Supplementary Materials and Methods

Steroid quantification by LC-MS/MS

The Agilent 1290 UPLC instrument is equipped with a binary solvent delivery system, an auto sampler (at 4°C), and a column oven, coupled to an Agilent 6490 triple quadrupole mass spectrometer equipped with a jet stream electrospray ionization interface (AJS-ESI) (Agilent Technologies, Basel, Switzerland). The chromatographic separation of the analytes was achieved using a Waters ACQUITY UPLC BEH C18, 1.7 µm, 2.1×150 mm, column (Waters, Wexford, Ireland). The column temperature was 65ºC for androgens and 40°C for other steroids. All analytes were separated using a mobile phase consisting of water-acetonitrile-formic acid (A) (95/5/0.1; v/v/v) and (B) (5/95/0.1; v/v/v). The injection volume was 1-2 µL per sample. Methanol in water (75/25 v/v) was used as needle and needle-seat flushing solvent for 10 s after sample aspiration. Samples were stored until analysis in the auto sampler (maintained at 4°C). Method A: 11OHT, 11OHA4, 11KT, 11KA4, testosterone, A4 were eluted by the gradient 25 - 70% of mobile phase B during 0 - 10 min, and 100% of mobile phase B at 10.1 min onwards at a constant flow rate of 0.63 mL/min. The run was stopped after 12.0 min, followed by re-equilibration of the column for 1.5 min. Method B: Cortisone, cortisol, and 20β-hydroxycortisone were eluted by the gradient 30 - 70% of mobile phase B during 0 - 2.3 min at a gradient flow rate from 0.5 mL/min to 0.47 mL/min, and 100% of mobile phase B at 3 min onwards at a constant flow rate of 0.5 mL/min. The run was stopped after 5 min, followed by re-equilibration of the column for 2 min. The measuring parameters for each steroid are outlined in Supplementary Tables 1 and 2.