Shali Mazaki-Tovi*, Adi L. Tarca, Edi Vaisbuch, Juan Pedro Kusanovic, Nandor Gabor Than, Tinnakorn Chaiworapongsa, Zhong Dong, Sonia S. Hassan and Roberto Romero*

Characterization of visceral and subcutaneous adipose tissue transcriptome in pregnant women with and without spontaneous labor at term: implication of alternative splicing in the metabolic adaptations of adipose tissue to parturition

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Abstract

Objective: The aim of this study was to determine gene expression and splicing changes associated with parturition and regions (visceral vs. subcutaneous) of the adipose tissue of pregnant women.

Study design: The transcriptome of visceral and abdominal subcutaneous adipose tissue from pregnant women at term with (n=15) and without (n=25) spontaneous labor was profiled with the Affymetrix GeneChip Human Exon 1.0 ST array. Overall gene expression changes and the differential exon usage rate were compared between patient groups (unpaired analyses) and adipose tissue regions (paired analyses). Selected genes were tested by quantitative reverse transcription-polymerase chain reaction.

Results: Four hundred and eighty-two genes were differentially expressed between visceral and subcutaneous fat of pregnant women with spontaneous labor at term (q-value <0.1; fold change >1.5). Biological processes enriched in this comparison included tissue and vasculature development as well as inflammatory and metabolic pathways. Differential splicing was found for 42 genes [q-value <0.1; differences in Finding Isoforms using Robust Multichip Analysis scores >2] between adipose tissue regions of women not in labor. Differential exon usage associated with parturition was found for three genes (*LIMS1*, *HSPA5*, and *GSTK1*) in subcutaneous tissues.

***Corresponding authors: Shali Mazaki-Tovi,** MD, Department of Obstetrics and Gynecology, Sheba Medical Center, Tel Hashomer, 52621 Israel, Tel.: (+972) 3-530-2169, Fax: (+972) 3-5302922, E-mail: shalimazaki@gmail.com; and Tel Aviv University, Tel Aviv, Israel; and **Roberto Romero,** MD, D(Med)Sci, Perinatology Research Branch, NICHD/NIH/ DHHS, Hutzel Women's Hospital, Box No. 4, 3990 John R, Detroit, MI 48201, USA, Tel.: (+313) 993-2700, Fax: (+313) 993-2694, E-mail: romeror@mail.nih.gov; Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI, USA; Department of Biostatistics and Epidemiology, Michigan State University, East Lansing, MI, USA; and Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA

Adi L. Tarca: Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, and Detroit, MI, USA; Department of Computer Science, Wayne State University, Detroit, MI, USA; and Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA

Edi Vaisbuch: Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, Israel

Juan Pedro Kusanovic: Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, and Detroit, MI, USA; Department of Obstetrics and Gynecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile; and Center for Research and Innovation in Maternal-Fetal Medicine (CIMAF), Department of Obstetrics and Gynecology, Sótero del Río Hospital, Santiago, Chile

Nandor Gabor Than: Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, and Detroit, MI, USA; Institute of Enzymology, Momentum Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary; and First Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary

Tinnakorn Chaiworapongsa and Sonia S. Hassan: Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, and Detroit, MI, USA; and Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA

Zhong Dong: Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, and Detroit, MI, USA

Conclusion: We show for the first time evidence of implication of mRNA splicing and processing machinery in the subcutaneous adipose tissue of women in labor compared to those without labor.

Keywords: Adipokines; delivery; fat depots; gestation; high dimensional biology; metabolism; obesity; pregnancy.

Introduction

Parturition imposes an increased energy demand on the laboring woman. Labor is characterized by increased concentrations of nutrients including glucose [1–5], free fatty acids [3, 6], ketone bodies [7], and lactic acid [8]. There is an approximate three-fold increase in whole body glucose utilization during labor and delivery and, as expected, energy expenditure of the parturient women in the second stage of labor is 40% higher compared to the first stage [9]. Additional support for the metabolic burden of labor can also be found in the examination of myometrial glycogen storage, which is significantly increased at term [10], but almost completely depleted during labor [11]. Consistent with these findings, examination of the human myometrial transcriptome revealed that biological processes related to metabolism were among the molecular functions enriched in the differentially expressed genes between pregnant women with and without spontaneous term labor [12].

The conventional view is that the energy expenditure of labor and delivery is equivalent to that of moderate exercise [1, 9] and that similar mechanisms (e.g. insulin and non-insulin dependent glucose uptake, enhanced hepatic gluconeogenesis, and direct sympathetic nervous system stimulation) govern the metabolic adaptation to parturition [9, 13, 14]. However, whether or not adipose tissue, the major energy reservoir, is affected by labor and delivery is still unknown. Assessment of the putative role of adipose tissue in human parturition may be of special importance considering the large body of evidence indicating that this endocrinal organ is powerful [15] and exerts autocrine, paracrine and endocrine effects by the production and secretion of highly active peptides and proteins collectively termed adipokines [16]. Importantly, adipokines have been implicated in physiological adaptations of normal gestation [17–28] as well as in the pathophysiology of preeclampsia [21, 29–50], gestational diabetes mellitus [51–65], preterm birth [66–68], delivery of large-for-gestational-age (LGA) newborns [69], small-for-gestational-age (SGA) neonates [70–76], pyelonephritis [77–79], and intrauterine infection and inflammation [80–83]. Of note is the well-established association between obesity and these complications of pregnancy [84–113].

It has been suggested that the implication of adipose tissue in physiological or pathological processes should take into account the region-specific differences between fat depots. Particularly, differences in function [114–116], gene expression [115, 117–144], and metabolic effect [145–150] between the visceral and subcutaneous adipose tissue are to be considered. Indeed, regional variations of adipose tissue in specific genes were reported in non-pregnant individuals using both high throughput techniques [131–133, 136, 151] and targeted approaches [115, 116, 131, 133, 152–166]. Overall gene expression in the adipose tissue of pregnant women has been previously reported [32, 117–123, 167–178]; however, adipose tissue gene expression, biological processes, molecular functions, and pathways associated with spontaneous term parturition have not been described. Furthermore, to our knowledge, exon-level changes that can inform on alternative promoter usage, alternative splicing, and alternative transcript termination [179] between the visceral and subcutaneous regions have not been reported in either fat or other tissue of parturient women.

We undertook this study in order to characterize the transcriptome of human visceral and subcutaneous adipose tissue during normal labor at term to gain understanding of the global changes in gene expression and splicing associated with adiposity using an unbiased approach. The aims of this study were: 1) to determine differences in visceral and subcutaneous gene expression between pregnant women with and without spontaneous labor at term; 2) to determine regional variations in the transcriptome of adipose tissue of patients with spontaneous labor at term; and 3) to identify depot-specific alternative splicing alterations in the adipose tissue of women with spontaneous labor at term.

Materials and methods

Study groups

A prospective study was performed in which visceral and subcutaneous adipose tissue samples were obtained from women undergoing cesarean section at term (≥37 weeks) in the following groups: 1) not in labor (n=25) and 2) spontaneous labor (n=15).

The inclusion criteria for both groups were as follows: 1) absence of medical complications; 2) no antibiotic administration prior to the sample collection; 3) normal post-operative course; 4) absence of meconium staining of the amniotic fluid; 5) neonatal Apgar scores >7 at 1 and 5 min; 6) absence of histologic chorioamnionitis; 7) absence of obstetric complications of pregnancy; and 8) normal pregnancy

outcome, including an infant who was of appropriate-weight-for-gestational-age (AGA) without congenital anomalies.

Eligible patients were enrolled at Hutzel Women's Hospital (Detroit, MI, USA). All women provided written informed consent prior to the collection of adipose tissue samples. The collection and utilization of the samples for research purposes was approved by the Institutional Review Boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD/NIH/DHHS, Bethesda, MD, USA), and the Human Investigation Committee of Wayne State University (Detroit, MI, USA). Samples obtained from pregnant women not in labor have been previously used to study the differences in transcriptome between pregnant and non-pregnant women.

Clinical definitions

Patients not in labor underwent a cesarean section secondary to a fetus in the non-cephalic presentation, previous uterine surgery, or classical cesarean section, or an elective cesarean section with no more than one previous cesarean section. Women in spontaneous labor underwent cesarean section due to a fetal malpresentation or for non-reassuring fetal status as determined by the clinical staff. Patients with clinical or histological chorioamnionitis and those undergoing induction of labor were excluded.

Labor was diagnosed in the presence of spontaneous regular uterine contractions occurring at a minimum frequency of two every 10 min with cervical changes that required hospital admission. Histologic chorioamnionitis was diagnosed using previously described criteria [180, 181]. An AGA neonate was defined by a birth weight between the 10^{th} and 90^{th} percentiles for the gestational age at birth [182]. Body mass index (BMI) was calculated according to the formula: weight (kg)/height^2 (m^2).

Sample collection

Paired visceral and subcutaneous adipose tissue samples were obtained from each participant. Subcutaneous adipose tissue samples were collected at the site of a transverse lower abdominal incision, in the middle of the Pfannenstiel incision, from the deeper strata of subcutaneous fat. Visceral samples were obtained from the most distal portion of the greater omentum [116, 183–186]. Visceral and subcutaneous adipose tissues were collected using Metzenbaum scissors and measured approximately 1.0 cm^3. Tissues were snap-frozen in liquid nitrogen and stored at −80°C until use.

RNA isolation

Total RNA was isolated from snap-frozen adipose tissue using TRI Reagent® combined with the Qiagen RNeasy Lipid Tissue kit protocol (Qiagen, Valencia, CA, USA), according to the manufacturers' recommendations. The RNA concentrations and the A260 nm/A280 nm ratio were assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA integrity numbers were determined using the Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE, USA).

Microarray analysis and quantitative real-time polymerase chain reaction

The Affymetrix GeneChip Human Exon 1.0 ST array (Affymetrix Inc., Santa Clara, CA, USA) platform was used to measure the expression levels in each unpooled specimen, per manufacturer's instructions (http://www.affymetrix.com). The array contains approximately 5.4 million 5-µm features (probes) grouped into 1.4 million probesets interrogating more than one million exon clusters [187–189]. To verify the results from microarray-based analysis, 24 genes were selected for quantitative real-time polymerase chain reaction (qRT-PCR) assays in the same set of samples used for microarrays.

Statistical analyses

Differential expression: The raw microarray probe intensity data were background corrected, quantile normalized [190] and summarized into one expression value for each transcript using a robust multi-array average implemented in the *aroma.affymetrix* package [191]. A paired moderated *t*-test [192] was used to test for differential expression with a false discovery rate (FDR) [193] correction of P-values to obtain q-values. Gene significance was inferred using $q<0.1$ and fold-change in expression >1.5 [194]. Gene ontology analysis was performed with algorithms previously described [195]. Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG) [196] pathway database (96 pathways with three or more genes on our microarray platform) with an overrepresentation analysis [197]. Alternatively, the Pathway Analysis with Down-weighting of Overlaping Genes (PADOG) [198] was applied on the canonical pathways collection from the MSigDB database [199] (831 pathways with at least 20 genes represented on our microarray platform). Differential expression between adipose tissue regions of the same subjects based on qRT-PCR data was performed with a paired *t*-test on $-\Delta Ct$ values.

Differential exon usage (splicing): To identify differential exon usage between the groups of samples, we used the method Finding Isoforms using Robust Multichip Analysis (FIRMA) [200] to quantify how far (above or below) a given exon's expression level was compared to the expected (average) transcript level in a given sample. Criteria for inclusion of transcripts and exons are described in the supplementary material. We applied a *t*-test for each probeset (typically one per exon) in each transcript based on the FIRMA scores, and inferred significance when the difference in mean FIRMA scores between groups was 2.0 or more combined with a threshold of 0.1 on the FDR-adjusted P-values (q-values). This was a more stringent approach than described in another study [200] in which positive results were identified based only on the difference in mean FIRMA scores above 1.5 units. Plotting of the probe-level expression data at exon levels vs. genomic coordinates was performed using functionality provided by the *GenomeGraphs* package with known isoforms in the ENSEMBL database retrieved with *biomaRt* [200]. All microarray analyses were performed using the R language and environment and Bioconductor [200, 201].

Demographic data analysis: The Student's *t*, Mann-Whitney *U*, and χ^2 tests were used to identify significant differences in patient

demographics between women in the microarray and qRT-PCR groups. SPSS software (version 14.0; SPSS Inc, Chicago, IL, USA) was used for statistical analysis of demographic data. A probability value of <0.05 was considered statistically significant.

Results

Demographics

Table 1 displays the demographic characteristics of patients who were included in the microarray and qRT-PCR analyses.

Regional differences in the transcriptome of adipose tissue of women with and without labor

Differential expression

Microarray analysis demonstrated 485 transcripts corresponding to 482 unique genes differentially expressed between the visceral and subcutaneous adipose tissue of pregnant women in spontaneous labor at term (q-value <0.1; fold change >1.5). A total of 329 genes had decreased expression, and 153 genes had increased expression in the subcutaneous, compared to visceral, adipose tissue. A "volcano plot" shows the differential expression of all annotated probesets on the Affymetrix GeneChip Human Exon 1.0 ST array with the log (base 10) of q-values (y-axis) plotted against the log (base 2) fold changes (x-axis) between the visceral and subcutaneous adipose tissue (Figure 1). The heatmap in Figure 2 uses a color scale to show the consistency of the expression levels within each group of samples as well as the differences between the groups that led to positive test results. A list of the top 100 genes differentially expressed between visceral and subcutaneous adipose tissue of patients with and without

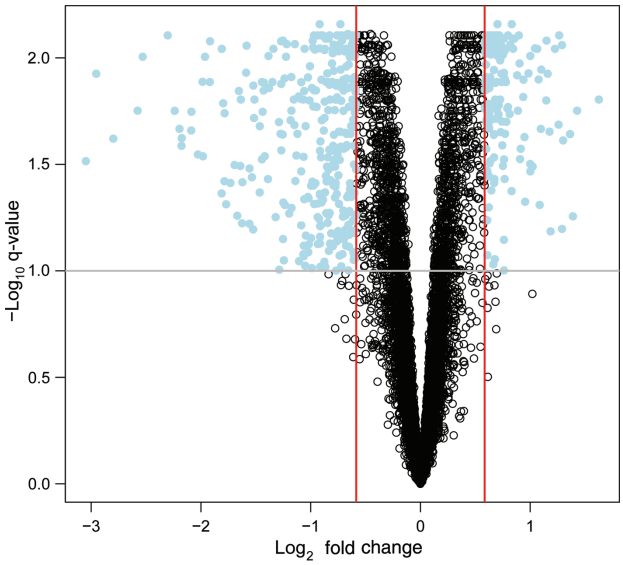

Figure 1: Differential expression of visceral versus subcutaneous adipose tissue transcripts in pregnant women in labor.
Volcano plot showing differential expression evidence between subcutaneous and visceral adipose tissue of women in labor. The x-axis represents the $\log_2$ fold changes in expression with positive values representing over-expression in the subcutaneous region compared to visceral. Transcripts outside the vertical red bars have fold change >1.5. The y-axis represents the q-values ($-\log_{10}$ of), with values above 1.0 corresponding to q<0.1.

Table 1: Demographic and clinical characteristics of the study population.

	Term labor (n=15)	Term not in labor (n=25)	P-value
Maternal age (years)	26 (24–38)	27 (25–39)	0.2
Gestational age at delivery (weeks)	39.7 (39–40.6)	39.1 (38.9–39.4)	0.2
Pre-gestational BMI (kg/m^2)	35.3 (30.9–38.5)	37.5 (26.2–40.2)	0.5
BMI at sampling (kg/m^2)	36.9 (32.5–39.7)	37.2 (27.8–45.4)	0.8
Gravidity	3 (2–3)	3 (2–4)	0.5
Parity	2 (1–3)	2 (2–3)	0.2
Ethnic origin (%)			1.0
African American	91.7	83.3	
Caucasian	8.3	16.7	
Systolic blood pressure (mm Hg)	124 (117–127)	121 (115–126)	0.4
Diastolic blood pressure (mm Hg)	75 (67–79)	66 (62–77)	0.3
Cervical dilatation at sampling	5 (4–7)	1 (1–2)	<0.001
Fasting glucose (mg/dL)	93 (87–98)	94 (88–97)	0.7
Birth weight (g)	3320 (3155–3825)	3275 (3105–3500)	0.7

Data are presented as median and interquartile range (IQR). BMI=Body mass index.

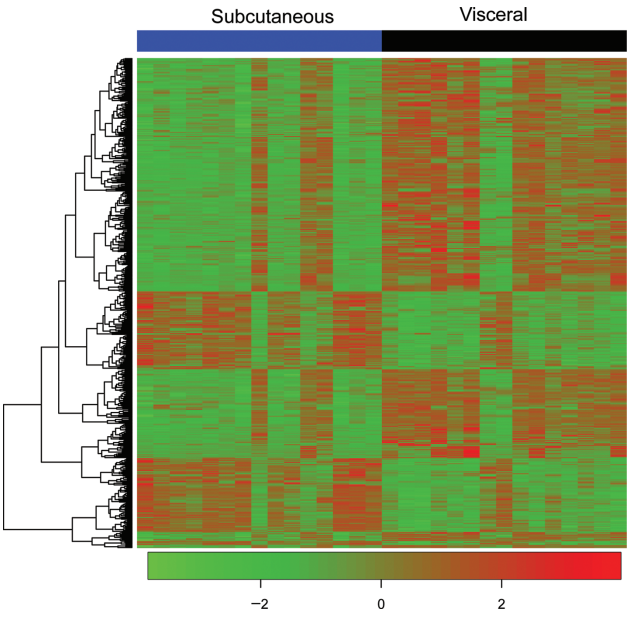

Figure 2: Heat map representing fat depot-specific differences in gene expression of pregnant women in labor.
Heatmap showing the consistency of gene expression levels between subcutaneous and visceral regions of the adipose tissue of women in labor. Log_2 transformed transcript expression values are centered and scaled row-wise.

spontaneous labor at term is presented in Table 2; the complete list of differentially expressed probes is available as supplementary material (Supplementary Table 1).

Among the 482 genes differentially expressed between visceral and subcutaneous adipose tissue in patients with spontaneous labor at term, 91 were not part of the 632 genes differentially expressed in the not in labor group (ENTREZ IDs suffixed by a * in Table 2 and Supplementary Table 1).

In order to gain further insight into the biology of the differential gene expression, Gene Ontology enrichment analysis was employed. A total of 94 biological processes were associated with regional differences in the spontaneous term labor group ($q<0.05$) (Table 3). Pathway analysis performed using an over-representation on the KEGG database resulted in seven significant pathways in this comparison ($q<0.05$): complement and coagulation cascades, cytokine-cytokine receptor interaction, focal adhesion, steroid hormone biosynthesis, ECM-receptor interaction, African trypanosomiasis, and protein digestion and absorption.

qRT-PCR analysis

The results of qRT-PCR confirmed the differential expression of nine of 29 genes found to be significant on the microarray analysis: lipoprotein lipase (*LPL*), retinol binding protein 4 (*RBP4*), leptin (*LEP*), complement component 4B (Chido blood group) (*C4B*), insulin-like growth factor binding protein 2 (*IGFBP2*), monoglyceride lipase (*MGLL*), annexin A8 (*ANXA8*), klotho beta (*KLB*), and prolactin (*PRL*).

Differential splicing

Using the Affymetrix GeneChip Human Exon 1.0 ST array that probes individual exons of known genes, we compared the exon usage (inclusion) rates between adipose tissue regions. Significant differences in exon usage were found for 42 genes between visceral and subcutaneous adipose tissue of pregnant women not in labor (Table 4) but not in the labor group.

Patients with spontaneous term labor versus pregnant women not in labor

Differential expression

We did not find significant differences in gene expression in either visceral or subcutaneous adipose tissue of pregnant women with and without spontaneous labor using our predefined gene selection criteria. However, when applying PADOG pathway analysis, four KEGG pathways (spliceosome, snare interactions in vesicular transport, pathogenic *Escherichia coli* infection, DNA replication) and three Reactome database [202] pathways (processing of capped intron containing pre-mRNA, mRNA processing, mRNA splicing) were found to be significantly perturbed in the presence of labor in the subcutaneous region of the adipose tissue (see enrichment plots for two of these pathways in Figure 3). Unlike the over-representation approach requiring gene selection as a first step, PADOG determines whether the differential expression *t*-scores of a given pathway are higher (in absolute value) than those of all genes profiled on the array and, hence, detects potentially smaller but systematic differential expression in a given pathway compared to all genes on the array (Figure 3). When comparing the visceral region of the women in labor to those without labor, the PADOG identified the Reactome asparagine N-linked glycosylation pathway to be associated with parturition (see Figure S1).

Differential splicing

Significant differences in exon usage were found between subcutaneous adipose tissue of pregnant women with and without spontaneous labor at term for three genes:

Table 2: A list of the top 100 differentially expressed genes between visceral and subcutaneous adipose tissue of patients with and without spontaneous labor at term.

ENTREZ	Symbol	Name	Fold change	q-Value
364	*AQP7*	Aquaporin 7	1.6	0.007
355	*FAS*	Fas (TNF receptor superfamily, member 6)	−1.9	0.007
100293763	*AQP7P1*	Aquaporin 7 pseudogene 1	1.8	0.007
9871*	*SEC24D*	SEC24 family, member D (*Saccharomyces cerevisiae*)	−1.7	0.007
83666*	*PARP9*	Poly(ADP-ribose) polymerase family, member 9	−1.6	0.008
729085*	*CCBP2*	Chemokine binding protein 2	−1.6	0.008
6285	*S100B*	S100 calcium binding protein B	1.6	0.008
10555	*AGPAT2*	1-Acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	1.6	0.008
6574	*SLC20A1*	Solute carrier family 20 (phosphate transporter), member 1	−1.9	0.008
54566	*EPB41L4B*	Erythrocyte membrane protein band 4.1 like 4B	1.6	0.008
58477*	*SRPRB*	Signal recognition particle receptor, B subunit	−1.8	0.008
54988*	*ACSM5*	Acyl-CoA synthetase medium-chain family member 5	1.6	0.008
9180	*OSMR*	Oncostatin M receptor	−1.9	0.008
284221	*FAM38B2*	Family with sequence similarity 38, member B2	−2.0	0.008
2819	*GPD1*	Glycerol-3-phosphate dehydrogenase 1 (soluble)	1.7	0.008
9052	*GPRC5A*	G protein-coupled receptor, family C, group 5, member A	−1.7	0.008
10973*	*ASCC3*	Activating signal cointegrator 1 complex subunit 3	−1.5	0.008
6517	*SLC2A4*	Solute carrier family 2 (facilitated glucose transporter), member 4	1.6	0.008
6713	*SQLE*	Squalene epoxidase	−1.6	0.008
83716	*CRISPLD2*	Cysteine-rich secretory protein LCCL domain containing 2	−1.9	0.008
10249	*GLYAT*	Glycine-N-acyltransferase	2.4	0.008
23555	*TSPAN15*	Tetraspanin 15	1.6	0.008
8908	*GYG2*	Glycogenin 2	1.5	0.008
5578	*PRKCA*	Protein kinase C, alpha	−1.5	0.008
54884	*RETSAT*	Retinol saturase (all-trans-retinol 13,14-reductase)	1.6	0.008
5055	*SERPINB2*	Serpin peptidase inhibitor, clade B (ovalbumin), member 2	−4.9	0.008
57568*	*SIPA1L2*	Signal-induced proliferation-associated 1 like 2	−1.5	0.008
5207	*PFKFB1*	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	1.9	0.008
60559*	*SPCS3*	Signal peptidase complex subunit 3 homolog (*S. cerevisiae*)	−1.6	0.008
9197*	*SLC33A1*	Solute carrier family 33 (acetyl-CoA transporter), member 1	−1.5	0.008
3991	*LIPE*	Lipase, hormone-sensitive	1.6	0.008
26064*	*RAI14*	Retinoic acid induced 14	−1.6	0.008
8542*	*APOL1*	Apolipoprotein L, 1	−1.6	0.008
374969	*CCDC23*	Coiled-coil domain containing 23	1.5	0.008
8639	*AOC3*	Amine oxidase, copper containing 3 (vascular adhesion protein 1)	1.6	0.008
11098	*PRSS23*	Protease, serine, 23	−1.7	0.008
2822	*GPLD1*	Glycosylphosphatidylinositol specific phospholipase D1	1.7	0.008
8659	*ALDH4A1*	Aldehyde dehydrogenase 4 family, member A1	1.8	0.008
4718	*THRSP*	Thyroid hormone responsive (SPOT14 homolog, rat)	1.8	0.008
55024*	*BANK1*	B-cell scaffold protein with ankyrin repeats 1	1.6	0.008
6782*	*HSPA13*	Heat shock protein 70 kDa family, member 13	−1.8	0.008
65983	*GRAMD3*	GRAM domain containing 3	−1.8	0.008
4137	*MAPT*	Microtubule-associated protein tau	1.6	0.008
1009	*CDH11*	Cadherin 11, type 2, OB-cadherin (osteoblast)	−2.0	0.008
10130*	*PDIA6*	Protein disulfide isomerase family A, member 6	−1.5	0.008
51602*	*NOP58*	NOP58 ribonucleoprotein homolog (yeast)	−1.6	0.008
81539	*SLC38A1*	Solute carrier family 38, member 1	−2.4	0.008
51716	*CES1*	Carboxylesterase 1 (monocyte/macrophage serine esterase 1)	2.2	0.008
9643*	*MORF4L2*	Mortality factor 4 like 2	−1.5	0.008
666	*BOK*	BCL2-related ovarian killer	1.6	0.008
9945	*GFPT2*	Glutamine-fructose-6-phosphate transaminase 2	−2.4	0.008
55254*	*TMEM39A*	Transmembrane protein 39A	−1.5	0.008
22915	*MMRN1*	Multimerin 1	−3.8	0.008
84293	*C10orf58*	Chromosome 10 open reading frame 58	1.6	0.008
64757	*MOSC1*	MOCO sulphurase C-terminal domain containing 1	1.6	0.008

Table 2 (continued)

ENTREZ	Symbol	Name	Fold change	q-Value
64805*	*P2RY12*	Purinergic receptor P2Y, G-protein coupled, 12	1.6	0.008
91607	*SLFN13*	Schlafen family member 13	−1.5	0.009
220	*ALDH1A3*	Aldehyde dehydrogenase 1 family, member A3	−2.3	0.009
212*	*ALAS2*	Aminolevulinate, delta-, synthase 2	1.7	0.009
10962	*MLLT11*	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*); translocated to, 11	−2.2	0.009
358*	*AQP1*	Aquaporin 1 (Colton blood group)	−1.5	0.009
23612*	*PHLDA3*	Pleckstrin homology-like domain, family A, member 3	1.5	0.009
6578	*SLCO2A1*	Solute carrier organic anion transporter family, member 2A1	−1.7	0.009
286753*	*TUSC5*	Tumor suppressor candidate 5	1.5	0.009
5271*	*SERPINB8*	Serpin peptidase inhibitor, clade B (ovalbumin), member 8	−1.7	0.009
63924	*CIDEC*	Cell death-inducing DFFA-like effector c	1.8	0.009
4189*	*DNAJB9*	DnaJ (Hsp40) homolog, subfamily B, member 9	−1.7	0.009
56265	*CPXM1*	Carboxypeptidase X (M14 family), member 1	−1.7	0.009
58528*	*RRAGD*	Ras-related GTP binding D	1.5	0.009
262*	*AMD1*	Adenosylmethionine decarboxylase 1	−1.5	0.009
222166	*C7orf41*	Chromosome 7 open reading frame 41	1.6	0.009
338	*APOB*	Apolipoprotein B [including Ag(x) antigen]	2.4	0.009
158295	*MGC24103*	Hypothetical MGC24103	−1.5	0.009
1805*	*DPT*	Dermatopontin	1.5	0.009
10237*	*SLC35B1*	Solute carrier family 35, member B1	−1.5	0.009
623	*BDKRB1*	Bradykinin receptor B1	−3.0	0.009
5740	*PTGIS*	Prostaglandin I2 (prostacyclin) synthase	−2.0	0.009
6272	*SORT1*	Sortilin 1	1.7	0.009
1645	*AKR1C2*	Aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-α hydroxysteroid dehydrogenase, type III)	2.1	0.009
5649	*RELN*	Reelin	−1.6	0.009
6446	*SGK1*	Serum/glucocorticoid regulated kinase 1	−1.9	0.009
51330	*TNFRSF12A*	Tumor necrosis factor receptor superfamily, member 12A	−1.8	0.009
388403*	*YPEL2*	Yippee-like 2 (*Drosophila*)	1.7	0.009
80704	*SLC19A3*	Solute carrier family 19, member 3	1.5	0.009
5140	*PDE3B*	Phosphodiesterase 3B, cGMP-inhibited	1.7	0.009
3036	*HAS1*	Hyaluronan synthase 1	−1.7	0.009
1646	*AKR1C1*	Aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-α (3-α)-hydroxysteroid dehydrogenase)	2.3	0.009
1962	*EHHADH*	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	1.5	0.009
90355*	*C5orf30*	Chromosome 5 open reading frame 30	1.6	0.009
283383	*GPR133*	G protein-coupled receptor 133	−2.1	0.009
4199	*ME1*	Malic enzyme 1, NADP(+)-dependent, cytosolic	1.7	0.009
6366	*CCL21*	Chemokine (C-C motif) ligand 21	−3.5	0.009
4023	*LPL*	Lipoprotein lipase	1.6	0.009
6385	*SDC4*	Syndecan 4	−2.5	0.009
84649	*DGAT2*	Diacylglycerol O-acyltransferase homolog 2 (mouse)	1.6	0.009
80339*	*PNPLA3*	Patatin-like phospholipase domain containing 3	1.6	0.009
783	*CACNB2*	Calcium channel, voltage-dependent, beta 2 subunit	−1.7	0.009
7086	*TKT*	Transketolase	1.5	0.009
63895*	*FAM38B*	Family with sequence similarity 38, member B	−1.6	0.009
4883	*NPR3*	Natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	1.8	0.009

Glutathione S-transferase kappa 1 (*GSTK1*), heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) (*HSPA5*), and LIM and senescent cell antigen-like-containing domain protein 1 (*LIMS1*). None of the three genes were differentially expressed between visceral and subcutaneous adipose tissue of parturient women as the change in mRNA abundance was present only for one exon of each gene (Figures 4 and 5 illustrate the differential exon

Table 3: Bological processes associated with regional differences in the spontaneous term labor group.

Biological process	Term size	DE genes	Odds ratio	q-Value
Response to external stimulus	951	74	2.7	<0.001
Retinal metabolic process	9	8	216.8	<0.001
Circulatory system development	739	61	2.6	<0.001
Regulation of complement activation	18	9	27.1	<0.001
Blood vessel morphogenesis	347	35	3.2	<0.001
Multicellular organismal process	4870	232	1.7	<0.001
Positive regulation of cellular component movement	283	30	3.3	<0.001
Anatomical structure formation involved in morphogenesis	846	61	2.2	<0.001
Regulation of cell motility	498	42	2.6	<0.001
Terpenoid metabolic process	63	13	7.2	<0.001
Retinol metabolic process	17	7	18.9	<0.001
Phototransduction, visible light	70	13	6.2	<0.001
Vasculature development	393	34	2.7	0.001
Triglyceride catabolic process	25	8	12.7	0.001
Positive regulation of signal transduction	905	60	2.1	0.001
Glomerular filtration	19	7	15.8	0.001
Neutral lipid catabolic process	29	8	10.3	0.002
Regulation of inflammatory response	123	16	4.2	0.002
Cell motility	461	35	2.5	0.002
Positive regulation of cell-substrate adhesion	60	11	6.1	0.002
Detection of light stimulus	86	13	4.9	0.003
Acute inflammatory response	75	12	5.2	0.003
Reproductive system development	321	28	2.6	0.004
Striated muscle cell differentiation	164	18	3.4	0.005
Regulation of hormone levels	122	15	3.9	0.005
Tissue morphogenesis	498	37	2.2	0.005
Death	1510	84	1.7	0.006
Glycerolipid catabolic process	36	8	7.7	0.007
Cellular response to jasmonic acid stimulus	3	3	Inf	0.008
Positive regulation of phosphate metabolic process	776	50	1.9	0.008
Receptor-mediated endocytosis	173	18	3.2	0.008
Cellular developmental process	2884	140	1.5	0.008
Protein activation cascade	49	9	6.1	0.009
Tube development	407	31	2.3	0.010
Urogenital system development	211	20	2.9	0.011
Positive regulation vascular endothelial growth factor production	21	6	10.8	0.011
Cell junction assembly	177	18	3.1	0.011
Positive regulation of angiogenesis	102	13	4.0	0.011
Response to lipid	607	41	2.0	0.012
Positive regulation of macrophage derived foam cell differentiation	14	5	14.9	0.012
Cell chemotaxis	181	18	3.0	0.013
Single-organism process	634	29	2.7	0.013
Response to oxygen-containing compound	948	56	1.8	0.013
Terpenoid biosynthetic process	8	4	26.8	0.013
Embryonic limb morphogenesis	106	13	3.8	0.014
Regulation of response to stress	847	52	1.8	0.014
Negative regulation of protein processing	234	21	2.7	0.014
Positive regulation of epithelial cell proliferation	123	14	3.5	0.016
Regulation of behavior	155	16	3.1	0.017
Regulation of multicellular organismal development	1167	66	1.7	0.017
Response to wounding	168	16	3.1	0.017
Regulation of phosphorylation	1016	59	1.7	0.019
Complement activation, alternative pathway	9	4	21.5	0.019
Negative regulation of cardiac muscle tissue development	16	5	12.2	0.019
Epithelium development	624	40	2.0	0.020
Muscle cell migration	46	8	5.7	0.021

Table 3 (continued)

Biological process	Term size	DE genes	Odds ratio	q-Value
Oxoacid metabolic process	849	51	1.8	0.022
Regulation of transport	1300	71	1.6	0.023
Regulation of leukocyte chemotaxis	72	10	4.4	0.023
Positive regulation of focal adhesion assembly	17	5	11.2	0.023
Regulation of protein metabolic process	152	15	3.1	0.024
Small molecule metabolic process	1919	97	1.5	0.024
Endothelial cell morphogenesis	10	4	17.9	0.025
Negative regulation of heart growth	10	4	17.9	0.025
Response to acid chemical	233	20	2.6	0.026
Negative regulation of endopeptidase activity	150	15	3.0	0.028
Establishment of localization	3464	158	1.4	0.028
Cellular response to tumor necrosis factor	90	11	3.8	0.031
Endodermal cell differentiation	39	7	5.9	0.034
Retinoic acid biosynthetic process	5	3	40.3	0.034
Protein secretion	352	26	2.2	0.035
Cellular lipid metabolic process	480	32	2.0	0.035
Cellular response to endogenous stimulus	836	49	1.7	0.035
Regulation of cell adhesion	418	29	2.1	0.035
Positive regulation of locomotion	193	17	2.7	0.035
Appendage morphogenesis	123	13	3.2	0.035
Negative regulation of muscle tissue development	29	6	7.0	0.035
Positive regulation of cell proliferation	487	32	2.0	0.036
Regulation of striated muscle tissue development	79	10	3.9	0.036
Lung development	110	12	3.4	0.038
Triglyceride biosynthetic process	53	8	4.8	0.039
Positive regulation of mesenchymal cell proliferation	30	6	6.7	0.040
Negative regulation of muscle organ development	30	6	6.7	0.040
Positive regulation of leukocyte migration	81	10	3.8	0.042
Neutral lipid biosynthetic process	54	8	4.7	0.042
Peptide transport	248	20	2.4	0.043
Peptide hormone secretion	195	17	2.6	0.045
Inflammatory response	227	18	2.5	0.047
Cell adhesion	493	31	2.0	0.047
Response to toxic substance	129	13	3.0	0.047
Positive regulation of MAPK cascade	236	19	2.4	0.047
Regulation of cell-matrix adhesion	69	9	4.1	0.047
Daunorubicin metabolic process	6	3	26.8	0.049
Doxorubicin metabolic process	6	3	26.8	0.049

usage for *LIMS1* and *GSTK1*). For all three genes, the exon showing differential usage had lower expression in the group of women in labor compared to the not-in-labor group. These three genes were not among the 42 genes with differential exon usage between visceral and subcutaneous adipose tissue of pregnant women not in labor (Table 4).

Discussion

The principal findings of this study include the following: 1) Visceral and subcutaneous adipose tissue transcriptome of pregnant women with spontaneous labor at term were different: i) 482 genes were differentially expressed between the two fat depots; ii) Gene Ontology analysis indicated specific biological processes (e.g. cell adhesion, vasculature development, and circulatory system development); iii) the KEGG pathways enriched in differentially expressed genes were: complement and coagulation cascades, cytokine-cytokine receptor interaction, focal adhesion, steroid hormone biosynthesis, ECM-receptor interaction, African trypanosomiasis, and protein digestion and absorption. 2) Significant differences in alternative spliced genes were found between the subcutaneous adipose tissue of pregnant women with and without spontaneous labor at term; three genes affected by alternative splicing were LIM and senescent

Table 4: A list of the alternative splicing events associated with the regional differences of the adipose tissue of pregnant women not in labor.

ENTREZ	SYMBOL	Name	Exon ID[a]	Diff. FIRMA[b]	P-value	q-Value
5376	*PMP22*	Peripheral myelin protein 22	887424	5.2	<0.001	<0.001
6711	*SPTBN*	Spectrin, beta, non-erythrocytic 1	103031	−5.1	<0.001	<0.001
25818	*KLK5*	Kallikrein-related peptidase 5	960481	−4.6	<0.001	<0.001
85442	*KNDC1*	Kinase non-catalytic C-lobe domain (KIND) containing 1	596822	−3.5	<0.001	<0.001
388610	*TRNP1*	TMF1-regulated nuclear protein 1	7232	−3.1	<0.001	<0.001
1612	*DAPK1*	Death-associated protein kinase 1	538110	3.1	<0.001	<0.001
9201	*DCLK1*	Doublecortin-like kinase 1	742860	−3.0	<0.001	<0.001
9214	*FAIM3*	Fas apoptotic inhibitory molecule 3	84252	−2.9	<0.001	<0.001
25891	*PAMR1*	Peptidase domain containing associated with muscle regeneration 1	656516	2.9	<0.001	<0.001
3983	*ABLIM1*	Actin binding LIM protein 1	619043	2.9	<0.001	<0.001
286204	*CRB2*	Crumbs homolog 2 (*Drosophila*)	544486	−2.8	<0.001	<0.001
11343	*MGLL*	Monoglyceride lipase	236116	−2.7	<0.001	0.0017
25891	*PAMR1*	Peptidase domain containing associated with muscle regeneration 1	656516	2.7	<0.001	<0.001
1674	*DES*	Desmin	131889	−2.7	<0.001	0.0068
10231	*RCAN2*	Regulator of calcineurin 2	399076	2.6	<0.001	<0.001
23524	*SRRM2*	Serine/arginine repetitive matrix 2	826444	−2.6	<0.001	<0.001
23524	*SRRM2*	Serine/arginine repetitive matrix 2	826444	−2.5	<0.001	<0.001
157506	*RDH10*	Retinol dehydrogenase 10 (all-trans)	491273	2.5	<0.001	0.0017
85442	*KNDC1*	Kinase non-catalytic C-lobe domain (KIND) containing 1	596819	−2.5	<0.001	<0.001
79804	*HAND2*	Heart and neural crest derivatives expressed 2	298919	−2.4	<0.001	<0.001
65108	*MARCKSL1*		55081	−2.4	<0.001	<0.001
4071	*TM4SF1*	Transmembrane 4 L six family member 1	240134	−2.4	<0.001	0.006
4837	*NNMT*	Nicotinamide N-methyltransferase	644508	−2.3	<0.001	0.0099
51090	*PLLP*	Plasma membrane proteolipid (plasmolipin)	855189	2.3	<0.001	<0.001
1674	*DES*	Desmin	131895	−2.3	<0.001	0.0028
152	*ADRA2C*	Adrenergic, α-2C-, receptor	249900	−2.3	<0.001	0.0037
81539	*SLC38A1*	Solute carrier family 38, member 1	707206	2.3	<0.001	<0.001
57121	*LPAR5*	Lysophosphatidic acid receptor 5	701054	−2.3	<0.001	0.001
56920	*SEMA3G*	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G	224606	−2.3	<0.001	<0.001
9945	*GFPT2*	Glutamine-fructose-6-phosphate transaminase 2	359171	2.2	<0.001	<0.001
2627	*GATA6*	GATA binding protein 6	908232	−2.2	<0.001	<0.001
4824	*NKX3-1*	NK3 homeobox 1	506935	−2.1	<0.001	<0.001
3036	*HAS1*	Hyaluronan synthase 1	960742	−2.1	<0.001	<0.001
5420	*PODXL*	Podocalyxin-like	472183	−2.1	<0.001	<0.001
5420	*PODXL*	Podocalyxin-like	472213	2.1	<0.001	<0.001
3339	*HSPG2*	Heparan sulfate proteoglycan 2	52523	−2.1	<0.001	0.0746
23555	*TSPAN15*	Tetraspanin 15	582734	−2.1	<0.001	<0.001
23428	*SLC7A8*	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	772193	−2.1	<0.001	<0.001
3913	*LAMB2*	Laminin, beta 2 (laminin S)	223487	−2.0	<0.001	<0.001
23428	*SLC7A8*	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	772200	−2.0	<0.001	<0.001
89932	*PAPLN*	Papilin, proteoglycan-like sulfated glycoprotein	763903	−2.0	<0.001	<0.001
255743	*NPNT*	Nephronectin	263882	2.0	<0.001	<0.001

[a]Exon Identifier based on annotation provided HuEx-1_0-st-v2.na30.hg19.probeset.csv file from www.affymetrix.com. [b]FIRMA scores are a measure of the exon abundance relative to the overall gene level in a given sample. Positive differences in FIRMA scores represent a higher exon usage rate in subcutaneous compared to visceral adipose tissue of women not in labor.

cell antigen-like-containing domain protein 1 (*LIMS1*), heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) (*HSPA5*), and Glutathione S-transferase kappa 1 (*GSTK1*); and 3) visceral and subcutaneous adipose tissue transcriptome of pregnant women with and without spontaneous labor at term did not differ significantly.

Visceral versus subcutaneous adipose tissue in pregnant women with spontaneous labor at term

This study describes, for the first time, the transcriptome of visceral and subcutaneous adipose tissue of pregnant

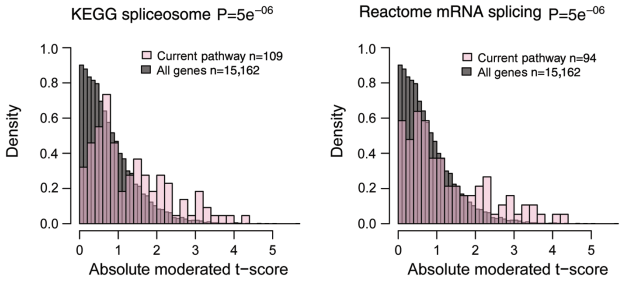

Figure 3: Pathway perturbation associated with parturition in subcutaneous tissue.
PADOG pathway enrichment plots showing evidence of pathway perturbation associated with parturition in subcutaneous tissue. The distribution of moderated t-scores of genes in KEGG spliceosome and reactome mRNA splicing is superimposed on the distribution of all genes on the array, and shows more differential expression in these pathways than in the pool of all genes.

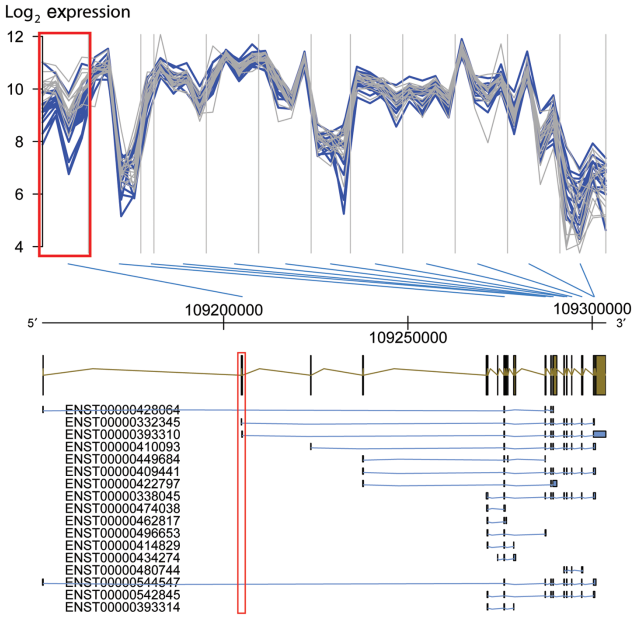

Figure 4: Differential exon usage for *LIMS1* gene in subcutaneous adipose tissue of women with and without labor.
The top panel shows the $\log_2$ expression of probes targeting 12 exonic regions of the *LIMS1* gene (separated by vertical gray lines). There are 1–4 probes per probeset. Each line corresponds to a sample, with colors blue and gray denoting one patient with and without labor, respectively. The second exon from the 5′ end targeted by Affymetrix probeset ID 2499062 (see red rectangles), shows systematically lower expression in women in labor, while the expression level for all other exons is very similar between groups, hence resulting in significantly lower FIRMA scores for this probeset between groups. The middle panel shows the genomic region and the gene model with each exon represented by one olive-colored rectangle. ENSEMBL transcripts that do or do not include the exon with differential usage are represented in blue with their corresponding identifiers.

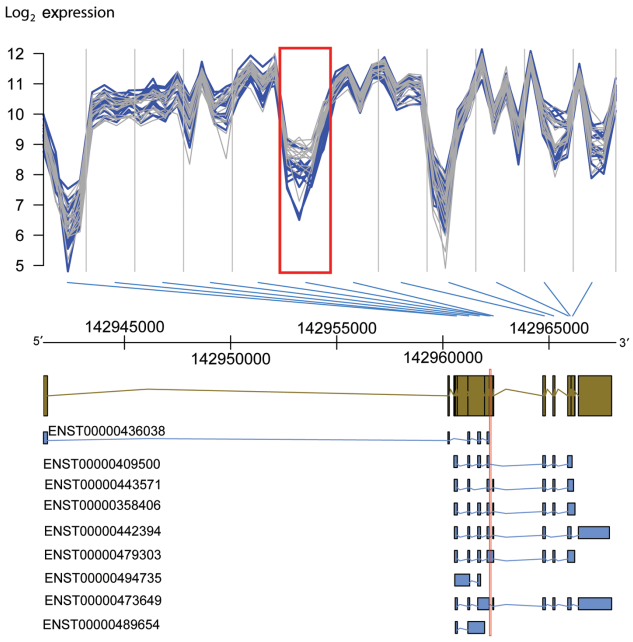

Figure 5: Differential exon usage for the *GSTK1* gene in subcutaneous adipose tissue of women with and without labor.
See Figure 3 legend for layout details. Affymetrix probeset ID 3028993 (see red rectangles), shows systematically lower expression in women in labor, while the expression level for all other exons is very similar between groups, hence resulting in significantly lower FIRMA scores for this probeset between groups. The only ENSEMBL transcript that includes the exonic region with differential usage between groups is ENST00000479303, and an imbalance of this isoform with respect to the other isoforms can explain the observed differences.

women with spontaneous labor at term. High throughput technology has been employed in obstetrics [203–208]. Specifically, the transcriptome of the uterine cervix [209–217], myometrium [12, 218–224], chorioamniotic membranes [225, 226], amniotic fluid [227–236], maternal blood [237], and umbilical cord blood [238] have been reported. Region-specific differences were extensively investigated in non-pregnant individuals using both targeted and high-dimensional biology techniques [124–142, 145, 148–151, 153, 239–242]. In contrast, previous reports concerning gene expression in adipose tissue of pregnant women have used only the targeted approach [32, 117–123, 167–176] with two exceptions [178]. Resi et al. investigated the transcriptome of subcutaneous adipose tissue obtained from the gluteal depot. Participants in that study included healthy non-obese women and healthy women not in labor [178]. This is the first report to use either a high-dimensional biological technique or a targeted approach in the investigation of fat depots during normal human labor.

Bashiri et al. [243] have determined alterations in genome-wide transcription expression in visceral and abdominal subcutaneous fat depots in obese and lean

pregnant women (four in each group) using the Affymetrix Human Exon 1.0 ST platform. The authors reported that global alteration in gene expression was identified in pregnancy complicated by obesity and the identification of indolethylamine N-methyltransferase, tissue factor pathway inhibitor-2, and ephrin type-B receptor 6 that were not previously associated with fat metabolism during pregnancy. In addition, subcutaneous fat of obese pregnant women demonstrated increased coding protein transcripts associated with apoptosis as compared to lean pregnant women. Of note, all participants in Bashiri et al. [243] were not in labor.

Comparison between the transcriptome of visceral and subcutaneous adipose tissue in pregnant women with and without spontaneous labor at term: evidence for an active role of adipose tissue response in the metabolic adaptation to parturition

An additional novel finding reported herein is the implication of alternative splicing in subcutaneous adipose tissue of pregnant women in spontaneous labor at term. Alternative splicing is a major biological process by which a relatively limited number of genes can be expended into elaborate proteomes [244]. It has been estimated that approximately two-thirds to three-quarters of all human genes undergo alternative splicing [201, 244–246]. This process allows cells to include or exclude different selective sections of pre-mRNA during RNA processing [247]. The altered transcripts result in closely related proteins expressed from a single locus [247]. The splicing process may affect function, localization, binding properties, and stability of the encoded proteins [244, 248] as well as degradation of the transcript [244, 249, 250]. It is an important regulatory mechanism that has been shown to be involved in several molecular pathways including angiogenesis and differentiation [247, 251]. To our knowledge, this is the first report implicating alternative splicing in parturition-related differences of subcutaneous adipose tissue or any other tissue.

While we did not find significant differences in gene expression between either visceral or subcutaneous adipose tissue of pregnant women with and without spontaneous labor at term, we identified three genes affected by alternative splicing: Glutathione S-transferase kappa 1 (*GSTK1*), heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa) (*HSPA5*), and LIM and senescent cell antigen-like-containing domain protein 1 (*LIMS1*). The Kappa class of glutathione S-transferases (*GSTK*) was first identified in the mitochondrial matrix from rat liver [252]. The human glutathione S-transferase kappa 1 (*GSTK1*) gene and protein were first characterized less than a decade ago [253]. Further studies of human *GSTK1-1* have confirmed its presence in mitochondria and peroxisomes [253–255]. *GSTK1-1* is highly expressed in adipose tissue, and its expression level was negatively correlated with obesity in humans and mice [256]. Importantly, *GSTK1-1* plays a critical and selective role in regulating adiponectin biosynthesis. Specifically, suppression of *GSTK1-1* inhibits adiponectin multimerization, probably by functioning as protein disulfide isomerase that regulates adiponectin disulfide bond formation, which is essential for multimerization.

Adiponectin, identified independently by four groups [257–260], is the most abundant gene *(AMP1)* product of adipose tissue; it circulates at a relatively high concentration [261]. Adiponectin has an important role in the pathophysiology of insulin resistance and diabetes [262], atherosclerosis [263], hypertension [264], dyslipidemia [265], and angiogenesis [266]. A solid body of evidence supports the role of adiponectin in normal gestation and pregnancy complications: 1) circulating maternal adiponectin correlates with insulin resistance indices during pregnancy [267]; 2) patients with gestational diabetes mellitus (GDM) have a lower concentration of adiponectin compared to those without GDM [51, 53, 268, 269]; 4) overweight pregnant patients have a lower adiponectin concentration than pregnant women of normal weight; and 5) preeclampsia is associated with altered maternal adiponectin concentrations [21, 29, 32–34, 36, 38, 45, 46]. Collectively, these findings suggest that adiponectin may play a regulatory role in metabolic and vascular complications of pregnancy. Adiponectin circulates in human plasma in distinct forms: 1) low-molecular-weight (LMW) trimers; 2) medium-molecular-weight (MMW) hexamers; and 3) high-molecular-weight (HMW) oligomers (12–18 subunits) [270]. These adiponectin multimers can exert distinct biological effects [270], activate different single transduction pathways [271, 272], and may have different affinities to the adiponectin receptors [273]. Consistent with these findings, the ratio of HMW to total adiponectin [270] has a better correlation with insulin resistance [270], obesity [274], cardiovascular diseases [275], and other impaired metabolic states [276, 277] than total adiponectin. Alterations in the relative distribution of adiponectin have been reported in normal gestation [17, 22, 26, 278, 279] as well as in preeclampsia [31, 280], gestational diabetes [52, 281], and delivery of SGA neonates [17, 71, 72, 278–281]. We have previously determined concentrations of circulating maternal adiponectin multimers in women with normal

pregnancy and in those with preterm labor, with and without intra-amniotic inflammation/infection [66]. We have found that labor, *per se*, regardless of the presence of infection/inflammation, is associated with significant quantitative and qualitative alterations in adiponectin multimers. Taken together, the results of our previous and present studies suggest that the differences in the expression of *GSTK1* in the subcutaneous adipose tissue between pregnant women with and without labor may provide a molecular mechanism for the altered regulation of adiponectin and adiponectin multimers associated with labor. This, in turn, may be important for the regulation of energy expenditure associated with parturition.

Heat shock 70 kDa protein 5 (HSPA5), also known as 78 kD glucose-regulated protein (GRP78) or immunoglobulin heavy chain-binding protein (BiP) [282, 283], is an ER-resident multifunctional molecular chaperone [284] belonging to the Hsp70 family of heat shock proteins [285]. HSPA5 is a key component of the unfolded protein response (UPR) signaling pathway that plays an important role in ER homeostasis [286]. HSPA5 increases the ER protein folding capacity by forming multiprotein complexes with other ER chaperones and regulates the activity of the ER-transmembrane sensor proteins PERK, IRE1, and ATF6 by sequestering them in inactive complexes [287, 288]. Recently, several studies proposed that increased endoplasmic reticulum stress may represent the proximal cause of the association between obesity and adipocyte insulin resistance [289–291]. Moreover, studies examining human adipose tissue have indicated that there is an increase in the ER stress transcript HSPA5 as a function of increased BMI [292, 293]. Thus, it can be hypothesized that parturition imposes increased metabolic demands and results in ER stress which, in turn, is attenuated by overproduction of HSPA5 in subcutaneous adipose tissue. Further studies are needed to test this hypothesis.

The additional gene affected by alternative splicing in subcutaneous adipose tissue of pregnant women with spontaneous labor at term is LIM and senescent cell antigen-like-containing domain protein 1 (LIMS1). LIM domain proteins contain at least one double zinc-finger motif, and they express mainly in mammalian hearts, particularly in cardiomyocytes [294]. These proteins contain between one and five LIM domains and have been implicated in the development of the heart and heart disorders. There are two members in the five-domain LIM family: LIMS1 and LIMS2. They act as adaptor proteins forming ternary complexes and participate in cell-cell, cell-matrix adhesion, migration, growth, and cell survival [295, 296]. LIMS1 and LIMS2 also function as stress sensors that enable the heart to detect mechanical stretch and respond by increasing contractile force. Other members in this large family have been implicated in the development of the heart [297–302], kidney [303–305], and liver [306] as well as in cancer [307–313] and in neurodegenerative disease [312, 314]. Interestingly, a member of the LIM family, four and a half LIM domains (FHL1), was found to be differentially expressed between visceral and omental adipose tissue in humans. To our knowledge, this report represents the first evidence that LIMS1 is expressed in human adipose tissue. Based on previous reports concerning the physiological role of this gene in other organs, it is tempting to postulate that LIMS1 is involved in the remodeling of the subcutaneous adipose tissue.

Strengths and limitations of the study

The major strengths of this study include the novel findings reported herein: 1) the implementation of a high throughput technique in the investigation of different adipose tissue depots, 2) the evaluation of paired specimens, 3) the inclusion of well-matched controls, and 4) the relatively large sample size. Our results include the first description of the transcriptome of adipose tissue – visceral and subcutaneous – in parturient women. Significant differences in alternative spliced genes were found in the subcutaneous adipose tissue between pregnant women with and without labor, implicating that alternative splicing in labor may be associated with differences in subcutaneous adipose tissue for the first time. We have identified the *LIMS1* gene, previously unrecognized, to be expressed in subcutaneous adipose tissue. Several limitations of our study should also be acknowledged. The cross-sectional nature of this study does not allow us to determine either a temporal or a causal relationship between labor and alterations in adipose tissue region-specific gene expression. In addition, as most of the participants in the study were African American, the generalization of our findings to pregnant women of different ethnic origins will require future investigation.

Conclusion

We provide evidence for the association between labor and changes in gene expression in adipose tissue. Specifically, alternative splicing has been implicated in human parturition for the first time, providing a putative molecular mechanism by which regulation of adipose tissue metabolic adaptations to the increased energy demand

associated with labor occurs. In addition, we provide evidence that human parturition is characterized by a unique pattern of adipose tissue region-specific alterations in gene expression. Collectively, our data indicate that adipose tissue may play a role in the metabolic regulation of human parturition.

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