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SEX-RELATED DIFFERENCES OF FATTY ACID-BINDING PROTEIN 4 AND LEPTIN LEVELS IN ATRIAL FIBRILLATION

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ABSTRACT

Aims

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Methods and results

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Conclusion

The major finding of the present study is the sex-based differences of FABP4 and leptin levels according to AF burden. These adipokines are associated with oxidative stress, inflammatory and autonomic indirect markers, indicating that they may play a role in AF perpetuation.

Keywords: Atrial fibrillation; Adipose tissue; Adipokines; FABP4; Gender; Ablation.

SEX-RELATED DIFFERENCES OF FATTY ACID-BINDING PROTEIN 4 AND LEPTIN LEVELS IN ATRIAL FIBRILLATION

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What´s new?

1- **Sex-related differences of FABP4 and leptin levels according to atrial fibrillation burden.**
2- **The adipose tissue and neutrophil biomarkers differed between male and female in paroxysmal and persistent atrial fibrillation.**
3- **The association of FABP4 with oxidative stress marker in persistent but not in paroxysmal atrial fibrillation.**
4- **Adipokines had higher importance than oxidative or inflammatory biomarkers for predicting recurrence after catheter ablation.**

ABSTRACT

Aims

Adiposity plays a key role in the pathogenesis of atrial fibrillation (AF). Our aim was to study the sex differences in adipokines levels according to AF burden.

Methods and results

Two independent cohorts of patients were studied: A) consecutive patients with AF undergoing catheter ablation (n=217) and B) a control group (n=105). 1) Adipokines, oxidative stress, indirect autonomic markers and leukocytes mRNA levels were analyzed; 2) Correlation between biomarkers was explored with heatmaps and Kendall correlation coefficients; 3) Logistic regression and random forest model were used to determine predictors of AF recurrence after ablation. Our results showed that: 1) FABP4 and leptin levels were higher in women than in men in both cohorts ($p<0.01$). In women, FABP4 levels were higher on AF cohort (20±14 control, 29±18 paroxysmal AF and 31±17 ng/mL persistent AF; $p<0.01$). In men, leptin levels were lower on AF cohort (22±15 control, 13±16 paroxysmal AF and 13±11 ng/mL persistent AF; $p<0.01$); 2) In female with paroxysmal AF there was a lower acetylcholinesterase

and higher carbonic anhydrase levels with respect to men ($p<0.05$); 4) Adipokines have an important role on discriminate AF recurrence after ablation. In persistent AF, FABP4 was the best predictor of recurrence after ablation (1.067, 95% CI 1-1.14; p=0.046).

Conclusion

The major finding of the present study is the sex-based differences of FABP4 and leptin levels according to AF burden. These adipokines are associated with oxidative stress, inflammatory and autonomic indirect markers, indicating that they may play a role in AF perpetuation.

Keywords: Atrial fibrillation; Adipose tissue; Adipokines; FABP4; Gender; Ablation.

INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia worldwide and a major public health problem whose prevalence is increasing in parallel with ageing and obesity (1). These factors predispose to accumulation of epicardial fat tissue (EAT), cardiac structure and function changes. The effects of this fat tissue may be local or systemic and a strong body of evidence highlights its ability to modulate the cardiovascular system and contribute to the development of arrhythmias (2). Although some effects of adipocytes, such as regulation of inflammation, oxidative stress or autonomic dysfunction have been postulated, the exact mechanisms of how EAT may drive AF is still not well described. Subsequently, it has been suggested that some adiposity markers might play a role in the pathogenesis of AF and explain this interaction (3). The intensification of research has evidenced its potential in obesity-related cardiovascular disease prevention and treatment. Proteomic studies have identified a fatty acid-binding protein 4 (FABP4), also known as adipocyte protein 2 (aP2), as a predictor of metabolic disorders and a new biomarker for AF risk (4). The main role of FABP4 is to be involved in the intracellular trafficking of fatty acids and lipid signals. But, in macrophages it can also improve the neutrophils recruitment and oxidative stress. Accordingly, FABP4 has been reported to contribute to structural heart disease and cardiac contractile dysfunction (5), explaining the relationship between FABP4 and AF perpetuation (6). In addition, FABP4 is co-regulated with leptin during adipose inflammatory process (7). Interestingly, the plasma levels of both proteins differ between males and females. This phenomenon likely might explain, among others, the sex differences in AF structural and electrophysiological mechanisms (8). Indeed, an increased risk of stroke, reduced catheter ablation efficacy and major complications have been reported in women compared to men (9).

Our study aimed to analyze a) the role of adipokines on AF and its sex-related differences; b) their relationship with inflammatory, oxidative and autonomic markers to elucidate potential mechanisms underlying obesity-AF and; c) their role on AF perpetuation.

METHODS

Subjects

Two independent cohorts have been used for this study. The case cohort belongs to a cross-sectional study where consecutive patients with paroxysmal or persistent AF were referred for pulmonary vein radiofrequency catheter ablation. The control cohort belongs to a cross-sectional study in which consecutive subjects with suspected coronary artery disease were referred for a CT scan. In the control cohort, subjects with history of AF or at very high risk of silent AF (patients with organic valvular disease, prosthetic valves, more than moderate mitral regurgitation secondary to left ventricular dilatation, pulmonary hypertension, or treated with oral anticoagulant) were excluded. In both groups, the exclusion criteria were age under 18 years, pregnancy, any latent infectious condition and an active oncology disease. Final analyses were thus based on 322 subjects, of which, 217 belonged to the case cohort (35% women and 65% men) and 105 to the control cohort (51% women and 49% men). All of the patients signed the informed consent. The study protocol follows the ethical guidelines of Declaration of Helsinki and approved by Ethical Committee of Clinical Research of our region according to Helsinki Declaration.

Blood sample collection

Case patients

During the ablation procedure (after a night of fasting), immediately after the transseptal puncture and previous to heparin administration, blood samples were obtained from the left

atrium (LA) through the transseptal sheath. At the same time, a peripheral blood sample was obtained from an ante-cubital vein using an 18-G butterfly cannula with a two-syringe technique, discarding the first 5 mL of blood and using the second 5 mL for measures (6). LA and peripheral blood samples were collected in EDTA-tubes. Electrical cardioversion was systematically performed at the end of the procedure.

Control patients

Peripheral blood sample collected in EDTA-tubes was obtained by venipuncture from an ante-cubital vein after a night of fasting and before the cardiac CT and contrast administration.

From cases and controls, glucose, creatinine and lipid profile were recorded and considered for the analysis.

Plasma and leukocytes measurements

1) FABP4, leptin, CAIX levels

After centrifuging at 1800xg for 10 minutes, the atrial and peripheral plasma samples were stored at -80 ° C until used. A magnetic Luminex multiplex test kit (R&D Systems, MN, USA) was used. The manufacturer's instructions were followed when analyzing plasma levels of FABP4, leptin and carbonic anhydrase IX (CAIX). The sensitivity for FABP4, Leptin and CAIX was 95.7, 10.2 and 2.11 pg/mL respectively.

2) Acetylcholinesterase (AChE) activity

The hydrolysis of acetylthiocholine by plasma AChE after 30 minutes of incubation was provided by a colorimetric assay to detect mU/mL as it is recommended in the manufacturer's instructions (abcam, Cambridge, UK).

3) Glycerol and H_2O_2 levels

Plasma glycerol levels were measured by a colorimetric assay based on glycerol kinase and glycerol phosphatase oxidase. The linear range of detection for this kit was 0.01-1 mM (Sigma-

Aldrich, St Louis, MO, USA). H_2O_2 levels were determined by a colorimetric assay that utilizes the chromogenic Fe3+ −xylenol orange reaction. The kit has a detection range of 0.2–30 M of H2O2 (Sigma-Aldrich).

4) IL-6 and DEFA3 mRNA expression levels

Atrial blood leukocytes were isolated after centrifuging. Then, erythrocytes were lysed by 155 mM NH_4Cl. Afterwards, RNA was isolated by Allprep RNA/protein kit (Qiagen, Gilden, GE) and complementary DNA was performed by Maxima Reverse Transcriptase activity (Thermo Scientific, Walthan, MA, USA). Real time polymerase chain reaction with the previous described primers was used for quantifying the mRNA expression levels with respect to β-actin levels as it was previously described (10).

Ablation procedure and patient follow-up

Patients underwent point-by-point radiofrequency catheter ablation (without contact force sensing, SmartTouch, Biosense Inc.). The procedural endpoint was ipsilateral pulmonary vein isolation (PVI). Most of the patients were discharged 24-36 hours after the ablation procedure. Oral anticoagulation (OA) was maintained for at least 3 months (until the first medical review). Then, OA was continued lifelong in those patients with a CHA2DS2-VASc score ≥ 2. During the blanking period (3 months), it is the standard of care in our center to continue or restart previously antiarrhythmic drug therapy (ADT). If the patient is free of recurrence after these 3 months, as evidenced by clinical evaluation and 24h Holter recording, patients are encouraged to discontinue ADT and only restart them in case of relapse. In case of a second recurrence after ADT or electrical cardioversion if needed, and always outside the blanking period, patients are advised for a *Redo* procedure. Medical visits were systematically performed at 3, 6 and 12 months after the index procedure. Each visit comprised detailed history, physical examination

and 12-lead electrocardiogram (ECG). Moreover, 24h Holter recording was routinely performed at 3, 6 and 12 months (6).

Statistical analyses

Numerical data were tested for normality using the Shapiro-Wilk test, and for homoscedasticity with the Levene's test, then summarized with mean, and standard deviation (SD). Bivariable analysis was performed either with the Wilcoxon rank-sum, or with the Pearson's Chi-squared test where appropriate. Kruskal-Wallis test was used for comparison multiple groups. Biomarker data were standardized prior to profile analyses and graphs. Data was matched by propensity scores for the graphical representation of biomarker profiles. Biomarker profiles were graphically explored with boxplots matched for age and body mass index (BMI). Correlation between biomarkers was explored by Kendall correlation coefficients. Logistic regressions and generalized additive models were used to test variables associated to either dichotomic or continuous dependent variables respectively. A random forest model based on the Breiman and Cutler's method was used to measure the within-study variable importance for classifying patients with or without recurrence 12 months after PVI. Random forests are regression and classification trees (CART) combined with bootstrap feature selection that provides increased classification performance and robustness in trained/validation data pairs with multiple variables. Multiple null hypothesis testing was addressed with false discovery ratio (FDR) control by the Benjamini-Hochberg procedure.

All analyses were programmed in R 3.5 (R Core Team, Vienna, Austria), using the packages ggplot, dplyr, purrr (Henry, 2019), Hmisc (Harrell, 2019) and $p < 0.05$ was considered with statistical significance.

RESULTS

Population characteristics

The study population included 322 participants of which 217 belonged to the case cohort (49% paroxysmal AF and 51% persistent AF) and 105 to the control cohort. After classifying patients in the case cohort according to AF pattern, we observed a younger population (57±14 vs. 63±13, $p<0.05$) and higher male percentage (80% vs. 49%, $p<0.05$) in the persistent AF group respect to the control cohort. Nonetheless, with respect to age and gender, the control cohort and the paroxysmal AF group were very similar. The mean BMI was higher in the case than in the control cohort (30±5 vs. 28±5 kg/m^2, $p<0.05$). There were no differences regarding the percentage of active smokers, arterial hypertension (AHT) or type 2 diabetes mellitus (T2DM). Neither the mean glucose, total and LDL cholesterol levels or percentage of statins prescription were different among cohorts. However, lower level of HDL cholesterol were observed in the persistent AF group ($p<0.05$). Although the levels of creatinine were higher in the case cohort ($p<0.01$), there were no differences in the percentage of chronic kidney disease (estimated glomerular filtration rate less than 60 ml/min/1.73 m^2). No between-group differences were observed in left ventricular ejection fraction (LVEF). Beta-blocker intake was higher in the case cohort. About 60% of patients in the case cohort were receiving ADT at baseline, most frequently Class I in paroxysmal AF and Class III ADT in patients with persistent AF. Baseline characteristics of patients included according AF pattern are presented in Table 1.

In 69% of the control cohort, coronary atherosclerosis was identified on cardiac CT (50% non-obstructive (≥20 but < 50% stenosis) and 19% obstructive).

All but one female from case cohort had reached menopause at the time of the ablation. This information was missing in the control cohort.

Peripheral plasma FABP4 and leptin levels in control cohort, paroxysmal AF and persistent AF groups

Higher peripheral plasma FABP4 levels were detected in patients with paroxysmal AF (23±16 ng/mL) as compared to the control cohort (17.5±12 ng/mL) ($p<0.05$). Leptin levels were lower in AF cohort ($p<0.001$) (Table 1).

While age was the main predictor for FABP4 levels in the control cohort, gender and BMI were in the AF cohort (Supplementary Table 1a). Regarding leptin levels, gender and BMI were the main predictors in the control and the AF cohort (Supplementary Table 1b).

Peripheral plasma FABP4 and leptin levels in women and men

Gender, BMI or ageing were the main factors associated with FABP4 or leptin levels in AF. Compared to men, women presented higher FABP4 and leptin levels in all cohorts (Figure 1). In women, despite of similar age and BMI among control, paroxysmal and persistent AF groups, the FABP4 levels were 20±14 ng/mL, 29±18 ng/mL and 31±17 ng/mL, respectively. So, the higher FABP4 levels were dependent on AF burden ($p=0.007$) (Supplementary Table 2). In men, there were not differences among groups regarding FABP4 levels. A decline in leptin levels was detected on AF cohort (Supplementary Table 3).

In the multivariable analysis, higher FABP4 levels were independently associated with ageing and higher BMI in male and female. However, only in female patients it was dependent on AF (Table 2a). Leptin levels were dependent on BMI, FABP4 levels and history of AF in male but not in female (Table 2b).

Relationship between FABP4 and leptin with inflammatory, oxidative stress, lipid transport and metabolism and indirect autonomic markers on atrial blood samples

As previously stated, we observed a clear positive association between FABP4 and leptin levels in patients with paroxysmal or persistent AF. Similarly, on leukocytes, defensin-3 (DEFA-3) levels, expressed mainly by neutrophils, were associated with interleukin 6 (IL-6). Also, there

was a significative association among hydrogen peroxide (H_2O_2) levels, which induces oxidative stress, and IL-6. However, FABP4 was inversely associated with glycerol, lipolytic metabolite, in persistent AF (Figure 2). The gender and AF type matched by age and BMI showed that the adipose tissue and neutrophil biomarkers differed between male and female in persistent and paroxysmal AF. In women with paroxysmal AF, there was a lower acetylcholinesterase, indirect parasympathetic activity, and higher CAIX (an intrinsic markers of hypoxia-acidosis) (Figure 3).

Variable importance to classify patients with recurrence after (PVI)

In a random forest model fitted to assess covariable importance, adipocyte markers were the highest ranked variables in predicting AF recurrence after PVI (Figure 4).

Logistic regression analysis was also performed and showed that FABP4 was the stronger predictor for persistent AF recurrence (adjusted by BMI, age, gender and leptin levels) ($p<0.05$), but not in patients with paroxysmal AF. Its interaction with leptin improved the predictive value of FABP4 (Table 3).

DISCUSSION

The major findings of the present study are the sex-related differences of two fat markers, FABP4 and leptin, on AF patients. This sex-dependent behavior might be related to differences in the pro-inflammatory potential of leukocytes in the context of AF. Importantly, FABP4 was the best predictor for persistent AF recurrence after catheter ablation in our sample population. These findings portray an association between adiposity and AF, even in terms of AF severity with specific differences amongst gender. These findings would give rise to further insights regarding the role of the adipose tissue in AF development, to design directed therapies

according to sex and finally it may help select individuals most likely to benefit from invasive strategies.

a) Adipocyte biomarker and its relationship with inflammatory and oxidative markers in patients with and without AF according to gender.

We found a positive relationship between plasma FABP4 and leptin levels with BMI and female sex. It is assumed that the concentration of these adipokines increases with obesity due to the greater amount of body fat. This argument is also used to explain the highest levels in women, but sex-specific associations between sexual hormones and adipose tissue secretion have also been described (11). Accordingly, with the same amount of visceral fat, there are differences in the secretome from adipose tissue between women and men, which could be one of the reasons for the important sex differences detected in AF patients. In the present study, plasma FABP4 and leptin levels were different among cohorts despite fairly similar cardiovascular risk factors and irrespective of age, BMI and sex. FABP4 levels were higher and leptin levels were lower in the AF cohort compared to the control cohort. After splitting the population according to gender, we found significant differences on plasma FABP4 levels in women according to AF burden. The lack of differences in female mean age among groups (64 ± 13 in the control group, 64 ± 8 in paroxysmal AF patients and 64 ± 8 year old in persistent AF patients; p=0.9) could indicate that this finding is not due to hormonal differences (menopausal), which as it has been described has influence on FABP4 levels (11).

A similar behavior was observed in coronary atherosclerosis, where women presented higher levels of FABP4 than men (12). A higher predominance of the parasympathetic nervous system in women and the antilipolytic effect of beta-blockers (ADT Class II) treatment might explain, at least in part, a higher fat accumulation and fatty acid transport into the adipose tissue storage

in women as compared to men. Whether this potential different response is due to specific fat metabolism is unknown but under our point of view would deserve further investigations.

On the other hand, in men, leptin levels were lower in patients with persistent AF as compared to those with paroxysmal AF and the control group, but without significant changes on FABP4 levels. Though, it has to be emphasized that the high proportion of coronary heart disease (69%) in the control cohort may justify pathological FABP4 levels in this subgroup. These findings could have masked the difference of FABP4 levels among groups since this protein increases with age and is related also to atherosclerosis. Nevertheless, it does not seem to be the explanation for the differences detected in leptin levels because is decreased with aging and increased with obesity. These results suggest a new mechanism associated with the gender-dependent increased FABP4 and decreased leptin levels in AF patients. One of the potential explanations for the differences seen in FABP4 and leptin levels according to AF type could be based on the pro-inflammatory substances release by the adipose tissue. For instance, epicardial fat becomes dysfunctional in obesity, resulting in an increased production of proinflammatory factors and cytokines targeting the vascular wall, inducing endothelial dysfunction and inflammation. The results of the present study show that FABP4 levels were positively correlated with H_2O_2 (oxidative stress radical) and inversely with glycerol (lipolysis metabolite) in persistent AF. The increment of fat accumulation suggests a lower lipolytic activity, which can be associated with autonomic disbalance, higher oxidative stress and pro-inflammatory activity. This mechanism might favor the arrhythmogenic substrate on the atrium and perpetuate AF. It is known the important role of obesity therapeutic effectiveness of sodium vs. potassium channels blocker antiarrhythmics drugs. One of the mechanisms suggested is the oxidative stress. In line with this hypothesis, our data showed an increment of hydrogen peroxide in the left atrium (as compared to peripheral blood) (see supplementary Table 4). Importantly, this difference was higher in persistent than paroxysmal AF (13). It might indicate

that the oxidative stress could be a potential mechanism involved in AF perpetuation, from paroxysmal to persistent forms. Likewise, the consistent associations of DEFA-3, IL-6 and H_2O_2, which promote endothelial dysfunction and prompt alterations in vascular structure, reinforced this potential relationship between pro-inflammatory and oxidative stress environment and AF progression.

While FABP4 and leptin levels differed among male and females in paroxysmal or persistent AF, there was a lower acetylcholinesterase and higher carbonic anhydrase levels in female patients with paroxysmal AF respect to men. These findings might explain in part the differential mechanism underlying paroxysmal AF between male and female.

b) *Value of these biomarkers as predictors of recurrence after AF catheter ablation*

Myocardial lipidosis, inflammation and proliferation of fibroblasts induced by epicardial fat-secreted adipokines contribute to the progressive fibrotic remodeling of the atrium (14). This disorganization and loss of homogeneity of the atrial myocardium is considered the substrate for the development and maintenance of electrophysiological disturbances (15). AF is a clinically manifestation of this pathological changes. As a matter of fact, the EHRAS classification (EHRAS Class I–IV) is a first attempt to characterize these atrial pathologies/stages into discrete cohorts. Specifically, based on the adipocyte infiltration into the myocardium and atrial fibrosis, cardiomyopathy due to obesity was classified as EHRAS Class IVf and EHRAS III as collagen depositions are also present (16). The biomarkers herein analyse could help characterizing the atrial substrate according to sex and this characterization is very important since the different predictive capacity according to AF type seems to vary over time and be different according to the underlying substrate: the presence of pulmonary vein triggers frequently seen in paroxysmal AF versus a heavier weight placed on a modified and complex substrate, with extrapulmonary vein triggers, seen in persistent AF. According

with this rationality and in agreement with our prior study (6), the random forest determined that the adipocyte markers were the most important variable for discriminating long-term AF recurrence after catheter ablation, being FABP4 levels were the best predictor of recurrence in persistent but not in paroxysmal AF.

Clinical implications and future directions

Based on the fact that adipokines can provide information about both the amount and activity of fat, these results could open the door to an intensification of research to exploit a more precise relationship between adipose tissue and AF. In fact, the sex-related differences in adipokines may provide an explanation for the important gender differences in the epidemiology, pathophysiology and prognosis of AF. Several studies found that FABP4 levels increase drastically after menopause (17), which would justify the later presentation of AF in this population. Other studies showed that high levels of FABP4 are associated with a worse prognosis after a stroke (18), which would explain why women might have an increased risk of stroke/TIA and all-cause mortality compared with men. Herein we describe the potential role of FABP4 as a predictor of success after catheter ablation in patients with persistent AF. From our point of view this is not a negligible point since at the present time there are few and poorly studies markers of atrial disease beyond atrial size. This may help select individuals most likely to benefit from invasive strategies.

On the other hand, changes in lifestyle and increasing aerobic physical activity can decrease FABP4 levels (19), and therefore it could reduce the adverse events and improve the efficacy of catheter ablation. Whether it could be used for monitoring changes in fat activity is still uncertain and will deserve further investigations.

Limitations of the study

We acknowledge that our study has several limitations. The lack of association with some other variable could be caused by a lack of statistical power due to the presence of missing data. Two independent cohorts were used in this study. Importantly, although none of the patients in the control cohort reported previous episodes of AF, silent episodes cannot be strictly ruled out. We tried to minimize this limitation by excluding patients at very high risk for AF (patients with organic valvular disease, prosthetic valves, more than moderate mitral regurgitation secondary to left ventricular dilatation, pulmonary hypertension, or treated with oral anticoagulant), a very similar profile of patients referred for AF ablation. Hence, although this is a limitation that has to be taken into account, from our point of view it should not have altered the conclusions of the present study.

The sex ratio was different between cohorts. Due to the inclusion of consecutives patients (not selected) the proportion of females was higher in the control cohort than in the case cohort (51% vs. 35%). Moreover, the vast majority of women referred for AF ablation experience menopause at the time of the procedure and this information was missing in the control cohort. Subsequently, the conclusions of the present study could not be applicable to non-menopausal females.

This study found that higher FABP4 levels were independently associated with ageing and higher BMI in male and female. However, we did not get the body fat percentage by Dual-Energy X-ray Absorptiometry (DEXA). Thus, although in our previous study FABP4 levels where associated with the amount of left atrial adipose tissue (LAAT), we cannot rule out the possibility that FABP4 levels from our patients are associated with both LAAT and total body fat volumes. Because there is a higher extracardiac adiposity, the FABP4 levels were also higher in peripheral than in atrial blood. However, the correlation between atrial FABP4 levels and hydrogen peroxide, levels that were higher in atrial than in peripheral blood (Supplementary

Table 4), might explain the therapeutic inefficiency against adiposity-oxidative stress and the AF perpetuation.

Long treatments with atorvastatin can reduce the FABP4 expression levels induced by oxidized-LDL (20). It is conceivable hence that baseline level of adipocytes marker can be affected by baseline medical treatment. However, due to the lack of baseline differences (Table 1), this fact should not have distorted the conclusions of the study.

In a high proportion of the subjects enrolled in the control cohort (69%) a coronary artery disease was detected by CT scan. As it has been described, perivascular adipose tissue is involved in atherosclerosis pathogeny via several possible mechanisms, among other due to the pro-atherogenic effect of FABP4 and leptin. Consequently, it is likely that if the study had it been done in patients without CAD, the differences seen in adipocytes markers could have been even higher.

In the present study, sex-specific differences in the associations between various adipokines and AF burden has been pursuit. However, mechanistic studies aiming at revealing the mechanistic role of these adipokines for AF perpetuation have not yet been performed and are clearly warranted to better appreciate the relevance of these findings for sex differences in AF.

CONCLUSION

The major finding of the present study is the sex-related differences of FABP4 and leptin according to AF burden and its relationship with oxidative stress, inflammatory and autonomic indirect markers.

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DISCLOSURES

Nothing to declare.

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Figure legends

Figure 1. Box plots represents median and interquartile range of adipokines and lipoproteins regarding gender and AF burden, data matched for age and BMI.

Figure 2. Heatmaps of Kendall´s correlation among levels of biomarkers on atrial plasma.

Figure 3. Box plots represents median and interquartile range of biomarkers levels on atrial plasma regarding gender and AF burden, data matched for age and BMI.

Figure 4. Random forest plot represents the main important variables for discriminating AF recurrence after catheter ablation.

Figure

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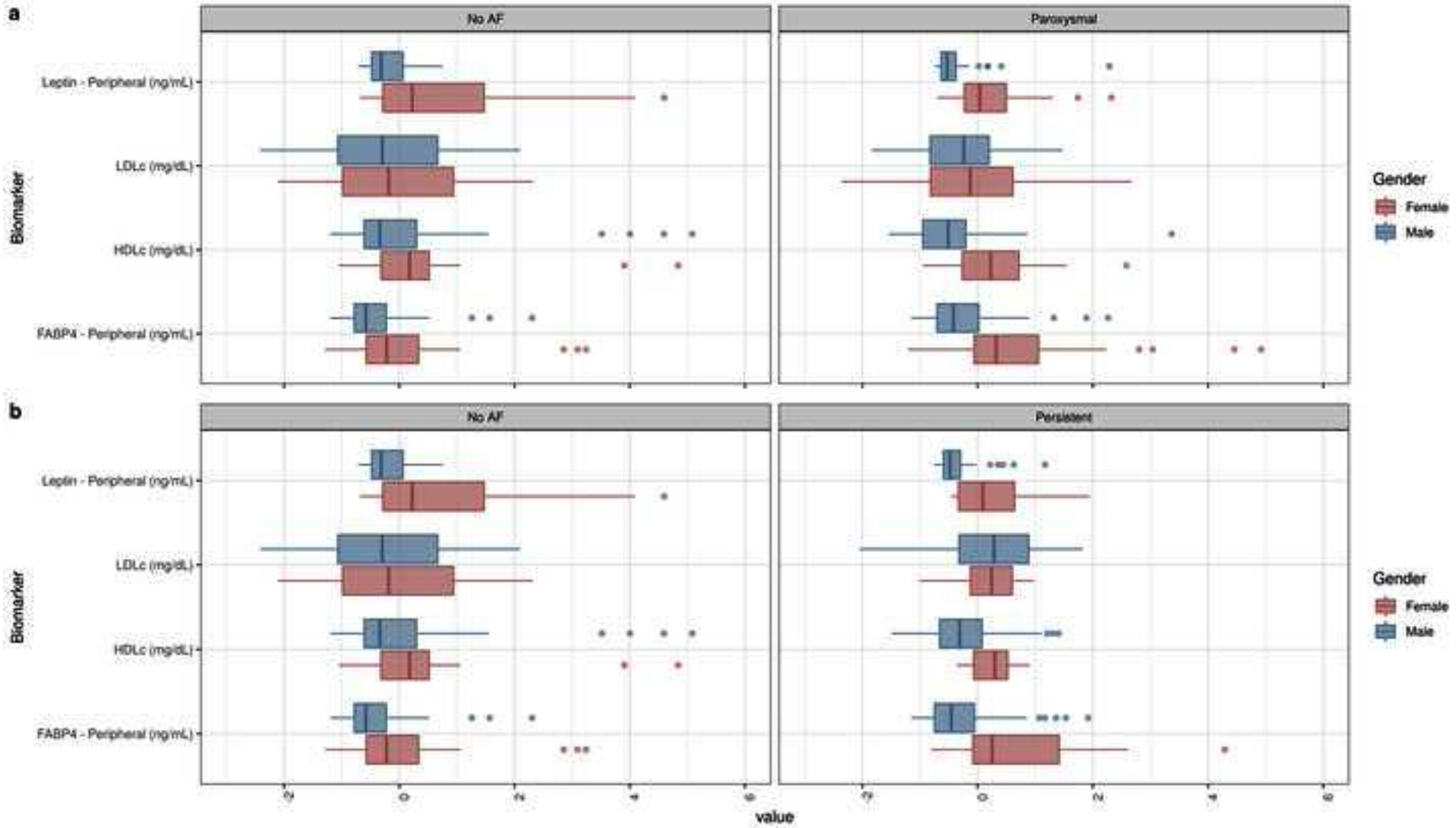

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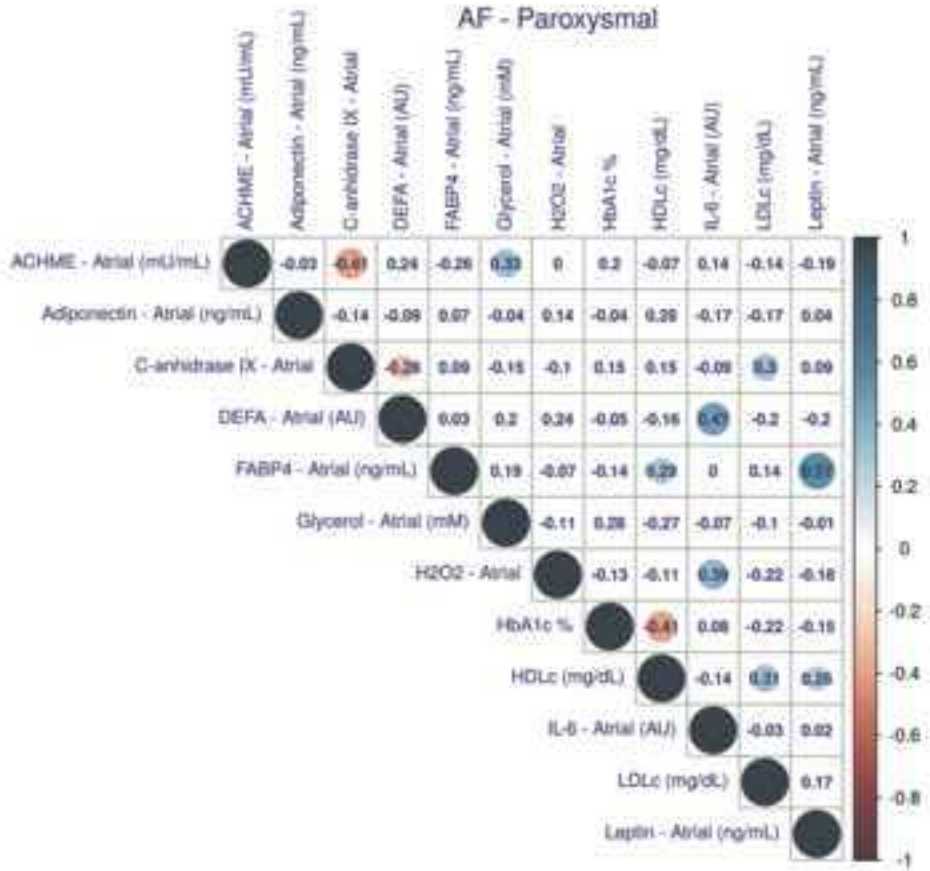

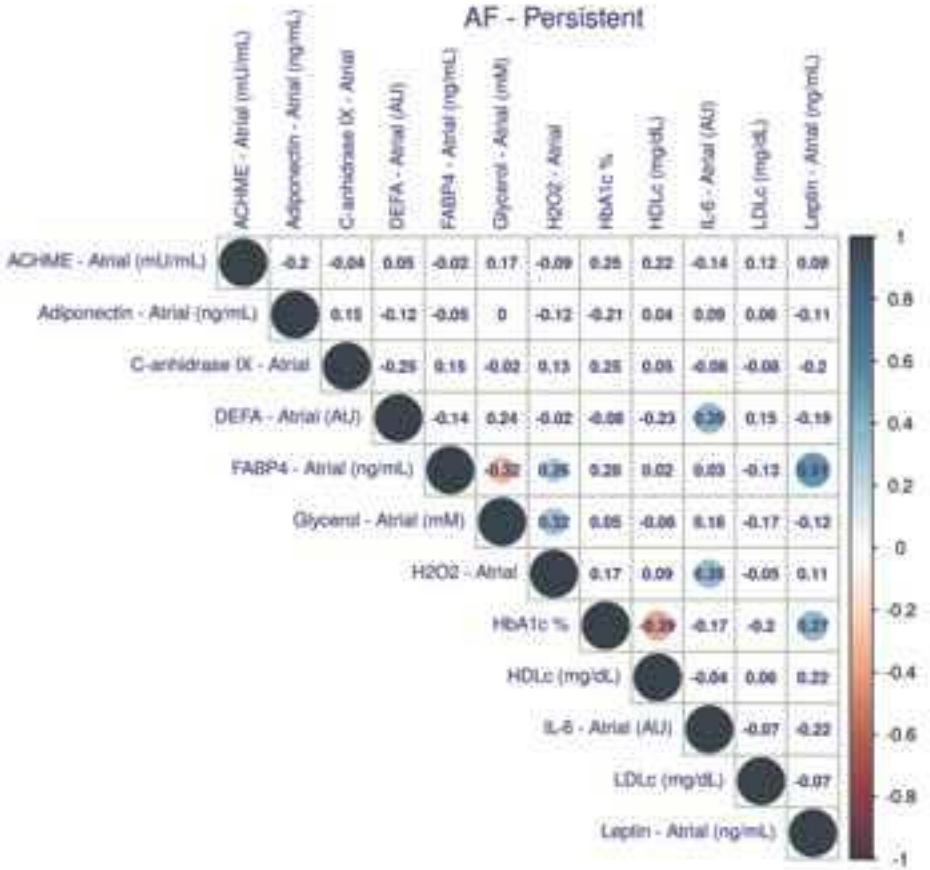

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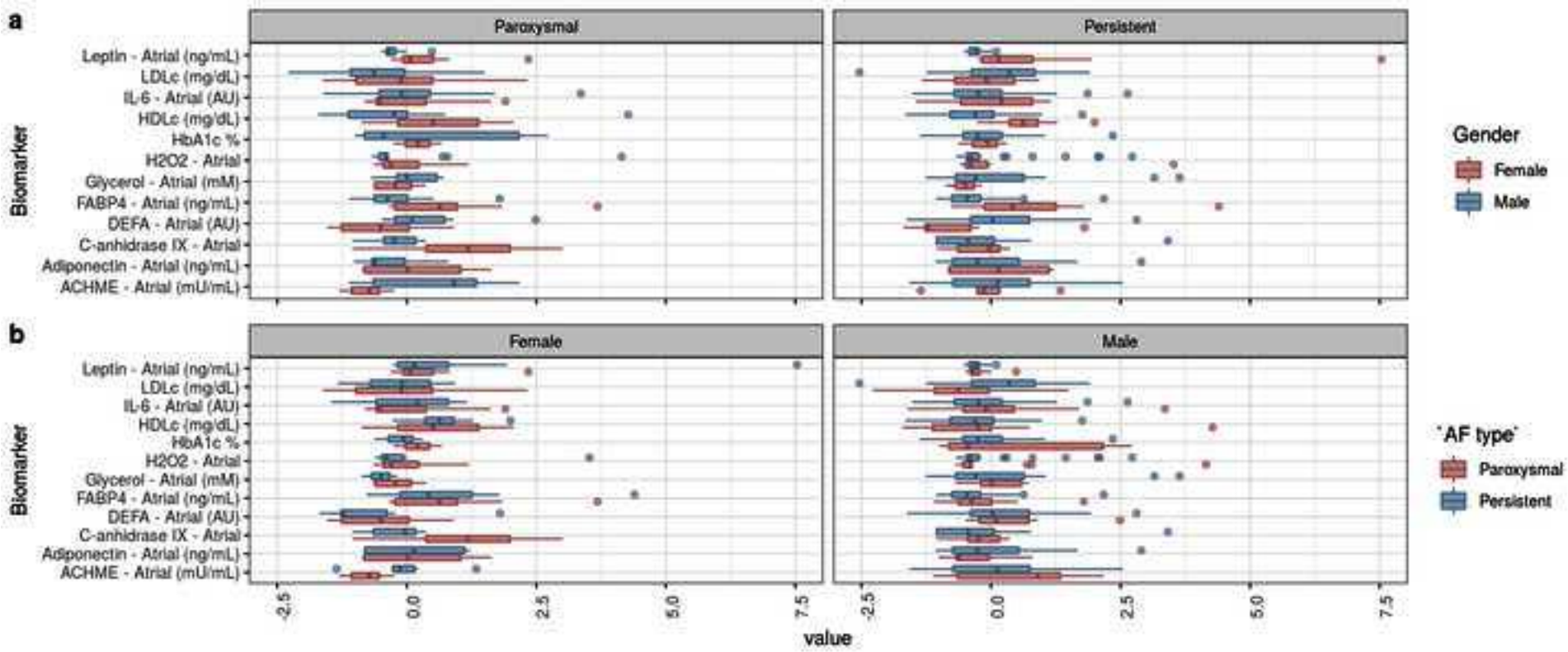

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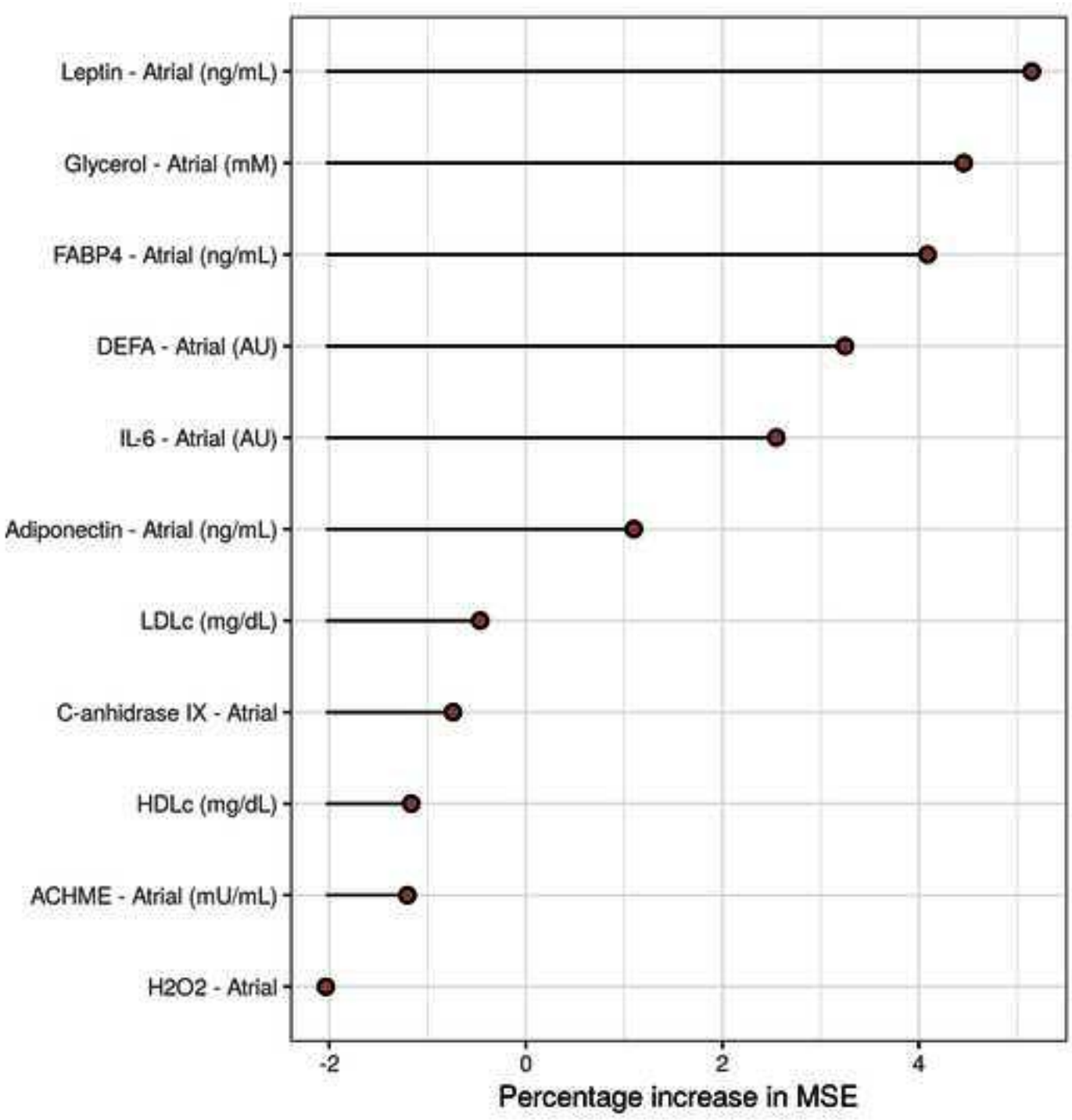

Table 1

Table 1. Differential characteristics among control, paroxysmal and persistent AF at baseline.

	Control (n=105)	**Paroxysmal AF (n=107)**	**Persistent AF (n=110)**	**P Value**
Age (years)	63±13	60±10	57±14*	**< 0.001**
BMI (kg/m2)	28±5	30±5*	30±5*	**0.002**
Gender male/female (%)	51/54 (49%)	54/53 (50%)	88/22 (80%)*≠	**< 0.001**
AHT (%)	56 (53%)	41 (38%)	53 (48%)	0.083
Active smokers (%)	32 (31%)	30 (28%)	36 (32%)	0.750
T2DM (%)	23 (22%)	12 (11%)	14 (13%)	0.064
Obesity (%)	31 (30%)	46 (43%)*	51 (46%)*	**0.028**
CKD (%)	4 (4%)	11 (10%)	11 (10%)	0.148
Creatinine (mg/dL), (mean ± SD)	0.82±0.20	0.95±0.27*	1.06±0.28*≠	**< 0.001**
Glucose (mg/dL), (mean ± SD)	106±35	108±25	109±30	0.702
TC (mg/dL), (mean ± SD)	199±42	190±45	198±37	0.307
LDL-C (mg/dL), (mean ± SD)	114±41 (105-122)	112±36 (104-121)	125±30(118-131)	0.076
HDL-C (mg/dL), (mean ± SD)	59±26 (53-64)	52±18 (48-56)	51±13 (48-54)*	**0.030**
FABP4 (ng/mL), (mean ± SD)	17.5±12 (15-20)	23±16 (20-26)*	19±13 (17-21)	**0.015**
Leptin (ng/mL), (mean ± SD)	39±42 (31-48)	24±22 (20-28)*	21±37 (14-28)*	**< 0.001**
LVEF (%)	63±7	64±6	62±8	0.220
Statin yes/no (%)	48/57 (46%)	32/44 (42%)	30/56 (35%)	0.313
Beta-blocker n (%)	23 (22%)	59 (56%)*	72 (67%)*	**< 0.001**
ADT Class I n (%)	----	43 (41%)	23 (21%)≠	**0.002**
ADT Class III n (%)	----	23 (22%)	39 (36%)≠	**0.020**

BMI: Body Mass Index; **AHT:** Arterial Hypertension; **T2DM:** Type 2 Diabetes Mellitus; **CKD:** Chronic Kidney Disease (eGFR < 60 ml/min/1.73 m2); **TC:** Total Cholesterol; **FABP4:** Fatty Acid-Binding Protein 4; **LVEF:** Left Ventricular Ejection Fraction; **ADT:** Antiarrhythmic Drug Therapy. Post hoc differences between paroxysmal or persistent AF vs. control* or paroxysmal vs. persistent AF≠.

Table 2a. Logistic regression. Dependent variable: FABP4 levels on women or men

	Women			*Men*		
	COEFF.	**SE**	**p**	**COEFF.**	**SE**	**p**
(Intercept)		-1.69	0.092		-2.62	**0.009**
Age	0.181	2.23	**0.027**	0.217	3.30	**0.001**
BMI	0.215	2.41	**0.017**	0.246	3.12	**0.002**
AF presence/control	0.232	2.81	**0.006**	0.129	1.80	0.074
Leptin	0.147	1.65	0.101	0.261	3.28	**0.001**

Table 2b. Logistic regression. Dependent variable: Leptin levels on women or men

	Women			*Men*		
	COEFF.	**SE**	**p**	**COEFF.**	**SE**	**p**
(Intercept)		-1.33	0.185		-4.74	**0.000**
Age	0.019	0.196	0.845	0.096	1.39	0.165
BMI	0.413	4.40	**0.000**	0.595	8.73	**0.000**
AHT	0.062	0.634	0.528	-0.078	-1.10	0.273
AF presence/control	-0.141	-1.50	0.136	-0.413	-6.23	**0.000**
Total cholesterol	-0.037	-0.403	0.688	.0.034	0.541	0.589

Table 3. Logistic regression analysis for persistent AF recurrence after PVI

	COEFF	95% CI	p Value
(Intercept)	1.0603195	0 - 480.6	0.9849
FABP4	1.0673804	1 - 1.14	**0.0466**
Leptin	0.9584996	0.9 - 1	0.1204
Gender	0.2758803	0.05 - 1.72	0.1527
BMI	1.0092720	0.84 - 1.2	0.9180
Age	0.9767280	0.92 - 1.03	0.4149

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Supplementary file

Supplementary Tables.R1.FINAL.docx