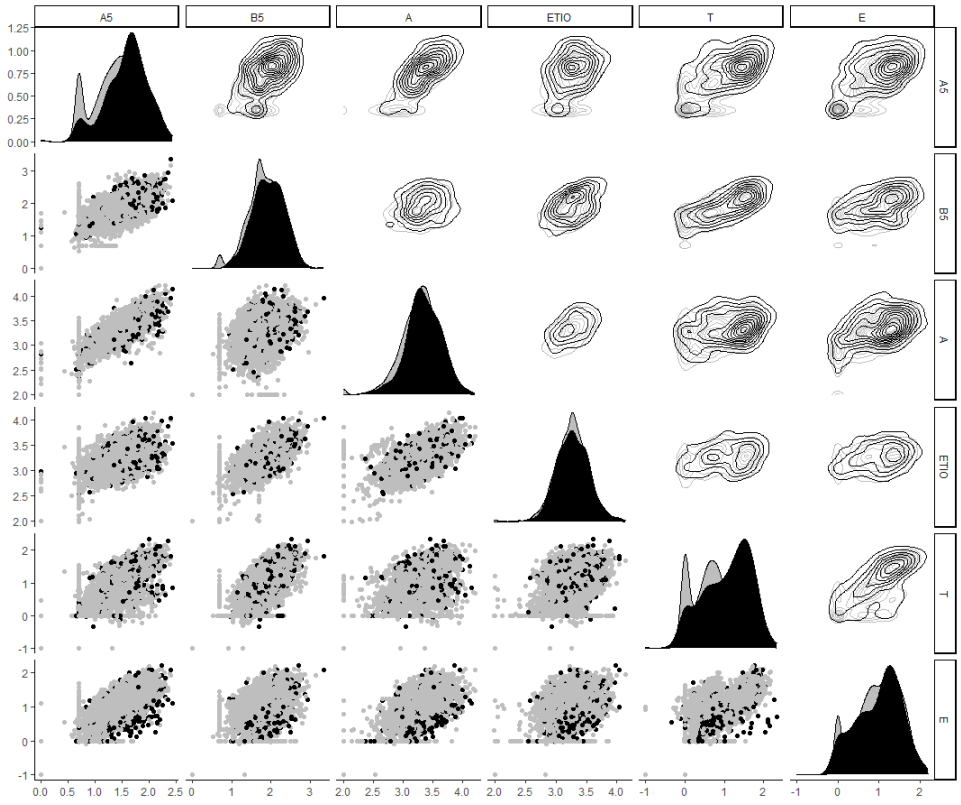
Supplementary Material for a multivariate Bayesian learning approach for improved detection of doping in athletes using urinary steroid profiles

Dimitra Eleftheriou*[1], Thomas Piper[2], Mario Thevis[2,3], and Tereza Neocleous[4]

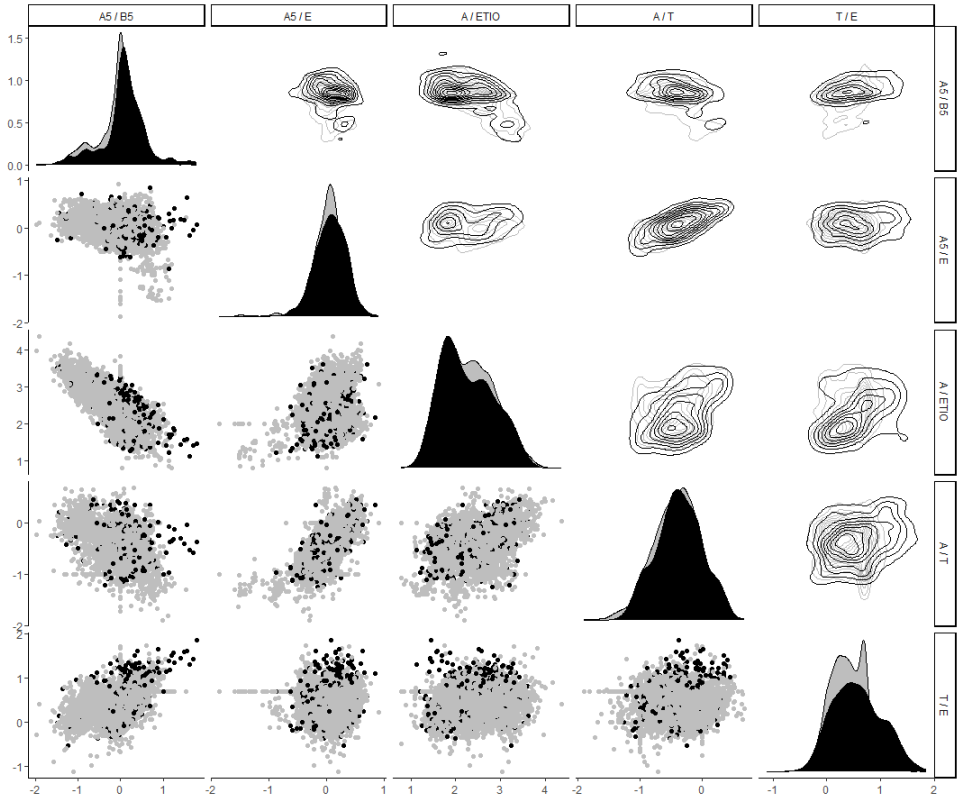
[1]Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands
[2]Center for Preventive Doping Research - Institute of Biochemistry, German Sport University Cologne, Cologne, Germany
[3]European Monitoring Center for Emerging Doping Agents, Cologne/Bonn, Germany
[4]School of Mathematics and Statistics, University of Glasgow, Glasgow, United Kingdom

***Corresponding author: Dimitra Eleftheriou,** Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands, E-mail: d.eleftheriou@lacdr.leidenuniv.nl. https://orcid.org/0000-0003-4377-6426

A Figures

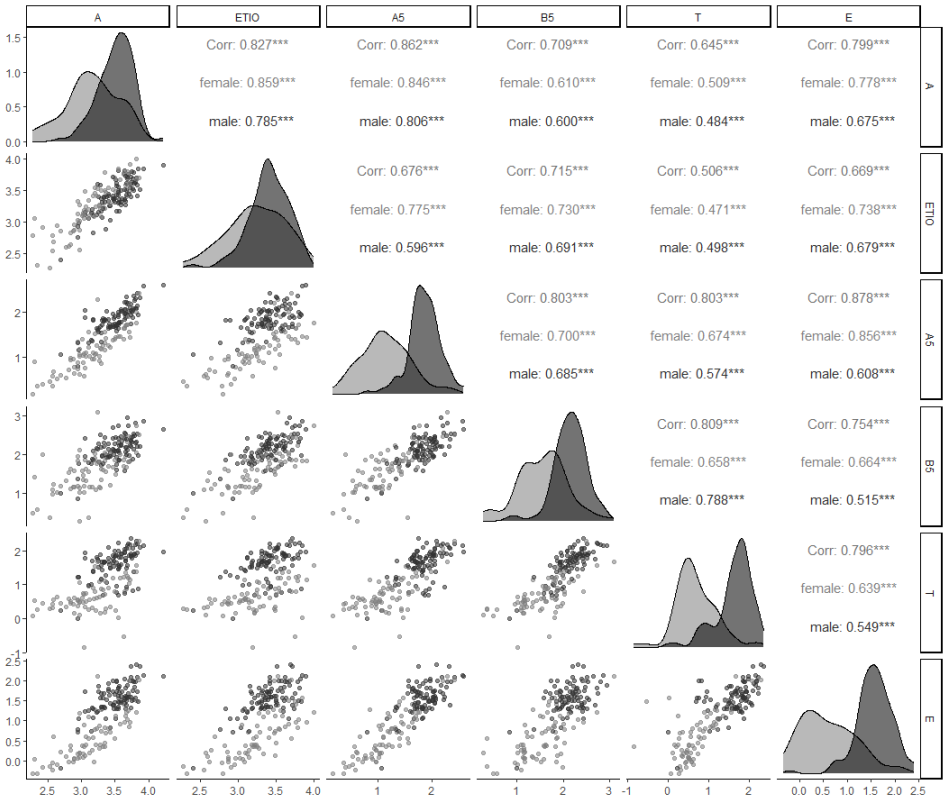


(a)

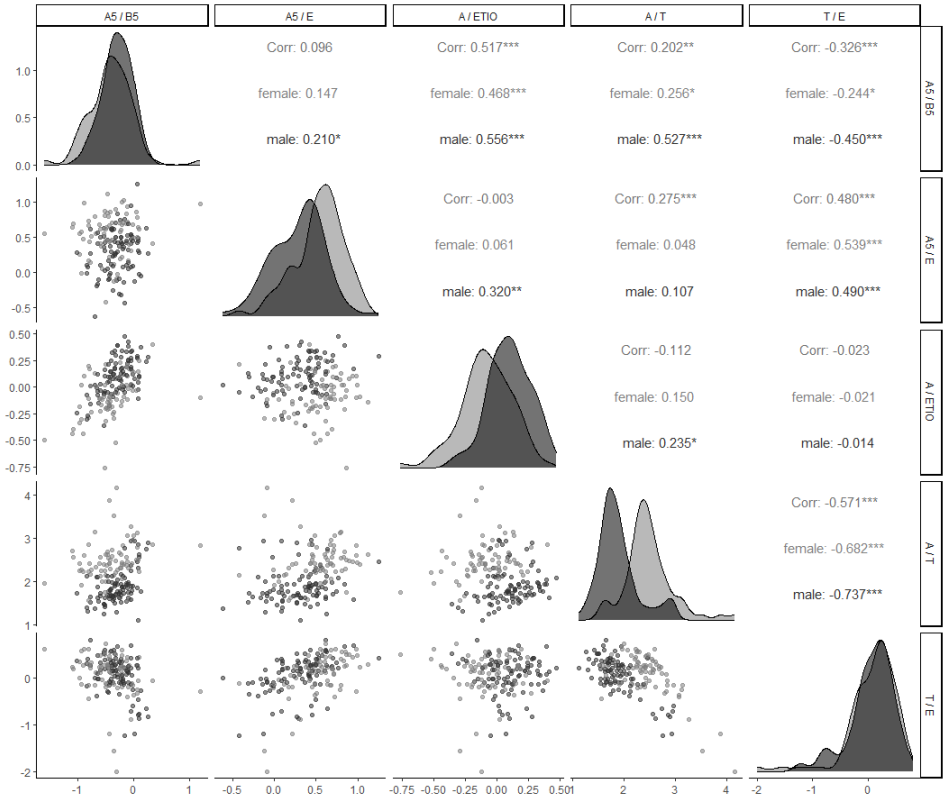


(b)

Figure A.1: Plots of normal (grey) vs non-normal (black) samples including the scatter, density and contour plots for: (a) the six markers, and (b) their five ratios in the logarithmic scale using the full longitudinal dataset (4399 urine samples from 229 athletes).

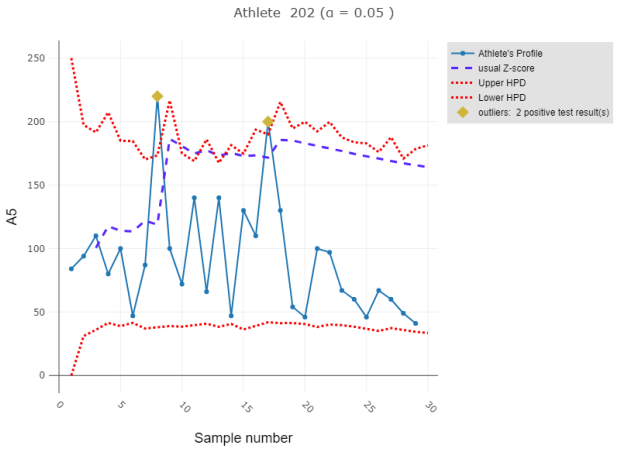


(a)

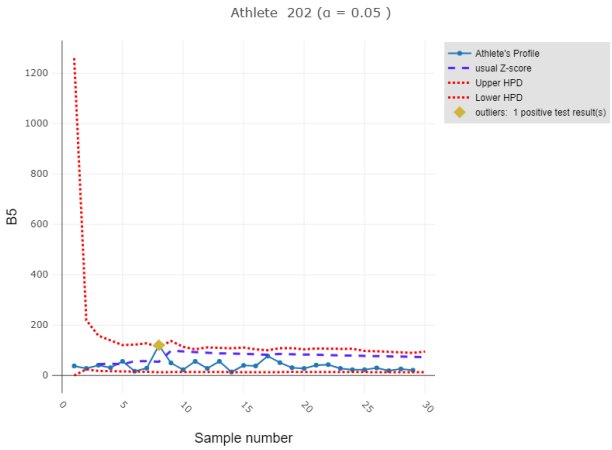


(b)

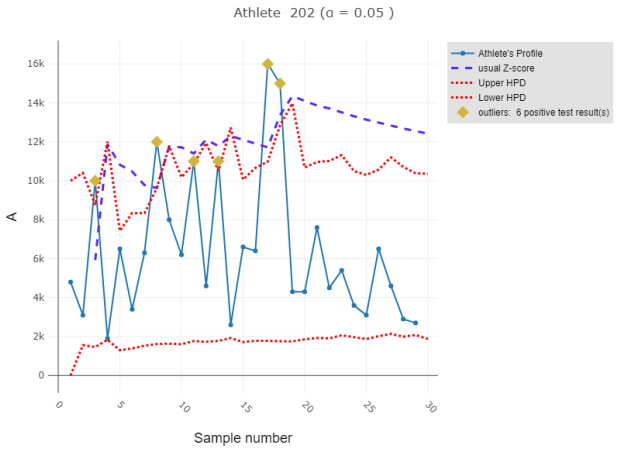
Figure A.2: Scatter and density plots by sex accompanied by the correlation coefficients for: (a) the six markers, and (b) their five ratios in the logarithmic scale using the full longitudinal dataset (4399 urine samples from 229 athletes).


Athlete 202 (α = 0.05)
A5
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 2 positive test result(s)

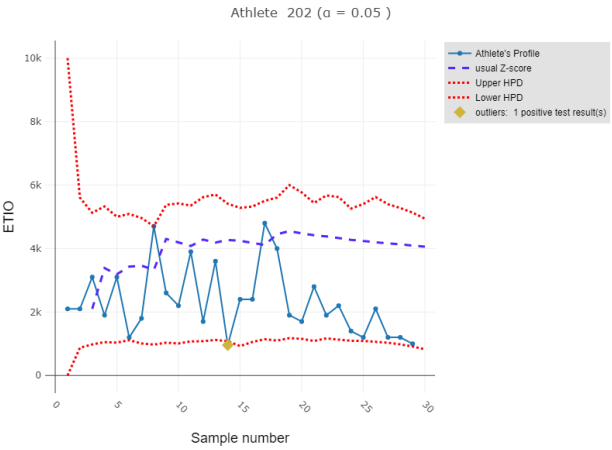
(a)


Athlete 202 (α = 0.05)
B5
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 1 positive test result(s)

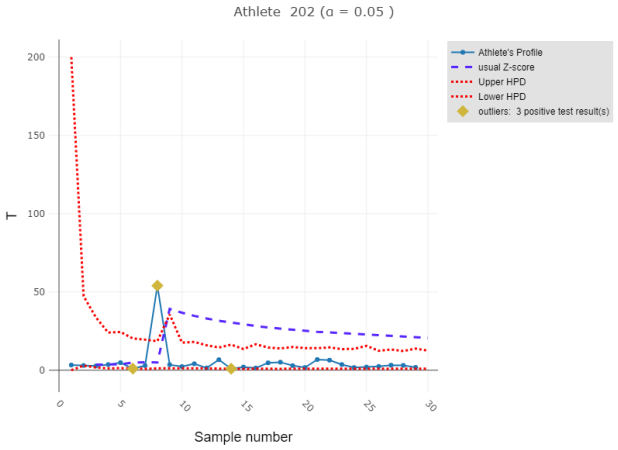
(b)


Athlete 202 (α = 0.05)
A
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 6 positive test result(s)

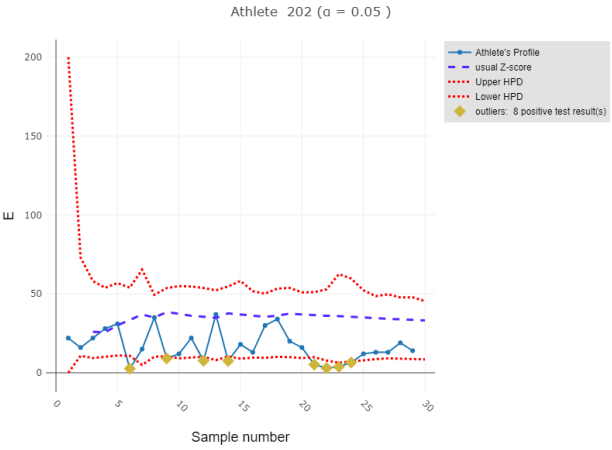
(c)


Athlete 202 (α = 0.05)
ETIO
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 1 positive test result(s)

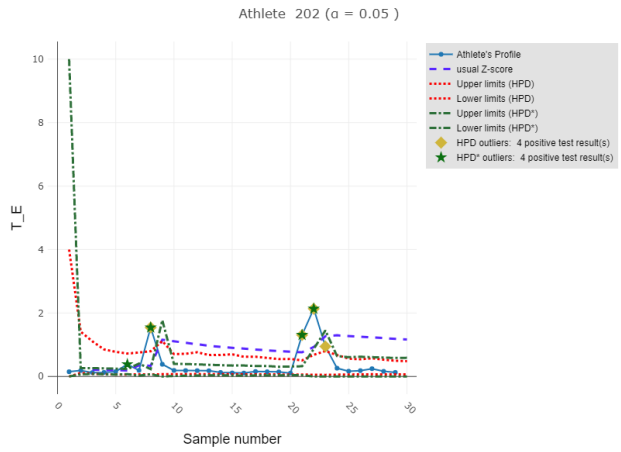
(d)


Athlete 202 (α = 0.05)
T
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 3 positive test result(s)

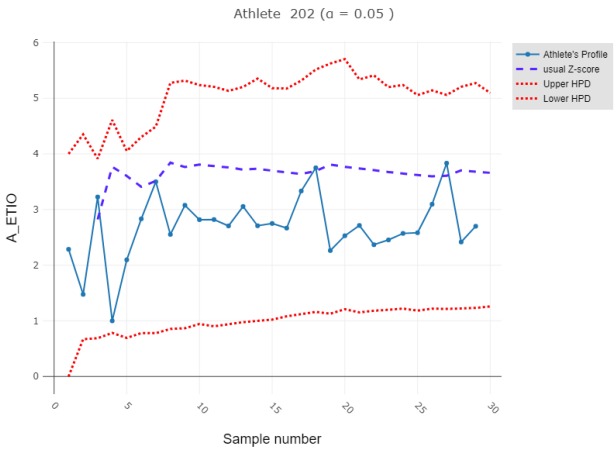
(e)


Athlete 202 (α = 0.05)
E
Sample number
Athlete's Profile
usual Z-score
Upper HPD
Lower HPD
outliers: 8 positive test result(s)

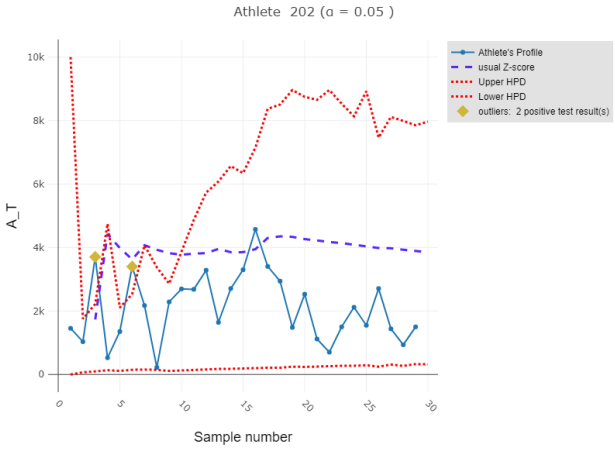
(f)



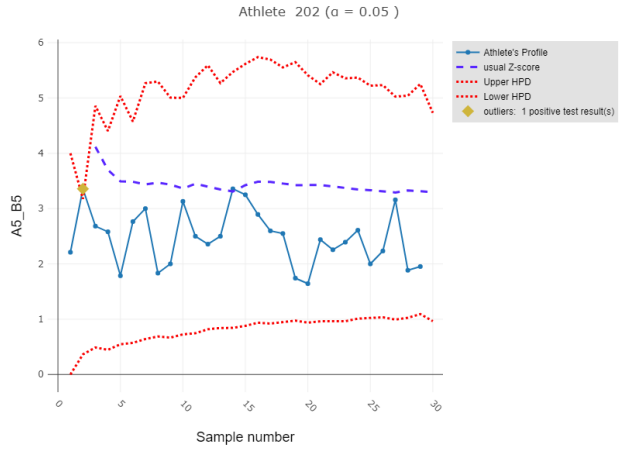
(g)



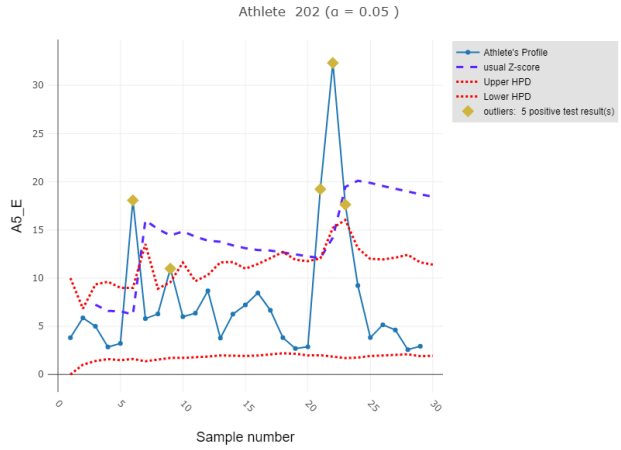
(h)



(i)



(j)



(k)

Figure A.3: (a-k) A series of 29 longitudinal values of the six EAAS and their five ratios (blue solid-dotted line) obtained from a doped athlete; upper and lower limits (red dotted lines) are calculated using the 95% HPD intervals of the predictive distribution from the univariate Bayesian model; upper limits assuming a usual Z-score (purple dashed line); suggested abnormal values are denoted by the gold diamonds. (g) Upper and lower limits (green dashed-dotted lines) are calculated using the 95% HPD interval of the predictive distribution from the T/E model of Sottas et al. [1]; suggested abnormal values based on the T/E model are denoted by the green stars.

B Tables

Trivial name	Abbreviation	Systematic name	Formula
5α-Adiol	A5	5α-Androstane-3α,17β-diol	$C_{19}H_{32}O_2$
5β-Adiol	B5	5β-Androstane-3α,17β-diol	$C_{19}H_{32}O_2$
Androsterone	A	3α-Hydroxy-5α-androstan-17-one	$C_{19}H_{30}O_2$
Epitestosterone	E	17α-Hydroxy-androst-4-en-3-one	$C_{19}H_{28}O_2$
Etiocholanolone	ETIO	3α-Hydroxy-5β-androstan-17-one	$C_{19}H_{30}O_2$
Testosterone	T	17β-Hydroxy-androst-4-en-3-one	$C_{19}H_{28}O_2$
5α-Adiol/5β-Adiol	A5/B5		
5α-Adiol/Epitestosterone	A5/E		
Androsterone/Etiocholanolone	A/ETIO		
Androsterone/Testosterone	A/T		
Testosterone/Epitestosterone	T/E		

Table B.1: Urinary steroid metabolites quantitated in this study.

Target Metabolite	Min (ng/mL)	Q1 (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Q3 (ng/mL)	Max (ng/mL)	SD (ng/mL)
A5	1.46	14.49	61.7	46.62	84.56	388.14	64.68
B5	1.88	40.46	139.92	89.14	180.92	1,232.95	164.27
A	187.7	1,315.9	2,997.3	2,517.2	4,428.1	16,674.1	2,169.41
E	0.47	4.31	33.71	20.52	42.22	252.96	42.94
ETIO	187.5	1,379.8	2,719.4	2,337.9	3,614	9,819.9	1,803.23
T	0.14	4.17	39.37	20.39	61.43	229.03	46.27
A5/B5	0.03	0.3	0.64	0.46	0.74	15.57	1.22
A5/E	0.24	1.47	3.29	2.73	4.23	17.72	2.54
A/ETIO	0.17	0.78	1.17	1.05	1.47	3	0.53
A/T	13.13	53.97	374.49	125.08	282.92	14,154.24	1,269.35
T/E	0.01	0.76	1.63	1.44	2.19	6.48	1.16

Table B.2: Descriptive summaries (minimum, 1st quartile; Q1, mean, median, 3rd quartile; Q3, maximum and standard deviation (SD)) of the metabolites and ratios of the baseline cross-sectional population of non-doped and healthy individuals (91 men and 73 women).

Target Metabolite	Min (ng/mL)	Q1 (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Q3 (ng/mL)	Max (ng/mL)
A5	1	11	35.08	25	49	210
B5	1	34	96.25	64	120	910
A	100	1,200	2,097	1,800	2,600	16,000
E	0.10	4	17.48	11	26	130
ETIO	98	1,100	1,863	1,700	2,400	7,200
T	0.10	3.30	20.17	9.60	31	150
A5/B5	0.013	0.24	0.49	0.40	0.61	4.8
A5/E	0.13	1.41	4.77	2.54	4.65	160
A/ETIO	0.06	0.80	1.21	1.11	1.48	8.16
A/T	6.25	72.41	365.25	159.46	412.7	23,000
T/E	0.012	0.75	1.35	1.0	1.6	13

Table B.3: Descriptive summaries (minimum, 1st quartile; Q1, mean, median, 3rd quartile; Q3, and maximum) of the metabolites and ratios of 100 athletes with normal samples (subset of the longitudinal dataset).

Target Metabolite	Min (ng/mL)	Q1 (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Q3 (ng/mL)	Max (ng/mL)
A5	1.0	14	36.61	28.0	50	250
B5	3.3	43	105.1	73	140	1,400
A	100	1,200	2,318	2,000	3,000	13,000
E	0.10	4.50	18.53	12.0	27	160
ETIO	270	1,300	2,199	1,900	2,700	14,000
T	0.10	3.3	19.44	9.25	33	150
A5/B5	0.02	0.19	0.48	0.35	0.65	4.6
A5/E	0.08	1.42	3.38	2.37	4.6	53.33
A/ETIO	0.014	0.7	1.16	1.04	1.46	6.91
A/T	6.252	67.66	454.63	183.33	525.65	9,200
T/E	0.01	0.62	1.46	1	1.64	35

Table B.4: Descriptive summaries (minimum, 1st quartile; Q1, mean, median, 3rd quartile; Q3, and maximum) of the metabolites and ratios of 100 athletes with atypical samples (subset of the longitudinal dataset).

Target Metabolite	Min (ng/mL)	Q1 (ng/mL)	Mean (ng/mL)	Median (ng/mL)	Q3 (ng/mL)	Max (ng/mL)
A5	3.7	21	50.53	39.5	65	270
B5	4.9	24	82.69	50	97	2,200
A	210	1,600	3,152	2,400	3,900	16,000
E	1	5.90	14.41	11	19	120
ETIO	100	932.5	1,747	1,500	2,175	11,000
T	0.50	1	12.51	3.50	15	220
A5/B5	0.085	0.47	0.76	1	1.42	4.2
A5/E	0.46	1.83	5.59	3.55	5.91	72.22
A/ETIO	0.50	1.17	1.92	1.69	2.53	5.08
A/T	13.18	177.55	1,262.99	632.46	1,800	14,828
T/E	0.03	0.15	1.58	0.34	1.3	61.11

Table B.5: Descriptive summaries (minimum, 1st quartile; Q1, mean, median, 3rd quartile; Q3, and maximum) of the metabolites and ratios of 29 athletes with abnormal samples (subset of the longitudinal dataset).

Target Metabolite	Max (M) (ng/mL)	Max (F) (ng/mL)	WADA TD 2021 (M) (ng/mL)	WADA TD 2021 (F) (ng/mL)
A5	652	263	250	150
B5	1,260	471		
A	20,700	17,500	10,000	10,000
E	391	51.9	200	50
ETIO	11,400	9,030	10,000	10,000
T	249	219	200	50
A/ETIO			4	4
T/E			4	4
Target Metabolite	**IUL (M) (ng/mL)**	**IUL (F) (ng/mL)**		
A5/B5	4	4		
A5/E	10	10		
A/T	10000	10000		

Table B.6: Maximum EAAS values and their ratios measured in a Caucasian population consisting of 2,027 male (M) and 1,004 female (F) athletes [2] along with the available WADA's threshold limits by sex [3, 4]. The initial upper limits (IUL) for ratios, in cases where there is no available population-specific information, are determined from the upper limit of the inter-quartile range (Q3) observed in the corresponding ratios within the cross-sectional dataset.

References

[1] Sottas, P.-E., Baume, N., Saudan, C., Schweizer, C., Kamber, M., and Saugy, M. (2007) Bayesian detection of abnormal values in longitudinal biomarkers with an application to T/E ratio. *Biostatistics 8*, 285–296.

[2] Van Renterghem, P., Van Eenoo, P., Geyer, H., Schänzer, W., and Delbeke, F. T. (2010) Reference ranges for urinary concentrations and ratios of endogenous steroids, which can be used as markers for steroid misuse, in a Caucasian population of athletes. *Steroids 75*, 154–163.

[3] World Anti-Doping Agency. Technical Document - TD2021EAAS [Online]. Available from: https://www.wada-ama.org/sites/default/files/2022-01/td2021eaas_final_eng_v_2.0.pdf. [Accessed 19 Feb 2024].

[4] World Anti-Doping Agency. WADA Technical Document – TD2021APMU [Online]. Available from: https://www.wada-ama.org/sites/default/files/resources/files/td2021apmu_final_eng_0.pdf. [Accessed 19 Feb 2024].