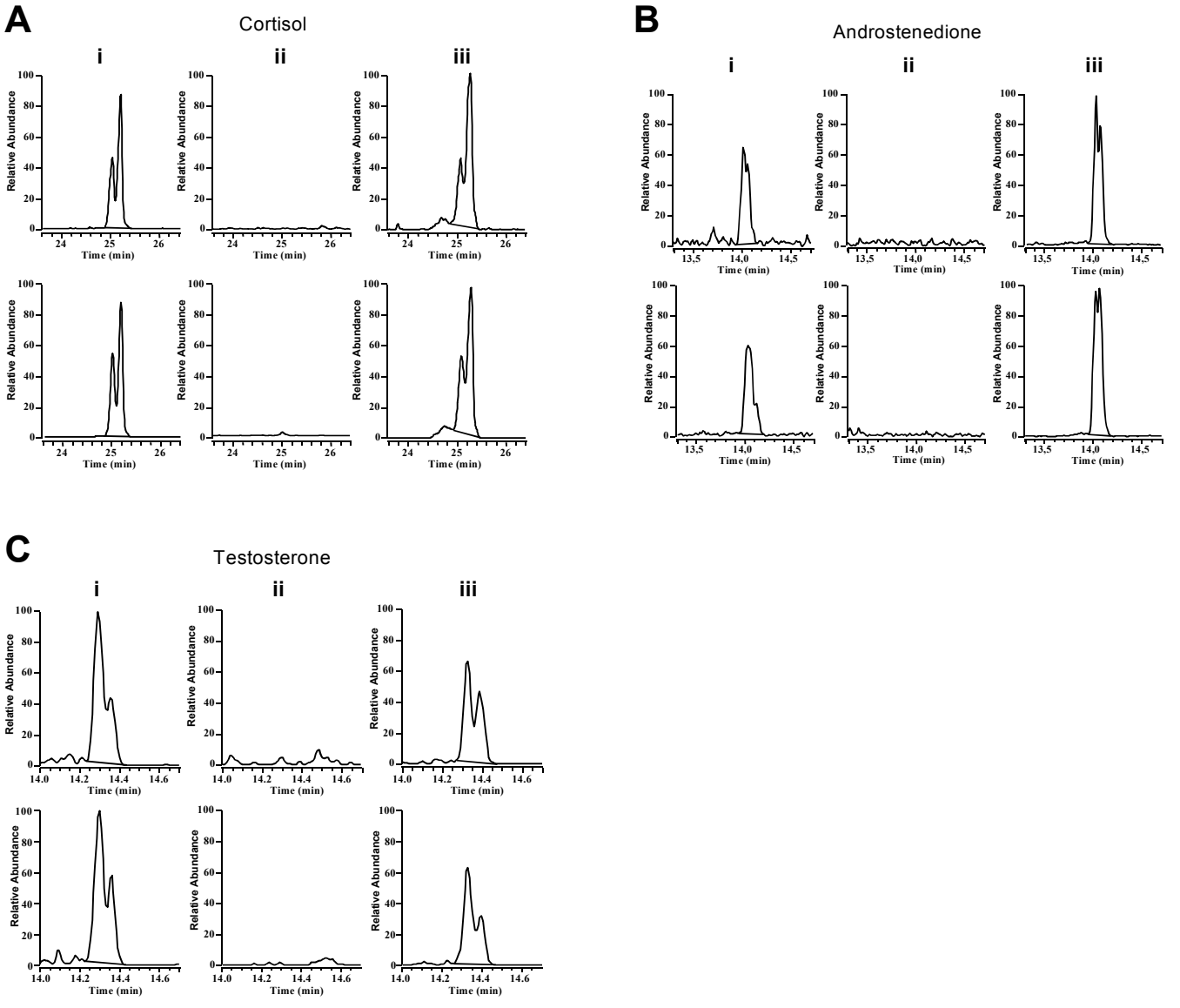
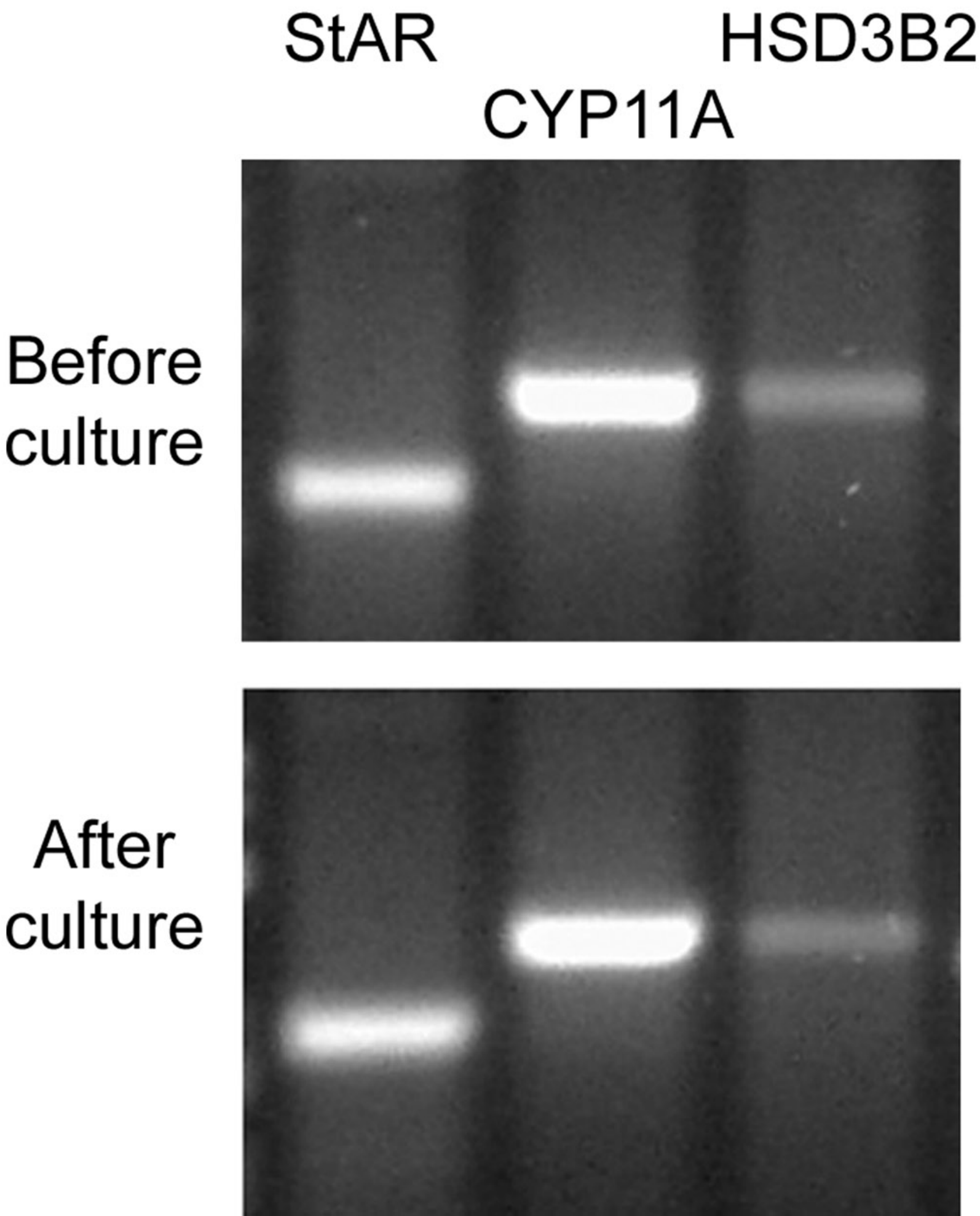
Supplementary methods. Identification of cortisol, androstenedione and testosterone by gas chromatography/ mass spectrometry (GC/MS).

Adrenal glands were cultured overnight in media. This media was collected and analyzed alongside a control (i.e. media unconditioned by adrenal glands) and three calibration samples (control media spiked with different amounts of testosterone, androstenedione and cortisol). An internal standard (400 ng of 17 α-methyltestosterone) was added to all samples. Samples were processed for analysis according to methodology described in *Shackleton CH, Profiling Steroid Hormones and Urinary Steroids (J Chromatogr 1986, 379, 91-156)* and *Griffiths W, Shackleton CHL, Sjövall J, Mass Spectrometry of Steroids (In: The Encyclopedia of Mass Spectrometry, Vol. 7, ed. Caprioli R, Elsevier: Amsterdam, 2005).* Extracts were analyzed by a GC Top 8000 gas chromatograph coupled with a PolarisQ ion trap mass spectrometer and AI3000S autosampler (Finnigan MAT, San Jose, CA, USA). The steroids were separated on a DB-1 cross-linked methyl-silicone column (15 m x 0.25 mm i.d., film thickness 0.25 μm; J&W Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a constant pressure of ~35 kPa. A 3 μl aliquot of the final derivatized extract was injected into the system operated in splitless mode (valve opened at 2 min). The GC temperature was ramped as follows: initial 50°C, held for 3 min, increased to 230°C at 30°C min^{-1}, thereafter increased to 285°C at 2°C min^{-1}. The injector and transfer line were kept at 260°C and 280°C, respectively. The ion source temperature was 225°C. Under these GC conditions, the retention times for androstenedione, testosterone, 17α-methyltestosterone and cortisol were: 14.0, 14.3, 15.6 and 25.3 min, respectively.


A
Cortisol
i
ii
iii
B
Androstenedione
i
ii
iii
C
Testosterone
i
ii
iii
Relative Abundance
Time (min)


StAR
CYP11A
HSD3B2
Before culture
After culture

Supplementary figure 1. GC/MS spectra for (A) cortisol, (B) androstenedione and (C) testosterone.

In A-C, chromatographs are shown for (i) calibration samples, (ii) control media and (iii) adrenal media. There was no detection in the control media. To facilitate comparison between the different samples, the same scale has been used. **A.** Cortisol. Upper chromatograms, molecular ion m/z 636; lower, m/z 605. **B.** Androstenedione. Upper chromatograms, molecular ion m/z 344; lower, m/z 313. **C.** Testosterone (precursor ion; m/z 389). Upper chromatograms, product ion m/z 358; lower, product ion m/z 268.

Supplementary figure 2. HSD3B2 is not induced by culture.

Reverse transcription followed by 24 cycles of PCR on total RNA isolated from human adrenal gland (8 wpc) before and after culture for 24 h. HSD3B2 is not increased by culture.

Gene	Forward primer	Reverse primer	Product (bp)	Annealing temperature (˚C)
StAR	GTGGGGCCCCGTGACTTTGTGA	CGCTTGCGCAGGTGGTTGG	287	61.5
CYP11A	CCGCCCTGGGTCGCCTATCA	GCATGGGGACGCTGGTGTGGA	373	61.2
CYP17	GGCCACCCACAACGGACAGT	CCACGCCAGCCCCAAAGATG	443	56.7
HSD3B2	GAGACATTCTGGATGAGCC	CGCACAAGTGTACAAGGTAT	370	54.9
HSD3B1	GAGACATTCTGGATGAGCC	GCACAAGTGTACAGGGTGC	370	54.9
CYP21	GGCAGGCCAGTGGAGGGACAT	TGGGGCAAGGCTAAGGGCACAA	348	62.5
CYP11B1	TCGTGGCGGAGCTCCTGTTGAATG	CTGGGACCCTGGGTGCAGAGACGT	672	63.2
CYP11B2	CCGCCGCAGCCAGCATCAG	GGGACCCTGGGTGCAGATGCAA	501	62.0
ST	CCCCATCCAGTTATTCCCCAAGTC	TTTTTCCAGTCCCCAGATACACCT	468	54.7
MC2R	GTGTGGTTTTGCCGGAGGAGAT	GTGGGCACATGATGGGAGAAGA	454	57.8
HSD17B3	GCCGGACGCTGGAAAAACTA	CTCTTGCAGGGCCTTGGAAAAT	407	55.9
HSD17B5/AKR1C3 (1)	GTAAAGCTTTGGAGGTCAC	CACCCATCGTTTGTCTCGT		
HSD17B5/AKR1C3 (2)	CGAACTCCCCGGTGCTCTT	TCTTTCTGGCCTATGGACTC	440	54.9

Supplementary table 1. RT-PCR primers and conditions.

The data shown in Figure 8 is derived from the second set of AKR1C3 primers located in the 3' UTR but was also validated by using the additional primer pair listed above [AKR1C3 (1)] from Penning et al, *Biochemical J*, 351, 67-77, 2000.