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Role of Calcitriol and Cortisol on Human Adipocyte Proliferation and Oxidative and Inflammatory Stress: A Microarray Study

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Key Words
Apoptotic gene expression · Calcitriol · Calcitriol/cortisone, microarray analysis · Cortisol · Gene expression, patterns · Gene expression, unique regulation · Human adipocyte proliferation · 11β-Hydroxysteroid dehydrogenase type 1 · Oxidative and inflammatory stress · Real-time PCR

Abstract
Dietary calcium inhibits adiposity, and a key underlying mechanism is suppression of calcitriol, which modulates Ca^{2+} signaling and mitochondrial uncoupling in adipocytes. We demonstrated that calcitriol directly regulates adipocyte 11β-HSD-1 expression and cortisol production in human adipocytes in vitro and dietary calcium inhibits visceral adipose tissue 11β-HSD-1 expression in mice, indicating an interaction of calcitriol and cortisol in obesity. Consequently, we have evaluated the gene expression profile of human subcutaneous adipocytes treated with calcitriol and/or cortisone. Data analysis demonstrated significant calcitriol modulation of gene expression toward inhibition of the adipocyte apoptosis (e.g., VEGF and STC-2) and promotion of adipocyte proliferation (e.g., IGF-1 and IGF-1R). Calcitriol also up-regulated oxidative stress and inflammatory genes such as NOX-4 and TLR-3. The calcitriol/cortisone combination resulted in significant additional up-regulation of 11β-HSD-1 and down-regulation of adiponectin expression, while cortisone exerted little independent effect in the absence of calcitriol. Overall, calcitriol stimulated a pattern of adipocyte gene expression which favored adipocyte proliferation, oxidative and inflammatory stress and visceral adiposity, and these effects were amplified in the presence of cortisone; however, this conclusion must be tempered by the adipocyte source (subcutaneous) and requires confirmation in visceral adipocytes.

Introduction

Excessive central fat deposition in obesity may result from the greater capacity for regeneration of active glucocorticoids in the visceral fat depot [1–5]. Local adipose tissue glucocorticoid levels and intracellular glucocorticoid availability are controlled by the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) to generate active cortisol from inactive cortisone [6, 7]. Previous studies from this laboratory demonstrate an anti-obesity effect of dietary calcium [8], and that this effect is associated with preferential loss of central adipose tissue [9, 10]. Increasing dietary calcium inhibits lipogenesis [11], stimulates energy metabolism [12, 13] and increases adipocyte apoptosis [14]. These effects are mediated by suppression of calcitriol, which stimulates Ca^{2+} influx via a non-genomic effect [11] and by suppression of uncoupling protein 2 (UCP2) gene expression [15], resulting in increased mitochondrial potential and ATP production [14]. We recently demonstrated that calcitriol also directly regulates adipocyte 11β-HSD-1 expression and, consequently, correspondingly effects local cortisol levels [16], indicating a potential role for calcitriol in visceral adiposity. However, little else is known regarding

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the independent and cooperative roles of calcitriol and cortisol in modulating adipocyte gene expression. Consequently, we conducted a microarray analysis of the effects of calcitriol and cortisone on the gene expression profile of differentiated human adipocytes.

Material and Methods

Cell Culture

Human preadipocytes used in this study were supplied by Zen-Bio (Research Triangle, N.C., USA) and cells were originally pooled from subcutaneous fat of 6 healthy female subjects whose BMI ranged from 26.56 to 32.77. Preadipocytes were inoculated in DMEM/Ham's F-10 medium (DMEM-F10) (1:1, vol/vol) containing 10% FBS, 15 mmol/l HEPES, and antibiotics at a density of 30,000 cells/cm^2. Confluent monolayers of preadipocytes were induced to differentiate with a standard differentiation medium consisting of DMEM-F10 (1:1, vol/vol) medium supplemented with 15 mmol/l HEPES, 3% FBS, 33 μmol/l biotin, 17 μmol/l pantothenate, 100 nmol/l insulin, 0.25 μmol/l methylisobutylxanthine, 1 μmol/l dexamethasone, 1 μmol/l BRL49653, and antibiotics. Preadipocytes were maintained in this differentiation medium for 3 days and subsequently cultured in adipocyte medium in which BRL49653 and methylisobutylxanthine were omitted. Cultures were re-fed every 2–3 days until 85–90% differentiation was attained.

Cells were washed with fresh adipocyte medium, re-fed with medium containing the different treatments (10 nM calcitriol, 10 nM cortisone or 10 nM calcitriol + 10 nM cortisone combination, each treatment had 2–3 replicates), and incubated at 37°C in 5% CO_2 in air before analysis. Cell viability was measured via trypan blue exclusion.

Total RNA Extraction

A total cellular RNA isolation kit (Ambion, Austin, Tex., USA) was used to extract total RNA from cells according to manufacturer's instruction. The concentration and purity of the isolated RNA were measured spectrophotometrically (A_{280}/A_{260} between 1.9 and 2.1).

Screening of mRNA by Affymetrix GeneChip

Human Genome U133A Arrays (P/N 510681, Lot # 3002124) were obtained from Affymetrix (Santa Clara, Calif., USA). The Affymetrix protocol for One-Cycle cDNA synthesis and labeling was followed. First, 4 μg of total RNA was converted into single-stranded cDNA using SuperScript II (Invitrogen) and a T7-Oligo (dT) primer (Affymetrix). Second-strand cDNA was synthesized using dNTPs, second-strand reaction buffer, *Escherichia coli* DNA ligase, *E. coli* DNA polymerase I, and *E. coli* RNase H (all reagents were from Invitrogen). One cycle cDNA synthesis was followed by cleanup using Affymetrix GeneChip Sample Cleanup Modules. Biotin-labeled cRNA was prepared using an ENZO BioArray High Yield RNA Transcript Labeling Kit (ENZO Life Sciences, Inc., Farmingdale, N.Y., USA). After cleanup of the in vitro transcription products using Affymetrix GeneChip Sample Cleanup Modules for IVT reactions, the purified cRNA was fragmented to a size ranging from 35 to 200 bases using fragmentation buffer at 94°C for 35 min. 4 μg of the fragmented cRNA was mixed into a hybridization cocktail containing hybridization buffer, B2 oligo control RNA (Affymetrix), herring sperm DNA, and BSA (both from Invitrogen). The solution was hybridized to a GeneChip at 45°C for 16 h at a setting of 60 rpm. After hybridization, the cocktail was removed from the GeneChip and stored for potential future analyses. Using an Affymetrix Fluidics 450 wash station (Affymetrix Fluidics Protocol EUKGE_WS2v4), the GeneChip was washed and stained with streptavidin-phycoerythrin (Molecular Probes), followed by a wash with biotinylated antibody (Vector Labs) and goat IgG (Sigma) then another staining with streptavidin-phycoerythrin. The GeneChips were immediately scanned with a GeneChip 3000 high-resolution scanner. The individual GeneChip scans were quality checked for the presence of image anomalies, outliers, control genes and background signal values. The GeneChips were processed at the University of Tennessee Knoxville Affymetrix Core Facility.

Analysis of Array Data

The data obtained from the GeneChip scanner were further analyzed using Affymetrix® Microarray Suite software. Hybridization of B2 oligo served as a positive control by the software to place a grid over the scanned image. Noise, which is the pixel-to-pixel variations of probe cells on the array and the average background (varies between 20 and 100) were subtracted from the signal intensity value. The control genes were analyzed (poly A controls and hybridization controls) for appropriate signal intensity.

The software uses a detection algorithm to call the expression of each gene as present (P), marginal (M) or absent (A). This is based on the target-specific intensity of the probe pair relative to the overall hybridization intensity (i.e., perfect match-mismatch/perfect match + mismatch). The genes that are considered as present were selected and their signal intensities were converted into a signal log base 2 value (log2) using the web tool on UTMD (http://genome.ws.utk.edu/). The array data of the control sample were compared to the array data from the treatment sample. The genes that were up- or down-regulated in the test sample relative to the control samples were examined by setting a cut-off value of two for the signal log ratio. To more thoroughly characterize sets of functionally related genes differentially expressed, we used the web-based analysis tool Onto-Express (http://vortex.cs.wayne.edu/ontoexpress/) to classify genes according to the following Gene-Ontology (GO) categories: biological process and cellular components.

All microarray data were submitted in compliance with the Minimal Information about Microarray Experiments (MIAME) [17] to the UTMD data repository under the title 'Human Cell Line' and may be accessed from that site.

Quantitative Real-Time PCR

Adipocyte and muscle 18S, stanniocalcin 1 (STC-1) and stanniocalcin 2 (STC-2) were quantitatively measured using an ABI 7300 Real-Time PCR System (Applied Biosystems, Branchburg, N.J., USA) with a TaqMan 1000 Core Reagent Kit (Applied Biosystems). The primers and probe sets were obtained from Applied Biosystems TaqMan® Assays-on-Demand™ Gene Expression primers and probe set collection according to manufacture's instruction. Pooled adipocyte total RNA were serial-diluted in the range of 1.5625–25 ng and used to establish a standard curve; total RNAs for unknown samples were also diluted in this range. Reactions of quantitative RT-PCR for standards and unknown samples were also performed according to the instructions of ABI 7300 Real-Time PCR System and TaqMan Real-Time PCR Core Kit. The mRNA quantitation for each sample were further normalized using the corresponding 18S quantitation.

Statistics

Real-time PCR data were expressed as mean ± SEM. Data from studies were evaluated by one-way ANOVA, and significantly different group means ($p < 0.05$) were then separated by the least significant difference test using SPSS (SPSS Inc., Chicago, Ill. USA).

Results

Patterns of Gene Expression Associated with Treatment with Calcitriol and/or Cortisone

There were 237, 141 and 360 genes that responded to treatment with calcitriol, cortisone and calcitriol/cortisone (calcitriol + cortisone combination), respectively. Figure 1 represents the numbers of differentially expressed genes from the three treatments. Notably, 94 genes were altered in common by calcitriol and calcitriol/cortisone treatments while only 31 genes in common between cortisone and calcitriol/cortisone. In addition, 7 genes in total were commonly up-regulated across all three treatments, with the calcitriol/cortisone combination exerting the greatest effect on four of these (fig. 2).

Treatment with calcitriol and the calcitriol/cortisone combination resulted in regulation of an additional 86 genes that were not also regulated by cortisone. Among these genes, calcitriol up-regulated those involved in cell proliferation, angiogenesis, cell cycle, inflammation and response to oxidative stress, while the calcitriol/cortisone combination exerted a similar effect but to lesser extent (table 1). Cortisone and the calcitriol/cortisone combination also commonly regulated a smaller number of genes (23 genes) in addition to the 7 gene described previously. Notably, cortisone down-regulated those associated with cell proliferation and cell cycle and the calcitriol/cortisone combination exhibited a similar effect (table 2).

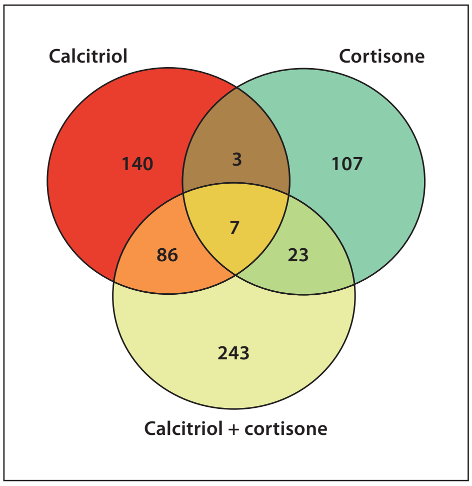

Fig. 1. Number of genes regulated by calcitriol, cortisone, or calcitriol/cortisone combination. Numbers of significant genes (log2-fold changes >1, $p < 0.05$) regulated by 10 nM calcitriol, 10 nM cortisone or 10 nM calcitriol/10 nM cortisone combination. Numbers of genes commonly regulated by these three treatments are presented in Venn diagram.

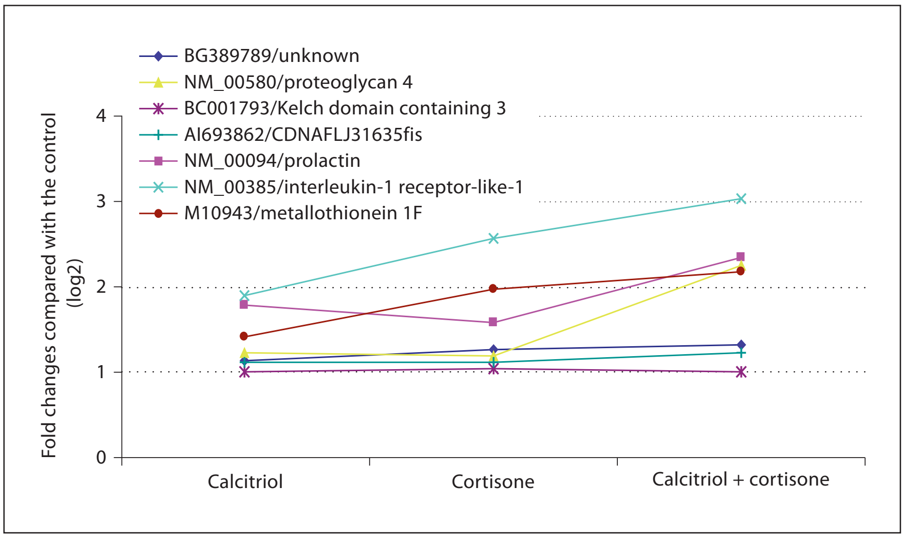

Fig. 2. Genes commonly regulated by calcitriol, cortisone and calcitriol/cortisone combination.

Table 1. Profile of commonly regulated genes by calcitriol and calcitriol/cortisone combination

Gene name	Affymetrix ID	Fold change (log2)	
		1,25-D	1,25-D+ cortisone
Hyperosmotic response			
Toll-like receptor 3	206271_at	1.005	–1.194
Immune response			
Myxovirus (influenza virus) resistance 2 (mouse)	204994_at	–2.042	–1.366
Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	202086_at	–2.837	–1.494
ISG15 ubiquitin-like modifier	205483_s_at	–2.867	–1.328
Interferon-induced protein with tetratricopeptide repeats 3	204747_at	–2.566	–1.683
Interferon-induced protein with tetratricopeptide repeats 1	203153_at	–2.544	–1.012
Interferon-induced protein with tetratricopeptide repeats 2	226757_at	–3.04	–1.973
Interferon-induced protein 35	209417_s_at	–1.821	–1.077
2′,5′-Oligoadenylate synthetase 2, 69/71 kDa	204972_at	–2.289	–1.437
Guanylate-binding protein 1, interferon-inducible, 67 kDa	231577_s_at	–1.468	–1.063
Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	203717_at	1.897	2.481
Response to virus			
Myxovirus (influenza virus) resistance 2 (mouse)	204994_at	–2.042	–1.366
Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	202086_at	–2.837	–1.494
Interferon-induced protein 35	209417_s_at	–1.821	–1.077
Synaptic vesicle to endosome fusion			
Early endosome antigen 1, 162 kDa	1560680_at	1.306	1.73
Positive regulation of interferon-β biosynthesis			
Toll-like receptor 3	206271_at	1.005	–1.194
Collagen fibril organization			
Collagen, type XIV, α1 (undulin)	212865_s_at	1.143	1.07
Bicarbonate transport			
Solute carrier family 4, sodium bicarbonate cotransporter, member 7	209884_s_at	1.725	1.536
Metabolism			
Branched chain aminotransferase 1, cytosolic	225285_at	1.036	1.626
ATPase, Ca^{2+} transporting, plasma membrane 1	215716_s_at	1.018	1.639
Isocitrate dehydrogenase 2 ($NADP^{+}$), mitochondrial	210046_s_at	1.741	1.383
Solute carrier family 27 (fatty acid transporter), member 2	205768_s_at	1.563	1.326
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism			
2′,5′-Oligoadenylate synthetase 2, 69/71 kDa	204972_at	–2.289	–1.437
Cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	206227_at	3.792	3.213
Protein amino acid phosphorylation			
G-protein-coupled receptor kinase 5	204396_s_at	1.844	2.241
Mitogen-activated protein kinase 13	210059_s_at	1.686	2.024
Wnt receptor signaling pathway			
Secreted frizzled-related protein 1	202037_s_at	1.104	1.501
Response to oxidative stress			
Oxidation resistance 1	222553_x_at	1	1.481
Apoptosis			
Full-length cDNA clone CS0DB001YB20 of Neuroblastoma Cot 10-normalized of *Homo sapiens* (human)	225816_at	–1.255	–1.091
Response to stress			
Mitogen-activated protein kinase 13	210059_s_at	1.686	2.024
Full-length cDNA clone CS0DB001YB20 of Neuroblastoma Cot 10-normalized of *Homo sapiens* (human)	225816_at	–1.255	–1.091
Regulation of cell shape			
ras homolog gene family, member J	235489_at	1.399	1.432
Negative regulation of adenylate cyclase activity			
Endothelin receptor type B	204273_at	1.547	2.477
Intracellular signaling cascade			
Triggering receptor expressed on myeloid cells 1	219434_at	2.022	2.315
DEP domain containing 6	218858_at	–1.492	–1.651

Table 1 (continued)

Gene name	Affymetrix ID	Fold change (log2)	
		1,25-D	1,25-D+ cortisone
Skeletal development			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
B-cell differentiation			
Kruppel-like factor 6	208961_s_at	–1.108	–1.37
Branched chain family amino acid metabolism			
Branched chain aminotransferase 1, cytosolic	225285_at	1.036	1.626
Perception of sound			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Endothelin receptor type B	204273_at	1.547	2.477
Cell proliferation			
c-fos-induced growth factor (vascular endothelial growth factor D)	206742_at	1.665	1.369
Branched chain aminotransferase 1, cytosolic	225285_at	1.036	1.626
Cytoskeleton organization and biogenesis			
Advillin	205539_at	1.353	1.77
Angiogenesis			
c-fos-induced growth factor (vascular endothelial growth factor D)	206742_at	1.665	1.369
Vesicle fusion			
Early endosome antigen 1, 162 kDa	1560680_at	1.306	1.73
Transcription			
Kruppel-like factor 6	208961_s_at	–1.108	–1.37
Nuclear receptor interacting protein 1	202600_s_at	1.115	1.157
Glyoxylate cycle			
Isocitrate dehydrogenase 2 (NADP$^+$), mitochondrial	210046_s_at	1.741	1.383
Development			
Thrombospondin 1	201109_s_at	–1.264	–1.11
Integrin-mediated signaling pathway			
Integrin, α11	222899_at	–1.162	–1.337
Regulation of cell cycle			
c-fos-induced growth factor (vascular endothelial growth factor D)	206742_at	1.665	1.369
Positive regulation of cell proliferation			
c-fos-induced growth factor (vascular endothelial growth factor D)	206742_at		1.369
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Regulation of G-protein-coupled receptor protein signaling pathway			
G-protein-coupled receptor kinase 5	204396_s_at	1.844	2.241
L-Glutamate transport			
Solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system Xag), member 1	213664_at	1.016	2.089
Induction of apoptosis			
Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	202086_at	–2.837	–1.494
Early endosome to late endosome transport			
Early endosome antigen 1, 162 kDa	1560680_at	1.306	1.73
Inflammatory response			
Toll-like receptor 3	206271_at	1.005	–1.194
Blood coagulation			
Plasminogen activator, tissue	201860_s_at	1.043	1.189
Thrombospondin 1	201109_s_at	–1.264	–1.11
Very-long-chain fatty acid metabolism			
Solute carrier family 27 (fatty acid transporter), member 2	205768_s_at	1.563	1.326
Protein biosynthesis			
Elongation factor Tu GTP-binding domain containing 1	218973_at	2.329	3.093
Mitochondrial ribosomal protein S6	212944_at	1.699	2.437
Solute carrier family 5 (inositol transporters), member 3	213164_at	1.734	1.98
G-protein signaling (phospholipase C activating)			
Endothelin receptor type B	204273_at	1.547	2.477

Table 1 (continued)

Gene name	Affymetrix ID	Fold change (log2)	
		1,25-D	1,25-D+ cortisone
G-protein signaling, coupled to cAMP nucleotide second messenger			
G-protein-coupled receptor kinase 5	204396_s_at	1.844	2.241
Protein kinase cascade			
Mitogen-activated protein kinase 13	210059_s_at	1.686	2.024
Cell cycle			
Mitogen-activated protein kinase 13	210059_s_at	1.686	2.024
Cell-cell signaling			
ISG15 ubiquitin-like modifier	205483_s_at	–2.867	–1.328
Cholesterol biosynthesis			
Transmembrane 7 superfamily member 2	210130_s_at	2.445	1.283
NAD(P)-dependent steroid dehydrogenase-like	215093_at	1.125	1.219
ION transport			
Ceruloplasmin (ferroxidase)	204846_at	1.598	2.005
Defense response to bacteria			
Toll-like receptor 3	206271_at	1.005	–1.194
Electron transport			
Cytochrome P450, family 3, subfamily A, polypeptide 5	214234_s_at	1.683	1.9
Cytochrome P450, family 3, subfamily A, polypeptide 5	205765_at	2.079	2.101
Flavin containing monooxygenase 1	205666_at	1.285	1.687
Carbohydrate metabolism			
Isocitrate dehydrogenase 2 ($NADP^+$), mitochondrial	210046_s_at	1.741	1.383
Humoral immune response			
Triggering receptor expressed on myeloid cells 1	219434_at	2.022	2.315
Proteolysis and peptidolysis			
Plasminogen activator, tissue	201860_s_at	1.043	1.189
Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	203717_at	1.897	2.481
Branched chain family amino acid biosynthesis			
Branched chain aminotransferase 1, cytosolic	225285_at	1.036	1.626
Glycolate metabolism			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Regulation of transcription, DNA-dependent			
Kruppel-like factor 6	208961_s_at	–1.108	–1.37
Nuclear receptor interacting protein 1	202600_s_at	1.115	1.157
Full-length cDNA clone CS0DB001YB20 of Neuroblastoma Cot 10-normalized of *Homo sapiens* (human)	225816_at	–1.255	–1.091
Transport			
Solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system Xag), member 1	213664_at	1.016	2.089
Cytochrome P450, family 3, subfamily A, polypeptide 5	214234_s_at	1.683	1.9
Biological process unknown			
Williams-Beuren syndrome chromosome region 16	221247_s_at	1.026	1.339
Physiological process			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Negative regulation of cell growth			
Full-length cDNA clone CS0DB001YB20 of Neuroblastoma Cot 10-normalized of *Homo sapiens* (human)	225816_at	–1.255	–1.091
Dicarboxylic acid transport			
Solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system Xag), member 1	213664_at	1.016	2.089
Small GTPase-mediated signal transduction			
Ras homolog gene family, member J	235489_at	1.399	1.432
Xenobiotic metabolism			
Cytochrome P450, family 3, subfamily A, polypeptide 5	214234_s_at	1.683	1.9

Table 1 (continued)

Gene name	Affymetrix ID	Fold change (log2)	
		1,25-D	1,25-D+ cortisone
DNA replication			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Negative regulation of osteoclast differentiation			
Toll-like receptor 3	206271_at	1.005	–1.194
Cell adhesion			
Collagen, type XIV, α1 (undulin)	212865_s_at	1.143	1.07
Thrombospondin 1	201109_s_at	–1.264	–1.11
Regulation of translation			
Mitogen-activated protein kinase 13	210059_s_at	1.686	2.024
Nanos homolog 1 *(Drosophila)*	228523_at	2.429	2.913
Synaptic transmission			
Solute carrier family 1 (neuronal/epithelial high-affinity glutamate transporter, system Xag), member 1	213664_at	1.016	2.089
Detection of virus			
Toll-like receptor 3	206271_at	1.005	–1.194
Ubiquitin cycle			
F-box and leucine-rich repeat protein 7	241457_at	1.029	1
Neurogenesis			
Advillin	205539_at	1.353	1.77
Endothelin receptor type B	204273_at	1.547	2.477
Thrombospondin 1	201109_s_at	–1.264	–1.11
Protein amino acid dephosphorylation			
Protein phosphatase 2 (formerly 2A), regulatory subunit B″, α	209633_at	1.141	1.853
Activation of NF-κB-inducing kinase			
Toll-like receptor 3	206271_at	1.005	–1.194
Anion transport			
Solute carrier family 4, sodium bicarbonate cotransporter, member 7	209884_s_at	1.725	1.536
Protein modification			
Plasminogen activator, tissue	201860_s_at	1.043	1.189
ISG15 ubiquitin-like modifier	205483_s_at	–2.867	–1.328
Actin cytoskeleton organization and biogenesis			
Advillin	205539_at	1.353	1.77
Ras homolog gene family, member J	235489_at	1.399	1.432
Muscle development			
Integrin, α11	222899_at	–1.162	–1.337
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Ubiquitin-dependent protein catabolism			
F-box and leucine-rich repeat protein 7	241457_at	1.029	1
Signal transduction			
Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	202086_at	–2.837	–1.494
G-protein-coupled receptor kinase 5	204396_s_at	1.844	2.241
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Toll-like receptor 3	206271_at	1.005	–1.194
Cell growth			
Kruppel-like factor 6	208961_s_at	–1.108	–1.37
Glycerol metabolism			
Glycerophosphodiester phosphodiesterase domain containing 5	213343_s_at	1.235	1.399
Cell differentiation			
c-fos-induced growth factor (vascular endothelial growth factor D)	206742_at	1.665	1.369
Secreted frizzled-related protein 1	202037_s_at	1.104	1.501
Steroid metabolism			
Cytochrome P450, family 3, subfamily A, polypeptide 5	214234_s_at	1.683	1.9

Table 1 (continued)

Gene name	Affymetrix ID	Fold change (log2)	
		1,25-D	1,25-D+ cortisone
Main pathways of carbohydrate metabolism			
Isocitrate dehydrogenase 2 (NADP$^+$), mitochondrial	210046_s_at	1.741	1.383
Cell-matrix adhesion			
Integrin, α11	222899_at	–1.162	–1.337
Nidogen 2 (osteonidogen)	204114_at	1.006	1.482
G1/S transition of mitotic cell cycle			
Branched chain aminotransferase 1, cytosolic	225285_at	1.036	1.626
Ras protein signal transduction			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Barbed-end actin filament capping			
Advillin	205539_at	1.353	1.77
Lipid metabolism			
Solute carrier family 27 (fatty acid transporter), member 2	205768_s_at	1.563	1.326
Copper ion transport			
Ceruloplasmin (ferroxidase)	204846_at	1.598	2.005
Phosphate transport			
Collagen, type XIV, α1 (undulin)	212865_s_at	1.143	1.07
Cation transport			
ATPase, Ca^{2+} transporting, plasma membrane 1	215716_s_at	1.018	1.639
Calcium ion transport			
ATPase, Ca^{2+} transporting, plasma membrane 1	215716_s_at	1.018	1.639
Iron ion homeostasis			
Ceruloplasmin (ferroxidase)	204846_at	1.598	2.005
Tricarboxylic acid cycle			
Isocitrate dehydrogenase 2 (NADP$^+$), mitochondrial	210046_s_at	1.741	1.383
Cell wall catabolism			
Oxidation resistance 1	218197_s_at	1.014	1.306
Oxidation resistance 1	222553_x_at	1	1.481
Cell motility			
Insulin-like growth factor 1 (somatomedin C)	209540_at	1.206	1.151
Thrombospondin 1	201109_s_at	–1.264	–1.11

Table 2. Profile of commonly regulated genes by cortisone and calcitriol/cortisone combination

Gene name	Affymetrix ID	Fold change (log2)	
		cortisone	1,25-D+ cortisone
Nucleosome assembly			
Solute carrier family 22 (organic cation transporter), member 4	205896_at	1.059	1.227
Metabolism			
Fibronectin 1	214701_s_at	–3.951	–1.559
Arylacetamide deacetylase (esterase)	205969_at	1.515	1.757
Response to wounding			
Fibronectin 1	214701_s_at	–3.951	–1.559
Protein amino acid phosphorylation			
Cyclin-dependent kinase 6	231198_at	–1.027	–1.034
Sodium ion transport			
Solute carrier family 22 (organic cation transporter), member 4	205896_at	1.059	1.227

Table 2 (continued)

Gene name	Affymetrix ID	Fold change (log2)	
		cortisone	1,25-D+ cortisone
Unknown P			
Interleukin-13 receptor, α2	206172_at	1.429	2.14
Chromosome 9 open reading frame 26 (NF-HEV)	209821_at	1.806	3.382
Blottin	238222_at	–1.546	–1.392
Premature ovarian failure, 1B	219756_s_at	–1.307	–1.314
Transcribed locus, strongly similar to NP_612442.1 hypothetical protein BC009980 *(Homo sapiens)*	236285_at	–1.025	–1.107
Poly(ADP-ribose)glycohydrolase	205060_at	–1.018	–1.123
Collagen, type I, α1	217430_x_at	–2.786	–1.032
Metallothionein 1M	216336_x_at	1.074	1.6
Cell proliferation			
Cyclin-dependent kinase 6	231198_at	–1.027	–1.034
Transcription			
Juxtaposed with another zinc finger gene 1	225800_at	–1.051	–1.051
Acute-phase response			
Fibronectin 1	214701_s_at	–3.951	–1.559
Regulation of cell cycle			
Cyclin-dependent kinase 6	231198_at	–1.027	–1.034
Cell surface receptor linked signal transduction blood coagulation			
Tissue factor pathway inhibitor 2	209278_s_at	1.263	1.637
Tissue factor pathway inhibitor 2	209277_at	1.185	1.634
Protein biosynthesis			
Arginyl-tRNA synthetase	201330_at	1.175	2.041
Triacylglycerol biosynthesis			
Diacylglycerol O-acyltransferase homolog 2 (mouse)	226064_s_at	–1.104	–1.99
Ion transport			
Solute carrier family 22 (organic cation transporter), member 4	205896_at	1.059	1.227
Regulation of transcription, DNA-dependent			
Juxtaposed with another zinc finger gene 1	225800_at	–1.051	–1.051
Biological process unknown			
Metallothionein 1F (functional)	213629_x_at	1.254	1.364
Transmembrane 4 L six family member 1	215034_s_at	1.183	1.393
Arginyl-tRNA aminoacylation			
Arginyl-tRNA synthetase	201330_at	1.175	2.041
Transmembrane receptor protein tyrosine kinase signaling pathway			
Fibronectin 1	214701_s_at	–3.951	–1.559
Cell division			
Cyclin-dependent kinase 6	231198_at	–1.027	–1.034
Cell adhesion			
Fibronectin 1	214701_s_at	–3.951	–1.559
Cell migration			
Fibronectin 1	214701_s_at	–3.951	–1.559
Organic cation transport			
Solute carrier family 22 (organic cation transporter), member 4	205896_at	1.059	1.227
Collagen catabolism			
Matrix metallopeptidase 3 (stromelysin 1, progelatinase)	205828_at	1.717	2.904
Matrix metallopeptidase 1 (interstitial collagenase)	204475_at	1.626	3.043
G1 phase of mitotic cell cycle			
Cyclin-dependent kinase 6	231198_at	–1.027	–1.034
Fatty acid biosynthesis			
Stearoyl-CoA desaturase (Δ9-desaturase)	211708_s_at	–1.766	–1.341

Cellular components classification indicated that the most frequently modulated genes for all three treatments were associated with membrane function and unclassified genes (fig. 3). Notably, calcitriol and cortisone regulated more genes associated with nuclear function than those associated with cytoplasm function, but this was not the case for the calcitriol/cortisone combination.

Unique Regulation of Gene Expression by Calcitriol, Cortisone and Calcitriol/Cortisone

Calcitriol, cortisone, and the calcitriol/cortisone combination also uniquely regulated 140, 107 and 243 genes respectively. Calcitriol modulated gene expression in a pattern which predicts reduced adipocyte apoptosis and increased adipocyte proliferation, and up-regulated genes involved in oxidative stress (table 3). Cortisone down-regulated genes associated with inflammation (table 4) and the calcitriol/cortisone combination resulted in significant additional up-regulation of 11β-HSD-1 and down-regulation of adiponectin expression (table 5).

Real-Time PCR of Apoptotic Gene Expression

To confirm the effects of calcitriol and/or cortisol on genes regulating adipocyte apoptosis components (in addition to those we have previously published [14]), we further studied STC-1 and STC-2 gene expression in human adipocytes using RT-PCR. Consistent with our microarray data, calcitriol attenuated expression of STC-2 by 43% ($p < 0.01$) versus control and cortisone, and the calcitriol/cortisone combination exerted a greater effect, such that STC-2 expression was reduced to only 20% of control ($p < 0.01$) (fig. 4). Consistent with this, calcitriol and the calcitriol/cortisone combination stimulated STC-1 expression by 80% ($p < 0.001$) and 180% ($p < 0.01$) respectively, but cortisone exerted no effect on STC-1 expression (fig. 5).

Discussion

Gene expression patterns from the present study indicate the potential for significant calcitriol modulation of adipocyte number by inhibition of the adipocyte apoptosis and promotion of adipocyte proliferation, as well as potential for calcitriol stimulation of oxidative stress and inflammation. Consistent with our previous direct observations [16], calcitriol and the calcitriol/cortisone combination resulted in significant additional up-regulation of 11β-HSD-1, which plays a key role in generating active cortisol from inactive cortisone, while cortisone exerted little independent effect in the absence of calcitriol.

Previous data from our laboratory demonstrate that calcitriol modulates adipocyte lipid and energy metabolism via both genomic and non-genomic pathways [11, 15, 18]. We have reported that calcitriol plays a direct role in the modulation adipocyte Ca^{2+} signaling, resulting in an increased lipogenesis and decreased lipolysis [11–13]. In addition, calcitriol also plays a role in regulating human adipocyte UCP2 mRNA and protein levels [15, 19], indicating that the suppression of calcitriol and the resulting up-regulation of UCP2 may contribute to increased rates of lipid oxidation. In addition, we also demonstrate that physiological doses of calcitriol inhibit apoptosis in differentiated human and 3T3-L1 adipocytes [14], and that the suppression of calcitriol in vivo by increasing dietary calcium stimulates adipocyte apoptosis in aP2 transgenic mice [14], suggesting that the stimulation of adipocyte apoptosis contributes to the observed reduction in adipose tissue mass after administration of high calcium diets. Moreover, we also recently demonstrated that calcitriol increases oxidative stress by inhibiting UCP2 expression [9] and increasing $[Ca^{2+}]_i$, and consequently favors adipocyte proliferation [20]. Accordingly, the suppression of calcitriol by increasing dietary calcium attenuates adipocyte triglyceride accumulation and causes a net reduction in fat mass in both mice and humans in the absence of caloric restriction [21], a marked augmentation of body weight and fat loss during energy restriction in both mice and humans [21–23], and a reduction in the rate of weight and fat regain following energy restriction in mice [19]. Results of the present study suggest multiple additional calcitriol-responsive genes which may contribute to these responses.

Central obesity is a principle causative factor in the development of the metabolic syndrome [24, 25]. Excess adipose tissue in the visceral compartment is associated with increased transcription and activity of 11β-HSD-1 [1, 2, 26], which functions as a high-affinity reductase to generate active cortisol [6], suggesting that the pattern of central fat deposition associated with chronic glucocorticoid excess. Previous data from this laboratory indicated that calcitriol stimulates human adipocyte expression of 11β-HSD-1 and increases the conversion of cortisone to cortisol, which is subsequently released by adipocytes [16]. In addition, the rapid non-genomic action of 1,25-dihydroxyvitamin D_3 also stimulates cortisol release in adipocytes by increasing $[Ca^{2+}]i$ through the 1,25-D_3-MARRS and consequently increases the amount of cortisol available to bind to activate glucocorticoid receptors [16].

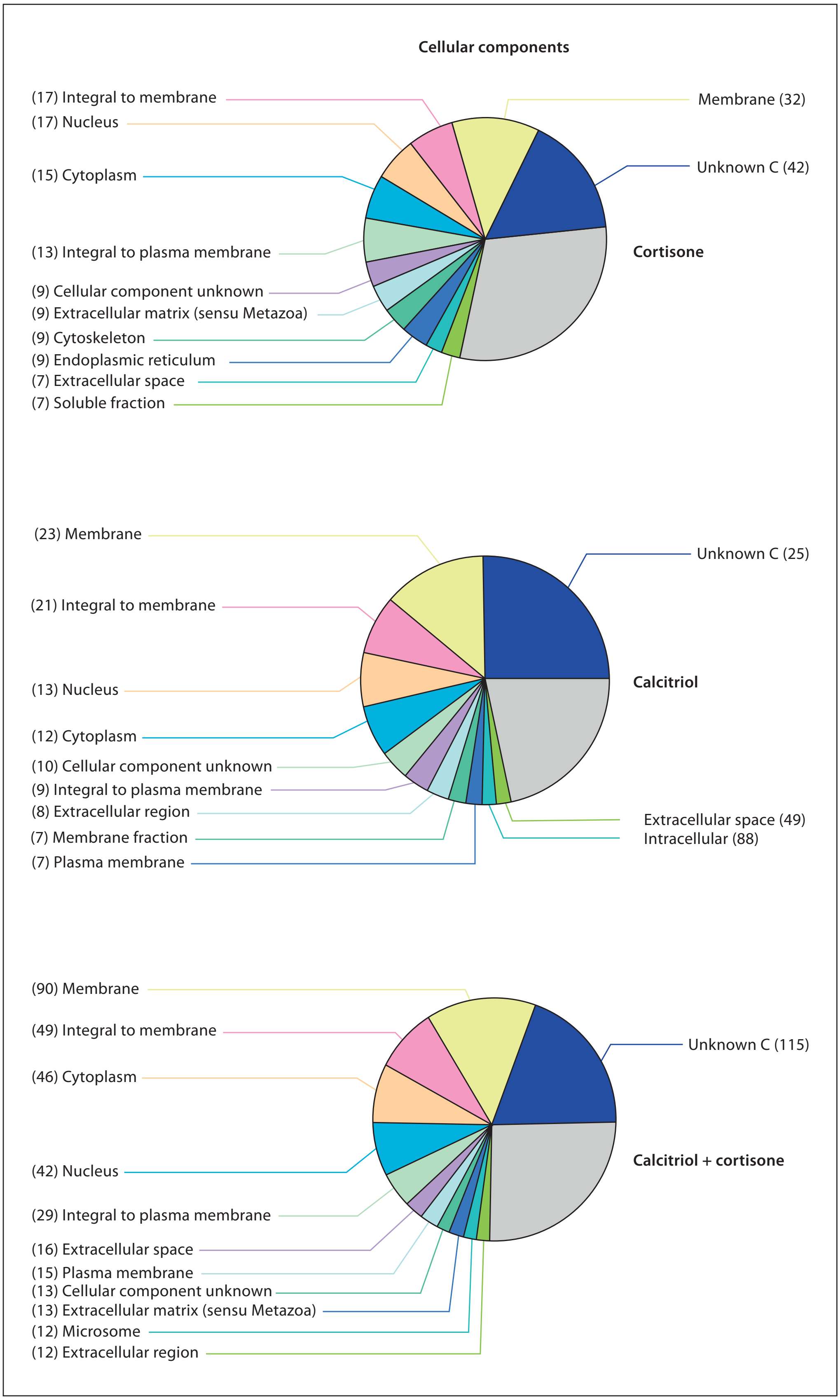

Fig. 3. Cellular components classification of significantly regulated genes by calcitriol, cortisone and calcitriol/cortisone combination.

Table 3. Unique genes regulated by calcitriol

Gene name	Affymetrix ID	Fold change (log2)
Regulation of cell redox homeostasis		
Thioredoxin domain containing 4 (endoplasmic reticulum)	208957_at	−1.087
Calcium ion sensing		
Kv channel interacting protein 2	223727_at	1.306
Metabolism		
Aldehyde dehydrogenase 1	212224_at	−1.318
ATPase, Na^+/K^+ transporting, α2 (+) polypeptide	203296_s_at	−1.012
Activation of MAPK		
Adrenergic, α2A-, receptor	209869_at	−1.074
Wnt receptor signaling pathway		
CDNA FLJ14388 fis, clone HEMBA1002716	229802_at	−1.574
Sodium ion transport		
ATPase, Na^+/K^+ transporting, α2 (+) polypeptide	203296_s_at	−1.012
STAT protein nuclear translocation		
Signal transducer and activator of transcription 1, 91 kDa	209969_s_at	−1.833
Response to stress		
Vascular endothelial growth factor	210512_s_at	1.882
Hemocyte development		
Prolactin	205445_at	1.794
Negative regulation of adenylate cyclase activity		
Adrenergic, α2A-, receptor	209869_at	−1.074
Intracellular signaling cascade		
Signal transducer and activator of transcription 1, 91 kDa	209969_s_at	−1.833
Positive regulation of vascular endothelial growth factor receptor signaling pathway		
Vascular endothelial growth factor	210512_s_at	1.882
Regulation of heart contraction rate		
Kv channel interacting protein 2	223727_at	1.306
Anti-apoptosis		
Insulin-like growth factor 1 receptor	203628_at	1.425
Ossification		
Cadherin 11, type 2, OB-cadherin (osteoblast)	207172_s_at	−1.028
Cell proliferation		
Proteoglycan 4	206007_at	1.235
Vascular endothelial growth factor	210512_s_at	1.882
Insulin-like growth factor 1 receptor	203628_at	1.425
Angiogenesis		
Vascular endothelial growth factor	210512_s_at	1.882
Regulation of nitric-oxide synthase activity		
Nitric oxide synthase 1 (neuronal) adaptor protein	1563512_at	1.083
Caspase activation		
Signal transducer and activator of transcription 1, 91 kDa	209969_s_at	−1.833
Regulation of cell cycle		
Insulin-like growth factor 1 receptor	203628_at	1.425
Positive regulation of cell proliferation		
Cell division cycle 2-like 5 (cholinesterase-related cell division controller)	232266_x_at	1.452
Adrenergic, α2A-, receptor	209869_at	−1.074
Vascular endothelial growth factor	210512_s_at	1.882
Insulin-like growth factor 1 receptor	203628_at	1.425
Cell surface receptor linked signal transduction		
Stanniocalcin 2	203438_at	−1.336
Inflammatory response		
N-myc (and STAT) interactor	203964_at	−1.136
Interferon regulatory factor 7	208436_s_at	−1.368
Response to nutrients		
Stanniocalcin 2	203438_at	−1.336

Table 3 (continued)

Gene name	Affymetrix ID	Fold change (log2)
Protein secretion		
Secretogranin II (chromogranin C)	204035_at	–1.223
Protein biosynthesis		
Protein tyrosine phosphatase, receptor type, G	242188_at	1.014
ATP hydrolysis coupled proton transport		
ATPase, Na^+/K^+ transporting, α2 (+) polypeptide	203296_s_at	–1.012
Lipoprotein metabolism		
Apolipoprotein L, 2	221653_x_at	–1.179
Cholesterol biosynthesis		
NAD(P)-dependent steroid dehydrogenase-like	209279_s_at	1.015
Ion transport		
Potassium voltage-gated channel, Isk-related family, member 4	1552508_at	1.149
Calcium channel, voltage-dependent, β1 subunit	206996_x_at	–1.1
Induction of positive chemotaxis		
Vascular endothelial growth factor	210512_s_at	1.882
Glycosaminoglycan catabolism		
Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	203676_at	1.035
Electron transport		
NADPH oxidase 4	219773_at	1.018
Cholesterol metabolism		
Apolipoprotein L, 2	221653_x_at	–1.179
Proteolysis and peptidolysis		
Cathepsin C	225646_at	–1.172
Platelet activation		
Phospholipid scramblase 1	202430_s_at	–1.751
Clustering of voltage-gated potassium channels		
Kv channel interacting protein 2	223727_at	1.306
Retinol metabolism		
Dehydrogenase/reductase (SDR family) member 9	219799_s_at	1.009
I-κB kinase/NF-κB cascade		
Signal transducer and activator of transcription 1, 91 kDa	209969_s_at	–1.833
Regulation of Wnt receptor signaling pathway		
Transcription factor 7-like 2 (T-cell specific, HMG-box)	236094_at	1.225
Neurogenesis		
Advillin	205539_at	1.353
Vascular endothelial growth factor	210512_s_at	1.882
Potassium ion transport		
Potassium voltage-gated channel, Isk-related family, member 4	1552508_at	1.149
Kv channel interacting protein 2	223727_at	1.306
ATPase, Na^+/K^+ transporting, α2 (+) polypeptide	203296_s_at	–1.012
Regulation of apoptosis		
Interferon induced with helicase C domain 1	219209_at	2.231
Negative regulation of apoptosis		
Cardiotrophin-like cytokine factor 1	1556385_at	1.013
Vascular endothelial growth factor	210512_s_at	1.882
Signal transduction		
Kv channel interacting protein 2	223727_at	1.306
Phosphodiesterase 4C, cAMP-specific (phosphodiesterase E1 dunce homolog, *Drosophila*)	206792_x_at	1.047
Muscle contraction		
Kv channel interacting protein 2	223727_at	1.306
Calcium channel, voltage-dependent, β1 subunit	206996_x_at	–1.1
Neuropeptide signaling pathway		
Polycystic kidney disease 1-like 2	1559261_a_at	1.01
Calcium ion transport		
Calcium channel, voltage-dependent, β1 subunit	206996_x_at	–1.1

Table 4. Unique gene regulated by cortisone

Gene name	Affymetrix ID	Fold change (log2)
Inflammatory response		
Interleukin-1 receptor, type I	215561_s_at	–1.707
Chemokine (C-C motif) ligand 2	216598_s_at	–1.011
Glucose metabolism		
Pyruvate dehydrogenase kinase, isozyme 4	225207_at	1.315
Positive regulation of I-κB kinase/NF-κB cascade		
Filamin A, α (actin-binding protein 280)	213746_s_at	–1.117
Immune response		
Interleukin-1 receptor-like 1	234066_at	1.594
Skeletal development		
Fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	210973_s_at	–1.438
Collagen, type XII, α1	231766_s_at	–1.086
Ion transport		
ATPase, H^+ transporting, lysosomal 70 kDa, V1 subunit A	201971_s_at	–1.461
Lipid catabolism		
Platelet-activating factor acetylhydrolase 2, 40 kDa	205232_s_at	–1.264
Anti-apoptosis		
Chemokine (C-C motif) ligand 2	216598_s_at	–1.011
Cholesterol biosynthesis		
3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	205822_s_at	–1.128
Digestion		
ATPase, class I, type 8B, member 1	214594_x_at	–1.282
Signal transduction		
Insulin-like growth factor 2 receptor	201392_s_at	–1.105
Platelet-derived growth factor receptor, β polypeptide	202273_at	–1.286
Steroid biosynthesis		
Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	211019_s_at	–1.073
Muscle contraction		
Caldesmon 1	201615_x_at	–1.424
Cation transport		
ATPase, class I, type 8B, member 1	214594_x_at	–1.282
Negative regulation of apoptosis		
Cardiotrophin-like cytokine factor 1	235525_at	–1.032
Endocytosis		
Low-density lipoprotein-related protein 1 (α2-macroglobulin receptor)	200784_s_at	–1.056
Proteolysis and peptidolysis		
Complement component 1, s subcomponent	1555229_a_at	–1.167
Lipid metabolism		
3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	205822_s_at	–1.128
Platelet-activating factor acetylhydrolase, isoform Ib, α subunit 45 kDa	200813_s_at	–1.183
ATPase, class I, type 8B, member 1	214594_x_at	–1.282
Low-density lipoprotein-related protein 1 (α2-macroglobulin receptor)	200784_s_at	–1.056
Phosphatidic acid phosphatase type 2B	209355_s_at	–1.098
Tricarboxylic acid cycle		
Succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa	216591_s_at	–1.01
Metabolism		
Retinol dehydrogenase 10 (all-trans)	1552378_s_at	–1.316
G2/M transition of mitotic cell cycle		
Anaphase promoting complex subunit 5	208721_s_at	–1.071
Proton transport		
ATPase, H^+ transporting, lysosomal 70 kDa, V1 subunit A	201971_s_at	–1.461

Table 5. Unique genes regulated by calcitriol/cortisone combination

Gene name	Affymetrix ID	Fold change (log2)
Mitosis		
Cell division cycle 2, G1 to S and G2 to M	203213_at	1.208
Sensory perception		
Chemokine (C-X-C motif) ligand 10	204533_at	–1.333
Glucocorticoid metabolism		
Hydroxysteroid (11β) dehydrogenase 1	205404_at	1.116
Androgen receptor signaling pathway		
Transmembrane, prostate androgen-induced RNA	222449_at	1.111
Frizzled signaling pathway		
Frizzled homolog 8 *(Drosophila)*	227405_s_at	1.189
Metabolism		
Acyl-CoA synthetase short-chain family member 2	234312_s_at	–1.164
1-Acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, β)	32837_at	–1.293
Hydroxysteroid (11β) dehydrogenase 1	205404_at	1.116
Response to wounding		
Coagulation factor II (thrombin) receptor	203989_x_at	–1.163
Fibronectin 1	214701_s_at	–1.559
Circulation		
Chemokine (C-X-C motif) ligand 10	204533_at	–1.333
Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	244661_at	1.237
Wnt receptor signaling pathway		
Secreted frizzled-related protein 1	202037_s_at	1.501
Response to oxidative stress		
Sulfiredoxin 1 homolog *(S. cerevisiae)*	225252_at	1.136
Dual specificity phosphatase 1	201041_s_at	1.293
Sodium ion transport		
Solute carrier family 9 (sodium/hydrogen exchanger), member 6	203909_at	1.28
Solute carrier family 22 (organic cation transporter), member 4	205896_at	1.227
Serum/glucocorticoid regulated kinase	201739_at	1.6
Apoptosis		
Homeodomain interacting protein kinase 2	219028_at	1.154
Coagulation factor II (thrombin) receptor	203989_x_at	–1.163
Tumor necrosis factor receptor superfamily, member 21	218856_at	1.039
Serum/glucocorticoid regulated kinase	201739_at	1.6
ATG5 autophagy related 5 homolog *(S. cerevisiae)*	202511_s_at	1.104
Cell death-inducing DFFA-like effector c	219398_at	–1.578
Sulfatase 1	212354_at	1.445
Lectin, galactoside-binding, soluble, 12 (galectin 12)	223828_s_at	–1.457
Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α	201502_s_at	1.279
Serine/threonine kinase 17a (apoptosis-inducing)	202693_s_at	1.003
Response to stress		
Vascular endothelial growth factor	210512_s_at	1.735
Serum/glucocorticoid regulated kinase	201739_at	1.6
Eukaryotic translation initiation factor 1	228967_at	1.364
Positive regulation of vascular endothelial growth factor receptor signaling pathway		
Vascular endothelial growth factor	210512_s_at	1.735
Prostaglandin biosynthesis		
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	215813_s_at	1.52
Anti-apoptosis		
Tumor necrosis factor, α-induced protein 8	208296_x_at	–1.162
Fatty acid metabolism		
Acyl-CoA synthetase long-chain family member 3	201662_s_at	1.089

Table 5 (continued)

Gene name	Affymetrix ID	Fold change (log2)
Cell proliferation		
DIP13 β	218218_at	1.134
Cysteine-rich protein 1 (intestinal)	205081_at	1.438
Chemokine (C-X-C motif) ligand 1 (melanoma growth-stimulating activity, α)	204470_at	1.489
Branched chain aminotransferase 1, cytosolic	225285_at	1.626
Vascular endothelial growth factor	210512_s_at	1.735
Met proto-oncogene (hepatocyte growth factor receptor)	203510_at	1.312
Cyclin-dependent kinase 6	231198_at	–1.034
Angiogenesis		
Vascular endothelial growth factor	210512_s_at	1.735
Glyoxylate cycle		
Isocitrate dehydrogenase 2 (NADP$^+$), mitochondrial	210046_s_at	1.383
Chemotaxis		
Chemokine (C-X-C motif) ligand 1 (melanoma growth-stimulating activity, α)	204470_at	1.489
Chemokine (C-X-C motif) ligand 10	204533_at	–1.333
Chemokine (C-C motif) receptor 1	205098_at	1.573
Positive regulation of cell proliferation		
Chemokine (C-X-C motif) ligand 10	204533_at	–1.333
Vascular endothelial growth factor	210512_s_at	1.735
FOS-like antigen 1	204420_at	1.036
Transforming growth factor, α receptor II (70/80 kDa)	207334_s_at	1.151
Inflammatory response		
Chemokine (C-X-C motif) ligand 1 (melanoma growth-stimulating activity, α)	204470_at	1.489
Chemokine (C-X-C motif) ligand 10	204533_at	–1.333
Chemokine (C-C motif) receptor 1	205098_at	1.573
Cell redox homeostasis		
Thioredoxin reductase 1	201266_at	1.114
Phospholipid biosynthesis		
Glycerol-3-phosphate acyltransferase, mitochondrial	225420_at	–1.269
Regulation of blood pressure		
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	215813_s_at	1.52
Cholesterol biosynthesis		
NAD(P) dependent steroid dehydrogenase-like	215093_at	1.219
Induction of positive chemotaxis		
Vascular endothelial growth factor	210512_s_at	1.735
Positive regulation of cytosolic calcium ion concentration		
Chemokine (C-C motif) receptor 1	205098_at	1.573
Carbohydrate metabolism		
Glycerol-3-phosphate dehydrogenase 1 (soluble)	213706_at	–1.315
Aldo-keto reductase family 1, member B1 (aldose reductase)	201272_at	1.122
Phosphoglucomutase 2	225367_at	1.701
Cholesterol metabolism		
Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	244661_at	1.237
Transport		
ATP-binding cassette, sub-family C (CFTR/MRP), member 1	202804_at	1.055
Generation of precursor metabolites and energy		
Adiponectin, C1Q and collagen domain containing	207175_at	–1.124
Cytoplasmic sequestering of NF-κB		
Nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, α	201502_s_at	1.279
Negative regulation of signal transduction		
Regulator of G-protein signalling 2, 24 kDa	202388_at	1.646
Potassium ion transport		
Potassium inwardly-rectifying channel, subfamily J, member 8	205304_s_at	1.7
Regulation of apoptosis		
Caspase-1 dominant-negative inhibitor pseudo-ICE	1552701_a_at	1.003

Table 5 (continued)

Gene name	Affymetrix ID	Fold change (log2)
Negative regulation of apoptosis		
Vascular endothelial growth factor	210512_s_at	1.735
Steroid metabolism		
Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	244661_at	1.237
Lipid metabolism		
Perilipin	205913_at	–1.312
Acyl-CoA synthetase long-chain family member 3	201662_s_at	1.089
Sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1	244661_at	1.237
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	215813_s_at	1.52
Hydroxysteroid (11β) dehydrogenase 1	205404_at	1.116
Thyroid hormone responsive (SPOT14 homolog, rat)	229476_s_at	–1.76
Copper ion transport		
Ceruloplasmin (ferroxidase)	204846_at	2.005
Gluconeogenesis		
Phosphoenolpyruvate carboxykinase 1 (soluble)	208383_s_at	–1.969
Fatty acid biosynthesis		
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	215813_s_at	1.52
Stearoyl-CoA desaturase (Δ9-desaturase)	211708_s_at	–1.341

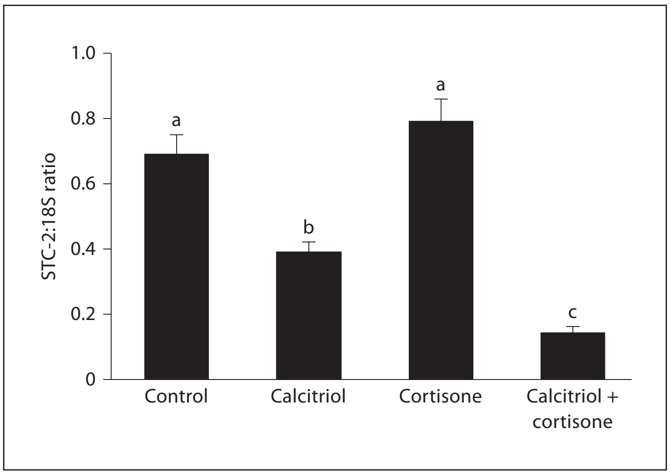

Fig. 4. Effect of calcitriol and/or cortisone on STC-2 to 18S expression ratio in human adipocytes. Values are presented as mean ± SEM, n = 6. Means with different letter differ with $p < 0.05$.

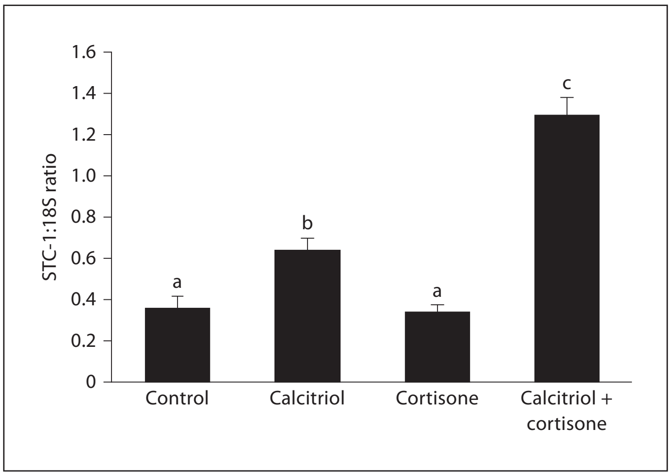

Fig. 5. Effect of calcitriol and/or cortisone on STC-1 to 18S expression ratio in human adipocytes. Values are presented as mean ± SEM, n = 6. Means with different letter differ with $p < 0.05$.

Furthermore, addition of cortisone greatly increases the capacity of calcitriol to stimulate 11β-HSD expression and cortisol production [16]. This effect may result from an increase in substrate availability for 11β-HSD in generating active glucocorticoid. Consistent with this, the present microarray analysis further confirmed that the combination of calcitriol and cortisone increased 11β-HSD expression in human subcutaneous adipocytes. If comparable mechanisms operate in visceral adipocytes, these data suggest that calcitriol may co-function with glucocorticoid precursor and contribute to the increased visceral fat accumulation and associated disorders, and that strategies designed to suppress circulating calcitriol levels may suppress adipocyte cortisol levels and mediate the preferential loss of visceral fat in obese humans.

Excess fat mass may result, in part, from recruitment of new adipocytes, and the adipogenic potential of precursor cells may be an important determinant in fat mass regulation and obesity-associated disorders [27, 28]. We have previously shown that calcitriol stimulates adipocyte proliferation and this effect was associated with a stimulation of oxidative stress [9, 20]. Indeed, suppression of calcitriol by increasing dietary calcium suppressed fat mass accumulation in humans and in a rodent model of obesity [8]. The present study provides further supporting evidence for a potential role of calcitriol in adipogenesis, as calcitriol stimulated gene expression associated with cell proliferation, angiogenesis, cell cycle, neurogenesis, DNA replication and cell differentiation in human adipocytes. Consistent with this, calcitriol alone or combined with cortisone also increased anti-apoptotic gene expression and suppressed apoptotic genes. Notably, further RT-PCR analysis confirmed that calcitriol stimulated STC-1 and suppressed apoptotic STC-2 gene expression while addition of cortisone resulted in a greater effect on both genes. These observations are consistent with our previous study that physiological levels of calcitriol inhibit the expression of several apoptotic genes in human adipocytes [14], suggesting that calcitriol may also contribute to increasing adipocyte number by attenuating adipocyte apoptosis.

Excess adiposity is characterized by low-grade systemic inflammation and increased oxidative stress, and obese subjects exhibit elevated production of inflammatory markers [29–32]. Adipocytes produce a variety of biological molecules, including both inflammatory cytokines, such as TNF-α, and anti-inflammatory factors, such as adiponectin [33]. Dysregulated production of these adipocytokines contributes to the pathogenesis of obesity-associated metabolic syndrome. Although the mechanism responsible for the development of systemic inflammation is not clear, multiple previous studies indicated that increased oxidative stress in accumulated fat is an underlying cause of dysregulation of adipocytokines [34–37]. We have shown previously that calcitriol was associated with oxidative stress and inflammation and stimulated the expression of multiple inflammatory cytokines in human adipocytes, while suppression of calcitriol by dietary calcium suppressed ROS and inflammatory cytokines independent of its anti-obesity effects [9]. Consistent with these observations, data from the present study indicate that calcitriol may increase multiple inflammatory cytokine and decreased anti-inflammatory cytokine transcription. In addition, data from the present study suggest that calcitriol also favors cytoplasmic oxidative stress by increasing NADPH oxidase expression, and our previous data demonstrate a regulatory effect of calcitriol on NAPDH oxidase expression [9]. However, this effect was absent with the combination of calcitriol with cortisone; attenuation of this effect by cortisone may contribute to the anti-inflammatory function of glucocorticoid.

In conclusion, we found 7 genes commonly regulated by calcitriol, cortisone and the calcitriol/cortisone combination. Moreover, calcitriol regulated additional 86 genes in common with the calcitriol/cortisone combination, many of which are associated with adipogenesis and apoptosis. Calcitriol also uniquely regulated 140 genes largely related to inflammation and oxidative stress, while the calcitriol/cortisone combination markedly upregulated 11β-HSD. Cortisone alone regulated only a relatively small number of genes and this may due to its inactive form. This study provides supporting evidence for an important role of calcitriol in regulation of adipocyte number and metabolism, suggesting that strategies to suppress calcitriol may promote preferential loss of visceral adipose tissue, although this conclusion must be tempered by the source of the cells utilized in the present study (subcutaneous depot in origin).

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