UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

ABP Steroidal Module in blood

Prof. Martial Saugy, REDs - University of Lausanne
Federico Ponzetto, Swiss Laboratory for Doping Analysis - University of Lausanne

05.11.18
ABP Symposium - Rome

A step forward…

URINE DRAWBACKS

Confounding factors

ENDOGENOUS	EXOGENOUS
UGT2B17 polymorphism	Bacterial contamination Ethanol consumption

GC-MS Sensitivity

T patch & gel administration

BLOOD MATRIX

- **Not easy to manipulate**
- **Reduced bacterial contamination**
- **Snapshot of athletes' physiological condition**
- **Accurate pharmacokinetics information**
- **Trend in clinical analyses** (evaluation/expertise)
- **1 Sample -> 2 ABP modules**

- **Few data in doping context**
- **Invasive sampling** (ethics)
- **Sample stability** (48h)
- **Transportation strategy**
- **Small sample volume** (4mL)

T. Kuuranne et al.
Confounding factors and genetic polymorphism in the evaluation of individual steroid profiling.
Br. J. Sports Med. (2014) 48(10):848-55

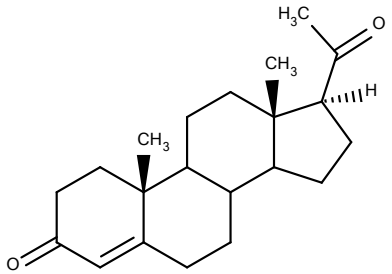
UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Blood Steroid Profiling – Target Compounds

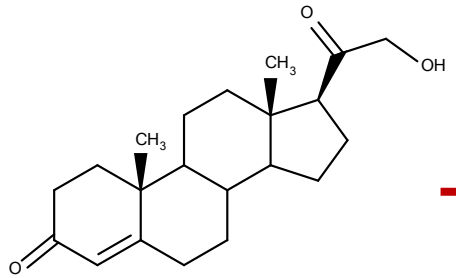
UHPLC-MS/MS

Acquity BEH C_{18} 100 x 2,1 mm , ID 1.7 µm

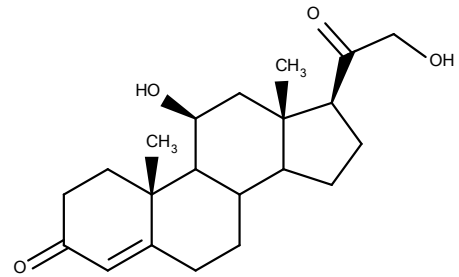
Flow rate	400 µL/min
Temperature	30°C
Inj. volume	10µL
Phase A	H_2O + 0.1% FA
Phase B	ACN + 0.1% FA
Gradient	0.5 min 25% B
	6 min 58% B
	8 min 98% B
Total Run Time	11 min



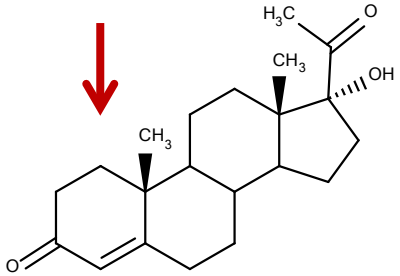
Progesterone



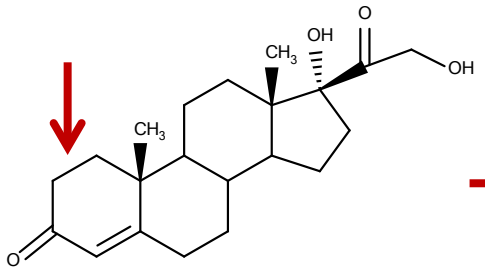
Deoxycorticosterone



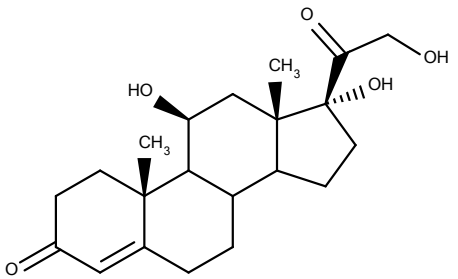
Corticosterone



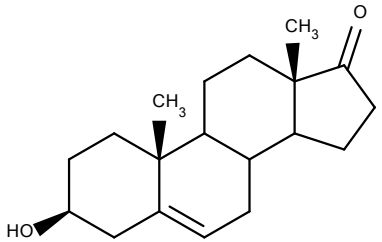
17α-Hydroxyprogesterone



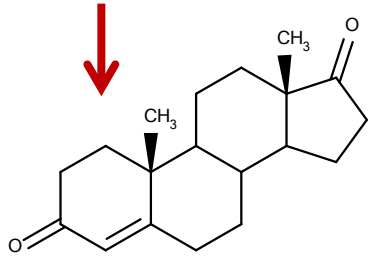
11-Deoxycortisol



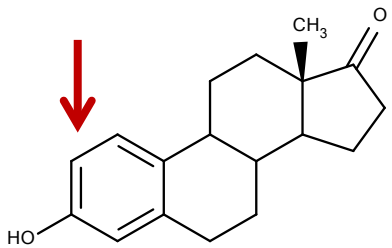
Cortisol



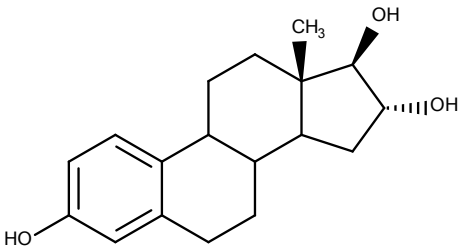
DHEA



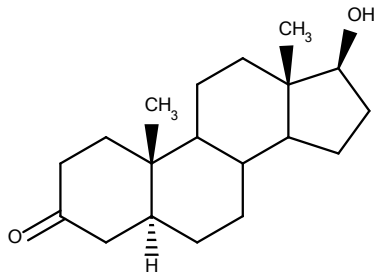
Androstenedione



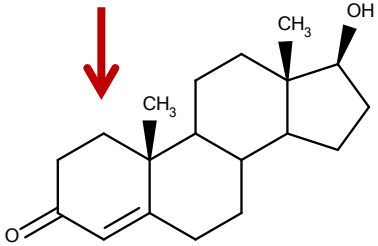
Estrone



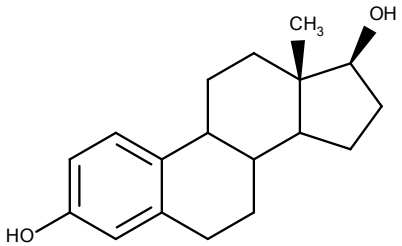
Estriol



DHT



Testosterone



Estradiol

UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

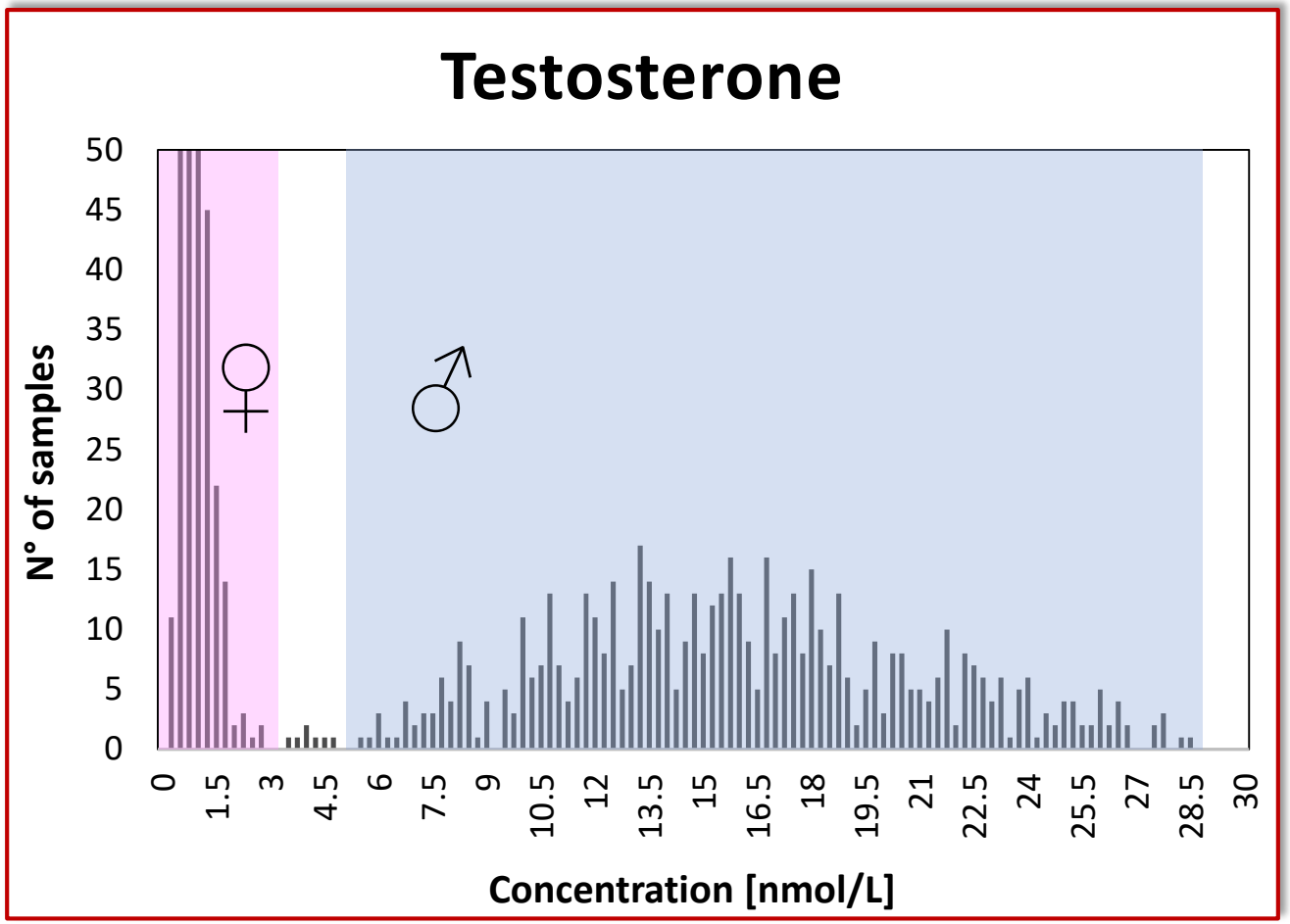
Reference Intervals

COMPOUND	LLOQ (nmo/L)	REFERENCE INTERVAL (nmol/L)
Testosterone	0.07	6.67 – 30.53 ♂ 0.26 – 1.80 ♀
Androstenedione	0.17	1.08 – 6.36
Progesterone	0.05	0.05 – 0.32 ♂ 0.06 – 39.59 ♀
17αOH-progesterone	0.30	0.34 – 7.71
DHEA	1.73	3.04 – 22.41
DHT	0.17	0.46 – 2.76 ♂ n.d. – 0.92 ♀
Corticosterone	0.29	0.85 – 38.47
Cortisol	2.76	64.82 – 696.69
11-deoxycortisol	0.07	n.d. – 2.20

IAAF World Championships MOSCOW 2013

1879 Elite Athletes

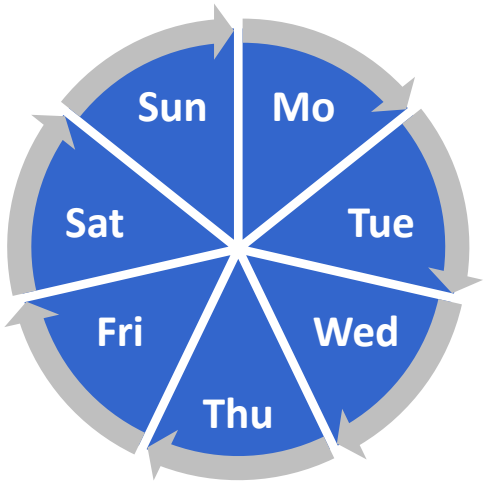
(1056 M, 824 F)



UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

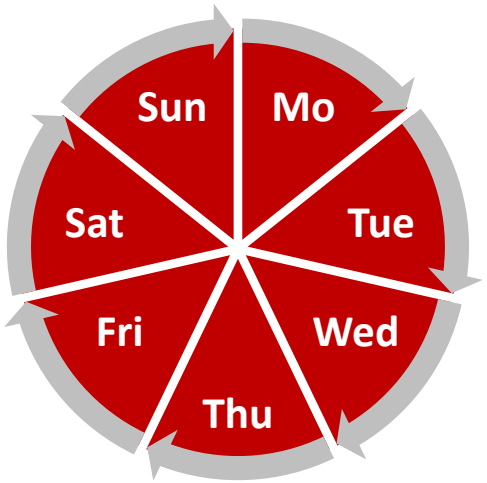
Testosterone Clinical Study

Population: 19 healthy men (UGT2B17: 7 ins/ins, 7 ins/del, 5 del/del)



CONTROL

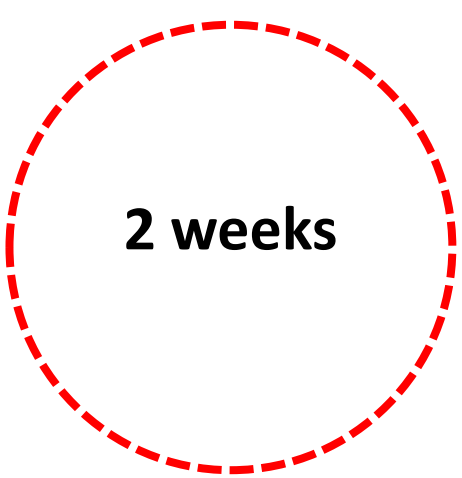
0h, 2h, 4h, 8h, 12h
24h



PATCH

0h, 2h, 4h, 8h, 12h
24h

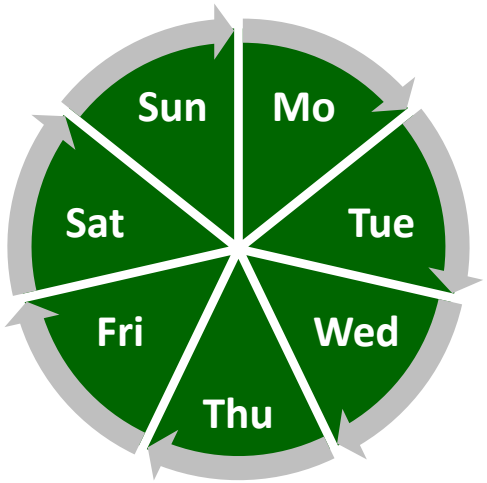
48h, 60h
72h
96h



WASH OUT

= 2 x 2.4mg/24h T

= 2 x 40mg TU



ORAL

0h, 2h, 4h, 8h, 12h
24h

48h, 60h
72h
96h

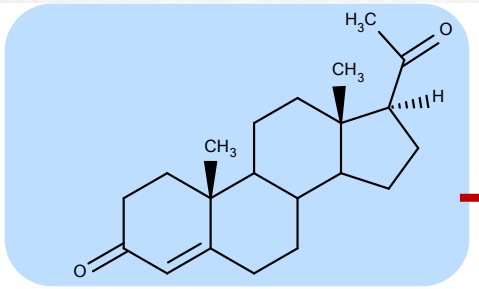
UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Multivariate Statistical Analysis

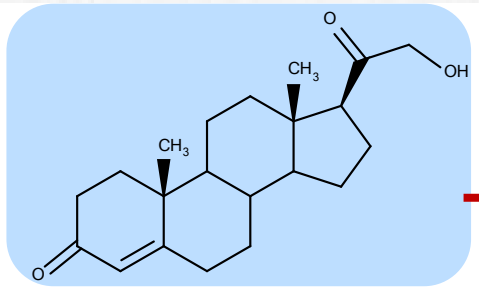
Parallel Factor Analysis (PARAFAC)

Circadian Rhythm

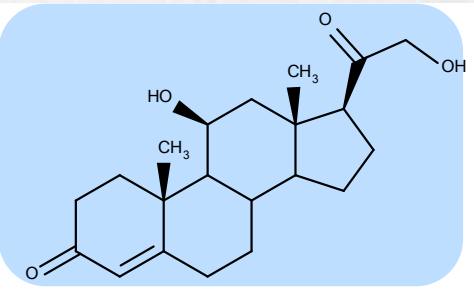
Testosterone Intake



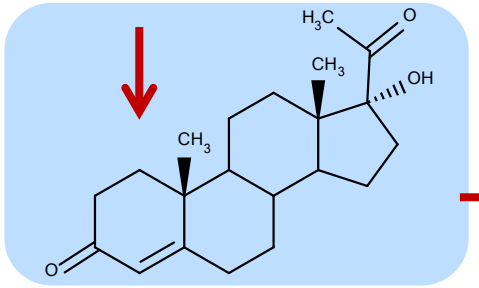
Progesterone



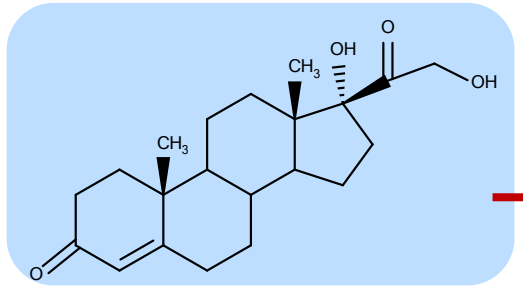
Deoxycorticosterone



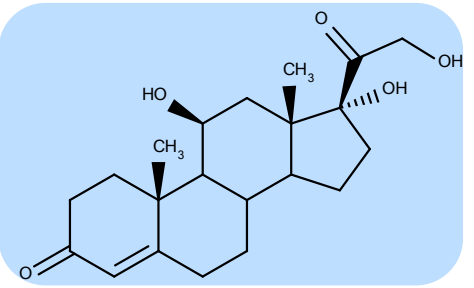
Corticosterone



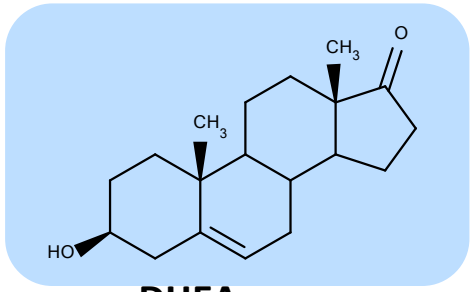
17α-Hydroxyprogesterone



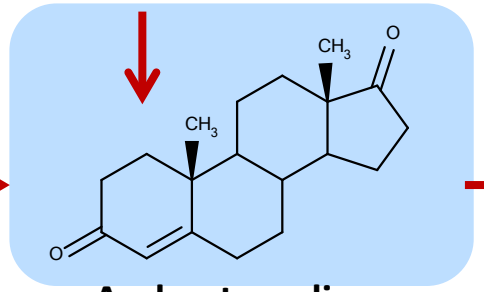
11-Deoxycortisol



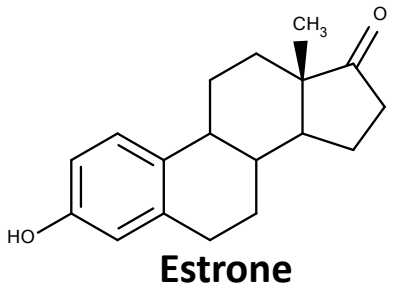
Cortisol



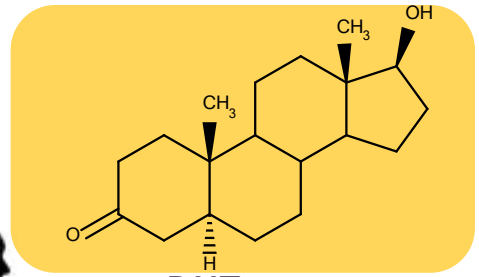
DHEA



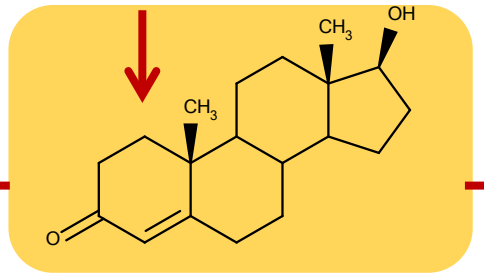
Androstenedione



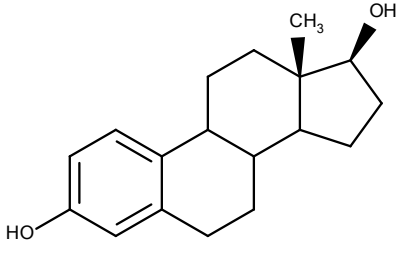
Estrone



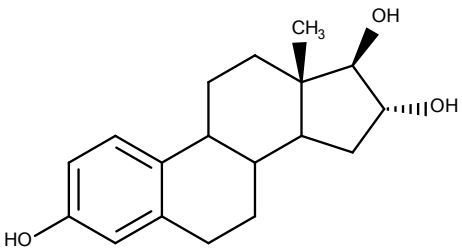
DHT



Testosterone



Estradiol



Estriol

UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

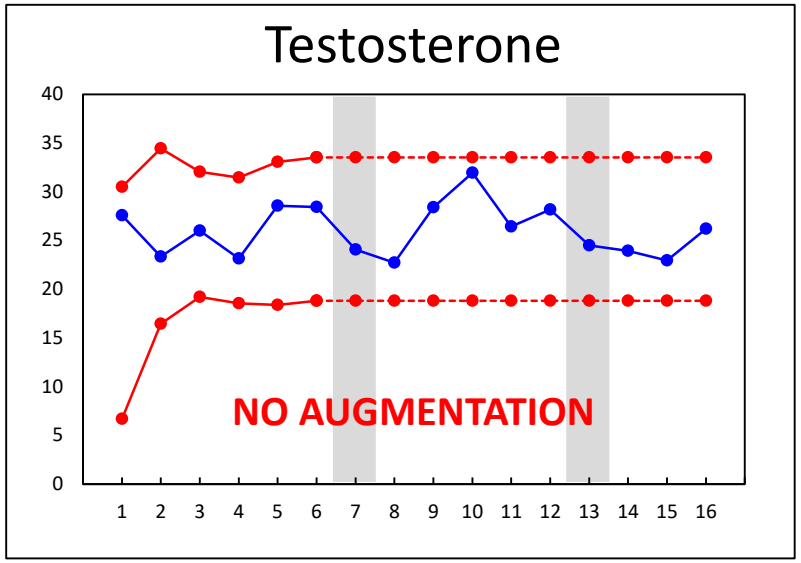
Longitudinal Monitoring

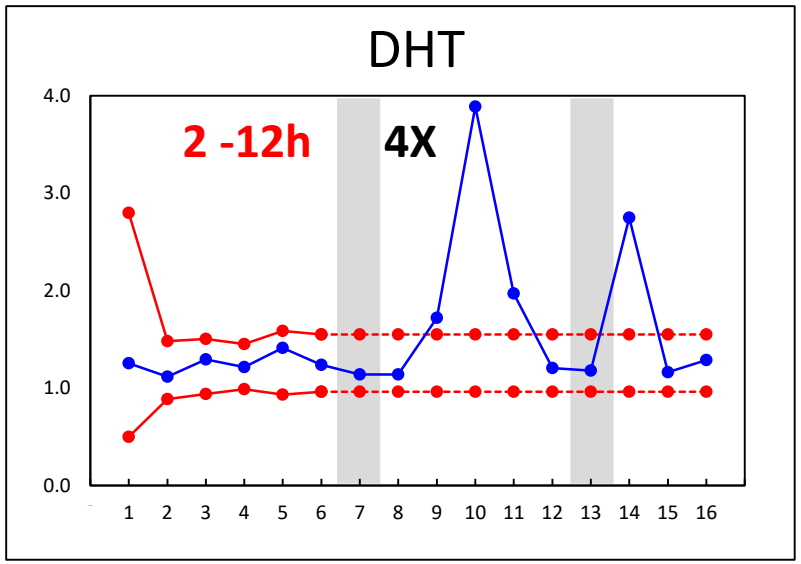
ins/ins
ins/del
del/del

F. Ponzetto et al.
Longitudinal monitoring of endogenous steroids in human serum by UHPLC-MS/MS as a tool to detect testosterone abuse in sports.
Anal. Bioanal. Chem.l. (2016), 408 (3):705-719

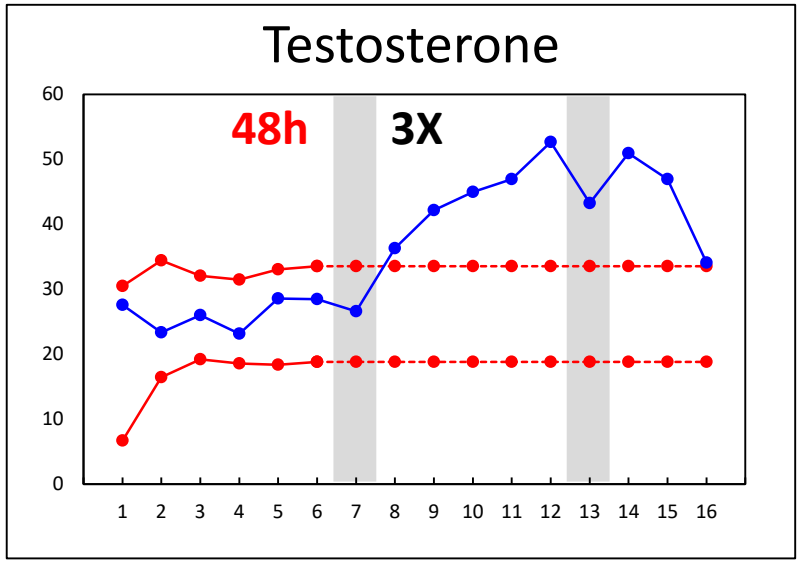
T intake

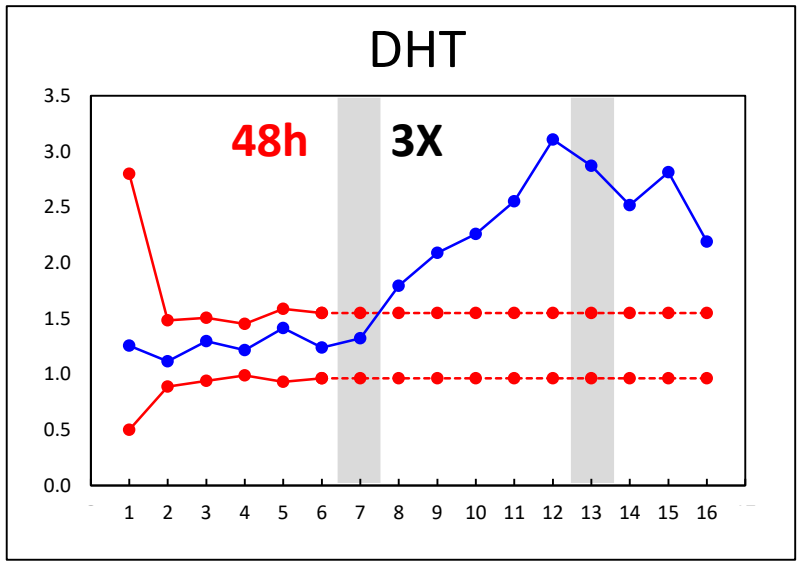
ORAL



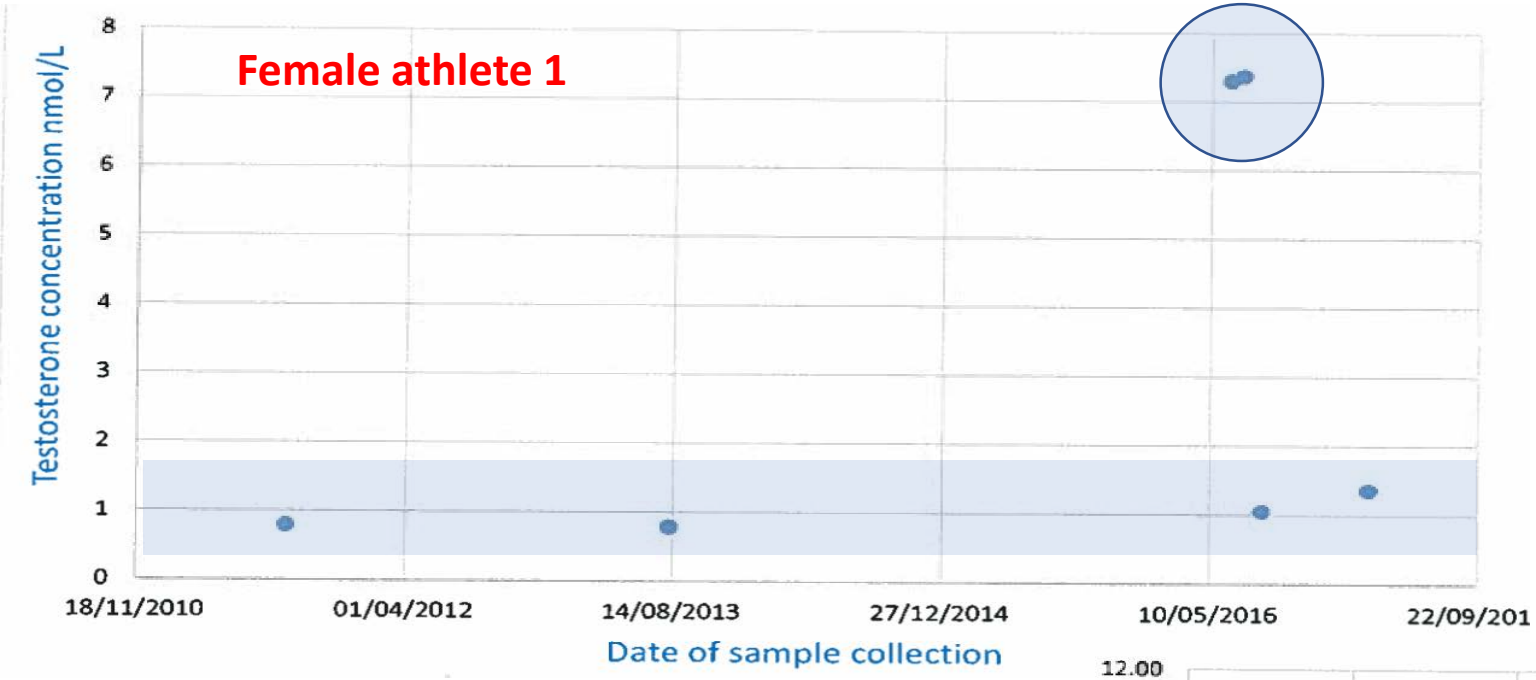


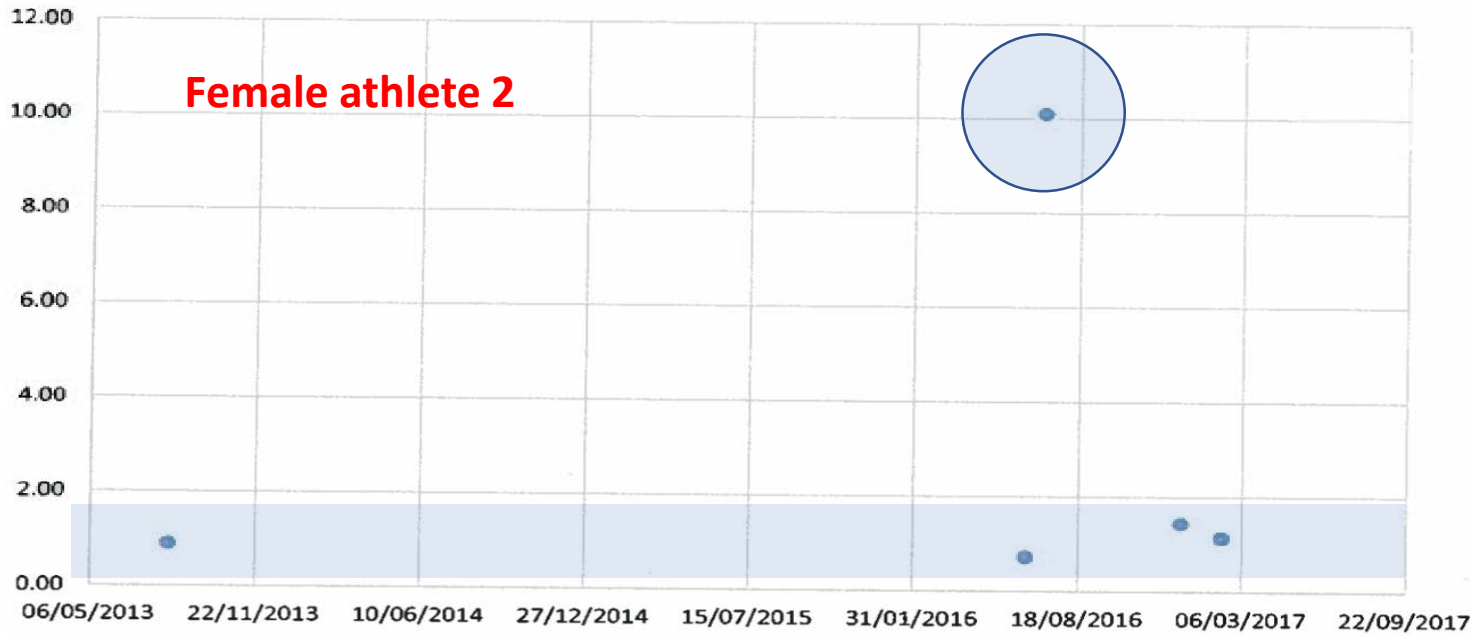
TRANSDERMAL





2 case reports





UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Untargeted Approach - Steroidomics

Sample Preparation

SPE

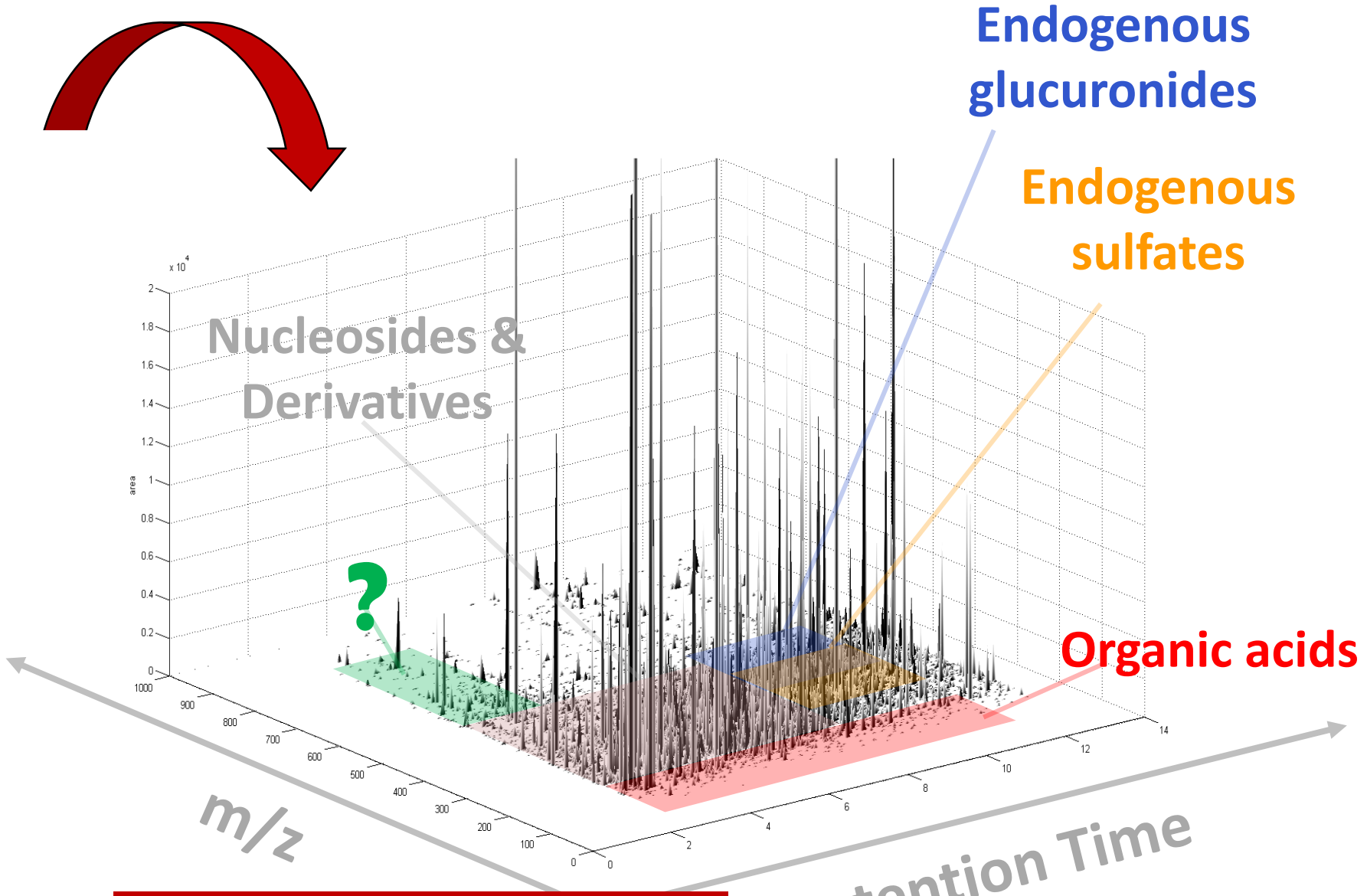
OASIS HLB (Waters)

Loading 200 µL serum + 200µL H_3PO_4 4%
Washing 400 µL H_2O/MeOH 95/5 (v/v) + 0.1% NH_4OH
Elution 50 µL DCM
Reconstitution 50 µL MeOH/H_2O 90/10 (v/v)

UHPLC-HRMS

Kinetex C_{18} 150 x 2,1 mm , ID 1.7 µm

Flow rate	300 µL/min
Temperature	30°C
Inj. volume	10µL
Phase A	H_2O + 0.1% FA
Phase B	ACN + 0.1% FA
Gradient	0.5 min 25% B
	16.8 min 95% B
Total Run Time	27 min



About 40'000 detected peaks

UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Focus on Steroid Compartment

FILTRATION

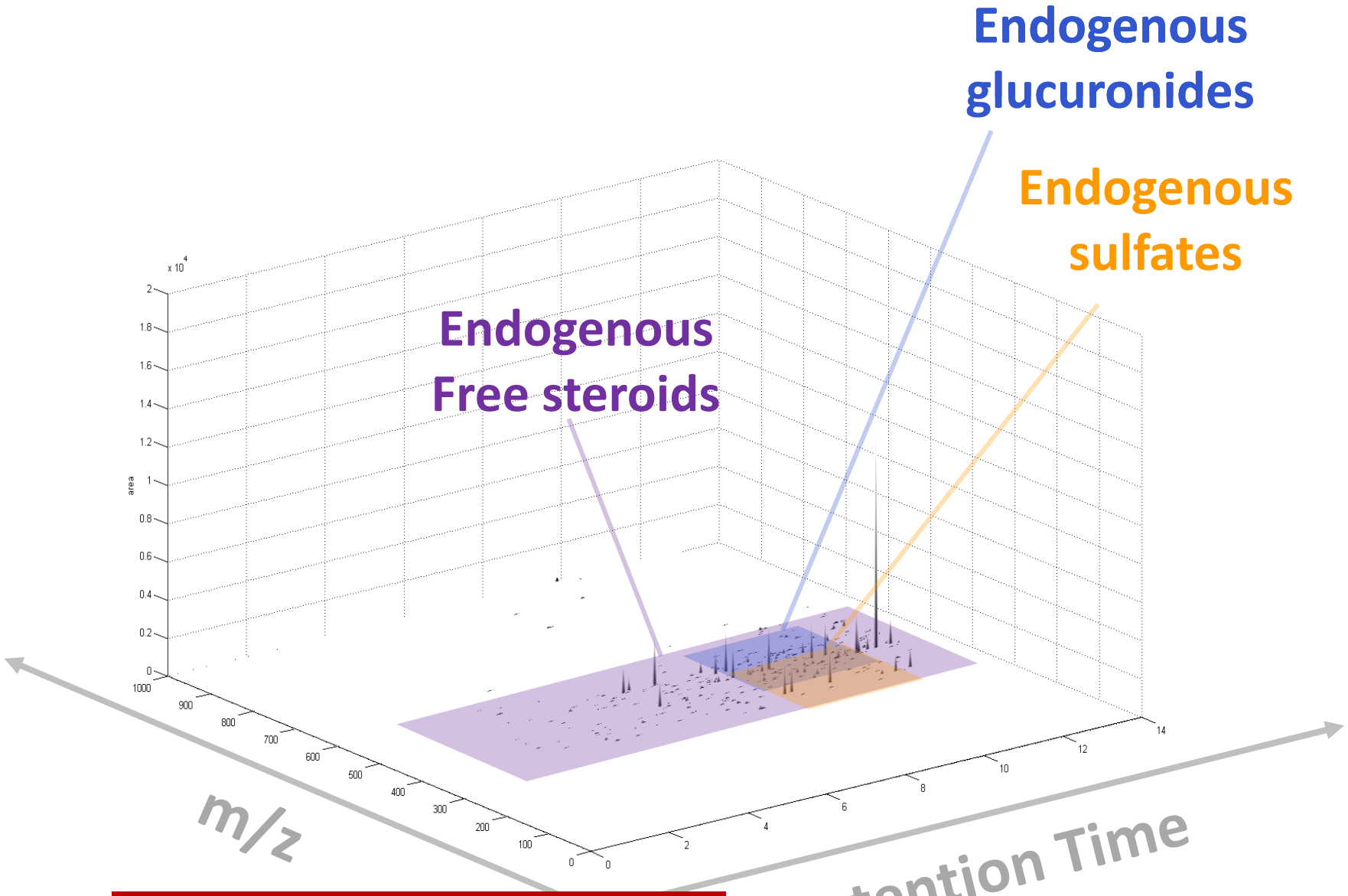
In-house Database
(130 steroidal compounds)

Online Database

(Ret. Time prediction)

Literature

Scientific Knowledge



About 250 putative steroids

UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Biomarkers

Feature	m/z	rT	Molecular formula	Annotation Level	Adduct	ID	Rank
N			O8	1	[M-H]-	**Androsterone Gluc**	1
			9	3	[M-H]-	Hydroxyandrosterone/Hydroxyetiocholanolone Gluc isomer	2
			9	3	[M-H]-	Hydroxyandrosterone/Hydroxyetiocholanolone Gluc isomer	3
			9	3	[M-H]-	Hydroxyandrostenedione Gluc isomer	4
			8	1	[M-H]-	**Etiocholanolone Gluc**	5
			9	3	[M-H]-	Hydroxyestradiol Gluc isomer	6
			8	1	[M-H]-	**5(a/b)-Androstan-3a,17b-diol-17-Gluc ***	7
N39	479.2295	6.15	C25H36O9	3	[M-H]-	Hydroxytestosterone/HydroxyDHEA Gluc isomer	8
N61	477.2126	6.67	C25H34O9	3	[M-H]-	Hydroxyandrostenedione Gluc isomer	9
N77					[M-H]-	**Testosterone Gluc**	
N28					[M-H]-	Hydroxytestosterone/HydroxyDHEA	
N62					[M-H]-	Hydroxyandrosterone/Hydroxyetioch	
N66					[M-H]-	Hydroxytestosterone/HydroxyDHEA	
N58					[M-H]-	Hydroxyandrostenedione Sulf isome	
N74					[M-H]-	11-Ketoetiocholanolone Gluc	
N123	369.1743	8.57	C19H30O5S	1	[M-H]-	**Androsterone/Etiocholanolone Sulf***	
N111	463.2339	7.86	C25H36O8	3	[M-H]-	Androstanedione Gluc isomer	17
			8	3	[M-H]-	17b-hydroxy-5b-estran-3-one Gluc	18
			9	3	[M-H]-	Hydroxytestosterone/HydroxyDHEA Gluc isomer	19
			S	3	[M-H]-	Hydroxyandrostenedione Sulf isomer	20
			S	3	[M-H]-	11b,20-Dihydroxy-3-oxopregn-4-en-21-oic acid Sulf	21
			S	1	[M-H]-	**Epiandrosterone Sulf**	22
			S	3	[M-H]-	11b,20-Dihydroxy-3-oxopregn-4-en-21-oic acid Sulf	23
			11	3	[M-H]-	Tetrahydrocortisol Gluc isomer	24
N50	385.1696	6.45	C19H30O6S	3	[M-H]-	Hydroxyandrosterone/Hydroxyetiocholanolone Sulf isomer	25

Phase II metabolites

increase more than

Free steroids

Glucuronides

increase more than

Sulfates

High presence of

Hydroxylated compounds

Urinary Steroidal Markers

also increasing in blood

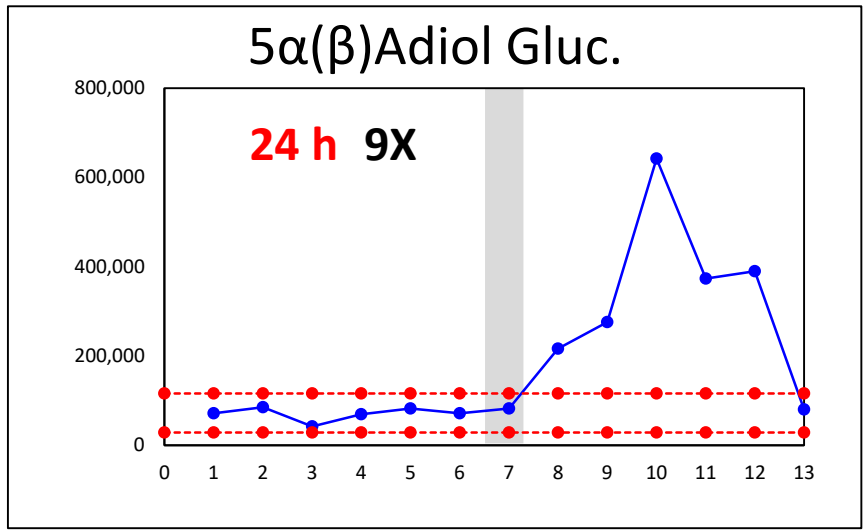
UNIL | Université de Lausanne

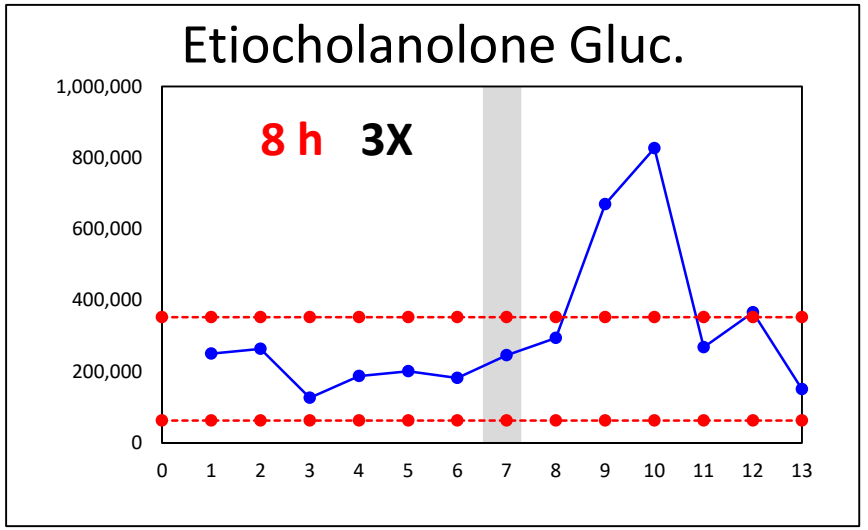
REDs - Research & Expertise in antiDoping sciences

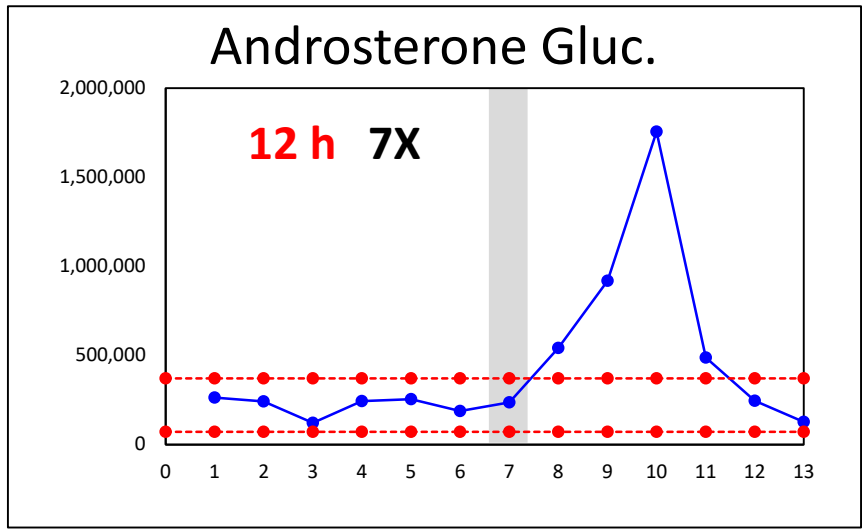
Longitudinal Monitoring

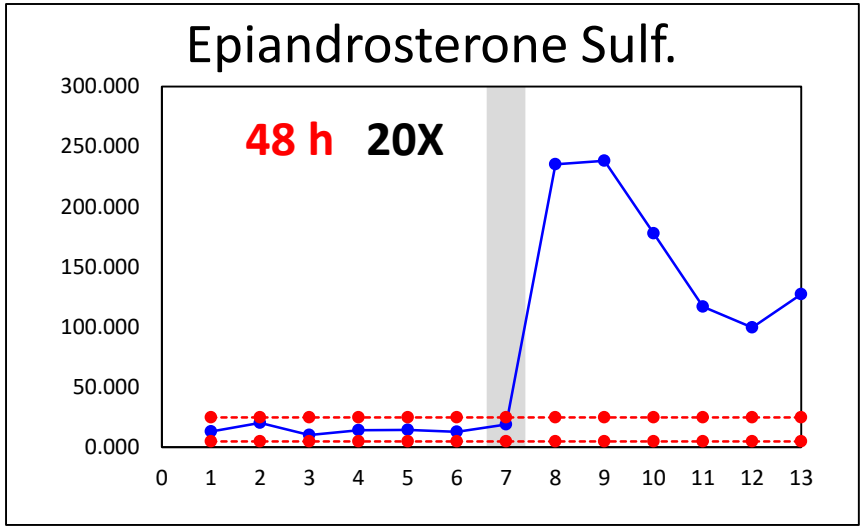
T intake

NEW BIOMARKERS









UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Conclusions & Perspectives

Longitudinal monitoring of blood T & DHT showed no differences between UGT2B17 genotypes

Compared to urinary T/E, increased sensitivity for transdermal administration

Steroidomics highlighted additional biomarkers of T intake (phase II met.)

Development and validation of a new quantitative UHPLC-MS/MS method

Application of the method on athletes' samples to obtain reference values (first point ABP) and check stability across time

UNIL | Université de Lausanne
REDs - Research & Expertise in antiDoping sciences

Aknowledgements

COLLABORATORS

Dr. Tiia Kuuranne
Dr. Raul Nicoli

UNIVERSITÉ DE GENÈVE

Dr. Julien Boccard
Prof. Serge Rudaz

FUNDING

WORLD ANTI-DOPING AGENCY

PARTNERSHIP FOR clean competition

CCCTA

FIFA FOOTBALL FOR HEALTH F-MARC