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Human epicardial adipokine messenger RNAs: comparisons of their expression in substernal, subcutaneous, and omental fat

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Abstract

We compared the gene expression of inflammatory and other proteins by real-time quantitative polymerase chain reaction in epicardial, substernal (mediastinal) and subcutaneous sternal, upper abdominal, and leg fat from coronary bypass patients and omental (visceral) fat from extremely obese women undergoing bariatric surgery. We hypothesized that (1) epicardial fat would exhibit higher expression of inflammatory messenger RNAs (mRNAs) than substernal and subcutaneous fat and (2) epicardial mRNAs would be similar to those in omental fat. Epicardial fat was clearly different from substernal fat because there was a far higher expression of haptoglobin, prostaglandin D_2 synthase, nerve growth factor β, the soluble vascular endothelial growth factor receptor (FLT1), and α1 glycoprotein but not of inflammatory adipokines such as monocyte chemoattractant protein–1, interleukin (IL)-8, IL-1β, tumor necrosis factor α, serum amyloid A, plasminogen activator inhibitor–1, or adiponectin despite underlying coronary atherosclerosis. However, the latter inflammatory adipokines as well as most other mRNAs were overexpressed in epicardial fat as compared with the subcutaneous depots except for IL-8, fatty acid binding protein 4, the angiotensin II receptor 1, IL-6, and superoxide dismutase–2. Relative to omental fat, about one third of the genes were expressed at the same levels, whereas monocyte chemoattractant protein–1, cyclooxygenase-2, plasminogen activator inhibitor–1, IL-1β, and IL-6 were expressed at far lower levels in epicardial fat. In conclusion, epicardial fat does not appear to be a potentially more important source of inflammatory adipokines than substernal mediastinal fat. Furthermore, the expression of inflammatory cytokines such as IL-6 and IL-1β is actually higher in omental fat from obese women without coronary atherosclerosis. The data do not support the hypothesis that most of the inflammatory adipokines are expressed at high levels in epicardial fat of humans.

1. Introduction

Human intrathoracic fat consists of epicardial fat (EAT) internal to the pericardium and fat external to the pericardium (paracardial fat or mediastinal fat), which includes substernal fat [1,2]. The expression of a number of inflammatory genes has been reported in EAT and subcutaneous adipose tissue (SAT) from the thorax, upper abdomen, thigh, and leg [3-6], but not from the substernal area. It is unclear why prior studies have not investigated gene expression in substernal fat because this depot is also in the visceral intrathoracic region.

In cross-sectional studies, the *volume of pericardial fat* defined as EAT plus paracardial fat has been directly associated with coronary atherosclerosis (CAD) as assessed by calcified and noncalcified plaque burden [7-9]. The current paradigm postulates that EAT is in direct contact with the coronary arteries and paracardial fat separated by the fibrous pericardial membrane and may contribute to atherogenesis [1]. In this context, whether substernal fat expresses more or less inflammation than EAT has not been determined; but its area is increased in overweight men with hypertension and correlates directly with insulin resistance and intraabdominal fat [2,10]. Lipogenesis and lipolysis have not been directly measured in human EAT but are

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increased 2-fold in guinea pig EAT compared with SAT [11]. The expression of messenger RNAs (mRNAs) regulating adipocyte lipid metabolism and other functions in EAT as compared with substernal adipose tissue has not been investigated.

The purpose of this study was to measure the gene expression of a wide variety of proteins thought to be important in either inflammation or adipose tissue biology in EAT; substernal fat; and thoracic, abdominal, and leg SAT from the same coronary artery bypass graft (CABG) patients and compare gene expression in EAT of CABG patients to that in omental fat from bariatric surgery patients. We hypothesized that (1) EAT would exhibit greater expression of inflammatory mRNAs than substernal fat or SAT and (2) EAT mRNAs would be expressed at higher levels than in omental fat.

2. Materials and methods

Omental adipose tissue was obtained from 32 to 70 extremely obese but otherwise healthy women undergoing either laparoscopic adjustable gastric banding surgery or laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of extreme obesity. Fat samples were trimmed of connective tissue and superficial blood vessels, bisected, and stored separately at −80°C. The omental adipose tissue was transported to the laboratory within 15 to 30 minutes of its removal from the donor and handled as previously described [12,13].

The clinical and metabolic characteristics of the 21 to 44 patients from whom EAT; intrathoracic substernal fat; and mandibular sterni region (sternal), abdominal, and leg SAT was obtained at the start of CABG surgery have been described previously [14]. All had angiographic evidence of critical CAD with stenosis greater than 50% left main or greater than 75% other arteries involving 1, 2, or 3 vessels deemed necessary for CABG surgery by the attending cardiologist and cardiovascular surgeon [14]. Exclusion criteria included any history of HIV and/or being viral hepatitis positive, a previous CABG or heart valve operation, chronic coexistent inflammatory disease such as systemic lupus erythematosus or rheumatoid arthritis, pharmacologic glucocorticoid and/or immunosuppressive therapy, or congestive cardiac failure. The studies had the approval of local institutional review boards, and all patients involved gave their informed consent.

2.1. Isolation and assay of mRNA

The isolation of RNA and assay of mRNA involved real-time quantitative polymerase chain reaction (PCR) [12,14,15]. The complementary DNA (cDNA) was prepared using the Transcriptor First Strand cDNA Synthesis Kit from Roche Diagnostics (Indianapolis, IN). The quantification of mRNA was accomplished using the Roche Lightcycler 480 Real-Time RT-PCR System and their Universal Probe Library of short hydrolysis locked nucleic acid probes in combination with the primers suggested by the Web-based assay design center (www.universalprobelibrary.com). Integrated DNA Technologies of Coralville, IA, synthesized the primers. Approximately 70 ng per tube of total RNA was used, and the ratio of the right to left primers was 1 for each assay. The data are obtained as crossing point values (Cp) obtained by the second derivative maximum procedure. Samples with higher copy number of cDNA have lower Cp values, whereas those with lower copy numbers have the reverse. The ratios were calculated using the comparative Cp method, which eliminates the need for standard curves. The arithmetic formula to calculate ratios from ΔCp is based on a $\log_2$ scale ($2^{-\Delta Cp}$). This method is identical to the comparative C_T procedure described in the ABI PRISM 7700 Sequence Detection System User Bulletin #2 for quantitative reverse transcriptase PCR except that Cp is used because Roche uses the crossing point procedure instead of crossing threshold to calculate cycles required for detection of mRNA.

In the tables, those mRNAs found in fat cells at levels at least 3-fold greater than in nonfat cells of human omental adipose tissue are indicated in bold, as previously described [12], except for the following mRNAs where the first number is the ratio of mRNA in fat cells over nonfat cells and the second number is the ΔΔCp as the mean and SEM of 4 different humans: uncoupling protein (UCP)–1, 3.7X and −1.9 ± 0.3; zinc α2 glycoprotein (ZAG), 7.5 X and −2.9 ± 0; adrenomedullin, 4.3 and −2.1 ± 0.6; fatty acid binding protein (FABP)–4, 5.3X and −2.4 ± 0.3; sirtuin1/SIRT1, 1.0 and 0 ± 0.3; leucine rich protein 130 (LRP-130), 1.0 and 0 ± 0.3; heme oxygenase–1 (HMOX-1), 0.6 and 0.8 ± 0.4; mitochondrial superoxide dismutase–2 tv 1 (SOD-2), 0.4 and 1.5 ± o.4; α1 glycoprotein, 0.2 and 2.3 ± 0.4; prostaglandin D_2 synthase (PGD2), 0.2 and 2.5 ± 0.7; collagen type VI/PBEF 1α3 tv1 (collagen VI α3), 0.1 and 2.8 ± 0.9; and vascular endothelial growth factor receptor LT (FLT) 0.07 and 3.9 ± 0.6. Because cellular subfractionation of EAT has not been reported, the assumption is that EAT has a similar enrichment of the fat cell mRNAs as omental fat.

Statistical analyses were carried out using a 2-tailed Student *t* test to determine whether differences were significant at a P value < .05. The Pearson correlation coefficients were determined using the GraphPad Prism program (San Diego, CA), assuming a Gaussian population and a 2-tailed P value.

3. Results

3.1. Characteristics of patient populations

The omental adipose tissue was obtained from 32 to 70 obese women undergoing bariatric surgery with a mean body mass index (BMI) of 46. The average age of the women was 40 years. The 21 to 44 CABG patients from whom EAT;

intrathoracic substernal fat; and mandibular sterni region (sternal), abdominal, and leg SAT were obtained had an average age of 68 years and BMI of 31.1. Approximately 60% were men, and comparisons of mRNAs between these tissues used patients from whom all 5 tissues were obtained.

3.2. The use of cyclophilin A as the recovery standard for comparisons of different fat depots

Comparisons between tissues were derived from the ΔCp values based on cyclophilin (the A form) as the recovery standard. The recovery of cyclophilin in tissues was based on the absolute Cp values that were the same in EAT as in substernal fat (Table 1). However, they were lower in EAT as compared with the 3 kinds of SAT in the open heart surgery patients (Table 2); and the data in Table 2 were corrected for these differences. In the comparisons of omental fat with EAT from 43 open heart surgery patients, the mean Cp ± SEM for cyclophilin was 25.88 ± 0.18, which was not significantly different from that in omental fat for 70 female bariatric surgery patients of 25.82 ± 0.13; so no correction was necessary in comparing EAT ΔCp values with those of omental fat (Table 3).

3.3. Comparison of 45 mRNAs in EAT as compared with substernal fat

Haptoglobin; prostaglandin D_2 synthase (PGDS); nerve growth factor β (NGFβ); VEGFR/FLT1; and, to a lesser extent, α1 glycoprotein, collagen VIα3, intercellular adhesion molecule–1 (ICAM-1), and sirtuin-1/SIRT1 were elevated in EAT as compared with substernal fat (Table 1). We include data for 24 other mRNAs as controls to demonstrate that many proteins are expressed at the same levels in both fat depots. Furthermore, there were 12 mRNAs expressed at significantly lower levels in EAT than in substernal fat. Except for tumor necrosis factor α (TNFα), IL-6, and angiotensin II receptor-1 (ATR_1), these proteins are preferentially expressed in fat cells of human adipose tissue [12].

3.4. Comparison of mRNAs in EAT with 3 types of SAT

The most striking finding was that the expression of most mRNAs was similar in the 3 types of SAT (Table 2). Most of the 45 mRNAs examined were expressed at higher levels in EAT than in SAT depots. The expression of cyclooxygenase (COX)-2, interleukin (IL)-8, and FABP4 (except in abdominal and leg SAT where it was lower) was the same in EAT as in SAT. The expression of IL-6, cyclophilin A, and SOD-2 was significantly lower in all SAT depots. In contrast, the other 36 mRNAs were expressed at far higher levels in EAT than in SAT (Table 2). This was especially striking in the case of haptoglobin, PGDS, NGFβ, and VEGFR/FLT1, which were also expressed at far higher levels in EAT as compared with substernal fat (Tables 1 and 2).

Table 1
Comparison of 45 mRNAs in epicardial as compared with substernal intrathoracic fat

mRNA	mRNAs elevated in EAT	mRNAs the same in EAT (ratio of epicardial to substernal)	mRNAs lower in EAT
Haptoglobin	21.0 ± 2.0‡		
PGDS	6.1 ± 0.9‡		
NGF-β	5.3 ± 0.7‡		
VEGFR/FLT1	5.3 ± 0.7‡		
α1 glycoprotein	1.9 ± 0.3†		
Collagen VI α3	1.6 ± 0.3*		
ICAM-1	1.6 ± 0.3*		
Sirtuin-1/SIRT-1	1.5 ± 0.2†		
SOD-2		1.4 ± 0.3	
ZAG		**1.4 ± 0.4**	
Adrenomedullin		**1.4 ± 0.4**	
11β HSD-1		**1.3 ± 0.2**	
MCP-1		1.2 ± 0.2	
IL-1β		1.2 ± 0.2	
IL-8]		1.2 ± 0.4	
Cyclophilin		1.0 ± 0.1	
LRP-130		1.0 ± 0.1	
COX-2		1.0 ± 0.4	
Angiotensinogen		**1.0 ± 0.1**	
Perilipin		**1.0 ± 0.3**	
PAI-1		1.0 ± 0.2	
CIDEA		1.0 ± 0.1	
Apelin		1.0 ± 0.1	
NFκB1 (p50)		0.9 ± 0.1	
Adipsin		0.9 ± 0.1	
HMOX-1		0.9 ± 0.2	
PPARγ		0.9 ± 0.2	
eNOS		0.9 ± 0.1	
Osteopontin		0.9 ± 0.2	
CD68		0.9 ± 0.2	
Endothelin-1		0.8 ± 0.1	
AMPK α2 catalytic subunit		0.8 ± 0.1	
Leptin		**0.8 ± 0.2**	
LPL			**0.7 ± 0.1**‡
RBP-4			**0.7 ± 0.1**†
TNFα			0.7 ± 0.1†
GPX-3			**0.6 ± 0.1**‡
FABP4			**0.6 ± 0.2***
ATR_1			0.6 ± 0.2‡
Amyloid A			**0.6 ± 0.1**‡
Adiponectin			**0.5 ± 0.1**‡
IL-6			0.5 ± 0.2†
HSL			**0.4 ± 0.1**‡
UCP-2			**0.4 ± 0.13**‡
Insulin receptor			**0.4 ± 0.1**‡

The values are based on the relative amount of each mRNA in EAT as compared with that of substernal adipose tissue from the same CABG patients based on the values relative to cyclophilin that was the recovery standard. The values for cyclophilin are based on the Cp values and were the same in epicardial as in substernal fat. The data are expressed as the ratio of the amount of mRNA in EAT as compared with that in substernal fat from 11 to 29 patients and are ranked from the highest to the lowest values, with the mRNAs enriched in fat cells by at least 3-fold shown in bold. eNOS indicates endothelial nitric oxide synthase; RBP-4, retinol binding protein 4; GPX-3, .glutathione peroxidase 3.

Statistically significant differences are denoted as follows:

* $P < .05$.

† $P < .01$.

‡ $P < .005$.

Table 2
Comparison of 45 mRNAs in epicardial as compared with 3 types of SAT

mRNA	Ratio of mRNA in EAT to that in		
	SAT sternal	SAT abdominal	SAT leg
Haptoglobin	111.0 ± 7‡	48.0 ± 3.2‡	64.0 ± 3.6‡
PGDS	17.0 ± 1‡	24.0 ± 1.0‡	16.0 ± 1.9‡
NGF-β	18.0 ± 2‡	24.0 ± 2.5‡	18.0 ± 1.6‡
Apelin	6.3 ± 0.5‡	3.0 ± 2.5‡	1.8 ± 0.2‡
ICAM-1	6.1 ± 0.9‡	5.3 ± 0.9‡	7.0 ± 1.0‡
VEGFR/FLT1	5.6 ± 0.3‡	2.2 ± 0.2‡	4.6 ± 0.4‡
Angiotensinogen	**5.5 ± 0.2‡**	**5.5 ± 0.2‡**	**5.8 ± 0.2‡**
Sirtuin-1/SIRT-1	5.3 ± 0.4‡	2.8 ± 0.2‡	2.0 ± 0.2‡
LRP-130	4.9 ± 0.5‡	3.7 ± 0.4‡	2.6 ± 0.3‡
Amyloid A	**4.3 ± 0.6‡**	**1.5 ± 0.2†**	**4.0 ± 0.5‡**
ZAG	**4.0 ± 0.2‡**	**3.0 ± 0.5‡**	**4.0 ± 0.4‡**
Endothelin-1	3.7 ± 0.3‡	1.6 ± 0.2†	0.6 ± 0.2†
Perilipin	3.7 ± 0.5‡	1.7 ± 0.4*	2.3 ± 0.4‡
Collagen VI α3	3.5 ± 0.4‡	2.3 ± 0.3‡	1.4 ± 0.2
CIDEA	3.5 ± 0.6‡	2.1 ± 0.2‡	1.8 ± 0.4
Osteopontin	3.5 ± 0.6‡	1.7 ± 0.2‡	1.0 ± 0.2
Insulin receptor	**3.2 ± 0.7‡**	**1.9 ± 0.5**	**2.5 ± 0.6†**
Adrenomedullin	**3.0 ± 0.3‡**	**2.2 ± 0.3‡**	**1.6 ± 0.3***
LPL	**3.0 ± 0.5‡**	**1.6 ± 0.3**	**2.1 ± 0.5***
Adipsin	**3.0 ± 0.4‡**	**1.8 ± 0.3‡**	**2.0 ± 0.3‡**
HMOX-1	3.0 ± 0.3‡	1.5 ± 0.3	1.2 ± 0.3
MCP-1	3.0 ± 0.4‡	2.5 ± 0.4‡	2.0 ± 0.3‡
GPX-3	**2.8 ± 0.2‡**	**1.3 ± 0.3**	**2.1 ± 0.2‡**
IL-1β	2.6 ± 0.4‡	4.9 ± 0.8‡	3.5 ± 0.6‡
eNOS	2.6 ± 0.4‡	1.9 ± 0.2‡	2.5 ± 0.3‡
NFκB1 (p50)	2.6 ± 0.2‡	2.5 ± 0.2‡	2.8 ± 0.3‡
α1 glycoprotein	2.5 ± 0.4†	2.5 ± 0.4†	2.5 ± 0.4†
11β HSD-1	**2.5 ± 0.5‡**	**2.6 ± 0.5‡**	**2.5 ± 0.5‡**
Leptin	2.3 ± 0.1‡	0.9 ± 0.2	1.1 ± 0.3
RBP-4	**2.2 ± 0.2‡**	**1.2 ± 0.1**	**1.0 ± 0.2**
PPARγ	**2.2 ± 0.3‡**	**1.7 ± 0.3†**	**2.0 ± 0.3†**
CD68	2.2 ± 0.3‡	1.6 ± 0.3*	1.4 ± 0.2
Adiponectin	**2.2 ± 0.2‡**	**1.2 ± 0.2**	**1.8 ± 0.3***
PAI-1	2.0 ± 0.4†	1.8 ± 0.2‡	0.7 ± 0.2
AMPK α2 subunit	2.0 ± 0.2‡	3.2 ± 0.3‡	1.3 ± 0.3
TNFα	2.0 ± 0.2‡	1.6 ± 0.1‡	1.7 ± 0.2‡
HSL	**1.9 ± 0.3***	**1.0 ± 0.2**	**1.2 ± 0.3**
UCP-2	**1.7 ± 0.3‡**	**1.2 ± 0.2**	**1.4 ± 0.2**
COX-2	1.5 ± 0.5	2.2 ± 0.6*	2.5 ± 0.4†
IL-8	1.2 ± 0.4	0.8 ± 0.3	0.9 ± 0.3
FABP4	**1.0 ± 0.1**	**0.6 ± 0.2†**	**0.6 ± 0.1†**
ATR_1	1.0 ± 0.4	1.2 ± 0.2	1.0 ± 0.2
IL-6	0.4 ± 0.2†	0.3 ± 0.2†	0.6 ± 0.1‡
Cyclophilin A	0.4 ± 0.2‡	0.5 ± 0.1‡	0.6 ± 0.1†
SOD-2	0.2 ± 0.1‡	0.4 ± 0.1‡	0.5 ± 0.1†

The values are based on the relative amount of each mRNA in EAT as compared with that of SAT samples from the mandibular sterni (sternal), abdominal, and leg depots from the same CABG patients based on the values relative to cyclophilin that was the recovery standard. All values are corrected from the lower amount of cyclophilin in each subcutaneous fat. The values for cyclophilin are based on the Cp values. The data are ranked from the highest to the lowest values, which are expressed as ratio ± SEM of the amount of mRNA. The mRNAs enriched in fat cells by at least 3-fold are shown in bold.

Statistically significant differences are denoted as follows:

* $P < .05$.

† $P < .01$.

‡ $P < .005$.

3.5. Comparison of mRNAs in EAT with omental fat

The data in Table 3 compares the expression of 36 mRNAs between EAT of 22 to 43 CABG surgery patients and omental adipose tissue of 32 to 70 bariatric surgery patients. Although the bariatric surgery patients did not have cardiovascular disease, were younger, and were much more obese than the CABG patients, the relative amounts of 13 mRNAs were not statistically different between the 2 tissues. Twelve mRNAs in EAT were 0.01- to 0.62-fold lower than those in omental fat, and these differences were significant. In contrast, there were 12 mRNAs whose gene expression in EAT was significantly greater than in omental fat. The UCP-1 mRNA in EAT was 9.2-fold higher than that in omental fat, whereas omentin was 4.3-fold higher than that in omental fat (Table 3). The amounts of PGDS, glycerol channel aquaporin 7, lipoprotein lipase (LPL), angiotensinogen, leptin, ZAG, haptoglobin, osteoprotegerin (OPG), sirtuin 1, and the insulin receptor were also significantly overexpressed by 16- to 21-fold in EAT as compared with omental fat.

The comparisons of gene expression used EAT from both men and women, approximately 60% men, but omental fat from women. However, the data in Table 3 demonstrate that in this population of elderly CABG patients there was no statistically significant effect of sex on mRNA levels in EAT.

The BMI values were lower and the age was higher in the CABG patients from whom EAT was obtained than in the bariatric surgery patients from whom omental fat was obtained. The data in Table 4 demonstrate that age did not affect the amount of mRNA in EAT except for haptoglobin and COX-2, where the older the fat donor was, the greater was the amount of mRNA. This could account for all or part of the greater amount of haptoglobin mRNA in EAT as compared with omental fat. However, for COX-2, it would have the opposite effect of enhancing the relative differences between EAT and omental fat. There was no statistically significant effect of BMI on the amount of any mRNA in EAT (Table 4). These data indicate that neither the age nor BMI differences account for the differences in mRNA expression between EAT and omental fat.

4. Discussion

The present studies compared the distribution of 36 to 45 mRNAs between different human fat depots. The novel finding of this work is that, contrary to expectations, inflammatory adipokines were not expressed at higher levels in EAT than substernal mediastinal adipose tissue. Actually, the levels of gene expression for the inflammatory adipokines TNFα and IL-6 as well as that of adiponectin were lower in EAT than substernal fat, whereas IL-1β, monocyte chemoattractant protein–1 (MCP-1), plasminogen activator inhibitor–1 (PAI-1), and the macrophage marker CD68 were similar in these sites.

Table 3
Relative expression of 36 mRNAs in epicardial as compared with omental adipose tissue and effect of sex on expression in EAT

	Ratio in epicardial to omental			Men	Women
	Increased	No change	Decreased	ΔCp in EAT	ΔCp in EAT
UCP-1	**9.2 ± 0.8**‡			**−4.54 ± 0.36**	**−4.19 ± 0.34**
Omentin	4.3 ± 0.9‡			5.67 ± 0.42	5.36 ± 0.58
PGDS	2.1 ± 0.3‡			3.50 ± 0.35	3.77 ± 0.13
LPL	**2.0 ± 0.3**‡			**4.53 ± 0.25**	**4.91 ± 0.41**
Angiotensinogen	**1.9 ± 0.2**‡			**−2.23 ± 0.20**	**−2.06 ± 0.23**
Leptin	**1.9 ± 0.3**‡			**2.08 ± 0.30**	**2.58 ± 0.37**
ZAG	**1.7 ± 0.2**‡			**−0.34 ± 0.19**	**0.31 ± 0.26**
α1 glycoprotein	1.7 ± 0.4*			**−9.12 ± 0.46**	**−8.66 ± 0.46**
Haptoglobin	1.6 ± 0.3*			1.54 ± 0.30	2.01 ± 0.45
OPG	1.6 ± 0.3*			−3.01 ± 0.32	−2.38 ± 0.24
SIRT-1	1.6 ± 0.2‡			−2.22 ± 0.15	−2.04 ± 0.17
INS-R	**1.6 ± 0.3**†			**−1.06 ± 0.29**	**−0.84 ± 0.29**
eNOS		1.3 ± 0.3		−1.54 ± 0.23	−1.22 ± 0.25
Adipsin		**1.3 ± 0.4**		**2.13 ± 0.31**	**2.64 ± 0.38**
Apelin		1.1 ± 0.2		−5.45 ± 0.21	−4.98 ± 0.23
CIDEA		**1.1 ± 0.2**		**0.90 ± 0.16**	**1.11 ± 0.25**
β_1-Adrenergic receptor		1.1 ± 0.2		−1.64 ± 0.23	−1.82 ± 0.21
NFκB1 (p50)		1.1 ± 0.1		−3.09 ± 0.12	−3.13 ± 0.19
Adiponectin		**1.1 ± 0.1**		**3.99 ± 0.21**	**4.44 ± 0.29**
VEGFR/FLT1		1.1 ± 0.1		1.04 ± 0.17	0.93 ± 0.16
RBP-4		**1.0 ± 0.2**		**4.01 ± 0.16**	**4.60 ± 0.27**
TNFα		1.0 ± 0.2		−4.05 ± 0.29	−4.61 ± 0.28
PPARγ		**1.0 ± 0.2**		**1.72 ± 0.15**	**1.53 ± 0.20**
ACE		1.0 ± 0.2		−1.21 ± 0.21	−1.10 ± 0.18
GPX-3			**0.62 ± 0.11**‡	**2.68 ± 0.21**	**3.29 ± 0.26**
ATR_1			0.57 ± 0.10‡	−0.17 ± 0.21	−0.43 ± 0.24
NGF-β			0.47 ± 0.12‡	−2.02 ± 0.19	−1.76 ± 0.18
HIF-1α			0.47 ± 0.10‡	0.66 ± 0.20	0.56 ± 0.13
UCP-2			**0.44 ± 0.09**‡	**0.81 ± 0.20**	**0.51 ± 0.18**
MCP-1			0.38 ± 0.18‡	1.54 ± 0.53	1.16 ± 0.28
FABP4			**0.35 ± 0.09**‡	**6.62 ± 0.19**	**6.80 ± 0.18**
Osteopontin			0.35 ± 0.22‡	−7.54 ± 0.31	−7.30 ± 0.25
COX-2			0.23 ± 0.29‡	−3.14 ± 0.56	−3.49 ± 0.92
PAI-1			0.23 ± 0.15‡	−4.30 ± 0.35	−4.28 ± 0.31
IL-1β			0.14 ± 0.15‡	−4.54 ± 0.44	−4.63 ± 0.42
IL-6			0.01 ± 0.16‡	−8.86 ± 0.44	−10.0 ± 0.57

The ratio values are based on the relative amount of each mRNA in samples of EAT from 21 to 44 CABG patients as compared with that of visceral omental adipose tissue from 32 to 70 women undergoing bariatric surgery based on the values relative to cyclophilin that was the recovery standard. The EAT values in male as compared with female patients are the ΔCps ± the SEM relative to cyclophilin. The mRNAs enriched in fat cells by at least 3-fold are shown in bold. Statistically significant differences are denoted as follows:

* $P < .05$.
† $P < .01$.
‡ $P < .001$.

We confirmed that IL-1β, TNFα, and MCP-1 mRNAs were higher in EAT than in leg SAT [3]; but similar results were seen for almost every other protein, including many not previously reported in EAT. In contrast, IL-6, SOD-2, cyclophilin A, and FABP4 were expressed at higher levels in sternal, abdominal, and leg SATs than in EAT. Baker et al [4] similarly reported higher levels of IL-6 in thigh and abdominal SAT than EAT. Levels of IL-6 and IL-1β in EAT from CABG patients were also lower than those in omental fat from obese women. Adiponectin was approximately 2-fold higher in EAT than abdominal and leg but not sternal SAT, in agreement with Kremen et al [5]. Despite the differences in age and BMI, one third of the proteins were expressed at the same level in EAT as in omental fat. In contrast, many proteins involved in the inflammatory response such as NGF-β, hypoxia inducing factor−1α (HIF-1α), MCP-1, COX-1, and PAI-I were expressed at lower levels in EAT than omental fat. This suggests that the omental fat of obese women has a greater inflammatory profile than does EAT from CABG patients.

Some of the proteins overexpressed in EAT as contrasted to omental and substernal fat are not those associated with inflammation but of special functions in EAT, one example being local thermoregulation for cardiac protection against hypothermia. Recently, we reported that UCP-1 expression is 5-fold higher in EAT than in substernal fat and 27-, 41-, and 90-fold higher in EAT than in SAT from the sternal, abdominal, and leg regions [14]. In the present study, the

Table 4
Effect of age and BMI on expression of mRNAs in EAT

	Correlation coefficient for age		Correlation coefficient for BMI		n
	Pearson *r* value	*P* value	Pearson *r* value	*P* value	
Omentin	0.19	.26	−0.19	.30	35
PGDS	0.09	.55	−0.28	.07	43
LPL	**0.21**	**.32**	**−0.28**	**.17**	**26**
Angiotensinogen	**0.04**	**.82**	**−0.04**	**.80**	**36**
Leptin	**0.08**	**.67**	**−0.17**	**.36**	**31**
ZAG	**0.21**	**.19**	**−0.30**	**.06**	**40**
α1 glycoprotein	**−0.03**	**.84**	**−0.12**	**.49**	**35**
Haptoglobin	0.38	.02*	−0.25	.14	38
OPG	0.26	.14	−0.23	.20	34
SIRT-1	0.05	.80	−0.17	.34	34
INS-R	**0.15**	**.44**	**−0.14**	**.50**	**26**
eNOS	−0.18	.32	−0.03	.87	33
Adipsin	**0.02**	**.92**	**−0.30**	**.18**	**21**
Apelin	−0.07	.75	−0.16	.45	25
CIDEA	**0.03**	**.86**	**−0.20**	**.20**	**44**
β_1-Adrenergic receptor	0.11	.50	0.01	.94	38
NFκB1 (p50)	−0.04	.82	0.01	.94	38
Adiponectin	**0.18**	**.24**	**−0.23**	**.14**	**43**
VEGFR/FLT1	−0.01	.96	−0.08	.64	40
RBP-4	**−0.15**	**.42**	**0.09**	**.64**	**31**
TNFα	0.08	.67	−0.09	.61	35
PPARγ	**0.03**	**.86**	**0.02**	**.89**	**41**
ACE	−0.32	.12	0.09	.66	25
GPX-3	**0.06**	**.72**	**−0.15**	**.37**	**35**
ATR_1	−0.11	.60	−0.09	.67	25
NGF-β	0.13	.47	−0.02	.91	34
HIF-1α	0.02	.89	0.02	.89	35
UCP-2	**0.13**	**.58**	**0.22**	**.34**	**21**
MCP-1	0.15	.37	0.05	.77	39
FABP4	**0.07**	**.70**	**0.07**	**.70**	**35**
Osteopontin	−0.12	.58	−0.01	.95	25
COX-2	0.38	.03*	0.07	.70	33
PAI-1	0.24	.15	−0.10	.54	37
IL-1β	−0.09	.55	0.14	.35	44
IL-6	0.07	.71	0.16	.38	33

The Pearson correlation coefficients (*r*) were based on the relative amount of each mRNA in samples of EAT from 21 to 44 CABG patients as compared with age or BMI for each individual. The mRNAs enriched in fat cells by at least 3-fold are shown in bold.

* Statistically significant differences based on a $P < .05$.

expression of UCP-1 in EAT was 9-fold higher than that in omental fat, indicating that of the visceral fat depots the highest expression of UCP-1 is in the epicardial intrathoracic fat depot rather than intrathoracic substernal fat or visceral omental fat. It appears more likely that EAT has special functions involved in thermoregulation rather than inflammation because UCP-1 expression positively correlates with BMI [14]. Further support for this hypothesis is the lack of a significant positive correlation between BMI and gene expression in EAT for inflammatory cytokines and in fact for any other protein we have measured to date in EAT other than UCP-1.

One significant difference between the bariatric surgery patients and the CABG patients was their age. There was a significant negative correlation between age and UCP-1 mRNA in EAT [14], which means that with age the amount of UCP1 mRNA goes down. The present results demonstrate that when age was correlated with mRNA expression in EAT, none of the 35 mRNAs examined had a significant negative correlation between age and mRNA expression. However, for haptoglobin and COX-2, there were significant positive associations between age and their gene expression in EAT. The effect of age resulted in an underestimation of the difference between the amount of UCP-1 mRNA in EAT and omental fat because the average age of the bariatric surgery patients was 40 years whereas that of the open heart surgery patients was 64 years. Another difference was that the visceral adipose tissue was obtained only from women, whereas EAT was from a mixed population of men and women. However, there was no effect of sex on the expression of any of the mRNAs in EAT. A third difference was the lower BMI of the CABG patients. However, we were unable to find any correlation between BMI and gene expression in EAT. We conclude that neither age, sex, nor BMI can account for the differences between gene expression in EAT and omental fat.

Substernal fat expressed the lipolytic proteins LPL, hormone sensitive lipase (HSL), and FABP4 mRNAs to a significantly greater extent than EAT, suggesting the possibility that mediastinal fat might contribute much more to overall systemic free fatty acid (FFA) turnover than EAT [16]. The expression of LPL and HSL was higher in EAT than SAT, implying that EAT lipolysis might not be insignificant because upper body nonvisceral fat stores, which include thoracic SAT, are a major contributor to systemic circulating FFAs in lean, obese, diabetic, and nondiabetic humans [17]. This might serve as another example of a special functional adaptation of EAT to permit the rapid availability of FFAs for myocardial energy needs were systemic FFAs to decline during starvation.

Haptoglobin is an acute phase protein primarily produced by the liver under the influence of IL-6 that scavenges hemoglobin and acts as a powerful antioxidant [18]. The positive association between haptoglobin gene expression in EAT and age is of interest but is not due to obesity because there was no correlation between BMI and haptoglobin expression in EAT. Thus, one can hypothesize that increased haptoglobin expression in EAT with age could act to reduce local oxidative stress by dampening the increased amounts of reactive oxygen species formed in CAD [19] and in the myocardium of patients with the metabolic syndrome and/or type 2 diabetes mellitus [20]. This notion that EAT may offer cardiovascular protection rather than being a harmful contributor to CAD [1] or myocardial disease could be yet another example of its special functional relationship to the heart. By contrast, in human omental adipose tissue, there appears to be little formation or release of haptoglobin as compared with liver [21]. Although haptoglobin is not an adipokine, it is probably an inhibitor of inflammation whose formation by the liver under the influence of IL-6 acts as a

feedback regulation of inflammation. Further evidence will be needed to demonstrate that EAT releases amounts of haptoglobin that would have any influence on the high circulating systemic levels of haptoglobin.

Previously, we have shown that omentin is preferentially expressed in EAT as compared with substernal fat [15]. Likewise, we now show a 4-fold increase of omentin expression in EAT as compared with omental fat. Omentin is primarily found in nonfat cells such as endothelial cells, but its functional role in endothelium and the reason for its enrichment in EAT are unknown.

What is striking about the overexpression of UCP-1 in EAT is that UCP-1 mRNA is primarily found in omental fat cells. None of the other mRNAs specifically enriched in EAT as compared with substernal fat such as haptoglobin, PGDS, VEGFR/FLT1, α1 glycoprotein, collagen VI α3, ICAM-1, and sirtuin-1 are preferentially found in omental fat cells; and their functional roles in EAT remains to be determined. In comparing EAT to omental fat, the ratio of UCP-1 expression was 9.1, whereas the only mRNAs specifically enriched in fat cells at higher levels in EAT than omentin were LPL, angiotensinogen, leptin, ZAG, and the insulin receptor. Of these, elevated angiotensinogen could potentially reduce coronary flow and exacerbate atherogenesis in the underlying coronaries [22] by diffusion through the porous adventitia into the closely apposed media. However, angiotensin-converting enzyme (ACE), which is the enzyme that forms angiotensin II, was expressed at the same levels in EAT as in omental fat; and the ATR_1 was actually expressed at lower levels in EAT than omental fat.

The finding that the PGDS gene expression is enriched in EAT is of interest because circulating PGDS is a biomarker for the severity of stable coronary artery disease [23]. We also observed a weak positive correlation between PGDS gene expression in EAT and BMI that was not statistically significant ($P = .07$). This enzyme metabolizes COX-derived PGH_2 to PGD_2, which is converted to 15-deoxy-PGJ that is a putative ligand for peroxisome proliferator-activated receptor (PPAR) γ activation [24]. The role of PGDS in obesity and inflammation is unclear, but its deficiency induces obesity and facilitates atherosclerosis in mice [25]. Although the physiologic role of 15-deoxy-PGJ is unresolved because it has both pro- and anti-inflammatory effects, its expression at higher levels in EAT suggests that it may play a special role in defining the function of this fat depot in relation to the adjacent myocardium and coronary vessels. The 5- to 24-fold higher expression of NGFβ in EAT than in substernal fat or the 3 types of SAT from CABG patients offers a physiologic explanation whereby EAT could provide a protective framework for maintaining the functional integrity of the cardiac autonomic nerves and ganglia [26-28]. The elevation of NGFβ expression in EAT is possibly related to the equally enhanced expression of PGDS because PGD_2, but not inflammatory adipokines, enhanced the expression of NGF in cultured murine 3T3L1 adipocytes [29].

One limitation of this and other fat depot studies [4-6] is that it is not ethically permissible to obtain simultaneous samples of omental and intrathoracic fat from CABG patients. Consequently, omental fat biopsies were taken from different patients undergoing elective abdominal surgery. Despite the fact that the CABG patients were heterogeneous in etiology, older, and not excessively obese, it is significant that EAT gene expression of one third of the proteins was the same in EAT as in omental fat from the bariatric surgery patients. Secondly, all fat samples were removed from patients at the beginning of the CABG surgery; but surgical stress elevates the circulating levels of inflammatory adipokines [5]. We assume that their effects, if any, on gene expression in adipose tissue would affect all adipose tissues similarly. Kremen et al [5] found 500-fold increases in IL-6 mRNA in both SAT sternal and EAT between the start of CABG surgery and the end of surgery 6 hours later. The stress resulting from CABG surgery is presumably greater than that resulting from bariatric surgery because of both the greater length and the nature of the CABG procedure. Actually, we found that the expression of IL-6 mRNA is far higher in omental fat of bariatric surgery patients than in EAT of CABG patients, whereas IL-6 expression is higher in substernal intrathoracic fat as well as 3 types of SAT than in EAT.

A caveat with regard to the present studies is that what was measured was mRNA expression, which may not necessarily correspond with the level of protein. However, in studies using adipose tissue from humans, especially from CABG patients, it is difficult to obtain sufficient tissue and specific antibodies to measure the amount of protein.

In conclusion, the gene expression of about 20% of 45 proteins was higher in EAT than in substernal fat, which is also located in the intrathoracic region; but none of these were inflammatory cytokines. Most of the proteins examined were expressed at the same or higher levels in EAT as compared with 3 types of SAT from CABG patients. Uncoupling protein–1 was expressed in far higher levels in EAT as compared with omental fat, whereas one third of the proteins were expressed at equivalent levels in omental as compared with EAT and another third at lower levels in EAT, this being most pronounced for IL-1β and IL-6. The data do not support the hypothesis that most of the inflammatory adipokines are expressed at high levels in human EAT surrounding severely atherosclerotic coronary arteries. We conclude that the present gene expression studies do not suggest that the EAT has a unique inflammatory function in CABG patients.

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