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Brown fat like gene expression in the epicardial fat depot correlates with circulating HDL-cholesterol and triglycerides in patients with coronary artery disease ☆

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ABSTRACT

Background: Recent evidence indicates that epicardial adipose tissue (EAT) expresses uncoupling protein-1 (UCP1), a marker of brown adipocytes. However, the putative effects of the presence of brown adipocytes in EAT remain unknown.
Methods: The mRNA expression of genes related to brown adipocyte-mediated thermogenesis was measured in the fat samples collected from the epicardial-, mediastinal- and subcutaneous-depots of patients undergoing coronary artery bypass grafting. Both univariate and multivariate analyses were then utilized to determine any association between gene expression and the anthropometrics and fasting blood chemistries of these patients.
Results: EAT exhibited significantly higher expression of UCP1 and cytochrome c oxidase subunit-IV (COX-IV) compared to mediastinal- and subcutaneous-fat depots ($P \leq 0.05$). EAT expression of UCP1 ($r = 0.50$), COX-IV ($r = 0.37$) and lipoprotein lipase (LPL) ($r = 0.58$) positively associated with circulating levels of HDL-cholesterol ($P \leq 0.05$). In addition, EAT expression of LPL, acyl coA dehydrogenase-short, -medium and -long chain genes associated negatively with circulating TG levels ($P \leq 0.05$).
Conclusions: Abundance of UCP-1 in the EAT relative to other fat depots confirms the presence of brown adipocytes in human EAT. Furthermore, the correlations among the EAT expression of thermogenesis-related genes with the circulating HDL and TG levels indicate that presence of active brown adipocytes shares a functional association with the circulating plasma lipids in humans.

1. Introduction

Epicardial adipose tissue (EAT) represents a visceral fat depot that has not been fully characterized. The current interest in EAT however has gained momentum in the light of reports that point towards its direct association with central obesity [1,2] and coronary artery disease (CAD) [3,4]. EAT is known to express various pro- and anti-inflammatory cytokines and adipokines, thereby supporting its putative role in altering CAD status under obese conditions [5,6]. Functionally, EAT has been proposed to provide metabolic-, especially fatty acid-, buffering to the underlying myocardium [7], to provide mechanical buffering to the coronary arteries against pulse wave torsion [8] and to serve as the anatomic site for the cardiac nervous system [9]. Moreover, recent studies reported that human EAT expresses uncoupling protein-1 (UCP1), a marker of brown adipocytes, thereby suggesting that EAT might also play a role in thermogenesis [10,11]. However, it remains to be determined whether presence of brown adipocytes in EAT have any metabolic significance. We have addressed this question by 1) analyzing the mRNA expression of genes related to brown adipose tissue (BAT) in EAT and related fat depots, and then 2) determining the association between gene expression and various anthropometric and biochemical markers in our cohort of patients undergoing coronary artery bypass grafting (CABG). Since BAT activity is known to control TG clearance and lipoprotein homeostasis [12,13], we hypothesized that presence of brown adipocytes in EAT would alter circulating lipid levels in humans.

2. Methods

2.1. Subjects

Between October 2007 to August 2009, a total of 33 predominantly overweight men (only 2 female subjects) undergoing a CABG procedure at the Quebec Heart and Lung Institute were recruited for the study. Subjects with impaired renal function, chronic inflammatory or auto-immune diseases, aortic or mitral valve replacement, cancer, insulin treatment, and/or chronic obstructive pulmonary disease were excluded from the study. A written informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of the Quebec Heart and Lung Institute.

2.2. Data collection and risk factors assessment

Data related to age, gender, anthropometrics including waist circumference (WC), body surface area (BSA), body mass index (BMI), systolic- and diastolic-blood pressure

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(SBP; DBP), mean arterial pressure (MAP) as well as fasting blood biochemistries including plasma total-, low-density lipoprotein (LDL)-, high-density lipoprotein (HDL)-cholesterol, triglycerides (TG) and fasting plasma glucose (FPG) were obtained from Quebec Heart and Lung Institute records that were collected prior to the CABG procedure. In addition, information on the clinical risk factors such as diabetes, hypertension, metabolic syndrome, medication regime of the patients (use of statins, angiotensin converting enzyme- (ACE) inhibitors and angiotensin receptor blockers (ARBs)) was collected for the study participants. BMI was calculated as weight in kilograms divided by height in meters squared. Obesity, overweight, and normal weight were defined as BMI ≥ 30 kg/m^2, 25 to 29.9 kg/m^2, and 20 to 24.9 kg/m^2, respectively. Waist circumference was obtained using a measuring tape directly on the skin with the subject standing. Measurements were taken at the end of expiration at the level midway between the lower rib margin and the iliac crest. The diagnosis of hypertension was based on a resting systolic or diastolic blood pressure > 140 or > 90 mm Hg, respectively, or an actual hypertensive treatment.

2.3. Adipose tissue collection

Adipose tissues from 3 compartments, namely epicardial, mediastinal, and subcutaneous, were taken from the chest of each individual during the CABG procedure. EAT was defined as the adipose depot in direct contact with the heart located between the myocardium and the visceral pericardium. Mediastinal adipose tissue (MAT) was sampled from the fat within the mediastinum, outside the pericardial sac. Subcutaneous adipose tissue (SAT) was sampled from the anterior thorax after sternotomy. Immediately after collection, the tissues were snap-frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

2.4. RNA extraction, reverse transcription and quantitative PCR

Total RNA was isolated from hundred mg of tissue using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Mississauga, Ontario) according to manufacturer's instructions. Purity of total RNA was determined as 260/280 nm absorbance ratio with expected values between 1.8 and 2.0 using GeneQuant pro UV/Vis Spectrophotometer (Biochrom, Cambridge, UK). In addition, RNA integrity of randomly selected samples (n = 12) was assessed using the Agilent 2100 Bioanalyzer (Agilent technologies, Santa Clara, California). Five hundred nanograms of extracted total RNA was reverse-transcribed using Expand Reverse Transcriptase (Roche Diagnostics, Montreal, QC, Canada) according to the manufacturer's instructions. The cDNA was diluted 1:20 in DNase-free water before using for quantification by real-time quantitative PCR (qPCR). The real-time PCR mixture was prepared using SYBR® Green JumpStart™ Taq ReadyMix™ (#S5193, Sigma Aldrich, USA) according to the manufacturer's instructions. The primers for qPCR were designed using AutoPrime software and synthesized commercially (Invitrogen, USA). Primer specificity was confirmed using the NCBI Blast tool against all available mRNA sequences. The sequence for each set of primers is given in Table 1. The qPCR was performed in a 96-well plate format using ABI-7900 HT Fast Real-time system (Applied Biosystems, USA). At the end of each run, melting curve analysis was performed to validate product specificity, and a few samples representative of each experimental group were run on agarose gel to verify specificity of the amplification. All samples were amplified in duplicates from the same RNA preparation and the mean values were used for further analysis. A normalization factor calculated from the geometric mean of expression levels of cyclophilin A and glyceraldehyde-3 phosphate dehydrogenase was used for the normalization process, which was derived from the validation methods described elsewhere [14].

2.5. Statistical analysis

Data are expressed as mean (n = 33) ± SEM. Relative mRNA expression of each gene was compared among EAT, MAT and SAT using one-way ANOVA followed by Tukey's post hoc tests. Differences exhibiting a $P \leq 0.05$ were considered significant. The potential association between expression of various genes in the EAT, MAT and SAT depots with the metabolic parameters was evaluated using univariate Pearson's correlation analysis (Graphpad prism 5.0 software, La Jolla, CA, USA). All the variables exhibiting a $P \leq 0.05$ in the univariate analysis were then included in the stepwise regression analysis (JMP 9.0 statistical package, SAS Institute, Cary, NC, USA).

3. Results

3.1. Clinical characteristics of the patients

The average age of the subjects in our cohort was 60 years (Table 2). The average BMI, waist circumference and body surface area were 27.3 kg/m^2, 101.7 cm and 1.9 m^2, respectively (Table 2). All of the subjects were dyslipidemic and on statin therapy. In addition, 54.5% of the subjects were hypertensive, 30.3% were diabetic, 24.2% had metabolic syndrome, 24.2% had peripheral vascular disease and 30% of the subjects smoked (Table 3). In addition to the statins, all of the subjects were kept on antiplatelet therapy, 78.8% on beta-blockers, 51.5% on ACE-inhibitors and 9% on ARBs. In addition, 24.2% of the subjects were on oral hypoglycemics (Table 3). The patient condition or use of any drug did not affect the expression of the tested genes (Supplementary Fig. 1).

3.2. Comparison of gene expression among EAT, MAT and SAT depots

In order to identify the BAT-like properties of EAT, the mRNA expression of genes related to BAT including uncoupling protein 1 (UCP1), PR domain containing 16 (PRDM16), peroxisome proliferator-activated receptor ϒ coactivator-1α (PGC-1α), lipoprotein lipase (LPL), carnitine palmitoyl transferase-1(CPT1), cytochrome c oxidase-subunit IV (COX-IV), acyl CoA dehydrogenase-short chain (ACAD-S), -medium chain (ACAD-M) and -long chain (ACAD-L) were compared among EAT, MAT and SAT depots. The mRNA expression of UCP1 was significantly higher in the EAT depot compared to both MAT (4-fold) and SAT (10-fold) depots ($P \leq 0.05$) (Fig. 1A). In addition, EAT had the highest expression of COX-IV compared to both MAT and SAT ($P \leq 0.05$) (Fig. 1F). MAT had significantly lower expression of LPL (Fig. 1D) and ACAD-L (Fig. 1I) compared to EAT and SAT, whereas SAT had the lowest expression of PGC-1α (Fig. 1C) and CPT1 (Fig. 1E) compared to EAT and MAT ($P \leq 0.05$). EAT had significantly higher expression of both ACAD-S and ACAD-M when compared to MAT but not SAT ($P \leq 0.05$) (Fig. 1G, H). No differences were observed for the mRNA expression of PRDM16 among EAT, MAT or SAT depots.

3.3. Association between gene expression in the EAT, MAT and SAT depots with metabolic parameters

In order to identify the association between the BAT-like properties of EAT and peripheral metabolism, both univariate and multivariate analyses were performed between gene expression in each depot and circulating markers of systemic metabolism i.e. plasma total-, LDL-, HDL-cholesterol, TG, FPG and anthropometric parameters including age, BMI, WC, BSA as well as systolic and diastolic hypertension.

Univariate analysis revealed a significant positive correlation between EAT expression of UCP1 (r = 0.50, $P < 0.05$) (Fig. 2) as well as COX-IV (r = 0.37, $P < 0.05$) with the circulating HDL-cholesterol (Table 4). EAT expression of LPL, ACAD-S, -M and -L also correlated positively with the circulating HDL-cholesterol and negatively with the circulating TG levels in these patients ($P < 0.05$) (Table 4). In

Table 1
Gene symbol, accession number and primer sequences for the studied genes.

Gene symbol	Accession number	Forward primer	Reverse primer
UCP1	NM_021833	CTCTCAGGATCGGCCTCTAC	GAGTAGTCCCTTTCCAAAGACC
COX-IV	NM_001861	GCAGAAGCACTATGTGTACGG	CCAGTAAATAGGCATGGAGTTG
LPL	NM_000237	CGCTCCATTCATCTCTTCATC	GTGGAAACTTCAGGCAGAGTG
CPT1	NM_004377	CTCTTCCAGAAGGCTGCTAAG	CATCTGCTACAGGGCCAAAG
PRDM16	NM_022114	CAGCACGGTGAAGCCTTTC	GCGTGCATCCGCTTGTG
PGC1α	NM_013261	GGGATGATGGAGACAGCTATG	ATACTTGCTCTTGGTGGAAGC
ACAD-S	NM_000017	GGGTTCTGAATGGAACCAAAG	AGGGTTTGCATGGCTATCTTG
ACAD-M	NM_000016	GCCATTGATGTGTGCTTATTG	GCCCATGTTTAATTCCTTTCTC
ACAD-L	NM_001608	AGGATTTATCAAGGGACGAAAG	TGTTGCACTGTCTGTAGGTGAG