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Atrial fibrillation and rapid acute pacing regulate adipocyte/adipositas-related gene expression in the atria

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ABSTRACT

Purpose: Atrial fibrillation (AF) has been associated with increased volumes of epicardial fat and atrial adipocyte accumulation. Underlying mechanisms are not well understood. This study aims to identify rapid atrial pacing (RAP)/AF-dependent changes in atrial adipocyte/adipositas-related gene expression (AARE).
Methods: Right atrial (RA) and adjacent epicardial adipose tissue (EAT) samples were obtained from 26 patients; 13 with AF, 13 in sinus rhythm (SR). Left atrial (LA) samples were obtained from 9 pigs (5 RAP, 4 sham-operated controls). AARE was analyzed using microarrays and RT-qPCR. The impact of diabetes/obesity on gene expression was additionally determined in RA samples (RAP ex vivo and controls) from 3 vs. 6 months old ZDF rats.
Results: RAP in vivo of pigs resulted in substantial changes of AARE, with 66 genes being up- and 53 down-regulated on the mRNA level. Differential expression during adipocyte differentiation was confirmed using 3T3-L1 cells. In patients with AF (compared to SR), a comparable change in RA mRNA levels concerned a fraction of genes only (*RETN*, *IGF1*, *HK2*, *PYGM*, *LOX*, and *NR4A3*). RA and EAT were affected by AF to a different extent. In patients, concomitant disease contributes to AARE changes.
Conclusions: RAP, and to lesser extent AF, provoke significant changes in atrial AARE. In chronic AF, activation of this gene panel is very likely mediated by AF itself, AF risk factors and concomitant diseases. This may facilitate the development of an AF substrate by increasing atrial ectopic fat and fat infiltration of the atrial myocardium.

1. Introduction

Obesity and metabolic syndrome are important risk factors for the development and progression of atrial fibrillation (AF) [1–5]. Previous studies showed an up to 8% increased risk for new-onset AF with each unit increment of the body mass index (BMI) [1,3]. In the general population, obesity leads to a 49% increased risk for AF [2]. Total visceral, epicardial, and intrathoracic fat exert, however, different effects on the cardiovascular system [6,7]. Recent work revealed that the epicardial adipose tissue (EAT) volume is highly associated with paroxysmal and persistent AF and this is independent of "classical" risk factors such as left atrial diameter (LAD) [8]. Abundance of EAT is independently related to AF recurrence after ablation [9]. Total and LA EAT volumes as well as left periatrial EAT thickness are greater in persistent AF versus paroxysmal or no AF [9–12]. EAT distribution around the LA is uneven with most EAT located within regions superior vena cava, right pulmonary artery, right-sided roof of the LA, aortic root, pulmonary trunk, left atrial appendage, and between left inferior PV and left atrioventricular groove [9]. EAT locations were associated with high dominant frequency (DF) sites, providing a mechanism for EAT's contribution to AF maintenance [11,13]. Recent data suggests that the contribution of EAT to the AF substrate may differ between LA and RA [13]. By releasing e.g. adipokines and pro-inflammatory cytokines, pericardial adipose tissue contributes to the established association of AF with inflammation and obesity [12,14–17]. Elevated levels of CRP, interleukin-6 (IL-6), IL-8, tumor necrosis factor 1α (TNF1α), and of the adipokine resistin

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have been associated with AF [18]. Elevated post-operative serum levels of resistin seem to increase the risk of AF after coronary artery bypass graft surgery [19]. In addition to these humoral effects, epicardial adipocytes can modulate the electrophysiological properties of neighboring cardiomyocytes by direct interaction [20]. Adipocyte accumulation within the myocardium may disturb atrial conduction and favor the development and persistence of re-entry circuits [21]. The origin of myocardial adipose infiltration is not fully understood. Adipocytes may develop from resident or recruited progenitor cells, or invade the myocardium from the adjacent ectopic epicardial fat. Both mechanisms may be supported by AF-dependent changes in atrial (or cardiac) gene expression. The activation of the local renin–angiotensin-system and in particular its classical angiotensin II (AngII) — angiotensin II type I receptor (AT1R) axis represents a hallmark of AF [22–25]. Similarly, increased local and systemic AngII levels have been associated with all four determinants of the metabolic syndrome, namely hypertension, hyperglycemia, obesity, and hyperlipidemia [26–28]. AngII affects the differentiation of progenitor cells and pre-adipocytes [29–32]. Accordingly, AT1R blockers and ACE inhibitors were shown to influence adipogenesis and adipocyte function, including the production and release of adipokines [33–38].

This study aimed (I) to determine the effect of acute rapid atrial pacing (RAP) in vivo on adipocyte-specific atrial gene expression, (II) to assess to what extent similar expression changes could be detected in ex vivo RA and EAT tissue samples from patients with AF or in SR, and (III) to determine the corresponding expression changes during adipocyte differentiation in vitro.

2. Materials and methods

2.1. Patients

After written informed consent, right atrial appendages (RA) and epicardial adipose tissue (EAT) were obtained from patients undergoing cardiac bypass surgery or mitral/aortic valve replacement. EAT was harvested directly from the aortic root and at the lateral wall of the right atrium close to the terminal crest. Tissue samples were removed from 13 consecutive patients with AF and from 13 matched patients with no history of AF suffering from different diseases (SR) (Table 1). The study was approved by the local ethics committee of the University Medicine Greifswald (BB049/13).

2.2. Rapid atrial pacing (RAP) model

The tissue samples used in this study were from the same animals as described previously [39]. The animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Magdeburg. Briefly, pigs were subjected to closed chest RAP. In five animals RAP was performed for 7 h at a rate of 600 bpm (twice diastolic threshold, 2-ms pulse duration; RAP-group), and four pigs were instrumented without further intervention (sham). After the 7 h pacing period, chest and pericardial sac were opened and the heart was exposed. The tissue samples were immediately frozen in liquid nitrogen until further use.

2.3. ZDF rats and rapid pacing ex vivo of atrial tissue slices

Male Zucker diabetic fatty (ZDF-Leprfa/Crl) were purchased from Charles River Laboratories (France) and housed in a constant temperature room with a 12-h dark/12-h light cycle. ZDF rats received high fat Purina 5008 diet (Charles River France) to accelerate the development of the disease. Animals were studied at 3 (early diabetic stage) and 6 (advanced diabetic stage) months of age. Animal care and treatments were conducted in conformity with the current version of the German Law on the Protection of Animals and according to the guidelines for ethical care of experimental animals of the European Union. RA tissue culture and rapid pacing were performed as described recently [40–42]. RA tissue slices were paced at 4 Hz (RAP) or 0.6 Hz (control)

Table 1
Patient characteristics.

Pat.-Nr.	Rh.	Age	Sex	BMI	CAD	VD	NYHA	LVEF	D/H	ACEi/ATRB	Statin	CRP
2	1	77	m	23	3	–	II–III	35	H	0/1	1	18.6
4	2	75	m	23.2	3	–	–	38	H	1/0	0	6.9
21	2	74	m	33.9	3	MR	III	55	D + H	1/0	1	2.5
33	2	73	m	32.8	3	AS	I	50	H	0/1	1	8.9
42	1	75	f	45.2	–	AS	II	55	H	0/0	0	8.9
50	1	66	m	27.8	3	–	II	55	H	1/0	1	<5
59	3	76	f	23	–	AS + MR	II	45	H	1/0	0	7.2
65	2	74	m	30.8	–	AS	III	55	H	1/0	0	2.5
66	1	73	m	35.2	3	–	II	50	H	1/0	0	7.5
68	3	50	f	37.1	–	AS	II–III	30	H	0/1	1	7.1
69	2	74	f	21.8	–	TR + MR	II	55	D	1/0	0	4.2
73	1	68	m	25.6	3	–	II	35	D	1/0	1	4.2
77	3	74	m	25.5	3	–	II–III	50	D + H	0/1	1	1.2
	13	71.5	9/4	29.6	8	7			2/11	8/4	7	
5	SR	72	f	28.2	3	–	II	55	H	0/0	1	3.3
10	SR	78	f	31.2	3	–	II–III	55	D	1/0	0	2.5
11	SR	73	m	28.3	3	–	III	55	H	1/0	1	2.5
17	SR	57	m	34.8	2	–	III	60	–	1/0	1	2.5
18	SR	64	m	25.9	2	–	III	60	H	1/0	1	2.5
29	SR	74	m	25.9	3	–	II	70	D	1/0	1	2.5
34	SR	76	f	21.7	–	AS	II	55	H	1/0	0	2.5
37	SR	76	f	30.5	3	–	II	58	H	1/0	1	10.9
51	SR	76	m	32.6	3	–	III	60	H	1/0	1	8.3
53	SR	72	f	23.7	–	AS	II	55	H	0/1	1	2.5
57	SR	70	m	33.5	2	–	II	40	D + H	1/0	1	10.9
62	SR	73	m	27.3	–	AR	II	50	H	0/1	1	2.5
72	SR	78	m	23.0	2	–	III	55	–	1/0	0	1
	13	72.2	8/5	28.2	10	5			3/9	10/2	10	

BMI = body mass index (kg/m^2), CAD = coronary artery disease, number of diseased coronary arteries; D = diabetes; H = hypertension; LVEF = left ventricular ejection fraction; Rh. = rhythm: SR = sinus rhythm, 1 = paroxysmal, 2 = persistent, 3 = permanent AF; sex: f = female, m = male; VD = valve disease requiring valve replacement: AS = aortic stenosis, AR = aortic regurgitation, MR = mitral regurgitation, MS = mitral stenosis, TR = tricuspid regurgitation.