Upregulation of AKR1C3 expression by insulin in a human differentiated preadipocyte cell line

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Background

- Polycystic ovary syndrome (PCOS) is a triad of anovulation, insulin resistance and hyperandrogenism.
- Adipose androgen generation of testosterone from androstenedione by aldo-ketoreductase type 1C3 (AKR1C3) may contribute to hyperandrogenism.
- We hypothesised that insulin may upregulate adipose AKR1C3 expression in vitro in a human differentiated preadipocyte cell line.

Methods

- Preadipocytes from the SGBS cell line were differentiated into adipocytes under optimised conditions over 14 days.
- We used RT-qPCR to characterise gene expression of steroidogenic enzymes and insulin-cascade proteins in SGBS cells across differentiation (Days 0, 7 and 14).
- Differentiated adipocytes were exposed to increasing concentrations of insulin, androstenedione and testosterone in serum-free media for 20 hours before changes in gene expression were analysed using RT-qPCR.
- Experiments were performed in triplicate. Data are expressed as arbitrary units (AU) with standard error of the mean bars (SEM). Statistical significance was calculated by two-tailed t-test.

Results

![Day 0, Day 7, Day 14 images](image)

**Figure 1:** SGBS cells on days 0, 7 and 14 of differentiation. Increased lipid droplet formation can be appreciated.

![PPARγ, LPL expressions](image)

**Figure 2:** Change in mRNA expression of fat markers of differentiation across differentiation of SGBS cells. PPARγ: peroxisome-proliferator activated receptor γ; LPL: lipoprotein lipase. * represents significant difference from day 0 (p<0.05).

![AKR1C3, IRS2, PIK3CA expressions](image)

**Figure 4:** Change in mRNA expression of insulin-signaling cascade proteins across differentiation of SGBS cells. IRS2: insulin receptor substrate 2; PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase. * represents significant difference from day 7 (p<0.05)

![AKR1C3, SRD5A1 expressions](image)

**Figure 5:** Change in mRNA expression of AKR1C3 and SRD5A1 in differentiated SGBS cells after 20 hour incubation with varying concentrations of insulin (0-20nM), and different androgens (T, A4 and DHT). While insulin can be seen to stimulate expression of these steroidogenic enzymes, co-incubation with androgens largely blunts this effect (except in the case of A4 on SRD5A1). Significance was not reached between SRD5A1 treatments. T: testosterone; A4: androstenedione; DHT: dihydrotestosterone. Lines between columns represent statistical significance (p<0.05).

Conclusions

- AK1C3 expression in a differentiated human preadipocyte cell line is upregulated by insulin.
- Adipose tissue may be a key site of cross-talk between insulin signalling and androgen metabolism.
- Local adipose androgen generation in PCOS may be increased in hyperinsulinaemic conditions, and represent a possible target for therapeutic intervention.