Hexose-6-phosphate dehydrogenase confers oxo-reductase activity upon 11β-hydroxysteroid dehydrogenase type 1

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Abstract

Two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) interconvert active cortisol and inactive cortisone. 11β-HSD2 (renal) acts only as a dehydrogenase, converting cortisol to cortisone. 11β-HSD1 (liver) is a bi-directional enzyme in cell homogenates, whereas in intact cells it typically displays oxo-reductase activity, generating cortisol from cortisone. We recently established that cortisone reductase deficiency is a digenic disease requiring mutations in both the gene encoding 11β-HSD1 and in the gene for a novel enzyme located within the lumen of the endoplasmic reticulum (ER), hexose-6-phosphate dehydrogenase (H6PDH). This latter enzyme generates NADPH, the co-factor required for oxo-reductase activity. Therefore, we hypothesized that H6PDH expression may be an important determinant of 11β-HSD1 oxo-reductase activity. Transient transfection of Chinese hamster ovary (CHO) cells with 11β-HSD1 resulted in the appearance of both oxo-reductase and dehydrogenase activities in intact cells. Co-transfection of 11β-HSD1 with H6PDH increased oxo-reductase activity whilst virtually eliminating dehydrogenase activity. In contrast, H6PDH had no effect on reaction direction of 11β-HSD2, nor did the cytosolic enzyme, glucose-6-phosphate dehydrogenase (G6PD) affect 11β-HSD1 oxo-reductase activity. Conversely in HEK 293 cells stably transfected with 11β-HSD1 cDNA, transfection of an H6PDH siRNA reduced 11β-HSD1 oxo-reductase activity whilst simultaneously increasing 11β-HSD1 dehydrogenase activity. In human omental preadipocytes obtained from 15 females of variable body mass index (BMI), H6PDH mRNA levels positively correlated with 11β-HSD1 oxo-reductase activity, independent of 11β-HSD1 mRNA levels. H6PDH expression increased 5.3-fold across adipocyte differentiation (P < 0.05) and was associated with a switch from 11β-HSD1 dehydrogenase to oxo-reductase activity. In conclusion, H6PDH is a crucial determinant of 11β-HSD1 oxo-reductase activity in intact cells. Through its interaction with 11β-HSD1, H6PDH may represent a novel target in the pathogenesis and treatment of obesity.

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Introduction

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1, EC 1·1·1·146) inter-converts active cortisol and inactive cortisone, and therefore represents an important regulatory mechanism for tissue specific glucocorticoid receptor (GR) activation. 11β-HSD1 resides within the lumen of the endoplasmic reticulum (ER) (Ozols 1995, Odermatt et al. 1999) and is bi-directional, being able to catalyze both the dehydrogenase (cortisol to cortisone) and oxo-reductase (cortisone to cortisol) reactions (Agarwal et al. 1989). In vivo and in intact cells, oxo-reductase activity predominates (Bujalska et al. 1997a, Jamieson et al. 2000, Masuzaki et al. 2001), but in cellular homogenates or in purified preparations, the enzyme behaves as a dehydrogenase (Lakshmi & Monder 1988); oxo-reductase activity can only be recovered in these situations upon the addition of an artificial NADPH re-generation system, such as can be provided by the activity of glucose-6-phosphate dehydrogenase (G6PD) (Agarwal et al. 1990, Walker et al. 2001).

11β-HSD1-mediated oxo-reductase activity in liver and adipose tissue may modulate development of the metabolic syndrome. Exposure to cortisol increases hepatic glucose output (Jones et al. 1993) and adipocyte differentiation (Hauner et al. 1987). Selective inhibitors have shown beneficial results in improving insulin sensitivity and causing weight loss (Alberts et al. 2003, Livingstone & Walker 2003), but the factors determining oxo-reductase activity per se have remained elusive.

Hexose-6-phosphate dehydrogenase (H6PDH, EC 1·1·1·47) performs the first two steps of a pentose phosphate pathway which is believed to exist within the ER lumen (Bublitz & Steavenson 1988). It is responsible for converting glucose-6-phosphate to...
6-phospho-gluconolactone, thereby generating NADPH (Kimura et al. 1979, Stegeman & Klotz 1979). Based on genetic evidence, we have argued that reduced H6PDH activity, together with reduced expression of 11β-HSD1, underlies cortisone reductase deficiency (CRD). Women affected with CRD have polycystic ovary syndrome (PCOS) with menstrual irregularity, hirsutism and infertility. They are unable to generate cortisol from cortisone due to defective 11β-HSD1 oxo-reductase activity (Nikkila et al. 1993, Jamieson et al. 1999) resulting in activation of the hypothalamic–pituitary–adrenal axis and adrenocorticotrophin (ACTH) driven adrenal androgen excess (Biason-Lauber et al. 2000, Laing et al. 2002). These patients carry coding-sequence mutations in the gene encoding H6PDH (H6PD) in association with intronic mutations in the 11β-HSD1 gene (HSD11B) that reduce gene transcription (Draper et al. 2003).

Here we show that H6PDH has the potential to profoundly influence 11β-HSD1 by affecting the reaction direction in intact cells and also demonstrate that in primary cultures of human omental preadipocytes, levels of H6PDH expression predict oxo-reductase activity.

Materials and methods

Patients

Omental adipose tissue biopsies were obtained from 15 female patients undergoing elective abdominal surgery (median age 40 years, range 28–60 years, median body mass index (BMI) 29·3 kg/m², range 19·7–39·2). All gave their full informed, written consent. The study had the approval of the local research ethics committee.

Primary culture

Omental preadipocytes were isolated as previously reported (Bujalska et al. 1997b), seeded in a 24-well tissue culture dish and cultured to confluence (up to 6 days) in DMEM/F12 media supplemented with 10% fetal calf serum. At this point, 11β-HSD1 oxo-reductase assays were performed, protein concentration measured and total RNA extracted. In a further set of experiments, omental preadipocytes were isolated from adipose tissue obtained at elective surgery (n=12). Preadipocytes were differentiated into mature adipocytes according to an established protocol (Hauner et al. 1987). Briefly, after cell washing (day 1), 11β-HSD1 oxo-reductase and dehydrogenase activities were measured and total RNA extracted. Cells were then differentiated for a further 14 days, 11β-HSD1 activity measured, total RNA extracted and H6PDH and 11β-HSD1 expression measured by real-time PCR as described below.

Transient transfection of chinese hamster ovary (CHO) cells

Full length cDNA encoding rat 11β-HSD1 was subcloned into pDR2 (Clontech). The pDR2 derivative encoding human 11β-HSD2 cDNA has been previously described (Brown et al. 1996). The coding region of human H6PDH cDNA (Mason et al. 1999) was subcloned into pcDNA3·1 (Invitrogen). PBADHuG6PD, encoding human G6PD (Persico et al. 1986), was kindly provided by Dr P Mason (Imperial College, London, UK). Chinese hamster ovary (CHO) cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin. Twenty-four hours prior to transfection, CHO cells were seeded at 2×10³ cells/60 mm dish and transfected with a total of 21 µg DNA using the calcium phosphate procedure (Voice et al. 1996). DNA consisted of 10 µg pDR2 vector or 11β-HSD expression plasmid, 10 µg pcDNA3·1, H6PDH or PBADHuG6PD plasmid and 1 µg pCH110 (Amersham) encoding β-galactosidase; the last plasmid was an internal control for transfection efficiency. In each experiment, transfections were independently carried out in sextuplet; three for each assay of 11β-HSD activity in oxo-reductase or dehydrogenase direction after adding 3H-steroid to the medium 2 h after transfection. Forty two hours after transfection, cells were lysed and β-galactosidase activity assayed, as previously described (Williams et al. 2000), to verify that transfection efficiency was similar between transfections.

siRNA experiments

HEK 293T1 cells were used for siRNA transfection. They were derived from human embryonic kidney cells (HEK 293) by stable transfection with human 11β-HSD1 cDNA in the vector pCR3 (Invitrogen, UK) and display both dehydrogenase and oxo-reductase activities (Bujalska et al. 1997a). Cells were plated at 3·0×10⁴ cells per well in a 24-well tissue culture plate. Following 24 h in culture, cells were transfected with 100 nM H6PDH siRNA (Ambion siRNA ID 14371), 11β-HSD1 siRNA (Ambion siRNA ID 16305), or SilencerTM Negative Control #1 siRNA (Ambion, Huntingdon, Cambs, UK) using 3 µl per well siPORT Amine Transfection Agent (Ambion) according to the manufacturer’s protocol. Forty eight hours after transfection, 11β-HSD1 assays were performed as described below and the cells were subsequently harvested for protein and total RNA. H6PDH and 11β-HSD1 mRNA levels were measured by real-time RT-PCR with 18S rRNA as the internal standard. Target gene expression was calculated as fold change compared with the negative control siRNA gene expression. All experiments were carried out in triplicate.
RNA extraction and reverse transcription

Total RNA was extracted using a single step extraction method (Tri Reagent, Sigma). One microgram of total RNA was reverse transcribed using random hexamers in 20 µl volume according to manufacturer’s protocol (Promega).

Real-time PCR

11β-HSD1 and H6PDH mRNA levels were measured by real-time PCR using an ABI 7700 system (Perkin-Elmer, Biosystems, Warrington, UK). PCR was performed in 25 µl reactions on 96-well plates. Reactions contained TaqMan universal PCR master mix (Applied Biosystems, Warrington, Cheshire, UK), 900 nmol primers, 100–200 nmol TaqMan probe and 25–50 ng cDNA. All reactions were multiplexed with primers specific for 18S rRNA (provided as a pre-optimized mix; Perkin-Elmer, Beaconsfield, Bucks, UK) as an internal reference. All target gene probes were labeled with the fluorescent label FAM, and the 18S probe with the reference. All target gene probes were labeled with the fluorescent label VIC. Reactions were as follows: 50 °C for 2 min, 95 °C for 10 min, and then 44 cycles of 95 °C for 15 sec and 60 °C for 1 min. Data were analyzed according to the manufacturer’s guidelines and were obtained as Ct values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine dCt values (dCt=Ct of the target gene minus Ct of the internal reference, 18S).

Sequences of oligonucleotide primers and probes for 11β-HSD1 and H6PDH were as follows; 11β-HSD1: forward 5′-AGGAAAGCTCATTGGAGGACTAG-3′, reverse 5′-ATGGTGAATATCATGAAAAAGAATCC-3′, and probe 5′-CATGCTCATTTCTACAACCACATCACCACA-3′. H6PDH: forward 5′-AGAACCTGGGACCTTTTTCCA-3′, reverse 5′-GCGCCACAATCGTGTTC-3′, and probe 5′-TAAGTAATGGTCCACCGGTACATCTCCTCC-3′.

Western blot analysis

Twenty micrograms of protein from cell lysates (siRNA experiments) was analysed by SDS-PAGE using 12.5% gels on a Bio-Rad Mini-Protein II apparatus (Bio-Rad, Richmond, CA, USA). Ten micrograms of human liver homogenate, produced as previously reported (Ricketts et al., 1998), was loaded as a control. Western blotting was performed as reported previously (Ricketts et al., 1998). Membranes were incubated with validated polyclonal antibody to human 11β-HSD1 (The Binding Site, Birmingham, UK) at a dilution of 1:75 000 for 1·5 h at room temperature. Bound peroxidase-conjugated Ig was visualized using ECL detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) by exposing membranes to X-ray film (Kodak).

11β-hydroxysteroid dehydrogenase assay

Assays of 11β-HSD1 activity were performed by incubating intact cells with 1 ml of serum-free culture media with 100 nM cortisone (oxo-reductase) or cortisol (dehydrogenase) and tritiated tracer for 3–16 h, or (for transfected CHO cells) with 25 nM corticosterone or 11-dehydrocorticosterone for 42 h. After incubation, media was transferred to a glass tube and steroids were extracted with 5 ml of dichloromethane. Next the aqueous phase was removed and dichloromethane phase concentrated to 100 ul which was then spotted on a TLC silica plate. Steroids were separated by thin layer chromatography using a mobile phase of ethanol and chloroform (8:92) and quantitated using a Bioscan 2000 image analyzer (Lablogic, Sheffield, UK) or a phosphorimager (Fuji FLA-2000, Raytek Scientific Ltd, Sheffield, UK). Protein levels were assayed using a 96-well plate assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). All experiments were carried out in triplicate.

Data analysis

Values are given as means ± s.e. of (n) separate experiments. Statistical analysis of comparisons among groups was undertaken using the one-way analysis of variance (for normal distribution) or Mann-Whitney rank sum test (for non-normal distribution). Non-parametric Spearman correlation has been used to analyze adipocyte data. P values of less than 0·05 were accepted as statistically significant.

Results

Overexpression of H6PDH, G6PD and 11β-HSD in CHO cells

In transfected cell lines, 11β-HSD1 is predominantly an o xo-reductase (Low et al., 1994). The important exception to this is CHO cells, in which transiently transfected 11β-HSD1 encodes both o xo-reductase and dehydrogenase activities at approximately equal levels (Agarwal et al., 1989). Co-transfection of CHO cells with an expression plasmid encoding H6PDH together with a 11β-HSD1 plasmid increased o xo-reductase activity compared with controls transfected with 11β-HSD1 plasmid and empty pcDNA3-1 vector; 68·7 ± 3·0 vs 42·4 ± 2·4% respectively, P < 0·05 (Fig. 1A). Simultaneously, dehydrogenase activity was virtually eliminated; 4·0 ± 0·7 vs 27·2 ± 1·9% in controls,
P<0.05 (Fig. 1B). In contrast, H6PDH had no effect on reaction direction of 11β-HSD2; 0.8 ± 0.3% vs 1.2 ± 0.5% in controls (oxo-reductase) and 97.5 ± 1.6% vs 91.0 ± 5.2% in controls (dehydrogenase) (Figs 1A and B). Furthermore co-transfection of CHO cells with an expression plasmid encoding the cytosolic enzyme G6PD failed to produce any differences in oxo-reductase (32.7 ± 2.4% vs controls 42.4 ± 2.4%) or dehydrogenase (19.0 ± 1.2% vs controls 27.2 ± 1.9%) activities (Figs 1A and B).

Suppression of 11β-HSD1 and H6PDH in HEK 293T1 cells by siRNA

Introduction of either 11β-HSD1 or H6PDH siRNA in HEK 293T1 cells caused a significant decrease in the target mRNA levels compared with cells transfected with a control/scrambled siRNA (Silencer Negative Control #1); 11β-HSD1 mRNA fold change: 0.48 ± 0.07, n=6, P<0.01, H6PDH mRNA fold change: 0.46 ± 0.12, n=6, P<0.01, (Fig. 2A). Furthermore, 11β-HSD1 protein levels were significantly decreased in cells transfected with 11β-HSD1 siRNA compared with cells transfected with H6PDH siRNA or a scrambled siRNA control (Fig. 2B). Both 11β-HSD1 dehydrogenase and oxo-reductase activities were similarly decreased in intact cells from 11β-HSD1 siRNA-transfected cells compared with scrambled siRNAs: dehydrogenase; 0.64 ± 0.11, n=6, P<0.001, and oxo-reductase; 0.85 ± 0.14, n=6, P<0.05 (Fig. 2C).

Correlation between 11β-HSD1 activity and H6PDH expression in human omental preadipocytes

To investigate whether H6PDH similarly regulates 11β-HSD1 reaction direction in intact primary cultures endogenously expressing 11β-HSD1, oxo-reductase activity and H6PDH mRNA levels were assayed in human omental preadipocytes. Significant oxo-reductase activity was seen in all cultured preadipocyte preparations.

A significant (P<0.05) negative correlation between dCt for H6PDH [Median 11.04; 10.64 (25%), 11.6 (75%) CI] and 11β-HSD1 oxo-reductase activity [Median 37.0; 21.02 (25%), 59.60 (75%) CI] in omental human preadipocytes was observed. This illustrates a positive correlation between H6PDH mRNA and 11β-HSD1 oxo-reductase activity. To present the positive directionality of the correlation, H6PDH mRNA levels have been expressed as −dCt, and its relation with 11β-HSD1 oxo-reductase has been shown.
in Fig. 3A. The change in 11β-HSD1 o xo-reductase levels occurred despite no significant difference in 11β-HSD1 mRNA levels (dCt: 12·34 ± 0·25, mean ± s.e.).

As previously reported in a larger cohort (Tomlinson et al. 2002), we observed a significant (P<0·05) inverse correlation between BMI [Median 29·3; 26·2 (25%), 33·1 (75%) CI] and 11β-HSD1 oxo-reductase activity [Median 37·0; 21·02 (25%), 59·60 (75%) CI] in cultured preadipocytes (Fig. 3B), but importantly no correlation between BMI and 11β-HSD1 mRNA levels (P=0·18) (data not shown).

Moreover, H6PDH mRNA levels inversely correlated with BMI as reflected by a positive correlation (P<0·05) between –dCt for H6PDH expression [Median 11·04; 10·64 (25%), 11·6 (75%) CI] and BMI [Median 29·3; 26·2 (25%), 33·1 (75%) CI]. Again, H6PDH mRNA levels have been expressed as –dCt and the correlation between –dCt and BMI has been shown in Fig. 3C.

Across adipocyte differentiation, 11β-HSD1 oxo-reductase activity increased (11·8 ± 3·6 vs 66·1 ± 21·9 pmol/mg/h, day 1 vs day 14, P<0·05) whilst dehydrogenase activity decreased (104·0 ± 25·1 vs 17·3 ± 5·0 pmol/mg/h, day 1 vs day 14, P<0·01), (Fig. 4A). Concomitantly, H6PDH mRNA levels increased 5·3-fold as measured by quantitative real-time PCR (dCt 13·5 ± 0·7 vs 11·5 ± 0·5, day 1 vs day 14, P<0·05) (Fig. 4B). There was no significant difference in 11β-HSD1 mRNA expression during omental preadipocytes differentiation (Fig. 4B).

**Discussion**

The oxo-reductase activity of 11β-HSD1 has emerged as an important mechanism by which glucocorticoid concentrations can be regulated in a tissue-specific fashion. By generating cortisol in hepatocytes (Jamieson et al. 1995, Ricketts et al. 1998), adipocytes (Bujalska et al. 1997b), osteoblasts (Bland et al. 1999, Cooper et al. 2000) and ocular tissues (Rauz et al. 2001), this enzyme may be involved in the pathogenesis of obesity, metabolic syndrome/diabetes mellitus, glucocorticoid-induced osteoporosis and glaucoma. In liver for example, 11β-HSD1-mediated oxo-reductase activity stimulates hepatic gluconeogenesis in fasted mice (Kotelevtsev et al. 1997). Mice lacking 11β-HSD1 resist hyperglycemia upon stress or feeding (Kotelevtsev et al. 1997). Conversely, transgenic over-expression of 11β-HSD1 in liver leads to insulin resistance and hypertension, but without obesity (Paterson et al. 2004). The role of 11β-HSD1 in the regulation of adipose tissue mass is an area of much scientific interest and speculation. The profound effects of cortisol upon adipose tissue biology are exemplified in patients with Cushing’s syndrome who develop marked and reversible central obesity (Rebuffe-Screve et al. 1988). We have demonstrated enhanced 11β-HSD1 expression in omental compared with subcutaneous depots, and have suggested that central obesity might arise from the autocrine generation of cortisol within omental adipose tissue – ‘Cushing’s disease of the omentum’ through the expression of 11β-HSD1.
Figure 3 (A) Significant positive correlation between H6PDH mRNA levels and 11β-HSD1 oxo-reductase activity in human omental preadipocytes. (B) 11β-HSD1 oxo-reductase activity in omental preadipocytes in relation to BMI from 15 female patients, showing a significant negative association. (C) H6PDH mRNA expression versus BMI from 15 female patients, showing a significant negative correlation.
11β-HSD1 (Bujalska et al. 1997b). However, subsequent studies have shown, if anything, a 10–20% global reduction in 11β-HSD1 expression in obese subjects, although expression within adipose tissue in obesity is still debated (Stewart et al. 1999, Tomlinson et al. 2002). Nevertheless, recent animal data have highlighted the beneficial effects of administration of selective 11β-HSD1 inhibitors upon insulin sensitivity and body weight (Alberts et al. 2003). The effects of 11β-HSD1 upon adipose tissue biology depend upon site of expression and enzyme directionality. Cortisol inhibits proliferation of preadipocytes yet stimulates differentiation to adipocytes (Tomlinson et al. 2002). Immediately following isolation of preadipocytes, dehydrogenase activity predominates (Bujalska et al. 2002) which would enhance proliferation. However, once established in cell culture, activity is predominantly oxo-reductase (Bujalska et al. 1997b) and serves to promote adipogenesis (Bujalska et al. 1999). This is most elegantly demonstrated in transgenic mice over-expressing 11β-HSD1 under the adipocyte specific aP2 promoter who develop central obesity as a consequence of increased adipocyte size (Masuzaki et al. 2001). Determining the factors regulating directionality of 11β-HSD1 activity in human adipose tissue is therefore of crucial importance. In other tissues, 11β-dehydrogenase activity has been reported in intact cell preparations with the direction of 11β-HSD1 catalysis appearing to vary according to physiological or developmental status of a particular cell type. In Leydig and neuronal cells, as well as in human bone, both 11β-dehydrogenase and oxo-reductase activities have been observed (Ge et al. 1997, Leckie et al. 1998, Jellinck et al. 1999, Cooper et al. 2000). Only oxo-reductase activity has been detected in kidney cells of monkey origin (Cos-7) transiently transfected with rat 11β-HSD1 cDNA (Low et al. 1994). However, in most

Figure 4  (A) Change from 11β-HSD1 dehydrogenase activity to oxo-reductase in human omental preadipocytes differentiated for 14 days, n=12. (B) Increase in H6PDH mRNA expression (but not in 11β-HSD1) concomitant with an increase in oxo-reductase activity in differentiated preadipocyte cultures, n=12. *P<0.05; **P<0.01.
cell models, stably transfected with human 11β-HSD1 cDNA into HEK 293 cells (Bujalska et al. 1997a) or transiently transfected with rat 11β-HSD1 into CHO cells (Agarwal et al. 1989), both oxo-reductase and dehydrogenase activity are reported. These studies have used different cell models and cDNA/expression constructs that might explain data inconsistency. Yet in every case where oxo-reductase activity is identified, it is reduced or lost when cells are disrupted or the enzyme purified (Monder & Lakshmi 1989, Monder et al. 1991). This striking change in directionality between intact cells and homogenates is apparently a consequence of the specific intracellular localization of 11β-HSD1 within the lumen of the ER (Ozols 1995), where H6PDH generates the reduced co-substrate, NADPH (Ozols 1993). Although NADPH generation within the ER lumen is potentially of critical importance to cellular function, at present there is little known about the cellular localization of the catalytic domains of other ER reductases, with the exception of 11β-HSD1. H6PDH is present at high levels within tissues known to express 11β-HSD1 (Beutler & Morrison 1967), and blocking the transport of glucose-6-phosphate into isolated rat liver microsomes inhibits both H6PDH and 11β-HSD1 activities (Gerrin & Van Schaftingen 2002, Banhegyi et al. 2004). These findings complement the observation that patients with CRD have functional mutations in H6PD in association with intronic mutations in HSD11B1 that attenuate 11β-HSD1 expression (Draper et al. 2003).

Here we show unequivocally that H6PDH is able to regulate 11β-HSD1 enzyme direction. Co-expression of 11β-HSD1 with H6PDH in CHO cells increased oxo-reductase activity whilst reducing dehydrogenase activity. A parallel study (Atanasov et al. 2004) also showed a 6-fold increase in oxo-reductase with simultaneous 6-fold decrease in dehydrogenase in transiently transfected HEK 293 cells with 11β-HSD1 and H6PDH, confirming our earlier concept of a close inter-relationship between these two enzymes within the ER. In this study, we have further shown the specificity of 11β-HSD1 for H6PDH, since co-transfection with a cytosolic NADPH-provider, G6PD, failed to modulate 11β-HSD1 reaction direction. In contrast, the overwhelmingly oxidative reaction direction of 11β-HSD2 was unaffected by co-transfection with H6PDH, as predicted given that the active site of this enzyme faces toward the cytosol (Naray-Fejes-Toth & Fejes-Toth 1996). This finding is indicative of a pool of co-factors localized within the ER lumen, separated from the cytosolic compartment.

Additionally, we have further endorsed these findings through siRNA ‘knock-down’ experiments in HEK 293T1 cells, where a 50% reduction in 11β-HSD1 or H6PDH mRNA expression was achieved. Reduction in 11β-HSD1 mRNA altered dehydrogenase and oxo-reductase activities to a similar degree, with the concurrent decrease in 11β-HSD1 protein expression. Silencing of H6PDH, however, preferably reduced oxo-reductase activity whilst increasing dehydrogenase activity.

Here, we have shown an increase in oxo-reductase 11β-HSD1 activity and H6PDH expression in differentiating human omental preadipocytes. In a parallel study, using the 3T3-L1 mouse cell line, a modest increase in H6PDH mRNA levels has been shown to occur across adipogenesis, associated with a highly significant increase in 11β-HSD1 oxo-reductase activity (Atanasov et al. 2004). However, in this cell system, levels of 11β-HSD1 mRNA also increased more than 100-fold across adipogenesis. This alone could have accounted for the observed significant increase in 11β-HSD1 oxo-reductase activity which is not the case in our human cell model where the increase in 11β-HSD1 oxo-reductase occurred despite unchanged levels of 11β-HSD1 expression.

In addition, in this study, we demonstrate that expression H6PDH mRNA levels decreased with increasing BMI, perhaps explaining the observed decrease in 11β-HSD1 oxo-reductase activity in omental preadipocytes in obese patients (Tomlinson et al. 2002). Our earlier studies have shown that glucocorticoids inhibit preadipocyte proliferation (Tomlinson et al. 2002); thus the impaired oxo-reductase activity may increase the number of proliferating preadipocytes in visceral depots. Because 11β-HSD1 mRNA levels in human omental adipose tissue are poorly correlated with BMI (Tomlinson et al. 2002), we hypothesize that the ‘set-point’ of 11β-HSD1 reaction direction may be contributing more to the pathogenesis of central obesity than its absolute expression levels. On this note, it is interesting to speculate that earlier studies that inferred increased oxo-reductase activity in obese subjects, based upon an increase in measured dehydrogenase activity, might not represent the in vivo situation as this reflects the amount of enzyme present but not its directionality (Rask et al. 2001, Wake et al. 2003). To summarize, in this study we now demonstrate a direct relationship between 11β-HSD1 oxo-reductase activity and H6PDH expression in human omental preadipocytes and cell line models.

In terms of human disease, the targeted inhibition of 11β-HSD1 as a therapeutic strategy in patients with the metabolic syndrome is an exciting advance (Alberts et al. 2002, Andrews et al. 2003, Sandeep et al. 2004, Rauz et al. 2003). In turn, the identification of H6PDH as a determinant of oxo-reductase activity in adipose tissue raises the possibility that H6PDH itself could be a promising therapeutic target in treating central obesity. Indeed it is tempting to speculate that some of the ‘selective 11β-HSD1’ inhibitors obtained through cell-based high throughput screening assays, might actually be inhibitors of H6PDH rather than 11β-HSD1.
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