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Changes in lipid transport-involved proteins of epicardial adipose tissue associated with coronary artery disease

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ABSTRACT

Objective: Recent studies have focused on the potential role of epicardial adipose tissue (EAT) in the physiopathology of several metabolic and cardiovascular diseases, especially coronary artery disease (CAD). We aimed to study whether there are differences in the proteome and the secretome between epicardial and subcutaneous adipose tissue (SAT) from patients with and without CAD.
Methods: EAT and SAT samples were collected from 64 patients undergoing elective cardiac surgery either for coronary artery bypass grafting or valve surgery. One or two-dimensional electrophoresis were performed on tissue samples and media collected at 3, 6, 24 or 48 of tissue culture. Protein identification was performed with mass spectrometry, and the results were then validated with Western blot or enzyme immunoassay. mRNA expression levels were analysed by real time polymerase chain reaction.
Results: The release of several proteins was found to be higher in EAT that in SAT. Remarkably, there were higher levels of apolipoprotein A-I and glutation S-transferase P release, whereas mRNA expression of fatty acid binding protein 4 was lower in EAT. Although apolipoprotein A-I protein quantity in EAT was similar between CAD and non CAD patients, its released levels from this fat pad were lower in CAD.
Conclusion: EAT and SAT show different profiles of protein release and a different pattern was also found in samples from patients with CAD. These findings might support the hypothesis that EAT plays an interesting role in the physiopathology of atherosclerosis and CAD.
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1. Introduction

We currently know that there is a close relationship between obesity and cardiovascular disease. Very interestingly, total body adiposity correlates well with the amount of adipose tissue around the myocardium and the coronary arteries, the so-called epicardial adipose tissue (EAT) [1]. Several cardiac imaging studies using magnetic resonance, computed tomography or echocardiography found a strong association between the amount of EAT and the presence of the metabolic syndrome or coronary artery disease (CAD) [2–4]. The location of EAT is peculiar in that it lacks a fascia separating it from the myocardium and the coronary arteries and it shares their vasculature, which would suggest the existence of a close interaction between them.

In patients with CAD, EAT was found to express higher levels of proinflammatory cytokines such as tumour necrosis factor-α, interleukin-6, visfatin and leptin [5,6], blood pressure–related peptides such as angiotensinogen [7] and adrenomedulin [8] and free fatty acid producers such as type II secretory phospholipase A2 [9]. In contrast, lower levels of adiponectin were observed in EAT from patients with hypertension or CAD but not in patients with established diabetes mellitus [10–13]. Overall, these data support the role of EAT as a complex endocrine organ producing peptides which might modulate the process of atherosclerosis.

Proteomic analysis is a valuable tool to assess the protein composition of tissues or groups of cells in different physiological or pathophysiological scenarios and it can provide information on inflammation [14], protein degradation [15] or fibrogenesis [16]. The analysis of the secretome of tissue explants can also be extremely helpful for the study of their pathophysiological role, but its interpretation has several difficulties: a) the complexity of

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distinguishing whether the proteins obtained come from the tissue cells themselves or from the microvascular circulation, b) dealing with cell rupture, and c) the potential secretion of extracellular matrix-involved proteins due to long incubation times.

In the present study, we hypothesize that there are differences in the proteome and the secretome between EAT and SAT. We also aimed to assess whether protein expression and secretion patterns are different in samples from patients with CAD as compared to those from patients without CAD. These analyses could possibly shed light on the knowledge of the implication of EAT in the physiopathology of atherosclerosis and CAD.

2. Methods

2.1. Patients

Sixty-four patients undergoing elective heart surgery–coronary artery bypass grafting, valve surgery or both- were included in the study. Exclusion criteria were previous heart surgery and/or severe infective diseases. The study protocol complied with the principles of the Declaration of Helsinki and was approved by the local ethical committee (Galician Clinical Investigation Committee). All participants gave written informed consent.

Anthropometric measurements and clinical and demographic data are shown in Table 1. CAD was defined according to the presence of at least one coronary stenosis $\geq$ 50% of luminal diameter as measured by coronary angiogram.

2.2. Samples

EAT samples (0.2–0.5 g wet weight) were obtained from the upper region of the right ventricle and SAT samples (0.4–2 g wet weight) were harvested from the thorax. Adipose tissue explants were carried from the operating theatre to the laboratory in 5 mL of physiological saline solution (PSS) containing 0.5 mM EDTA, 5 mM KCl, 10 mM hydroxyethyl-piperazineethanesulfonic acid (HEPES), 2 mM $MgCl_2$, 10 mM $NaHCO_3$, 0.5 mM KH_2PO_4, 0.5 mM NaH_2PO_4, 10 mM Glucose, 110 mM NaCl, 0.16 mM $CaCl_2$, pH = 7.4 (Sigma–Aldrich, St. Louis, MO, USA). After centrifugation at 300 $\times$ g for 5 min in order to remove blood cells, fat samples were dried with sterile surgical cotton dressing and processed for their immediate analysis or frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Culture of tissue explants

EAT and SAT samples (100 mg each) were rinsed in PSS and placed into two different wells of 24-well culture plate containing 0.5 ml of medium 199 (M − 199) (Sigma–Aldrich, St. Louis, MO, USA) and antibiotics (100UI/mL penicillin, 100 μg/mL streptomycin from Laboratorios Normon, Madrid, Spain) at 37 °C and 5% CO_2. Culture media from EAT or SAT explants were collected at different times (0–3 h, 3–6 h, 6–24 h, 24–48 h after culture) as it was described previously [17].

2.4. One dimensional electrophoresis (1DE)

EAT and SAT explants from a subsample of 12 patients (age 70 $\pm$ 9 years, 50% male, body mass index [BMI] 31 $\pm$ 3 kg/m^2, 83% hypertensive, 58% diabetic, 67% hyperlipidemic and 50% with CAD) were cultured for 48 h and used for the 1DE approach.

Culture media collected from the tissue explants were concentrated from 0.5 mL to 0.05 mL using Amicon Ultra 0.5 mL 3K column system (Merck Millipore, Billerica, MA, USA). Subsequently, 1DE was performed simultaneously for all samples in order to reduce inter-assay variability. Fifteen microlitres of concentrated culture media were used for protein separation with SDS gradient gel (10–20%) at 100 V during 20 h. The gels were stained with Coomassie brilliant blue and scanned with a GS800 system (Bio-Rad, Hercules, CA, USA). The resulting images were processed and analysed using Quantity One 4.6.5 (Bio-Rad, Hercules, CA, USA) software. The differences between both adipose tissue secretion patterns were analysed using two different methods after background subtraction: a) based on the peak of optical density of each band (POD), and b) based on the relative quantity (RQ). Quantity One generates a peak intensity (pixel optical density) for each band and with RQ the band intensity is corrected by total intensity data in each lane. This last analysis takes into consideration the total bands in each lane of the gel.

Table 1
Clinical characteristics of patients regarding analysis and validations.

	1D-gel analysis (n = 12)		WB-Secretion (n = 16)		WB-protein tissue (n = 24)		RT-PCR (n = 32)	
	NCAD (n = 6)	CAD (n = 6)	NCAD (n = 8)	CAD (n = 8)	NCAD (=12)	CAD (=12)	NCAD (n = 15)	CAD (n = 17)
Age (yrs)	73(6)	66(10)	72(6)	65(9)	74(9)	73(8)	71(7)	69(9)
BMI (kg/m^2)	30(2)	31(4)	30(2)	32(3)	27(5)	30(4)	29(3)	31(3)
Male (n)	3	3	5	5	5	7	9	14
TG (mmol/L)	1.4(0.3)	2.3(0.5)	1.4(0.3)	2.0(0.7)	1.2(0.3)	1.5(0.7)	1.3(0.4)	1.7(0.7)
TC (mmol/L)	4.9(0.7)	4.7(0.4)	4.9(0.7)	5.0(0.5)	***5.5(1.0)***	***3.8(0.9)***^{b}	4.6(0.9)	4.5(1.0)
Glucose (mmol/L)	6.4(1.6)	6.1(1.6)	6.3(1.4)	6.1(1.2)	5.6(0.8)	6.9(2.3)	6.2(1.1)	6.0(0.9)
HT (n)	5	5	6	7	9	10	12	15
T2DM (n)	3	4	3	4	1	4	5	6
HLP (n)	4	4	4	5	5	8	8	10
VR (n)	6	0	8	0	12	1	14	0
CABG (n)	0	5	0	7	0	9	0	15
VR + CABG (n)	0	1	0	1	0	2	0	2
ACEI (n)	1	4	2	5	4	3	3	8
ARB (n)	4	1	4	1	1	3	5	2
Statins (n)	2	4	3	5	6	7	6	9
β–Blockers (n)	1	4	***1***	***6***^{a}	2	5	***3***	***10***^{a}
Oral anti-diabetics (n)	2	3	2	3	1	3	4	4

WB: Western Blot; TG: Triglycerides; TC: Total cholesterol; HT: Hypertension; T2DM: Type 2 Diabetes Mellitus; HLP: Hyperlipidaemia; VR: Valve replacement; CABG: Coronary artery bypass grafting; ACEI: Angiotensin converting enzyme inhibitors; ARB: Angiotensin II receptor blockers. β–Blockers: Beta-adrenergic blocking agents; Significant differences in characteristics between NCAD (non coronary artery disease) and CAD (coronary artery disease) are indicated with superscript bold and italic letters ($a = p < 0.05$; $b = p < 0.001$).

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