Supplementary figure 1

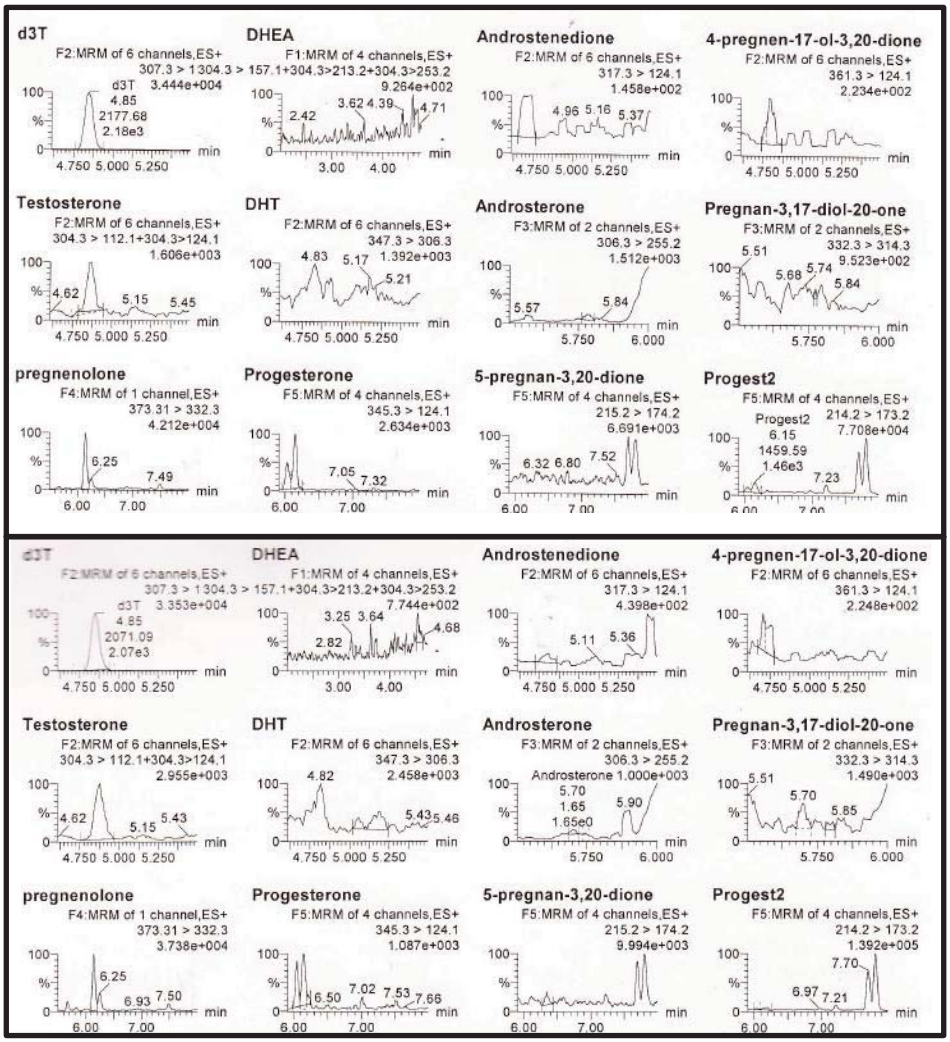
A

Intracellular steroid concentrations (raw integrated peak values)				
(16hrs)	**Control**		**Insulin**	
Steroid	**ng/ml (off Mass Spec)**	**ng/g cells**	**ng/ml (off Mass Spec)**	**ng/g cells**
DHEA	0.000974	0.001542	0.017793	0.028172
Androstendione	0.073581	2.61E-12	0.106509	0.168639
17-OH Progesterone	0.011366	0.017997	0.164881	0.261062
Testerosterone	0.006694	0.010599	0.412208	0.6527
DHT	NA	NA	NA	NA
Androsterone	0.0	0.0421	0.0578	0.0915
Pregnan-3,17-diol-20-one	0.04406	0.0698	0.1647	0.261
pregnenolone	2.124	3.364	5.369	8.502
progesterone	1.36	2.15	1.585	2.510
pregnan3,30dione	NA	NA	NA	NA

B

Steroid concentrations (secreted) in media (raw integrated peak values)				
(16hrs)	**Control**		**Insulin**	
Steroid	**ng/ml (off Mass Spec)**	**mol/L (Media)**	**ng/ml (off Mass Spec)**	**mol/L (Media)**
DHEA	0.0449	4.94E-12	0.109	1.2E-11
Androstendione	0.024	2.61E-12	0.070	7.75E-12
17-OH Progesterone	0.082	7.91E-12	0.147	1.41E-11
Testerosterone	0.211	1.68E-11	0.312	2.49E-11
DHT	0.313	2.49E-11	0.465	3.7E-11
Androsterone	0.38	4.18E-11	0.549	5.99E-11
Pregnan-3,17-diol-20-one	0.053	5.08E-12	0.122	1.17E-11
pregnenolone	0.22	2.42E-11	0.354	3.89E-11
progesterone	0.21	2.12E-11	0.293	2.96E-11
pregnan3,30dione	0.019	2.12E-11	0.035	3E-12

C



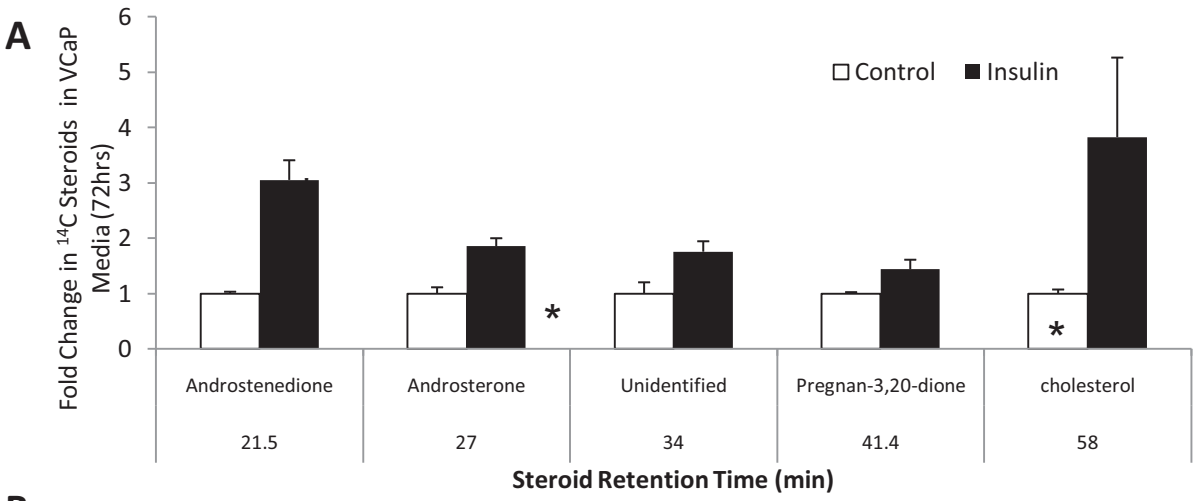
Supplementary figure 1.

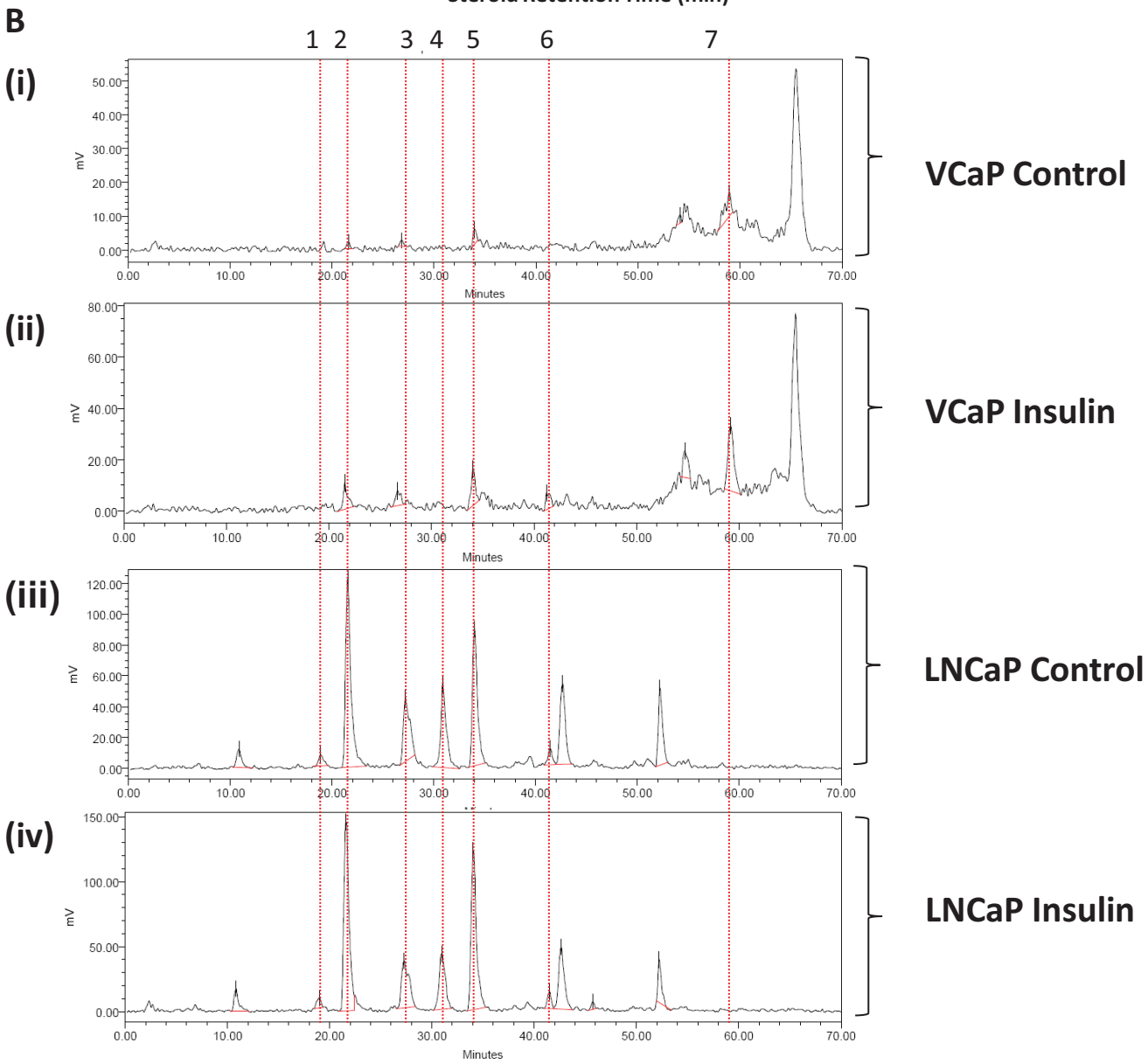
(A) Table of values from a representative LC/MS/MS spectra shown in ng/ml isolated from approximately 0.060g of LNCaP cells. Cellular steroids were extracted by MTBE extraction into 95µl 0.2M hydroxylamine HCl, and concentrations of that volume were measured by LC/MS/MS. The amount for the whole sample (ng) was calculated, then divided by 0.06g to give ng/g cell pellet.

(B) For media steroid concentrations, 3mL of media were extracted into 95µl MTBE. The total grams of steroid was calculated then converted to moles and divided by the original volume to get the original concentration of steroids in the media. The concentrations of T (cells), and T and DHT (media) have been included in the paper.

(C) This method of steroid quantitation measures unlabelled steady-state steroid concentrations (in contrast to *de novo* synthesised steroids measured in Figure 3d and 3e), therefore a spectrum of peaks has been provided. Spectrum from vehicle control appear in the top panel and insulin treated in the bottom panel.

Supplementary figure 2





1(18.88mins)	2 (21.5mins)	3 (27.7mins)	4 (30.8mins)	5 (34mins)	6 (41mins)	7 (58mins)
Testosterone std	Androstenedione std	Androsterone std	Pregnan-3,17diol-20-one std	Steroid peak (no std)	Pregnan-3,20-dione std	Cholesterol s td

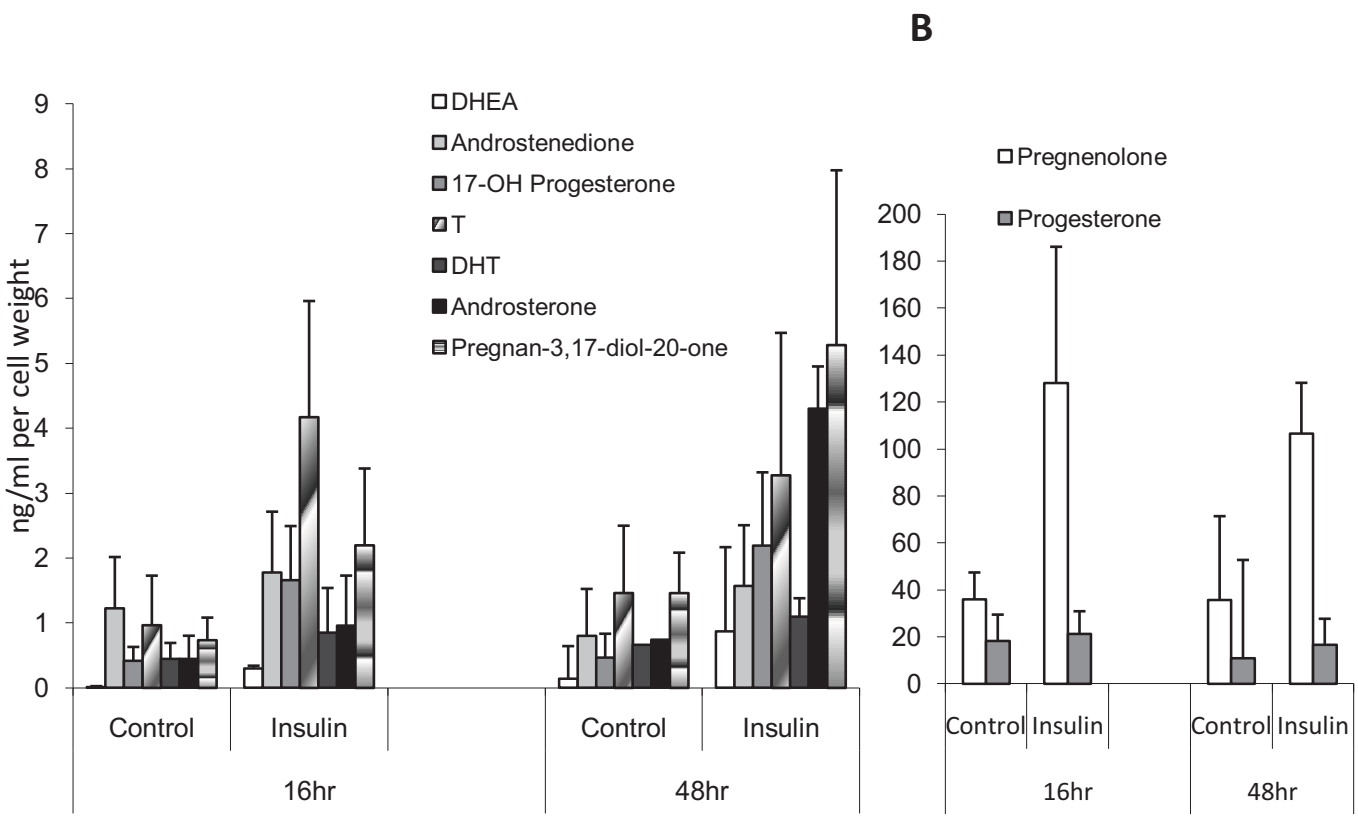
Supplementary figure 2

Supplementary figure 2

LNCaP and VCaP cells were treated with ^{14}C acetate for 72hours $\pm$10nM insulin. Steroids were extracted from media with equi-volume hexane/ ethanoloactetate (75:25), dried down and resuspended in 75µl of 50% methanol. Mix 10 standards (Sigma) were used to identify steroid retention times (Supp figure 2b) and peaks were quantified by calculating area under the curve and normalizing to cell pellet weight. Treated samples were then normalized to controls. (a) In VCaPs, androstendione, the step before testosterone in the classical steroid pathway, increased approximately 3-fold ($p<0.05$); whereas, androsterone, a steroid of the backdoor pathway, increased approximately 2-fold ($p<0.05$). The 34 min steroid peak which elutes at a time between progesterone and pregnan-3,20-dione, and pregan-3,30-dione steroid increased 1.75 and 1.5-fold, respectively ($p<0.05$). Cholesterol was detected in VCaP cells and furthermore, was increased approximately 4-fold by insulin treatment. Intriguingly, steroids beyond androstenedione and androsterone in the pathways were below the limit of detection in VCaP cells at 72hrs.

(b) Representative spectra of steroids isolated from 2mL of media following treatment with ^{14}C acetate for 72hours $\pm$10nM insulin in VCaP (a, b) or LNCaP cells (c, d). For VCaP cells, the control (a) was compared to 10nM insulin treated (b) to calculate fold change. The same was done for LNCaPs, vehicle-treated control cells (c) being used to normalise insulin induced peaks (d). Steroid retention times were comparable in both LNCaP and VCaP cells and are identified from 1 to 7, with testosterone the first to elute from the column and cholesterol the last. De novo synthesised testosterone was detected in LNCaPs. The total amount of radiolabelled steroids after 72 hours insulin treatment in the presence of ^{14}C acetate was substantially lower in VCaPs. Peaks were also present in the steroid region which could not be definitively identified (Peak 5; 34 mins) as there was no corresponding peak in the Mix 10 standards.

Supplementary figure 3



Supplementary figure 3

LNCaP cells were treated with 10nM insulin for 16 or 48 hours and steroids were extracted (see methods and materials) and prepared for quantitation by LC/MS/MS. Graphs (a, b) depict the difference in intracellular steroids, shown in ng/ml (controlled to weight of cell pellet) and show accumulation of androgens over time.