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PROINSULIN LEVEL AS A PREDICTOR OF METABOLIC SYNDROME IN THE ROMANIAN POPULATION

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Abstract

Background and Aims: *The aim of the present study was to determine discriminating values of proinsulin (FPP), proinsulin to insulin ratio (PIR), proinsulin to C-peptide ratio (PCPR) and Homeostasis model of assessment of insulin resistance (HOMA-IR) for the metabolic syndrome (MetS) as well as discovering sex-specific cutoff points of these parameters in the Romanian population.* ***Material and Methods:*** *We analyzed data from 224 patients. Circulating levels of fasting plasma glucose (FPG), fasting plasma insulin (FPI), FPP, fasting plasma C-peptide, HbA1c, and lipid profile were measured.* ***Results:*** *Among the 224 patients (87 males) MetS was diagnosed in 97 patients (43.3%) according to International Diabetes Federation (IDF) criteria. After stratification by gender, 43 men (49.4%) and 54 women (39.4%) had MetS. There were statistically significant differences between sexes for body mass index (BMI), % body fat, FPG, FPP, PIR, PCPR (all p<0.05). On multivariate logistic regression analysis, only age, BMI, FPP, and HOMA-IR were the independent factors associated with the presence of MetS.* ***Conclusions:*** *The present study showed that FPP and HOMA-IR were the best predictors for MetS in this sample of the Romanian population. Our results suggest that, regardless of gender, HOMA-IR and FPP could be the preferred parameters for predicting MetS.*

key words: *metabolic syndrome****,*** *C-peptide, proinsulin, proinsulin to insulin ratio, proinsulin to C-peptide ratio*

Background and Aims

Metabolic syndrome (MetS) is known to be a cluster of a variety of metabolic-related factors such as obesity, elevated blood pressure, glucose metabolism disturbances, and increased lipid levels leading to an increased risk of mortality and morbidity [1].

Considering that proinsulin concentrations are disproportionately elevated in subjects with diabetes [2-7] and with impaired glucose tolerance (IGT) [8], it is conceivable that this

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prohormone may have particularly detrimental metabolic effects in the pathogenesis of cardiovascular disease (CVD) or metabolic syndrome.

Among both diabetic and nondiabetic subjects, proinsulin has exhibited moderate but significant associations with blood pressure (BP) and concentrations of total cholesterol, triglycerides, LDL and HDL cholesterol [9-11], independent of other factors included in multivariate analyses.

Several studies have suggested that plasma proinsulin concentrations and the proinsulin-to-insulin ratio may be better related to cardiovascular risk factors (in particular, increased blood pressure and higher triglyceride levels) as compared to plasma insulin concentrations [12,13]. However, the role of elevated proinsulin in atherogenesis remains controversial [14].

The aim of the present study was to determine the discriminating values of proinsulin, proinsulin-to-insulin ratio (PIR), proinsulin-to-C-peptide ratio (PCPR) and HOMA-IR as predictors for the metabolic syndrome as well as discovering the sex-specific cutoff points of these parameters in Romanian population.

Materials and Methods

Study population and sampling methods

A cross-sectional, population-based screening campaign whose main objective was the screening for diabetes took place between November-December 2011 in Bucharest, Romania. During this campaign a total of over 15,000 people were assessed. Only data from patients who gave their informed consent were analyzed and processed. The exclusion criteria were: patients with a previous diagnosis of diabetes, pregnancy, patients having an alcohol consumption of more than 20 g/day for women and 30 g/day for men, history of pancreatitis, chronic liver disease, autoimmune liver disease, hemochromatosis, HIV infection, patients with history of hepatotoxic or steatosis-inducing drug use, currently on interferon treatment or during the last 12 months, recent surgery, inflammatory or malignant disease, anticoagulant therapy, steroid therapy, postmenopausal women on estrogen replacement therapy. Finally, a random population-based sample (n=656) of Romanians (26–80 years) was studied. Of these, 432 persons had diabetes and they were not analyzed for this paper so that the study group finally included 224 subjects.

Procedures and Measurements

Participants underwent an extensive interview for information on current medications, medical history, smoking, physical activity, etc. Anthropometric indices were measured with participants lightly dressed and barefoot. Body mass index (BMI) was calculated as weight (in kilograms)/height (in meters) squared. Waist and hip circumferences were measured at the level of the umbilicus, and at the level of the maximum perimeter between the iliac crest and the crotch, respectively.

The presence of MetS (score ≥3/5) was defined according to the joint harmonized International Diabetes Federation (IDF), National Heart, Lung, and Blood Institute (NHLBI), American Heart Association (AHA), World Heart Federation (WHF), International Atherosclerosis Society (IAS), International Association for the Study of Obesity (IASO) criteria [15,16]. Metabolic circumference (metabolic WC) was defined as waist circumference ≥94 cm for men and ≥80 cm for women.

Percentage body fat (BF%) was predicted using an equation developed in Dutch Caucasians which include BMI, age, and sex [17]: $BF\% = 1.2xBMI + 0.23xage – 10.8xsex –5.4$, where age is in years and sex = 0 in females and 1 in males.

Laboratory assays

Fasting blood samples were drawn between 7:00 a.m. and 10:00 a.m. The biochemical analyses, including fasting plasma glucose (FPG), fasting plasma insulin (FPI), fasting plasma proinsulin (FPP), fasting plasma C-peptide, HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), creatinine, urea, were measured after an overnight fasting period of 12h, using routine clinical chemistry methods and then documented.

Intact proinsulin was measured using an ELISA kit (Demeditec Diagnostics GmbH, Germany). The inter- and intra-assay CVs was 4.3% and 5.5% for proinsulin. Serum insulin and C-peptide were determined by chemiluminescence enzyme immunoassay (Architect, Abbott). The cross-reactivity of insulin with proinsulin, and with C-peptide was 0.1%, respectively 0.001%.

Insulin resistance (IR) was determined using the Homeostasis model assessment for insulin resistance (HOMA-IR) formula: fasting insulin level (mUI/l)x fasting glucose level (mg/dl)/405 [18]. A HOMA-IR index value of more than 2.0 was considered as the criteria of insulin resistance. The homeostatic model for assessment of B-cell function (HOMA-B) was calculated using the formula [18]: 20×FPI (µU/ml)/(FPG (mmol/l) – 3.5. The quantitative insulin sensitivity check index (QUICKI) was calculated using the formula: 1/(log (FPI) (µU/ml)+log (FPG) (mg/dl)) [19].

Statistical Analyses

Values were expressed as means ± SD for normally distributed data or median (range) for skewed data. Log transformation was also applied to skewed data for insulin, triglyceride (TG), HOMA-IR, C-peptide, proinsulin, PIR, and PCPR. Comparisons among groups were made by use of ANOVA for quantitative variables and the χ^2 test of independence for categorical variables.

The area under the Receiver's Operating characteristic Curve (ROC) – AUROC - and the 95% confidence intervals (CIs) were used to compare the predictive ability of the tested parameters. The AUC is a measure of the degree of separation between case and control subjects.

Spearman correlation test was used to summarize the association between FPP, FPI, PIR, PCPR and MetS components. Logistic regression model was used to estimate odds ratios ($OR_{s)}$) and 95 percent confidence intervals. Multivariate logistic regression analysis was used to estimate the adjusted odds ratios (OR) and the 95% confidence intervals of FPP, FPI, PIR, PCPR, HOMA-IR in predicting MetS. The level of significance was set at $p \leq 0.05$.

Results

Among the 224 patients (87 males and 137 females), MetS was diagnosed in 97 patients (43.3%) according to the modified IDF criteria [15,16]. After stratification by gender, 43 men (49.4%) and 54 women (39.4%), had MetS. Table 1 summarizes the general characteristics of the study group according to gender, including anthropometric and lab parametres. There were statistically significant differences between sexes for BMI, %BF, FPG, HDL-C, FPP, PIR, PCPR, HOMA-B (all $p<0.05$).

The presence of the metabolic syndrome components was similar in women in comparison to men (Table 2).

Baseline and demographic characteristics of the patients with MetS versus patients without MetS (according to the IDF criteria) [15,16] are summarized in Table 3. The prevalence of MetS increased with age ($p<0.0001$). Patients presenting MetS were older, and they had a higher BMI, % BF, and systolic blood pressure

(SBP), higher values of TG, FPG, HbA1c, FPI, C-Peptide, FPP, PIR, PCPR, HOMA-IR and lower values of HDL-C, Quicki index (all p<0.05).

Table 1. Baseline characteristics and clinical parameters in men and women

Variables	Men (n=87) Mean±SD/Median	Women (n=137) Mean±SD/Median	Total (n=224) Mean±SD/Median	p*
Age (years)	59.3±15.3	56.1±13.4	57.4±14.2	NS
BMI (kg/m^2)	27.7±4.5	27.8±5.2	27.8±4.9	NS
SBP (mmHg)	143.1±21.8	142.3±19.9	142.6±20.7	NS
TC (mg/dl)	213.9±52.5	220.3±44.1	217.8±47.5	NS
Creatinine (mg/dl)	1.1±0.2	0.9±0.1	1±0.2	0.0001
eGFR (ml/min/1.73 m^2)	87.5±28.6	79.0±19.9	82.3±24	0.009
FPG (mg/dl)	105.1±12.9	100.7±13.8	102.4±13.6	0.016
HDL-C (mg/dl)	50.6±14.8	58.3±12	55.3±13.7	0.0001
TG (mg/dl)	133 993-208)	122 (94.5-164.5)	126 (94.2-176)	0.087
HbA1c (%)	5.8±0.5	5.8±0.6	5.8±0.5	NS
FPI (uU/ml)	8.2 (5.9-11.2)	8.8 (6.3-11.9)	8.55 (6.2-11.5)	NS
C-Peptide (ng/ml)	2.23 (1.71-3.02)	2.1 (1.63-2.79)	2.13 (1.6-2.93)	NS
FPP (pmol/l)	5.1 (3.18-8.64)	3.6 (2.16-6.59)	4.33 (2.53-7.16)	0.039
PIR	4.27 (3.15-6.67)	3.28 (2.06-4.87)	3.65 (2.29-5.49)	0.002
PCPR	2.24 (1.53-2.99)	1.72 (1.17-2.69)	1.9 (1.31-1.9)	0.037
HOMA-IR	2.14 (1.54-3.05)	2.15 (1.41-3.04)	2.14 (1.45-3.04)	NS
Quicki Index	0.34 (0.32-0.35)	0.34 (0.32-0.36)	0.34 (0.32-0.36)	NS
HOMA-B	73.7 (49.7-106.4)	91.2 (60.5-119.5)	84 (55.9-115.3)	0.004

* between men and women

Table 2. Presence of metabolic syndrome components in women and men.

	Men	Women	Total	p
Metabolic WC	74.7% (n=65)	67.2% (n=92)	70.1% (n=157)	0.14
FPG>100 mg/dl	57.5% (n=50)	46% (n=63)	50.4% (113)	0.062
HypoHDL-C	21.8% (n=19)	24.8% (n=34)	23.7% (n=53)	0.365
Hypertension	44.8% (n=39)	48.9% (n=67)	47.3% (n=106)	0.324
TG>150 mg/dl	39.1% (n=34)	32.1% (n=44)	34.8% (n=78)	0.178

Table 3. Baseline and demographic characteristics of the patients with MetS versus patients without MetS.

	MetS - (n=127)			MetS + (n=97)			
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**	**p**
Age (years)	53.66	15.72	55.00	62.23	10.04	62.00	0.0001
Weight (kg)	71.52	12.75	70.00	85.59	15.34	85.00	0.0001
BMI (kg/m2)	25.65	4.01	25.21	30.58	4.56	30.12	0.0001
%BF	33.98	8.50	34.00	40.82	7.76	41.44	0.0001
SBP (mmHg)	133.94	17.95	127.00	153.95	18.41	160.00	0.0001
FPG (mg/dl)	96.35	12.35	94.00	110.34	10.96	114.00	0.0001
HDL-C (mg/dl)	59.58	12.81	58.00	49.68	12.75	48.00	0.0001
TG (mg/dl)	114.22	55.36	108.00	191.53	94.57	171.00	0.0001
HbA1c (%)	5.58	0.48	5.50	6.01	0.50	6.10	0.0001
FPI (uU/ml)	7.90	3.52	7.30	11.24	5.57	10.20	0.0001
C-Peptide (ng/ml)	2.09	1.12	1.83	2.82	1.18	2.60	0.0001
FPP (pmol/l)	4.42	5.11	3.08	7.38	4.90	6.18	0.0001

PIR	4.83	9.08	3.23	5.09	2.89	4.43	0.0020
PCPR	2.45	4.20	1.67	2.60	1.23	2.48	0.0010
HOMA-IR	1.91	0.95	1.76	3.05	1.52	2.70	0.0001
Quicki index	0.36	0.03	0.35	0.33	0.02	0.33	0.0001
HOMA-B	93.44	47.28	88.50	92.98	59.17	77.43	0.6600

Table 4. Areas under the ROC curves (95% CI) for potential markers of MetS, categorized by gender.

	Total			Women			Men		
Variables	AUROC (95% CI)	SE	p	AUROC (95% CI)	SE	p	AUROC (95% CI)	SE	p
HOMA-IR*	0.756 (0.695-0.818)	0.032	<0.001	0.735 (0.651-0.818)	0.034	<0.001	0.797 (0.707-0.888)	0.046	<0.001
FPP (pmol/l)*	0.755 (0.693-0.818)	0.032	<0.001	0.731 (0.646-0.815)	0.043	<0.001	0.786 (0.690-0.882)	0.049	<0.001
TG (mg/dl)*	0.788 (0.727-0.0.850)	0.031	<0.001	0.845 (0.778-0.912)	0.043	<0.001	0.707 (0.597-0.818)	0.052	<0.001
PCPR*	0.686 (0.616-0.756)	0.036	<0.001	0.671 (0.579-0.764)	0.047	0.001	0.692 (0.581-0.804)	0.057	0.002
PIR*	0.662 (0.590-0.734)	0.037	<0.001	0.668 (0.575-0.762)	0.048	0.001	0.626 (0.508-0.744)	0.057	0.001
FPI (uU/ml)*	0.7 (0.632-0.768)	0.035	<0.001	0.685 (0.595-0.775)	0.046	<0.001	0.734 (0.631-0.837)	0.060	0.043

*these variables were log-transformed before analysis

Comparison of areas under ROC curves (95% CI) for predictors of MetS, categorized by gender

In all patients, ROC analysis showed that HOMA-IR (AUROC = 0.756 (95% CI: 0.695, 0.818)) was the strongest predictor of MetS, followed by FPP (AUROC = 0.755 (95% CI: 0.693, 0.818)) and TG (AUROC=0.788 (95% CI: 0.727, 0.850)) (overall p< 0.0001) (Table 4; Figure 1).

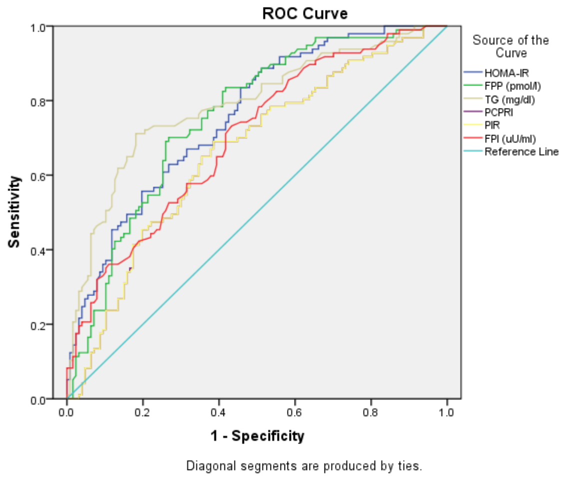

Figure 1. *Receiver operating characteristics (ROC) curves in all patients.*

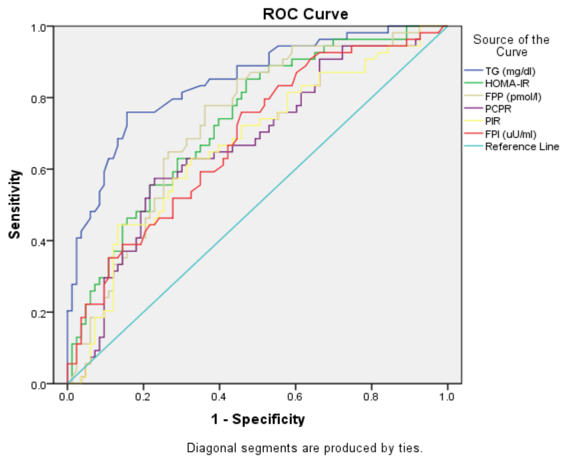

Figure 2. *Receiver operating characteristics (ROC) curves in women.*

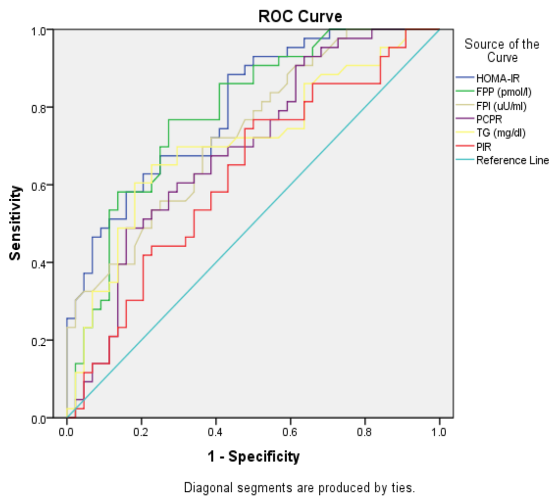

Figure 3. *Receiver operating characteristics (ROC) curves in men.*

In women all tested variables showed discriminative ability, with an AUROC significantly different from 0.5. The test with the largest area under the curve was TG (AUROC=0.845, (95% CI: 0.778, 0.912), followed by HOMA-IR (AUROC=0.735 (95% CI: 0.651, 0.818), and FPP (AUROC=0.731 (95% CI: 0.646, 0.815) (Table 4; Figure 2).

In men HOMA-IR (AUROC = 0.797 (95% CI: 0.707, 0.888)) was the strongest predictor of MetS followed by FPP (AUROC = 0.786 (95% CI: 0.690, 0.882)), and FPI (AUROC=0.734 (95% CI: 0.631, 0.837)) (overall $p < 0.05$) (Table 4; Figure 3).

The cutoff value for TG was 138.5 mg/dl (137 mg/dl in women and 139 mg/dl in men), for HOMA-IR was 1.85 (1.85 in women and 1.67 in men) and for FPP was 4.75 pmol/L (3.52 pmol/L in women and 4.78 pmol/L in men). All these parameters have a high sensitivity but low specificity for identifying MetS as shown in Table 5.

Table 5. Cutoff, sensitivity and specificity values for predicting of MetS, categorized by gender.

	Total			Women			Men		
Variables	Cutoff	Sensitivity	Specificity	Cutoff	Sensitivity	Specificity	Cutoff	Sensitivity	Specificity
HOMA-IR*	1.853	0.835	0.457	1.85	0.852	0.470	1.670	0.884	0.432
FPP (pmol/l)*	4.7550	0.701	0.268	3.52	0.778	0.361	4.780	0.767	0.273
TG (mg/dl)*	138.50	0.711	0.181	137.00	0.759	0.157	139.000	0.651	0.227
PCPR*	2.19	0.619	0.283	2.21	0.574	0.229	2.840	0.488	0.154
PIR*	3.58	0.691	0.386	4.83	0.444	0.133	3.580	0.767	0.500
FPI (uU/ml)*	7.75	0.732	0.433	7.75	0.759	0.458	7.650	0.721	0.386

*these variables were log-transformed before analysis

Table 6. Spearman correlations between FPP, FPI, PIR, PCPR and anthropometric and metabolic variables.

	FPP (pmol/l)	**FPI (uU/ml)**	**PIR**	**PCPR**
FPP (pmol/l)	1	0.452**	0.767**	0.871**
FPI (uU/ml)	0.452**	1	–0.135*	0.117
PIR	0.767**	–0.135*	1	0.891**
PCPR	0.871**	0.117	0.891**	1
Age (years)	0.295**	0.039	0.358**	0.229**
BMI (kg/m^2)	0.378**	0.479**	0.108	0.189**
%BF	0.256**	0.380**	0.048	0.094
WC (cm)	0.307**	0.378**	0.095	0.154*
SBP (mmHg)	0.312**	0.190**	0.309**	0.240**
TG (mg/dl)	0.330**	0.343**	0.154*	0.203**
HDL-C (mg/dl)	–0.275**	–0.230**	–0.164*	–0.215**
FPG (mg/dl)	0.365**	0.266**	0.267**	0.277**
HOMA-IR	0.513**	0.964**	–0.042	0.189**

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 6 shows the Spearman correlations between FPP, FPI, PIR, PCPR and the anthropometric and metabolic variables. FPP and FPI correlated positively with BMI, %BF, WC, SBP, TG, FPG, HOMA-IR and negatively with HDL-C, all with statistical significance. The correlations of FPP were stronger with age, SBP, FPG, whereas FPI correlated more strongly with BMI, %BF, WC, TG. PIR was significantly correlated with age, SBP, TG, FPG but not with BMI, %BF, WC. PCPR correlated with age, BMI, WC, SBP, TG, FPG. Only FPP and PCPR were correlated significantly with HOMA-IR.

A significant risk for MetS was observed among the patients over 57.7 years, with higher BMI, %BF, FPI, FPP, PIR, PCPR, HOMA-IR (Table 7). On multivariate logistic regression analysis, only age, BMI, FPP, and HOMA-IR were the only independent factors associated with presence of MetS (Table 7).

Table 7. Unadjusted and adjusted OR of predictors for MetS.

	Unadjusted OR (95% CI)	p	Adjusted OR (95% CI)†	p
Age> 57.7 years	4.609 (2.551-8.324)	<0.001	1.042 (1.008-1.078)	0.013
BMI>28.3 kg/m^2	8.28 (4.509-15.232)	<0.001	1.315 (1.167-1.481)	<0.001
%BF>39.6	4.055 (2.297-7.157)	<0.001	0.920 (0.403-2.096)	0.583
FPI>7.75 uU/ml*	3.575 (2.021-6.322)	<0.001	1.412 (0.454-4.398)	0.472
FPP>4.75 pmol/l*	6.414 (3.57-11.523)	<0.001	1.239 (1.121-1.474)	<0.001
PIR>3.58*	3.389 (1.943-5.913)	<0.001	0.495 (0.159-1.547)	0.564
PCPR>2.19*	3.944 (2.252-6.91)	<0.001	0.52 (0.184-1.495)	0.227
HOMA-IR>1.85*	6.023 (3.176-11.42)	<0.001	1.279 (0.133-1.589)	<0.001

† Model 1 - adjusted for age, BMI, %BF
*these variables were log-transformed before analysis

Discussions

The metabolic syndrome is an important risk factor for type 2 diabetes (T2DM) and cardiovascular diseases. Thus, the main clinical implication of a MetS diagnosis is the identification of patients who need aggressive lifestyle modification [20,21]. The results of this study showed that the prevalence of metabolic syndrome is high in the studied population and it increases significantly with age and BMI. Thus, the prevalence of MetS in this study (according to the modified IDF criteria [15,16]) was 43.3%. After stratification by gender, 43 men (49.4%) and 54 women (39.4%) had MetS. The prevalence of MetS in our study was higher than previously published in Romania [22] and in most European countries [23]. Patients included in this study were randomly selected following a screening campaign for diabetes. A possible explanation for this increased prevalence is the participation in a higher percentage of patients already presenting other metabolic disturbances.

The prevalence of obesity in our study was 30.4% (n=68), 25.3% (n=22) in men and 33.6% (n=46) in women. We also observed a higher prevalence of obesity than previously reported [24].

In the present study FPP and HOMA-IR were the best predictors in multivariate analysis for MetS; FPI, PIR, and PCPR were not associated with metabolic syndrome in multivariate models.

There are multiple definitions of the metabolic syndrome according to cutoff values for its components. In our study the best predictor for MetS in all patients and in women was TG, followed by HOMA-IR and FPP; in men the best predictor was HOMA-IR, followed by FPP and FPI. All these parameters have a high sensitivity but low specificity for identifying metabolic syndrome.

In our study the median fasting plasma proinsulin concentration was 4.33 (2.53-7.16) pmol/L, higher in men than in women (5.1 pmol/L (3.18-8.64) vs. 3.6 pmol/L (2.16-6.59)). These results are similar to those previously reported [25-30].

Similar with our study, Haffner et al. found that FPP was significantly correlated with TG levels, increased BP, and decreased HDL-C in non-diabetic patients [10]. Similar results were obtained by Nagi et al. but in diabetic subjects [9]. Haffner et al. and Nagi et al. found that FPI was more strongly associated with BMI than proinsulin concentrations [9,10]. Similar with other studies, we found that FPP was strongly associated with insulin resistance [31].

Our study has several limitations. First, this was a non-randomized study, with a cross-sectional design and it has a limited ability to eliminate causal relationships between FPP, FPI, HOMA-IR and MetS. Second, our definition of insulin resistance is based on HOMA-IR.

Conclusions

In conclusion, the present study showed that FPP and HOMA-IR were the best predictors in multivariate analysis for MetS; FPI, PIR, and PCPR were not associated with metabolic syndrome in multivariate models. Our results indicate that regardless of gender HOMA-IR and FPP could be the preferred parameters for predicting metabolic syndrome. These parameters enable the early identification of MetS, allow precocious treatment and therefore represent a major issue for the health care professionals.

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