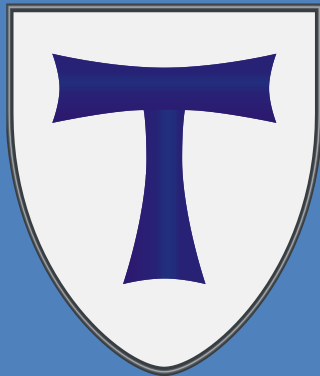


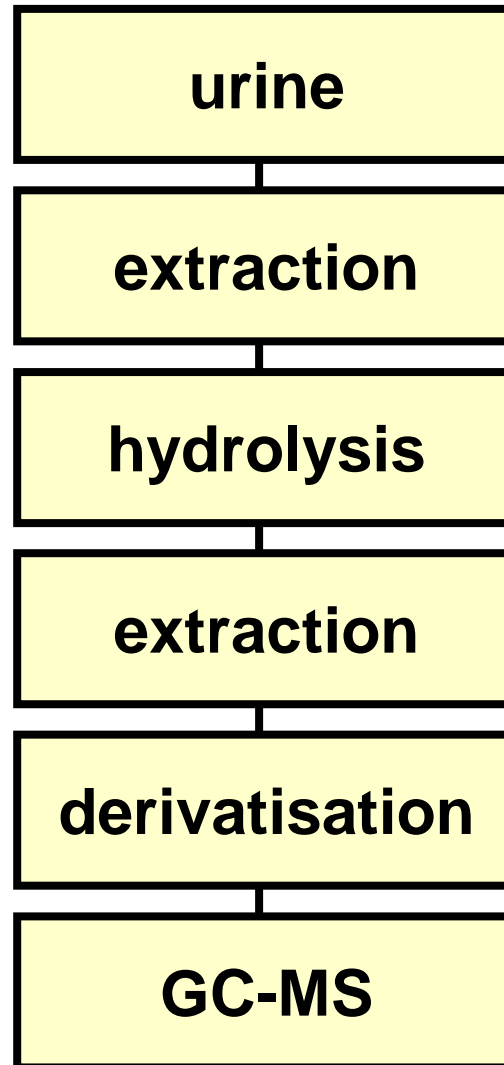
# Seminar Metabolomics



Michaela F. Hartmann  
Steroid Research and Mass Spectrometry Unit  
Pediatric Endocrinology & Diabetology  
Justus Liebig University  
Giessen



# GC-MS Urinary steroid profiling

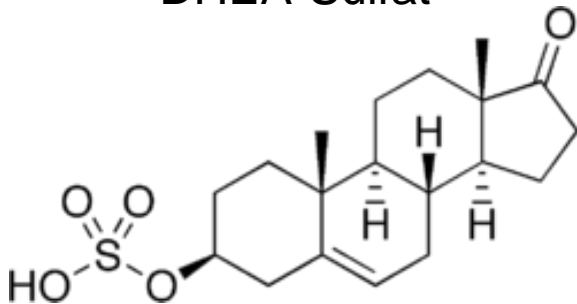


# Prinzip der GC-MS – Scan Techniken

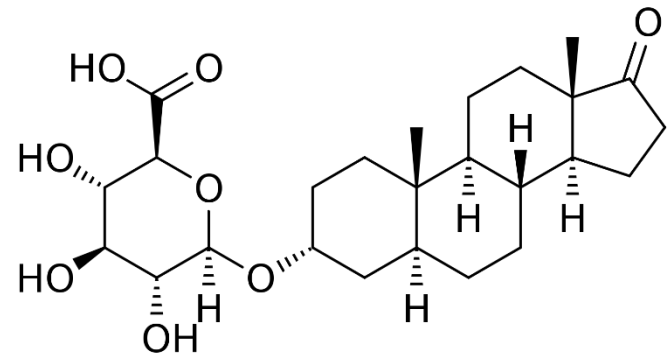
- Steroide im Urin:

- Frei
- Sulfate
- Glucuronide

DHEA-Sulfat



Androsteron-Glucuronid

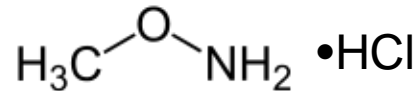


# Prinzip der GC-MS – Scan Techniken

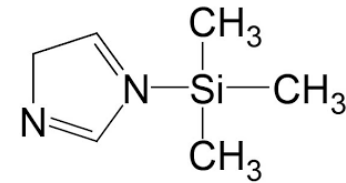
- Derivatisierung zu Methyloxim-Trimethylsilyl-Derivaten:

1. Schritt: Bildung von Methyloximen aus den Ketogruppe

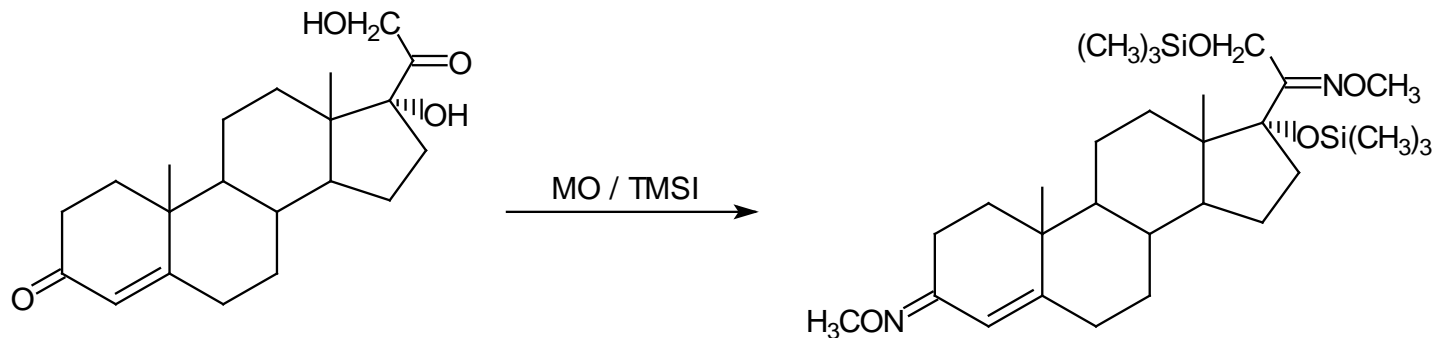
2. Schritt: Bildung von Trimethylsilylethern aus den Hydroxylgruppen



Methoxyamin Hydrochlorid (MO)

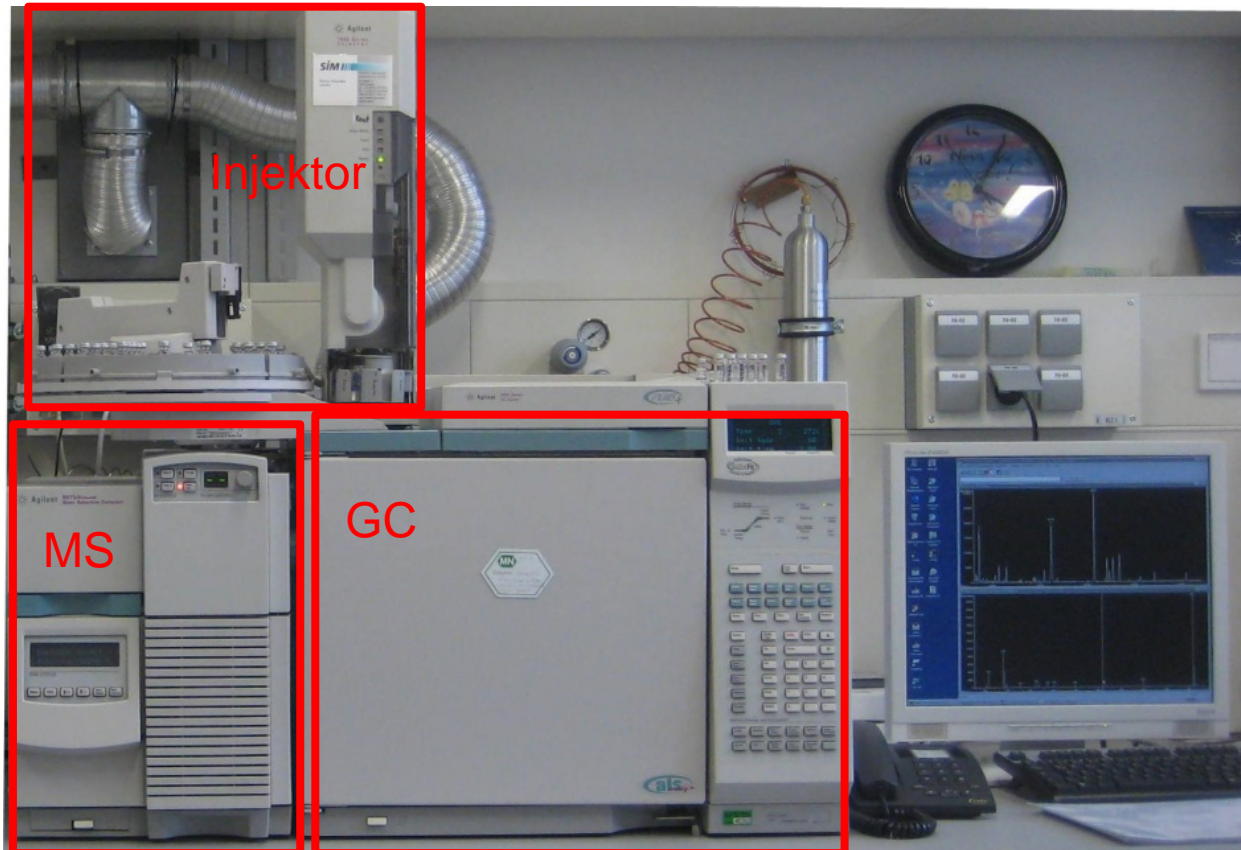


Trimethylsilylimidazol (TMSI)



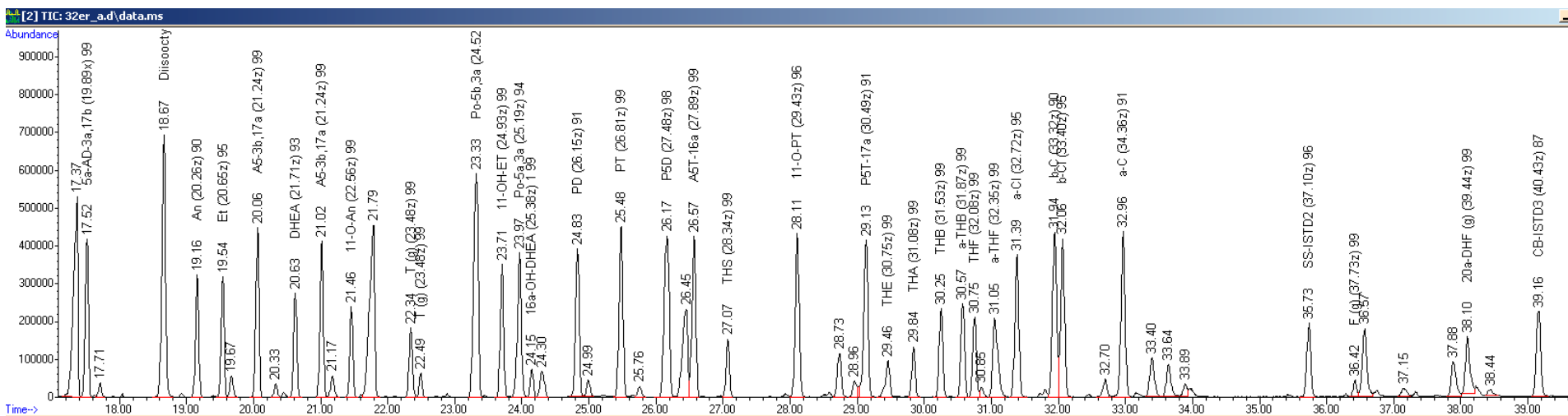
# Kopplungstechnik GC-MS

- In der Abbildung wird ein GC-MS System gezeigt.



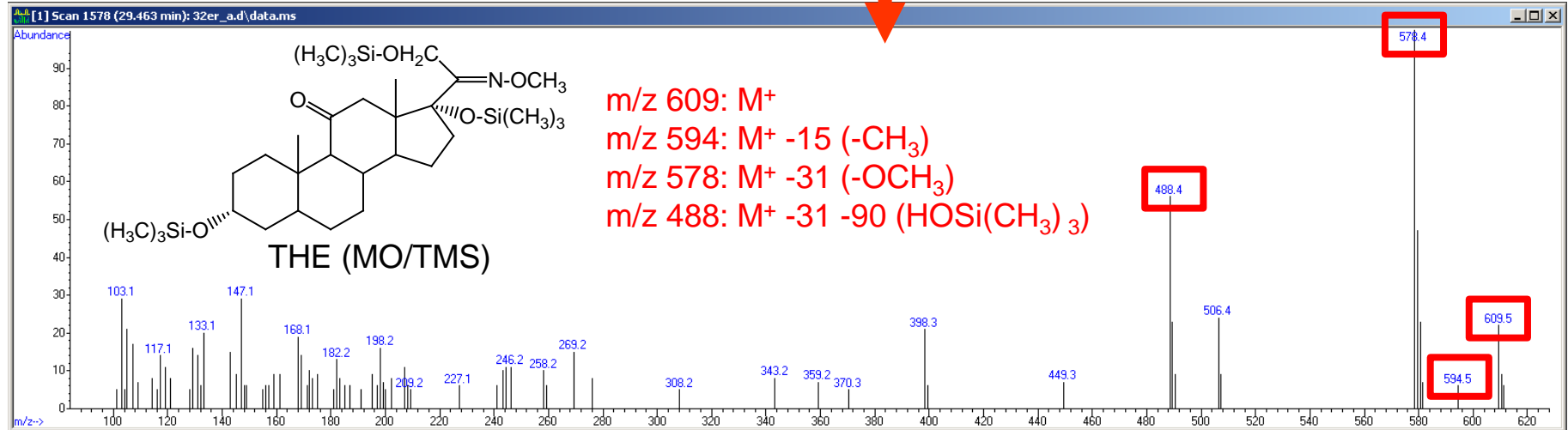
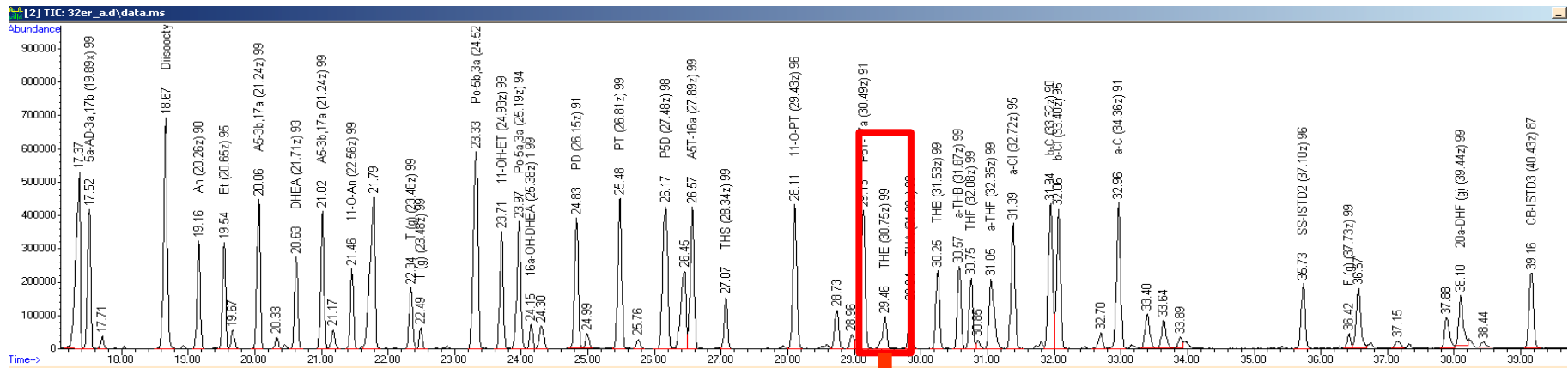
# Prinzip der GC-MS – Scan Techniken

- Scan-Lauf der Methyloxim-Trimethylsilylether-Derivate eines Steroidgemisches mit Abgleich der gemessenen Spektren mit der Bibliothek
    - „non targeted metabolic profiling“
- In der Abbildung ist das TIC zu sehen.



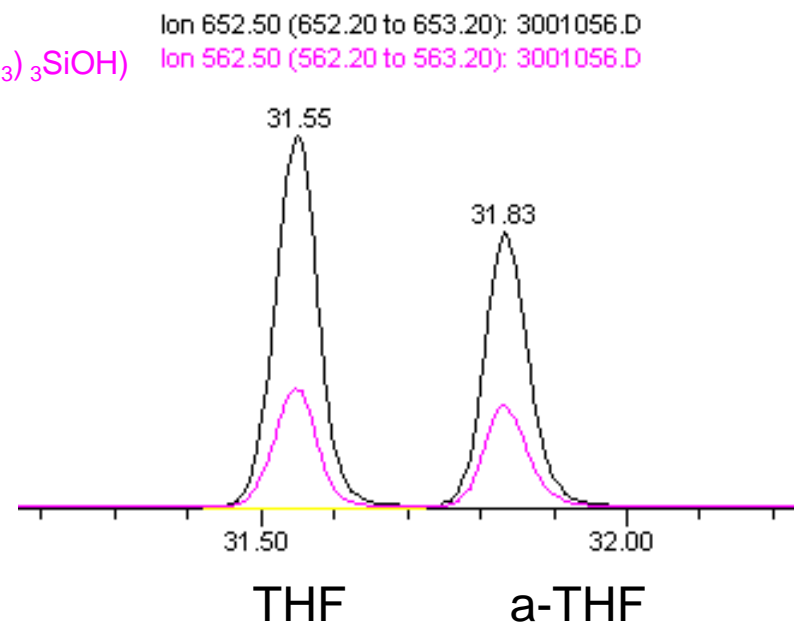
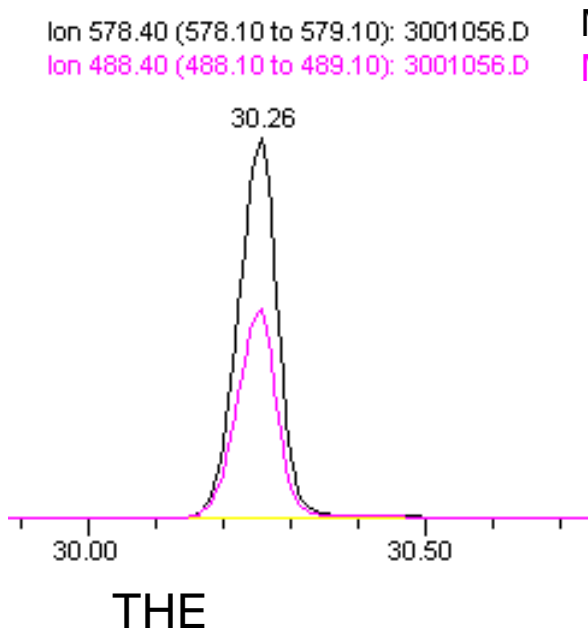
# Prinzip der GC-MS – Scan Techniken

Scan 1578 bei 29.46 min entspricht dem Massenspektrum von Tetrahydrocortison (THE) als Methyloxim-Trimethylsilylether-Derivat

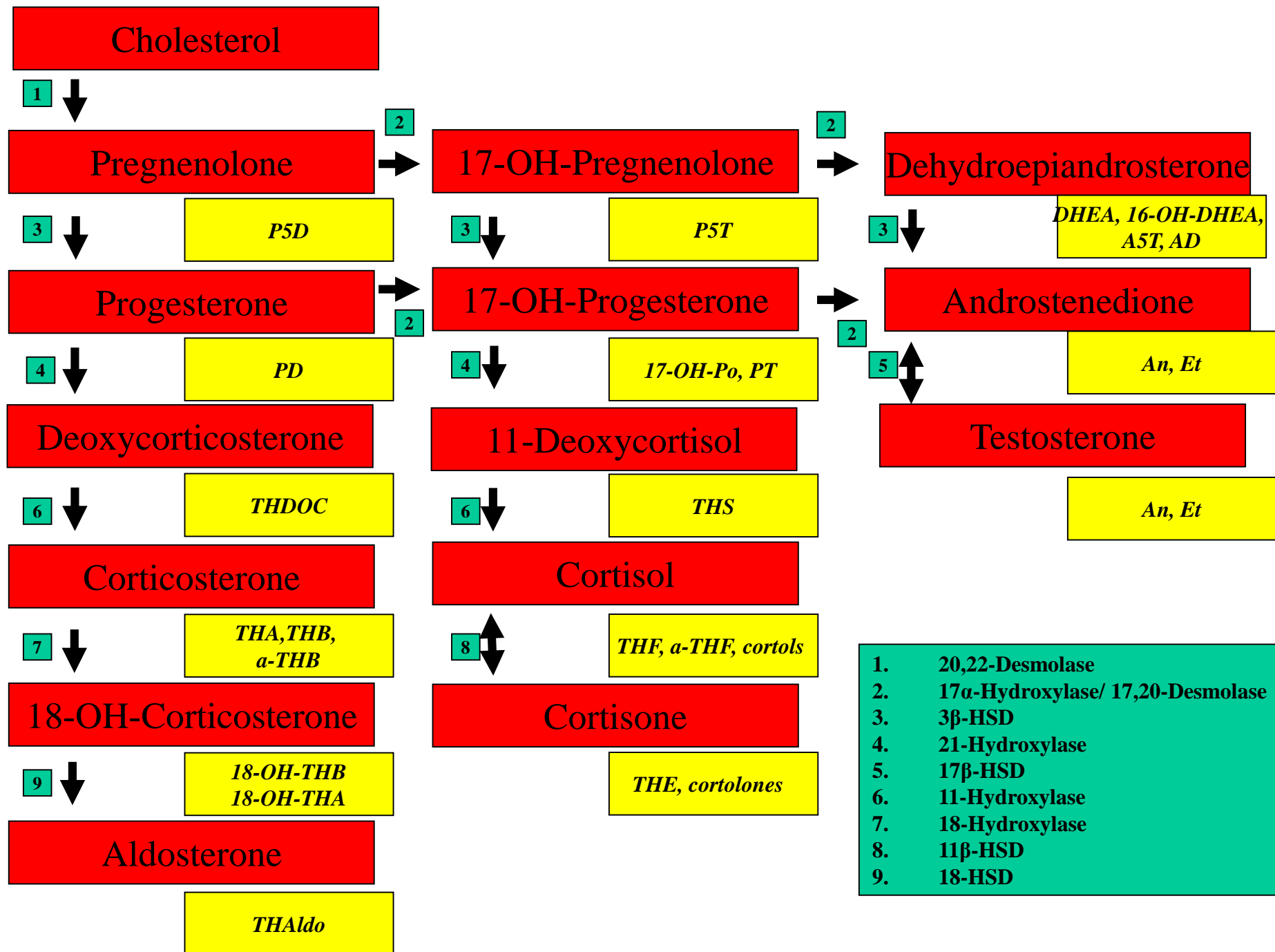


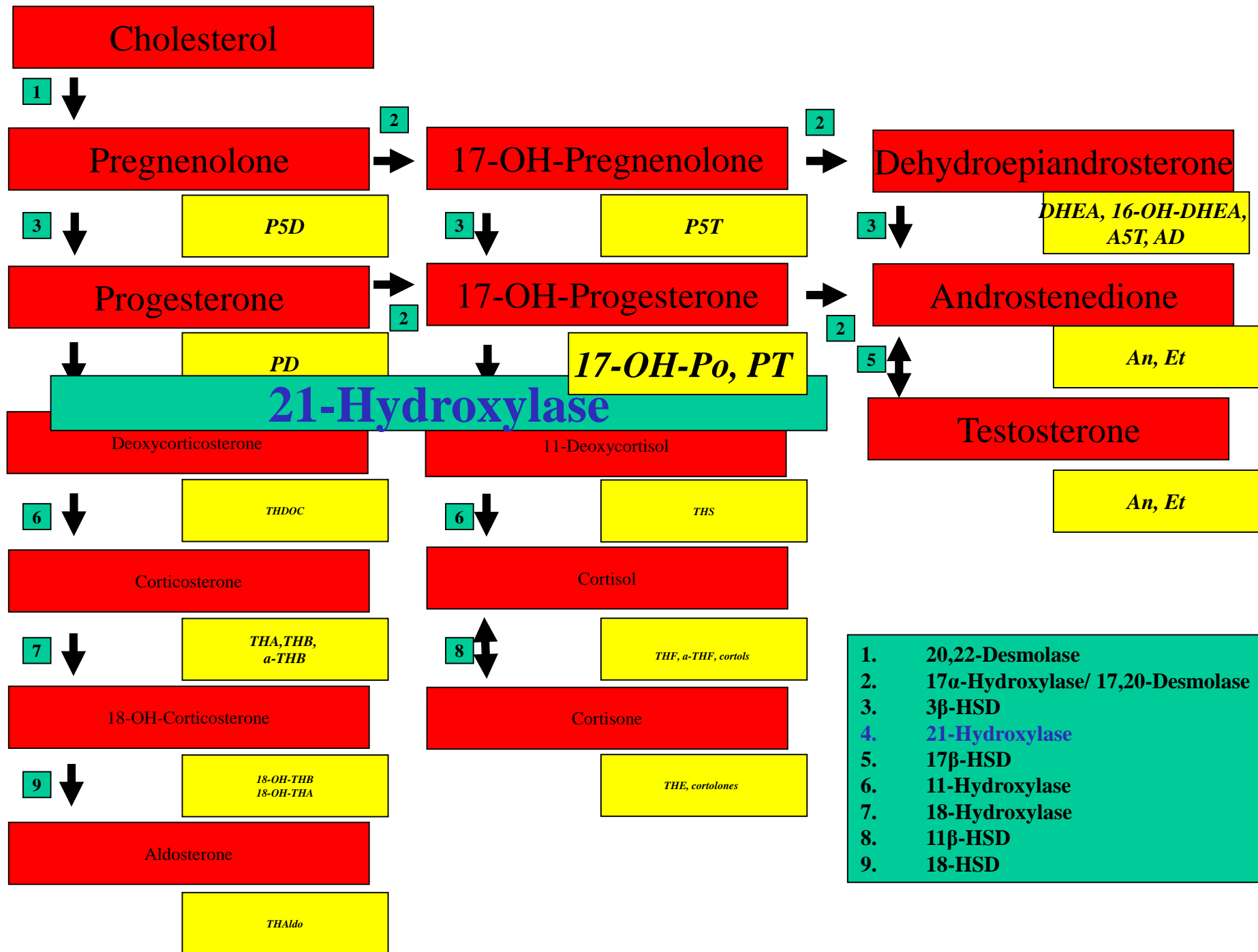
# Prinzip der GC-MS – SIM

- MS im „selected ion monitoring mode“ (SIM) – „targeted metabolic profiling“:
  - Es werden für einen Analyten nur ein oder zwei spezifische Ionen aufgezeichnet.
  - Die Empfindlichkeit der Detektion ist erhöht.
  - SIM wird hauptsächlich zur Quantifizierung verwendet.

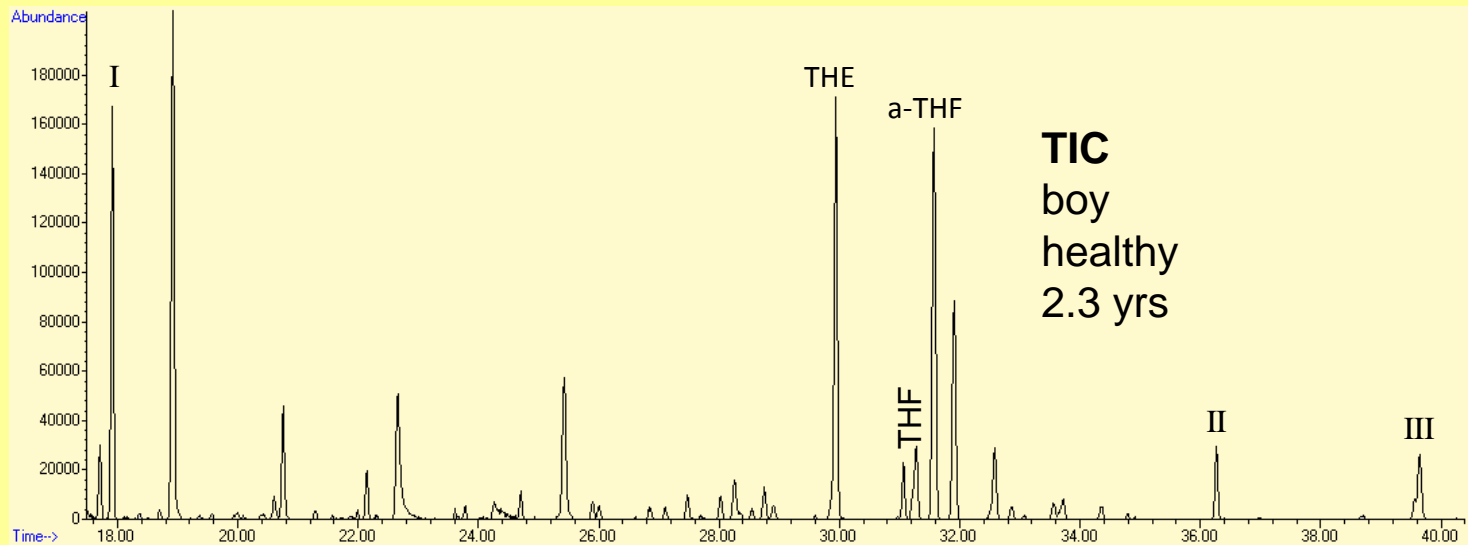
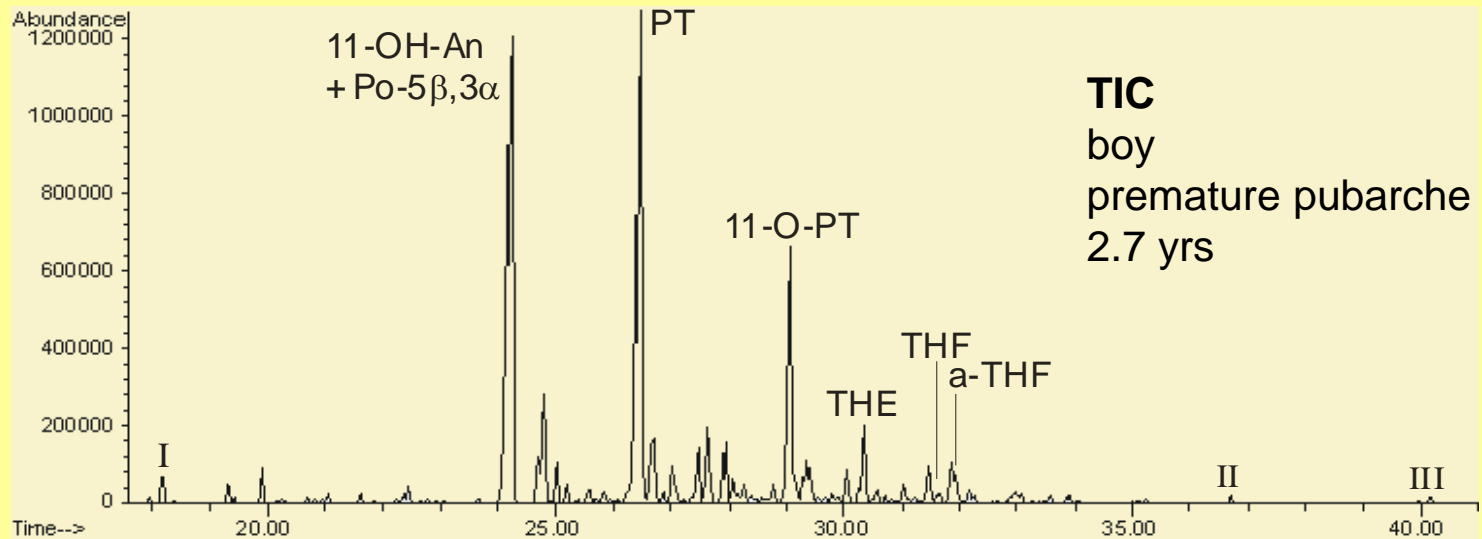




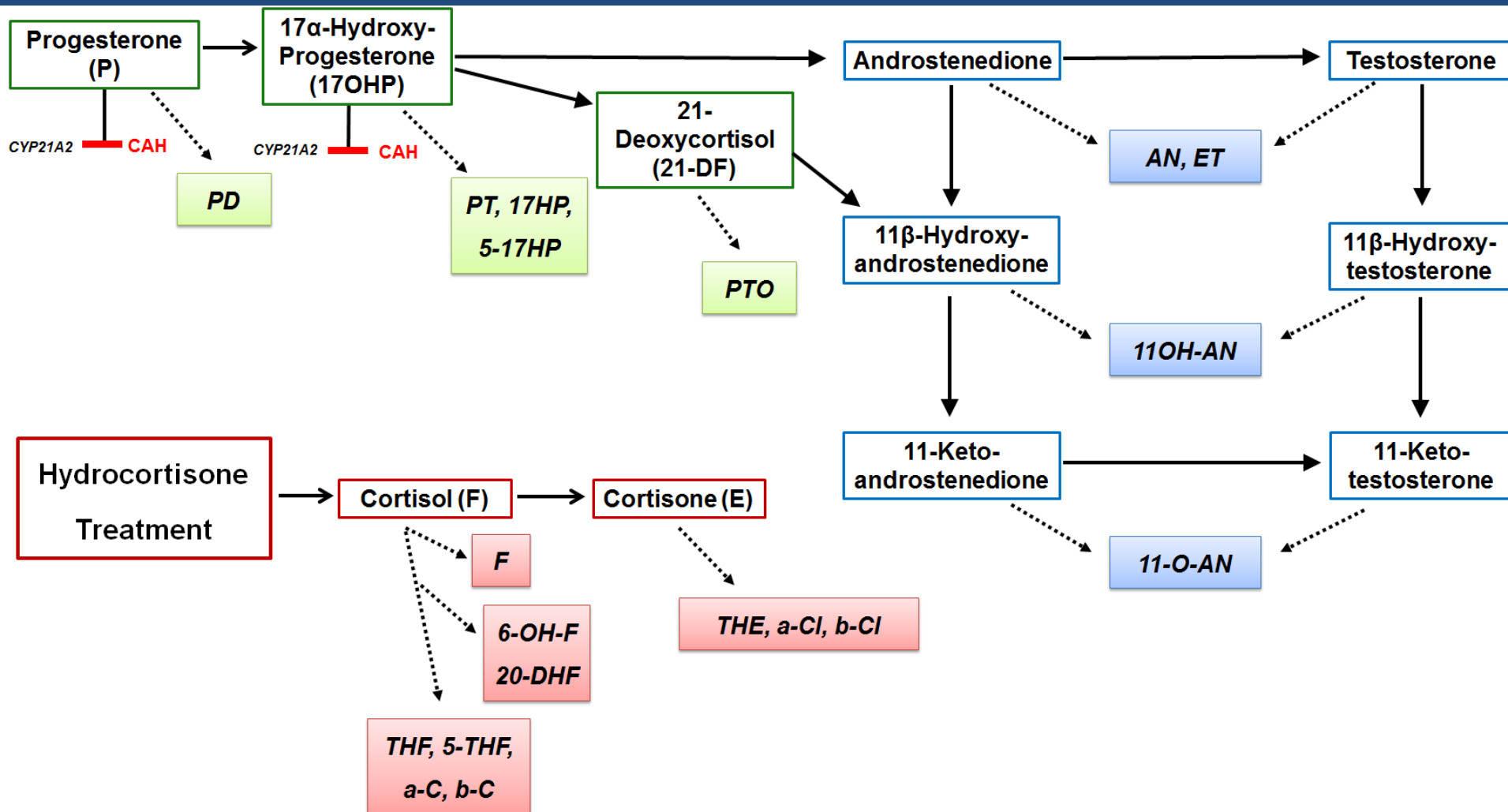




# GC-MS urinary steroidomics: 21-hydroxylase deficiency



# Androgen-Synthese und Steroid Metabolismus bei Kindern mit AGS (CAH)



C. Kamrath et al, Urinary GC-MS Steroid Metabotyping in treated Children with Congenital Adrenal Hyperplasia; Metabolism, Metabolism Clinical and Experimental 112 (2020) 154354.

# Steroid-Datenbank

# Steroid-Datenbank

31 Steroide, ca. 300 Kontrollen, Patienten mit verschiedenen Enzymdefekten der Steroidbiosynthese

| [0-m 1-w |     | [a-h Konzentrationen in µg/L] |         |         |         |         |             |           |           |         |          |        |         |          |          |          |         |         |         |         |         |        |        |        |         |         |          |         |          |         |        |        |        |        |
|----------|-----|-------------------------------|---------|---------|---------|---------|-------------|-----------|-----------|---------|----------|--------|---------|----------|----------|----------|---------|---------|---------|---------|---------|--------|--------|--------|---------|---------|----------|---------|----------|---------|--------|--------|--------|--------|
| Pat.-Nr. | Sex | Alter                         | hA      | An      | Et      | DHA     | 16a-OH-DHEA | A5-3b,17a | A5-3b,17b | A5T-16a | PD       | PT     | P5D     | P5T-17a  | Po-5b,3a | Po-5a,3a | F       | THE     | THF     | a-THF   | a-Cl    | b-Cl   | a-C    | b-C    | 6b-OH-F | 20a-DHF | 11-OH-An | 11-O-An | 11-OH-Et | 11-O-Pr | THA    | THB    | a-THB  | THS    |
| 1306     | 1   | 16.92                         | 455.83  | 539.46  | 21.13   | 178.44  | 13.13       | 16.80     | 48.46     | 98.79   | 196.41   | 3.25   | 11.43   | 29.16    | 7.04     | 30.40    | 614.15  | 333.01  | 290.48  | 289.88  | 220.43  | 34.16  | 157.88 | 3.25   | 19.68   | 283.35  | 19.57    | 113.41  | 2.65     | 24.87   | 23.53  | 34.82  | 16.78  |        |
| 1307     | 0   | 16.93                         | 406.49  | 549.16  | 165.23  | 503.13  | 16.77       | 53.55     | 164.49    | 247.72  | 326.72   | 3.25   | 11.75   | 89.29    | 7.25     | 48.48    | 1533.82 | 638.32  | 250.08  | 891.06  | 427.98  | 103.51 | 279.16 | 78.15  | 35.24   | 222.08  | 17.78    | 35.97   | 3.06     | 65.30   | 64.33  | 56.64  | 26.43  |        |
| 1308     | 1   | 16.93                         | 445.00  | 822.23  | 202.98  | 1741.80 | 19.62       | 57.54     | 454.06    | 786.64  | 516.18   | 3.25   | 55.61   | 189.99   | 15.85    | 76.28    | 2623.34 | 655.70  | 405.65  | 74.16   | 478.60  | 113.03 | 282.14 | 110.51 | 25.16   | 313.81  | 39.21    | 234.41  | 4.34     | 83.76   | 57.19  | 74.93  | 38.87  |        |
| 1309     | 1   | 16.97                         | 1473.74 | 1599.60 | 124.10  | 521.56  | 44.91       | 82.52     | 777.97    | 151.16  | 488.48   | 72.44  | 223.30  | 86.03    | 13.19    | 144.35   | 4146.50 | 1526.63 | 793.43  | 1484.16 | 623.41  | 271.22 | 588.09 | 240.91 | 85.79   | 636.58  | 57.80    | 390.90  | 13.12    | 215.58  | 241.25 | 256.79 | 67.09  |        |
| 1310     | 0   | 17.05                         | 3376.28 | 3289.35 | 200.42  | 581.25  | 90.95       | 194.20    | 839.01    | 93.21   | 1449.46  | 153.54 | 268.12  | 70.42    | 34.29    | 142.19   | 8434.72 | 2685.62 | 2965.38 | 3231.25 | 1492.85 | 633.08 | 679.64 | 231.62 | 60.67   | 2727.27 | 224.17   | 1898.73 | 40.50    | 436.22  | 399.59 | 765.88 | 194.60 |        |
| 1311     | 1   | 17.07                         | 1540.12 | 927.01  | 1577.63 | 1446.09 | 55.31       | 258.13    | 1023.48   | 44.41   | 366.96   | 79.33  | 288.10  | 136.66   | 13.14    | 288.34   | 1703.24 | 1342.75 | 1390.09 | 1401.86 | 423.42  | 454.31 | 559.93 | 505.72 | 280.41  | 989.51  | 57.44    | 346.47  | 8.15     | 71.67   | 93.39  | 331.59 | 93.63  |        |
| 1312     | 1   | 17.10                         | 1129.93 | 681.72  | 982.93  | 535.86  | 22.78       | 99.43     | 234.34    | 48.42   | 221.54   | 25.80  | 95.43   | 23.51    | 7.02     | 69.06    | 616.88  | 341.39  | 288.48  | 564.05  | 221.48  | 94.82  | 172.82 | 125.59 | 87.81   | 248.59  | 18.76    | 48.98   | 3.05     | 40.26   | 29.97  | 56.81  | 21.64  |        |
| 1313     | 1   | 17.13                         | 1595.16 | 1417.16 | 117.28  | 390.24  | 33.95       | 102.62    | 327.92    | 234.96  | 964.37   | 54.37  | 138.21  | 200.86   | 28.10    | 28.61    | 2020.40 | 671.31  | 766.21  | 614.27  | 538.73  | 81.08  | 250.57 | 47.56  | 17.20   | 40.03   | 39.02    | 310.96  | 9.45     | 47.21   | 51.91  | 107.45 | 59.69  |        |
| 1314     | 1   | 17.18                         | 1385.74 | 2270.60 | 251.04  | 580.84  | 39.11       | 143.26    | 432.68    | 133.49  | 375.66   | 88.46  | 206.38  | 82.09    | 8.77     | 207.24   | 4289.55 | 1449.31 | 550.18  | 1713.79 | 754.64  | 219.71 | 517.10 | 341.72 | 123.80  | 617.28  | 38.29    | 449.08  | 5.71     | 97.17   | 147.39 | 112.74 | 98.69  |        |
| 1315     | 1   | 17.33                         | 1253.24 | 848.08  | 107.61  | 498.74  | 26.84       | 103.25    | 103.65    | 551.98  | 864.75   | 17.99  | 218.12  | 267.79   | 44.76    | 83.96    | 3930.18 | 1132.05 | 1142.35 | 1296.81 | 791.26  | 202.93 | 260.91 | 86.50  | 27.47   | 35.37   | 26.67    | 25.55   | 34.43    | 209.80  | 129.21 | 312.16 | 58.44  |        |
| 1316     | 1   | 17.52                         | 1409.40 | 1362.57 | 365.53  | 755.29  | 61.51       | 167.99    | 389.62    | 154.35  | 898.87   | 45.09  | 212.02  | 88.22    | 15.45    | 123.66   | 4130.19 | 1285.28 | 1203.19 | 1385.95 | 810.30  | 260.83 | 314.77 | 218.49 | 43.42   | 706.09  | 62.90    | 884.84  | 42.07    | 462.33  | 401.98 | 655.45 | 102.90 |        |
| 1317     | 0   | 17.56                         | 1156.06 | 1068.14 | 1354.66 | 450.98  | 36.26       | 163.19    | 165.92    | 93.89   | 888.66   | 73.01  | 190.58  | 58.68    | 14.12    | 31.63    | 1284.22 | 643.52  | 632.86  | 463.90  | 305.29  | 89.39  | 269.68 | 70.10  | 20.03   | 52.97   | 53.45    | 361.52  | 6.64     | 54.31   | 35.78  | 109.42 | 47.41  |        |
| 1318     | 1   | 17.66                         | 919.25  | 1222.67 | 360.91  | 568.13  | 32.51       | 68.05     | 204.02    | 212.00  | 311.32   | 24.37  | 162.46  | 60.17    | 10.32    | 80.00    | 1648.87 | 695.99  | 386.08  | 633.13  | 385.46  | 90.95  | 295.30 | 85.04  | 45.92   | 323.79  | 28.17    | 156.82  | 4.75     | 101.51  | 110.22 | 112.39 | 48.48  |        |
| 1319     | 0   | 17.68                         | 1067.01 | 765.28  | 867.42  | 1125.88 | 28.86       | 256.16    | 1034.32   | 42.54   | 385.25   | 197.87 | 297.09  | 110.93   | 19.79    | 55.99    | 2683.95 | 1260.48 | 1710.87 | 951.91  | 325.43  | 226.64 | 740.68 | 88.87  | 31.22   | 391.53  | 18.06    | 26.43   | 7.36     | 59.10   | 78.60  | 213.40 | 55.48  |        |
| 1320     | 1   | 17.75                         | 642.84  | 834.72  | 930.96  | 379.84  | 27.87       | 124.10    | 137.49    | 109.51  | 270.68   | 79.28  | 145.44  | 45.10    | 8.72     | 36.37    | 1104.86 | 537.07  | 422.17  | 422.72  | 250.72  | 71.96  | 179.42 | 62.84  | 20.82   | 349.89  | 31.68    | 257.85  | 4.93     | 3.25    | 40.65  | 72.25  | 31.13  |        |
| 1321     | 1   | 17.76                         | 1328.25 | 1105.00 | 486.55  | 916.42  | 43.12       | 149.43    | 376.37    | 1007.96 | 672.69   | 101.29 | 329.32  | 192.70   | 27.02    | 54.30    | 1972.52 | 811.06  | 814.94  | 792.89  | 447.06  | 113.34 | 308.23 | 107.68 | 29.13   | 524.04  | 40.23    | 236.26  | 5.99     | 83.54   | 106.18 | 288.83 | 44.19  |        |
| 1322     | 1   | 17.85                         | 630.77  | 539.36  | 30.24   | 288.38  | 12.36       | 24.77     | 155.88    | 123.09  | 289.52   | 3.25   | 11.26   | 24.26    | 7.54     | 91.84    | 767.24  | 433.39  | 390.14  | 565.53  | 278.32  | 94.38  | 236.95 | 110.88 | 43.93   | 243.73  | 29.53    | 151.75  | 5.23     | 41.28   | 36.03  | 90.00  | 21.25  |        |
| 1323     | 0   | 18.04                         | 3226.20 | 1631.52 | 294.01  | 561.22  | 48.19       | 78.10     | 355.85    | 274.01  | 1132.61  | 86.38  | 269.08  | 202.05   | 55.84    | 76.58    | 3797.54 | 1369.07 | 2063.41 | 1254.80 | 888.63  | 253.74 | 470.07 | 139.00 | 29.49   | 581.80  | 85.34    | 493.00  | 6.41     | 125.74  | 182.22 | 423.30 | 71.18  |        |
| 1324     | 1   | 18.06                         | 656.46  | 1027.43 | 48.78   | 290.19  | 25.61       | 27.31     | 336.77    | 90.73   | 219.56   | 28.72  | 44.28   | 29.05    | 6.63     | 62.66    | 1150.59 | 644.48  | 314.58  | 600.92  | 296.90  | 102.12 | 220.57 | 116.28 | 41.96   | 343.40  | 32.58    | 271.56  | 3.64     | 42.60   | 87.90  | 67.64  | 28.65  |        |
| 2002     | 1   | 2.72                          | 1248.44 | 296.70  | 87.08   | 2206.75 | 122.14      | 32.57     | 1393.42   | 161.77  | 15225.73 | 49.142 | 810.33  | 16911.26 | 2963.81  | 62.34    | 1589.95 | 465.25  | 1019.15 | 350.37  | 317.43  | 104.03 | 269.89 | 135.38 | 38.10   | 7907.22 | 490.07   | 116.51  | 8873.66  |         | 429.96 | 232.49 |        |        |
| 2003     | 1   | 3.29                          | 219.32  | 120.02  | 16.02   | 209.69  | 10.71       | 3.25      | 39.42     | 62.87   | 3618.75  | 279.69 | 60.76   | 2343.52  | 580.03   | 24.23    | 461.50  | 51.69   | 147.74  | 89.55   | 78.94   | 15.62  | 3.25   | 3.25   | 3.25    | 51.33   | 47.64    | 17.24   | 1520.06  |         |        | 37.14  | 43.55  |        |
| 2004     | 1   | 4.66                          | 206.08  | 176.44  | 18.45   | 200.12  | 14.18       | 18.68     | 174.61    | 44.74   | 1794.76  | 15.12  | 53.81   | 1690.74  | 265.03   | 49.62    | 2426.63 | 737.32  | 1047.00 | 801.27  | 433.83  | 172.33 | 207.87 | 282.28 | 37.57   | 860.05  | 69.71    | 267.34  | 884.08   | 51.67   | 84.10  | 124.46 | 152.35 |        |
| 2005     | 0   | 5.12                          | 2163.02 | 780.01  | 635.91  | 956.12  | 85.53       | 100.05    | 393.84    | 274.60  | 10097.56 | 962.53 | 1743.30 | 9478.40  | 2445.71  | 109.08   | 494.83  | 1367.28 | 2858.29 | 1799.67 | 824.07  | 416.79 | 240.46 | 454.40 | 79.45   | 4329.60 | 162.47   | 82.43   | 9594.96  |         |        | 350.30 | 398.58 |        |
| 2006     | 1   | 6.19                          | 646.38  | 319.51  | 1059.25 | 480.83  | 19.88       | 64.75     | 139.55    | 77.50   | 2206.04  | 284.04 | 592.20  | 1659.23  | 419.00   | 29.72    | 1184.41 | 337.24  | 479.09  | 310.96  | 257.04  | 38.99  | 151.56 | 69.26  | 3.25    | 1008.85 | 49.70    | 51.79   | 1334.38  |         |        | 48.41  | 35.02  |        |
| 2007     | 1   | 6.57                          | 1700.69 | 809.91  | 1285.80 | 1344.66 | 72.57       | 201.75    | 385.40    | 225.48  | 5667.96  | 535.51 | 1103.96 | 2881.32  | 635.63   | 48.24    | 2104.54 | 534.46  | 797.71  | 817.61  | 654.34  | 100.44 | 453.78 | 244.02 | 30.57   | 1555.05 | 114.81   | 62.57   | 1929.05  | 54.60   | 149.75 | 92.52  | 110.27 |        |
| 2008     | 0   | 6.65                          | 375.31  | 238.39  | 54.03   | 289.09  | 16.21       | 28.10     | 134.49    | 38.83   | 1417.45  | 102.93 | 88.31   | 555.41   | 125.13   | 57.24    | 2166.97 | 756.82  | 953.79  | 743.28  | 621.56  | 166.13 | 435.01 | 429.12 | 32.88   | 664.99  | 58.85    | 181.76  | 291.95   |         |        | 86.35  | 116.35 |        |
| 2008     | 1   | 6.97                          | 196.72  | 154.00  | 31.24   | 166.79  | 9.52        | 10.66     | 20.73     | 40.38   | 893.13   | 22.58  | 30.48   | 430.51   | 72.13    | 78.39    | 2979.87 | 1172.46 | 1202.72 | 1019.60 | 71.19   | 177.77 | 486.34 | 329.39 | 49.06   | 342.80  | 31.75    | 140.43  | 268.59   | 50.51   | 49.27  | 110.12 | 102.30 |        |
| 2009     | 1   | 7.14                          | 1022.50 | 319.84  | 868.27  | 1142.51 | 25.16       | 176.49    | 373.30    | 35.33   | 941.66   | 212.08 | 516.06  | 194.39   | 104.41   | 64.45    | 2899.83 | 676.35  | 2177.22 | 1083.75 | 613.00  | 245.75 | 589.01 | 328.39 | 32.66   | 970.22  | 66.79    | 33.32   | 242.42   |         |        | 88.80  | 57.46  |        |
| 2010     | 1   | 7.15                          | 400.16  | 243.96  | 55.70   | 3.25    | 23.97       | 37.10     | 39.76     | 32.75   | 1123.18  | 165.99 | 85.57   | 493.69   | 87.25    | 65.96    | 2394.31 | 710.13  | 1207.67 | 894.13  | 514.53  | 178.22 | 342.95 | 367.13 | 27.65   | 765.50  | 38.30    | 54.62   | 548.80   | 24.25   | 22.42  | 75.93  | 72.46  |        |
| 2011     | 1   | 7.16                          | 715.13  | 785.73  | 90.71   | 535.62  | 46.66       | 55.03     | 245.16    | 66.45   | 2080.57  | 144.33 | 163.69  | 1297.66  | 205.98   | 48.94    | 3181.60 | 654.10  | 875.64  | 829.87  | 497.09  | 98.40  | 210.04 | 194.39 | 28.96   | 1500.78 | 113.71   | 207.57  | 1143.76  | 49.55   | 67.23  | 104.60 | 82.04  |        |
| 2012     | 1   | 8.11                          | 807.84  | 685.25  | 218.66  | 2006.57 | 91.08       | 97.39     | 151.87    | 164.06  | 4851.86  | 609.02 | 645.67  | 2071.03  | 269.51   | 98.58    | 3966.85 | 929.69  | 1007.96 | 901.91  | 731.92  | 152.02 | 361.66 | 699.84 | 37.89   | 2760.88 | 161.30   | 400.41  | 2592.24  |         |        | 84.68  | 149.58 |        |
| 2013     | 0   | 8.22                          | 1604.39 | 623.31  | 3877.74 | 6564.38 | 68.19       | 301.33    | 1398.57   | 157.66  | 5034.19  | 484.50 | 5040.50 | 1777.44  | 2393.10  | 646.96   | 17.43   | 1179.32 | 461.10  | 998.19  | 539.04  | 325.01 | 469.49 | 258.42 | 183.79  | 2847    | 4381.17  | 177.99  | 233.45   | 1155.65 | 3.25   | 3.25   | 85.59  | 113.05 |
| 2014     | 1   | 8.50                          | 2027.76 | 1973.68 | 109.62  | 381.35  | 92.11       | 61.12     | 389.44    | 199.18  | 6814.00  | 229.35 | 277.16  | 6556.91  | 395.84   | 97.50    | 2384.75 | 739.22  | 555.84  | 860.91  | 528.16  | 146.58 | 347.92 | 482.42 | 38.67   | 3688.32 | 211.65   | 465.37  | 2561.30  |         |        | 81.36  | 161.11 |        |
| 2015     | 1   | 8.85                          | 1557.36 | 968.09  | 348.87  | 626.47  | 51.61       | 118.30    | 346.53    | 136.79  | 3465.06  | 128.88 | 221.89  | 756.38   | 157.87   | 32.72    | 2558.89 |         |         |         |         |        |        |        |         |         |          |         |          |         |        |        |        |        |

# Urinary Steroids: Sums'n Ratios I

## Sums

**C18-steroids** (estrogens):

$E1 + E2 + E3$

**C19-steroids** (androgens)

DHEA and its 16-hydroxylated downstream metabolites reflecting major adrenarcheal secretion products:

$DHEA + 16\alpha\text{-OH-DHEA} + A5T\text{-}16\alpha$

Overall androgen metabolite secretion ( $\Sigma C19$ ):  $An + Et + A5\text{-}3\beta,17\alpha + A5\text{-}3\beta,17\beta + DHEA + 16\alpha\text{-OH-DHEA} + A5T\text{-}16\alpha$

**C21-steroids** (cortisol (F) metabolites)

Major cortisol metabolites:  $5\alpha\text{-THF} + THF + THE$

Overall cortisol metabolite secretion ( $\Sigma Fs$ ):  $5\alpha\text{-THF} + THF + THE + a\text{-C} + b\text{-C} + a\text{-Cl} + b\text{-Cl}$

## Ratios

**Relative overall androgen production:**

$(An + Et) / (5\alpha\text{-THF} + THF + THE)$

$(An + Et + A5\text{-}3\beta,17\alpha + A5\text{-}3\beta,17\beta + DHEA + 16\alpha\text{-OH-DHEA} + A5T\text{-}16\alpha) / (5\alpha\text{-THF} + THF + THE)$

**Relative adrenal androgen production:**

$DHEA / (5\alpha\text{-THF} + THF + THE)$

$(DHEA + 16\alpha\text{-OH-DHEA} + A5T\text{-}16\alpha) / (5\alpha\text{-THF} + THF + THE)$

# Urinary Steroids: Sums'n Ratios II

## 3 $\beta$ -hydroxysteroiddehydrogenase (3 $\beta$ HSD) activity

Indicator of 3 $\beta$ HSD deficiency: P5T-17 $\alpha$

Relative overproduction of 3 $\beta$ -hydroxy-5-ene steroids or relative deficiency of 3 $\beta$ HSD:

DHEA / (An + Et)

(DHEA + 16 $\alpha$ -OH-DHEA + A5T-16 $\alpha$ ) / (5 $\alpha$  -THF + THF + THE)

(DHEA + 16 $\alpha$ -OH-DHEA + A5T-16 $\alpha$ ) / (An + Et)

P5T-17 $\alpha$  / (5 $\alpha$  -THF + THF + THE)

P5T-17 $\alpha$  / PT

## 21-hydroxylase-activity

17-hydroxyprogesterone metabolites are indicators of 21-hydroxylase deficiency: 11-O-PT, PT, Po-5 $\beta$ 3 $\alpha$ , Po-5 $\alpha$ 3 $\alpha$

ratios of relative 21-hydroxylase deficiency:

(PT + Po-5 $\beta$ 3 $\alpha$  + Po-5 $\alpha$ 3 $\alpha$ ) / (5 $\alpha$  -THF + THF + THE)

(11-O-PT + PT + Po-5 $\beta$ 3 $\alpha$  + Po-5 $\alpha$ 3 $\alpha$ ) / (5 $\alpha$  -THF + THF + THE)

11-O-PT / (5 $\alpha$  -THF + THF + THE)

11-O-PT / a-CL

# Urinary Steroids: Sums'n Ratios III

## 11 $\beta$ -hydroxylase activity

Indicator of 11 $\beta$ -hydroxylase activity: THS

Ratios of relative 11 $\beta$ -hydroxylase activity:

$\text{THS} / (5\alpha\text{-THF} + \text{THF} + \text{THE})$

$(\text{An} + \text{Et}) / (11\beta\text{-OH-An} + 11\beta\text{-OH-Et})$

## 17-hydroxylase/17,20-lyase activity

Global activity:

$(\text{THA} + \text{THB} + 5\alpha\text{-THB}) / (\text{An} + \text{Et})$

17 $\alpha$ -hydroxylase activity:

$\Delta 5\text{-pathway: P5D} / \text{P5T-17}\alpha$

$\Delta 4\text{-pathway: PD} / \text{PT}$

global:  $(\text{THA} + \text{THB} + 5\alpha\text{-THB}) / (5\alpha\text{-THF} + \text{THF} + \text{THE})$

17,20-lyase activity:

$\Delta 5\text{-pathway: P5T-17}\alpha / \text{A5-3}\beta 17\beta$

$\Delta 4\text{-pathway: PT} / (\text{An} + \text{Et}); (\text{PT} + \text{Po-5}\beta 3\alpha + \text{Po-5}\alpha 3\alpha) / (\text{An} + \text{Et});$

global:  $(5\alpha\text{-THF} + \text{THF} + \text{THE}) / (\text{An} + \text{Et})$