Expression profiling of 11 β -hydroxysteroid dehydrogenase type-1 and glucocorticoid-target genes in subcutaneous and omental human preadipocytes

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Abstract

Obesity is associated with increased morbidity and mortality from cardiovascular disease, diabetes and cancer. Although obesity is a multi-factorial heterogeneous condition, fat accumulation in visceral depots is most highly associated with these risks. Pathological glucocorticoid excess (i.e. in Cushing's syndrome) is a recognised, reversible cause of visceral fat accumulation. The aim of this study was to identify depot-specific glucocorticoid-target genes in adipocyte precursor cells (preadipocytes) using Affymetrix microarray technique. Confluent preadipocytes from subcutaneous (SC) and omental (OM) adipose tissue collected from five female patients were treated for 24 h with 100 nM cortisol (F), RNA was pooled and hybridised to the Affymetrix U133 microarray set. We identified 72 upregulated and 30 downregulated genes by F in SC cells. In OM preadipocytes, 56 genes were increased and 19 were decreased. Among the most interesting were transcription factors, markers of adipocyte differentiation and glucose metabolism, cell adhesion and growth arrest protein factors involved in G-coupled and Wnt signalling. The Affymetrix data have been confirmed by quantitative real-time PCR for ten specific genes, including *HSD11B1*, *GR*, *C/EBP α* , *C/EBP β* , *IL-6*, *FABP4*, *APOD*, *IRS2*, *AGTR1* and *GHR*. One of the most upregulated genes in OM but not in SC cells was *HSD11B1*. The GR was similarly expressed and not regulated by glucocorticoids in SC and OM human preadipocytes. *C/EBP α* was expressed in SC preadipocytes and upregulated by F, but was below the detection level in OM cells. *C/EBP β* was highly expressed both in SC and in OM preadipocytes, but was not regulated by F. Our results provide insight into the genes involved in the regulation of adipocyte differentiation by cortisol, highlighting the depot specifically in human adipose tissue.

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Introduction

The magnitude and severity of the obesity epidemic (Kopelman 2000) have provided the impetus to further understand its genetic basis as well as focussing attention on the neuroendocrine regulation of appetite control and energy expenditure and adipocyte cell biology. Crucially, fat distribution is of pivotal importance with central or visceral adiposity conferring the greatest risk of premature mortality (Jensen 1997), and therefore, there is a need to define factors that regulate adipose distribution. Human adipose tissue exhibits different properties depending on their anatomical localisation, and has different sensitivity to glucocorticoids (Bujalska *et al.* 1997, Fried *et al.* 1998).

Clinical observations in patients with Cushing's syndrome who develop reversibly central obesity highlight the importance of glucocorticoids in this regard (Rebuffe-Scrive 1988). Glucocorticoids (GCs) induce adipocyte differentiation (Hauner *et al.* 1987) preferentially within visceral adipose tissue due to increased

glucocorticoid receptor (GR) expression (Bronnegard *et al.* 1990), although other tissue-specific factors may be important.

In human adipose tissue, cortisol availability to bind and activate GR is controlled by 11 β -hydroxysteroid dehydrogenase type-1 (11 β -HSD1). 11 β -HSD1 amplifies glucocorticoid action by reducing inactive cortisone (E) to active cortisol (F) (Lakshmi & Monder 1988) and is highly expressed in omental (OM) compared with subcutaneous (SC) fat. Furthermore in primary adipose cultures, 11 β -HSD1 expression and activity are induced by F itself (Bujalska *et al.* 1997), thus representing a novel mechanism whereby cells can generate F locally independent of circulating concentrations. Several factors are known to regulate 11 β -HSD1 expression and activity in the liver (Jamieson *et al.* 1995) and adipocytes; the most potent inducers are glucocorticoids, cytokines (leptin, tumour necrosis factor (TNF) α , interleukin (IL)-1 β , IL-4, IL-6 and IL-14; Handoko *et al.* 2000, Tomlinson *et al.* 2001) and peroxisome proliferator activated receptor (PPAR γ) agonists (Berger *et al.*

2001, Laplante *et al.* 2003). Additionally, a significant role for C/EBP transcription factors in regulating rat hepatic 11 β -HSD1 has been reported (Williams *et al.* 2000). Amongst the factors suppressing 11 β -HSD1 activity are: growth hormone, insulin-like growth factor-I (IGF-I) (Moore *et al.* 1999, Tomlinson *et al.* 2001), liver X receptors (Stulnig *et al.* 2002), PPAR α agonists (Hermanowski-Vosatka *et al.* 2000) and pituitary hormones like corticotrophin-releasing hormone and adrenocorticotrophin hormone (Friedberg *et al.* 2003).

Microarray approaches have been increasingly used to study gene profiling during adipocyte differentiation; however, most of these studies were carried out on mouse cell lines and not on human primary preadipocyte cultures (Burton *et al.* 2002, Jessen & Stevens 2002, Wang *et al.* 2004). On this background, we aimed to identify factors that might regulate 11 β -HSD1 gene transcription in human OM versus SC adipose tissue. We have also hypothesised that the identification of GR-induced target genes in human OM versus SC adipose tissue will increase our knowledge base of the predilection of glucocorticoids to increase visceral fat mass and might reveal exciting candidate targets involved in the pathogenesis of central obesity.

Materials and methods

Human preadipocyte cell culture

Paired SC and OM abdominal adipose tissue was obtained from five females (mean age 44.8 years, mean body mass index 29.4 kg/m²; patient details shown in Table 1). All patients were undergoing hysterectomy and not on any hormonal treatment. Tissues were dispersed with collagenase 1 as described earlier (Bujalska *et al.* 1999). Preadipocytes were seeded in Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F-12 (DMEM/F12) media with 10% FCS into six-well tissue culture dish and cultured to confluence for 4–6 days. Media were changed every other day. The study had the approval of the local research ethics committee, and all patients had given written informed consent prior to study inclusion.

Table 1 Details of subjects who participated in the study

	Sex	Age	Body mass index (BMI)	Procedure
pat 34	F	46	28.5	Hysterectomy
pat 36	F	50	23.3	Hysterectomy
pat 39	F	54	39.7	Hysterectomy
pat 41	F	29	26.2	Hysterectomy
pat 43	F	45	28.8	Hysterectomy
Mean		44.8	29.3	
S.D.		9.5	6.2	

Glucocorticoid treatment

Confluent SC and OM preadipocytes were treated with 100 nM F for 24 h in serum-free DMEM/F12 media. In control cells, F was omitted.

RNA isolation

Total RNA was extracted from primary cultures using TriReagent (Sigma). Total RNA was extracted from five confluent primary cultures of human SC and OM preadipocytes treated with or without 100 nM F. Total RNA was treated with DNaseI to remove any genomic DNA contamination (DNaseI, Invitrogen) and its quantity and quality were assessed spectrophotometrically at OD_{260/280} and by electrophoresis on 1% agarose gel.

Affymetrix microarray experiments

All experiments were performed using Affymetrix human HgU133A and HgU133B oligonucleotide array set, as described at <http://www.affymetrix.com/products/arrays/specific/hgu133.affy> and complied with Minimum Information About a Microarray Experiment (MIAME) standard. Total RNA was used to prepare biotinylated-target RNA with minor modifications from the manufacturer's recommendations (http://www.affymetrix.com/support/technical/manual/expression_manual.affy). Briefly, 10 μ g mRNA (pool of 2 μ g total RNA from each cell preparation) were used to generate first-strand cDNA by using a T7-linked oligo(dT) primer (SuperScript Double Stranded cDNA Synthesis Kit, Invitrogen). After second-strand synthesis, *in vitro* transcription was performed with biotinylated UTP and CTP (Enzo Life Sciences, Farmingdale, NY, USA), resulting in approximately 100-fold amplification of RNA. A complete description of procedures is available at http://bioinf.picr.man.ac.uk/mbcf/downloads/GeneChip_Target_Prep_Protocol_CRUK_v_2.pdf. The target cDNA generated from each sample was processed as per manufacturer's recommendation using an Affymetrix GeneChip Instrument System (http://www.affymetrix.com/support/technical/manual/expression_manual.affy). Briefly, spike controls were added to 15 μ g fragmented cDNA before overnight hybridisation. Arrays were then washed and stained with streptavidin–phycoerythrin, before being scanned on an Affymetrix GeneChip scanner. A complete description of these procedures is available at http://bioinf.picr.man.ac.uk/mbcf/downloads/GeneChip_Hyb_Wash_Scan_Protocol_v_2_web.pdf. Additionally, quality and amount of starting RNA were confirmed using an agarose gel. After scanning, array images were assessed by eye to confirm scanner alignment and the absence of significant bubbles or scratches on the chip surface. 3'/5' ratios for glyceraldehyde-3-phosphate dehydrogenase

(GAPDH) and β -actin were confirmed to be within acceptable limits (0.96–1.14), and BioB spike controls were found to be present on all chips, with BioC, BioD and CreX also present in increasing intensity. When scaled to a target intensity of 100, scaling factors for all arrays were within acceptable limits (0.8–1.1), as were background, Q values and mean intensities.

Microarray data analysis

Data from absolute and comparison files (F treated versus control) in SC and OM preadipocytes were normalised and analysed using Gene Expression Pattern Analysis Suite 2.0 software (<http://gepas.bioinfo.cipf.es>). Methods used: background correction: Robust Multi-array Analysis (RMA); normalisation: quantiles; Perfect Match (PM) correction: pmonly; summary: medianpolish (<http://gepas.bioinfo.cipf.es/cgi-bin/tools>). A total of 22 283 (U133A) and 22 645 (U133B) entries in comparison analysis were sorted out according to criteria: (1) genes with absent call in control and treated cell arrays were deleted; (2) genes with difference call of no change were deleted; (3) genes with absolute signal below 100 were eliminated; (4) genes with signal log ratio (SLR) below 1 for I (increase) or above -1.0 for D (decrease) were deleted (arbitrary cut-off point of twofold change) and (5) expressed sequence tags (ESTs) and hypothetical protein entries were not analysed.

This filtering procedure generated two datasets: SC preadipocytes treated with F versus control and OM preadipocytes treated with F versus control. Genes were analysed according to their metabolic function, such as glucocorticoid metabolism, transcription factors, genes involved in growth arrest, extracellular matrix (ECM) modulation, adipocyte-specific genes, immune responses, glucose metabolism and metabolic enzymes using NetAffx Analysis Center (www.affymetrix.com).

Reverse transcription

Total RNA (1 μ g) in total volume of 20 μ l was reverse transcribed using avian myeloblastosis virus reverse transcriptase (AMV-RT) and random hexamers according to manufacturer's protocol (Promega). Fifty nanograms of cDNA (1 μ l reverse transcriptase (RT) reaction) were used for real-time PCR to confirm the expression of ten genes.

Real-time PCR

To confirm that the Affymetrix DNA microarray accurately identified the gene expression changes, we carried out quantitative RT-PCR of ten genes with varying degree of change, using the Applied Biosystems 7700 Real-Time PCR System (PE Applied Biosystems,

UK). Briefly, PCRs were carried out in 25 μ l volumes on 96-well plates, in a reaction buffer containing 2 $\times$ TaqMan Universal PCR Master Mix. All reactions were singleplexed with the housekeeping gene 18S rRNA on the same plate, provided as a preoptimised control probe (PE Biosystems, Warrington, UK), enabling data to be expressed in relation to an internal reference, to allow for differences in RT efficiency. Measurements were carried out at least three times for each sample. All target gene probes were labelled with FAM, and the housekeeping gene with VIC. Reactions were as follows: 50 °C for 2 min, 95 °C for 10 min; then 44 cycles of 95 °C for 15 s and 60 °C for 1 min. According to the manufacturer's guidelines, data were expressed as Ct values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine Δ Ct values (Δ Ct = Ct of the target gene minus Ct of the housekeeping gene). To exclude potential bias due to averaging data, which had been transformed through the equation $2^{-\Delta\Delta Ct}$ to give a fold change in gene expression, all statistics were performed with Δ Ct values. Five sequences of primers and probes used for real-time PCR were designed using PrimerExpress software (Applied Biosystems, UK) and shown in Table 2. Five other genes; *IL-6*, *IRS2*, *AGTR1*, *APOD* and *FABP4* were validated using commercially available Assay on Demand (Applied Biosystems, Applera, UK). Inter-patient and site differences of gene expressions were tested using individual cDNA preparation. These confirmed the same pattern of change as pooled cDNA used for Affymetrix arrays (data not shown).

Statistical analysis

In comparison analysis where baseline array were the control cells, statistical significance of signal log ratio (SLR; quantitative measure of the relative change in transcript abundance) was calculated. The Wilcoxon's signed rank test was used to compute change P value for each probe set. The P values range in scale from 0.0 to 1.0 and provide the likelihood of change and direction. P values close to 0.0 (<0.001) and 1.0 (>0.998) were used for significant increase and significant decrease respectively (detailed information can be found in GeneChip Expression Analysis, Data Analysis Fundamentals, Affymetrix). For quantitative RT-PCR of the gene validation, statistical software (SPSS for Windows 11.5, SPSS, Inc., UK) was used for statistical analysis. Data are presented as mean values with standard error and P value of <0.05 was accepted as statistically significant.

Results

We segregated genes of known function into three groups (Fig. 1): (a) SC specific – 43 upregulated and 21

Table 2 Primer and probe sequences (5' to 3' orientation) used for real-time PCR. Primers and probes for IL-6, IRS 2, AGTR1, APOD, FABP4 were purchased from Applied Biosystems (TaqMan Gene Expression Assays, Applied Biosystems, UK)

Gene	Gene Bank no.	Forward	Reverse	Probe
GH-receptor	NM_000163	GAGGCCACAGCAGCTATCCT	ATTTGTCTTTAGGCTGGATTAAACA	AGCAGAGCACCCCTGGAGTCTGCAAA
GR	NM_000176	GCGATGGTCTCAGAAACCAAC	GAGATTACAGAGGAAGTTATCCTCTGC	TGCAGTGAAGGTTGCTGAGGCTCTGA
C/EBP α	NM_004364	TGGACAAGAACACAGCAACGAG	TTGTCACTGGTCAGCTCCAG	CACCTTCTGCTGCTCCACGTT
C/EBP β	NM_005194	ATCTTGGCCCTTGTCGGGCTCTT	GACAAGCACAGCGACGAGTA	GTGCTGCTCTCCAGGTT
HSD11B1	NM_005525	AGGAAAGCTCATGGGAGGACTAG	ATGGTGAATATCATGTGAAAAGATTCC	CATGGTCATTCTCAACCCACATCACCACACA

Figure 1 Number of genes with (A) increased or (B) decreased expression ($\geq$ twofold) after 24 h cortisol (F) treatment in SC, OM preadipocytes or in both adipose depots (adipose specific).

downregulated; (b) OM specific – 27 upregulated and 10 downregulated and (c) adipose specific – 29 upregulated and 9 downregulated in both SC and OM depots. The list of genes with their symbol, GeneBank accession number, description, fold change and function is shown in Table 3 (for increased expression) and Table 4 (for decreased expression).

SC preadipocytes

Genes that were upregulated by cortisol included those associated with negative regulation of cell growth (*BTG1*, *PMP22*, *CD9*, *RAI2* and *CUGBO2*) and apoptosis-related proteins (e.g. *CDO1*, *AMIGO2*, *CFLAR* and *CUGBP2*) as well as genes involved in cell matrix rearrangements (e.g. *CHL1*, *DPT*, *ABMLIM*, *PRELP* and *KIAA1011*; Table 2). Three transcription factors linked to cell growth, C/EBP α , FOS (part of the AP-1 complex) and GFB were upregulated 2·0-, 2·2- and 2·1-fold (respectively). Additionally, a number of genes linked to lipid metabolism were upregulated – *FACL2*, *LPL* and *APOB*. Significant upregulation of DF (adipsin), FABP4 and AGTR1 was also seen in SC preadipocytes, changes that were absent in OM cells. Expression levels of three of these genes (*C/EBP* α , *FABP4* and *AGTR1*) as well as C/EBP β and glucocorticoid receptor (GR) were validated by real-time PCR (Figs 2–4).

A number of genes associated to G-protein coupled receptor signalling (*EDNRB*, *GPRC5B*, *RABGAP1* and *TM7SF1*) and calcium action (*ITPR1* and *SPARCL1*) were upregulated in depot-specific way (Table 3).

Genes that were specifically decreased in SC adipose stromal cells following incubation with cortisol (Table 4) included those positively regulating cell proliferation (*VEGF* and *WARS*), oncogenes (*ENPP2*), anti-apoptotic (*OXTR*). Interestingly, in SC preadipocytes F-treatment decreased expression of two genes

Table 3 Overview of the genes that were significantly increased after 24 h 100 nM cortisol treatment (F) compared to control (ctr) in subcutaneous (SC) omental (OM) preadipocytes or both fat deposits. Abbreviations are given

	Acc no.	SC	Gene	Function
Symbol				
SC specific (fold increase)				
ABLM	NM_006720	2.5	Actin-binding LIM protein 1	Cytoskeletal protein
ADARB1	NM_015833	2.1	Adenosine deaminase	RNA processing
AGTR1	NM_000685	2.4	Angiotensin receptor 1, transcript variant 1	Renin/angiotensin system
ALDH6A1	NM_005589	2.1	Aldehyde dehydrogenase 6 family, member A1	Oxidative decarboxylation
AMIGO2	NM_181847	2.3	Adhesion molecule with Ig-like domain 2	Anti-apoptotic
ANPEP	NM_001150	2.0	Alanyl (membrane) aminopeptidase	Metabolism of regulatory peptides
APOB	NM_000384	2.0	Apolipoprotein B	Metabolism (lipid)/transport
BRUNOL3	NM_006561	2.7	RNA-binding protein BRUNOL3	RNA processing
BTG1	NM_001731	2.0	B-cell translocation gene 1	Anti-proliferative
CD9	NM_001769	2.1	CD9 antigen	Cell growth
CDO1	NM_001801	2.7	Cysteine dioxygenase, type-I	Apoptosis-related protein
C/EBP α	NM_004364	2.0	CCAAT enhancer-binding protein	Transcription factor/growth arrest
CFLAR	NM_003879	2.1	CASP8 and FADD-like apoptosis regulator	Anti-apoptotic
CHL1	NM_006614	2.7	Cell adhesion molecule with homology to L1CAM	Cell adhesion
CLEC3B	NM_003278	2.3	C-type lectin domain family 3, member B	Plasminogen-binding protein
CUGBO2	NM_006403	2.7	Crk-associated substrate-related protein Cas-L	Cell growth/cell cycle
CUGBP2	NM_006561	3.1	CUG triplet repeat, RNA-binding protein 2	RNA processing/apoptosis-related protein
DF	NM_001928	2.1	D component of complement (adipsin)	Adipose-specific peptide
DPT	NM_001937	2.7	Dermatopontin	Cell adhesion
EDNRB	NM_003991	2.3	Endothelin receptor type-B variant 2	G-protein-coupled receptor signalling
FABP4	NM_001442	4.1	Fatty acid-binding protein 4	Adipose-specific peptide
FACL2	NM_021122	2.0	Fatty acid-coenzyme A ligase, long-chain 2	Metabolism (lipids)
FOS	NM_005252	2.2	v-fos FBJ murine osteosarcoma viral oncogene	Cell proliferation/transcription factor
GBF	NM_001300	2.1	DNA-binding zinc finger	Transcription factor (anti-oncogene)
GCHFR	NM_005258	2.0	GTP cyclohydrolase I feedback regulatory protein	Metabolism (nitric oxide biosynthesis)
GCNT1	NM_001490	2.1	Glucosaminyl (<i>N</i> -acetyl) transferase 1	<i>N</i> -acetyl-glucosaminyl transferase
GPRC5B	NM_016235	3.5	G-protein-coupled receptor, family C group5 member B	G-protein-coupled receptor signalling
ITPR1	NM_002222	2.5	Inositol 1,4,5-triphosphate receptor, type-1	Ca ⁺⁺ mobilisation from endoplasmic reticulum
KIAA1011	NM_015180	2.5	Synaptic nuclei expressed gene 2	Cytoskeletal protein
KIAA1017	NM_007216	2.3	α integrin-binding protein 63	Metabolism (organelle biogenesis)
LEP	NM_000230	2.0	Leptin (murine obesity homologue)	Adipose-specific peptide
LPL	NM_000237	5.5	Lipoprotein lipase	Metabolism (lipids)
MAFF	NM_012323	2.1	Novel MAFF oncogene family, protein F	Protooncogene
MMD	NM_012329	2.4	Monocyte to macrophage differentiation associated	Macrophage-specific gene
NDRG1	NM_006096	2.0	<i>N</i> -myc downstream regulated	Macrophage-specific gene
PMP22	NM_000304	2.0	Peripheral myelin protein 22	Cell growth (negative)
PRELP	NM_002725	2.0	Proline arginine-rich end leucine-rich repeat protein	Extracellular matrix (ECM) protein
RABGAP1	NM_012197	2.1	GTPase-activating protein	G-protein-coupled receptor signalling
RAI2	NM_021785	2.6	Retinoic acid induced 2	Cell growth
RNASE4	NM_002937	2.6	DnaJ (Hsp40) homologue, subfamily C, member 8	Ribonuclease
SPARCL1	NM_004684	2.2	SPARC-like 1 (mast9, hevin)	Calcium binding
TM7SF1	NM_003272	2.1	Transmembrane 7 superfamily member 1	G-protein-coupled receptor signalling
ZNF294	NM_015565	2.0	Zinc finger protein 294	Transcription factor
	Acc no.	OM	Gene	Function
Symbol				
OM specific (fold increase)				
ABC1	NM_005502	2.0	ATP-binding cassette, subfamily A member1	Steroid metabolism/transport
ADM	NM_001124	2.0	Adrenomedullin (ADM)	Electrolyte homeostasis (secreted)
AKAP2	NM_007203	2.1	A kinase (PRKA) anchor protein 2	Scaffold protein (kinase)
AKR1C1	NM_001353	2.2	Aldo-keto reductase family 1, member C1	Metabolism (progesterone)
ALCAM	NM_001627	2.1	Activated leucocyte cell adhesion molecule	Cell adhesion
ALDH1	NM_000689	2.4	Aldehyde dehydrogenase 1	Metabolism (alcohol)
AOX1	NM_001159	3.0	Aldehyde oxidase 1	Metabolism (aldehyde oxidation)
ARHGAP6	NM_013427	2.0	GTPase-activating protein 6	GTPase activator

(continued)

Table 3 Continued

	Acc no.	OM	Gene	Function
CITED2	NM_006079	2:1	GluAsp-rich carboxy-terminal domain, 2	Transcription factor
DIAPH2	NM_006729	2:0	Diaphanous (<i>Drosophila</i> , homologue) 2	Cell differentiation
EDG2	NM_001401	2:3	Lysophosphatidic acid G-protein-coupled receptor 2	G-protein-coupled receptor signalling
FMO2	NM_001460	2:4	Flavin-containing monooxygenase 2	Metabolism (N-oxidation of alkylamines)
FN	NM_002026	2:3	Migration stimulation factor MSF-FN70	Immune response/ECM protein
FOXO1A	NM_002015	2:3	Forkhead box O1A	Transcription factor
GABRA2	NM_000810	2:1	γ -aminobutyric acid A receptor $\alpha 5$	Transport channel (chloride)
GAS1	NM_002048	2:4	Growth arrest-specific 1	Cell proliferation (negative)
HPS5	NM_007216	2:1	α -integrin-binding protein 63	Organelle biogenesis
HSD11B1	NM_005525	3:3	Hydroxysteroid (11- β) dehydrogenase 1	Metabolism (glucocorticoids)
ID1	NM_002165	2:6	Inhibitor of DNA binding 1	Transcription factor/cell growth (negatively)
KIAA0992	NM_016081	2:0	Palladin (KIAA0992)	Cell adhesion
LAMA2	NM_000426	2:3	Laminin, $\alpha 2$	ECM protein
OPN3	NM_014322	2:1	Opsin 3 (encephalopsin)	G-protein-coupled receptor signalling
PDE1A	NM_005019	2:0	Phosphodiesterase 1A, calmodulin dependent	Cyclic nucleotide phosphodiesterase
PDGFD	NM_025208	2:1	Platelet-derived growth factor D	Proliferation/growth factor
PIK3R1	NM_181523	2:0	Phosphoinositide-3-kinase (p85 α)	Insulin receptor signalling
SRPX	NM_006307	2:3	Sushi-repeat-containing protein, X chromosome	Cell adhesion
WISP1	NM_003882	2:1	WNT1-inducible-signalling pathway protein 1	Wnt receptor signalling/cell proliferation

	Acc no.	SC	OM	Gene	Function
Symbol					
Adipose specific (fold increase)					
ADAMTS1	NM_006988	5:7	2:0	Metalloproteinase with thrombospondin type-1	Cell proliferation (negative)
ADH2	NM_000668	4:2	11:5	Alcohol dehydrogenase $\beta 1$	Metabolism (ethanol)
APOD	NM_001647	5:1	2:4	Apolipoprotein D	Metabolism (lipids)
C10orf10	NM_007021	4:0	4:9	Decidual protein induced by progesterone	Unknown/increased expression in fasting
CD163	NM_004244	3:9	3:6	CD163 antigen	Immune response
CDKN1C	NM_000076	2:2	2:4	Cyclin-dependent kinase inhibitor 1C	Cell cycle (G1 phase arrest)
CHST2	NM_004267	2:7	2:3	Carbohydrate sulphotransferase 2	Immune response
CPM	NM_001874	9:1	2:5	Carboxypeptidase M	Immune response
ENNRB	NM_000115	3:9	3:0	Endothelin receptor type B, variant 1	G-protein-coupled receptor signalling
FBN2	NM_001999	5:0	2:5	Fibrillin 2 (congenital contractural arachnodactyly)	ECM protein
GHR	NM_000163	3:0	3:0	Growth hormone receptor	Insulin/IGF-signalling pathway
GLUL	NM_002065	2:5	2:1	Glutamate-ammonia ligase (glutamine synthase)	Metabolism (glutamate/nitrogen)
GPX3	NM_002084	5:6	9:3	Glutathione peroxidase 3 (plasma)	Metabolism (anti-oxidative)
HSPA2	NM_021979	3:1	2:4	Heat shock 70kD protein 2	Chaperone protein (glucose regulated)
HXL1	NM_021958	2:4	2:2	<i>Drosophila</i> -like homeo box 1	Transcription factor
IMPA2	NM_014214	2:8	2:5	Inositol(myo)-1(or 4)-monophosphate 2	Phosphatidyl inositol-signalling pathway
IRS2	NM_003749	3:3	2:2	Insulin receptor substrate 2	Metabolism (glucose metabolism)
LDO3	NM_018640	3:7	2:3	LIM domain only 3	Transcription factor
MAOA	NM_000240	5:1	4:5	Monoamine oxidase A	Metabolism (neurotransmitters catabolism)
MGP	NM_000900	3:5	2:6	Matrix Gla protein	ECM protein
NRCAM	NM_005010	2:4	2:7	Neuronal cell adhesion molecule	Cell adhesion
PDK4	NM_002612	2:4	2:0	Pyruvate dehydrogenase kinase, isoenzyme 4	Metabolism (glucose metabolism)
REV3L	NM_002912	2:2	3:3	REV3-like, catalytic subunit of DNA polymerase ζ	Transcription factor
RGC32	NM_014059	11:9	6:0	RGC32 protein	Cell proliferation (growth arrest)
RNAASE4	NM_002937	2:3	2:0	Ribonuclease, RNase A family, 4	Ribonuclease activity
SAA2	NM_030754	2:1	3:7	Serum amyloid A2- α	Immune response
STC2	NM_003714	2:0	2:0	Stanniocalcin 2	Calcium and phosphate homeostasis
TSC22D3	NM_198057	4:4	3:3	δ sleep-inducing peptide, immunoreactor	Transcription factor
ZNF145	NM_006006	6:4	2:8	Zinc finger protein 145	Transcription factor/growth arrest

Table 4 Overview of the genes that were significantly decreased after 24 h 100 nM cortisol treatment (F) compared to control (ctr) in subcutaneous (SC), omental (OM) preadipocytes or both fat deposits. Abbreviations are given

	Acc no.	SC	Gene	Function	
Symbol					
SC specific (fold decrease)					
C5orf13	NM_004772	2·4	Chromosome 5 open reading frame 13	Unknown	
CDH2	NM_001792	2·7	N-cadherin .1	Cell adhesion protein	
CRABP2	NM_001878	2·2	Cellular retinoic acid-binding protein 2	Retinoic acid action on cell differentiation	
DKFZP564I1922	AF245505	2·8	Adican DKFZP564I1922 protein	Vascular endothelial growth	
ENPP2	NM_006209	2·1	Ectonucleotide pyrophosphatasephosphodiesterase 2	Oncogene activity/G-protein-coupled signalling	
FZD7	NM_003507	3·5	frizzled (<i>Drosophila</i>) homologue 7	Wnt-receptor-signalling pathway (receptor)	
GATA6	NM_005257	2·0	GATA-binding protein 6	Transcription factor (proliferation/differentiation)	
IGFBP5	NM_000599	2·4	Insulin-like growth factor-binding protein 5	IGF signalling (cell growth)	
IL-6	NM_000600	2·3	Interleukin 6 (interferon, β2)	Immune response/cytokine	
LDB2	NM_001290	5·0	LIM domain binding 2	Transcription factor	
NOV	NM_002514	2·9	Nephroblastoma overexpressed gene	Cell growth	
OXTR	NM_000916	2·0	Oxytocin receptor	G-protein-coupled IP3 signalling	
PRSS23	NM_007173	2·2	Protease, serine, 23	Protein degradation	
PSA	NM_021154	2·0	Phosphoserine aminotransferase	Metabolism (amino acid synthesis)	
PSG5	NM_002781	2·6	Pregnancy-specific β-1-glycoprotein 5	Unknown	
SERPINE2	NM_006216	3·0	Nexin, plasminogen activator inhibitor type-1	Serine protease inhibitor	
SFRP4	NM_003014	3·3	Secreted frizzled-related protein 4	Wnt-receptor-signalling pathway/differentiation	
SGNE1	NM_003020	2·1	Secretory granule, neuroendocrine protein 1	Facilitate active transport from ER	
VEGF	NM_003376	2·0	Vascular endothelial growth factor	Cell proliferation (positive)/angiogenesis	
WARS	NM_004184	2·4	Tryptophanyl-tRNA synthetase	Cell proliferation	
ZNF423	NM_015069	2·1	Zinc finger protein 423	Nucleic acid binding/developmental	
	Acc no.	OM	Gene	Function	
Symbol					
OM specific (fold decrease)					
AMIGO2	NM_181847	2·1	Adhesion molecule with Ig-like domain 2	Anti-apoptosis	
CCL11	NM_002986	4·5	Small inducible cytokine subfamily A member 11	Immune response	
HAS2	NM_005328	3·0	Hyaluronan synthase 2	ECM protein	
LIF	NM_002309	2·2	Leukaemia inhibitory factor (LIF)	Cell proliferation (positive)/immune response	
MMP1	NM_002421	2·3	Matrix metalloproteinase 1	ECM remodelling (enzyme)	
NGFB	NM_002506	2·0	Nerve growth factor, β polypeptide	Growth factor activity	
NPPB	NM_002521	2·7	Natriuretic peptide precursor B	Electrolyte homeostasis	
PRAD1	M73554	2·0	bcl-1 cyclin D1	Cell cycle (G1/S) transition control	
SLC22A3	NM_021977	3·1	Solute carrier family 22, member 3	Monoamine transporter	
Smurf2	NM_022739	2·8	E3 ubiquitin ligase	Transforming growth factor β signalling (negative)/protein catabolism	
	Acc no.	SC	OM	Gene	Function
Symbol					
Adipose specific (fold decrease)					
ADAM12	NM_003474	2·7	2·4	Metalloproteinase domain 12 transcript 1	Metalloprotease/cell–cell interaction
C1orf24	NM_022083	2·4	2·1	Chromosome 1 open reading frame 24	Cell growth (positive)(?)
DACT1	NM_016651	2·6	2·1	Hepatocellular carcinoma novel gene 3 protein	Wnt-signalling pathway
DSCR1	NM_004414	3·1	2·7	Down syndrome critical region gene 1	Transcription factor
FGF2	NM_002006	2·7	2·2	Fibroblast growth factor 2 (basic)	Cell proliferation (positive)
GDF15	NM_004864	2·1	2·0	Prostate differentiation factor 1	Tissue differentiation/growth factor
RGS4	NM_005613	2·4	4·3	Regulator of G-protein signalling 4	G-protein couped receptor signalling pathway
TNFAIP6	NM_007115	2·8	2·0	Tumour necrosis factor, α-induced protein 6	Immune response
TRIB3	NM_021158	2·4	2·4	Kinase domains-containing protein	Cell growth (positive)

Figure 2 Fold change in mRNA expression in OM and SC preadipocytes after 24 h cortisol (100 nM F) or control (ctr) treatment measured by quantitative real-time PCR. (A) 11β-Hydroxysteroid dehydrogenase type-1 (11β-HSD-1); (B) glucocorticoid receptor (GR); (C) CCAAT enhancer-binding protein α (CEBPα) and (D) CCAAT enhancer-binding protein β (CEBPβ).

associated with Wnt-signalling pathways (*FZD7* and *SFRP4*; 3.5- and 3.3-fold respectively; Fig. 3).

F-treatment significantly decreased IL-6 expression only in SC preadipocytes (2.3-fold), however, the absolute signal of IL-6 was much higher in OM

preadipocytes. This finding was confirmed and validated by real-time PCR (Fig. 3).

The expression levels of two transcription factors, LDB2 and GATA6, were lowered by cortisol (5.0- and 2.0-fold respectively). Additionally, SGNE1 (associated

Figure 3 Fold change in mRNA expression in OM and SC preadipocytes after 24 h cortisol (100 nM F) or control (ctr) treatment measured by quantitative real-time PCR. (A) Interleukin 6 (IL-6); (B) fatty acid-binding protein 4 (FABP4); (C) insulin receptor substrate 2 (IRS2) and (D) apolipoprotein D (Apolipo D).

Figure 4 Fold change in mRNA expression in OM and SC preadipocytes after 24 h cortisol (100 nM F) or control (ctr) treatment measured by quantitative real-time PCR. (A) Growth hormone receptor (GHR) and (B) angiotensin receptor 1 (AGTR1).

with active transport from the ER) and IGFBP5 (IGF-signalling pathway) were downregulated by F (2.1- and 2.4-fold).

OM preadipocytes

Several genes were specifically upregulated by cortisol but only in OM depots: *GAS1*, *PDGFD* and *ID1* negatively associated with cell growth (2.4-, 2.1- and 2.6-fold respectively), positively with cell differentiation (*DIAPH2*, 2.0-fold), and ECM proteins (*LAMA2*, *AKAP2*, *ACKLAM*, *SPRX* and *KIAA0992*). A number of metabolic enzymes were upregulated in OM cells only: *ALDH1* (2.4-fold), *AOX1* (3.0-fold) and *AKR1C1* (2.1-fold). Interestingly, among metabolic enzymes involved in glucocorticoid metabolism, *HSD11B1* was upregulated in OM preadipocytes (3.3-fold), encoding 11 β -HSD1 enzyme. This was confirmed using real-time PCR (Fig. 2). The gene encoding adrenomedullin (ADM) was upregulated 2.0-fold. Fibronectin (FN) involved in the immune response was upregulated in OM cells (2.3-fold) but not in SC cells. One member of Wnt-signalling pathway, *WISP1*, was upregulated in OM cells (2.1-fold) as well as two transcription factors, *CITED2* and *FOXO1A* (2.1- and 2.3-fold respectively).

Downregulated OM-specific genes included *PRAD1* (controlling G1/S transition) and ECM proteins (*HAS2* and *MMP1*). A gene associated with transforming growth factor β signalling (*SMURF2*) was 2.8-fold. Small inducible cytokine subfamily A, member 11 (*CCL11*) and leukaemia inhibitory factor involved in immune responses were downregulated 4.5- and 2.2-fold respectively in OM cells. Interestingly, natriuretic peptide B (NPB), gene involved in electrolyte homeostasis and lipolysis in human adipocytes was decreased 2.7-fold by cortisol and was below detection levels in SC cells.

Common adipose-specific genes

Twenty-eight genes were upregulated by cortisol in both SC and OM depots, including those involved in cell cycle regulation and growth (*CDKN1C*, *RGC32* and *ADAMTS1*), one associated with cell adhesion (*NRCAM*) and rearrangement of cell matrix (*FBN2* and *MGP*). A number of transcription factors were upregulated in cells from both depots: *HXL1*, *LDO3*, *TSC22D3*, *REV3L* and *ZFP145*. Components of insulin-signalling pathway: *IRS2*, *GHR*, *PDK4* and glucose-regulated chaperone protein (*HSPA2*) were significantly upregulated by cortisol in SC and OM preadipocytes as apolipoprotein D (ApoD) implicated in lipid metabolism (Table 3). *IRS2* and *GHR* expression profiles were validated by real-time PCR (Figs 3 and 4).

In terms of genes related to the immune response, expression of *CD163*, *CHST2* and *CPM* all increased in both depots. Plasma glutathione peroxidase 3 precursor (*GPX3*), catalyses the reduction of hydrogen peroxide, organic hydroperoxide and lipid peroxides, protecting cells against oxidative damage. It was expressed at very high levels in control SC cells and was significantly upregulated in both SC and OM depots by F. Additionally, genes encoding many metabolic enzymes like *GLUL* (glutamate/nitrogen metabolism), *ADH2* (ethanol metabolism) and *MAOA* (neurotransmitters catabolism) were upregulated in preadipocytes from both depots (Table 3).

Most of the genes downregulated by cortisol in both SC and OM preadipocytes were associated with cell cycle control and proliferation/growth processes; e.g. *FGF2*, *TRIB3* and oncogene (*C1orf24*). As expected, pro-inflammatory gene such as TNF α induced protein 6 (TNFAIP6) was decreased by cortisol in both depots. Transcription factor *DSCR1* was decreased in SC and OM cells (3.1- and 2.7-fold respectively). Cortisol treatment decreased expression of one component of Wnt-signalling pathway (*DACT1*) in SC and OM preadipocytes (2.6- and 2.1-fold respectively), Table 4.

Discussion

We have identified several glucocorticoid-regulated genes in human adipose tissue, many of which show site specificity between OM and SC depots that may contribute to the established predilection of glucocorticoids for visceral adiposity. Additionally, these candidates may be informative as we look towards an explanation for the basis of the deleterious distribution of visceral fat.

In terms of the GR itself, earlier studies had reported increased expression in OM adipose tissue when compared with SC fat (Bronnegard *et al.* 1990). Here, we show very similar levels of GR mRNA in preadipocytes of both depots and report that incubation with F for 24 h had no significant effect on GR expression in human SC or OM preadipocytes. By contrast, the study does confirm our earlier reports of increased expression of the 'prereceptor' regulator of GR action, the *HSD11B1* gene which encodes the enzyme 11 β -hydroxysteroid dehydrogenase type-1 (11 β -HSD1) in OM compared with SC preadipocytes and significant upregulation of its expression by F only in OM sites (Bujalska *et al.* 1997). The enzyme 11 β -HSD2 was absent confirming previous observations (Bujalska *et al.* 1997). The explanation for the increased *HSD11B1* expression in OM versus SC sites remains unclear. Rodent studies had indicated marked induction of expression by the transcription factors CCAAT enhancer-binding protein α (C/EBP α ; Williams *et al.* 2000). However, in this human-based study C/EBP α expression was absent in OM preadipocytes and C/EBP β , whilst being highly expressed in SC and OM preadipocytes, was not regulated by F. It seems unlikely based on these data that C/EBPs play a major role in the site-specific transcriptional regulation of *HSD11B1* or GR in human preadipocytes.

Growth arrest/extracellular matrix

Whilst GCs provide a crucial signal for preadipocyte differentiation (Hauner *et al.* 1987), the first step defining adipogenesis is cell growth arrest (Ailhaud *et al.* 1989). This study looked comprehensively on the effect of GCs on genes associated with cell cycle control. We identified a number of genes such as *CDKN1C*, which overexpression caused complete cell arrest in G1 phase (Lee *et al.* 1995), and *RGC32* negatively controlling cell growth (Badea *et al.* 1998) as novel GC-target genes in SC and OM preadipocytes. Furthermore, we found *ADAMTS1*, gene which disruption in knockout mice caused adipose tissue malformation (Shindo *et al.* 2000), being upregulated by GC-target in human preadipocytes together with downregulation of FGF2 providing positive proliferation signal (Kurokawa *et al.* 1987). We identified different sets of genes

regulating cell growth in depot-specific manner. Only in OM cells, we see increased expression of *GAS1* (involved in growth suppression) and decreased of *PRAD1* (a G1 marker gene decreased by dexamethasone in airway smooth muscle cells (Fernandes *et al.* 1999)), but increased expression of *BTG1* in SC cells. Our findings imply site-specific pathway of cell arrest in human preadipocytes.

Interestingly, downregulation of genes, *FZD7* and *SFRP4* associated with the Wnt-signalling pathway was observed only in SC preadipocytes while upregulation of *WISP1*, down-stream target of Wnt pathway in hepatocellular carcinoma cells (Cervello *et al.* 2004), was seen only in OM cells. Downregulation of Wnt pathway was recently shown to be crucial for the adipogenesis in mouse cell line (3T3L1; Bennett *et al.* 2002), however, its role in human preadipocytes still needs to be evaluated.

Preadipocyte growth arrest is accompanied by reorganisation of extracellular matrix (ECM; Gregoire *et al.* 1991). We found several novel GC-target genes associated with cell-cell adhesion proteins in human preadipocytes, e.g. *FBN2*, *MGP* and *NRCAM*. However, several ECM GC-target genes were site specific and their role in site-specific adipogenesis remains to be elucidated. For example, in SC cells, *CLEC3B* (tetranectin) was highly expressed and upregulated by F but below detection levels in OM cells. *CHL1*, *ABLIM*, dermatopontin (*DPT*) and *PRELP* were GC-target genes of the ECM upregulated only in SC cells. *DPT*, cartilage matrix component, was recently shown to be upregulated by dexamethasone in chondrogenic differentiation from human bone marrow mesenchymal stem cells (Derfoul *et al.* 2006). Conversely, expression of *ALCAM*, *KIAA0992* (identified as risk factor for myocardial infarction; Shiffman *et al.* 2005) and *SRPX* was upregulated in OM cells.

Hereby, we demonstrate that GCs induce a site-specific block on the cell cycle progression together with site-specific differences in ECM remodelling. These would indicate unique pathways of preadipocyte commitment to adipogenesis in these two human adipose tissue depots.

Metabolic enzymes

Human alcohol dehydrogenase 2 (*ADH2*) exhibits high activity for unsaturated and aromatic alcohols and aldehydes in the metabolism of noradrenaline, retinoids and lipid peroxidation thus plays an important role in detoxification in the liver, reviewed by Higuchi *et al.* (2004). This study revealed high *ADH2* expression in human preadipocytes (much higher in SC than OM) and positive regulation by GCs in both adipose depots. Dexamethasone has been shown to upregulate *ADH2* expression in the HepG2 cell line (Dong *et al.* 1988);

however, ADH2 expression and its role in the human adipose tissue have not yet been elucidated. On the other hand, aldo-keto reductase family 1, member C1 (AKR1C1), a major enzyme in progesterone metabolism was also highly expressed in SC and OM preadipocytes (again more in SC than OM), but was only upregulated by GCs in OM preadipocytes. AKR1C1 has been shown to be expressed in human SC and OM adipose tissue (Blanchette *et al.* 2004) and positively correlated with visceral obesity. Our findings may provide an explanation for this correlation, since more cortisol is generated in OM tissue due to increased expression of 11 β -HSD1.

Adipose-specific genes

In keeping with previous findings (Masuzaki *et al.* 1995), leptin expression was undetectable in SC and OM preadipocytes; GCs increased its expression only in SC preadipocytes. It has been shown that C/EBP α upregulates human (Miller *et al.* 1996) leptin mRNA expression and we confirmed a parallel induction of leptin and C/EBP α by GCs in SC cells. Lipoprotein lipase, an early differentiation marker of adipogenesis has been shown to be expressed at higher levels and upregulated by GCs in SC human female adipose tissue (Fried *et al.* 1993), a pattern that was confirmed in preadipocytes in this study. In rat, insulin is required for the induction of early differentiation genes such as *LPL* (Gregoire *et al.* 1991), but we showed that a relatively short exposure to GCs to SC preadipocytes induced *LPL* expression without the requirement of insulin.

FABP4, product of the *aP2* gene, is regulated by dexamethasone in mouse 3T3L1 cells (Cook *et al.* 1988) as well as in differentiating preadipocytes from rat bone marrow (Atmani *et al.* 2003). This study verified *FABP4* gene as a glucocorticoid-target gene but only in SC preadipocytes. Upregulation of *aP2* gene in response to GCs as well as transcription factors-binding sites for C/EBP and c-fos (forming AP-1 complex) in the promoter region of mouse *aP2* gene was shown previously (Ross *et al.* 1990). Our findings, demonstrating increased expression of these two transcription factors together with *FABP4* expression in SC preadipocytes, might reflect different sensitivity of human SC and OM preadipocytes to adipogenic effect of GCs.

ApoD is involved in cellular transport of small hydrophobic ligands, such as progesterone, cholesterol, pregnenolone, bilirubin and arachidonic acid but its exact role is still not known. ApoD was expressed at similar levels in SC and OM preadipocytes but these fat compartments responded differently to GC (upregulation SC > OM). On the other hand, ApoB, component for VLDL associated with type-2 diabetes and insulin resistance reviewed by Ginsberg *et al.* (2005) and

increased risk of coronary heart disease (Despres 1998), was positively regulated only in SC cells. Although our finding provides evidence for glucocorticoid regulation of ApoB in human SC preadipocyte, its significance needs to be evaluated.

Natriuretic peptides have been shown to have a potent lipolytic effect in human adipocytes (Sengenès *et al.* 2000). Moreover, significantly reduced levels of NPB have been found in obese patients with heart failure (Mehra *et al.* 2004). Our finding shows NPB expression in OM preadipocytes as well as GC-target gene (but absent in SC) could provide a novel mechanism explaining the relationship between glucocorticoids, central obesity and heart failure. Moreover, increased expression of ADM has been positively correlated with cardiovascular disease and hypertension reviewed in Nikitenko *et al.* (2002). Here, we found ADM as a novel GC-target gene only in OM preadipocytes; its angiogenic and anti-lipolytic effects (Harmancey *et al.* 2005) might be an important factor explaining depot specificity of GC action in human adipose tissue.

Genes involved in glucose metabolism/insulin signalling

Many genes were similarly expressed and regulated by GCs in preadipocytes from both depots. Among them was the growth hormone receptor (GHR). GH has an anti-insulin activity (Roupas *et al.* 1991) and the upregulation of its receptor by GCs may be one mechanism underpinning GC-induced insulin resistance.

Insulin receptor substrate (IRS) plays a crucial role in adipogenesis; IRS2 (–/–) embryonic mouse fibroblasts retain only 15% of their ability to differentiate to adipocytes (Miki *et al.* 2001). IRS2 knockout mice develop progressive deterioration in glucose homeostasis through impaired insulin signalling in the liver and skeletal muscle leading to type-2 diabetes (Withers *et al.* 1998). IRS2 expression was higher in OM than SC preadipocytes, but was induced to a similar extent by GCs in both depots.

PDK4, an enzyme involved in glucose metabolism and fatty acid synthesis is known to be expressed in skeletal muscle and adipose tissue (Rowles *et al.* 1996). Decreased PDK4 in muscle has been associated with increased insulin sensitivity (Rosa *et al.* 2003) and increased expression with insulin resistance, diabetes and hyperthyroidism reviewed by Sugden (2003). The relationship between PDK4 and glucocorticoids has been studied in HepG2 cells but not in human adipose tissue. Elevation of PDK4 expression in human preadipocytes might be an additional factor in GC-induced insulin resistance.

Anti-inflammatory

In general, primary preadipocyte cultures isolated from human adipose tissue represent heterogeneous cell populations, some of which can be a part of the immune system (Kershaw & Flier 2004). In this study, we observed GC effects on genes associated with immune responses, such as *IL-6*, *TNFAIP6* and *CD163*. Activation of anti-inflammatory protein, TNFAIP6 (TNF α -stimulated gene 6) gene by NF/IL-6 was previously demonstrated (Klampfer *et al.* 1995). The simultaneous GC-induced downregulation of the TNFAIP6 and IL-6 in human preadipocytes might reflect interaction between these two genes in adipose tissue inflammation. GPX3, plasma glutathione peroxidase 3 precursor, catalyses the reduction of hydrogen peroxide, organic hydroperoxide and lipid peroxides, thus protecting cells against oxidative damage. GPX3 was reported to be present in human adipose tissue (Maeda *et al.* 1997), and here we identified GPX3 expression as being one of the most highly expressed genes in preadipocyte compartment of human adipose tissue as well as a novel GC-target gene.

Recently, adipose tissue has been identified as a major production site of serum amyloid A (ASSA; Sjöholm *et al.* 2005), a known risk factor for coronary artery disease (Johnson *et al.* 2004). In this study, we have shown that ASSA is a GC-target gene in OM preadipocytes. Together, these findings contribute to the role of OM tissue as a potential link between an inflammatory response and coronary disease.

Transcription factors

Several transcription factors are known to be key regulators of the adipogenic processes, reviewed by MacDougald & Mandrup (2002). In this study, we identified distinctively different as well as common sets of transcription factors involved in early glucocorticoid-induced adipogenesis in human SC and OM adipose tissues. Downregulation of Wnt-signalling pathway during adipogenesis has been shown in 3T3 cells (Bennett *et al.* 2002). Here, we demonstrate that the downregulation of Wnt-signalling pathway, as well as the induction of C/EBP α occurs only in SC preadipocytes. Different transcription factors, FOXO1A (upregulated in OM cells) and FOS (upregulated in SC cells), were the depot-specific early GC-targets genes. FOXO1 is induced in early stages of adipocyte differentiation (Nakae *et al.* 2003); our finding confirmed this but only in OM depots. Recently, involvement of two members of the zinc finger proteins family of the transcription factors, KLF2 and KLF15 has been reported in adipogenesis (Gray *et al.* 2002, Banerjee *et al.* 2003). Our study identified another member of this family, zinc finger protein 145 (ZFP145) as a novel, common,

GC-target in human preadipocytes. Moreover, the pattern of induction from undetectable levels suggests its important role in GCs-induced adipogenesis; however, the functional role of ZFP145 in adipose tissue needs to be further evaluated.

Summary

In summary, we have identified novel tissue-specific GC-target genes in SC and OM abdominal preadipocytes. The profile and intensity of fold changes in the expression of ten genes was confirmed by quantitative real-time PCR, indicating that the data obtained from our DNA array analysis are robust. The different sensitivity to GCs of SC and OM preadipocytes might explain heterogeneity as well as metabolic differences between these adipose depots in man.

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