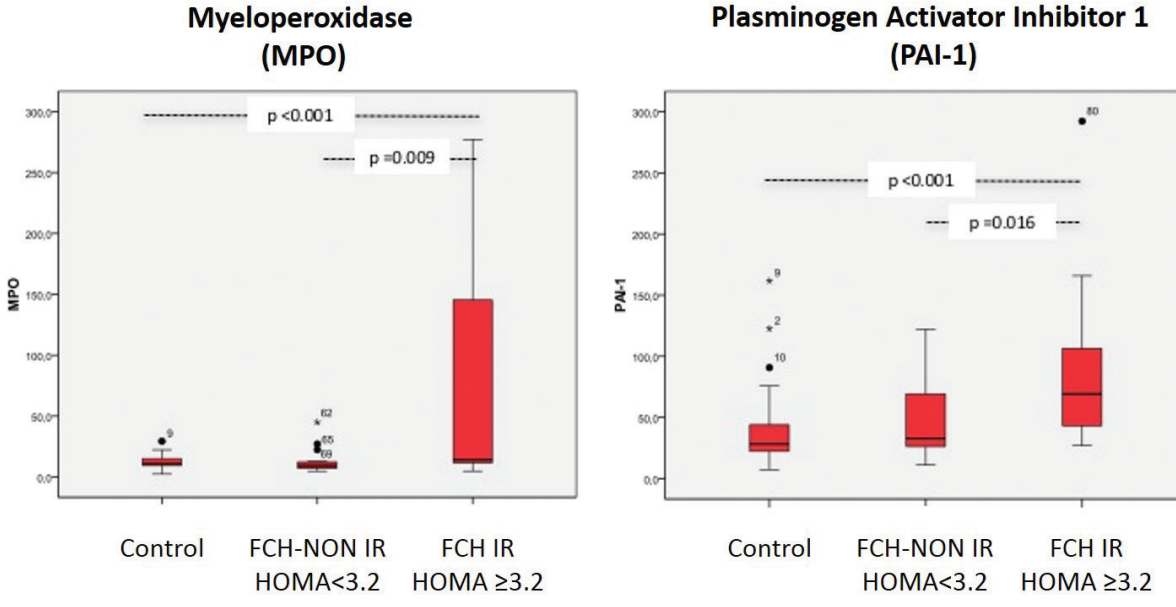

Figure 1 MPO and PAI-1 levels according to the group of the study (control, FCH without insulin resistance and FCH with insulin resistance). FCH, familial combined hyperlipidemia; IR, insulin resistance (homeostasis model assessment (HOMO) ≥3,2); MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor type-1.

Table 3 Correlations between PAI-1 and MPO values and study parameters

	PAI-1 (ng/mL)	MPO (ng/mL)
Waist (cm)	**r 0.520, p<0.001**	**r 0.346, p=0.005**
TG (mg/dL)	**r 0.548, p<0.001**	r 0.229, p=0.053
HDL-C (mg/dL)	**r −0.290, p=0.014**	**r −0.252, p=0.033**
LDL-C (mg/dL)	**r 0.374, p=0.001**	r 0.132, p=0.268
ApoB (mg/dL)	**r 0.439, p<0.001**	r 0.177, p=0.136
Glucose (mg/dL)	**r 0.435, p<0.001**	r 0.079, p=0.508
Insulin (µU/mL)	**r 0.460, p<0.001**	**r 0.370, p=0.002**
HOMA index	**r 0.506, p<0.001**	**r 0.352, p=0.003**
IMT (mm)	**r 0.412, p=0.003**	r −0.069, p=0.632

Bold indicates statistically significant (p<0.05).
ApoB, apolipoprotein B; FCH, familial combined hyperlipidemia; HDL-C, high density lipoprotein cholesterol; HOMA, homeostasis model assessment; IMT, intima media thickness; LDL-C, low density lipoprotein cholesterol; MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor type-1; TC, total cholesterol; TG, triglycerides; waist, abdominal circumference.

cells formation.[14] In addition, MPO activates catalytic processes in the plaque, generating oxidative products and the oxidation of apoB.[15–17] Moreover, MPO activates leucocytes chemotaxis,[18] is involved in the transformation of HDL into dysfunctional particles,[19–21] and promotes endothelial dysfunction and a prothrombotic state of endothelial and smooth vascular muscle cells.[22] As a consequence, MPO participates in the processes of plaque instability and thrombus formation.[23 24] In our study, plasma levels of MPO were significantly elevated in FCH subjects with insulin resistance compared with controls or FCH without insulin resistance.

Compared with the control group, the plasma values of PAI-1 were significantly elevated in FCH subjects. PAI-1 is associated with abdominal obesity and insulin resistance and has been considered as another component of the metabolic syndrome.[25] The atherothrombotic complications present in the insulin-resistance syndrome are partly attributed to disturbed fibrinolysis as a consequence of elevated PAI-1 levels in plasma.[26] Reduced fibrinolytic activity and increased procoagulant markers and PAI-1 levels have been described in hypertriglyceridemic patients with hepatic steatosis, including FCH subjects without hepatic steatosis.[27] Compared with controls of similar age and body weight, our group of FCH subjects showed a significant elevation of plasma PAI-1 levels. Such elevation was independent of body weight values and was significantly correlated with metabolic syndrome components.

In addition, we found a significant correlation between PAI-1 values, carotid IMT, and HOMA values of insulin resistance (table 3). These results could explain the elevated cardiovascular risk of FCH subjects. Along these lines, a causal role of elevated PAI-1 levels in atherosclerotic cardiovascular diseases has been suggested due to its prothrombotic activity.[28 29] Different studies have demonstrated an independent predictor value of PAI-1 in the progression of coronary atherosclerosis and thrombotic events following coronary revascularization.[30–33] On the other hand, other authors have not replicated these findings, raising the possibility that different PAI-1 polymorphisms may be implicated in the risk and progression of cardiovascular disease.[34]

In asymptomatic members of FCH families, Karásek *et al* have found that elevated plasma PAI-1 values correlated with IMT and components of the metabolic syndrome.[35] These results are in accordance with our findings: elevated PAI-1 levels and IMT measurements in asymptomatic FCH subjects in primary prevention, free of other cardiovascular risk factors. Our findings were interrelated with insulin resistance since no differences were found between controls and FCH without insulin resistance, as shown in table 2. Along this line, Ikeda *et al*, in a model using 3T3-L1 adipocytes, have recently reported that the relationship between insulin resistance and PAI-1 may be regulated by resistin.[36] Our results are in agreement with those published by other authors, who also found elevated PAI-1 values in FCH subjects with hyperinsulinemia, hypertriglyceridemia and insulin resistance.[37]

In conclusion, in addition to dyslipidemia, the cardiovascular risk of FCH subjects is incremented by insulin resistance and high PAI-1 plasma values, as shown by a positive correlation with IMT measurements. We have found that two proinflammatory and prothrombotic factors, MPO and PAI-1, are elevated in FCH subjects with insulin resistance. These findings help to explain the high cardiovascular risk of FCH subjects and may open the door to new therapeutic approaches in these patients.

Contributors AC and EB reviewed the charts and collected the data. ERB and GTS performed the statistical analysis. SMH, JTR, JFA conceived and designed the study. SMH, RC and JFA drafted the manuscript. All the authors contributed to the approval of the final version.

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Competing interests None declared.

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