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RESEARCH ARTICLE

ADIPOKINE CONCENTRACTIONS ARE SIMILAR IN FEMORAL ARTERY AND CORONARY VENOUS SINUS BLOOD: EVIDENCE AGAINST *IN VIVO* ENDOCRINE SECRETION BY HUMAN EPICARDIAL FAT

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Abstract

Human epicardial adipose tissue expresses and secretes *in vitro* hormones and inflammatory cytokines and chemokines collectively termed adipokines. We hypothesized that human epicardial fat did not secrete adipokines into coronary blood under basal conditions *in vivo*. Adiponectin, leptin, resistin, tumor necrosis factor-α, monocyte chemoattractant protein-1, active plasminogen-activator inhibitor-1, interleukin-1β,-6,-8 and vascular endothelial growth factor were measured simultaneously in femoral arterial (a surrogate for coronary arterial) blood and coronary sinus venous blood from eleven patients, mostly young non-obese women, without known heart disease undergoing cardiac catheterisation for radioablation of supraventricular tachycardia. Mean adipokine concentrations were not significantly different in both vessels. In contrast, free fatty acid levels were significantly higher in femoral arterial than coronary sinus blood in keeping with net uptake of free fatty acids by the myocardium. Femoral artery levels of monocyte chemoattractant protein-1, active plasminogen activator inhibitor-1, leptin and resistin showed positive correlations with BMI in descending order of significance but adiponectin showed no relationship. Values for the other adipokines were below the assay detection limit in several patients negating the use of regression analysis. As opposed to their secretion *in vitro*, the adipokines described above are not secreted into coronary blood by human epicardial adipose tissue under near-normal basal conditions *in vivo* and are more likely released into the interstitium of the myocardium and coronary vessels to function as local paracrine regulators.

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Key words: adipose tissue, adipokines, inflammation, paracrine, gradients

Introduction

The physiological functions of human epicardial adipose tissue (EAT) are not well-defined most likely because this strategically located adipose tissue depot is difficult to access and study, and most of the information about it comes from humans with severe cardiac disease or by inference from animal experiments (1,2). Hypothetically, EAT's functions include lipid storage for myocardial energy use, coronary artery mechanical buffering against arterial wave torsion, coronary artery vasomotion and remodeling, protection of the cardiac and coronary autonomic nerve supply, and secretion of adipokines, a collective definition for white adipose tissue-derived hormones, growth factors, coagulation mediators, and pro-and anti-inflammatory cytokines and chemokines (1-3).

The hormones, adiponectin and leptin, and the cytokines, tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin(IL)-1β and IL-6 are secreted *in vitro* by explants of EAT obtained from patients undergoing coronary artery bypass graft for severe

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coronary atherosclerotic disease (CAD) or heart valve replacements without CAD (4,5). mRNAs for these proteins and resistin, IL-8, active plasminogen-activator inhibitor-1 (aPAI-1), and vascular endothelial growth factor (VEGF) are expressed by EAT sampled intraoperatively (4-7). By definition, the designation of epicardial fat as an endocrine tissue requires that leptin and adiponectin or other adipokines are secreted from EAT into the coronary venous effluent. The results of experiments testing adiponectin handling across the human coronary vascular bed have been controversial. In subjects without angiographic CAD or type 2 diabetes mellitus (T2DM), there was a small (~5%) significant increase in coronary venous sinus (CVS) compared to coronary artery (CA) adiponectin suggesting release of adiponectin from EAT (8). In another report (9), aortic root adiponectin levels were significantly higher (~10-15%) than CVS adiponectin levels in non-diabetic patients without and with CAD suggesting cardiac uptake of this adipokine (9). Secretion of the other adipokines into human coronary blood has not been determined.

The purpose of this study was to measure simultaneous femoral artery (FA) and CVS blood concentrations of adiponectin, leptin, resistin, aPAI-1, MCP-1, TNF-α, IL-1β,-6,-8, VEGF, insulin, glucose, and free fatty acids (FFA) in patients undergoing cardiac catheterization to ablate supraventricular tachycardia (10). We hypothesized that adipokine levels would not be higher in CVS than in FA blood in this group of patients. The rationale for including insulin, glucose and FFA was to use them as comparators of hormone and metabolic substrate metabolism by the heart during the experiment.

Subjects and methods

Patients

Each patient had an established diagnosis of atrioventricular nodal re-entry supraventricular tachycardia (SVT) for which slow pathway radiofrequency catheter ablation (10) was deemed necessary by the cardiologist (EJ). Exclusion criteria were age 16 or under; a left ventricular ejection fraction equal to or less than 50%; evidence of coronary atherosclerosis, cardiomyopathy, chronic valvular heart disease and congestive cardiac failure; past or present cigarette smoking; the presence of acute or chronic pulmonary, hepatic, renal, collagen-vascular, gastrointestinal or neuromuscular disease and T2DM, defined as a fasting blood glucose of 126 or more (11). This study was approved by the local Institutional Review Board. All patients involved gave their informed consent.

Sampling proceedure

In the morning after an overnight fast, patients were given endotracheal general anesthesia in the cardiac catheterization laboratory.Electrocardiographic and hemodynamic monitoring were established. Lactated Ringers solution without glucose was infused via a peripheral vein. Catheters were inserted into a FA for hemodynamic monitoring and into the CVS. The position of the catheter tip in the CVS was confirmed fluoroscopically just before withdrawing blood samples to ensure no mixing of right atrial with CVS blood. Under stable hemodynamic conditions, the first 7-10 ml and 5 ml of blood drawn simultaneously from the FA and CVS catheters respectively were discarded to avoid contamination and 10 ml were drawn from the FA and the CVS over ~30 sec 10 and 5 minutes before the start of atrial and ventricular programmed electrical stimulation. The samples were immediately transferred into heparinised tubes in ice. Plasma was separated at 4C and samples stored at –80C until assayed.

Assays

Glucose was measured by autoanalyser in the hospital laboratory. FFA were measured by Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA, using an *in vitro* enzymatic colorimetric method that recognizes a variety of FFA including palmitic, stearic, arachidonic, oleic, palmitoleic, linolenic and linoleic. Total adiponectin, resistin, TNF-α, MCP-1, IL-1β,-6,-8, aPAI-1, and VEGF were measured by Bioscience Division Laboratories, Millipore Corporation, St Charles, MO using LINCOplex well plate immunoassays with specific antibody-immobilised fluorescent-labelled microsphere beads. Assay sensitivity (MinDC) was 145 pg/ml for adiponectin, 6.7 pg/ml for resistin and 1.3 pg/ml for aPAI-1, and per cent intra-assay and inter-assay variation (% cv) were respectively 3.4 and 13.7 for adiponectin, 2.2 and 17.2 for resistin, and 4.1 and 9.9 for aPAI-1. For each of TNF-α, MCP-1, IL-1β,-6,-8 and VEGF assays, Min DC was 3.2 pg/ml, intra-assay % cv was 5.8-10.5% and inter-assay % cv was 7.0-15.9. Insulin and leptin were measured by double antibody radioimmunassays. For insulin, Min DC was 2μU/ml, intra-assay % cv 3.2 and inter-assay % cv 3.9. For leptin, MinDC was 0.5 ng/ml, intra-assay % cv 5.0 and inter-assay % cv 4.5. Fractionated plasma catecholamines were measured by high pressure liquid chromatography with electrochemical detection (Esoterix Laboratories, Burlington, NC).

Statistical Analysis

FA and CVS concentrations of glucose, FFA, insulin and each adipokine drawn at 10 and at 5 minutes were averaged. For each substance, differences in FA and CVS concentrations were analysed using a 2-tailed Student's t test. A p value of <0.05 was considered to indicate a significant difference.

Results

Patients

The upper part of Table 1 shows the order patients were recruited to the study. In compliance with prespecified selection criteria and by chance, all patients except number 4 were female. Patient 1 (58 yr) and 8 (79 yr) were older, weighed more, had hypertension controlled with one or 2 blood pressure drugs and had normal chest xrays, normal echocardiograms (ECHO), normal ejection fractions (EF) of 60% and normal coronary angiograms.The remaining 9 younger non-obese patients had normal chest xrays, normal ECHO and normal EF (58-65%) and the performance of coronary angiography in them was deemed not to be clinically necessary. Patients 1, 5 and 11 were taking estrogen-containing medications.

Plasma glucose, FFA, insulin and adipokines in FA and CVS blood

Table 1 shows the average of the pre-ablation 10 and 5 min values for glucose, FFA, insulin and each adipokine in FA and CVS blood in each patient and the means for the group. Concentrations of glucose were barely but significantly higher in FA (mean +/-sem,103.3+/-1.7mg/dl) than CVS (102.3+/-1.6 mg/dl),p=0.03. Concentrations of FFA were significantly higher in FA (821+/-99 nmol/ml) than in CVS (703+/-95 nmol/ml), p=0.007. Adiponectin, leptin, resistin, aPAI-1 and MCP-1 were detected in FA and CVS in all subjects and despite variation in values from individual to individual, mean values were not significantly different. However,it should be noted that the p value for the aPAI-1 difference was 0.07 for eleven patients. TNF-α, IL-1β,-6,-8 and VEGF were not detectable in all subjects but for the available number of detected values, the means for FA and CVS blood showed no differences.

As an indicator of sympathetic adrenomedullary activation at the time of blood sampling during the procedure, FA epinephrine and nor-epinephrine concentrations in 8 patients (data not shown) were in the normal range (respectively <100 pg/ml and <400 pg/ml) confirming no catecholamine release under these experimental conditions.

Table 1. Glucose, FFA, insulin and adipokine concentrations in femoral artery (FA) and coronary venous sinus (CVS) blood.

Patient	1		2		3		4		5		6		7		8		9		10		11		Mean ± sem		p
Age/Gender	58	F	36	F	17	F	18	M	47	F	24	F	19	F	79	F	40	F	37	F	26	F	36.5 ± 5.8		
BMI	25.8		19.7		23.9		19.5		21.9		22.6		20.1		40		43.3		40.5		24.1		27.4 ± 2.8		
	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CVS	FA	CS	FA	CS	FA	CS	
glucose mg/dl	111	110	98	96	97	96	107	104	107	104	101	101	96	97	106	105	112	112	101	101	100	99.5	103.3 ± 1.7	102.3 ± 1.6	0.03
Insulin µU/ml	4.5	4.0	6.5	6.5	7.5	7.0	5.0	7.0	6.0	6.0	16.0	18.0	3.5	3.5	30.0	23.5	34.0	28.0	10.0	9.0	7.5	8.0	11.9 ± 3.2	11.0 ± 2.5	0.31
FFA nmol/ml	675	550	595	740	740	555	455	360	512	410	570	475	765	580	1135	845	975	740	1535	1425	1075	1060	821 ± 99	704 ± 95	0.007
Adiponectin µg /ml	64.3	56.5	12.6	11.7	31.7	28.8	38.0	46.8	20.2	27.4	16.8	15.8	24.1	24.0	18.8	18.8	9.5	8.6	9.9	9.8	14.7	14.7	23.7 ± 4.9	23.9 ± 4.7	.0.88
Leptin ng/ml	10.9	10.9	1.6	1.6	5.8	6.0	1.5	1.5	6.4	6.7	11.6	11.8	2.6	2.7	46.3	41.1	22.3	27.9	11.3	11.2	8.4	8.5	11.8 ± 3.7	11.7 ± 3.9	0.89
Resistin ng/ml	14.8	13.1	17.4	13.4	10.7	11.3	6.8	9.3	8.2	9.8	12.2	16.1	14.4	14.0	21.1	26.0	31.4	29.1	11.7	11.8	13.7	17.2	14.8 ± 2.1	15.6 ± 1.9	0.37
aPAI-1 ng/ml	13.2	13.0	8.7	8.9	14.5	13.2	15.0	21.5	10.8	11.8	5.7	6.8	4.8	5.6	40.3	42.0	28.9	29.6	50.5	53.9	7.4	7.6	18.2 ± 4.6	19.5 ± 4.8	0.07
MCP-1 pg/ml	180	182	137	141	108	109	92	101	196	199	195	189	143	162	375	379	270	280	362	347	166	190.0	202.2 ± 28.7	207.2 ± 27.5	0.15
TN F - α pg/ml	15	14	5	5	234	207	5	5	10	6	ND	ND	ND	ND	ND	ND	7	6	ND	ND	5	5	40.1 ± 32.3	35.4 ± 28.6	0.26
IL-1β pg/ml	160.5	155.0	ND	ND	91.0	73.0	5.5	5.0	18.0	13.5	6.5	5.5	ND	ND	ND	ND	6.5	7.0	ND	ND	ND	ND	48.0 ± 26.4	43.2 ± 24.8	0.15
IL-6 pg/ml	260.0	250.0	9.0	8.0	440.0	386.0	10.0	9.0	254.0	238.0	16.5	8.5	ND	ND	55.5	52.5	30.0	28.0	5.5	4.4	ND	ND	120.1 ± 52.7	109.3 ± 47.8	0.109
IL-8 pg/ml	103.0	93.0	6.0	5.0	162.0	144.0	ND	ND	118.0	107.0	ND	ND	ND	ND	25.5	26.5	4.5	5.5	5.0	6.0	ND	ND	60.5 ± 24.8	55.2 ± 22	0.12
VEGF pg/ml	667	613	14	10	998	943	59	67	543	494	129	75	ND	ND	376	407	ND	ND	277	236	ND	ND	382.9 ± 119.6	355.6 ± 114.1	0.06

ND = values below the assay detection limit (see methods for details)

Regression analysis

Table 2 shows the relationship between BMI and FA concentrations of adipokines, insulin and FFA from 11 patients. In descending order of statistical significance, there were positive correlations between MCP-1, aPAI-1, insulin, FFA, leptin and resistin. Adiponectin and glucose showed no correlation with BMI. Correlations of BMI with the remaining adipokines were not performed because results fell below assay detection limits in several patients invalidating the analysis due to inadequate numbers of data.

Table 2. Regression analysis: femoral artery adipokines, insulin and FFA related to BMI

Substance	n	r	p
MCP-1	11	0.88	0.0003
aPAI-1	11	0.88	0.0003
Insulin	11	0.79	0.003
FFA	11	0.78	0.004
Leptin	11	0.75	0.008
Resistin	11	0.65	0.025

Discussion

In a group of otherwise healthy patients with SVT and no other cardiac abnormalities, we found no significant differences between fasting basal concentrations of adiponectin, leptin, resistin, aPAI-1, MCP-1, TNF-α, IL-1β,-6,-8 and VEGF in FA compared to CVS blood. Given the 7.1% higher aPAI-1 values in CVS than FA (p=0.07), the number of patients examined may not have been sufficient to exclude aPAI-1 release. With this provision, the data supports the hypothesis that these proteins are not secreted into the coronary vein effluent in relatively healthy people and that their physiological function in EAT is probably not endocrine but paracrine whereby they are secreted from EAT directly into the closely apposed myocardium and coronary vessels. The results also suggest that the contribution of EAT to overall adipokine turnover normally is negligible. This does not exclude the possibility that other hormones released from white adipose tissue depots into systemic blood that were not measured in this study such as retinol-binding protein 4 (12), angiotensin II (13) and omentin (14) might be secreted from EAT in endocrine fashion.

The lack of an increase in CVS adipokines relative to FA adipokines observed in this study might be due to the fact that EAT was not in a state of chronic inflammation which is characterized by increased expression of all the adipokines measured except for adiponectin (2,3) and nerve growth factor and brain-derived neurotrophic factor (15) which are decreased. Firstly, most (8 of 11) of the patients were non-obese and likely to have had normal EAT thickness defined by ECHO in a population of healthy women and men of similar BMI (16). In this situation, there would be no stimulus for macrophage infiltration into adipose tissue mediated by adipocyte hypertrophy from weight gain and no generation of inflammatory cytokines by adipocytes and inflammatory cells in the stromal-vascular fractions of EAT (2). It is of interest that we did not observe any step-up in adipokine gradients in 3 patients with stage III obesity but the significance of this finding remains unclear pending further studies. Secondly, at autopsy, macrophage density is low in EAT surrounding normal coronary arteries (17).We infer that this was also the case in our patients who were assumed not to have CAD by virtue of absence of risk factors such as age, smoking, hypertension or diabetes mellitus or by normal coronary angiograms in selected cases. By contrast, in patients with severe CAD, inflammatory adipokines are expressed and secreted in greater amounts from EAT (4,5,7) and therefore the potential exists that one or more adipokines might be released from EAT into coronary blood under pathophysiological circumstances. This issue requires further study.

Regression analysis and correlation of systemic blood adipokines with BMI was not the primary focus of this investigation. Despite this, we noted significant positive correlations between BMI and FA concentrations of leptin and aPAI-1 as previously reported (18,19), and between BMI and resistin. The positive correlation we found between BMI (mean 27.4 kg/m^2) and MCP-1 contrasts with no correlation in a study in which peripheral vein blood was obtained from obese (mean BMI 43.5) otherwise healthy women (20). The difference could be due to different body weights, selection criteria and number of subjects. Total adiponectin did not show the expected inverse relationship with BMI (21), probably because the numbers were too small. In several patients, values for the remaining adipokines were below the assay detection limit likewise reducing the numbers for analysis and limiting any definitive conclusions.

Mean FFA were significantly lower (14.0%) in the CVS than the FA. This difference is an underestimate of myocardial FFA extraction because radioactive tracer-labelled FFA infusions show that while FFA are being taken up, endogenous FFA are simultaneously released from EAT by lipolysis which raises the effluent FFA concentration (22,23).Conclusions about glucose flux across the heart in these experiments cannot be made without the use of radioactive glucose tracers (24).The barely perceptible higher glucose concentration in FA than CS implies glucose turnover at much lower rates compared to FFA, in keeping with the normal myocardial preference for FFA over glucose as en-

ergy substrate in the basal fasting state (25,26). Different results might be observed under non-fasting or hyperglycemic and/or hyperinsulinemic circumstances (24) or under conditions of increased cardiac work and oxygen consumption (25).There was no observed uptake of insulin even though the heart has insulin receptors (27) which mediate insulin removal from circulating blood. Our study has several methodological limitations. Firstly, we assumed that concentrations of adipokines in the FA and the CVA are the same. Ethical constraints did not permit us to place a catheter in the CVA or the aortic root next to the CA for blood sampling. However, sampling of FA blood instead of CVA blood has been used in human heart metabolism experiments during cardiac catheterization (25). Human aortic root and peripheral vein adiponectin concentrations are not significantly different (28) so that sampling the FA closer "upstream" to the coronary orifice would more likely reflect CVA blood than would a peripheral vein sample. These lines of evidence support FA as a surrogate for CVA blood. Secondly, we assumed that blood flow across the coronary vascular bed remained constant during sampling because direct measurements of coronary flow were not made. This was likely to be the case because during the procedure, general hemodynamic parameters were stable and the rate of blood withdrawal from the CVS was slow (24). Thirdly, measurements of arteriovenous differences across the coronary vascular bed are simple representations of net flux and cannot accurately quantitate release or uptake of each substance. For example, we cannot exclude the possibility that adiponectin and leptin may be taken up by the myocardium from the coronary influx at the same time as they are released into the efflux from EAT, resulting in no net change in their transmyocardial values. Lastly, EAT thickness or volumes were not measured but this should not detract from the principle findings of the study. On the contrary, if the effluent concentrations of one or more coronary adipokines had been higher, it would have been essential to quantitate the amount of EAT to explain the out-versus-in differences between them.

Conclusion

We were unable to demonstrate any differences between FA and CVS levels of adiponectin, leptin, resistin, aPAI-1, MCP-1, TNF-α, IL-1β,-6,-8 and VEGF in blood traversing the hearts of subjects under basal conditions. In this context, EAT is not an endocrine organ. Adipokines expressed by human EAT are more likely released into the interstitium of the myocardium and coronary vessels to act as local paracrine regulators *in vivo*.

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