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Pregnancy Complicated by Obesity Induces Global Transcript Expression Alterations in Visceral and Subcutaneous Fat

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Abstract

Maternal obesity is a significant risk factor for development of both maternal and fetal metabolic complications. Increase in visceral fat and insulin resistance is a metabolic hallmark of pregnancy, yet little is known how obesity alters adipose cellular function and how this may contribute to pregnancy morbidities. We sought to identify alterations in genome-wide transcription expression in both visceral (omental) and abdominal subcutaneous fat deposits in pregnancy complicated by obesity. Visceral and abdominal subcutaneous fat deposits were collected from normal weight and obese pregnant women (n=4/group) at time of scheduled uncomplicated cesarean section. A genome-wide expression array (Affymetrix Human Exon 1.0 st platform), validated by quantitative real-time PCR, was utilized to establish the gene transcript expression profile in both visceral and abdominal subcutaneous fat in normal weight and obese pregnant women. Global alteration in gene expression was identified in pregnancy complicated by obesity. These regions of variations lead to identification of indolethylamine N-methyltransferase (INMT), tissue factor pathway inhibitor-2 (TFPI-2), and ephrin type-B receptor 6 (EPHB6), not previously associated with fat metabolism during pregnancy. In addition, subcutaneous fat of obese pregnant women demonstrated increased coding protein transcripts associated with apoptosis compared to lean counterparts. Global alteration of gene expression in adipose tissue may contribute to adverse pregnancy outcomes associated with obesity.

Keywords

gene expression; subcutaneous; omental; obesity; pregnancy

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Introduction

By 2030, over 2.16 billion people worldwide are projected to be overweight and an additional 1.12 million people will be obese (Kelly et al. 2008; Han et al. 2010). Obesity inflicts adverse health consequences, such as type 2 diabetes mellitus (T2DM), cardiovascular, respiratory, musculoskeletal, reproductive, and psychological complications (Catalano and Ehrenberg 2006). Increased maternal and fetal morbidities have also been attributed to the parallel rise in prevalence of maternal obesity (Guelinckx et al. 2008). Pre-pregnancy obesity is associated with increased maternal risk of infertility, miscarriage, pregnancy-related hypertension, preeclampsia, gestational diabetes, venous thromboembolism, and cesarean deliveries (Wang et al. 2002; Barau et al. 2006; Ehrmann et al. 2006; Frederick et al. 2006; Larsen et al. 2007). Fetal morbidities can also occur during all developmental phases and includes increased risk of stillbirth, perinatal death, fetal anomalies, excessive fetal growth, and significant long-term health implications (Catalano 2003; Boney et al. 2005; Kristensen et al. 2005; Barau et al. 2006; Catalano and Ehrenberg 2006). Fetal intrauterine exposure to maternal obesity also leads to increased risk of developing childhood obesity, metabolic syndrome and age related diseases later on in life with subsequent generations being affected earlier and more severely by a disorder (Catalano 2003; Boney et al. 2005; Catalano and Ehrenberg 2006). In addition, obesity in combination with diabetes would only further acts synergistically to enhance these morbidities (Moore et al. 2000; Catalano 2003; Callaway et al. 2006; Catalano and Ehrenberg 2006). Despite efforts to understand this major public health concern, the characteristic maternal metabolic profile that mediates the adverse intrauterine environment has not been well identified.

Normal pregnancy results in significant accretion of visceral fat and associated accumulation of hepatic triglycerides, both important contributors to pregnancy-related hepatic insulin resistance (Bjorntorp 1991). Little is known about the biology of adipose tissue in pregnancy and how the rapid expansion of adipose tissue influences the metabolic homeostasis in the mother. More importantly, much less is known of the effects of increase in maternal visceral fat secondary to obesity in pregnancy. Visceral fat has been shown to have greater impact compared to subcutaneous fat in altering insulin action (Bjorntorp 1991; Kissebah 1991; Rexrode et al. 1998). Longitudinal human studies demonstrate a higher risk of developing impaired glucose tolerance, T2DM and coronary heart disease in subjects with greater visceral adiposity (Atzmon et al. 2002). Further, decreases in visceral fat with weight loss has been associated with increases in insulin sensitivity, favorable lipid profiles (high HDL and low triglycerides) and reduced blood pressure, emphasizing the physiologic significance of this tissue. The importance of characteristic differences between subcutaneous and visceral fat was also exemplified in animals studies demonstrating approximately 20% variation in global gene expression (Einstein et al. 2005), with visceral fat demonstrating more robust changes in fat derived peptides (FDP) expression in response to nutrients (Okoniewski et al. 2007a).

Although there is abundant evidence concerning characterization of subcutaneous and visceral fat and their metabolic relevance in non-pregnant individuals, such information is

currently spare with respect to pregnancy. In this study, we applied a validated human genome-wide approach (Affymetrix Human Exon 1.0 ST Array)(Okoniewski et al. 2007b) to interrogate variation in gene expression between visceral (omental) and subcutaneous (abdominal) adipose tissue collected from normal weight and obese pregnant women at time of scheduled cesarean delivery to determine if obesity significantly alters adipose gene expression. The GeneChip array employed in this study contains approximately 5.5 million probes, targeting approximately 1.2 million known and predicted exons(Okoniewski et al. 2007a). Rather than focus on known candidate loci, we used an unbiased approach to identify potentially novel loci and then validated our exon array data with quantitative real time PCR in order to ascertain the dysregulatory effect of obesity in pregnancy on adipose tissue expression.

Materials and Methods

This study was approved by the institutional review board (IRB) of Soroka University Medical Center and the Committee on Clinical Investigation at the Albert Einstein College of Medicine. Written informed consent was obtained from all subjects prior to participation in accordance with regulatory guidelines.

Clinical Data Collection and Identification of Cases and Controls

Biological samples and clinical information were collected from consenting women who underwent cesarean delivery based on the clinical judgment of women's health care provider at Soroka University Medical Center during 2010. Maternal pre-pregnancy body mass index (BMI) was used to identify cases and controls. Normal weight and obesity were defined as pre-pregnancy BMI between 18.9 to 25.0 kg/m² and BMI >30 kg/m² respectively. Pertinent clinical data, such as maternal medical co-morbidities, pregnancy complications and neonatal outcomes, were collected. Glucose tolerance test (GTT) was also administrated month throughout the pregnancy period to rule out pregestational and gestational diabetes in participating subjects. Pregnant women with abnormal glucose tolerance screening, preterm delivery less than 36 weeks' gestation and those carrying a fetus with a known congenital anomaly, multiple gestations, or intrauterine fetal demise were excluded from the study.

Fat Tissue Collection

At the time of scheduled cesarean delivery approximately 10g of abdominal (subcutaneous) fat and 10g of omental (visceral) fat were collected after closure of the uterus and assurance of hemostasis. Specimens were placed immediately in sterile protected containers and stored in liquid nitrogen until they were transferred to a -80°C freezer.

RNA extraction and labeling

Total RNA was extracted from both subcutaneous (SC) and visceral fat (VF) depots by direct homogenization in 1.0 ml of Trizol reagent (Life Technologies, Grand Island, NY). The isolation process was performed in accordance with the manufacturer's recommended protocol. Subsequent purification methods included silica-column-based procedures using RNeasy columns (Qiagen, Valencia, CA) according to manufacturer's protocol. Confirmation of RNA quality was assessed using Agilent 2100 Bioanalyzer. All samples

had high quality and showed no signs of DNA contamination or RNA degradation. RNA samples were immediately frozen and stored at 80°C until further analysis.

Chip Hybridization and Expression

The Affymetrix Human Exon 1.0 ST Array (Lockstone 2011), a validated high-density gene expression microarray platform with close to 1.2 million probe sets, was used in this study. cDNA was generated with GeneChip WT cDNA Synthesis and Application Kit (Affymetrix) as per manufacturer's recommendation. GeneChip WT Terminal Labeling Kit (Affymetrix) was used to end label fragmented cDNA. Approximately 6.0ug of cDNA was hybridized to the Affymetrix Gene Chip Human Exon 1.0 ST Array. Distribution of the transcript cluster differentially expressed between VF and SC in normal weight patients and obese patients were identified.

Data processing (Einstein et al. 2010)

Raw signal intensity data provided in .CEL files was processed in *JMP* genomic to generate normalized (including chip to chip adjustment by implementing Robust Multi-array Average (RMA) procedure) exon- and gene-level intensity estimates. Probesets were further grouped into transcript clusters enabling a gene-level estimate to be computed by summarizing data from all probes within the transcript cluster. Our selection was restricted to the original transcript signals as they are more reliable and are recommended for the gene-level estimates. For quality control, we adopted the Affymetrix quality assessment. Briefly, high mean absolute deviations (MAD) were interrogated to identify single arrays that could skew results since the number of arrays is small. Bonferroni tests were used for multiple comparisons correction setting a strict p-value ($p < 1.9 \times 10^{-7}$) to minimal false positive results.

Validation of chip hybridization assays using quantitative real time PCR analysis

The next component of our two-stage experimental design was to validate the Affymetrix Human *Exon* 1.0 ST Array results with quantitative real-time PCR experiments to confirm the degree of differences in expression within the loci. Utilizing the same samples from the initial array assay, we applied quantitative real-time PCR experiments with LightCycler 480 systems (Roche) and Sybergreen master mix (Roche) with the capacity to run 384 samples simultaneously. All primers were synthesized by Invitrogen and were designed with PRIMER 3 software (<http://frodo.wi.mit.edu/primer3/input.htm>). The default parameters of the program were: product size 150–600, primer size 18–25, Tm 55–62 with max difference of 2°C. The mRNA copy number for each unknown sample was calculated from the standard curve of serial dilution of a known sample's concentration by the instrument software. The LightCycler software determined a threshold cycle (Ct), which represented the lowest number of cycles necessary to measure a fluorescent signal that is statistically significant above background. Samples were run in duplicate. The Ct values were normalized against GAPDH expression. Ct variations (Ct) are expressed as the differences between Ct Normal and Ct Obese. To analyze the absolute variation of Ct values, we calculated the mean and the standard error of the mean of absolute values of Ct variation for each gene.

Ingenuity Pathway Analysis

After qRT-PCR validation, the most significantly differentially expressed loci were mapped to RefSeq gene identifiers by position within 10 kb upstream/downstream of the gene body. The List of RefSeq identifiers was then uploaded to the Ingenuity Pathway Analysis (IPA) software (Redwood City, CA) to explore potential molecular interaction networks. Core networks were constructed for both direct and indirect interactions using default parameters and the focus genes with the highest connectivity to other locus genes were selected as seed elements for network generation (Jacob and Presti 2005). The ranking score for each network was then computed by a right-tailed Fisher's exact test as the negative log of the probability that the number of focus genes in the network is not due to random chance. Similarly significances for functional enrichment of specific genes were also determined by the right-tailed Fisher's exact test, using all input genes as a reference set.

Statistical Analysis

Descriptive statistics (chi-square tests for categorical variables and independent samples t-test for continuous variables) was used (JMP genomics v 6) to describe differences between VF and SC fat. We applied STD normalization (standardizes values to the mean with a scale equivalent to the standard deviation) followed by ANOVA and set the false discovery rate (FDR) of 0.1 -level. The Bonferroni test was used for multiple comparison correction in both the initial unbiased genomic expression screening with the chip array and the validation of the candidate genes with qRT-PCR.

Results

Gestational and Neonatal Clinical Parameters in Obese and normal weight controls

Abdominal fat (SC) and omental fat (VF) samples were collected from obese ($n = 4$) and normal weight ($n = 4$) consenting individuals, matched for gestational age at delivery, infant gender, and ethnicity. By design, obese subjects had higher BMI compared to controls (31.2 ± 1.1 vs 22.8 ± 0.7 kg/m², $p < 0.01$) and weighed more at time of delivery (96.5 ± 5.7 vs 67.5 ± 7.7 kg, $p < 0.05$) (Table 1). Total maternal weight gain and birth weight were not significantly different between groups. In all cases, cesarean deliveries were uncomplicated and all of the participants and their infants were healthy. None of the participants have significant co-morbidities, such as hypertensive disorders, diabetes, chronic renal disease or autoimmune disorders.

Patterns of Gene Expression in Obese and Normal Weight Subjects

To detect variations in patterns of global gene expression in subcutaneous and omental fat of both normal weight and obese pregnant women, we performed the Affymetrix Human Exon 1.0 ST array on the collected samples of subcutaneous and omental fat. Figure 1 represents a heat map showing the top 479 loci with fat specific variations in transcripts created using an unsupervised clustering analysis. The heatmap reveals not only clear differences between VF and SC fat in normal weight women, but also changes associated with maternal obesity. Of the total 265,000 transcript sites screened, transcript expression variation between subcutaneous and omental fat reached a significant threshold in 89 loci in the normal weight

group (Table 1s) and 202 in the obese group (Table 2s) with $p < 1.9 \times 10^{-7}$ using multiple comparison analysis. In Figure 2, we show volcano plots demonstrating the distributions of visceral vs subcutaneous fat transcript expression variations along with the degree of significance associated with each of these changes for both normal weight and obese women. Using stringent thresholds to determine the most widely divergent and highly significant loci in each tissue, we found that in normal weight women, visceral fat demonstrates higher overall transcript expression compared to subcutaneous fat (64 vs 25 transcripts, $p < 0.003$) (Figure 2). In obese women, overall expression between subcutaneous and omental fat was not significantly different (115 vs 87, $p = \text{NS}$), demonstrating that while more gene loci are altered in obese pregnant women, the over expression in visceral fat that was demonstrated in normal weight women was not longer seen.

Of the sites that reached the statistical threshold for variation in gene expression between visceral and subcutaneous fat, 36 overlapping transcripts were identified in both normal weight and obese pregnant groups (Figure 1s, Tables 2 and 3s) and again a tendency toward over expression in visceral compared to subcutaneous fat (27 vs 9, $p < 0.03$) was found. To confirm the exon array data, we performed a quantitative real time PCR analysis on the thirty-six overlapping transcripts. A total of 3 genes were identified that passed significance for the strict multiple comparison threshold in gene expression (Figure 3). Two loci (INMT, TFPI2) with variable gene expression between subcutaneous and visceral fat were found in the normal weight pregnant group and one locus (EPHB6) in the obese group ($p < 0.01$) (Figure 3). All three loci demonstrated over expression in visceral compared to subcutaneous fat ($p < 0.01$).

Molecular Interaction Network Analysis of Genes

Using Ingenuity Pathway Analysis (IPA) software (Redwood City, CA), we carried out a network analysis to investigate the functional role and significance of the three identified genes (Figure 4, Table 2). The Network analysis of the validated genomic candidates revealed several predominant pathways highly important in embryonic development, as well as cellular and tissue function and regulation (Figures 2s and Figure 3s). TFPI2 promotes regulation of cell-to-cell signaling and interaction, as well as cellular growth and proliferation. INMT is also important for cellular development and function and in addition plays an important role in cell cycle and proliferation. These genes were typically over expressed in visceral fat of normal weight pregnant women, yet they appear to be suppressed in visceral fat of obese women potentially implicating deregulation of cell-to-cell signaling and cellular growth in visceral fat of obese individuals in pregnancy.

EPHB6 is important in cellular signaling, growth and proliferation and is also deregulated in obese pregnant women with over expression in the visceral compared to subcutaneous fat. However, in this specific case, the significant alterations appears to be attributable to the decrease in expression in the subcutaneous adipose of the obese women compared to subcutaneous fat of normal weight group ($p < 0.05$) (Figure 3).

Differential Patterns of Subcutaneous Gene Expression in Obese and Normal Weight Subjects

Variations in patterns of transcript expression in subcutaneous fat of normal weight and obese pregnant women were further assessed. Following multiple comparison adjustment, fourteen transcripts were identified, with majority having significantly higher expression in the obese group compared to the lean. Of these, 6 were of coding proteins: ATP-Binding Cassette, SubFamily C (CFTR/MRP), Member 3 (ABCC3), Chromosome 9 Open Reading Frame 116 (C9orf116), NAD(P)H Dehydrogenase Quinone 1 (NQO1), Toll-Like Receptor 7 (TLR7), Triggering Receptor Expressed On Myeloid Cells 2 (TREM2), and Parvin Gamma (PARVG) (Table 3). The Network analysis of these genomic candidates, using Ingenuity Pathway Analysis (IPA) software (Redwood City, CA), revealed alterations in shared pathways mediating cellular function, molecular transport and nucleic acid metabolism in subcutaneous fat of obese pregnant women (Figure 4). TNF appeared as central effector gene in these pathways also implicating dysfunctional cellular apoptosis as a mediator of abnormal cellular metabolism.

Discussion

We present evidence of alterations in global gene expression in subcutaneous and visceral fat of obese pregnant women using a genome-wide expression array, which has not previously been employed to aid in the study of maternal adipose tissue biology. In normal weight women with a term pregnancy, a tendency for over expression of gene transcripts in visceral fat compared to subcutaneous fat (62 VF vs 25 SC) reaffirms the importance of visceral adiposity in metabolic regulation during normal pregnancy. In obesity complicating pregnancy, a greater global variation in transcript expression in both subcutaneous and visceral fat is seen compared to normal weight controls. The over expression of transcripts in the subcutaneous fat appear to dramatically reduce the dominance in expression in visceral fat (115 VF vs 87 SC) in the obese patients as compared to normal weight controls.

Using real time PCR of the top 36 transcripts identified by the expression array, we validated over expression of INMT and TFPI2 genes in visceral compared to subcutaneous fat in normal weight pregnant women and over expression of EPHB6 in the obese group. Network analysis of the overlapping transcripts in both groups shows important involvement of the two genes in embryonic, cellular, and nervous system development and function. Indolethylamine N-methyltransferase (INMT) is implicated in cell cycle regulation and replication by serving as a methylation catalyst for tryptamine during the production of N-dimethyltryptamine (Larkin et al. 2012) and is associated with pathways of neuromodulation and neurotransmission. More recent studies have demonstrated a link between decreased expression of INMT and cancers. Larkin et al. found decrease expression of INMT in more aggressive prostate cancers (Kopantzev et al. 2008). In another study, cancerous lung tissue had decreased expression of INMT compared to normal lung (Catalano et al. 2012; Djelantik et al. 2012; McIntyre et al. 2012). Although the precise mechanism is unknown, ingenuity pathway analysis (IPA), <http://www.ingenuity.com/> reveals molecular interaction between INMT and E2F-1, a transcription activator found in the promoter region of a number of genes with products that are involved in cell cycle regulation and DNA

replication. INMT also mediates both cell proliferation and p53-dependent apoptosis. Further, INMT also interacts with CCAAT/enhancer binding protein beta (CEBPB), another important transcriptional activator involved in regulation of immune and inflammatory response genes. Visceral adipose tissue of obese pregnant women demonstrate a loss of normal INMT transcript over expression, which may lead to dysregulated proliferation of adipocytes and increase in inflammatory responses, and ultimately contribute to adverse pregnancy outcomes associated with obesity (Pou et al. 2011). Further, Nuño-Ayala et al. also demonstrated the importance of increase INMT expression with pregnancy maintenance while decrease in expression was associated with pregnancy loss (Nuno-Ayala et al. 2012).

Tissue factor pathway inhibitor-2 (TFPI-2) is an extracellular matrix-associated broad-spectrum Kunitz-type serine proteinase inhibitor and is another important modulator of immune and inflammatory response. TFPI-2 has also been associated with coagulation and fibrinolysis regulation (Zawadzki et al. 2009). TFPI-2 interferes with the tissue factor (TF) pathway through inhibition of the TF-factor VIIa complex and reduced expression has been linked to many pathophysiological processes, including atherosclerosis and tumor growth (Zhang et al. 2012), (Xiong et al. 2010). Lower maternal plasma levels of TFPI-2 have been associated with preeclampsia (Matsuoka et al. 1997; Hafner et al. 2004). Therefore, the loss of the typical over expression of TFPI-2 in visceral fat of obese pregnant women may signify a disassociation of cellular signaling and abnormal interaction and may manifest clinically as adverse pregnancy outcomes associated with maternal obesity.

Ephrin type-B receptor 6 (EPHB6) is a kinase-defective receptor that binds to ephrin-B1 and ephrin-B2 and modulates cell adhesion and migration, and inhibits JNK activation, T cell receptor-induced IL-2 secretion and CD25 expression (<http://www.genecards.org/>). EPHB6 is typically abundantly expressed in normal tissues with the strongest presence observed in the human thymus, brain, and pancreas (Tang et al. 2000; Muller-Tidow et al. 2005; Fox and Kandpal 2009; Truitt and Freywald 2011). EPHB6 expression is inversely linked to progression of several solid malignances in human (Truitt et al. 2010; Truitt and Freywald 2011). In addition EPHB6 has also been implicated in intercellular communication during embryonic development. In our study, the over expression of EPHB6 in visceral fat compared to subcutaneous fat was only seen in the obese group. However, the significant variation appears to be attributed to the decrease in expression in the subcutaneous fat of the obese group compared to that in the normal weight group ($p < 0.05$) (Figure 2). In pregnancy complicated by obesity, subcutaneous fat may play a more metabolically active role. The loss of expression of EPHB6 in the subcutaneous fat may promote cellular dysregulation analogous to metastatic cells by altering cellular signaling, growth and proliferation that is not apparent in normal weight pregnant women counterparts. It has also been demonstrated that restoration of EPHB6 expression in metastatic breast cancer cells reduces their invasive activity. EPHB6 has been identified as a potential prognostic marker and target for therapeutic approaches for various cancers and may ultimately lead to interventions for gestational diabetes and pregnancy complications associated with obesity in pregnancy.

Further comparison of subcutaneous fat between obese and normal weight pregnant women also demonstrated significant differences in gene transcripts, with prevalence of cell surface regulation elements. Interestingly, all of the 6 coding protein transcripts identified were

much more highly expressed in obese pregnant women compared to lean controls. Also, these transcripts all shared central gene (TNF) associated with apoptosis. Dysfunctional cellular apoptosis may contribute to abnormal cellular metabolism leading to maternal and fetal complications.

Fat cells turn over throughout the lifespan, and their characteristics have significant impact on fat tissue growth, plasticity, function, and distribution (Tchkonia et al. 2005). Pregnancy is associated with rapid expansion of fat deposits with metabolic sequelae. Studies have demonstrated that human subcutaneous and omental preadipocytes, which comprise 15–50% of cells in fat tissue (Cartwright et al. 2010), consists of two subtypes of preadipocytes with varying replicative and differentiating capacities (Tchkonia et al. 2005). Subcutaneous fat typically consists of higher ratio of preadipocytes with rapidly replicating and differentiating subtype compared to omental fat (Tchkonia et al. 2005). The observed transcript expression differences seen in both subcutaneous and visceral fat of obese pregnant women may represent differences in these preadipocytes cell dynamic and properties that alter their function and contributes to metabolic dysregulation that negatively influences both mother and the fetus.

Maternal obesity is a significant risk factor for development of both maternal and fetal metabolic complications. Increase in visceral fat and insulin resistance is a metabolic hallmark of pregnancy, yet little is known how obesity alters adipose cellular function and how this may contribute to pregnancy morbidities. Although our study sample size was small, given the small variability between the samples, we were able to identified potential genes that are dysregulated in the adipose tissue of obese pregnant women. This study represents one of the first such genome-wide surveys of the genomic expression in subcutaneous and visceral fat of normal weight and obese pregnant women demonstrating significant differences. Future human studies will be needed to access variations in subcutaneous and visceral fat in normal weight and obese women outside of pregnancy to further distinguish changes in transcript expression associated with obesity or pregnancy itself. However, we propose that these observed changes in transcript expression in obese pregnant women, regardless of a pregnancy or obesity related phenomenon, may contribute to pregnancy complications and requires further exploration. Continued investigation into changes in adipose tissue biology may provide further insights and lead to potential targets for therapeutic interventions to prevent the development of gestational diabetes and other pregnancy complications associated with pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Heat map representing global fat depot-specific differences in gene expression of normal weight and obese pregnant women

A heat map of the top 479 loci with significant differential expression is shown, with each row corresponding to data from a single locus, and each column representing a fat depot (o=omental and s=subcutaneous) obtained from each subject. The branching dendrogram at the bottom was generated from unsupervised clustering demonstrates relatedness of specific fat depots. Gene expression profiles show differentiation of omental and subcutaneous fat depots, as well as maternal BMI category in both fat depots.

Figure 2. Volcano plot representations to assess fat depot-specific changes in global gene expression measured by exon gene array in both normal and obese pregnant women
Volcano plots reveal differences in gene expression in omental and subcutaneous fat in both normal weight (A) and obese (B) pregnant women. Every locus analyzed corresponds to a dot, showing the average differences between subcutaneous and omental fat (on x-axis, values below zero represent higher expression of the omental fat, above zero higher expression of subcutaneous fat) with negative log-transformed significance (p-values) along the y-axis. Significance threshold was set at $-\log p$ value of $<1.9 \times 10^{-7}$ represented by the bold line and 0.05 the dash line.

Figure 3. Validated candidate genes with differential expression

Of the thirty-five transcripts identified on the human expression array, three candidate genes were validated to have differential gene expression using qRT-PCR in omental and subcutaneous fat and strict significance thresholds for multiple comparisons. These values were normalized with the housekeeping gene, GAPDH. NO-Normal weight Omental, NS-Normal weight Subcutaneous, OO-Obese Omental, OS-Obese Subcutaneous, * $p < 0.01$.

Figure 4. Ingenuity Pathway Analysis (IPA) from the top validated candidate genes reveal that many of the most differentially expressed genes form a molecular interaction network of particular relevance to cellular and tissue development

RefSeq identifiers from the 3 top sites (TFP12, EPHB6 and INMT) that mapped to gene bodies and promoters were analyzed using the “Core Analysis” tool of IPA.

Figure 5.
Ingenuity Pathway Analysis (IPA) of the top significant coding subcutaneous transcripts between Obese and normal pregnant women.

Table 1

Pregnancy and delivery characteristics of the mother and the newborn.

Subject #	Pre-Pregnancy BMI	Height (cm)	Maternal Weight (kg)			Infant	
			Before	End(BMI)	Gained	Gender	Birth Weight (g)
1	22.6	160	58	66(25.8)	8	M	2670
2	23.8	175	73	89(29.1)	16	F	2930
3	24	162	52	63(24.0)	11	M	2913
4	20.8	155	50	52(21.6)	2	M	2605
5	31.2	160	80	87(34.0)	7	M	2610
6	29.4	168	83	93(33.0)	10	M	3155
7	30.1	166	83	93(33.7)	10	M	3710
8	34.4	160	88	113(44.1)	25	F	4065

Top thirty-six candidate genes with the greatest variation in expression between subcutaneous and omental fat in both normal and obese pregnant women.

Table 2

Chr.	start	Gene code	Mean-Normal		Mean-Obese		-log (P value) O vs. S	
			Omental	Sub-Q	Omental	Sub-Q	Normal	Obese
1	62193452	INADL	336	323	356	291	12.75	9.56
1	107682639	NTNG1	263	173	298	162	10.47	10.61
1	178613738	RALGPS2	262	265	302	243	10.48	9.31
1	120404221	NOTCH2	1097	2003	1387	2048	10.95	8.04
1	196151109	KCNT2	439	201	432	180	11.67	9.18
2	150186499	LYPD6	253	194	261	169	10.84	8.93
3	118930508	B4GALT4	319	326	330	263	8.46	6.92
3	165471637	BCHE	476	177	383	123	11.68	6.90
4	35949853	ARAP2	291	256	310	224	9.35	7.29
4	176554108	GPM6A	259	198	267	166	15.67	13.36
5	78985667	CMYA5	262	252	321	251	8.50	9.08
6	165731167	PDE10A	282	271	331	246	8.31	9.86
7	30791767	INMT*	2492	673	2958	743	10.48	13.30
7	93,514,709	TFPI2 (close proximity)*	329	137	243	111	8.20	3.57
7	142540198	EPHB6*	438	396	514	363	12.66	16.67
7	103112243	RELN	350	274	383	207	8.16	10.45
8	110547872	---	369	120	344	107	11.72	9.16
8	120569329	ENPP2	1022	2428	957	2395	10.72	13.27
9	94672188	---	279	197	264	138	8.63	7.25
11	15922881	SOX6	256	233	295	212	7.99	7.41
11	35453383	PAMR1	534	325	604	249	9.88	13.79
12	8789967	MFAP5	829	3176	2038	4033	9.19	7.32
12	21949846	ABCC9	914	2259	1084	2238	10.55	9.62
12	44771321	NELL2	306	200	325	186	12.10	9.91
13	22242806	FGF9	262	226	314	204	11.89	13.89
13	78049977	SCEL	292	136	304	118	8.14	7.58

Chr.	start	Gene code	Mean-Normal		Mean-Obese		-log (P value) O vs. S	
			Omental	Sub-Q	Omental	Sub-Q	Normal	Obese
13	110818599	COL4A2	819	1699	1191	2151	11.39	18.57
13	110801320	COL4A1	940	2144	1157	2260	12.59	13.68
14	26884748	NOVA1	349	850	433	812	9.56	9.27
14	95837639	C14orf49	470	941	674	1099	6.96	10.21
15	57668676	CGNL1	319	283	330	224	14.91	13.85
17	57409040	YPEL2	463	847	589	862	7.54	8.78
18	53579716	---	331	251	399	204	6.89	9.56
19	49141214	CA11	398	347	412	300	8.62	7.06
19	51322404	KLK1	279	260	300	215	8.21	6.88
X	102659770	LOC100506901	213	244	242	205	13.66	12.84

The highlighted rows correspond to transcripts with over expression of subcutaneous fat compared to omental fat, while the un-highlighted rows represents over expression of omental fat compared to subcutaneous fat for both normal and obese pregnant women.

* Candidate genes with variation in gene expression validated using qRT-PCR

Table 3
Differential subcutaneous transcripts (highlighted coding protein) expression between obese and normal pregnant women

Chromosome	Position	Symbol	Obese_vs_Normal		
			Fold	Raw p	Adj p
17	48712246	ABCC3	5.474444	2.67E-06	0.040481
4	119990528	RP11-211110.2	-4.91965	2.91E-06	0.040481
12	69746983	RP11-1143G9.4	5.571975	3.63E-06	0.040481
10	37529838	RP11-20F24.2	3.666019	3.83E-06	0.040481
2	47562488	BCYRN1	3.430693	7.51E-06	0.047224
22	27617357	RP5-1172A22.1	3.57111	8.78E-06	0.047224
4	159120286	RP11-597D13.8	5.502205	9.62E-06	0.047224
8	57431224	RP11-16M8.2	2.339372	1.02E-05	0.047224
9	138387092	C9orf116	1.891656	1.17E-05	0.047224
16	69745196	NQO1	3.755799	1.18E-05	0.047224
7	38339423	TRGV10	2.577584	1.23E-05	0.047224
X	12885163	TLR7	3.421692	1.48E-05	0.047986
6	41126258	TREM2	2.962513	1.58E-05	0.047986
22	44567870	PARVG	2.900105	1.59E-05	0.047986