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Orosomucoid secretion levels by epicardial adipose tissue as possible indicator of endothelial dysfunction in diabetes mellitus or inflammation in coronary artery disease

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is associated with fat and autonomic system dysfunction. Epicardial adipose tissue (EAT) plays an endocrine role over the heart. Since orosomucoid (ORM) has local actions around the coronaries, our aim was to assess the relationship between its secretion profile by EAT and its catecholaminergic regulation in patients with T2DM and coronary artery disease (CAD).
Methods: We obtained EAT, subcutaneous adipose tissue (SAT) and plasma from 55 patients undergoing cardiac surgery. Fat explants were stimulated with isoproterenol (ISO) 1 μM for 6 h. After, the fat explants released-ORM and plasma levels were analyzed by ELISA. mRNA or protein expression was analyzed by real time PCR or western blot, respectively. The effects of ORM on endothelial cells were analyzed by impedance and wound healing assays.
Results: We observed that EAT-released ORM levels were higher than SAT (328 ± 185 *vs* 58 ± 45 ng/mL; $p < 0.001$). Interestingly, EAT secretion was lower in patients with than those without T2DM (260 ± 141 *vs* 370 ± 194 ng/mL; $p < 0.05$) and this difference was enhanced after ISO stimulation ($p < 0.01$). However, plasma levels (412 ± 119 *vs* 594 ± 207 μg/mL) and EAT-released ORM levels were higher in patients with than those without CAD (384 ± 195 *vs* 279 ± 159 ng/mL; $p < 0.05$). ISO stimulation, also reduced the EAT released-ORM levels in patients with CAD. On human endothelial cells, ORM induced an increase of healing and proliferation in a dose-dependent manner.
Conclusion: EAT-released ORM levels in patients with T2DM or CAD and its regulation by catecholamines might be the mirror of local endothelium dysfunction or inflammatory process in different cardiovascular disorders.

1. Introduction

Coronary arteries are the main affected vascular beds by atherosclerosis. In this process, epicardial adipose tissue (EAT) seems to play an important role because is in direct contact with coronaries without any separating fascia boundary [1]. In fact, segments of coronary arteries lacking pericardial fat are absent of atherosclerotic lesions in humans and animals [2]. Recently, it was found that EAT extension is related to coronary atherosclerosis severity [3]. Although this fat tissue participates in the energy homeostasis of the heart and vessels [2,4], in several pathological conditions, it shows a dysbalance of pro/anti-inflammatory adipokines production with deleterious effect on the coronary bed [5]. This is a noticeable local effect because higher pro-inflammatory and lower anti-inflammatory proteins secretion by EAT than subcutaneous adipose tissue (SAT) was described in several reports [6–8]. This dysbalance increases the potential to induce atherogenic process in monocytes and endothelial cells [9]. The relationship between EAT thickness and pathogenesis of diabetic

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coronary atherosclerosis was evidenced [10]. Although functional pericardial fat might play a protector role over the myocardium or endothelium [11,12], in patients with type 2 diabetes mellitus (T2DM), EAT-conditioned media is able to impair the contraction of cardiomyocytes [13]. In this sense, the released proteins by this fat pad might be the main connection between the diabetic state and complexity of coronary lesions in patients with CAD [14]. Accordingly, inflammation in EAT can precede insulin resistance and CAD. One of the proteins with immune regulatory functions [15] that contribute to maintain the high capillary permselectivity required for normal homeostasis [16] is orosomucoid (ORM). This glycoprotein (also known as alfa-1-glycoprotein) is a positive acute phase reaction (APR) protein induced selectively in the adipose tissue to suppress inflammation [17]. Thus, our aim was to study its release profile by EAT and SAT after catecholamines regulation in patients with T2DM and/or CAD.

2. Materials and methods

2.1. Subjects

Epicardial and subcutaneous fat biopsies and blood (5 mL) were obtained from fifty-five patients underwent coronary artery bypass grafting (CABG) or valve replacement (VR). The exclusion criteria were previous heart surgery or severe infective diseases. The study protocol was approved by the Galician Clinical Research Ethics Committee and carried out in accordance with the Declaration of Helsinki. Before extracorporeal circulation, EAT (0.5–1 g) was obtained from right ventricular upper and SAT (0.5–1 g) from thoracic region. Immediately, samples were collected with physiological saline solution (PSS) that contains (in mM): 0.5 EDTA, 5 KCl, 10 HEPES, 2 $MgCl_2$, 10 $NaHCO_3$, 0.5 KH_2PO_4, 0.5 NaH_2PO_4, 10 glucose, 110 NaCl, and 0.16 $CaCl_2$ (pH 7.4) (Sigma–Aldrich, St. Louis, MO, USA). Clinical characteristics of the study population were shown with respect to presence/absence of T2DM as it is shown in Table 1. We made also groups regarding presence/absence of CAD as it is shown in Table 2. The diagnosis of hypertension, T2DM and CAD was performed by the patients' general physicians as it was previously described [18–20]. Dyslipidemia was diagnosed according European Cardiology and Atherosclerosis societies guidelines [21].

2.2. Released ORM by EAT and SAT, and plasma levels

After splitting samples in 100 mg pieces and washing overnight with M-199 medium (Sigma–Aldrich, St. Louis, MO, USA) with antibiotics (100 UI/mL penicillin, 100 μg/mL streptomycin), fat pads were or not stimulated with isoproterenol (ISO) 1 μM (Sigma–Aldrich, St. Louis, MO, USA) for 6 h. Then, EAT and SAT biopsies and supernatants with/out treatment were collected and frozen at −80 °C until used. Treated biopsies were used for RNA expression and supernatants for protein analysis. Blood samples from patients were collected in EDTA-tubes after overnight fasting and plasma was stored at −80 °C with previous centrifugation for 15 min at 1000× *g* at 2–8 °C.

Table 1
Clinical characteristics of patients with and without T2DM.

	Total	No T2DM (36)	T2DM (19)	*p* Value
Demographic characteristics				
Age (years)	69 ± 10	68 ± 11	71 ± 8	0.305
Men *n* (%)	33 (60%)	22 (61%)	11 (58%)	0.817
BMI (kg/m^2)	29 ± 6	28 ± 6	32 ± 5	0.007*
Hypertension *n* (%)	39 (71%)	23 (64%)	16 (84%)	0.115
Dyslipidemia *n* (%)	38 (69%)	22 (63%)	16 (84%)	0.101
LVEF > 50% *n* (%)	47 (85%)	31 (86%)	16 (84%)	0.849
Atrial fibrillation *n* (%)	18 (33%)	12 (34%)	6 (32%)	0.840
HF *n* (%)	29 (53%)	19 (54%)	10 (53%)	0.907
CAD *n* (%)	27 (45%)	15 (42%)	12 (63%)	0.130
Drugs				
β-blockers *n* (%)	23 (42%)	14 (41%)	9 (56%)	0.318
ACEI *n* (%)	14 (25%)	8 (24%)	6 (37%)	0.305
ARB *n* (%)	19 (34%)	11 (32%)	8 (50%)	0.230
Diuretics *n* (%)	24 (44%)	15 (44%)	9 (56%)	0.423
Statins *n* (%)	32 (58%)	18 (50%)	14 (87%)	0.018*
Laboratory parameters				
Total cholesterol (mg/dL)	179 ± 39	183 ± 39	171 ± 43	0.395
LDL-cholesterol (mg/dL)	111 ± 38	112 ± 35	109 ± 48	0.874
HDL-cholesterol (mg/dL)	43 ± 15	45 ± 15	37 ± 15	0.254
Triglycerides (mg/dL)	125 ± 85	120 ± 94	136 ± 59	0.609
Glucose (mg/dL)	110 ± 30	95 [91–103]	125 [106–171]	0.000*
Leukocytes (cells/μL)	6447 ± 1778	6465 ± 1428	6403 ± 2523	0.917
ORM plasma (μg/mL)	521 ± 202	564 ± 241	510 ± 190	0.435
EAT released-ORM cont (ng/mL)	344 ± 203	370 ± 194	260 ± 141	0.040*
EAT released-ORM ISO (ng/mL)	321 ± 190	405 ± 251	238 ± 131	0.002*
SAT released-ORM cont (ng/mL)	60 ± 46	56 ± 51	69 ± 36	0.325
SAT released-ORM ISO (ng/mL)	68 ± 43	61 ± 45	80 ± 37	0.137
EAT mRNA-ORM cont (a.u.)	1.57 ± 0.11	1.57 ± 0.11	1.57 ± 0.11	0.998
EAT mRNA-ORM ISO (a.u.)	1.59 ± 0.14	1.59 ± 0.13	1.58 ± 0.17	0.873
SAT mRNA-ORM cont (a.u.)	1.52 ± 0.09	1.51 ± 0.06	1.53 ± 0.12	0.640
SAT mRNA-ORM ISO (a.u.)	1.51 ± 0.14	1.52 ± 0.10	1.50 ± 0.19	0.732
PAI-1 plasma (ng/mL)	5 [3–8]	5 [3–11]	5 [3–8]	1.000
EAT released-PAI-1 cont (ng/mL)	29 [11–45]	32 [11–46]	23 [12–43]	0.628
EAT released-PAI-1 ISO (ng/mL)	29 [13–41]	28 [14–40]	31 [9–43]	0.944

CAD: coronary artery disease, T2DM: type 2 diabetes mellitus, LVEF: left ventricule ejection fraction, HF: heart failure, ACEI: angiotensin converting-enzyme inhibitors, ARB: angiotensin receptor blockers, EAT: epicardial adipose tissue, SAT: subcutaneous adipose tissue, ORM: orosomucoid, PAI-1: plasminogen activator inhibitor 1, cont: control, ISO: isoproterenol, a.u: arbitrary units. *Statistical significance.