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Adipose Gene Expression Profile Changes with Lung Allograft Reperfusion

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Abstract

Obesity is a risk factor for primary graft dysfunction, a form of lung injury resulting from ischemia reperfusion after lung transplantation, but the impact of ischemia reperfusion on adipose tissue is unknown. We evaluated differential gene expression in thoracic visceral adipose tissue (VAT) before and after lung reperfusion. Total RNA was isolated from thoracic VAT sampled from 6 subjects enrolled in the Lung Transplant Body Composition study before and after allograft reperfusion and quantified using the Human Gene 2.0 ST array. KEGG pathway analysis revealed enrichment for genes involved in complement and coagulation cascades and Jak-STAT signaling pathways. Overall, 72 genes were upregulated and 56 genes were down-regulated in the post-

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reperfusion time compared with baseline. Long pentraxin-3 (PTX3), a gene and plasma protein previously associated with PGD, was the most upregulated gene (19.5 fold increase, p=0.04). Fibronectin leucine rich transmembrane protein (FLRT3), a gene associated with cell adhesion and receptor signaling, was the most down-regulated gene (4.3 fold decrease, p=0.04). Ischemia reperfusion has a demonstrable impact on gene expression in visceral adipose tissue in our pilot study of non-obese, non-PGD lung transplant recipients. Future evaluation will focus on differential adipose tissue gene expression and the development of PGD after transplant.

Introduction

Lung transplantation is an established therapy for severe incurable lung diseases, such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), and pulmonary hypertension. Obesity, as measured by the body mass index (BMI), has been linked to increased risk of primary graft dysfunction (PGD), a form of lung injury resulting from ischemia reperfusion after lung transplantation[1]. The impact of ischemia reperfusion on adipose tissue at the time of lung transplantation is unknown.

Obesity is known to produce a chronic, systemic pro-inflammatory milieu that likely plays a role in the development of disease[2,3]. Adipocytes have well-characterized endocrine functions and respond to inflammatory insults, including hypoxia and hypoxiareoxygenation (H-R)[2]. Cultured mature human adipocytes exposed to H-R demonstrate higher gene expression levels of pro-inflammatory cytokines and genes, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX2), interleukin-1 beta (IL-1β), vascular endothelial growth factor (VEGF) and heme oxygenase 1 (HO1), and tumor necrosis factor alpha (TNF-α), than normoxic cells[4]. In mouse models of acute respiratory distress syndrome, obesity is associated with higher levels of leukocyte adhesion markers in pulmonary vasculature, as measured by gene and protein expression levels of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM-1) and E-selectin in lung endothelial cells, and enhanced susceptibility to lipopolysaccharide induced lung injury[5].

Despite the association of obesity with significant outcomes after lung transplantation, BMI remains a poor surrogate measure for adiposity. BMI, as well as other anthropometric indices, poorly predicts internal body fat compartments, specifically the visceral adipose compartment. BMI may in fact strongly underestimate the risk impact of adiposity on disease states[6]. In order to better understand the association of adiposity with outcomes after lung transplantation, we evaluated the changes in gene expression levels in thoracic visceral adipose tissue (VAT) associated with ischemia reperfusion occurring at the time of lung transplantation.

Materials and Methods

Participant selection and cohort design

Subjects were enrolled from the prospective, multicenter Lung Transplant Body Composition Study cohort study between 2013 and 2014. The institutional review boards of

each site approved the study and all participants provided written informed consent. Clinical data on all study subjects was collected prospectively.

Adipose tissue sampling and Gene expression analysis

Approximately 5 grams of adipose tissue was removed from mediastinal structures at two time points: during initial dissection and following reperfusion of the allograft. Tissue was immediately placed in PBS and then cut into 3mm cubes. Following centrifugation at 500g for 5 minutes, tissue was placed in ALLprotect (Qiagen, Hilden, Germany) and stored at −20 C. Additional tissue was snap frozen and stored in liquid nitrogen. Total RNA was isolated from thoracic VAT using Qiagen's RNeasy Mini Kit (Qiagen, Hilden, Germany). Spectrophotometer was used to measure purity and concentration of isolated RNA. RNA expression was quantified using the Human Gene 2.0 ST array (Affymetrix, Santa Clara, CA).

Statistical analysis

Affymetrix GeneChip Human Gene 2.0 ST CEL files were normalized to produce gene-level expression values using the implementation of the Robust Multiarray Average (RMA) in the *affy* package (version 1.36.1) included within in the Bioconductor software suite (version 2.12) and an Entrez Gene-specific probeset mapping (version 16.0.0) from the Molecular and Behavioral Neuroscience Institute (Brainarray) at the University of Michigan[7-11]. Array quality was assessed by computing Relative Log Expression (RLE) and Normalized Unscaled Standard Error (NUSE) using the affyPLM Bioconductor package (version 1.34.0)[12]. Principal Component Analysis (PCA) was performed using the prcomp R function with expression values that had been normalized across all samples to a mean of zero and a standard deviation of one. Linear fold changes were computed in a paired manner for the whole-tissue analyses, i.e., the mean of the ratios of $2^{\log2(\text{expression})}$ computed within each patient between timepoints. These fold changes were converted to a signed metric by taking the negative reciprocal of any fold changes less than 1. Linear mixed-effects modeling and the associated analyses of variance were carried out using the lme and anova.lme functions, respectively, in the nlme package (version 3.1-108). Paired or unpaired Student ttests were computed using the limma package (version 3.14.4) by creating simple linear models with lmFit (with or without adjustment for patient, respectively), and performing ttests on the time point model coefficient of each model. Correction for multiple hypothesis testing was accomplished using the Benjamini-Hochberg false discovery rate (FDR)[13]. A false discovery rate-corrected p-value<0.05 was utilized to assess significance. All microarray analyses were performed using the R environment for statistical computing (version 2.15.1)[14,15]. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using Gene Annotation Tool to Help Explain Relationships (GATHER) utilizing inferences from networks[16]. Pathways with p>0.05 and Bayes factor≥6 were considered statistically significantly enriched.

Results

Due to the small number of patients with adipose tissue sample available both before and after allograft reperfusion and the low rate of PGD among those patients, for this pilot study

we restricted analysis to patients without PGD at 72 hours after transplant who had pre- and post-reperfusion samples available. Paired before and after reperfusion thoracic VAT samples were available for 6 patients and were assessed for differential gene expression. Clinical characteristics of those patients are presented in table 1. Of note, the mean BMI of study subjects was 23.6 kg/m^2.

In the 6 paired thoracic VAT samples, there were a total of 128 genes differentially expressed pre and post allograft reperfusion. Of those genes, 72 were upregulated in the post-reperfusion samples compared with the pre-reperfusion tissue (Table 2). 46 of these genes (64%) had a fold increase ≥1.5. The single most upregulated gene was long pentraxin-3 (*PTX3),* a secreted innate immune acute phase reactant, (19.5 fold increase, FDR p=0.04). Variation in genotype and protein expression of *PTX3* has previously been associated with altered risk of PGD after lung transplantation[17,18].

There were 56 genes significantly down-regulated in the post-reperfusion samples compared with the pre-reperfusion thoracic VAT biopsies (Table 3). 25 of these genes (45%) had a fold increase ≥1.5. The most down-regulated gene was fibronectin leucine rich transmembrane protein-3 (*FLRT3*) (−4.3 fold change, FDR p=0.04). FLRTs are involved in cell adhesion and receptors signaling, with *FLRT3* predominantly expressed in kidney, brain, pancreas, skeletal muscle, lung, liver, placenta, and heart[19].

Finally, we sought to understand which pathways were over represented by the differentially expressed gene expression patterns identified. KEGG pathway analysis on the differentially expressed genes with fold change ≥|1.5| revealed enrichment for genes involved in complement and coagulation cascades and Jak-STAT signaling pathways (Table 4).

Discussion

In a small pilot study of 6 lung transplant recipients without PGD, we identified 128 genes that were differentially expressed in thoracic VAT following lung allograft reperfusion compared to thoracic VAT obtained prior to transplantation, highlighting the impact of ischemia reperfusion on thoracic VAT. The most differentially expressed gene in this small sample was PTX3, which has been previously implicated in the development of PGD[17,18]. Obesity is a well-defined risk factor for both mortality and PGD after lung transplantation. While this study included normal weight subjects, the changes in gene expression we observed directly in visceral adipose tissue suggest that changes occurring in thoracic VAT during surgery might contribute to lung inflammation and/or injury. Additionally, in a study of potential lung transplant candidates, 62% of obese/sarcopenic subjects, as determined by whole body dual x-ray absorptiometry, had a normal BMI, highlighting both the limitation of BMI for the assessment of adiposity and the need for studies focused on direct evaluation of adiposity[20].

Our pathway analysis indicated that complement and coagulation cascade pathways were the most over represented among genes differentially expressed in thoracic VAT in the setting of lung transplantation. Plasma differences in proteins involved in these pathways have been associated with PGD risk after lung transplantation in prior studies[21,22]. Circulating levels of

the anti-coagulant protein C are lower in those with PGD compared to those without PGD while levels of the pro-inflammatory protein plasminogen activator inhibitor-1 (PAI-1) are higher in those with PGD[21]. Changes in plasma levels of complement protein 5a (C5a), a potent neutrophil and lymphocyte chemoattractant, are associated with PGD after transplant[22]. Plasma levels of these proteins are also excellent predictors of obesity, with PAI-1 being one of the top 5 circulating biochemical predictors of total, abdominal, visceral and liver fat[23]. Plasma PAI-1 activity is strongly correlated with elevated plasma triglyceride levels while plasma levels of complement protein 3a (C3a) increase with increasing level of obesity[24,25]. Given the separate association of coagulation and complement abnormalities with both adiposity and PGD after transplant, dysregulation of these pathways in thoracic VAT is an intriguing potential mechanism for the association of adiposity with PGD after lung transplantation.

PTX3 is highly evolutionarily conserved and plays a central role in innate immune responses, host defense, response to ischemia reperfusion injury, and activation and modulation of the complement cascade. *PTX3* was the most upregulated gene in thoracic VAT in the setting of lung allograft reperfusion, further highlighting the finding of complement pathway over representation in this study. We have previously demonstrated that genetic variation in PTX3 is significantly associated with risk of PGD after lung transplantation and that increased plasma protein levels of PTX were associated with increased risk of PGD, predominantly among subjects transplanted for interstitial lung disease[17,18]. PTX3 is also associated with obesity, with higher plasma levels found in obese adults with BMI>40 and obese children with obstructive sleep apnea compared to normal weight subjects[26,27]. Identification of increased PTX3 gene expression in the thoracic VAT of non-PGD, non-obese patients with lung reperfusion provides further credence to a systemic, recipient innate immune response to ischemia reperfusion injury in the setting of lung transplantation. Given the role of PTX3 in the pathogenesis of PGD, its association with obesity, and the observation that visceral adipose tissue gene expression of PTX3 significantly increases in the setting of ischemia reperfusion in the absence of clinically apparent PGD, future investigation should focus on the potential mechanistic role of PTX3 in the link between obesity and PGD as well as an evaluation of the association between obesity and circulating plasma PTX3 levels.

FLRT3, the most down-regulated gene in thoracic VAT, encodes a protein involved in cell adhesion and adipocytokine signaling pathways[19]. *FLRT3* was reported to be differentially expressed in omental fat compared to subcutaneous adipose tissue, although the functional implications of this are not well defined[28]. Future evaluation should focus on the potential impact of ischemia reperfusion injury on the integrity of adipose cell-cell interactions and adipocyte signaling.

There are limitations to our study. As the total number of patients available for study is small, we chose to focus on a homogeneous sample, limiting the analysis to non-obese single lung transplant recipients without PGD after lung transplantation. Conclusions made using this population cannot be directly applied to an obese population or to patients with PGD. While the patients included in this study were predominantly normal weight (mean BMI 23.6 kg/m^2), BMI is a poor surrogate for actual body composition[6]. One of the

strengths of this study is the focus on gene expression directly from thoracic visceral adipose tissue rather than a reliance on anthropometric measures of body composition. While the current study focuses only on non-PGD patients, we can conclude that ischemia reperfusion has a significant impact on visceral adipose tissue gene expression in lung transplant recipients. One resulting hypothesis is that the volume or amount of visceral adipose tissue present in the thorax of transplant recipients may lead to a dose response relationship with PGD risk. Future studies identifying potential causal mediators for the relationship between adiposity and poor outcomes after transplant and on the impact of more significant adiposity on PGD risk mediated via gene expression differences is an area of ongoing focus. While the sample size is small, similarities in genes and pathways identified in this study compared with previous studies add to the validity of our findings. Due to the small amount of thoracic VAT initially procured and the lack of sufficient residual RNA, quantitative PCR was not performed for technical replication of the identified microarray gene expression differences. While we are unable to provide technical replication, the similarity of our findings with previous studies of lung transplant recipients, specifically the role of long pentraxin-3 and complement and coagulation pathways, adds significant biological relevance to our findings.

In summary, ischemia reperfusion at the time of lung transplant has a demonstrable impact on gene expression in visceral adipose tissue in our pilot study of non-obese, non-PGD lung transplant recipients, highlighted by the finding of PTX3 upregulation and coagulation and complement pathways over representation in association with allograft reperfusion in lung transplant recipients. Future evaluation will focus on differential adipose tissue gene expression and the development of PGD. Differences in adipose tissue gene expression profiles may provide a link between recipient adiposity, PGD, and mortality risk after lung transplantation.

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Abbreviations

C3a	complement protein 3a
C5a	complement protein 5a
CF	cystic fibrosis
COPD	chronic obstructive pulmonary disease
COX2	cyclooxygenase-2
FLRT3	fibronectin leucine rich transmembrane protein-3
GATHER	Gene Annotation Tool to Help Explain Relationships
HO1	heme oxygenase 1
ICAM-1	intercellular adhesion molecule-1

IL-1β	interleukin-1 beta
iNOS	inducible nitric oxide synthase
IPF	idiopathic pulmonary fibrosis
KEGG	Kyoto Encyclopedia of Genes and Genomes
PAI-1	plasminogen activator inhibitor-1
PCA	Principal Component Analysis
PGD	primary graft dysfunction
PTX3	pentraxin-3
RMA	Robust Multiarray Average
TNF-α	tumor necrosis factor alpha
VAT	visceral adipose tissue
VCAM-1	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

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Table 1

Subject Demographics.

Covariate	Study Patients (n=6)
Donor Variables	
Male Gender, n (%)	4 (67)
Age, mean	41.6
Race, n (%)	
Caucasian	2 (33)
African American	3 (50)
Hawaiian/Pacific Islander	1 (17)
Any Smoking, n (%)	1 (17)
Any Alcohol, n (%)	3 (50)
Cause of Death, n %)	
Head Trauma	3 (50)
Cerebrovascular Accident	3 (50)
Recipient Variables	
Male Gender, n (%)	5 (83)
Age, mean	68.5
Race, n (%)	
Caucasian	6 (100)
BMI, mean	23.6
Pulmonary Diagnosis, n (%)	
Idiopathic pulmonary fibrosis (IPF)	2 (33)
Lymphangioleiomyomatosis	1 (17)
Non-specific interstitial pneumonia	1 (17)
Other interstitial lung disease	2 (33)
mPAP	41.5
Lung Allocation Score, median	40.8
Operative Variables	
Ischemic time, min	297
Transplant type, single, n (%)	6 (100)
Cardiopulmonary bypass use, yes, n (%)	0 (0)

BMI: Body mass index

mPAP: mean pulmonary artery pressure

FiO_2: Fraction of inspired oxygen

Percentages may not exactly equal 100% because of rounding.

Table 2

Upregulated genes with allograft reperfusion

Gene Symbol	Gene Name	Fold change	FDR p value
PTX3	pentraxin 3, long	19.5	0.044
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	8.7	0.046
LDLR	low density lipoprotein receptor	5.2	0.041
PIM1	pim-1 oncogene	4.6	0.044
CCRN4L	CCR4 carbon catabolite repression 4-like (S. cerevsiae)	4.0	0.041
SLC25A25	solute carrier family 25), member 25	3.8	0.044
GCLM	glutamate-cysteine ligase, modifier subunit	3.7	0.041
ITPKC	inositol-trisphosphate 3-kinase C	3.2	0.046
NOP16	NOP16 nucleolar protein homolog (yeast)	3.0	0.044
GADD45A	growth arrest and DNA-damage-inducible, alpha	2.9	0.041
MT1M	metallothionein 1M	2.9	0.046
PER2	period homolog 2 (Drosophila)	2.8	0.048
ELL2	elongation factor, RNA polymerase II, 2	2.5	0.046
CTPS1	CTP synthase 1	2.2	0.041
DCUN1D3	DCN1, defective in cullin neddylation 1, domain containing 3	2.2	0.041
YRDC	yrdC domain containing (E. coli)	2.2	0.041
FAM107A	family with sequence similarity 107, member A	2.1	0.046
SOX17	SRY (sex determining region Y)-box 17	2.1	0.044
BMP2	bone morphogenetic protein 2	2.0	0.048
SNORD5	small nucleolar RNA, C/D box 5	1.9	0.039
MT2A	metallothionein 2A	1.9	0.048
BYSL	bystin-like	1.9	0.041
KIAA0040	KIAA0040	1.9	0.044
NXT1	NTF2-like export factor 1	1.8	0.046
SOCS2	suppressor of cytokine signaling 2	1.8	0.041
MAK16	MAK16 homolog (S. cerevisiae)	1.8	0.041
FOXC1	forkhead box C1	1.7	0.044
FNIP2	folliculin interacting protein 2	1.7	0.046
PIRH2	peptidy-tRNA hydrolase 2	1.7	0.048
PIGW	phosphatidylinositol glycan anchor biosynthesis, class W	1.7	0.041
TUBB2A	tubulin, beta 2A class IIa	1.7	0.048
DDX28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28	1.6	0.041
ZNF259	zinc finger protein 259	1.6	0.039
TRMT10C	tRNA methytransferase 10 homolog C (S. cerevsiae)	1.6	0.041
RHOU	ras homolog family member U	1.6	0.048
AEN	apoptosis enhancing nuclease	1.6	0.044
AKAP12	A kinase (PRKA) anchor protein 12	1.5	0.041

Gene Symbol	Gene Name	Fold change	FDR p value
USP38	ubiquitin specific peptidase 38	1.5	0.046
DIS3	DIS3 mitotic control homolog (S. cerevisiae)	1.5	0.046
PSMG1	proteasome (prosome, macropain) assembly chaperone 1	1.5	0.014
TAF5L	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa	1.5	0.041
SNORA37	small nucleolar RNA, H/ACA box 37	1.5	0.041
TUSC1	tumor suppressor candidate 1	1.5	0.041
SLC41A1	solute carrier family 41, member 1	1.5	0.044
LDHA	lactate dehydrogenase A	1.5	0.044
MESDC1	mesoderm development candidate 1	1.5	0.035
AVPR1A	arginine vasopressin receptor 1A	1.4	0.039
IMPAD1	inositol monophosphatase domain containing 1	1.4	0.046
RRAGC	Ras-related GTP binding C	1.4	0.044
PSMD14	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	1.4	0.044
STRAP	serine/threonine kinase receptor associated protein	1.4	0.039
YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	1.4	0.041
EID2	EP300 interacting inhibitor of differentiation 2	1.3	0.046
SYT3	synaptotagmin III	1.3	0.044
TCP1	t-complex 1	1.3	0.044
HAUS2	HAUS augmin-like complex, subunit 2	1.3	0.048
PSME3	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	1.3	0.041
KPNA4	karyopherin alpha 4 (importin alpha 3)	1.3	0.044
FYTTD1	forty-two-three domain containing 1	1.3	0.046
SLC25A28	solute carrier family 25 (mitochondrial iron transporter), member 28	1.3	0.046
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)	1.3	0.046
RPUSD4	RNA pseudouridylate synthase domain containing 4	1.3	0.046
ZNF672	zinc finger protein 672	1.2	0.044
ACSL3	acyl-CoA synthetase long-chain family member 3	1.2	0.047
UBE2W	ubiquitin-conjugating enzyme E2W (putative)	1.2	0.044
LTK	leukocyte receptor tyrosine kinase	1.2	0.041
LHX1	LIM homeobox 1	1.2	0.044
CDV3	CDV3 homolog (mouse)	1.2	0.044
PSMD13	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	1.2	0.046
RASGRF1	Ras protein-specific guanine nucleotide-releasing factor 1	1.1	0.039
EIF4H	eukaryotic translation initiation factor 4H	1.1	0.048
WWTR1	WW domain containing transcription regulator 1	1.1	0.041

Genes with significantly upregulated gene expression in thoracic adipose tissue after lung reperfusion compared with prior to reperfusion.

Table 3

Down-regulated genes with allograft reperfusion

Gene symbol	Gene name	Fold change	FDR p value
FLRT3	fibronectin leucine rich transmembrane protein 3	−4.3	0.039
LRP2	low density lipoprotein receptor-related protein 2	−3.6	0.035
SLC28A3	solute carrier family 28 (sodium-coupled nucleoside transporter), member 3	−3.4	0.041
MS4A14	membrane-spanning 4-domains, subfamily A, member 14	−3.4	0.041
TGM1	transglutaminase 1 (K polypeptide epidermal type I, protein-glutamine-gamma-glutamyltransferase)	−3.1	0.035
RSPO1	R-spondin 1	−2.8	0.041
MMP24	matrix metallopeptidase 24 (membrane-inserted)	−2.7	0.014
LOC100287290	cytokine receptor CRL2	−2.4	0.041
MGARP	mitochondria-localized glutamic acid-rich protein	−2.3	0.041
NTNG1	netrin G1	−2.1	0.042
CLDN15	claudin 15	−2.1	0.046
GAL3ST2	galactose-3-O-sulfotransferase 2	−2.1	0.041
CALHM2	calcium homeostasis modulator 2	−1.9	0.046
ITLN2	intelectin 2	−1.9	0.039
GDPD1	glycerophosphodiester phosphodiesterase domain containing 1	−1.8	0.041
RASSF9	Rasassociation (RalGDS/AF-6) domain family (N-terminal) member 9	−1.8	0.044
LRRC33	leucine rich repeat containing 33	−1.8	0.041
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog	−1.7	0.041
GIMAP7	GTPase, IMAP family member 7	−1.7	0.046
CCDC64	coiled-coil domain containing 64	−1.6	0.035
ZKSCAN3	zinc finger with KRAB and SCAN domains 3	−1.6	0.046
CCDC141	coiled-coil domain containing 141	−1.6	0.041
CKAP2	cytoskeleton associated protein 2	−1.6	0.041
PKD1L3	polycystic kidney disease 1-like 3	−1.5	0.046
MIR553	microRNA553	−1.5	0.041
FANCF	Fanconi anemia, complementation group F	−1.4	0.046
STAB1	stabilin 1	−1.4	0.041
SASS6	spindle assembly 6 homolog (C. elegans)	−1.4	0.044
LOC100131564	uncharacterized LOC100131564	−1.4	0.005
NBPF1	neuroblastoma breakpoint family, member 1	−1.4	0.044
LOC100132707	uncharacterized LOC100132707	−1.4	0.044
KANSL1	KAT8 regulatory NSL complex subunit 1	−1.4	0.046
ZNF521	zinc finger protein 521	−1.4	0.044
NPC2	Niemann-Pick disease, type C2	−1.4	0.044
ITFG2	integrin alpha FG-GAP repeat containing 2	−1.4	0.046
TBX19	T-box 19	−1.4	0.039

Gene symbol	Gene name	Fold change	FDR p value
KIAA0528	KIAA0528	−1.3	0.041
UBA7	ubiquitin-like modifier activating enzyme 7	−1.3	0.041
SLCO2B1	solute carrier organic anion transporter family, member 2B1	−1.3	0.039
TULP3	tubby like protein 3	−1.3	0.046
HKR1	HKR1, GLI-Kruppel zinc finger family member	−1.3	0.039
ZNF225	zinc finger protein 225	−1.3	0.041
CAPRIN2	caprin family member 2	−1.3	0.041
PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2	−1.3	0.041
DNMT3A	DNA (cytosine-5-)-methytransferase 3 alpha	−1.3	0.041
RYR1	ryanodine receptor 1 (skeletal)	−1.3	0.046
WDR91	WD repeat domain 91	−1.3	0.041
PAN2	PAN2 poly(A) specific ribonuclease subunit homolog	−1.3	0.048
LOC646938	TBC1 domain family, member 2B pseudogene	−1.3	0.045
CTC1	CTS telomere maintenance complex component 1	−1.3	0.046
NR2C2	nuclear receptor subfamily 2, group C, member 2	−1.2	0.048
ASB6	ankyrin repeat and SOCS box containing 6	−1.2	0.041
ZMIZ1	zinc finger, MIZ-type containing 1	−1.2	0.041
LRCH3	leucine-rich repeats and calponin homology (CH) domain containing 3	−1.2	0.045
ZNF730	zinc finger protein 730	−1.2	0.046
LOC100133957	uncharacterized LOC100133957	−1.1	0.039

Genes with significantly downregulated gene expression in thoracic adipose tissue after lung reperfusion compared with prior to reperfusion.

Table 4

Results of KEGG pathway analysis using GATHER. All genes with fold-change ≥ |1.5| were included. Analysis included genes inferred from network but was restricted to human genes.

KEGG Pathway	p-value	Bayes Factor
Complement and coagulation cascades	0.009	17
Jak-STAT signaling pathway	0.03	6
Cytokine-cytokine receptor interaction	0.06	2