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RESEARCH ARTICLE

Characterization of Visceral and Subcutaneous Adipose Tissue Transcriptome and Biological Pathways in Pregnant and Non-Pregnant Women: Evidence for Pregnancy-Related Regional-Specific Differences in Adipose Tissue

Shali Mazaki-Tovi[1,2]*, Edi Vaisbuch[3], Adi L. Tarca[4,5,6], Juan Pedro Kusanovic[4,7,8], Nandor Gabor Than[4,9,10], Tinnakorn Chaiworapongsa[4,11], Zhong Dong[4], Sonia S. Hassan[4,11], Roberto Romero[4,11]*

1 Department of Obstetrics and Gynecology, Sheba Medical Center, Tel Hashomer, Israel, **2** Tel Aviv University, Tel Aviv, Israel, **3** Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, Israel, **4** 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, Maryland, and Detroit, Michigan, United States of America, **5** Department of Computer Science, Wayne State University, Detroit, Michigan, United States of America, **6** Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan, United States of America, **7** Department of Obstetrics and Gynecology, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile, **8** Center for Research and Innovation in Maternal-Fetal Medicine (CIMAF), Department of Obstetrics and Gynecology, Sótero del Río Hospital, Santiago, Chile, **9** Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary, **10** First Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary, **11** Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan, United States of America

* shalimazaki@gmail.com (SMT); romeror@mail.nih.gov (RR)

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Abstract

Objective

The purpose of this study was to compare the transcriptome of visceral and subcutaneous adipose tissues between pregnant and non-pregnant women.

Study Design

The transcriptome of paired visceral and abdominal subcutaneous adipose tissues from pregnant women at term and matched non-pregnant women (n = 11) was profiled with the Affymetrix Human Exon 1.0 ST array. Differential expression of selected genes was validated with the use of quantitative reverse transcription–polymerase chain reaction.

Results

Six hundred forty-four transcripts from 633 known genes were differentially expressed (false discovery rate (FDR) <0.1; fold-change >1.5), while 42 exons from 36 genes showed

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differential usage (difference in FIRMA scores >2 and FDR<0.1) between the visceral and subcutaneous fat of pregnant women. Fifty-six known genes were differentially expressed between pregnant and non-pregnant subcutaneous fat and three genes in the visceral fat. Enriched biological processes in the subcutaneous adipose tissue of pregnant women were mostly related to inflammation.

Conclusion

The transcriptome of visceral and subcutaneous fat depots reveals pregnancy-related gene expression and splicing differences in both visceral and subcutaneous adipose tissue. Furthermore, for the first time, alternative splicing in adipose tissue has been associated with regional differences and human parturition.

Introduction

Physiological adaptations of normal pregnancy include insulin resistance,[1–3] hyperlipidemia,[4,5] and, most notably, increased fat depot.[6,7] Teleologically, these profound metabolic changes are aimed to ensure adequate nutrient supply for the rapidly growing fetus and placenta. The ephemeral nature of the pregnancy-induced metabolic alterations, as well as empirical findings,[8–11] led to the conventional view that these physiologic adaptations stem solely from the "diabetogenic" effect of the placental hormones. This concept has been challenged by a large body of evidence suggesting that adipose tissue may play a regulatory role in both normal and abnormal gestations.

During the last decade, adipose tissue has emerged as a powerful endocrine organ [12–17] that exerts autocrine, paracrine, and endocrine effects by production and secretion of highly active peptides and proteins collectively termed adipokines.[15,18] The realization that fat is an important endocrine organ that is crucial for whole-body insulin sensitivity and energy homeostasis has rekindled the scientific interest in adipose tissue in both non-pregnant and pregnant individuals. Indeed, adipokines have been implicated in physiological adaptations of normal gestation,[12,19–34] as well as in the pathophysiology of preeclampsia,[35–54] gestational diabetes mellitus,[55–72] preterm birth,[73–75] delivery of large-for-gestational-age (LGA) newborns,[76] small-for-gestational-age (SGA)[77–84] neonates, pyelonephritis,[85–87] and intrauterine infection and inflammation.[88–91] Of note is the well established association between obesity and theses complications of pregnancy.[30–33,70–72,92–117]

An emerging concept is that fat accrual in different depots is associated with different metabolic consequences.[118] Specifically, accumulation of visceral (intra-abdominal) fat is associated with a much higher risk of diabetes, dyslipidemia, accelerated atherosclerosis, and metabolic syndrome than subcutaneous fat accretion.[119–122] Although the specific mechanism(s) by which an intra-abdominal fat depot exerts its detrimental effects has not been fully elucidated, it is clear that visceral and subcutaneous adipose tissues display distinct structural and functional properties, which include: 1) the size of adipocytes is smaller in visceral than in subcutaneous adipose tissue;[123] 2) visceral fat cells have an increased β-adrenoceptor-mediated lipolysis;[124] 3) visceral adipocytes display greater responsiveness to both adrenergic receptor- and postreceptor-acting agents compared with subcutaneous adipocytes[123]; 4) gene expression of β1- and β3-adrenoceptors is higher and β2-adrenoceptor lower in the visceral cells;[124] and 5) mRNA expression[125] and secretion of important adipokines such as

leptin,[125,126] adiponectin,[126,127] and retinol-binding protein-4 (RBP4),[126] is lower in visceral compared to subcutaneous adipocytes.

The mechanisms responsible for adipose tissue depot-specific structural and functional differences are unknown. Regional variations in specific genes coding for important functional proteins[124,126–148] have led several investigators to employ high throughput techniques to identify adipose tissue depot-specific gene differences in non-pregnant individuals. [128,131,149–151] However, to date, no studies have been published on the differences in the transcriptome of visceral and subcutaneous adipose tissues between pregnant and non-pregnant women. Furthermore, to our knowledge, alternative splice variants whose expression differs between visceral and subcutaneous have not been reported in either pregnant or non-pregnant individuals.

This study was undertaken to characterize the transcriptome of visceral and subcutaneous adipose tissues during human pregnancy to gain further insight into the molecular changes that are associated with normal gestation. The aims of this study were: 1) to determine the differences between visceral and subcutaneous gene expression in non-pregnant women; 2) to characterize, for the first time, regional variations in the transcriptome of adipose tissue during normal pregnancy; 3) to determine the differences in visceral and subcutaneous gene expression between pregnant and non-pregnant women; and 4) to identify depot-specific and pregnancy-related alternative splicing alterations in adipose tissue.

Materials and Methods

Study groups

A prospective study was performed in which visceral and subcutaneous adipose tissue samples were obtained from patients in the following groups: 1) pregnant women undergoing elective cesarean section at term (n = 25); and 2) non-pregnant women undergoing elective laparotomy for conservative myomectomy (n = 11). Patients were matched according to age, parity and body mass index (BMI) at sampling for the comparison between pregnant and non-pregnant women.

The inclusion criteria for both groups were: 1) absence of medical complications; 2) no antibiotic administration prior to the sample collection; and 3) normal post-operative course. The inclusion criteria for pregnant women also included: 1) absence of obstetric complications of pregnancy; 2) normal pregnancy outcome, including an infant who was of appropriate weight for gestational age (AGA) without congenital anomalies and had Apgar scores >7 at 1 and 5 minutes; 3) absence of meconium staining of the amniotic fluid; and 4) absence of histologic chorioamnionitis.

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 were approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), U.S. Department of Health and Human Services (DHHS), Bethesda, MD, and Detroit, MI, USA), and the Human Investigation Committee of Wayne State University (Detroit, MI, USA).

Clinical definitions

Patients not in labor underwent a cesarean section secondary to a fetus in non-cephalic presentation, a previous uterine surgery or classical cesarean section, or an elective cesarean section with no more than one previous cesarean section. Only women who delivered an AGA newborn were included. Acute histologic chorioamnionitis was diagnosed using previously

described criteria.[152,153] An AGA neonate was defined as having a birth weight between the 10th and 90th percentiles for the gestational age at birth.[154] Term gestation was defined as gestational age >37 completed weeks. BMI was calculated according to the formula: weight (kg)/height (m^2). Normal weight was defined as a BMI of 18.5–24.9 kg/m^2, overweight as a BMI 25–29.9 kg/m^2, and obesity as a BMI >30 kg/m^2, according to the definitions of the World Health Organization.[155]

Sample collection

Paired visceral and subcutaneous adipose tissue samples were obtained after an eight-hour fast. 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. [127,156–159] 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 were kept at –80°C until use.

RNA isolation

Total RNA was isolated from snap-frozen adipose tissue samples using TRI Reagent® (Ambion®, Life Technologies Corporation, Austin, TX, USA) 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 ratios were assessed using a NanoDrop® 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA integrity numbers were determined using the Agilent Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE, USA).

Microarray analysis and quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR)

The Affymetrix GeneChip Human Exon 1.0 ST array (Affymetrix, Santa Clara, CA, USA) platform was used to measure the expression levels in each unpooled specimen, according to the manufacturer's instructions. The array contains approximately 5.4 million 5-μm features (probes) grouped into 1.4 million probesets interrogating more than one million exon clusters. [160–162] To verify the results from microarray, 53 genes were selected for qRT-PCR assays from the original sample set (n = 11). A detailed description of the method and analysis is available as supplementary material (S1 File. Supplementary methods).

Statistical analyses

The raw gene expression data were preprocessed using Robust Multi-array Average (RMA). [163] A paired moderated *t* test[164] was used to test for differential expression with a false discovery rate (FDR)[165] threshold of 0.1 in conjunction with a threshold of 1.5 on the fold-change to assign gene significance.[166] Differential exon usage was tested using the FIRMA (Finding Isoforms Using Robust Multichip Analysis) method [167] adapted for multiple samples as described in the Supplementary methods (S1 File). Gene Ontology analysis was conducted with algorithms that were described previously.[168] Pathway analysis was performed on the Kyoto Encyclopedia of Genes and Genomes (KEGG)[169] pathway database with an overrepresentation analysis[170] Assessment of differential expression between experimental regions from qRT-PCR data was performed with a paired *t* test on –ΔCt values. The Student *t*, Mann-Whitney *U*, and X^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) was used for statistical analysis of the 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.

Results of the microarray analysis

Pregnant women: visceral versus subcutaneous. Microarray analysis demonstrated 644 transcripts that corresponded with 633 unique known genes that were differentially expressed between visceral and subcutaneous adipose tissue of pregnant women at term (q-value <0.1; fold change >1.5). A total of 391 genes had decreased expression, and 242 genes had increased expression in the subcutaneous, compared with visceral adipose tissue. A "volcano plot" shows the differential expression of all the transcripts tested in this comparison, with the log (base 10) of the FDR-adjusted probability values (q-value) (y-axis) plotted against the log (base 2) fold-changes (x-axis) between the visceral and subcutaneous adipose tissues (Fig 1). The heat map in Fig 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 10 differentially expressed transcripts between visceral and subcutaneous adipose tissues is presented in Table 2; the complete list of differentially expressed transcripts is available as supplementary material (S1 Table).

Gene ontology meta-analysis of the significantly up- and down-regulated genes was performed to identify gene ontology terms that were represented by the differentially expressed genes. In this analysis, 82 biological processes were enriched (q-value <0.05); the top 10 biological processes are presented in Table 3; the complete list of differentially expressed transcripts is available as supplementary material (S2 Table). Pathway analysis of the significant genes was undertaken with an overrepresentation method resulting in 12 KEGG pathways were significant (q-value <0.05) in the comparison between visceral and subcutaneous adipose tissues

Table 1. Demographic and clinical characteristics.

	Pregnant N = 25	Non-Pregnant N = 11	p
Maternal age (years)	34 (33–42)	32 (28–40)	0.6
BMI at Sampling (kg/m2)	30.4 (27.5–38.8)	31.1 (26.2–39.1)	0.9
Gravidity	4 (3–5)	4 (3–7)	0.7
Parity	3 (2–4)	3 (2–4)	0.6
Ethnic Origin (%)			0.5
African American	90.9	81.8	
Caucasian	9.1	18.2	
Systolic Blood Pressure (mmHg)	117 (114–120)	114 (111–117)	0.6
Diastolic Blood Pressure (mmHg)	74 (69–79)	66 (63–81)	0.4
Fasting Glucose (mg/dl)	87 (80–89)	88 (73–90)	0.3
Gestational Age at Delivery (weeks)	39.1 (38–39.3)	NA	NA
Birth Weight (grams)	3335 (2980–3555)	NA	NA

Data are presented as median and interquartile range (IQR). BMI—Body Mass Index.

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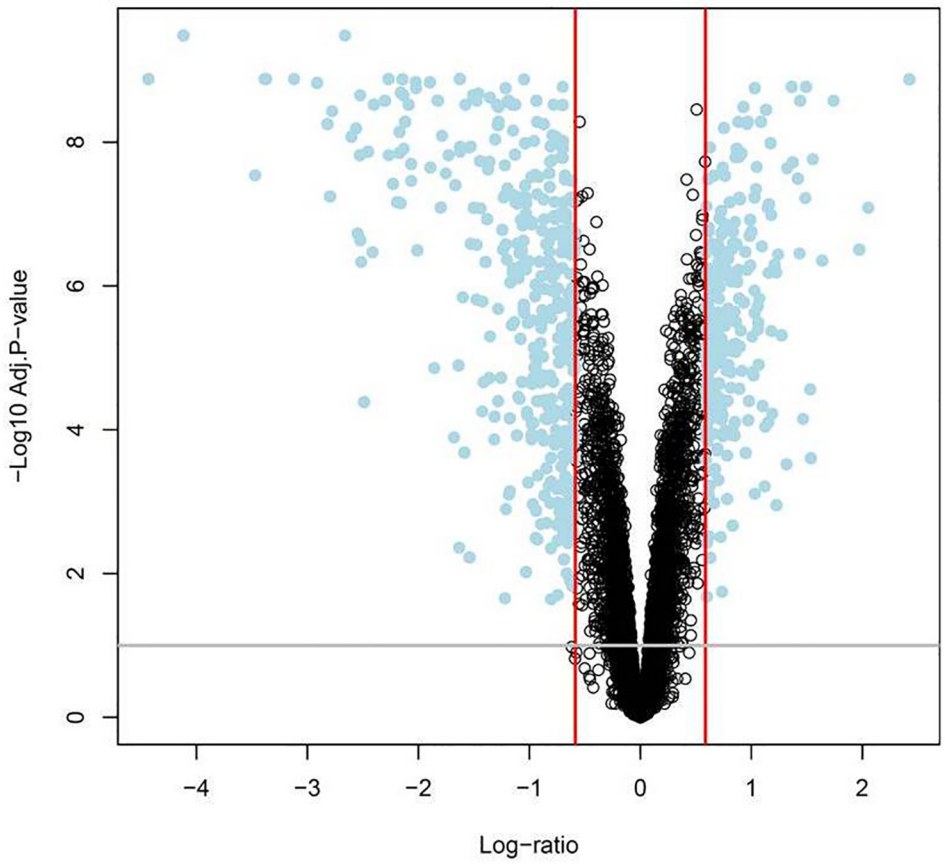

Fig 1. Pregnant women differential expression of visceral versus subcutaneous adipose tissue transcripts. A "volcano plot" shows the differential expression of all the transcripts tested in this comparison, with the log (base 10) of the FDR-adjusted probability values (q-value) (y-axis) plotted against the log (base 2) fold-changes (x-axis) between the visceral and subcutaneous adipose tissues.

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(Table 4); the complete list of differentially expressed transcripts is available as supplementary material (S3 Table). The five most significant pathways include: 1) the ECM-receptor interaction (S1 Fig); 2) the PPAR signaling pathway (S2 Fig); 3) protein digestion and absorption (S3 Fig); 4) focal adhesion; and 5) complement and coagulation cascades.

Non-pregnant women: visceral versus subcutaneous. Microarray analysis demonstrated significant changes in the transcriptome of visceral and subcutaneous adipose tissues of non-pregnant women. In total, 226 unique genes were differentially expressed (q-value <0.1; fold-change >1.5). A total of 147 genes had decreased expression, and 79 genes had increased expression in the subcutaneous, compared with visceral, adipose tissue. A list of the top 10 differentially expressed genes between visceral and subcutaneous adipose tissues is presented in Table 5; the complete list of differentially expressed transcripts is available as supplementary material (S4 Table).

Enrichment analyses identified 26 biological processes and five KEGG pathways that were significantly enriched in differentially expressed genes (q-value <0.05) in the comparison between visceral and subcutaneous adipose tissues. A list of the 10 enriched biological processes in the comparison between visceral and subcutaneous adipose tissues of non-pregnant women is presented in Table 6; the complete list is available as supplementary material (S5 Table). The significant pathways were: 1) complement and coagulation cascades; 2)

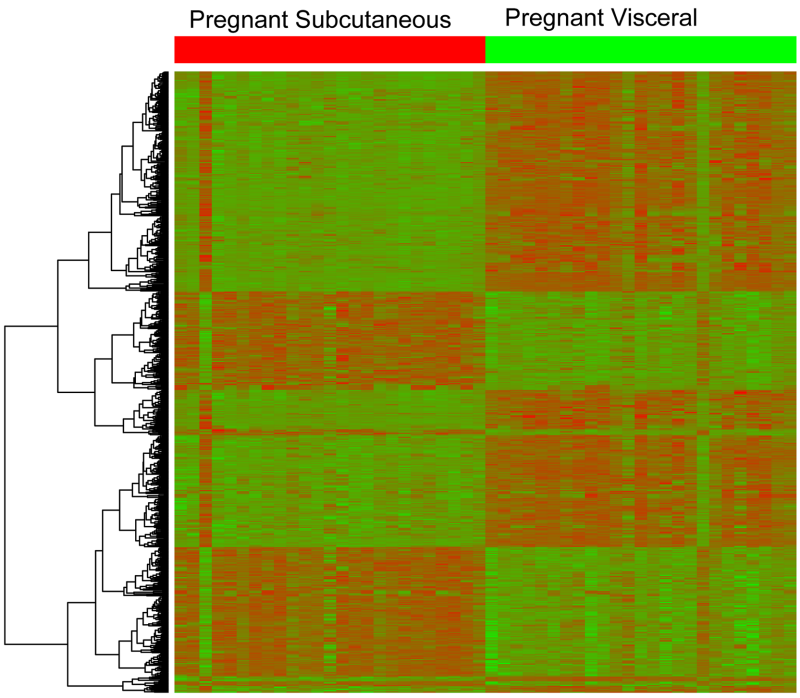

Fig 2. Heat map representing fat depot-specific differences in gene expression of pregnant women. The heat map uses a color scale to show the consistency of the expression levels within each group of samples as well as the differences between the groups that led to positive test results.

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Staphylococcus aureus infection; 3) prion diseases; 4) Chagas disease (American trypanosomiasis); and 5) retinol metabolism.

Pregnant versus non-pregnant women: subcutaneous adipose tissue. Microarray analysis demonstrated significant changes in the transcriptome of subcutaneous adipose tissue between pregnant and non-pregnant women. In total, 57 transcripts corresponding to 56 known genes were differentially expressed (q-value <0.1; fold-change >1.5). A total of 19 genes

Table 2. A list of the top 10 differentially expressed transcripts between visceral and subcutaneous adipose tissues of pregnant women.

ENTREZ ID	SYMBOL	Gene Name	Fold Change*
55600	ITLN1	intelectin 1 (galactofuranose binding)	-21.60
9076	CLDN1	claudin 1	-17.33
3250	HP	haptoglobin	-11.08
93035	PKHD1L1	polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	-10.45
244	ANXA8	annexin A8	-10.35
642826	ANXA8L2	annexin A8-like 2	-8.68
246	ALOX15	arachidonate 15-lipoxygenase	-7.52
10950	CXADR	coxsackie virus and adenovirus receptor	-7.06
3240	HPR	haptoglobin-related protein	-6.94
100294156	C4B	complement component 4B (Chido blood group)	-6.86

(*) The fold change represents the number of times the average expression in one group is higher than the one in the other group. Positive values mean higher expression in subcutaneous compared to visceral tissues, while negative values represent higher expression in visceral compared to subcutaneous tissues. Genes are ranked by absolute fold change. All q-values < 0.01.

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Table 3. A list of top 10 enriched biological processes in the comparison between visceral and subcutaneous adipose tissues of pregnant women.

Biological process	Genes in differentially expressed list, n	Genes in reference array, n	Odds Ratio	q-value
circulatory system development	85	657	3.43	0.000
multicellular organismal process	300	4412	2.15	0.000
localization of cell	83	765	2.77	0.000
response to wounding	86	905	2.37	0.000
retinal metabolic process	7	8	144.91	0.000
regulation of inflammatory response	19	103	4.83	0.000
multicellular organismal development	121	1894	1.80	0.000
cell adhesion	34	306	2.88	0.000
positive regulation of cellular component movement	28	212	3.23	0.000
locomotion	33	301	2.86	0.000

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Table 4. A list of the 10 KEGG pathways that were significant in the comparison between visceral and subcutaneous adipose tissues of pregnant women.

Map Name	Genes in differentially expressed list, n	Genes in reference array, n	Odds Ratio	q-value
ECM-receptor interaction	16	72	5.55	0.000
PPAR signaling pathway	12	55	5.34	0.001
Protein digestion and absorption	13	64	4.89	0.001
Focal adhesion	23	171	3.05	0.001
Complement and coagulation cascades	11	49	5.52	0.001
Cytokine-cytokine receptor interaction	23	195	2.61	0.004
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	11	63	4.02	0.007
Cell adhesion molecules (CAMs)	14	103	3.01	0.015
Malaria	8	40	4.71	0.015
Steroid hormone biosynthesis	6	28	5.11	0.039

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Table 5. A list of the top 10 differentially expressed transcripts between visceral and subcutaneous adipose tissues of non-pregnant women.

ENTREZ ID	SYMBOL	Name	Fold Change*	q-value
55600	ITLN1	intelectin 1 (galactofuranose binding)	-11.5	0.09
9076	CLDN1	claudin 1	-8.3	0.09
730	C7	complement component 7	-5.0	0.09
244	ANXA8	annexin A8	-5.0	0.09
93035	PKHD1L1	polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1	-4.6	0.09
100294156	C4B	complement component 4B (Chido blood group)	-4.4	0.09
642826	ANXA8L2	annexin A8-like 2	-4.3	0.09
5999	RGS4	regulator of G-protein signaling 4	-4.1	0.09
246	ALOX15	arachidonate 15-lipoxygenase	-3.9	0.09
7980	TFPI2	tissue factor pathway inhibitor 2	-3.9	0.09

(*) The fold change represents the number of times the average expression in one group is higher than the one in the other group. Positive values mean higher expression in subcutaneous compared to visceral tissues, while negative values represent higher expression in visceral compared to subcutaneous tissues. Genes are ranked by absolute fold change.

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Table 6. A list of the 10 enriched biological processes in the comparison between visceral and subcutaneous adipose tissues of non-pregnant women.

Biological process	Genes in differentially expressed list, n	Genes in reference array, n	Odds Ration	q-value
complement activation, classical pathway	7	23	26.80	0.0001
protein activation cascade	8	45	13.37	0.001
cell adhesion	25	515	3.45	0.001
retinol metabolic process	5	14	33.68	0.002
retinal metabolic process	4	8	60.31	0.003
single-multicellular organism process	83	3703	2.02	0.004
localization of cell	30	765	2.68	0.004
circulatory system development	27	657	2.79	0.005
cellular response to cAMP	5	22	17.82	0.009
developmental process	59	2336	2.02	0.010

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had decreased expression, and 37 genes had increased expression in the subcutaneous adipose tissue of pregnant compared with non-pregnant women. A "volcano plot" shows the differential expression of all tested transcripts, with the log (base 10) of the FDR-adjusted probability values (y-axis) plotted against the log (base 2) fold-changes (x-axis) between pregnant and non-pregnant groups (Fig 3). The heat map in Fig 4 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 the positive test results. A list of the top 10 differentially expressed transcripts in the

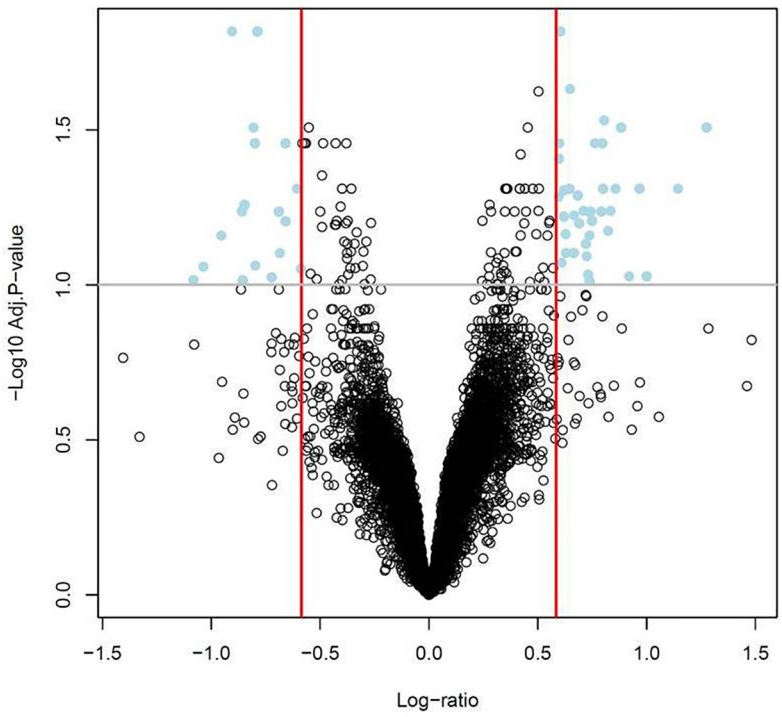

Fig 3. Differential expression of subcutaneous adipose tissue transcripts in pregnant vs. non-pregnant women. A "volcano plot" shows the differential expression of all tested transcripts, with the log (base 10) of the FDR-adjusted probability values (y-axis) plotted against the log (base 2) fold-changes (x-axis) between pregnant and non-pregnant groups.

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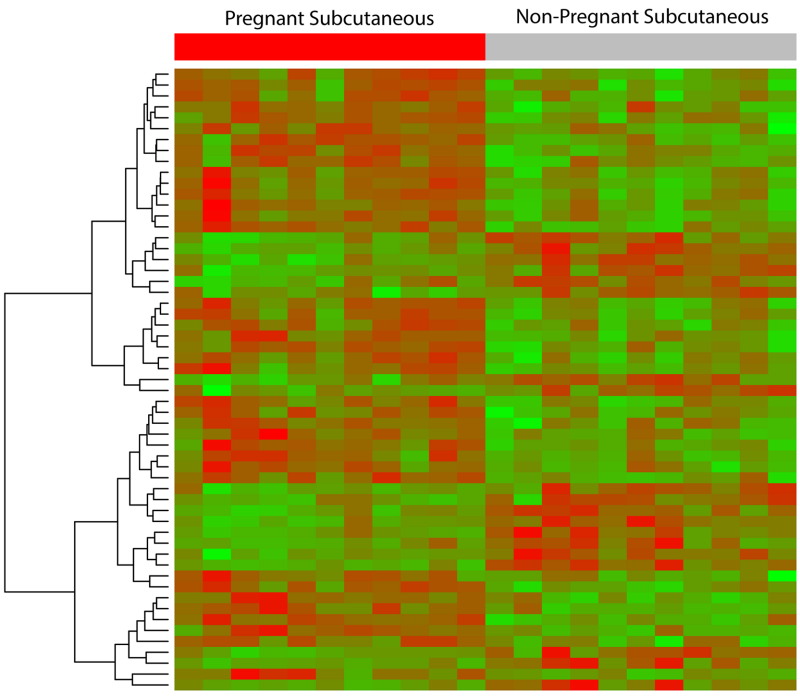

Fig 4. Heat map representing subcutaneous adipose tissue differences in gene expression of pregnant vs non-pregnant women. The heat map in Fig 4 uses a color scale to show the consistency of the expression levels within each group of samples as well as the differences between the groups that led to the positive test results.

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subcutaneous adipose tissues between the pregnant and non-pregnant women is presented in Table 7; the complete list is available as supplementary material (S6 Table).

Gene ontology meta-analysis revealed four biological processes that were enriched at q-value <0.05: 1) complement activation, classical pathway; 2) protein activation cascade; 3) immunoglobulin-mediated immune response; and 4) humoral immune response. Pathway

Table 7. A list of the 10 differentially expressed transcripts in the subcutaneous adipose tissues between the pregnant and non-pregnant women.

ENTREZ ID	SYMBOL	Gene Name	Fold Change*	q-value
729	C6	complement component 6	2.4	0.031
81617	CAB39L	calcium binding protein 39-like	2.2	0.049
81575	APOLD1	apolipoprotein L domain containing 1	-2.1	0.097
390075	OR52N5	olfactory receptor, family 52, subfamily N, member 5	-2.1	0.087
643616	MOP-1	MOP-1	2.0	0.094
11326	VSIG4	V-set and immunoglobulin domain containing 4	2.0	0.049
79473	OR52N1	olfactory receptor, family 52, subfamily N, member 1	-1.9	0.069
51338	MS4A4A	membrane-spanning 4-domains, subfamily A, member 4	1.9	0.094
3099	HK2	hexokinase 2	-1.9	0.015
10149	GPR64	G protein-coupled receptor 64	1.8	0.031

(*) The fold change represents the number of times the average expression in one group is higher than the one in the other group. Positive values mean higher expression in subcutaneous tissues of pregnant compared to non-pregnant women, while negative values represent higher expression subcutaneous tissues of non-pregnant women compared to pregnant women. Genes are ranked by absolute fold change.

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Table 8. A list of the 3 differentially expressed transcripts in the visceral adipose tissues between the pregnant and non-pregnant women.

SYMBOL	ENTREZ ID	Name	Fold Change	q-value
CA1	759	carbonic anhydrase I	2.4	0.07
GPR64	10149	G protein-coupled receptor 64	2.0	0.05
CXorf21	80231	chromosome X open reading frame 21	1.5	0.05

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analysis identified two KEGG pathways that were significantly enriched (q-value,<0.05) in the comparison between the subcutaneous adipose tissue samples of pregnant and non-pregnant women: 1) complement and coagulation cascades (S4 Fig), and 2) Prion diseases (S5 Fig).

Pregnant versus non-pregnant women: visceral adipose tissue. Microarray analysis demonstrated significant changes in the transcriptome of visceral adipose tissue between pregnant and non-pregnant women. In total, three unique genes had increased expression in the visceral adipose tissue of pregnant compared with non-pregnant women (Table 8).

Alternative splicing. The Affymetrix Human Exon 1.0 ST array that we used in this study allowed us to test for differential exon usage (a.k.a. differential/alternative splicing) in addition to the differential expression analysis described above. Significant differences in exon usage rates were found between visceral and subcutaneous adipose tissues of pregnant women and between pregnant and non-pregnant women in the subcutaneous region. Forty-two alternative splicing events in 36 unique genes were associated with the regional differences of the adipose tissue of pregnant women. For six of the 36 affected genes, the evidence for differential splicing was found for two distinct Affymetrix probesets that either targeted the same exon of the gene [peptidase domain containing associated with muscle regeneration 1 (*PAMR1*) and serine/arginine repetitive matrix 2 (*SRRM2*)] or different exons of the same gene [kinase non-catalytic C-lobe domain (KIND) containing 1 (*KNDC1*), the podocalyxin-like (*PODXL*), solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 (*SLC7A8*), and desmin (*DES*)]. A list of the top 10 alternative splicing events associated with the regional differences of the adipose tissue of pregnant women is presented in Table 9; the complete list is available as supplementary material (S7 Table). Fifty percent of the genes that were affected by alternative splicing were also differentially expressed.

Table 9. A list of the alternative splicing events associated with the regional differences of the adipose tissue of pregnant women.

Symbol	Gene Name	Transcript ID	Exon ID	Probeset ID	Diff. mean FIRMA	q-value
ABLIM1	actin binding LIM protein 1	3307939	619043	3308001	2.851	0.000
ADRA2C	adrenergic, alpha-2C-, receptor	2716328	249900	2716338	-2.288	0.004
CRB2 #	crumbs homolog 2 (Drosophila)	3188478	544486	3188501	-2.842	0.000
DAPK1 #	death-associated protein kinase 1	3177880	538110	3177903	3.061	0.000
DCLK1 #	doublecortin-like kinase 1	3509473	742860	3509602	-3.042	0.000
DES*	desmin	2528476	131889	2528483	-2.660	0.007
DES*	desmin	2528476	131895	2528491	-2.291	0.003
FAIM3	Fas apoptotic inhibitory molecule 3	2452977	84252	2452981	-2.944	0.000
GATA6 #	GATA binding protein 6	3781245	908232	3781284	-2.220	0.000
GFPT2#	glutamine-fructose-6-phosphate transaminase 2	2890660	359171	2890703	2.247	0.000

*Genes showing differential exon usage for two exons

Genes demonstrating differential exon usage that were also differentially expressed

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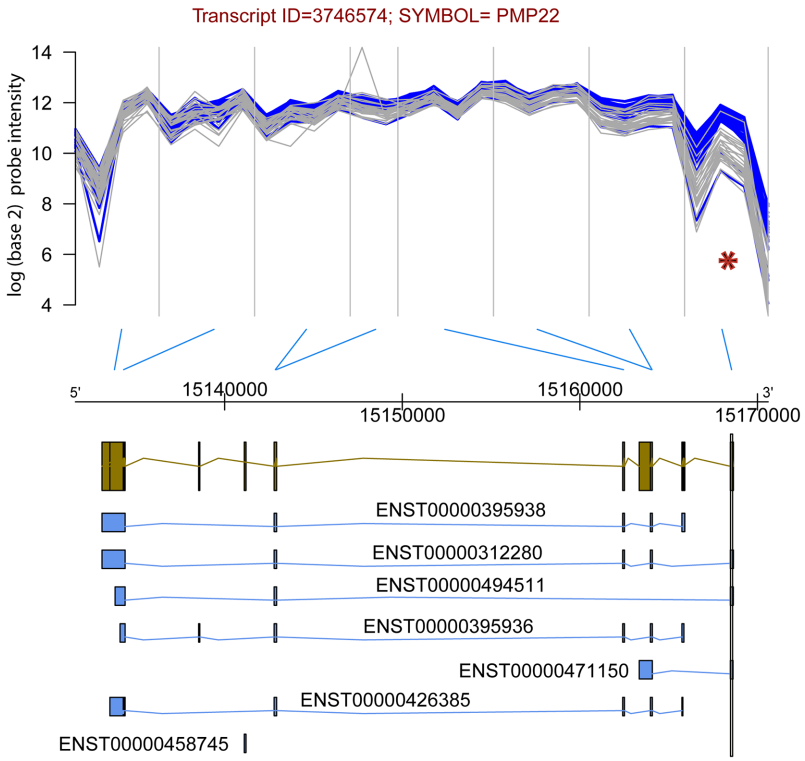

Fig 5. Normalized probe expression data against the genomic coordinates for gene PMP22. The figure shows the normalized probe intensity for gene PMP22 plotted as a function of the genomic coordinates from the 5' to the 3' end. Each line corresponds to one sample (blue: subcutaneous and grey: visceral). The probesets, each containing 4 probes, are separated by vertical grey lines. The ideogram under the genomic axis shown in gold color represents the gene model, with each vertical rectangle denoting one exon. Under the gene model are depicted known ENSEMBL database transcripts (in blue) that either include or exclude the exon that shows differential usage between groups, and which is highlighted with a vertical rectangle across all ideograms. For most probes used to target this gene, the expression level is about the same in the visceral and subcutaneous samples, except for the probes targeting the last exon for which the expression in the subcutaneous (blue) group, the expression is consistently higher.

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For an illustration of the differential exon usage between the visceral and subcutaneous adipose tissues of pregnant women see Fig 5 which depicts the normalized probe expression data against the genomic coordinates for gene PMP22. The figure shows the normalized probe intensity for gene PMP22 plotted as a function of the genomic coordinates from the 5' to the 3' end. Each line corresponds to one sample (blue: subcutaneous and grey: visceral). The probesets, each containing 4 probes, are separated by vertical grey lines. The ideogram under the genomic axis shown in gold color represents the gene model, with each vertical rectangle denoting one exon. Under the gene model are depicted known ENSEMBL database transcripts (in blue) that either include or exclude the exon that shows differential usage between groups, and which is highlighted with a vertical rectangle across all ideograms. For most probes used to target this gene, the expression level is about the same in the visceral and subcutaneous samples, except for the probes targeting the last exon for which the expression in the subcutaneous (blue) group, the expression is consistently higher. As a consequence the FIRMA scores, indicative of the exon usage, are significantly higher in the subcutaneous than in the visceral samples.]

Differential splicing associated with labor was found for three genes in the subcutaneous adipose tissue, namely LIMS1 (LIM and senescent cell antigen-like domains 1), YY1AP1 (YY1

associated protein 1) and GSTK1 (glutathione S-transferase kappa 1). S6 Fig (see legend of Fig 5 for details) shows that for one of the middle exons of the gene GSTK1, the usage rate is lower in the labor group than in the not in labor group, while this is not the case for any other of the exons. Such a phenomenon can be explain for instance by an imbalance in the abundance of the isophorm that includes the exon (ENSEMBLE ID: ENST00000479303) and the remaining ones documented in the ENSEMBLE database which skip this exon.

We did not find significant differences in exon usage rates between pregnant and non- pregnant women in the visceral adipose tissue.

Discussion

Visceral versus subcutaneous adipose tissue in pregnant women

To our knowledge, this is the first study that describes the transcriptome of visceral and subcutaneous adipose tissues in pregnant women. Previous reports regarding gene expression in adipose tissue of pregnant women have used a targeted approach.[171–189] High-dimensional biology techniques allow comprehensive and unbiased insight into complex physiologic events including the investigation of the reproductive tract.[190–202] Using high-throughput techniques, differential gene and protein expressions have been reported in pregnant women in the uterine cervix,[203–211] human myometrium,[212–218] chorioamniotic membranes,[219,220] amniotic fluid,[221–230] and umbilical cord blood.[231] While region-specific differences were extensively investigated in non-pregnant individuals using both targeted and high-dimensional biology techniques,[118–120,128,130,131,149–151,232–253] the comprehensive gene expression, biological processes, and pathways associated with gestational adiposity have not yet been described. We used an unbiased approach to characterize the transcriptome of visceral and subcutaneous adipose tissues in pregnant and non-pregnant women to gain an understanding of pregnancy-related global changes in adipose tissue depot-specific gene expression and splicing.

The findings of the present study provide evidence for pregnancy-associated differences between visceral and subcutaneous adipose tissues. Compared with non-pregnant women, the magnitude of regional differences in the transcriptome of pregnant women was larger, with the fold changes of the top ten genes ranging from 6.9 to 21.6 in pregnant women compared to 4.6 to 11.5 in non-pregnant women. These findings suggest that adipose tissue depot-specific differences in gene expression are more accentuated in human gestation. Remarkably, among the top five differentially expressed genes between visceral and subcutaneous adipose tissues, four are common for both pregnant and non-pregnant women. These genes encode for omentin (intelectin 1), claudin 1, polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1, and annexin A8. Consistent with this finding, among the top 100 differentially expressed genes between visceral and subcutaneous adipose tissues, 53 are common for both pregnant and non-pregnant women, suggesting that many of the depot-specific alterations are common for these groups yet the magnitude of changes is higher in pregnant women.

Omentin (also named intelectin) is a secretory protein that has been recently identified as a new depot-specific adipokine.[254–256] Several lines of evidence support the highly selective expression of omentin in human visceral adipose tissue: 1) omentin mRNA was predominantly expressed in visceral compared with subcutaneous fat;[255,257–259] 2) qRT-PCR demonstrated that omentin mRNA was expressed in stromal vascular cells, isolated from omental adipose tissue, with more than 150-fold less in subcutaneous cell fractions;[255] and 3) consistent with these findings, omentin was detected in a culture medium of omental, but not of subcutaneous, fat explants.[255] Importantly, qRT-PCR has demonstrated that omentin is expressed in stromal vascular cells but not in adipocytes.[255] Our results confirm that there is a

significant over-expression of omentin in visceral compared to subcutaneous adipose tissue. We were able to extend the abovementioned reports by demonstrating over-expression of this adipokine in visceral fat of pregnant women. Furthermore, while the fold-change increase of omentin expression in visceral adipose tissue was 11.5 in the non-pregnant state, during pregnancy the fold-change was approximately twice as high (21.6), suggesting that this adipokine may play a role in the metabolic adaptations of visceral fat to normal gestation.

Omentin enhances insulin-stimulated glucose uptake in human adipocytes and triggers AKT signaling.[255,256] This adipokine does not stimulate basal glucose transport on its own, indicating that omentin has no intrinsic insulin-mimic activity. Thus, it has been suggested that depot-dependent insulin action is subject to modulation by this adipokine. Omentin has been detected in maternal circulation;[188,260,261] however, how omentin concentrations change during pregnancy is not yet known. It can be speculated that the insulin-sensitizing effect of this insulin-sensitizing hormone may be of special importance during pregnancy in order to balance the "diabetogenic" effect of several placental hormones. Alterations in omentin expression and/or secretion may also account for the association between maternal obesity and complications of pregnancy. Indeed, pre-existing maternal obesity is associated with lower omentin-1 expression in adipose tissue and maternal plasma.[188] In conclusion, the dramatic over-expression of omentin in visceral adipose tissue during pregnancy may point to a regulatory role of this adipokine in the depot-dependent insulin action that may alleviate the pregnancy-related insulin resistance.

Gene ontology and pathway analysis revealed functional categories associated with the adipose tissue depot-specific expression changes in human pregnancy. Specifically, the biological process related to extracellular matrix–receptor interactions, inflammation, metabolism, and tissue development characterized the pregnancy-related adipose tissue depot-specific alterations. The extracellular matrix–receptor interaction and PPAR signaling pathway were among the most impacted signaling pathways in the visceral adipose tissue of pregnant women. Interestingly, the extracellular matrix–receptor interaction pathway was also the most impacted signaling pathway at the site of rupture in the chorionic membranes[220] and has been implicated in cervical ripening before the onset of labor at term in human pregnancy.[205] Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that are activated by fatty acids and their derivatives. PPARs are abundantly expressed in adipose tissue and central to the regulation of pre-adipocyte differentiation through transcriptional control of adipocyte-specific genes.[234,262,263] PPARs include three subtypes: PPAR -α, -β/δ, and -γ, and each is encoded in a separate gene.[264] PPAR-γ is considered the master adipogenic regulator,[263,265] and it has an essential role in maintaining mature adipocyte function.[265–267] This is of special importance as human pregnancy is characterized by increased accumulation of visceral fat. Indeed, using longitudinal ultrasound measurements, a significant increase in intraabdominal to subcutaneous ratio was observed during the third trimester when compared with the first and second trimesters.[268] Accordingly, parity is associated with increased abdominal fat retention for months and years after delivery.[269–271] Taken together, our findings suggest that, during pregnancy, biological processes aimed at the expansion and development of both components of adipose tissue, i.e., the adipocytes and stromal vascular cells, are activated in visceral adipose fat. Thus, the results of this study may provide a putative molecular mechanism(s) by which pregnancy-associated increase and expansion of fat accrual occurs.

Bashiri et al[272] have determined alterations in genome-wide transcription expression in visceral and abdominal subcutaneous fat deposits in obese and lean pregnant women (4 in each group) using 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. Several of the top 36 candidate genes with the greatest variation in expression between subcutaneous and omental fat in both normal and obese pregnant women reported in the study of Bashiri et al. [272] were also found to be differentially expressed in the present study including NTNG1, KCNT2, LYPD6 and others.

Pregnant versus non-pregnant states: gestational-related alterations in the subcutaneous and visceral adipose tissue transcriptome

To our knowledge, there has been no report comparing the visceral and subcutaneous adipose tissue transcriptome between pregnant and non-pregnant women. We report herein that 57 and three genes were differentially expressed between pregnant and non-pregnant women in subcutaneous and visceral adipose tissues, respectively. Intriguingly, the gene encoding G protein-coupled receptor 6 (*GPR64*) was over-expressed in both subcutaneous and visceral adipose tissues of pregnant women. GPR64 (also known as HE6—human epididymis-specific protein 6) is an orphan member of the LNB-TM7(B2) subfamily of G-protein-coupled receptors.[273–276] GPR64 mRNA is highly expressed in the epithelia of ductuli efferentes and proximal epididymis.[273–278] It has been suggested to function in the control of water balance and fluid reabsorption in the male excurrent ducts.[279–281] qRT-PCR studies with numerous tissue probes from mouse, rat, and human specimens as well as microarray analyses of essentially all human tissues and organs revealed a highly epididymis-restricted expression of HE6.[275] Thus, to our knowledge, this report represents the first evidence that GPR64 is expressed in human adipose tissue. The biological importance of GPR64 in adipose tissue, and specifically during gestation, has to be evaluated. HE6/GPR64 is an 'orphan' member of the adhesion GPCRs,[274–276] and an endogenous ligand(s) is presently unknown. Moreover, ligand prediction for GPCRs is extremely difficult since ligands for GPCRs are associated with remarkable variation.[275]

The findings of this study indicate that three out of four biological processes enriched in subcutaneous fat during pregnancy (i.e. complement activation, classical pathway, immunoglobulin mediated immune response and humoral immune response) are related to inflammation. This finding is consistent with a large body of evidence indicating that adipose tissue can orchestrate an inflammatory response, including: 1) knockout mice for IL-6,[282] TNF-α,[283] PAI-1,[284] IL-18,[285] IL-1α,[286] MCP-1,[287] and JNK1[288] are often obese or have a metabolic phenotype related to obesity; 2) adipose tissue is an important site for the production of inflammatory mediators including TNF-α,[13,289–291] IL-6,[292–294] monocyte chemoattractant protein (MCP)-1,[295,296] C-reactive protein (CRP),[23,24,293,297–299] serum amyloid A,[300] and plasminogen activator inhibitor-1 (PAI-1);[301] 3) adipocytokines such as resistin,[302–305] visfatin,[306–308] and adipsin[309] have been implicated in the regulation of the innate immune responses, and leptin[310–315] and adiponectin[316–322] are involved in the regulation of both innate and adaptive limbs of the immune system; and 4) obesity is associated with high-circulating pro-inflammatory and acute phase reactant adipocytokines such as TNF-α,[323] IL-6,[294] and CRP.[293] Normal pregnancy is considered a pro-inflammatory state. The total white blood cell count in maternal blood increases with advancing gestational age and leukocytes derived from normal pregnant women are phenotypically and metabolically activated.[324,325] During pregnancy, there is also an increased circulating concentration of acute phase proteins. Of note, a growing body of evidence suggests that

normal gestation is characterized by adipose tissue inflammation,[326]and this process seems to be accentuated toward the end of the pregnancy[327,328]. Collectively, our data suggest that pregnancy-related subcutaneous adipose inflammation may contribute to the generalized pro-inflammatory state that characterizes human gestation.

Resi V et al[329] have recently reported the results of a longitudinal study in which adipose tissue biopsies were obtained from the subcutaneous gluteal depot of healthy non-obese women who are in early (8–12 weeks of gestation) and late (36–38 weeks of gestation) pregnancy. Specimens obtained via liposuction were subjected to histologic examination and gene expression analysis using DNA microarray. The findings of our study are in agreement with the above-mentioned report[329] in which approximately 40% of pregnancy-associated changes were related to mediators of the immune response, extracellular matrix components. [329] In addition, comparison between pre-conception and early pregnancy gene expression revealed marked changes in genes regulating pathways for the inflammatory response and metabolism.[329] In contrast to the report by Resi V et al,[329] in which 26% of pregnancy-associated changes were related to lipid metabolism, we did not identify this biological process as significantly enriched in genes differentially expressed between pregnant and non-pregnant women. Several explanations can account for this discrepancy: 1) the methods by which specimens were obtained were different between the studies; and gene expression, as determined by microarray experiments, has been shown to be affected by the adipose tissue biopsy technique; [245] 2) subcutaneous adipose tissue gene expression varies as a function of the specific region from which specimens were obtained and 3) the microarray platforms used were different. A recent study has shown that a total of 2,890 transcripts were differentially expressed between four subcutaneous adipose depots: upper abdomen, lower abdomen, flank, and hip in normal weight women;[330] and 3) it has been proposed that gluteal and femoral adipocytes serve as energy stores during pregnancy and lactation to meet the increased need for energy during that time.[269,331] This view is supported by studies in humans demonstrating that the activity of lipoprotein lipase in adipocytes from the femoral region increases during pregnancy, whereas such a pattern was not detected for abdominal adipocytes.[331] Thus, different gene expression patterns among subcutaneous adipose depots (e.g. abdominal versus gluteal) may represent their diverse function during normal human pregnancy.

Alternative splicing: a novel pregnancy-associated mechanism for the regional differences between visceral and subcutaneous adipose tissues

Alternative splicing of mRNA transcripts is the process by which cells can selectively include or exclude different sections of pre-mRNA during RNA processing.[332] Once translated, these altered transcripts result in closely related proteins expressed from a single locus. [332,332,333] Alternative splicing is a major biological process by which a relatively limited number of genes can be expended into elaborate proteomes.[334] It has been estimated that approximately two-thirds to three-quarters of all human genes undergo alternative splicing. [334–337] The splicing process may affect function, localization, binding properties, and stability of the encoded proteins.[334,338] Moreover, alternative splicing can also lead to degradation of the transcript.[334,339,340] It is an important regulatory mechanism that has been shown to be involved in several molecular pathways including angiogenesis and differentiation.[332,341]

To the best of our knowledge, this is the first report implicating alternative splicing in regional differences of adipose tissue either in pregnant or non-pregnant individuals. We identified 42 exons in 36 genes showing differential usage in the comparison between visceral and

subcutaneous adipose tissues. Of note, significant results were found only for the comparison between adipose tissue depots of pregnant women, but not for non-pregnant individuals. This finding characterized pregnancy as a unique physiologic condition in which alternative splicing may account for the different biological functions of visceral versus subcutaneous adipose tissue. It can be postulated that the remodeling and adaptations of adipose tissue during gestation require a larger repertoire of proteins that can be achieved by alternative splicing.

Strengths and limitations of the study

The major strengths of this study are novel findings reported herein, the employment of a high throughput technique in the investigation of adipose tissue during pregnancy, the evaluation of paired specimens, and the inclusion of well-matched, non-pregnant controls.

This report represents the first description of the transcriptome of adipose tissue visceral and subcutaneous transcriptome in pregnant women and the comparison between pregnant and non-pregnant gene expression in these fat depots. Using the Illumina GeneChip Human Exon 1.0 ST array, we analyzed exon level expression data to determine differential usage associated with adipose tissue regions and pregnancy. Significant differences in exon usage were found between visceral and subcutaneous adipose tissues of pregnant women, implicating alternative splicing in regional differences in adipose tissue for the first time. We have identified novel genes previously unrecognized to be differentially expressed in visceral versus subcutaneous adipose tissues. Furthermore, we have demonstrated the expression of G protein-coupled receptor 6 (GPR64) in both visceral and subcutaneous. This gene was thought to be expressed exclusively and abundantly in epithelia of ductuli efferentes and proximal epididymis. Several limitations of our study should be acknowledged, and these include the racial polarity of our patient population. As the study population consists mainly of African-American women, the generalization of our findings to pregnant women of different ethnic origins will require future investigation. In addition, this study was specifically designed to delineate differences between visceral and abdominal subcutaneous adipose tissues. Thus, adipose tissues of other regions were not evaluated. Finally, we recognize that the cross-section natural of this study does not allow us to demonstrate neither a temporal nor a causal relationship between gestation and alterations in adipose tissue region-specific gene expression.

In conclusion, we have provided evidence that the adipose tissue region-specific alterations in gene expression established in non-pregnant individuals are enhanced during human gestation. Furthermore, unique pregnancy-related gene expression characterized both visceral and especially subcutaneous adipose tissues. Finally, alternative splicing has been implicated in regional differences in adipose tissue for the first time. Collectively, these novel findings may provide a molecular mechanism for the pregnancy-related adipose tissue remodeling, expansion, metabolic adaptations and the inflammatory response.

Supporting Information

S1 Fig. Rendering of the ECM-receptor interaction KEGG pathway (hsa04512) showing genes and genes complexes as colored rectangles (blue = down-regulation, magenta = up-regulation) between subcutaneous and visceral tissues of pregnant women. The genes COL4A1, COL4A2, COL5A2 and COL3A1 are shown as a single rectangle (Collagen). The same is true about LAMB1 and LAMB3, which are represented as the Laminin rectangle. (TIF)

S2 Fig. Rendering of the PPAR signaling KEGG pathway (hsa03320) showing genes and genes complexes as colored rectangles (blue = down-regulation, magenta = up-regulation)

between subcutaneous and visceral tissues of pregnant women.
(TIF)

S3 Fig. Rendering of the Protein digestion and absorption KEGG pathway (hsa04974) showing genes and genes complexes as colored rectangles (blue = down-regulation, magenta = up-regulation) between subcutaneous and visceral tissues of pregnant women. The genes COL12A1, COL15A1, COL3A1, COL4A1, COL4A2 and COL5A2 are shown as a single rectangle (Collagen). The same is true about ACE2, DPP4, MME and XPNPEP2, which are represented as the Peptidase rectangle.
(TIF)

S4 Fig. Rendering of the Complement and coagulation cascades KEGG pathway (hsa04610) showing genes and genes complexes as colored rectangles (blue = down-regulation, magenta = up-regulation) between subcutaneous tissues of pregnant and non-pregnant women. The genes C1QA, C1QB and C1QC are shown as a single rectangle (C1Q).
(TIF)

S5 Fig. Rendering of the Prion diseases KEGG pathway (hsa05020) showing genes and genes complexes as colored rectangles (blue = down-regulation, magenta = up-regulation) between subcutaneous tissues of pregnant and non-pregnant women. The genes C1QA, C1QB and C1QC are shown as a single rectangle (C1q).
(TIF)

S6 Fig. Normalized probe expression data against the genomic coordinates for gene GSTK1.
(TIF)

S1 File. Supplementary methods.
(DOC)

S1 Table. Subcutaneous vs. visceral: pregnant women.
(XLS)

S2 Table. Subcutaneous vs. visceral in pregnant women: enriched biological processes.
(DOC)

S3 Table. Subcutaneous vs. visceral in pregnant women: KEGG pathways.
(DOC)

S4 Table. Subcutaneous vs. visceral: non-pregnant women.
(XLS)

S5 Table. Subcutaneous vs. visceral in non-pregnant women: enriched biological processes.
(DOC)

S6 Table. Subcutaneous adipose in pregnant vs. non-pregnant women.
(DOC)

S7 Table. Alternative splicing.
(DOC)

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Author Contributions

Conceived and designed the experiments: SMT EV ALT RR JPK NGT TC SSH ZD. Performed the experiments: SMT EV ALT RR JPK NGT TC SSH ZD. Analyzed the data: SMT EV ALT RR JPK. Contributed reagents/materials/analysis tools: SMT EV ALT RR JPK. Wrote the paper: SMT EV ALT RR JPK NGT TC SSH ZD.

References

1. Buchanan TA, Metzger BE, Freinkel N, Bergman RN. (1990) Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. Am J Obstet Gynecol 162: 1008–1014. PMID: 2183610
2. Phelps RL, Metzger BE, Freinkel N. (1981) Carbohydrate metabolism in pregnancy. XVII. Diurnal profiles of plasma glucose, insulin, free fatty acids, triglycerides, cholesterol, and individual amino acids in late normal pregnancy. Am J Obstet Gynecol 140: 730–736. PMID: 7020420
3. Ryan EA, O'Sullivan MJ, Skyler JS. (1985) Insulin action during pregnancy. Studies with the euglycemic clamp technique. Diabetes 34: 380–389. PMID: 3882502
4. Desoye G, Schweditsch MO, Pfeiffer KP, Zechner R, Kostner GM. (1987) Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum. J Clin Endocrinol Metab 64: 704–712. PMID: 3546352
5. Knopp RH, Warth MR, Charles D, Childs M, Li JR, Mabuchi H, et al. (1986) Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes. Biol Neonate 50: 297–317. PMID: 3542067
6. Hytten FE, Chamberlain G (1991) Clinical physiology in obstetrics. Oxford, United Kingdom: Blackwell Scientific Publications.
7. Pipe NG, Smith T, Halliday D, Edmonds CJ, Williams C, Coltart TM, et al. (1979) Changes in fat, fat-free mass and body water in human normal pregnancy. Br J Obstet Gynaecol 86: 929–940. PMID: 118770
8. Beck P. (1969) Progestin enhancement of the plasma insulin response to glucose in Rhesus monkeys. Diabetes 18: 146–152. PMID: 4974771
9. Kalkhoff RK, Jacobson M, Lemper D. (1970) Progesterone, pregnancy and the augmented plasma insulin response. J Clin Endocrinol Metab 31: 24–28. PMID: 4316582
10. Polderman KH, Gooren LJ, Asscheman H, Bakker A, Heine RJ. (1994) Induction of insulin resistance by androgens and estrogens. J Clin Endocrinol Metab 79: 265–271. PMID: 8027240
11. Ryan EA, Enns L. (1988) Role of gestational hormones in the induction of insulin resistance. J Clin Endocrinol Metab 67: 341–347. PMID: 3292560
12. Catalano PM, Hoegh M, Minium J, Huston-Presley L, Bernard S, Kalhan S, et al. (2006) Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. Diabetologia 49: 1677–1685. PMID: 16752186
13. Hotamisligil GS, Shargill NS, Spiegelman BM. (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87–91. PMID: 7678183
14. Hutley L, Prins JB. (2005) Fat as an endocrine organ: relationship to the metabolic syndrome. Am J Med Sci 330: 280–289. PMID: 16355012
15. Kahn SE, Hull RL, Utzschneider KM. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444: 840–846. PMID: 17167471
16. Spiegelman BM, Flier JS. (2001) Obesity and the regulation of energy balance. Cell 104: 531–543. PMID: 11239410
17. Tilg H, Moschen AR. (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6: 772–783. PMID: 16998510
18. Rosen ED, Spiegelman BM. (2006) Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444: 847–853. PMID: 17167472
19. Mazaki-Tovi S, Kanety H, Sivan E. (2005) Adiponectin and human pregnancy. Curr Diab Rep 5: 278–281. PMID: 16033679
20. Kajantie E, Hytinantti T, Hovi P, Andersson S. (2004) Cord plasma adiponectin: a 20-fold rise between 24 weeks gestation and term. J Clin Endocrinol Metab 89: 4031–4036. PMID: 15292345
21. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Wiser A, Schiff E, et al. (2007) Maternal serum adiponectin levels during human pregnancy. J Perinatol 27: 77–81. PMID: 17262038

22. Cortelazzi D, Corbetta S, Ronzoni S, Pelle F, Marconi A, Cozzi V, et al. (2007) Maternal and foetal resistin and adiponectin concentrations in normal and complicated pregnancies. Clin Endocrinol (Oxf) 66: 447–453.
23. Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Vaisbuch E, Gotsch F, et al. (2008) Adiponectin multimers in maternal plasma. J Matern Fetal Neonatal Med 21: 796–815. doi: 10.1080/14767050802266881 PMID: 19031276
24. Nien JK, Mazaki-Tovi S, Romero R, Erez O, Kusanovic JP, Gotsch F, et al. (2007) Plasma adiponectin concentrations in non-pregnant, normal and overweight pregnant women. J Perinat Med 35: 522–531. PMID: 17919116
25. Mazaki-Tovi S, Romero R, Kusanovic JP, Vaisbuch E, Erez O, Than NG, et al. (2009) Maternal visfatin concentration in normal pregnancy. J Perinat Med 37: 206–217. doi: 10.1515/JPM.2009.054 PMID: 19284295
26. Haugen F, Drevon CA. (2007) Activation of nuclear factor-kappaB by high molecular weight and globular adiponectin. Endocrinology 148: 5478–5486. PMID: 17702846
27. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Yissachar E, Schiff E, et al. (2011) Insulin sensitivity in late gestation and early postpartum period: the role of circulating maternal adipokines. Gynecol Endocrinol 27: 725–731. doi: 10.3109/09513590.2010.500426 PMID: 21714696
28. Nien JK, Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Gotsch F, et al. (2007) Resistin: a hormone which induces insulin resistance is increased in normal pregnancy. J Perinat Med 35: 513–521. PMID: 17919114
29. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Yissachar E, Schiff E, et al. (2011) Insulin sensitivity in late gestation and early postpartum period: the role of circulating maternal adipokines. Gynecol Endocrinol 27: 725–731. doi: 10.3109/09513590.2010.500426 PMID: 21714696
30. Poole AT, Vincent KL, Olson GL, Patrikeev I, Saade GR, Stuebe A, et al. (2014) Effect of lactation on maternal postpartum cardiac function and adiposity: a murine model. Am J Obstet Gynecol 211: 424–427. doi: 10.1016/j.ajog.2014.06.004 PMID: 24905416
31. Sween LK, Althouse AD, Roberts JM. (2015) Early-pregnancy percent body fat in relation to preeclampsia risk in obese women. Am J Obstet Gynecol 212: 84–87. doi: 10.1016/j.ajog.2014.07.055 PMID: 25088867
32. Ceyhan ST, Safer U, Cintosun U. (2015) Bioelectric impedance analysis in pregnant women. Am J Obstet Gynecol 212: 120. doi: 10.1016/j.ajog.2014.09.033 PMID: 25281366
33. Karachaliou M, Georgiou V, Roumeliotaki T, Chalkiadaki G, Daraki V, Koinaki S, et al. (2015) Association of trimester-specific gestational weight gain with fetal growth, offspring obesity, and cardiometabolic traits in early childhood. Am J Obstet Gynecol 212: 502. doi: 10.1016/j.ajog.2014.12.038 PMID: 25557209
34. Brandt B, Mazaki-Tovi S, Hemi R, Yinon Y, Schiff E, Mashiach R, et al. (2014) Omentin, an adipokine with insulin-sensitizing properties, is negatively associated with insulin resistance in normal gestation. J Perinat Med.
35. D'Anna R, Baviera G, Corrado F, Giordano D, Di Benedetto A, Jasonni VM. (2005) Plasma adiponectin concentration in early pregnancy and subsequent risk of hypertensive disorders. Obstet Gynecol 106: 340–344. PMID: 16055585
36. D'Anna R, Baviera G, Corrado F, Giordano D, De VA, Nicocia G, et al. (2006) Adiponectin and insulin resistance in early- and late-onset pre-eclampsia. BJOG 113: 1264–1269. PMID: 17010118
37. Mazaki-Tovi S, Romero R, Vaisbuch E, Kusanovic JP, Erez O, Gotsch F, et al. (2009) Maternal serum adiponectin multimers in preeclampsia. J Perinat Med 37: 349–363. doi: 10.1515/JPM.2009.085 PMID: 19348608
38. Haugen F, Ranheim T, Harsem NK, Lips E, Staff AC, Drevon CA. (2006) Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression. Am J Physiol Endocrinol Metab 290: E326–E333. PMID: 16144822
39. Kajantie E, Kaaja R, Ylikorkala O, Andersson S, Laivuori H. (2005) Adiponectin concentrations in maternal serum: elevated in preeclampsia but unrelated to insulin sensitivity. J Soc Gynecol Investig 12: 433–439. PMID: 15979355
40. Lu D, Yang X, Wu Y, Wang H, Huang H, Dong M. (2006) Serum adiponectin, leptin and soluble leptin receptor in pre-eclampsia. Int J Gynaecol Obstet.
41. Mazaki-Tovi S, Romero R, Kim SK, Vaisbuch E, Kusanovic JP, Erez O, et al. (2010) Could alterations in maternal plasma visfatin concentration participate in the phenotype definition of preeclampsia and SGA? J Matern Fetal Neonatal Med 23: 857–868. doi: 10.3109/14767050903301017 PMID: 19900033

42. Naruse K, Yamasaki M, Umekage H, Sado T, Sakamoto Y, Morikawa H. (2005) Peripheral blood concentrations of adiponectin, an adipocyte-specific plasma protein, in normal pregnancy and preeclampsia. J Reprod Immunol 65: 65–75. PMID: 15694968

43. Nien JK, Mazaki-Tovi S, Romero R, Erez O, Kusanovic JP, Gotsch F, et al. (2007) Adiponectin in severe preeclampsia. J Perinat Med 35: 503–512. PMID: 17919115

44. Ramsay JE, Jamieson N, Greer IA, Sattar N. (2003) Paradoxical elevation in adiponectin concentrations in women with preeclampsia. Hypertension 42: 891–894. PMID: 14517227

45. Vaisbuch E, Romero R, Mazaki-Tovi S, Erez O, Kim SK, Chaiworapongsa T, et al. (2010) Retinol binding protein 4—a novel association with early-onset preeclampsia. J Perinat Med 38: 129–139. doi: 10.1515/JPM.2009.140 PMID: 19708829

46. Chen D, Dong M, Fang Q, He J, Wang Z, Yang X. (2005) Alterations of serum resistin in normal pregnancy and pre-eclampsia. Clin Sci (Lond) 108: 81–84.

47. Fasshauer M, Waldeyer T, Seeger J, Schrey S, Ebert T, Kratzsch J, et al. (2008) Circulating high-molecular-weight adiponectin is upregulated in preeclampsia and is related to insulin sensitivity and renal function. Eur J Endocrinol 158: 197–201. doi: 10.1530/EJE-07-0454 PMID: 18230826

48. Fasshauer M, Waldeyer T, Seeger J, Schrey S, Ebert T, Kratzsch J, et al. (2008) Serum levels of the adipokine visfatin are increased in pre-eclampsia. Clin Endocrinol (Oxf) 69: 69–73.

49. Seol HJ, Kim JW, Kim HJ. (2011) Retinol-binding protein-4 is decreased in patients with preeclampsia in comparison with normal pregnant women. J Perinat Med 39: 287–289. doi: 10.1515/JPM.2011.016 PMID: 21299359

50. Stepan H, Philipp A, Roth I, Kralisch S, Jank A, Schaarschmidt W, et al. (2011) Serum levels of the adipokine chemerin are increased in preeclampsia during and 6 months after pregnancy. Regul Pept 168: 69–72. doi: 10.1016/j.regpep.2011.03.005 PMID: 21477622

51. Suwaki N, Masuyama H, Nakatsukasa H, Masumoto A, Sumida Y, Takamoto N, et al. (2006) Hypoadiponectinemia and circulating angiogenic factors in overweight patients complicated with pre-eclampsia. Am J Obstet Gynecol 195: 1687–1692. PMID: 16769024

52. Hendler I, Blackwell SC, Mehta SH, Whitty JE, Russell E, Sorokin Y, et al. (2005) The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant women with and without preeclampsia. Am J Obstet Gynecol 193: 979–983. PMID: 16157097

53. Briana DD, Malamitsi-Puchner A. (2009) Adipocytokines in Normal and Complicated Pregnancies. Reprod Sci 16: 921–937. doi: 10.1177/1933719109336614 PMID: 19474287

54. Fasshauer M, Bluher M, Stumvoll M, Tonessen P, Faber R, Stepan H. (2007) Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. Clin Endocrinol (Oxf) 66: 434–439.

55. Kinalski M, Telejko B, Kuzmicki M, Kretowski A, Kinalska I. (2005) Tumor necrosis factor alpha system and plasma adiponectin concentration in women with gestational diabetes. Horm Metab Res 37: 450–454. PMID: 16034719

56. Mazaki-Tovi S, Romero R., Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. (2009) Maternal Serum Adiponectin Multimers in Gestational Diabetes. Journal Of Perinatal Medicine 37: 637–650. doi: 10.1515/JPM.2009.101 PMID: 19530957

57. Ranheim T, Haugen F, Staff AC, Braekke K, Harsem NK, Drevon CA. (2004) Adiponectin is reduced in gestational diabetes mellitus in normal weight women. Acta Obstet Gynecol Scand 83: 341–347. PMID: 15005780

58. Worda C, Leipold H, Gruber C, Kautzky-Willer A, Knofler M, e Bancher-Todesca D. (2004) Decreased plasma adiponectin concentrations in women with gestational diabetes mellitus. Am J Obstet Gynecol 191: 2120–2124. PMID: 15592301

59. Chan TF, Chen YL, Lee CH, Chou FH, Wu LC, Jong SB, et al. (2006) Decreased plasma visfatin concentrations in women with gestational diabetes mellitus. J Soc Gynecol Investig 13: 364–367. PMID: 16814166

60. Chan TF, Chen HS, Chen YC, Lee CH, Chou FH, Chen IJ, et al. (2007) Increased serum retinol-binding protein 4 concentrations in women with gestational diabetes mellitus. Reprod Sci 14: 169–174. PMID: 17636228

61. Chen D, Fang Q, Chai Y, Wang H, Huang H, Dong M. (2007) Serum resistin in gestational diabetes mellitus and early postpartum. Clin Endocrinol (Oxf) 67: 208–211.

62. Mazaki-Tovi S, Romero R, Kusanovic JP, Vaisbuch E, Erez O, Than NG, et al. (2009) Visfatin in human pregnancy: maternal gestational diabetes vis-a-vis neonatal birthweight. J Perinat Med 37: 218–231. doi: 10.1515/JPM.2009.053 PMID: 19099366

63. Haider DG, Handisurya A, Storka A, Vojtassakova E, Luger A, Pacini G, et al. (2007) Visfatin response to glucose is reduced in women with gestational diabetes mellitus. Diabetes Care 30: 1889–1891. PMID: 17416788

64. Krzyzanowska K, Krugluger W, Mittermayer F, Rahman R, Haider D, Shnawa N, et al. (2006) Increased visfatin concentrations in women with gestational diabetes mellitus. Clin Sci (Lond) 110: 605–609.

65. Krzyzanowska K, Zemany L, Krugluger W, Schernthaner GH, Mittermayer F, Schnack C, et al. (2008) Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. Diabetologia 51: 1115–1122. doi: 10.1007/s00125-008-1009-9 PMID: 18437353

66. Kuzmicki M, Telejko B, Szamatowicz J, Zonenberg A, Nikolajuk A, Kretowski A, et al. (2009) High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. Gynecol Endocrinol 25: 258–263. doi: 10.1080/09513590802653825 PMID: 19408175

67. Lewandowski KC, Stojanovic N, Press M, Tuck SM, Szosland K, Bienkiewicz M, et al. (2007) Elevated serum levels of visfatin in gestational diabetes: a comparative study across various degrees of glucose tolerance. Diabetologia 50: 1033–1037. PMID: 17334748

68. Lewandowski KC, Stojanovic N, Bienkiewicz M, Tan BK, Prelevic GM, Press M, et al. (2008) Elevated concentrations of retinol-binding protein-4 (RBP-4) in gestational diabetes mellitus: negative correlation with soluble vascular cell adhesion molecule-1 (sVCAM-1). Gynecol Endocrinol 24: 300–305. doi: 10.1080/09513590802141052 PMID: 18584408

69. Szamatowicz J, Kuzmicki M, Telejko B, Zonenberg A, Nikolajuk A, Kretowski A, et al. (2009) Serum visfatin concentration is elevated in pregnant women irrespectively of the presence of gestational diabetes. Ginekol Pol 80: 14–18. PMID: 19323054

70. Hermann M, Le RC, Blondel B, Goffinet F, Zeitlin J. (2015) The risk of prelabor and intrapartum cesarean delivery among overweight and obese women: possible preventive actions. Am J Obstet Gynecol 212: 241–249. doi: 10.1016/j.ajog.2014.08.002 PMID: 25108139

71. Acosta O, Ramirez VI, Lager S, Gaccioli F, Dudley DJ, Powell TL, et al. (2015) Increased glucose and placental GLUT-1 in large infants of obese nondiabetic mothers. Am J Obstet Gynecol 212: 227. doi: 10.1016/j.ajog.2014.08.009 PMID: 25132463

72. Daly N, Stapleton M, O'Kelly R, Kinsley B, Daly S, Turner MJ. (2015) The role of preanalytical glycolysis in the diagnosis of gestational diabetes mellitus in obese women. Am J Obstet Gynecol.

73. Mazaki-Tovi S, Romero R., Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T, et al. (2009) Dysregulation of maternal serum adiponectin in preterm labor. J Matern Fetal Neonatal Med 22: 887–904. doi: 10.1080/14767050902994655 PMID: 19579094

74. Mazaki-Tovi S, Romero R., Vaisbuch E, Erez O, Chaiworapongsa T, Mittal P, et al. (2009) Maternal Plasma Visfatin in Preterm Labor. J Matern Fetal Neonatal Med 22: 693–704. doi: 10.1080/14767050902994788 PMID: 19572235

75. Mazaki-Tovi S, Romero R, Vaisbuch E, Kim SK, Kusanovic JP, Chaiworapongsa T, et al. (2010) Evidence for differential regulation of the adipokine visfatin in the maternal and fetal compartments in normal spontaneous labor at term. J Perinat Med 38: 281–288. doi: 10.1515/JPM.2010.045 PMID: 20146661

76. Mazaki-Tovi S, Romero R, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Kim SK, et al. (2010) Retinol-binding protein 4: a novel adipokine implicated in the genesis of LGA in the absence of gestational diabetes mellitus. J Perinat Med 38: 147–155. doi: 10.1515/JPM.2010.044 PMID: 20146659

77. Briana DD, Boutsikou M, Baka S, Gourgiotis D, Marmarinos A, Hassiakos D,et al. (2008) Perinatal changes of plasma resistin concentrations in pregnancies with normal and restricted fetal growth. Neonatology 93: 153–157. PMID: 17878741

78. Mazaki-Tovi S, Romero R., Vaisbuch E, Erez O, Mittal P, Chaiworapongsa T,et al. (2009) Maternal Serum Adiponectin Multimers in Patients with a Small-For-Gestational-Age Newborn. J Perinat Med 37: 623–635. doi: 10.1515/JPM.2009.128 PMID: 19530958

79. Catov JM, Patrick TE, Powers RW, Ness RB, Harger G, Roberts JM. (2007) Maternal leptin across pregnancy in women with small-for-gestational-age infants. Am J Obstet Gynecol 196: 558. PMID: 17547894

80. Mazaki-Tovi S, Vaisbuch E, Romero R, Kusanovic JP, Chaiworapongsa T, Kim SK, et al. (2010) Maternal and neonatal circulating visfatin concentrations in patients with pre-eclampsia and a small-for-gestational age neonate. J Matern Fetal Neonatal Med 23: 1119–1128. doi: 10.3109/14767050903572190 PMID: 20121389

81. Briana DD, Malamitsi-Puchner A. (2010) The role of adipocytokines in fetal growth. Ann N Y Acad Sci 1205: 82–87. doi: 10.1111/j.1749-6632.2010.05650.x PMID: 20840257

82. Mazaki-Tovi S, Kasher-Meron M, Hemi R, Haas J, Gat I, Lantsberg D, et al. (2012) Chemerin is present in human cord blood and is positively correlated with birthweight. Am J Obstet Gynecol 207: 412–10. doi: 10.1016/j.ajog.2012.08.008 PMID: 22925375

83. Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Kuint J, Yinon Y, et al. (2011) Cord blood adiponectin and infant growth at one year. J Pediatr Endocrinol Metab 24: 411–418. PMID: 21932574

84. Darling AM, McDonald CR, Conroy AL, Hayford KT, Liles WC, Wang M, et al. (2014) Angiogenic and inflammatory biomarkers in midpregnancy and small-for-gestational-age outcomes in Tanzania. Am J Obstet Gynecol 211: 509–8. doi: 10.1016/j.ajog.2014.05.032 PMID: 24881826

85. Mazaki-Tovi S, Romero R., Vaisbuch E, Chaiworapongsa T, Erez O, Mittal P, et al. (2009) Low circulating maternal adiponectin in patients with pyelonephritis: adiponectin at the crossroads of pregnancy and infection. Journal Of Perinatal Medicine 38: 9–17.

86. Mazaki-Tovi S, Vaisbuch E, Romero R, Kusanovic JP, Chaiworapongsa T, Kim SK et al. (2010) Maternal plasma concentration of the pro-inflammatory adipokine pre-B-cell-enhancing factor (PBEF)/visfatin is elevated in pregnant patients with acute pyelonephritis. Am J Reprod Immunol 63: 252–262. doi: 10.1111/j.1600-0897.2009.00804.x PMID: 20085562

87. Vaisbuch E, Romero R, Mazaki-Tovi S, Kusanovic JP, Chaiworapongsa T, Dong Z. et al. (2010) Maternal plasma retinol binding protein 4 in acute pyelonephritis during pregnancy. J Perinat Med 38: 359–366. doi: 10.1515/JPM.2010.066 PMID: 20163326

88. Mazaki-Tovi S, Romero R., Vaisbuch E, Kusanovic JP, Erez O, Mittal P, et al. (2009) Adiponectin in amniotic fluid in normal pregnancy, spontaneous labor at term, and preterm labor: A novel association with subclinical intrauterine infection/inflammation. J Matern Fetal Neonatal Med 23: 120–130.

89. Vaisbuch E, Mazaki-Tovi S, Kusanovic JP, Erez O, Than GN, Kim SK, et al. (2009) Retinol Binding Protein 4: An Adipokine Associated with Intra-amniotic Infection / Inflammation. J Matern Fetal Neonatal Med: 19900011.

90. Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Gotsch F, Mittal P, et al. (2008) Visfatin/Pre-B cell colony-enhancing factor in amniotic fluid in normal pregnancy, spontaneous labor at term, preterm labor and prelabor rupture of membranes: an association with subclinical intrauterine infection in preterm parturition. J Perinat Med 36: 485–496. doi: 10.1515/JPM.2008.084 PMID: 18598235

91. Mazaki-Tovi S, Vaisbuch E, Romero R, Kusanovic JP, Chaiworapongsa T, Kim SK, et al. (2010) Hyperresistinemia—a novel feature in systemic infection during human pregnancy. Am J Reprod Immunol 63: 358–369. doi: 10.1111/j.1600-0897.2010.00809.x PMID: 20178460

92. Karatas A, Ozlu T, Erdem A. (2014) Maternal metformin, obesity, and metabolic syndrome: the contribution of autonomic nervous system function. Am J Obstet Gynecol 210: 282.

93. Marrs CC, Moussa HN, Sibai BM, Blackwell SC. (2014) The relationship between primary cesarean delivery skin incision type and wound complications in women with morbid obesity. Am J Obstet Gynecol 210: 319–4. doi: 10.1016/j.ajog.2014.01.018 PMID: 24560557

94. Subramaniam A, Jauk VC, Goss AR, Alvarez MD, Reese C, Edwards RK. (2014) Mode of delivery in women with class III obesity: planned cesarean compared with induction of labor. Am J Obstet Gynecol 211: 700–709. doi: 10.1016/j.ajog.2014.06.045 PMID: 24956550

95. Mackeen AD, Schuster M, Berghella V. (2015) Suture versus staples for skin closure after cesarean: a metaanalysis. Am J Obstet Gynecol 212: 621. doi: 10.1016/j.ajog.2014.12.020 PMID: 25530592

96. Mei-Dan E, Ray JG, Vigod SN. (2015) Perinatal outcomes among women with bipolar disorder: a population-based cohort study. Am J Obstet Gynecol 212: 367–368. doi: 10.1016/j.ajog.2014.10.020 PMID: 25446660

97. Sugerman HJ. (2014) Effect of obesity on incidence of preeclampsia. Am J Obstet Gynecol 210: 375.

98. Zera CA, Seely EW, Wilkins-Haug LE, Lim KH, Parry SI, McElrath TF. (2014) The association of body mass index with serum angiogenic markers in normal and abnormal pregnancies. Am J Obstet Gynecol 211: 247. doi: 10.1016/j.ajog.2014.03.020 PMID: 24631439

99. Kessous R, Shoham-Vardi I, Pariente G, Sergienko R, Holcberg G, Sheiner E. (2014) Recurrent pregnancy loss: a risk factor for long-term maternal atherosclerotic morbidity? Am J Obstet Gynecol 211: 414–11. doi: 10.1016/j.ajog.2014.05.050 PMID: 24905415

100. Bigelow CA, Pereira GA, Warmsley A, Cohen J, Getrajdman C, Moshier E, et al. (2014) Risk factors for new-onset late postpartum preeclampsia in women without a history of preeclampsia. Am J Obstet Gynecol 210: 338. doi: 10.1016/j.ajog.2013.11.004 PMID: 24211478

101. Yao R, Ananth CV, Park BY, Pereira L, Plante LA. (2014) Obesity and the risk of stillbirth: a population-based cohort study. Am J Obstet Gynecol 210: 457–459. doi: 10.1016/j.ajog.2014.01.044 PMID: 24674712

102. Harper LM, Jauk VC, Owen J, Biggio JR. (2014) The utility of ultrasound surveillance of fluid and growth in obese women. Am J Obstet Gynecol 211: 524–528. doi: 10.1016/j.ajog.2014.04.028 PMID: 24791732

103. Lesseur C, Armstrong DA, Paquette AG, Li Z, Padbury JF, Marsit CJ. (2014) Maternal obesity and gestational diabetes are associated with placental leptin DNA methylation. Am J Obstet Gynecol 211: 654–659. doi: 10.1016/j.ajog.2014.06.037 PMID: 24954653

104. Ahmadzia HK, Thomas SM, Dude AM, Grotegut CA, Boyd BK. (2014) Prediction of birthweight from third-trimester ultrasound in morbidly obese women. Am J Obstet Gynecol 211: 431–437. doi: 10.1016/j.ajog.2014.06.041 PMID: 24954654

105. Hancke K, Gundelach T, Hay B, Sander S, Reister F, Weiss JM. (2015) Pre-pregnancy obesity compromises obstetric and neonatal outcomes. J Perinat Med 43: 141–146. doi: 10.1515/jpm-2014-0069 PMID: 24964255

106. Kevane B, Donnelly J, D'Alton M, Cooley S, Preston RJ, Ni AF. (2014) Risk factors for pregnancy-associated venous thromboembolism: a review. J Perinat Med 42: 417–425. doi: 10.1515/jpm-2013-0207 PMID: 24334422

107. Farren M, Daly N, O'Higgins AC, McKeating A, Maguire PJ, Turner MJ. (2014) The interplay between maternal obesity and gestational diabetes mellitus. J Perinat Med.

108. Michels KB, Harris HR, Barault L. (2011) Birthweight, maternal weight trajectories and global DNA methylation of LINE-1 repetitive elements. PLoS One 6: e25254. doi: 10.1371/journal.pone.0025254 PMID: 21980406

109. Zhang YP, Liu XH, Gao SH, Wang JM, Gu YS, Zhang JY, et al. (2012) Risk factors for preterm birth in five Maternal and Child Health hospitals in Beijing. PLoS One 7: e52780. doi: 10.1371/journal.pone.0052780 PMID: 23300774

110. Oteng-Ntim E, Kopeika J, Seed P, Wandiembe S, Doyle P. (2013) Impact of obesity on pregnancy outcome in different ethnic groups: calculating population attributable fractions. PLoS One 8: e53749. doi: 10.1371/journal.pone.0053749 PMID: 23341993

111. Li N, Liu E, Guo J, Pan L, Li B, Wang P, et al. (2013) Maternal prepregnancy body mass index and gestational weight gain on pregnancy outcomes. PLoS One 8: e82310. doi: 10.1371/journal.pone.0082310 PMID: 24376527

112. Nohr EA, Timpson NJ, Andersen CS, Davey SG, Olsen J, Sorensen TI. (2009) Severe obesity in young women and reproductive health: the Danish National Birth Cohort. PLoS One 4: e8444. doi: 10.1371/journal.pone.0008444 PMID: 20041193

113. Macaulay S, Dunger DB, Norris SA. (2014) Gestational diabetes mellitus in Africa: a systematic review. PLoS One 9: e97871. doi: 10.1371/journal.pone.0097871 PMID: 24892280

114. Leng J, Shao P, Zhang C, Tian H, Zhang F, Zhang S, et al. (2015) Prevalence of gestational diabetes mellitus and its risk factors in Chinese pregnant women: a prospective population-based study in Tianjin, China. PLoS One 10: e0121029. doi: 10.1371/journal.pone.0121029 PMID: 25799433

115. Leng J, Li W, Zhang S, Liu H, Wang L, Liu G, et al. (2015) GDM Women's Pre-Pregnancy Overweight/Obesity and Gestational Weight Gain on Offspring Overweight Status. PLoS One 10: e0129536. doi: 10.1371/journal.pone.0129536 PMID: 26098307

116. Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X. (2013) Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: a systematic review and meta-analysis. PLoS One 8: e61627. doi: 10.1371/journal.pone.0061627 PMID: 23613888

117. Zhang J, Himes JH, Guo Y, Jiang J, Yang L, Lu Q, et al. (2013) Birth weight, growth and feeding pattern in early infancy predict overweight/obesity status at two years of age: a birth cohort study of Chinese infants. PLoS One 8: e64542. doi: 10.1371/journal.pone.0064542 PMID: 23755127

118. Despres JP, Lemieux I. (2006) Abdominal obesity and metabolic syndrome. Nature 444: 881–887. PMID: 17167477

119. Wajchenberg BL. (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 21: 697–738. PMID: 11133069

120. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, et al. (2007) Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 116: 39–48. PMID: 17576866

121. Einstein FH, Atzmon G, Yang XM, Ma XH, Rincon M, Rudin E, et al. (2005) Differential responses of visceral and subcutaneous fat depots to nutrients. Diabetes 54: 672–678. PMID: 15734842

122. Einstein FH, Fishman S, Muzumdar RH, Yang XM, Atzmon G, Barzilai N. (2008) Accretion of visceral fat and hepatic insulin resistance in pregnant rats. Am J Physiol Endocrinol Metab 294: E451–E455. PMID: 18073320

123. Tchernof A, Belanger C, Morisset AS, Richard C, Mailloux J, Laberge P, et al. (2006) Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. Diabetes 55: 1353–1360. PMID: 16644692

124. Dicker A, Astrom G, Wahlen K, Hoffstedt J, Naslund E, Wiren M, et al. (2009) Primary differences in lipolysis between human omental and subcutaneous adipose tissue observed using in vitro differentiated adipocytes. Horm Metab Res 41: 350–355. doi: 10.1055/s-0028-1112135 PMID: 19204889

125. Van H, V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonnqvist F, et al. (1998) Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 47: 913–917. PMID: 9604868

126. Samaras K, Botelho NK, Chisholm DJ, Lord RV. (2010) Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity (Silver Spring) 18: 884–889.

127. Drolet R, Belanger C, Fortier M, Huot C, Mailloux J, Legare D, et al. (2009) Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. Obesity (Silver Spring) 17: 424–430.

128. Gealekman O, Guseva N, Hartigan C, Apotheker S, Gorgoglione M, Gurav K, et al. (2011) Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. Circulation 123: 186–194. doi: 10.1161/CIRCULATIONAHA.110.970145 PMID: 21200001

129. Langin D, Holm C, Lafontan M. (1996) Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc Nutr Soc 55: 93–109. PMID: 8832784

130. Giusti V, Suter M, Verdumo C, Gaillard RC, Burckhardt P, Pralong FP. (2004) Molecular determinants of human adipose tissue: differences between visceral and subcutaneous compartments in obese women. J Clin Endocrinol Metab 89: 1379–1384. PMID: 15001637

131. Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G. (2004) Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. J Lipid Res 45: 148–154. PMID: 14563828

132. Vidal H. (2001) Gene expression in visceral and subcutaneous adipose tissues. Ann Med 33: 547–555. PMID: 11730162

133. Lihn AS, Bruun JM, He G, Pedersen SB, Jensen PF, Richelsen B. (2004) Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. Mol Cell Endocrinol 219: 9–15. PMID: 15149722

134. Ryden M, Elizalde M, Van H, V, Ohlund A, Hoffstedt J, Bringman S, et al. (2001) Increased expression of eNOS protein in omental versus subcutaneous adipose tissue in obese human subjects. Int J Obes Relat Metab Disord 25: 811–815. PMID: 11439294

135. Livingston JN, Lerea KM, Bolinder J, Kager L, Backman L, Arner P. (1984) Binding and molecular weight properties of the insulin receptor from omental and subcutaneous adipocytes in human obesity. Diabetologia 27: 447–453. PMID: 6391988

136. Dusserre E, Moulin P, Vidal H. (2000) Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. Biochim Biophys Acta 1500: 88–96. PMID: 10564721

137. Marette A, Mauriege P, Marcotte B, Atgie C, Bouchard C, Theriault G, et al. (1997) Regional variation in adipose tissue insulin action and GLUT4 glucose transporter expression in severely obese premenopausal women. Diabetologia 40: 590–598. PMID: 9165229

138. Atzmon G, Yang XM, Muzumdar R, Ma XH, Gabriely I, Barzilai N. (2002) Differential gene expression between visceral and subcutaneous fat depots. Horm Metab Res 34: 622–628. PMID: 12660871

139. Lefebvre AM, Laville M, Vega N, Riou JP, van GL, Auwerx J, et al. (1998) Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 47: 98–103. PMID: 9421381

140. Montague CT, Prins JB, Sanders L, Zhang J, Sewter CP, Digby J, et al. (1998) Depot-related gene expression in human subcutaneous and omental adipocytes. Diabetes 47: 1384–1391. PMID: 9726225

141. Lee MJ, Gong DW, Burkey BF, Fried SK. (2011) Pathways regulated by glucocorticoids in omental and subcutaneous human adipose tissues: a microarray study. Am J Physiol Endocrinol Metab 300: E571–E580. doi: 10.1152/ajpendo.00231.2010 PMID: 21189358

142. Gealekman O, Guseva N, Hartigan C, Apotheker S, Gorgoglione M, Gurav K, et al. (2011) Depot-specific differences and insufficient subcutaneous adipose tissue angiogenesis in human obesity. Circulation 123: 186–194. doi: 10.1161/CIRCULATIONAHA.110.970145 PMID: 21200001

143. Van H, V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, et al. (1998) Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 47: 913–917. PMID: 9604868

144. Engfeldt P, Arner P. (1988) Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Horm Metab Res Suppl 19: 26–29. PMID: 3069692

145. Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, et al. (1995) Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 44: 855–858. PMID: 7789654

146. Reynisdottir S, Dauzats M, Thorne A, Langin D. (1997) Comparison of hormone-sensitive lipase activity in visceral and subcutaneous human adipose tissue. J Clin Endocrinol Metab 82: 4162–4166. PMID: 9398732

147. Fried SK, Kral JG. (1987) Sex differences in regional distribution of fat cell size and lipoprotein lipase activity in morbidly obese patients. Int J Obes 11: 129–140. PMID: 3610466

148. Hube F, Lietz U, Igel M, Jensen PB, Tornqvist H, Joost HG, et al. (1996) Difference in leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. Horm Metab Res 28: 690–693. PMID: 9013743

149. Vohl MC, Sladek R, Robitaille J, Gurd S, Marceau P, Richard D, et al. (2004) A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. Obes Res 12: 1217–1222. PMID: 15340102

150. Kloting N, Berthold S, Kovacs P, Schon MR, Fasshauer M, Ruschke K, et al. (2009) MicroRNA expression in human omental and subcutaneous adipose tissue. PLoS One 4: e4699. doi: 10.1371/journal.pone.0004699 PMID: 19259271

151. von Eyben FE, Kroustrup JP, Larsen JF, Celis J. (2004) Comparison of gene expression in intra-abdominal and subcutaneous fat: a study of men with morbid obesity and nonobese men using microarray and proteomics. Ann N Y Acad Sci 1030: 508–536. PMID: 15659836

152. Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, et al. (2002) Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. J Matern Fetal Med 11: 18–25.

153. Redline RW. (2006) Inflammatory responses in the placenta and umbilical cord. Semin Fetal Neonatal Med 11: 296–301. PMID: 16621749

154. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. (1996) A United States national reference for fetal growth. Obstet Gynecol 87: 163–168. PMID: 8559516

155. Diet, nutrition and the prevention of chronic diseases. (2003) World Health Organ Tech Rep Ser 916: i–149, backcover. PMID: 12768890

156. Bashan N, Dorfman K, Tarnovscki T, Harman-Boehm I, Liberty IF, Bluher M, et al. (2007) Mitogen-activated protein kinases, inhibitory-kappaB kinase, and insulin signaling in human omental versus subcutaneous adipose tissue in obesity. Endocrinology 148: 2955–2962. PMID: 17317777

157. Harman-Boehm I, Bluher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, et al. (2007) Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab 92: 2240–2247. PMID: 17374712

158. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, et al. (2005) Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 54: 2911–2916. PMID: 16186392

159. Bluher M, Bashan N, Shai I, Harman-Boehm I, Tarnovscki T, Avinaoch E, et al. (2009) Activated Ask1-MKK4-p38MAPK/JNK stress signaling pathway in human omental fat tissue may link macrophage infiltration to whole-body Insulin sensitivity. J Clin Endocrinol Metab 94: 2507–2515. doi: 10.1210/jc.2009-0002 PMID: 19351724

160. Gardina PJ, Clark TA, Shimada B, Staples MK, Yang Q, Veitch J, et al. (2006) Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. BMC Genomics 7: 325. PMID: 17192196

161. Kurokawa K, Kuwano Y, Tominaga K, Kawai T, Katsuura S, Yamagishi N, et al. (2010) Brief naturalistic stress induces an alternative splice variant of SMG-1 lacking exon 63 in peripheral leukocytes. Neurosci Lett 484: 128–132. doi: 10.1016/j.neulet.2010.08.031 PMID: 20723581

162. Zhang W, Duan S, Bleibel WK, Wisel SA, Huang RS, Wu X, et al. (2009) Identification of common genetic variants that account for transcript isoform variation between human populations. Hum Genet 125: 81–93. doi: 10.1007/s00439-008-0601-x PMID: 19052777

163. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4: 249–264. PMID: 12925520

164. Smyth GK. (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 3: Article3. PMID: 16646809

165. Benjamini Y HY. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc B 57: 289–300.

166. Tusher VG, Tibshirani R, Chu G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 98: 5116–5121. PMID: 11309499

167. Purdom E, Simpson KM, Robinson MD, Conboy JG, Lapuk AV, Speed TP. (2008) FIRMA: a method for detection of alternative splicing from exon array data. Bioinformatics 24: 1707–1714. doi: 10.1093/bioinformatics/btn284 PMID: 18573797

168. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5: R80. PMID: 15461798

169. Draghici S, Khatri P, Tarca AL, Amin K, Done A, Voichita C, et al. (2007) A systems biology approach for pathway level analysis. Genome Res 17: 1537–1545. PMID: 17785539

170. Khatri P, Draghici S, Ostermeier GC, Krawetz SA. (2002) Profiling gene expression using onto-express. Genomics 79: 266–270. PMID: 11829497

171. Hojbjerre L, Alibegovic AC, Sonne MP, Dela F, Vaag A, Bruun JM, et al. (2011) Increased lipolysis but diminished gene expression of lipases in subcutaneous adipose tissue of healthy young males with intrauterine growth retardation. J Appl Physiol 111: 1863–1870. doi: 10.1152/japplphysiol.00960.2011 PMID: 21903886

172. Ma Y, Cheng Y, Wang J, Cheng H, Zhou S, Li X. (2010) The changes of visfatin in serum and its expression in fat and placental tissue in pregnant women with gestational diabetes. Diabetes Res Clin Pract 90: 60–65. doi: 10.1016/j.diabres.2010.06.010 PMID: 20621376

173. Kuzmicki M, Telejko B, Wawrusiewicz-Kurylonek N, Kalejta K, Lemancewicz A, Zdrodowski M, et al. (2011) The expression of transcription factor 7-like 2 (TCF7L2) in fat and placental tissue from women with gestational diabetes. Diabetes Res Clin Pract 94: e43–e46. doi: 10.1016/j.diabres.2011.07.033 PMID: 21855162

174. Kuzmicki M, Telejko B, Wawrusiewicz-Kurylonek N, Nikolajuk A, Zwierz-Gugala D, Jelski W, et al. (2011) Retinol-binding protein 4 in adipose and placental tissue of women with gestational diabetes. Gynecol Endocrinol 27: 1065–1069. doi: 10.3109/09513590.2011.579651 PMID: 21609194

175. Liu Y, Zhu L, Pan Y, Sun L, Chen D, Li X. (2012) Adiponectin levels in circulation and breast milk and mRNA expression in adipose tissue of preeclampsia women. Hypertens Pregnancy 31: 40–49. doi: 10.3109/10641955.2010.525273 PMID: 21174574

176. Ma Y, Cheng Y, Wang J, Cheng H, Zhou S, Li X. (2010) The changes of visfatin in serum and its expression in fat and placental tissue in pregnant women with gestational diabetes. Diabetes Res Clin Pract 90: 60–65. doi: 10.1016/j.diabres.2010.06.010 PMID: 20621376

177. Telejko B, Kuzmicki M, Zonenberg A, Modzelewska A, Niedziolko-Bagniuk K, Ponurkiewicz A, et al. (2010) Ghrelin in gestational diabetes: serum level and mRNA expression in fat and placental tissue. Exp Clin Endocrinol Diabetes 118: 87–92. doi: 10.1055/s-0029-1238313 PMID: 20180161

178. Colomiere M, Permezel M, Lappas M. (2010) Diabetes and obesity during pregnancy alter insulin signalling and glucose transporter expression in maternal skeletal muscle and subcutaneous adipose tissue. J Mol Endocrinol 44: 213–223. doi: 10.1677/JME-09-0091 PMID: 19955252

179. Telejko B, Kuzmicki M, Wawrusiewicz-Kurylonek N, Szamatowicz J, Nikolajuk A, Zonenberg A, et al. (2010) Plasma apelin levels and apelin/APJ mRNA expression in patients with gestational diabetes mellitus. Diabetes Res Clin Pract 87: 176–183. doi: 10.1016/j.diabres.2009.10.018 PMID: 19926159

180. Lappas M, Mitton A, Permezel M. (2010) In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes. J Endocrinol 204: 75–84. doi: 10.1677/JOE-09-0321 PMID: 19833719

181. Kleiblova P, Dostalova I, Bartlova M, Lacinova Z, Ticha I, Krejci V, et al. (2010) Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus. Mol Cell Endocrinol 314: 150–156. doi: 10.1016/j.mce.2009.08.002 PMID: 19682537

182. Telejko B, Kuzmicki M, Zonenberg A, Szamatowicz J, Wawrusiewicz-Kurylonek N, Nikolajuk A, et al. (2009) Visfatin in gestational diabetes: serum level and mRNA expression in fat and placental tissue. Diabetes Res Clin Pract 84: 68–75. doi: 10.1016/j.diabres.2008.12.017 PMID: 19185944

183. Orcy RB, Schroeder S, Martins-Costa SH, Ramos JG, Schechinger W, Klein H, et al. (2008) Signalization of Akt/PKB in the placenta, skeletal muscle and adipose tissue of preeclampsia patients. Gynecol Obstet Invest 66: 231–236. doi: 10.1159/000147169 PMID: 18645256

184. Morgan SA, Bringolf JB, Seidel ER. (2008) Visfatin expression is elevated in normal human pregnancy. Peptides 29: 1382–1389. doi: 10.1016/j.peptides.2008.04.010 PMID: 18524416

185. Zhou Y, Zhang M, Guo W, Yu M, Xue K, Huang S, et al. (2006) Expression of resistin protein in normal human subcutaneous adipose tissue and pregnant women subcutaneous adipose tissue and placenta. J Huazhong Univ Sci Technolog Med Sci 26: 288–291. PMID: 16961271

186. Haugen F, Ranheim T, Harsem NK, Lips E, Staff AC, Drevon CA. (2006) Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression. Am J Physiol Endocrinol Metab 290: E326–E333. PMID: 16144822

187. Okuno S, Akazawa S, Yasuhi I, Kawasaki E, Matsumoto K, Yamasaki H, et al. (1995) Decreased expression of the GLUT4 glucose transporter protein in adipose tissue during pregnancy. Horm Metab Res 27: 231–234. PMID: 7642174

188. Barker G, Lim R, Georgiou HM, Lappas M. (2012) Omentin-1 is decreased in maternal plasma, placenta and adipose tissue of women with pre-existing obesity. PLoS One 7: e42943. doi: 10.1371/journal.pone.0042943 PMID: 22952622

189. Okuno S, Akazawa S, Yasuhi I, Kawasaki E, Matsumoto K, Yamasaki H, et al. (1995) Decreased expression of the GLUT4 glucose transporter protein in adipose tissue during pregnancy. Horm Metab Res 27: 231–234. PMID: 7642174

190. Khoury MJ, Romero R. (2006) The integration of genomics into obstetrics and gynecology: a HuGE challenge. Am J Obstet Gynecol 195: 1503–1505. PMID: 17132472

191. Kolialexi A, Mavrou A, Spyrou G, Tsangaris GT. (2008) Mass spectrometry-based proteomics in reproductive medicine. Mass Spectrom Rev 27: 624–634. doi: 10.1002/mas.20181 PMID: 18618655

192. Romero R, Espinoza J, Gotsch F, Kusanovic JP, Friel LA, Yamasaki H, et al. (2006) The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome. BJOG 113 Suppl 3: 118–135. PMID: 17206980

193. Romero R, Tromp G. (2006) High-dimensional biology in obstetrics and gynecology: functional genomics in microarray studies. Am J Obstet Gynecol 195: 360–363. PMID: 16890547

194. Romero R, Kusanovic JP, Gotsch F, Erez O, Vaisbuch E, Mazaki-Tovi S, et al. (2009) Isobaric labeling and tandem mass spectrometry: A novel approach for profiling and quantifying proteins differentially expressed in amniotic fluid in preterm labor with and without intra-amniotic infection/inflammation. J Matern Fetal Neonatal Med: 19670042.

195. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. (2010) Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med 23: 1344–1359. doi: 10.3109/14767058.2010.482618 PMID: 20504069

196. Lassance L, Haghiac M, Leahy P, Basu S, Minium J, Zhou J, et al. (2015) Identification of early transcriptome signatures in placenta exposed to insulin and obesity. Am J Obstet Gynecol 212: 647. doi: 10.1016/j.ajog.2015.02.026 PMID: 25731694

197. Edlow AG, Slonim DK, Wick HC, Hui L, Bianchi DW. (2015) The pathway not taken: understanding 'omics data in the perinatal context. Am J Obstet Gynecol.

198. Rosa-e-Silva JC, Virginio LA Jr., Meola J, Dentillo DB, Ferriani RA, Giuliatti S. (2015) A system for storing, retrieving, and comparing gene expression information of patients with endometriosis. Am J Obstet Gynecol 212: 407. doi: 10.1016/j.ajog.2014.11.038 PMID: 25434836

199. Manuck TA, Esplin MS, Biggio J, Bukowski R, Parry S, Zhang H, et al. (2015) The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. Am J Obstet Gynecol 212: 487. doi: 10.1016/j.ajog.2015.02.010 PMID: 25687564

200. Parry S, Zhang H, Biggio J, Bukowski R, Varner M, Xu Y, et al. (2014) Maternal serum serpin B7 is associated with early spontaneous preterm birth. Am J Obstet Gynecol 211: 678–12. doi: 10.1016/j.ajog.2014.06.035 PMID: 24954659

201. Ghartey J, Bastek JA, Brown AG, Anglim L, Elovitz MA. (2015) Women with preterm birth have a distinct cervicovaginal metabolome. Am J Obstet Gynecol.

202. Bahado-Singh RO, Ertl R, Mandal R, Bjorndahl TC, Syngelaki A, Han B, et al. (2014) Metabolomic prediction of fetal congenital heart defect in the first trimester. Am J Obstet Gynecol 211: 240. doi: 10.1016/j.ajog.2014.03.056 PMID: 24704061

203. Hassan SS, Romero R, Haddad R, Hendler I, Khalek N, Tromp G, et al. (2006) The transcriptome of the uterine cervix before and after spontaneous term parturition. Am J Obstet Gynecol 195: 778–786. PMID: 16949412

204. Hassan SS, Romero R, Tarca AL, Draghici S, Pineles B, Bugrim A, et al. (2007) Signature pathways identified from gene expression profiles in the human uterine cervix before and after spontaneous term parturition. Am J Obstet Gynecol 197: 250–257. PMID: 17826407

205. Hassan SS, Romero R, Tarca AL, Nhan-Chang CL, Vaisbuch E, Erez O, et al. (2009) The transcriptome of cervical ripening in human pregnancy before the onset of labor at term: identification of novel molecular functions involved in this process. J Matern Fetal Neonatal Med 22: 1183–1193. doi: 10.3109/14767050903353216 PMID: 19883264

206. Hassan SS, Romero R, Tarca AL, Nhan-Chang CL, Mittal P, Vaisbuch E, et al. (2010) The molecular basis for sonographic cervical shortening at term: identification of differentially expressed genes and

the epithelial-mesenchymal transition as a function of cervical length. Am J Obstet Gynecol 203: 472. doi: 10.1016/j.ajog.2010.06.076 PMID: 20817141

207. Hassan SS, Romero R, Pineles B, Tarca AL, Montenegro D, Erez O, et al. (2010) MicroRNA expression profiling of the human uterine cervix after term labor and delivery. Am J Obstet Gynecol 202: 80–88. doi: 10.1016/j.ajog.2009.08.016 PMID: 19889381

208. Huber A, Hudelist G, Czerwenka K, Husslein P, Kubista E, Singer CF. (2005) Gene expression profiling of cervical tissue during physiological cervical effacement. Obstet Gynecol 105: 91–98. PMID: 15625148

209. Mowa CN, Li T, Jesmin S, Folkesson HG, Usip SE, Papka RE, et al. (2008) Delineation of VEGF-regulated genes and functions in the cervix of pregnant rodents by DNA microarray analysis. Reprod Biol Endocrinol 6: 64. doi: 10.1186/1477-7827-6-64 PMID: 19087268

210. Read CP, Word RA, Ruscheinsky MA, Timmons BC, Mahendroo MS. (2007) Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. Reproduction 134: 327–340. PMID: 17660242

211. Wang H, Stjernholm Y, Ekman G, Eriksson H, Sahlin L. (2001) Different regulation of oestrogen receptors alpha and beta in the human cervix at term pregnancy. Mol Hum Reprod 7: 293–300. PMID: 11228250

212. Mittal P, Romero R, Tarca AL, Gonzalez J, Draghici S, Xu Y, et al. (2010) Characterization of the myometrial transcriptome and biological pathways of spontaneous human labor at term. J Perinat Med 38: 617–643. doi: 10.1515/JPM.2010.097 PMID: 20629487

213. Bethin KE, Nagai Y, Sladek R, Asada M, Sadovsky Y, Hudson TJ, et al. (2003) Microarray analysis of uterine gene expression in mouse and human pregnancy. Mol Endocrinol 17: 1454–1469. PMID: 12775764

214. Bukowski R, Hankins GD, Saade GR, Anderson GD, Thornton S. (2006) Labor-associated gene expression in the human uterine fundus, lower segment, and cervix. PLoS Med 3: e169. PMID: 16768543

215. Chan EC, Fraser S, Yin S, Yeo G, Kwek K, Fairclough RJ, et al. (2002) Human myometrial genes are differentially expressed in labor: a suppression subtractive hybridization study. J Clin Endocrinol Metab 87: 2435–2441. PMID: 12050195

216. Esplin MS, Fausett MB, Peltier MR, Hamblin S, Silver RM, Branch DW, et al. (2005) The use of cDNA microarray to identify differentially expressed labor-associated genes within the human myometrium during labor. Am J Obstet Gynecol 193: 404–413. PMID: 16098862

217. Havelock JC, Keller P, Muleba N, Mayhew BA, Casey BM, Rainey WE, et al. (2005) Human myometrial gene expression before and during parturition. Biol Reprod 72: 707–719. PMID: 15509731

218. O'Brien M, Morrison JJ, Smith TJ. (2008) Upregulation of PSCDBP, TLR2, TWIST1, FLJ35382, EDNRB, and RGS12 gene expression in human myometrium at labor. Reprod Sci 15: 382–393. doi: 10.1177/1933719108316179 PMID: 18497345

219. Haddad R, Tromp G, Kuivaniemi H, Chaiworapongsa T, Kim YM, Mazor M, et al. (2006) Human spontaneous labor without histologic chorioamnionitis is characterized by an acute inflammation gene expression signature. Am J Obstet Gynecol.

220. Nhan-Chang CL, Romero R, Tarca AL, Mittal P, Kusanovic JP, Erez O, et al. (2010) Characterization of the transcriptome of chorioamniotic membranes at the site of rupture in spontaneous labor at term. Am J Obstet Gynecol 202: 462–41. doi: 10.1016/j.ajog.2010.02.045 PMID: 20452490

221. Bujold E, Romero R, Kusanovic JP, Erez O, Gotsch F, Chaiworapongsa T, et al. (2008) Proteomic profiling of amniotic fluid in preterm labor using two-dimensional liquid separation and mass spectrometry. J Matern Fetal Neonatal Med 21: 697–713. doi: 10.1080/14767050802053289 PMID: 19012186

222. Cho CK, Shan SJ, Winsor EJ, Diamandis EP. (2007) Proteomics analysis of human amniotic fluid. Mol Cell Proteomics 6: 1406–1415. PMID: 17495049

223. Cobo T, Palacio M, Navarro-Sastre A, Ribes A, Bosch J, Filella X, et al. (2009) Predictive value of combined amniotic fluid proteomic biomarkers and interleukin-6 in preterm labor with intact membranes. Am J Obstet Gynecol 200: 499–6. doi: 10.1016/j.ajog.2008.12.036 PMID: 19375569

224. Gravett MG, Novy MJ, Rosenfeld RG, Reddy AP, Jacob T, Turner M, et al. (2004) Diagnosis of intraamniotic infection by proteomic profiling and identification of novel biomarkers. JAMA 292: 462–469. PMID: 15280344

225. Michaels JE, Dasari S, Pereira L, Reddy AP, Lapidus JA, Lu X, et al. (2007) Comprehensive proteomic analysis of the human amniotic fluid proteome: gestational age-dependent changes. J Proteome Res 6: 1277–1285. PMID: 17373841

226. Park JS, Oh KJ, Norwitz ER, Han JS, Choi HJ, Seong HS, et al. (2008) Identification of proteomic biomarkers of preeclampsia in amniotic fluid using SELDI-TOF mass spectrometry. Reprod Sci 15: 457–468. doi: 10.1177/1933719108316909 PMID: 18579854

227. Park SJ, Yoon WG, Song JS, Jung HS, Kim CJ, Oh SY, et al. (2006) Proteome analysis of human amnion and amniotic fluid by two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proteomics 6: 349–363. PMID: 16294308

228. Romero R, Espinoza J, Rogers WT, Moser A, Nien JK, Kusanovic JP, et al. (2008) Proteomic analysis of amniotic fluid to identify women with preterm labor and intra-amniotic inflammation/infection: the use of a novel computational method to analyze mass spectrometric profiling. J Matern Fetal Neonatal Med 21: 367–388. doi: 10.1080/14767050802045848 PMID: 18570116

229. Ruetschi U, Rosen A, Karlsson G, Zetterberg H, Rymo L, Hagberg H, et al. (2005) Proteomic analysis using protein chips to detect biomarkers in cervical and amniotic fluid in women with intra-amniotic inflammation. J Proteome Res 4: 2236–2242. PMID: 16335971

230. Vuadens F, Benay C, Crettaz D, Gallot D, Sapin V, Schneider P, et al. (2003) Identification of biologic markers of the premature rupture of fetal membranes: proteomic approach. Proteomics 3: 1521–1525. PMID: 12923777

231. Madsen-Bouterse SA, Romero R, Tarca AL, Kusanovic JP, Espinoza J, Kim CJ, et al. (2010) The transcriptome of the fetal inflammatory response syndrome. Am J Reprod Immunol 63: 73–92. doi: 10.1111/j.1600-0897.2009.00791.x PMID: 20059468

232. Wronska A, Kmiec Z. (2012) Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol (Oxf) 205: 194–208.

233. Samaras K, Botelho NK, Chisholm DJ, Lord RV. (2010) Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity (Silver Spring) 18: 884–889.

234. Walker GE, Marzullo P, Verti B, Guzzaloni G, Maestrini S, Zurleni F, et al. (2008) Subcutaneous abdominal adipose tissue subcompartments: potential role in rosiglitazone effects. Obesity (Silver Spring) 16: 1983–1991.

235. Dolinkova M, Dostalova I, Lacinova Z, Michalsky D, Haluzikova D, Mraz M, et al. (2008) The endocrine profile of subcutaneous and visceral adipose tissue of obese patients. Mol Cell Endocrinol 291: 63–70. doi: 10.1016/j.mce.2008.05.001 PMID: 18565643

236. Alvehus M, Buren J, Sjostrom M, Goedecke J, Olsson T. (2010) The human visceral fat depot has a unique inflammatory profile. Obesity (Silver Spring) 18: 879–883.

237. Miranda M, Escote X, Ceperuelo-Mallafre V, Alcaide MJ, Simon I, Vilarrasa N, et al. (2010) Paired subcutaneous and visceral adipose tissue aquaporin-7 expression in human obesity and type 2 diabetes: differences and similarities between depots. J Clin Endocrinol Metab 95: 3470–3479. doi: 10.1210/jc.2009-2655 PMID: 20463097

238. Insenser M, Montes-Nieto R, Vilarrasa N, Lecube A, Simo R, Vendrell J, et al. (2012) A nontargeted proteomic approach to the study of visceral and subcutaneous adipose tissue in human obesity. Mol Cell Endocrinol 363: 10–19. doi: 10.1016/j.mce.2012.07.001 PMID: 22796336

239. Ibrahim MM. (2010) Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev 11: 11–18. doi: 10.1111/j.1467-789X.2009.00623.x PMID: 19656312

240. Avram MM, Avram AS, James WD. (2007) Subcutaneous fat in normal and diseased states 3. Adipogenesis: from stem cell to fat cell. J Am Acad Dermatol 56: 472–492. PMID: 17317490

241. Avram AS, Avram MM, James WD. (2005) Subcutaneous fat in normal and diseased states: 2. Anatomy and physiology of white and brown adipose tissue. J Am Acad Dermatol 53: 671–683. PMID: 16198791

242. Avram MM, Avram AS, James WD. (2005) Subcutaneous fat in normal and diseased states: 1. Introduction. J Am Acad Dermatol 53: 663–670. PMID: 16198790

243. Lefebvre AM, Laville M, Vega N, Riou JP, van GL, Auwerx J, et al. (1998) Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 47: 98–103. PMID: 9421381

244. Mathur SK, Jain P, Mathur P. (2011) Microarray evidences the role of pathologic adipose tissue in insulin resistance and their clinical implications. J Obes 2011: 587495. doi: 10.1155/2011/587495 PMID: 21603273

245. Mutch DM, Tordjman J, Pelloux V, Hanczar B, Henegar C, Poitou C, et al. (2009) Needle and surgical biopsy techniques differentially affect adipose tissue gene expression profiles. Am J Clin Nutr 89: 51–57. doi: 10.3945/ajcn.2008.26802 PMID: 19056587

246. Vidal H. (2001) Gene expression in visceral and subcutaneous adipose tissues. Ann Med 33: 547–555. PMID: 11730162

247. Zha JM, Di WJ, Zhu T, Xie Y, Yu J, Liu J, et al. (2009) Comparison of gene transcription between subcutaneous and visceral adipose tissue in Chinese adults. Endocr J 56: 935–944. PMID: 19564704

248. de Souza Batista CM, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J, et al. (2007) Omentin plasma levels and gene expression are decreased in obesity. Diabetes 56: 1655–1661. PMID: 17329619

249. Van H, V, Dicker A, Ryden M, Hauner H, Lonnqvist F, Naslund E, et al. (2002) Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. Diabetes 51: 2029–2036. PMID: 12086930

250. Guan H, Arany E, van Beek JP, Chamson-Reig A, Thyssen S, Hill DJ, et al. (2005) Adipose tissue gene expression profiling reveals distinct molecular pathways that define visceral adiposity in offspring of maternal protein-restricted rats. Am J Physiol Endocrinol Metab 288: E663–E673. PMID: 15562247

251. varez-Llamas G, Szalowska E, de Vries MP, Weening D, Landman K, et al. (2007) Characterization of the human visceral adipose tissue secretome. Mol Cell Proteomics 6: 589–600. PMID: 17255083

252. Lee YS. (2009) The role of genes in the current obesity epidemic. Ann Acad Med Singapore 38: 45–3. PMID: 19221670

253. Copland JA, Davies PJ, Shipley GL, Wood CG, Luxon BA, Urban RJ. (2003) The use of DNA microarrays to assess clinical samples: the transition from bedside to bench to bedside. Recent Prog Horm Res 58: 25–53. PMID: 12795413

254. Schaffler A, Neumeier M, Herfarth H, Furst A, Scholmerich J, Buchler C. (2005) Genomic structure of human omentin, a new adipocytokine expressed in omental adipose tissue. Biochim Biophys Acta 1732: 96–102. PMID: 16386808

255. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, et al. (2006) Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab 290: E1253–E1261. PMID: 16531507

256. Tan BK, Adya R, Randeva HS. (2010) Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med 20: 143–148. doi: 10.1016/j.tcm.2010.12.002 PMID: 21742269

257. Fain JN, Sacks HS, Buehrer B, Bahouth SW, Garrett E, Wolf RY, et al. (2008) Identification of omentin mRNA in human epicardial adipose tissue: comparison to omentin in subcutaneous, internal mammary artery periadventitial and visceral abdominal depots. Int J Obes (Lond) 32: 810–815.

258. Barth S, Klein P, Horbach T, Dotsch J, Rauh M, Rascher W, et al. (2010) Expression of neuropeptide Y, omentin and visfatin in visceral and subcutaneous adipose tissues in humans: relation to endocrine and clinical parameters. Obes Facts 3: 245–251. doi: 10.1159/000319508 PMID: 20823688

259. Auguet T, Quintero Y, Riesco D, Morancho B, Terra X, Crescenti A, et al. (2011) New adipokines vaspin and omentin. Circulating levels and gene expression in adipose tissue from morbidly obese women. BMC Med Genet 12: 60. doi: 10.1186/1471-2350-12-60 PMID: 21526992

260. Briana DD, Boutsikou M, Baka S, Gourgiotis D, Marmarinos A, Liosi S, et al. (2011) Omentin-1 and vaspin are present in the fetus and neonate, and perinatal concentrations are similar in normal and growth-restricted pregnancies. Metabolism 60: 486–490. doi: 10.1016/j.metabol.2010.04.014 PMID: 20488498

261. Lewandowski K, Nadel I, Lewinski A, Bienkiewicz M, Tan B, Randeva HS, et al. (2010) Positive correlation between serum omentin and thrombospondin-1 in gestational diabetes despite lack of correlation with insulin resistance indices. Ginekol Pol 81: 907–912. PMID: 21391440

262. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. (1994) Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology 135: 798–800. PMID: 8033830

263. Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, et al. (1999) PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell 4: 611–617. PMID: 10549292

264. Apostoli AJ, Nicol CJ. (2012) PPAR Medicines and Human Disease: The ABCs of It All. PPAR Res 2012: 504918. doi: 10.1155/2012/504918 PMID: 22919365

265. Cristancho AG, Lazar MA. (2011) Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 12: 722–734. doi: 10.1038/nrm3198 PMID: 21952300

266. Schupp M, Cristancho AG, Lefterova MI, Hanniman EA, Briggs ER, Steger DJ, et al. (2009) Re-expression of GATA2 cooperates with peroxisome proliferator-activated receptor-gamma depletion to revert the adipocyte phenotype. J Biol Chem 284: 9458–9464. doi: 10.1074/jbc.M809498200 PMID: 19136559

267. Liao W, Nguyen MT, Yoshizaki T, Favelyukis S, Patsouris D, Imamura T, et al. (2007) Suppression of PPAR-gamma attenuates insulin-stimulated glucose uptake by affecting both GLUT1 and GLUT4 in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 293: E219–E227. PMID: 17389706

268. Kinoshita T, Itoh M. (2006) Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. Gynecol Obstet Invest 61: 115–118. PMID: 16272815

269. Sohlstrom A, Forsum E. (1995) Changes in adipose tissue volume and distribution during reproduction in Swedish women as assessed by magnetic resonance imaging. Am J Clin Nutr 61: 287–295. PMID: 7840065

270. Blaudeau TE, Hunter GR, Sirikul B. (2006) Intra-abdominal adipose tissue deposition and parity. Int J Obes (Lond) 30: 1119–1124.

271. Gunderson EP, Sternfeld B, Wellons MF, Whitmer RA, Chiang V, Quesenberry CP Jr., et al. (2008) Childbearing may increase visceral adipose tissue independent of overall increase in body fat. Obesity (Silver Spring) 16: 1078–1084.

272. Bashiri A, Heo HJ, Ben-Avraham D, Mazor M, Budagov T, Einstein FH, et al. (2014) Pregnancy complicated by obesity induces global transcript expression alterations in visceral and subcutaneous fat. Mol Genet Genomics 289: 695–705. doi: 10.1007/s00438-014-0841-5 PMID: 24696292

273. Osterhoff C, Ivell R, Kirchhoff C. (1997) Cloning of a human epididymis-specific mRNA, HE6, encoding a novel member of the seven transmembrane-domain receptor superfamily. DNA Cell Biol 16: 379–389. PMID: 9150425

274. Kirchhoff C, Obermann H, Behnen M, Davies B. (2006) Role of epididymal receptor HE6 in the regulation of sperm microenvironment. Mol Cell Endocrinol 250: 43–48. PMID: 16413110

275. Gottwald U, Davies B, Fritsch M, Habenicht UF. (2006) New approaches for male fertility control: HE6 as an example of a putative target. Mol Cell Endocrinol 250: 49–57. PMID: 16442214

276. Kirchhoff C, Osterhoff C, Samalecos A. (2008) HE6/GPR64 adhesion receptor co-localizes with apical and subapical F-actin scaffold in male excurrent duct epithelia. Reproduction 136: 235–245. doi: 10.1530/REP-08-0078 PMID: 18469038

277. Kirchhoff C, Osterhoff C, Pera I, Schroter S. (1998) Function of human epididymal proteins in sperm maturation. Andrologia 30: 225–232. PMID: 9739419

278. Obermann H, Samalecos A, Osterhoff C, Schroder B, Heller R, Kirchhoff C. et al. (2003) HE6, a two-subunit heptahelical receptor associated with apical membranes of efferent and epididymal duct epithelia. Mol Reprod Dev 64: 13–26. PMID: 12420295

279. Davies B, Kirchhoff C. (2010) Adhesion-GPCRs in the male reproductive tract. Adv Exp Med Biol 706: 179–188. PMID: 21618837

280. Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, et al. (2004) Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. Mol Cell Biol 24: 8642–8648. PMID: 15367682

281. Davies B, Behnen M, Cappallo-Obermann H, Spiess AN, Theuring F, Kirchhoff C. (2007) Novel epididymis-specific mRNAs downregulated by HE6/Gpr64 receptor gene disruption. Mol Reprod Dev 74: 539–553. PMID: 17034053

282. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, et al. (2002) Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 8: 75–79. PMID: 11786910

283. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389: 610–614. PMID: 9335502

284. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, et al. (2004) Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. Diabetes 53: 336–346. PMID: 14747283

285. Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, et al. (2006) Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 12: 650–656. PMID: 16732281

286. Matsuki T, Horai R, Sudo K, Iwakura Y. (2003) IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. J Exp Med 198: 877–888. PMID: 12975454

287. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. (2006) CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest 116: 115–124. PMID: 16341265

288. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, et al. (2002) A central role for JNK in obesity and insulin resistance. Nature 420: 333–336. PMID: 12447443

289. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95: 2409–2415. PMID: 7738205

290. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. (1995) The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95: 2111–2119. PMID: 7738178

291. Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. (1996) The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest 97: 1111–1116. PMID: 8613535

292. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 82: 4196–4200. PMID: 9398739

293. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286: 327–334. PMID: 11466099

294. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 9: 414–417. PMID: 11445664

295. Sartipy P, Loskutoff DJ. (2003) Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci U S A 100: 7265–7270. PMID: 12756299

296. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, et al. (2003) Human epicardial adipose tissue is a source of inflammatory mediators. Circulation 108: 2460–2466. PMID: 14581396

297. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, et al. (1999) C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 99: 237–242. PMID: 9892589

298. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. (1996) C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ 312: 1061–1065. PMID: 8616412

299. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. (1999) Elevated C-reactive protein levels in overweight and obese adults. JAMA 282: 2131–2135. PMID: 10591334

300. Yang RZ, Lee MJ, Hu H, Pollin TI, Ryan AS, Nicklas BJ, et al. (2006) Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med 3: e287. PMID: 16737350

301. Lundgren CH, Brown SL, Nordt TK, Sobel BE, Fujii S. (1996) Elaboration of type-1 plasminogen activator inhibitor from adipocytes. A potential pathogenetic link between obesity and cardiovascular disease. Circulation 93: 106–110. PMID: 8616916

302. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. (2005) Resistin, an adipokine with potent proinflammatory properties. J Immunol 174: 5789–5795. PMID: 15843582

303. Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. (2005) Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochem Biophys Res Commun 334: 1092–1101. PMID: 16039994

304. Verma S, Li SH, Wang CH, Fedak PW, Li RK, Weisel RD, et al. (2003) Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. Circulation 108: 736–740. PMID: 12874180

305. Kusanovic JP, Romero R., Mazaki-Tovi S, Chaiworapongsa T, Mittal P, Gotsch F, et al. (2008) Resistin in Amniotic Fluid and its Association with Intra-amniotic Infection and Inflammation. The Journal of Maternal-Fetal and Neonatal Medicine.

306. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, et al. (2004) Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest 113: 1318–1327. PMID: 15124023

307. Mazaki-Tovi S, Romero R, Kusanovic JP, Erez O, Gotsch F, Mittal P, et al. (2008) Visfatin/Pre-B cell colony-enhancing factor in amniotic fluid in normal pregnancy, spontaneous labor at term, preterm labor and prelabor rupture of membranes: an association with subclinical intrauterine infection in preterm parturition. J Perinat Med.

308. Ognjanovic S, Bryant-Greenwood GD. (2002) Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes. Am J Obstet Gynecol 187: 1051–1058. PMID: 12389004

309. White RT, Damm D, Hancock N, Rosen BS, Lowell BB, Usher P, et al. (1992) Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. J Biol Chem 267: 9210–9213. PMID: 1374388

310. Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, et al. (1996) Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci U S A 93: 14564–14568. PMID: 8962092

311. Matarese G, Moschos S, Mantzoros CS. (2005) Leptin in immunology. J Immunol 174: 3137–3142. PMID: 15749839

312. Tian Z, Sun R, Wei H, Gao B. (2002) Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. Biochem Biophys Res Commun 298: 297–302. PMID: 12413939

313. Zhao T, Hou M, Xia M, Wang Q, Zhu H, Xiao Y, et al. (2005) Globular adiponectin decreases leptin-induced tumor necrosis factor-alpha expression by murine macrophages: involvement of cAMP-PKA and MAPK pathways. Cell Immunol 238: 19–30. PMID: 16438946

314. Howard JK, Lord GM, Matarese G, Vendetti S, Ghatei MA, Ritter MA, et al. (1999) Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. J Clin Invest 104: 1051–1059. PMID: 10525043

315. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394: 897–901. PMID: 9732873

316. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. (1999) Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 100: 2473–2476. PMID: 10604883

317. Saijo S, Nagata K, Nakano Y, Tobe T, Kobayashi Y. (2005) Inhibition by adiponectin of IL-8 production by human macrophages upon coculturing with late apoptotic cells. Biochem Biophys Res Commun 334: 1180–1183. PMID: 16039610

318. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. (2004) Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochem Biophys Res Commun 323: 630–635. PMID: 15369797

319. Yamaguchi N, Argueta JG, Masuhiro Y, Kagishita M, Nonaka K, Saito T, et al. (2005) Adiponectin inhibits Toll-like receptor family-induced signaling. FEBS Lett 579: 6821–6826. PMID: 16325814

320. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. (2000) Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 96: 1723–1732. PMID: 10961870

321. Yokota T, Meka CS, Kouro T, Medina KL, Igarashi H, Takahashi M, et al. (2003) Adiponectin, a fat cell product, influences the earliest lymphocyte precursors in bone marrow cultures by activation of the cyclooxygenase-prostaglandin pathway in stromal cells. J Immunol 171: 5091–5099. PMID: 14607907

322. Okamoto Y, Folco EJ, Minami M, Wara AK, Feinberg MW, Sukhova GK, et al. (2008) Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. Circ Res 102: 218–225. PMID: 17991878

323. Dandona P, Weinstock R, Thusu K, bdel-Rahman E, Aljada A, Wadden T. (1998) Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. J Clin Endocrinol Metab 83: 2907–2910. PMID: 9709967

324. Naccasha N, Gervasi MT, Chaiworapongsa T, Berman S, Yoon BH, Maymon E, et al. (2001) Phenotypic and metabolic characteristics of monocytes and granulocytes in normal pregnancy and maternal infection. Am J Obstet Gynecol 185: 1118–1123. PMID: 11717644

325. Soto E, Romero R, Vaisbuch E, Erez O, Mazaki-Tovi S, Kusanovic JP, et al. (2010) Fragment Bb: evidence for activation of the alternative pathway of the complement system in pregnant women with acute pyelonephritis. J Matern Fetal Neonatal Med 23: 1085–1090. doi: 10.3109/14767051003649870 PMID: 20218820

326. Lappas M, Permezel M, Rice GE. (2005) Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-kappaB, peroxisomal proliferator-activated receptor-gamma and extracellularly regulated kinase 1/2. Endocrinology 146: 3334–3342. PMID: 15905315

327. de CJ, Sevillano J, Marciniak J, Rodriguez R, Gonzalez-Martin C, Viana M, et al. (2011) Implication of low level inflammation in the insulin resistance of adipose tissue at late pregnancy. Endocrinology 152: 4094–4105. doi: 10.1210/en.2011-0068 PMID: 21914778

328. Zhang L, Sugiyama T, Murabayashi N, Umekawa T, Ma N, Kamimoto Y, et al. (2011) The inflammatory changes of adipose tissue in late pregnant mice. J Mol Endocrinol 47: 157–165. doi: 10.1530/JME-11-0030 PMID: 21697073

329. Resi V, Basu S, Haghiac M, Presley L, Minium J, Kaufman B, et al. (2012) Molecular inflammation and adipose tissue matrix remodeling precede physiological adaptations to pregnancy. Am J Physiol Endocrinol Metab 303: E832–E840. doi: 10.1152/ajpendo.00002.2012 PMID: 22811467

330. Rehrer CW, Karimpour-Fard A, Hernandez TL, Law CK, Stob NR, Hunter LE, et al. (2012) Regional differences in subcutaneous adipose tissue gene expression. Obesity (Silver Spring) 20: 2168–2173.

331. Rebuffe-Scrive M, Enk L, Crona N, Lonnroth P, Abrahamsson L, Smith U, et al. (1985) Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy, and lactation. J Clin Invest 75: 1973–1976. PMID: 4008649

332. Moller-Levet CS, Betts GN, Harris AL, Homer JJ, West CM, Miller CJ. (2009) Exon array analysis of head and neck cancers identifies a hypoxia related splice variant of LAMA3 associated with a poor prognosis. PLoS Comput Biol 5: e1000571. doi: 10.1371/journal.pcbi.1000571 PMID: 19936049

333. Gardina PJ, Clark TA, Shimada B, Staples MK, Yang Q, Veitch J, et al. (2006) Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. BMC Genomics 7: 325. PMID: 17192196

334. Thorsen K, Sorensen KD, Brems-Eskildsen AS, Modin C, Gaustadnes M, Hein AM, et al. (2008) Alternative splicing in colon, bladder, and prostate cancer identified by exon array analysis. Mol Cell Proteomics 7: 1214–1224. doi: 10.1074/mcp.M700590-MCP200 PMID: 18353764

335. Johnson JM, Castle J, Garrett-Engele P, Kan Z, Loerch PM, Armour CD, et al. (2003) Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. Science 302: 2141–2144. PMID: 14684825

336. Clark TA, Schweitzer AC, Chen TX, Staples MK, Lu G, Wang H, et al. (2007) Discovery of tissue-specific exons using comprehensive human exon microarrays. Genome Biol 8: R64. PMID: 17456239

337. Kampa D, Cheng J, Kapranov P, Yamanaka M, Brubaker S, Cawley S, et al. (2004) Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22. Genome Res 14: 331–342. PMID: 14993201

338. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, et al. (2005) Function of alternative splicing. Gene 344: 1–20. PMID: 15656968

339. Lareau LF, Inada M, Green RE, Wengrod JC, Brenner SE. (2007) Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. Nature 446: 926–929. PMID: 17361132

340. Ni JZ, Grate L, Donohue JP, Preston C, Nobida N, O'Brien G, et al. (2007) Ultraconserved elements are associated with homeostatic control of splicing regulators by alternative splicing and nonsense-mediated decay. Genes Dev 21: 708–718. PMID: 17369403

341. Venables JP. (2006) Unbalanced alternative splicing and its significance in cancer. Bioessays 28: 378–386. PMID: 16547952