Epicardial fat, gender, and cardiovascular risk

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ABSTRACT

Epicardial fat (EF) is considered an important risk factor and an active player in the pathogenesis of cardiovascular and metabolic diseases. EF is an endocrine organ that releases hormones and mediators, including the circulating C-reactive protein (CRP), and plays a vital role in modifying the vascular endothelial function and promoting the growth of coronary atherosclerosis. This study aimed to investigate the relationship between CRP concentrations and EF in a cohort of patients with metabolic syndrome at risk for coronary artery disease. In our study, carried out in primary prevention, we enrolled 36 subjects (M/F: 21/15; age: 59.3±0.79 yrs) diagnosed with metabolic syndrome. We have classified the patients into two groups: Men and Women. Besides anthropometric characterization and screening laboratory tests, the subjects performed a multidetector computed tomography scan, which allowed the EF quantification. Mean EF was 115.1 cc in the study population. The average EF of women was 111 cc; the average EF of men was 118 cc (P=0.18). CRP levels were strongly positively correlated with EF area in women (P=0.01), while the correlation was not found in men (P=0.4). Our findings suggest that, in women, the EF produces a greater amount of acute-phase proteins and increases the pro-inflammatory state in the epicardial region. For this reason, we can hypothesize, in women, a different role in the development of atherosclerotic plaque of the epicardial fat compared to men.

Introduction

Epicardial fat (EF) is considered an essential independent cardiovascular risk factor and may contribute, through local production of inflammatory cytokine and the development of a chronic, low-grade inflammatory state, to the progression of coronary atherogenesis.[1-3] Particularly, EF is the visceral fat depot with anatomical and functional contiguity to the myocardium. The EF has unique characteristics compared to others of visceral fat deposits, as it uses the same bloodstream of the myocardium and has a more remarkable ability to release free fatty acids (FFA), necessary for the myocardium to produce energy.[4,5] Furthermore, the EF shows a greater expression and secretion of adipokines and inflammatory chemokines [tumor necrosis factor (TNF)-alpha, interleukin (IL)-8, MCP-1, C-reactive protein (CRP), IL-1β, and IL-6] and infiltration of the chronic inflammatory cell as macrophage in pathological conditions.[6] For these features, EF plays an important role in modifying the vascular endothelial function and promoting the growth of coronary atherosclerosis.[7]

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Materials and Methods

We retrospectively studied 36 adult patients (M/F: 21/15; age: 55±1.5 years) diagnosed with metabolic syndrome (MetS) who underwent cardiac multidetector computed tomography (MDCT) for the calcium score evaluation. Patients were recruited at the Prevention of Cardiovascular Diseases ambulatory of 'Fatebenefratelli Hospital - Isola Tiberina'. According to the Adult Treatment Panel III (ATP III) report, the subjects were diagnosed with MetS in the presence of three or more of the following criteria: i) waist circumference higher or equal to 102 cm for men and 88 cm for women; ii) triglyceride levels higher or equal to 1.7 mmol/L; iii) high-density lipoprotein cholesterol level less than 1.03 mmol/L or 1.29 mmol/L for men and women, respectively; iv) systolic blood pressure higher than, or equal to 130 mmHg, or diastolic blood pressure higher than or equal to 85 mmHg; v) fasting blood glucose higher than or equal to 6.1 mmol/L.[8]

The recruited patients were males and females aged between 45 and 75 years, affected by MetS (according to ATP III) but without diabetes (glycemia ≤126). Our study excluded patients with renal impairment, previous cardiovascular events (ischemic heart disease, cerebrovascular disease) and history of cancer and/or diabetes, and women in pregnancy and/or breastfeeding. Moreover, to avoid selection bias, patients with a history of overt type 2 diabetes were excluded from the study. This study was carried out with the approval of the local ethics committee and the informed consent of all the participating patients.

Computed tomography imaging

Patients performed MDCT scans, according to the protocol generally used for calcium score. This method is a non-invasive approach for the research and quantification of EF (Figure 1). The imaging parameters for cardiac MDCT were: prospective sequential electrocardiogram gating; 64 channel detectors along the z-axis, scan FOV 15-21 cm (depending on the patient size), matrix 512×512, by means of axial scans detector collimation 3 mm; reconstruction 2.5 mm, gantry rotation time 0.25 ms, tube 9 current range 50-100 mA (depending on the patient size and with automated modulation), 100 kV or 120 kV [according to body mass index (BMI)]. CT images were reconstructed with a slice thickness of 2.5 mm and a slice distance of 0.5 mm with an overlap of 0.5 mm, both in the CAS and CTA images (Figure 2).

The epicardial fat was considered the adipose tissue accumulated between the visceral pericardium and the myocardium, without a structure or fascia separating it from the myocardium and the epicardial vessels.[9] For the EF quantification, DICOM images were transferred to a research workstation. The epicardial surface was computed by using an interactive procedure previously developed at the CNR Institute of Clinical Physiology. The procedure required tracing the pericardial contours both in contiguous axial slices. Furthermore, the procedure allowed splitting the cardiac region into left and right zones by tracing an interventricular plane in two axial slices. In order to evaluate the correlation between individuals, every single cardiac silhouette and the subsequent volume were analyzed in a blinded fashion by two radiologists with four and ten years' experience on thorax CT imaging, respectively. We decided to calculate the EF quantity as a volumetric measurement, to obtain higher reproducibility and inter-observer agreement than a distance measurement.[10]

Anthropometric measurements

Body height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was measured to the nearest 0.01 kg using calibrated electronic digital scales in barefoot subjects. BMI was calculated by dividing the body weight (in kilograms) by height squared (in meters) and was used to quantify obesity. Waist circumference was measured at the level of the umbilicus with quiet respiration. The calculation of body surface area (BSA) was done using the Mosteller formula ([Height (cm) × Weight (kg)]/ 3600)$^{(1/2)}$.[11]

Blood pressure

Blood pressure (systolic and diastolic) was measured, in the sitting position, after a 20-min rest period using a mercury manometer. We calculated the average value originated from two different measurements.

Biochemical analysis

Blood exams were put into 8-mL tubes containing thrombin- and heparin-neutralizing agents. Blood was

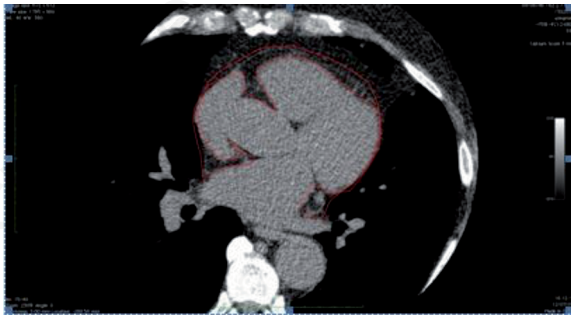

Figure 1. Epicardial fat on axial computed tomography images of the heart.

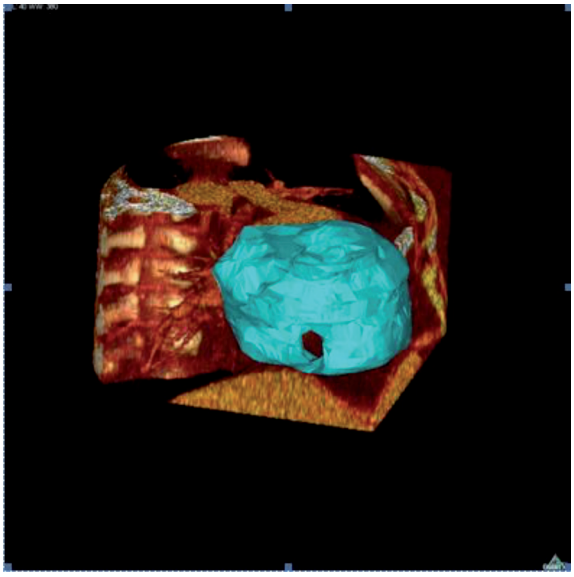

Figure 2. Measurement of epicardial fat volume: 3D reconstruction.

centrifuged at 3000 rpm for 30 min at 4°C to separate plasma. High-density lipoprotein cholesterol and triglyceride levels were determined in serum through the semiautomatic chemical analyzer Ekem Control Lab. The blood glucose level was measured using Cobas c111 automated chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany) *in vitro* test kits for the qualitative test of human serum and plasma. Plasma TG concentrations were determined by means of the enzymatic method using a TG kit, and plasma-free fatty acids were measured by the colorimetric method. CRP was measured using a sensitive double-antibody sandwich ELISA with rabbit anti-human CRP and peroxidase-conjugated rabbit anti-human CRP. The assay was linear up to 5 mg/L and logarithmic thereafter. The inter-assay and intra-assay coefficients of variation were <10% across the range of measured results. Low-density lipoprotein-cholesterol was calculated by Friedewald's formula.[12]

Statistical analysis

Statistical comparisons between groups (men and women) were performed using Student's *t*-test for two samples or Mann-Whitney U test for parametric or non-normally distributed data (Kolmogorov-Smirnov test), respectively. Unequal-Variance test was used when the variances of the two populations were not equal to the Aspin-Welch. Correlations between serum CRP levels and other variables were determined using Spearman's (rs) and Pearson's (rp) r correlation, while multivariate relationships were analyzed using multiple regression models. Data are shown as mean ± standard error mean or median (25th percentile; 75th percentile). P<0.05 were considered to indicate statistical significance. Statistical analysis was carried out using the NCSS software (Kaysville, UT, USA).[13]

Results

A total of 36 patients were evaluated, and we have classified the patients into two groups: men and women. 26.6% satisfied three criteria among women, and 50% of them had blood glucose >100 mg/dL. In 73.4% of cases, there were more than three criteria satisfied, and 33% had blood glucose values greater than 100 mg/dL. Among men, three metabolic syndrome diagnostic criteria were present in 22% of cases (there was an alteration in glucose control in 25% of cases), whereas the other patients (78%) had more than three diagnostic criteria (blood glucose >100 mg/dL were 33%). This distribution was homogeneous. The mean age of patients was 59.3±10.79 years, and 58.3% of the subjects were male. The average values of EF were 115.1 cc in the study population. The average EF of women was 111 cc; the average EF of men was 118 cc (P=0.18) (Figure 3). The main clinical and laboratory findings of the 2 groups were summarized in Table 1.

There was no significant difference between the Men and the Women group for BMI, waist circumference, blood pressure, fasting blood glucose, insulin levels, lipids, uric acid concentrations, smoking habit, and age.

The median levels of C-reactive protein were 2.71 mg/L in women compared with 1.74 mg/L in men

Figure 3. Epicardial fat measures in men and women (cc).

Table 1. Principal characteristics of the study population.

	Men	Women	P
Age (yrs)	57 (54; 61)	59 (54;66)	ns
Waist circumference (cm)	105 (101;110)	101 (100;115)	ns
BPs (mmHg)	140 (130;147)	143 (140;145)	ns
BPd (mmHg)	84 (81;91)	91 (90;95)	ns
Triglycerides (mmol/L)	1.5 (1.12;1.9)	2.12 (1.52;2.2)	ns
Glucose (mmol/L)	5.1 (4.3;5.8)	5.12 (4,8;5.78)	ns
HDL (mmol/L)	1.03(0.96;1.27)	1,03(0.97;1.27)	ns
Epicardial fat (ml)	118 (91.7; 129.8)	111 (106;150)	ns
HbA1c (mmol/mol)	42(31;48)	40 (35;60)	ns
Insulin (pmol/L)	80.7±8.8	85.9±15.2	ns
Uric acid (µmol/L)	52±3.4	59.8±4.2	ns

Data are mean ± standard error mean or median (25th percentile; 75th percentile). BPs, systolic blood pressure; BPd, diastolic blood pressure; HDL, high-density lipoproteins; ns, not significant.

(P=0.002) (Figure 4). Pearson's correlation showed a strong, positive correlation between epicardial fat obtained from MDCT and HS-CRP in women (r=0.426 P<0.01), but not in men (r=0.142 P=0.4) (Table 2). No differences between premenopausal and postmenopausal women were observed.

Discussion

EF is now considered an independent risk factor for cardiovascular risk due to its capacity to secrete a number of cytokines and chemokines. These molecules, known as adipokines, are produced from mature adipocytes and from the cells present within the adipose tissue (macrophages, fibroblasts, mast cells) and are correlated to the development of coronary atherosclerosis.[14,15] In pathological conditions, such as those found in patients with MetS, the EF undergoes many changes causing the invasion of macrophages and T lymphocytes in the adipose tissue.[16,17] Consequently, there is an increased secretion of pro-inflammatory cytokines and a reduction in the secretion of anti-atherosclerotic adipokines such as adiponectin. These modifications are related to the development of atherosclerosis and changes in plaque phenotype.[18] CRP is an acute-phase reactant that is a marker of inflammation in the body, and elevations of CRP concentrations are risk factors predictive of future cardiovascular events.[19] A high CRP value results to be a marker of increase in IL-6. IL-6 plays a vital role in the development of atherosclerosis, through the activation of endothelial cells and the coagulation cascade and the proliferation of lymphocyte and the activation of the local renin-angiotensin pathways, and this promotes vascular wall inflammation and damage.[20]

In addition, the increase in CRP may directly enhance inflammation in plaques, mediated binding to the complement factor C1q, and through the direct activation of different adhesion molecules.[21,22] Previous studies had already shown an association between CRP levels and visceral adipose tissue.[23,24] The reason for this correlation was identified in overproduction, by adipocytes, tumor necrosis factor messenger RNA (TNF-alpha). TNF-alpha induces IL-6 synthesis, which modulates the production of CRP.[25,26] In our study, we noted strong correlations between EF and CRP in the Women group only. A possible explanation for this result might be that the metabolic activity of adipose tissue is different in men and women due to the influence of sex steroids, which leads to a different response in the production of inflammation mediators.[27] Some experimental data suggest an inhibitory effect of estrogens on interleukin 6 gene expression, while some authors have suggested an increase in CRP with hormone replacement therapy (no patients recruited in our study, however, took on replacement therapy).[28-30] Anyway, although many studies show that estrogens play an important role in decreasing visceral fat, it is still unclear the role of sex hormones in the epicardial fat with unique specificity.[31,32] Gender differences are confirmed by other studies showing that the EF produces higher adiponectin concentrations and leptin in women than in men.[33]

Furthermore, our data found that the average abdominal circumference in the female population exceeds the basic values according to ATP III (101 *vs* 88 cm) than in the male population (105 *vs* 102 cm), although it is not statistically significant. Probably, that is due to the hormonal changes that occur with menopause, characterized by a reduction in estrogen (responsible for the accumulation of fat in the subcutaneous tissue, in particular in the gluteal and femoral regions), and by a relative increase in androgens (which instead promote the accumulation of fat in the abdomen). Consequently, the relative hyperandrogenism observed in menopause, caused by the decline in estrogen, becomes the cause of metabolically unfavorable fat redistribution prevalent in the abdominal area. On the one hand, this reflection allows us to interpret the results of our work better; on the other hand, it leads us to propose the introduction of a corrective measure for the age group that is useful for women, for the measurement of the waist, which includes at least a pre and postmenopausal cut-off. The metabolically unfavorable redistribution of fat prevalent in the abdominal area can also determine a higher value of

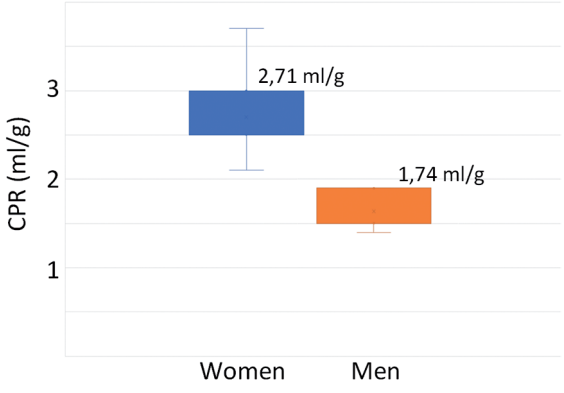

Figure 4. C-reactive protein (CRP) values in the men and women group.

Table 2. Correlation of high sensitivity C-reactive protein with percentage epicardial fat area in subjects with metabolic syndrome.

Epicardial fat area	Men (n=22)	Women (n=14)
hs-CRP	0.209	0.360*

Values are correlation coefficients, *P<0.01 hs-CRP, high sensitivity C-reactive protein.

triglyceridemia, as we found in the female sample, always in a not statistically significant way. In this last regard, it should also be emphasized that compared to measuring the waist, the triglyceridemia values are extremely variable and more difficult to read. Moreover, sex-specific adipose tissue distribution also may result from the secretion of sex hormones produced by adipose tissue itself. These data suggest that the role of sex hormones may have fundamental effects on the metabolic activity of epicardial fat.

Study limitations

This study has some limitations: the retrospective and cross-sectional nature of the survey does not allow conclusions to be drawn about the actual prognostic values and the molecular mechanisms underlying the relationships between EF, and CRP. Moreover, the small sample size may partly explain the lack of differences between groups (type I error).

Conclusions

The present study demonstrates that women patients affected by metabolic syndrome exhibit a significant increase in CRP levels, at EF equal, compared to men ones. Being recognized now EF as an independent cardiovascular risk factor, our study suggests that the pro-inflammatory coronary state can be increased in women, resulting in an increased risk of atherosclerosis development. Further studies are necessary to elucidate the true importance of the different activities of the sex-related epicardial fat. Determining the expression of steroidogenic metabolizing enzymes, different receptors of the EF behavior, and the expression of inflammatory proteins will allow us to understand better the mechanisms of the epicardial fat in the pathogenesis of atherosclerosis.

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