Uncoupling Protein-1 and Related Messenger Ribonucleic Acids in Human Epicardial and Other Adipose Tissues: Epicardial Fat Functioning as Brown Fat

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Context: Uncoupling protein-1 (UCP-1) is the inner mitochondrial membrane protein that is a specific marker for and mediator of nonshivering thermogenesis in brown adipocytes.

Objective: This study was performed to better understand the putative thermogenic function of human epicardial fat.

Design: We measured the expression of UCP-1 and brown adipocyte differentiation transcription factors PR-domain-missing 16 (PRDM16) and peroxisome-proliferator-activated receptor γ co-activator-1α (PGC-1α) in epicardial, substernal, and sc thoracic, abdominal, and leg fat.

Setting: The study was conducted at a tertiary care hospital cardiac center.

Patients: Forty-four patients had coronary artery bypass surgery, and six had heart valve replacement.

Interventions: Fat samples were taken at open heart surgery.

Results: UCP-1 expression was 5-fold higher in epicardial fat than substernal fat and barely detectable in sc fat. Epicardial fat UCP-1 expression decreased with age, increased with body mass index, was similar in women and men and patients on and not on statin therapy, and showed no relationship to epicardial fat volume or waist circumference. UCP-1 expression was similar in patients without and with severe coronary atherosclerosis and metabolic syndrome or type 2 diabetes. PRDM16 and PGC-1α expression was 2-fold greater in epicardial than sc fat. Epicardial fat UCP-1, PRDM16, and PGC1-α mRNAs were similar in diabetics treated with thiazolidinediones compared to diabetics not treated with thiazolidinediones.

Conclusion: Because UCP-1 is expressed at high levels in epicardial fat as compared to other fat depots, the possibility should be considered that epicardial fat functions like brown fat to defend the myocardium and coronary vessels against hypothermia. This process could be blunted in the elderly. (*J Clin Endocrinol Metab* 94: 3611–3615, 2009)

Abbreviations: BAT, Brown adipose tissue; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary atherosclerosis; Cp, crossing point; CT, computed tomography; CV, coefficient of variation; DM, type 2 diabetes; EAT, epicardial adipose tissue; m. sterni, manubrium sterni; MS, metabolic syndrome; PGC-1α, peroxisome-proliferator-activated receptor γ co-activator-1α; PRDM-16, PR-domain-missing 16; SAT, sc adipose tissue; TZD, thiazolidinedione; UCP-1, uncoupling protein-1.

Hypothetical functions of human epicardial adipose tissue (EAT) 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, as well as expression and production of adipokines (1–3). EAT has a light yellow appearance but has been described as brown adipose tissue (BAT) (1), yet thermoregulation as another function of EAT was not mentioned.

At autopsy, there is histological evidence for more BAT in the pericardium (*i.e.* EAT) in human infants and children under 10 yr than adults (4, 5). After age 25, the proportion of brown adipocytes decreases as more become unilocular white adipocytes (6) and may account for the pale yellow color of adult EAT (4, 7).

Uncoupling protein-1 (UCP-1) has classically been regarded as a marker of BAT (8). In mice, its presence at very high levels in the inner membrane of brown fat cell mitochondria is determined by brown adipocyte precursor cell differentiation along a pathway involving the critical dual presence of PR-domain-missing 16 (PRDM16) and peroxisome-proliferator-activated receptor γ co-activator-1α (PGC-1α) (9).

The goal of this study was to better understand the putative thermogenic function of human EAT as BAT using tissue obtained from patients undergoing open heart surgery. We hypothesized that UCP-1 mRNA in human adult EAT decreased with age. Additional aims were: 1) to relate UCP-1 mRNA expression in EAT to body mass index (BMI), to waist circumference, and to EAT volume; and 2) to determine the expression of UCP-1, PRDM16, and PGC-1α in EAT and in substernal and sc fat depots.

Patients and Methods

Fifteen patients had the metabolic syndrome (MS) (10), and 29 had type 2 diabetes (DM) (11) defined by published criteria. All had angiographic evidence of critical coronary atherosclerosis (CAD) with stenosis greater than 50% in left main or more than 75% in other arteries, involving one, two, or three vessels requiring elective coronary artery bypass grafting (CABG). A third group of six control patients without DM or MS (except for one woman with MS but no angiographic CAD), had chronic valvular heart disease without (n = 3) or with stenosis less than 50% in any vessel requiring valve replacement but not CABG. All patients had left ventricular ejection fractions of 50% or more. The decision to perform heart surgery was made by the attending cardiologist and cardiac surgeon. Exclusion criteria for each group were: any history of HIV and/or positive viral hepatitis; a previous CABG or heart valve operation; coexistent chronic inflammatory disease; and pharmacological glucocorticoid and/or immunosuppressive therapy. The local institutional review board approved the studies, and all patients involved gave their informed consent.

Fat samples

After the thorax had been opened and before heparinization and cardiopulmonary bypass, fat (0.2 to 1 g) was obtained from the following sites (12): 1) EAT over the right ventricle as close as possible to the origin of the right coronary artery at the base of the heart; 2) substernal fat from the inner chest wall around the internal mammary artery between its midcourse and the diaphragm; 3) anterior upper thoracic sc adipose tissue (SAT) from the sternotomy incision at the manubrium sterni (SAT m. sterni); 4) upper abdominal wall SAT at the lower sternotomy incision (SAT abdominal); and 5) leg SAT at vein harvesting, except in controls. Fat samples were trimmed of connective tissue and superficial blood vessels, bisected, and stored separately at −80 C.

mRNA isolation and assays

The methods have been previously described (12). The quantification of mRNA was accomplished using the Roche Lightcycler 480 Real-time RT-PCR system (Roche Diagnostics Corp., Indianapolis, IN) and the Universal Probe Library of short hydrolysis locked nucleic acid probes in combination with the primers suggested by their web-based assay design center (www.universalprobelibrary.com). Integrated DNA Technologies (Coralville, IA) synthesized the primers. The data were obtained as crossing point (Cp) values obtained by the second derivative maximum procedure. The data were normalized by the use of cyclophilin A as the recovery standard in each run. The data are shown with the sign of the ΔCp values reversed so that the greater the number the more mRNA. The mean Cp value for cyclophilin did not differ significantly from that for substernal fat, but for the SAT depots it differed by 1.4, 1.1, and 0.7 Cp units for m. sterni, abdominal, and leg fat, respectively. The mean values for these sc depots were corrected for the lower recovery of cyclophilin.

The arithmetic formula to calculate ratios in the other fat depots compared with epicardial fat from ΔΔCp is based on a log$_2$ scale ($2^{-\Delta\Delta Cp}$). The calculation of ratios assumes that the number of target molecules doubles with every PCR cycle.

Quantification of EAT volume

EAT was quantified on electrocardiogram-gated diagnostic cardiac computed tomography (CT) scans using a 16-speed GE Lightspeed scanner (GE Medical Systems, Milwaukee, WI). Truncated EAT volume was measured by the method of Gorter *et al.* (13) using the CT images reconstructed on a GE AW workstation. The intraobserver coefficient of variation (CV) for repeated independent measurements of EAT volume in 20 patients was 5.2%. The interobserver CV for repeated independent blinded measurements by two observers in 20 patients was 9.6%. CV was determined by implementing a randomized block design ANOVA model.

Blood assays

Fasting plasma glucose, glycosylated hemoglobin, lipids, and high-sensitivity C-reactive protein were measured by routine assays in the hospital laboratory.

Waist circumference

Waist circumference (in centimeters) was measured around the hospital gown-covered abdomen between the lower rib mar-

gin and iliac crest midwaist while standing up with heels together.

Statistical analysis

We used Excel (Microsoft Corp., Redmond, WA), GraphPad Prism (GraphPad Software, Inc., San Diego, CA), and SPSS software (SPSS, Inc., Chicago, IL) for linear regression analysis, ANOVA, correlation analysis, and the two-tailed t test. A P value of < 0.05 was statistically significant in all analyses.

Results

The percentages of CABG patients on therapy were, respectively: statins, 50%; angiotensin-converting enzyme inhibitors or angiotensin receptor-blockers, 50%; antiplatelet drugs, 48%; β-blockers, 47%; and calcium channel blockers, 27%. Percentages of DM patients on medications were: metformin, 69%; thiazolidinediones (TZDs), 28% (two rosiglitazone and six pioglitazone); sulfonylureas, 21%; insulin, 13%; and sitagliptin, 10%. Three controls took angiotensin-converting enzyme inhibitors or angiotensin receptor-blockers, and one took a statin.

Table 1 shows that controls were significantly younger than the other patients. mRNA for UCP-1 in EAT was similar in all four groups, whether expressed as the absolute Cp values or as the ΔCp value from cyclophilin A that was used as the recovery standard. The Cp values for cyclophilin A in EAT are also shown and did not differ between the four groups of patients. PRDM16 and PGC-1α mRNAs in EAT were similar in DM and DM patients on TZDs. However, PRDM16 expression in EAT was significantly lower in DM patients on TZDs than control or

MS patients, as was that of PGC-1α in MS and DM patients. Mean $\pm$ SEM absolute Cp values for EAT UCP-1 mRNA in patients on statins (29.55 $\pm$ 0.48) and not on statins (30.60 $\pm$ 0.40) were not different. The sex of the patients did not influence the absolute Cp values for cyclophilin A, which were 25.92 $\pm$ 0.22 for 27 men and 25.83 $\pm$ 0.24 for 23 women. Similar results were seen for UCP-1 mRNA in EAT, where the Cp values were 30.28 $\pm$ 0.44 in the men and 29.89 $\pm$ 0.44 in the women.

In Fig. 1, A–C, different symbols were used for men and women, but the data were pooled for calculation of correlation coefficients. Figure 1A shows a significant inverse relationship between UCP-1 mRNA in EAT in 50 patients ages 37 to 87 yr. BMI did not significantly decrease with age (r = -0.27; $P = 0.06$). The inverse relationship between UCP-1 with age held after adjusting for BMI and including TZD and statin use as additional covariates.

There was no significant difference between mean EAT volume (156 $\pm$ 20 cc in 13 MS patients, and 187 $\pm$ 22 cc in 19 DM patients). Controls had insufficient data for analysis (n = 2). UCP-1 mRNA did not correlate with increasing EAT volume (Fig. 1B). EAT volume was directly related to waist (r = 0.54; $P = 0.001$) and to BMI (r = 0.39; $P = 0.02$) but not to age (r = 0.24; $P = 0.2$).

UCP-1 mRNA in EAT positively correlated with BMI (Fig. 1C). There was no relationship between UCP-1 and the other two mRNAs in EAT with waist circumference (data not shown).

In CABG patients, UCP-1 expression was 5-fold higher in EAT than substernal fat and barely detectable in SATs

TABLE 1. Patient characteristics and expression of UCP-1, PRDM16, and PGC1α in epicardial fat

	Control	MS	Diabetic	Diabetic + TZD
Women/men (n)	5/1	7/8	9/12	2/6
Age (yr)	52 $\pm$ 5[a]	67 $\pm$ 3[b]	66 $\pm$ 2[b]	68 $\pm$ 2[b]
Weight (kg)	73 $\pm$ 5	92 $\pm$ 6	88 $\pm$ 3	92 $\pm$ 10
BMI (kg/m^2)	26 $\pm$ 2	31 $\pm$ 1	31 $\pm$ 1	29 $\pm$ 3
Waist (cm)	96 $\pm$ 6	107 $\pm$ 4	109 $\pm$ 1	110 $\pm$ 6
Glucose (mg/dl)	100 $\pm$ 4[c]	98 $\pm$ 3[c]	137 $\pm$ 6[a,d]	145 $\pm$ 22[b,d]
HbA1c (%)	5.6 $\pm$ 0.1[a,c]	6.0 $\pm$ 0.1[c]	7.5 $\pm$ 0.3[b,d]	7.4 $\pm$ 0.7[a,c,d]
Cholesterol (mg/dl)	189 $\pm$ 23	173 $\pm$ 8	162 $\pm$ 8	163 $\pm$ 21
LDL-cholesterol (mg/dl)	125 $\pm$ 17	96 $\pm$ 9	97 $\pm$ 8	101 $\pm$ 18
HDL-cholesterol (mg/dl)	46 $\pm$ 6	47 $\pm$ 7	37 $\pm$ 4	42 $\pm$ 6
Triglyceride (mg/dl)	111 $\pm$ 30	157 $\pm$ 25	214 $\pm$ 34	158 $\pm$ 43
hsCRP (mg/dl)	1.6 $\pm$ 0.6	1.61 $\pm$ 0.7	7.3 $\pm$ 4.2	6.0 $\pm$ 3.5
Cyclophilin A mRNA (Cp)	26.49 $\pm$ 0.35	25.75 $\pm$ 0.29	25.81 $\pm$ 0.28	25.84 $\pm$ 0.36
UCP-1 mRNA (Cp)	30.14 $\pm$ 1.07	29.93 $\pm$ 0.42	30.18 $\pm$ 0.56	29.94 $\pm$ 0.64
UCP-1 mRNA (Δ Cp)	-4.27 ± 1.00	-4.24 ± 0.28	-4.57 ± 0.43	-4.26 ± 0.68
PRDM16 mRNA (Δ Cp)	-4.11 ± 0.32[a]	-4.09 ± 0.21[a]	-4.77 ± 0.14[a,b]	-5.64 ± 0.41[b]
PGC-1α mRNA (Δ Cp)	-2.37 ± 0.28[a]	-3.55 ± 0.25[b]	-3.65 ± 0.16[b]	3.36 ± 0.46[a,b]

Patient categories with *superscripts in common* are not statistically significant from each other at $P = 0.05$, whereas those without common superscripts differ from each other at $P < 0.05$. HbA1c, Glycosylated hemoglobin; hsCRP, high-sensitivity C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
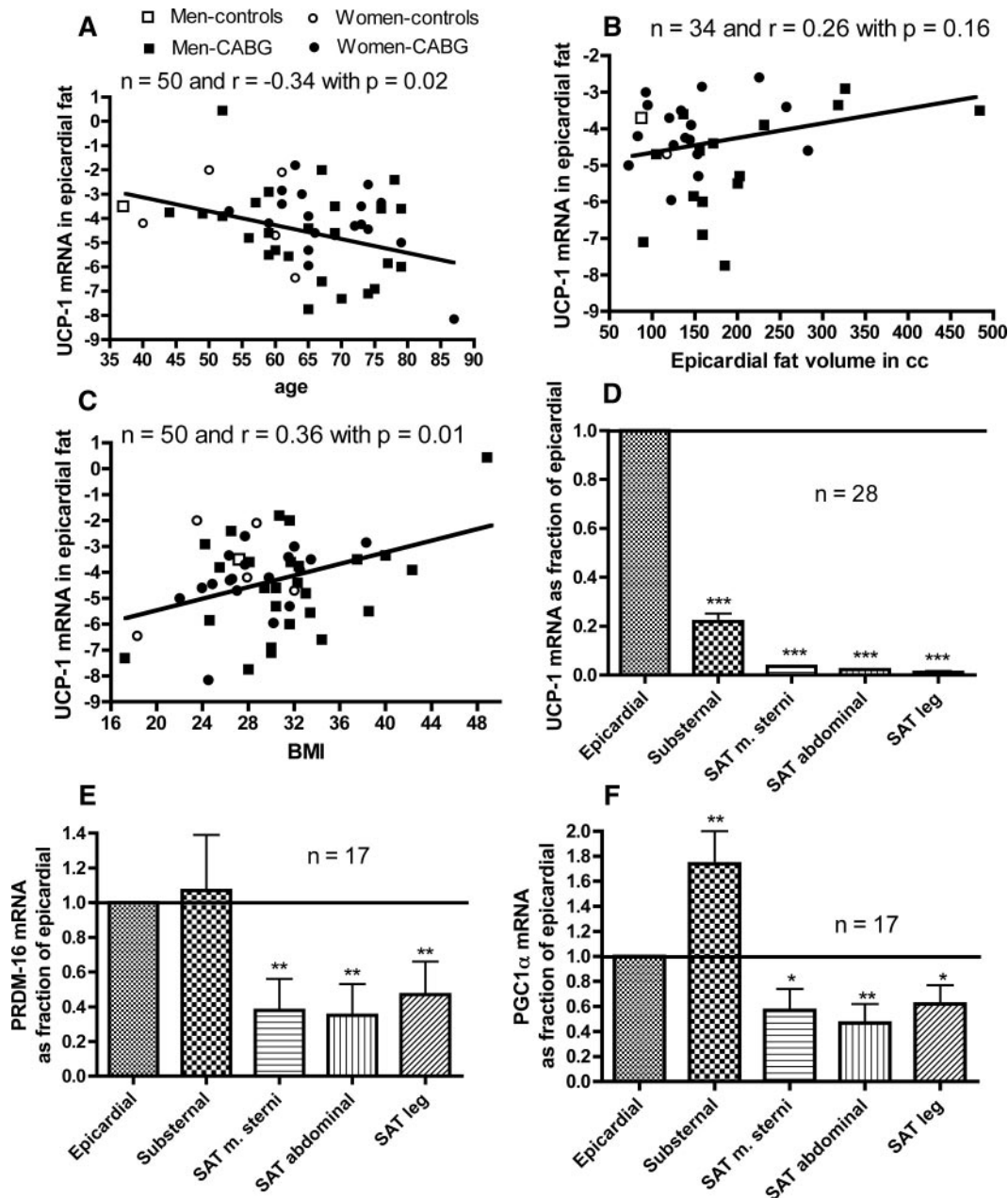
FIG. 1. A, Negative correlation of UCP-1 mRNA in epicardial fat with age; B, absence of correlation of epicardial fat UCP-1 mRNA with epicardial fat volume; C, positive correlation of epicardial fat UCP-1 mRNA with BMI. D–F, Comparison of UCP-1 (D), PRDM-16 (E), and PGC-1α (F) mRNAs in substernal and sc fat from the SAT m. sterni, anterior abdominal wall (SAT abdominal), and SAT leg as fractions of that in epicardial fat from the number of patients (n). Data in D–F are from CABG patients. In A–C, the Pearson correlation coefficient is given along with the *P* value for significance. In D–F, the *P* values for significant differences from epicardial fat were as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

(Fig. 1D). PRDM16 (Fig. 1E) and PGC-1α (Fig. 1F) mRNAs were similar in EAT and substernal fat but 2-fold greater than those mRNAs in all three SATs. PRDM16 (r = -0.23; $P = 0.12$) and PGC-1α (r = -0.12; $P = 0.4$) mRNAs did not correlate with age.

Discussion

Using UCP-1 mRNA as a specific marker of the brown adipocyte, our data agree with autopsy histological evi-

dence reported in the 1970s that brown adipocyte numbers in adult human EAT decrease with age (4, 5). EAT expression of UCP-1 increased with BMI but not with waist circumference or EAT volume. The significance of this finding remains to be determined, given the fact that in human adults BMI was negatively associated with BAT activity, measured by ^{18}F-fluorodeoxyglucose uptake using integrated positron-emission tomography-CT scans (14, 15). BAT produces heat on exposure to cold by nonshivering thermogenesis whereby activated UCP-1 uncou-

ples mitochondrial oxidative phosphorylation leading to the rapid conversion of electrochemical energy to heat instead of ATP (16). The presence of brown adipocytes in EAT led Lean and James (7) to postulate that "pericardial (epicardial) fat is well placed to provide direct heating to the myocardium." BAT in EAT could protect the myocardium from developing fatal ventricular arrhythmias during a drop in core body temperature from cold exposure. The age-dependent decline in brown epicardial adipocyte number (4) and UCP-1 could be one reason the elderly are prone to succumb from hypothermia (17).

EAT UCP-1 expression did not parallel the rise in EAT volume with increasing obesity, suggesting that white adipocytes in EAT were responsible for its expansion, although there is no histological evidence in humans that this is the case. The implied maintenance of a relatively constant proportion of BAT in EAT in human obesity could be protective by shielding the myocardium and coronary vessels from "overheating" during adaptive nonshivering thermogenesis. UCP-1 expression was similar in patients with no or minimal CAD, compared with severe CAD, suggesting no relationship between atherogenesis and EAT UCP-1.

We showed that PRDM16 and PGC-1α, which are essential molecular determinants of BAT formation in mouse studies (9, 18), are more expressed in EAT than SAT and are present in human SATs where UCP-1 is relatively deficient. Transcription factors including PRDM-16 and PGC-1α might regulate the transformation of brown adipocyte precursors into their mature cellular forms containing UCP-1 in humans.

In conclusion, the possibility should be considered that EAT functions like brown fat to defend the myocardium and coronary vessels against hypothermia.

Acknowledgments

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