Regional Differences in Subcutaneous Adipose Tissue Gene Expression

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Adipose tissue (AT) located in the viscera is considered to be functionally and metabolically different from that found in the subcutaneous depot. However, subcutaneous AT (SAT) in generalized regions is considered to be homogeneous in nature. Affymetrix GeneChip Human Exon 1.0 ST Arrays were used to determine differential gene expression in four subcutaneous adipose depots (upper abdomen, lower abdomen, flank and hip) in normal weight women. A total of 2,890/24,409 transcripts were differentially expressed between all sites. When comparing the hip and flank to the lower abdomen, 248 and 83 genes were differentially expressed, respectively. When comparing the hip and flank to the upper abdomen, 2,480 and 79 genes were differentially expressed, respectively. No genes were significantly different when the lower abdomen was compared to the upper abdomen and the hip to the flank. Genes involved in the complement and coagulation cascades and immune responses showed increased expression in the lower abdomen compared to the flank. In addition, two genes involved in the complement and coagulation cascade, CR1 and C7, were expressed more highly in the lower abdomen compared to the hip. Genes involved in basic biochemical metabolism including insulin signaling, the urea cycle, glutamate metabolism, arginine and proline metabolism and aminosugar metabolism had higher expression in the lower abdomen compared to the hip. These results in normal weight healthy women provide a new perspective on regional differences in SAT biology that may have pathophysiologic implications when adiposity increases.

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for small-volume "contouring" liposuction (<5,000 ml removal) were determined by the surgeon (C.K.L.). Premenopausal women between 18 and 50 years with a BMI of 22 and 27 kg/m² were considered eligible for the study. Eligible participants were weight stable for at least 3–6 months (<7% change from maximum weight) before entering the study. Participants were nonsmokers with no history of glucose intolerance, dyslipidemia, liver, kidney, or cardiac disease, hypertension, cancer or any disorder that might have interfered with a "normal" lifestyle (nutrition, physical activity). Women on thyroid hormone replacement were included if thyroid stimulating hormone levels were normal. Women on oral contraceptives were included if this form of contraceptive therapy was maintained throughout study participation. Further exclusions were: reduced-obesity (history weight loss >10% of maximum body weight); history of liposuction or gastric bypass surgery; evidence of body dysmorphic disorder; taking medications affecting carbohydrate and lipid metabolism; hematocrit, hemoglobin, white blood cells, platelet count, liver or renal function tests outside of the normal range; fasting glucose >110 mg/dl; triglycerides >200 mg/dl; high density lipoprotein cholesterol <35 mg/dl and low density lipoprotein cholesterol >160 mg/dl; proteinuria; blood pressure >140/90 mm/Hg; electrocardiographic abnormalities.

Abdominal and limb circumferences/subcutaneous skinfold measures

Circumferences were measured on the thigh, waist, abdomen, and hips using a standardized tape measure with tension regulator (Novel Products, Rockton, IL). Waist circumference was measured just under the ribcage and abdominal circumference at the level of the umbilicus. Suprailiac and periumbilical subcutaneous skinfolds were measured at standard locations (Lange Skinfold Calipers; Heathcheck Systems, Brooklyn, NY) (16). Anthropometric measurements were taken by the same investigator (T.L.H.) (15).

Regional AT biopsies

AT biopsies were collected from two subcutaneous sites at baseline (before randomization to the suction lipectomy group or the no suction lipectomy group), 6 weeks, and 6 months (Table 1). At baseline, the first site was chosen in a region where suction lipectomy would occur whereas the second site was a region wherein surgery would not ensue. Approximately 4mL of AT were removed from each site during the biopsy. Following local anesthesia (1% lidocaine, 0.25% bupivacaine, and sodium bicarbonate mixed in 100 mL of 0.9% normal saline), stab incisions were made and fat biopsies were obtained using Coleman's manual vacuum technique (17). Collection of tissue was accomplished using a 10-ml syringe system consisting of a cannula and locking device to hold the plunger back thereby creating a vacuum. The cannula was inserted into the fat and pulled back and forth as the plunger was pulled back to create the vacuum. This resulted in minimal bleeding, ecchymoses, and pain while obtaining substantial quantities of non-lysed AT.

Exon array analysis

Six subjects from the nonsurgery group were randomly chosen for microarray analysis at baseline, 6 weeks, and 6 months. Seven subjects were randomly chosen from the surgery group for microarray analysis at baseline. RNA was isolated from the AT and expression of 24,409 genes quantified using the Affymetrix GeneChip Human Exon 1.0 ST Array. The Exonmap package in R was used to analyze the expression data (16). This package supports a variety of routines for translating between probe sets, exons, genes, and transcripts, and makes use of a relational database (X:Map) to define these relationships for the current genome assembly. X:Map is an annotation database using Ensembl. Exonmap provided some basic functions to load expression data into R and uses RMA to normalize and generate expression summaries for the probe sets. The genes with the lowest overall variance and fold change were removed by the filter. A nonpaired t-test was used to detect differences between the sites. The P values associated with the t-test were adjusted for multiple testing by using the Benjamini & Hochberg approach (18). Genes with an adjusted P value (q value <0.05) were considered differentially expressed. Principal component analysis (19) was performed on our data using R (20). Non-negative matrix factorization was used to predict the classes within the significant genes that were predicted between the sites. The significant genes were assigned to functional pathways using GENE BROWSER2 (21,22).

Real-time PCR

RNA isolation from AT biopsies was performed using both TRIzol reagent (Invitrogen, Carlsbad, CA) and the RNaseasy Mini Kit (Qiagen, Valencia, CA). Total RNA (500 ng) was reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA), and quantitative PCR was performed using primer sets for genes of interest and reference genes and iQ SYBR Supermix (Bio-Rad) following manufacturer's protocols. Reactions were run in duplicate on an iQ5 real-time PCR detection system (Bio-Rad) along with a no-template control per gene. Validation experiments were performed to demonstrate that efficiencies of target and reference genes were approximately equal. Data were normalized to RPL13A (5′ CTC GGA GGA GAA GAG GAA AGA 3′, for CR1 were 5′ TGA CCG CCT GCG AGT CTG AGT 3′) and 5′ TCC GGC CTC TGT CT 3′, for C7 were 5′ TGG CAG AGA CCT GGG TAC 3′ and fold change was determined using the formula 2^−ΔΔCt. Primer sequences for plasminogen activator inhibitor-1 (PAI-1) were 5′ CTC GGC TTC AGA CTT CGG GTT 3′ and 5′ GGG CCA TGC CCT TCT TGT CAT CAA TCT T 3′, for CR1 were 5′ CCG GTG GCC TGG GTT CAA TG 3′ and 5′ CTT CTG CAC CTG TCC TTA GCA CC 3′, for F13A1 were 5′ GTG CTG ATC CAA GAC GCC GGG GA 3′ and ACC TGA GTG CCA CGG ACC TTG A 3′, for C7 were 5′ GGC AGT GGG ACT TCT ATG CCC CT 3′ and 5′ TGA CGG CCT GGG ATG CTG AGT 3′, for C2 5′ TTC TCC CTC CGG GGA CGT TCT AC 3′ and 5′ GCC AGA CCT GGG TAG AGG AAC AG 3′ and for C1qC were 5′ GGG CCA CCT TGG CCC TCC TTC TCT T 3′ and 5′ CCT GTG TGT GCT TGG CCC CTG 3′.

RESULTS

Subjects and randomization

Eligibility for the liposuction study was assessed in 75 women during the years 2004–2007 and 34 subjects were randomized...
into the study. Fourteen women were randomized to receive liposuction surgery and 18 to the control group. The biopsies from seven women in the liposuction group and six in the control group were randomly chosen for GeneChip analysis. Amongst the subjects chosen for GeneChip analysis, no significant differences in anthropometric measurements including weight, BMI, abdominal circumferences, and subcutaneous skinfold thickness measurements were observed over the course of the study (Table 2).

**Table 2 Anthropometric measurements at baseline, 6 weeks, and 6 months**

|                | BMI (kg/m²) | Waist (cm) | Abdomen (cm) | Hip (cm) | Thigh (cm) | Suprailiac (cm) | Periumbicularly (cm) |
|----------------|-------------|------------|--------------|----------|------------|-----------------|---------------------|
| Baseline n = 13| 23.9 ± 2.5  | 75.8 ± 6.86| 80.4 ± 9.45 | 98.43 ± 6.7| 53.97 ± 4.4 | 19.8 ± 5.1       | 20.4 ± 8.00         |
| 6 weeks n = 6 | 23.8 ± 2.8  | 74.9 ± 6.46| 79.8 ± 8.15 | 98.2 ± 6.9| 54.6 ± 4.92 | 18.1 ± 5.5       | 20.5 ± 7.2          |
| 6 months n = 6| 23.9 ± 2.8  | 75.2 ± 6.1 | 81.2 ± 8.3  | 99.1 ± 5.8| 54.9 ± 4.2  | 18.1 ± 7.5       | 21.1 ± 9.0          |
| **P value**    | 0.997       | 0.968      | 0.951        | 0.968    | 0.925      | 0.838           | 0.983               |

No significant changes were observed in anthropometric measurements over time. Baseline, 6 weeks, and 6 months measurements were analyzed using an ANOVA. Values displayed are the average ± SD.

We compared the variance/covariance features between genes at different time points by conducting principal component analysis. Our results showed there was no significant change in gene expression over time when biopsies taken at baseline were compared to those obtained at 6 weeks and 6 months (Figure 1). This suggests that gene expression is stable within SAT depots over time. However, we did find that SAT gene expression varied between sites in these normal weight, healthy women (Figure 2).

**Microarray analysis**

We compared the variance/covariance features between genes at different time points by conducting principal component analysis. Our results showed there was no significant change in gene expression over time when biopsies taken at baseline were compared to those obtained at 6 weeks and 6 months (ANOVA and principal component analysis) (Figure 1). This suggests that gene expression is stable within SAT depots over time. However, we did find that SAT gene expression varied between sites in these normal weight, healthy women (Figure 2).

When comparing the hip and flank to the lower abdomen, 248 and 83 genes were differentially expressed, respectively. When comparing the hip and flank to the upper abdomen, 2,480 and 79 genes were differentially expressed, respectively. No genes were differentially expressed when comparing the lower abdomen to the upper abdomen, and the hip to the flank (Figure 2).

From the 83 genes differentially expressed in the lower abdomen compared to the flank (see Supplementary Note S1 online), 10 genes clustered into 12 functional pathways with a P value <0.05 (Figure 3). Nine of the ten genes were expressed more highly in the lower abdomen with the exception of Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and Contactin 1 (CNTN1). Six of these 10 genes are involved in pathways related to the immune response. Of particular interest, three genes clustered into the complement and coagulation cascade, PAI-1, coagulation factor XII (F13A1), and complement receptor-1 (CR1) (P = 6.67 x 10^-4) and showed higher levels of expression in the lower abdomen compared to the flank. Although levels of complement component 2 (C2) were not significantly different (P = 0.09), C2 expression was highly correlated with the expression of F13A1 and CR1 (r = 0.86, 0.74, and 0.85, respectively). In addition, cluster of differentiation 4 (CD4) and B-cell linker (BLNK), genes that are involved in the development of T and B cells, were expressed higher in the lower abdomen compared to the flank.
Twenty of the 248 genes (see Supplementary Note S2 online) differentially expressed in the lower abdomen compared to the hip clustered into 11 functional pathways with a P value <0.05 (Figure 4). All of these 20 genes in these pathways were more strongly expressed in the lower abdomen with the exception of pyruvate kinase isozyme R/L (PKLR). Five of the eleven enriched pathways are related to basic biochemical metabolism, including insulin signaling, the urea cycle, glutamate metabolism, arginine and proline metabolism and aminosugar metabolism (Figure 4). Two genes involved in the complement and coagulation cascade, CR1 and complement component 7 (C7), were more highly expressed in the lower abdomen compared to the hip.

Real-time PCR
Four genes from the complement and coagulation cascade that were differentially expressed according to the microarray and two that approached significance were chosen for confirmation by real-time PCR. Of the five genes chosen for confirmation in the lower abdomen compared to the hip, PAI-1, F13A1, C2, and C1qC were concordant with the microarray data showing higher expression in the lower abdomen compared to the flank.
The discordance between the microarray and real-time PCR observed with CR1 is mostly likely explained by the increased sensitivity of real-time PCR analysis and the low expression levels of CR1. Thus, in a sampling of transcripts analyzed by real-time PCR, we feel that the regional SAT microarray data now set the stage for much more extensive pursuit of these pathways and the mechanism(s) by which anatomic region modify gene expression.

In obesity, increased AT mass is often associated with a proinflammatory state that may contribute to the development of insulin resistance and the metabolic syndrome (23). Increased adipocyte size and number are linked to higher levels of macrophage infiltration and activation through the release of monocyte chemotactic protein-1 (24–26). These macrophages are an important source of the inflammatory cytokines tumor necrosis factor-α and interleukin-6 (27). Of interest, cross-sectional data from the Framingham Heart Study show both VAT and SAT volumes are associated with elevated levels of the proinflammatory cytokines C-reactive protein, fibrinogen, interleukin-6, and tumor necrosis factor-α (28).

Our data demonstrate that genes related to the complement and coagulation cascades were dominantly expressed in the lower abdomen. The complement system is integral in the initiation and maintenance of the proinflammatory response (29). The complement components C2, C3, C4, C7, and factor B are expressed more highly in VAT compared to SAT (5). In our study, we found higher expression of C7 in the lower abdomen compared to the hip and higher expression of C2 and C1QC in the lower abdomen compared to the flank. C1Q, the first subcomponent of complement, is able to bind to endothelial cells, activate the complement pathway and illicit the production of interleukin-8, interleukin-6, and monocyte chemotactic protein-1 (30).

Increased levels of the prothrombotic protein PAI-1 are also associated with the insulin resistant state and the risk of cardiovascular disease and type-2 diabetes (31). PAI-1 produced by the stromal vascular cells present in VAT and circulating levels of PAI-1 are associated with the volume of VAT (32). In addition to abdominal VAT, the amount of abdominal SAT is related to circulating PAI-1 levels, but not femoral SAT (33). In our study, the increase in PAI-1 gene expression in the upper abdominal SAT compared to SAT located in the flank provides further evidence that the abdominal wall is primed for further contribution to the risk for type-2 diabetes and cardiovascular disease when central adiposity expands.

The current prevalence of overweight and obesity in the United States is 68% for both men and women (34). Overweight and obesity are undeniably risk factors for the development and progression of chronic metabolic diseases such as diabetes and cardiovascular disease. Identifying the changes that take place in the AT as weight gain begins will be beneficial in developing prevention and treatment strategies for obesity-related metabolic diseases. In weight stable healthy individuals we observed differences in the expression of genes related to the proinflammatory process and insulin signaling in SAT depots. This emphasizes the need for longitudinal studies in

**DISCUSSION**

The use of a pathway analysis tool provides a method to group the list of differentially expressed genes from our microarray data into functional pathways. Given the fact that the women in this study had an average BMI of 23.8 and all of the biopsies were from the SAT, large differences in gene expression were not expected. However, changes in the expression of groups of genes with related function are meaningful to compare possible functional differences between SAT depots.

In this study, gene expression in distinct SAT depots sampled did not change over the 6-month period. This suggests that in weight stable women without any perturbations, gene expression patterns in SAT were stable over time. However, differences in regional expression were consistent with abdominal SAT being associated with increased expression of genes that may contribute to insulin resistance and the metabolic syndrome, even before overweight/obesity commence.

Six genes that were upregulated in the lower abdomen were chosen for verification by real-time PCR, i.e., PAI-1, F13A1, and CR1 that were significantly higher in the lower abdomen compared to the flank according to the microarray and C2 and C1QC that approached significance. In the lower abdomen compared to the hip the microarray revealed C7 and CR1 expression to be higher in the lower abdomen. CR1 was the only gene identified in both sets of analyses. Based on the RT-PCR we were able to confirm the increased expression of PAI-1, F13A1 and C2 in the lower abdomen compared to the flank and increased C7 in the lower abdomen compared to the hip.

**Figure 5** Real-time PCR validation of microarray. Fold change was determined using the equation $2^{-\Delta\Delta Ct}$ with expression in the lower abdomen used as the reference between groups. Values displayed are the fold change ± SD.

![Figure 5](image-url)
subjects before and after weight gain to determine the timing and amount of increased expression within these gene family members across various SAT AT depots.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

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DISCLOSURE
The authors declared no conflict of interest. See the online ICMJE Conflict of Interest Forms for this article.