Case studies of products resulting in positive IRMS findings – Isotope signatures and detection windows

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SMRTL
2nd IRMS Workshop

Targets for big pharma testosterone

Kleinfelter syndrome

Kallman syndrome

Fertile-eunuch syndrome (Pasqualini and Bur)

Partial/Complete androgen insensitivity (Reifenstein)

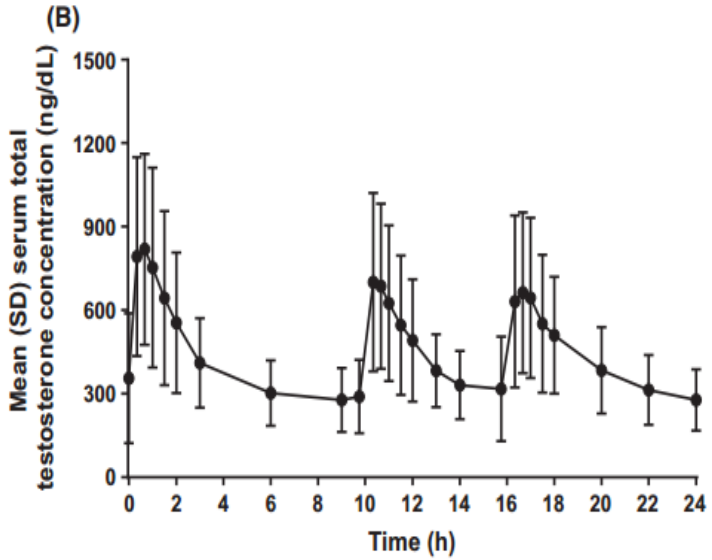
Sertoli-cell only syndrome (Del Castillo et al)

* Men of a certain age….. The male climacteric (controversial)

Testosterone products on the market

Route of administration	Dose	Frequency	Tmax
Nasal	11 mg	8 h	40 min
Buccal	30 mg	12 h	10-12 h
Transdermal patch	4 mg	Daily	8.2 h
Transdermal gel	~ 40 mg	Daily	4-24h
Subcutanous	50-100 mg	1 week	12-24 h
Intramuscular	50-750 mg	2-10 weeks	2-7 days
Subdermal	150 – 450 mg	3—6 months	1 month

Intranasal formulation - Natesto®



- Not an aerosol
- 11 mg per use – 3 times a day
- 5 days on – 2 days off (4 weeks total)
- Collections on off days and washout (3 days)

http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205488s000lbl.pdf
http://www.excelmale.com/showthread.php?2079-FDA-Approved-Natesto-Nasal-Gel-to-Treat-Men-with-Low-Testosterone

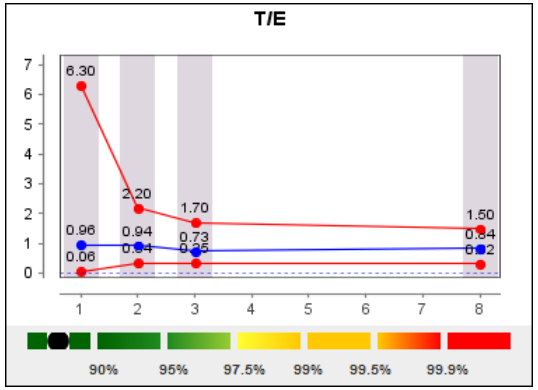
Results from adaptive model for T/E

Flagged atypical

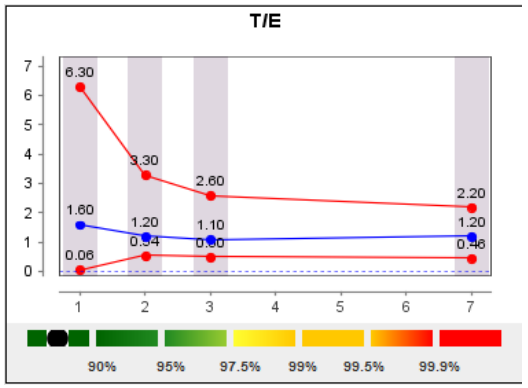
0-24 h	24-48 h
80 %	0 %

IRMS positive

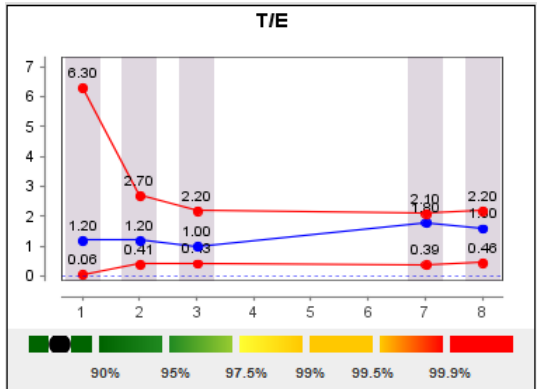
0-24 h	24-48 h
90 %	40 %



Subject 3

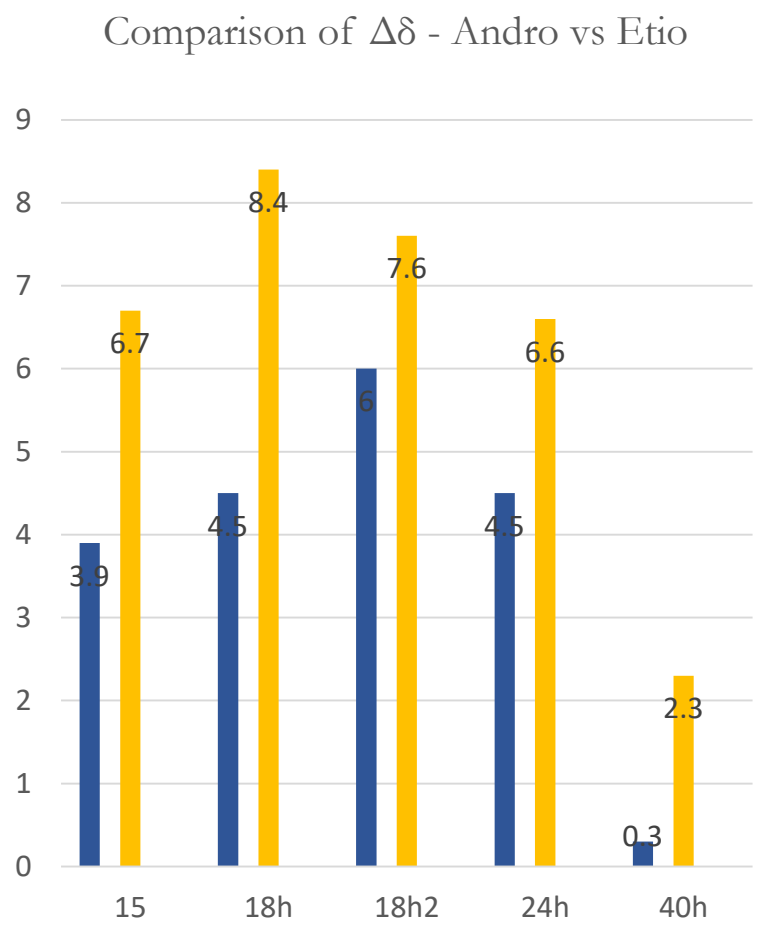


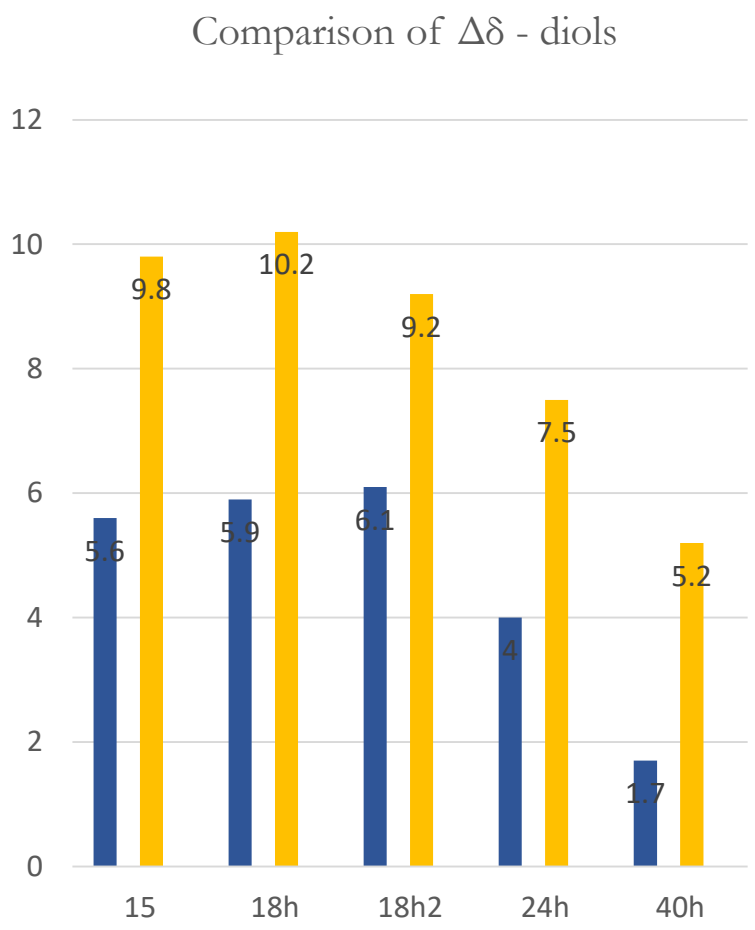
Subject 4



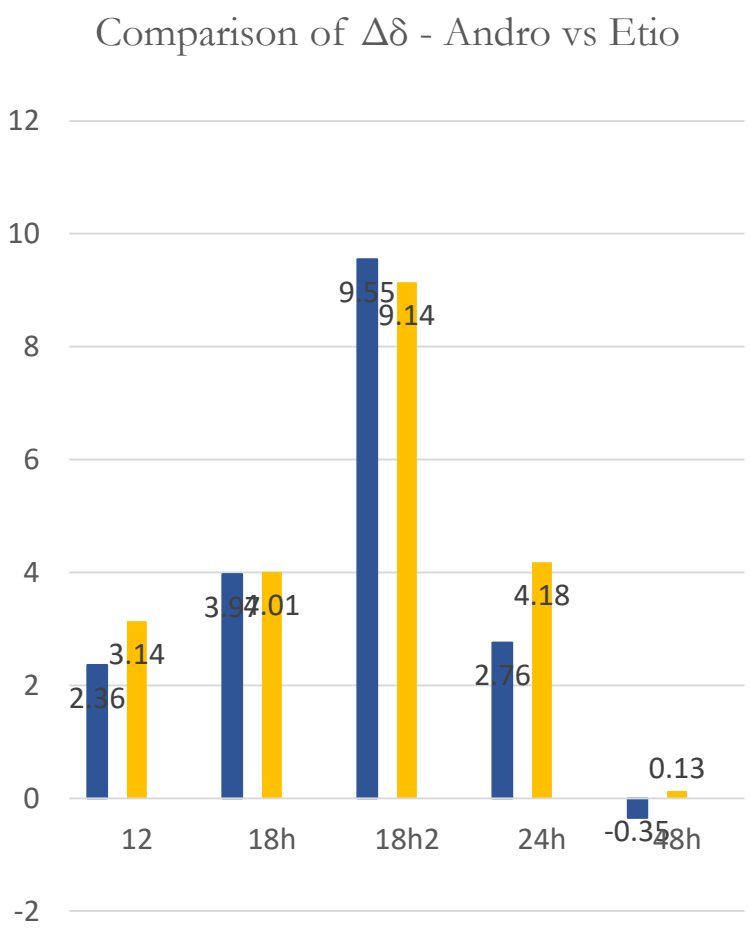
Subject 5

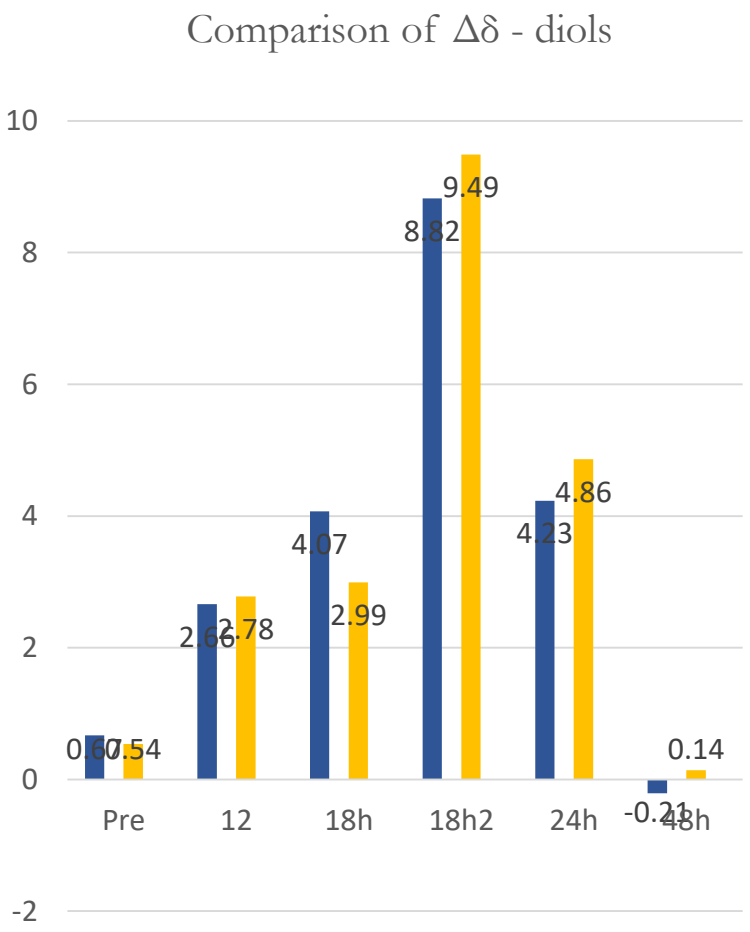
Characteristics of Irms data – subject 3



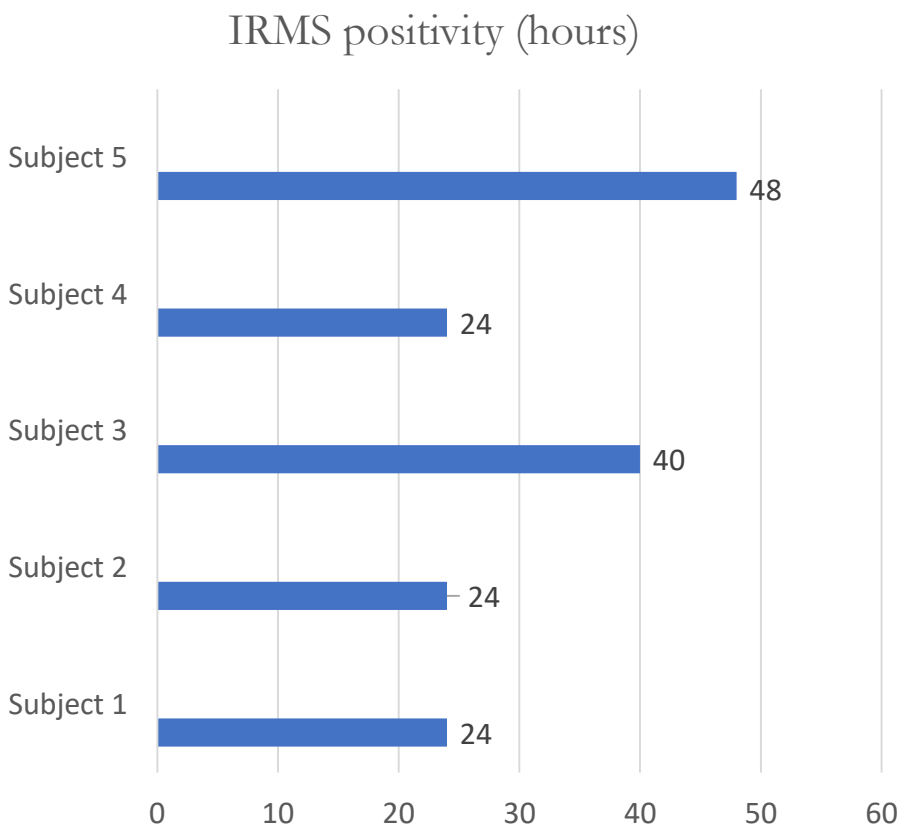


Irms data – subject 4





Detection windows



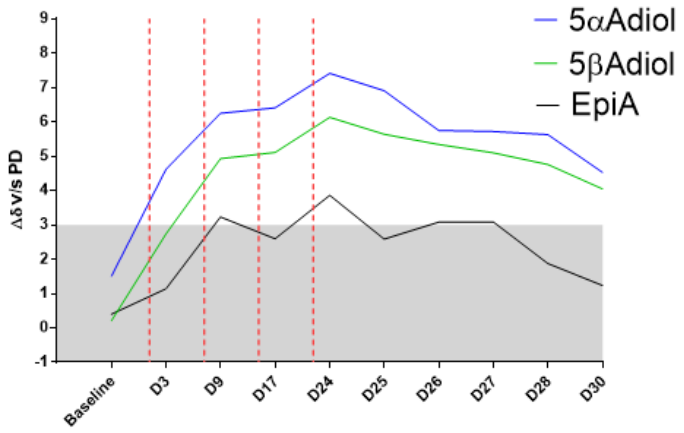
Subcutaneous self-administered formulation – Xyosted®

https://www.xyosted.com

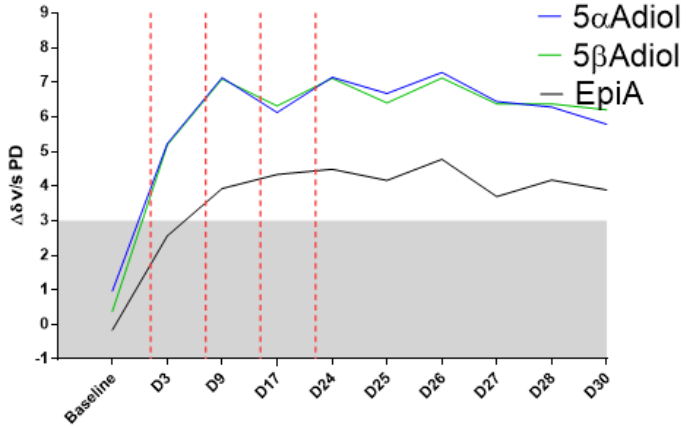
- Subjects were allocated different strength formulations
- Once a week administration
- Collections in between dosing and washout (10 days)

Subcutaneous admin

Subject B - SC (50 mg / week for 4 weeks)



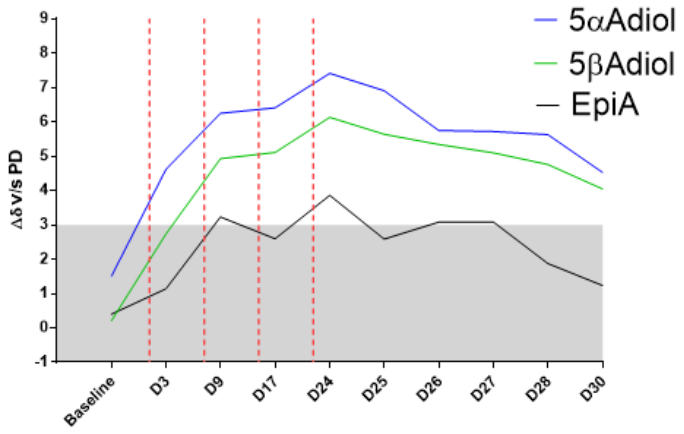
Subject A - SC (75 mg / week for 4 weeks)



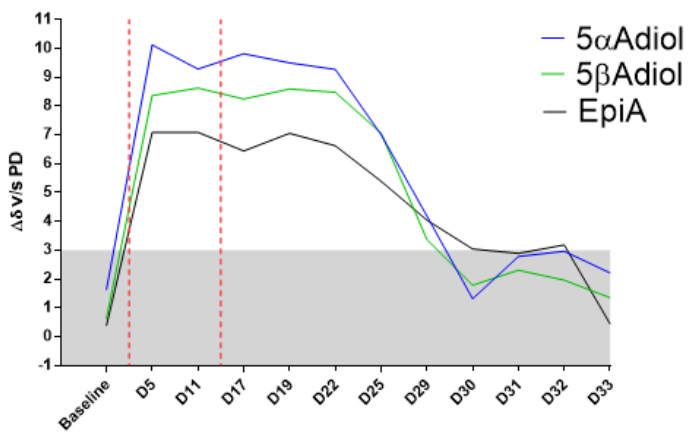
Evaluation of epiandrosterone as a TC

Subcutaneous adm v/s intramuscular

Subject B - SC (50 mg / week for 4 weeks)



Subject B - IM (200 mg / 2 weeks)



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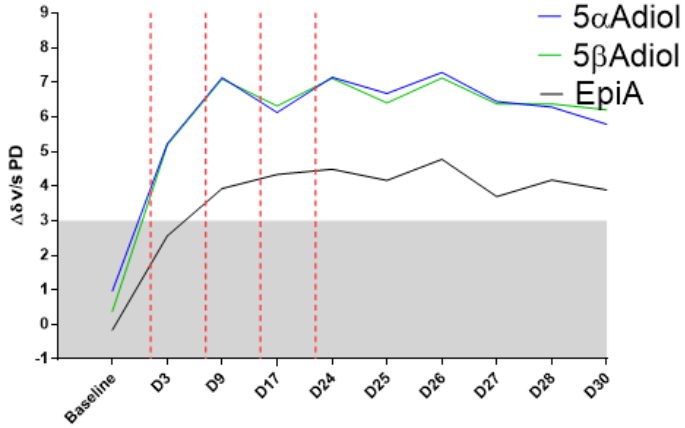

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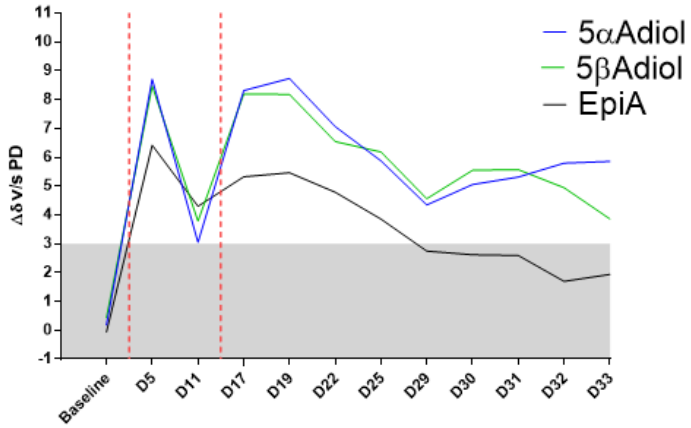
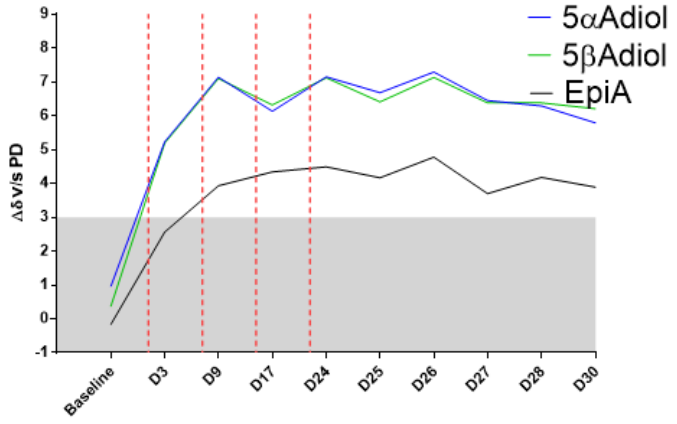


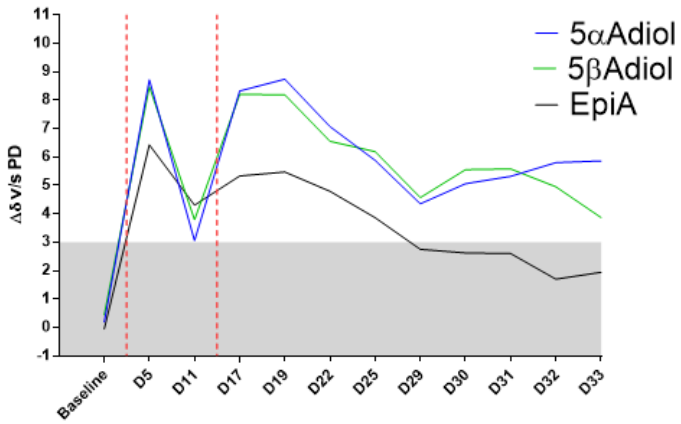
Evaluation of epiandrosterone as a TC

Subcutaneous adm v/s intramuscular – subject specific differences

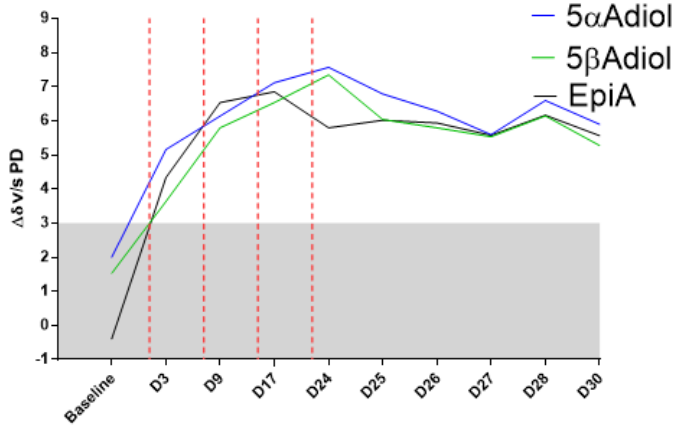
Subject A - SC (75 mg / week for 4 weeks)



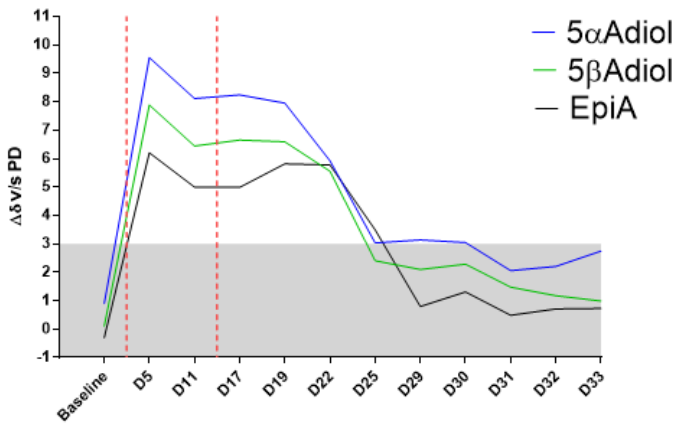
Subject A - IM (200 mg / 2 weeks)



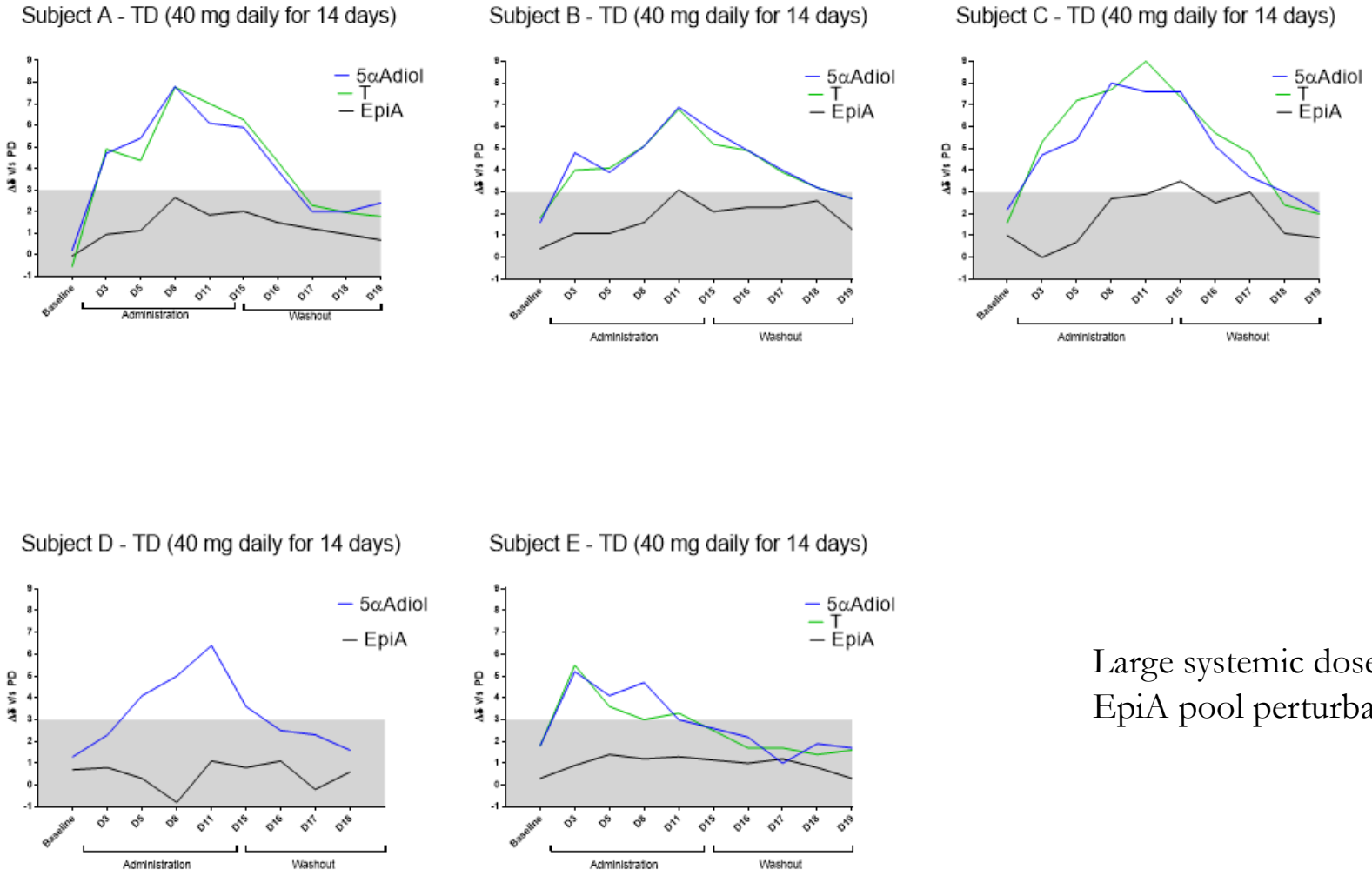
Subject C - SC (75 mg / week for 4 weeks)



Subject C - IM (200 mg / 2 weeks)



Comparison with TD gel use (40 mg/day for 14 days)



Large systemic dose appears necessary for EpiA pool perturbation

* Nair VS *et al.* Evaluation of epiandrosterone as a long term marker of testosterone use

Case study 1: adverse irms finding

- Professional league athlete
- Claim: caused by application of cream obtained from a pharmacy in the Dominican republic – no significant fault or negligence
- Website for cream makes no mention of testosterone
- Cream tested by private lab – found testosterone
- SMRTL contacted by RMA to assess cream

Case Study 1: Source of product causing finding

- Obtained from athlete
  - 1 container – tested by private lab
  - 1 container – from athlete's agent
- Obtained independently by RMA from pharmacy in the Caribbean
  - 3 containers – unopened

Case study 1: evaluating athlete's claim

- Do the creams contain testosterone?

Sample provided by	Agent	RMA sample 1	RMA sample 2	RMA sample 3	Athlete via private lab
Weight (g)	240	255	275	250	136
δ (‰)	- 31.3	- 31.5	- 31.8	- 32	- 31.6
Testosterone conc (ppm)	9000	7500	8000	9000	33500

- Are the IRMS findings consistent with topical application?

Case study 1: evaluating athlete's claim

Athlete

- ~ 6 g cream / day ≡ 200 mg T / day
- Used for ~ 50 days

- All δ ~ -30 ‰
- All Δδ ~ 11
- No discrimination between diols

Administration study[1]

- 2 x 5 g cream /day ≡ 100 mg T / day
- Administered for 42 days

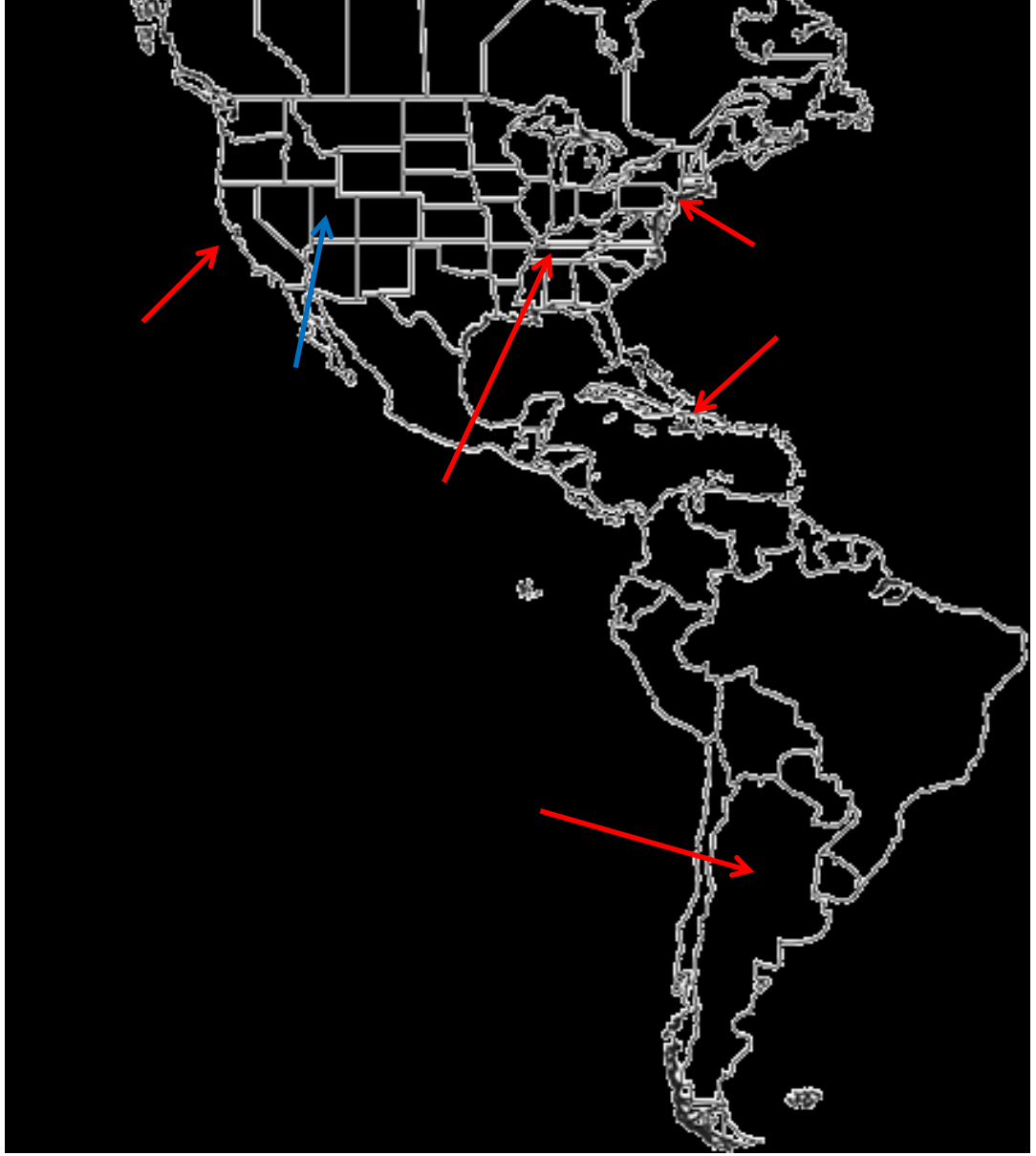
ERC - Target	Max obs Δδ
PD – A	3.1
PD – E	1.1
PD – 5α diol	6.3
PD – 5β diol	3.9

AAF extremely unlikely to be caused by cream alone.

[1] - Piper, T., Mareck, U., Geyer, H., Flenker, U., Thevis, M., Platen, P. and Schanzer, W – *Rapid Commun, Mass Spectrom.* 2008; 22:2161-2175

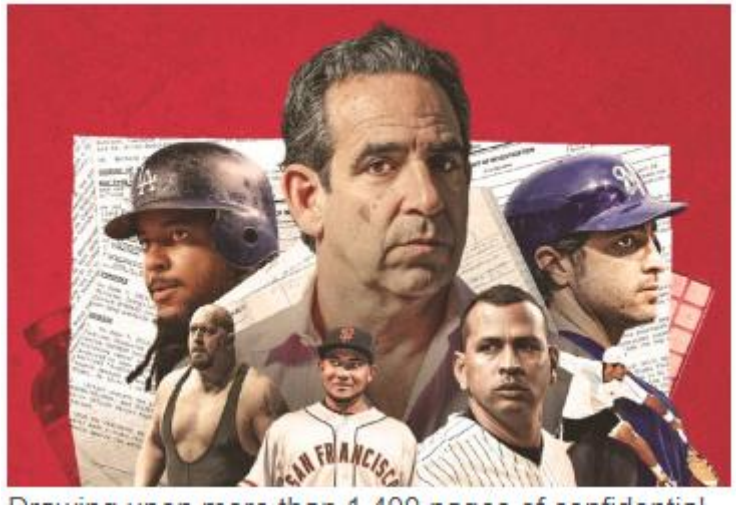
Case 1: Follow-up

- New investigation
- Website traced, created after date of positive test
- Label printer traced, printed after positive test
- Obscure pharmacy stocked with cream to lend credibility to story



Case study 1: Follow up

- Doping Protocol – Biogenesis clinic in Miami (Florida, USA)
- Designed by Antony Bosch (Dr T)
- Protocol apparently included GH, IGF-1 and T gel and injections
- Multiple high-level athletes linked to this clinic



Case study 2

Steroid Profile

Andro	**2000**
Etio	1800
5α diol	27
5β diol	125
DHEA	33
T/E	0.54

IRMS results

ERC	**δ (‰)**
PD	-26.8
11-OHA	-18.9

ERC - TC	**Δδ**
ERC – A	4.4
ERC – E	4.7
ERC - 5α diol	< 3
ERC – 5β diol	< 3
ERC – T	< 3

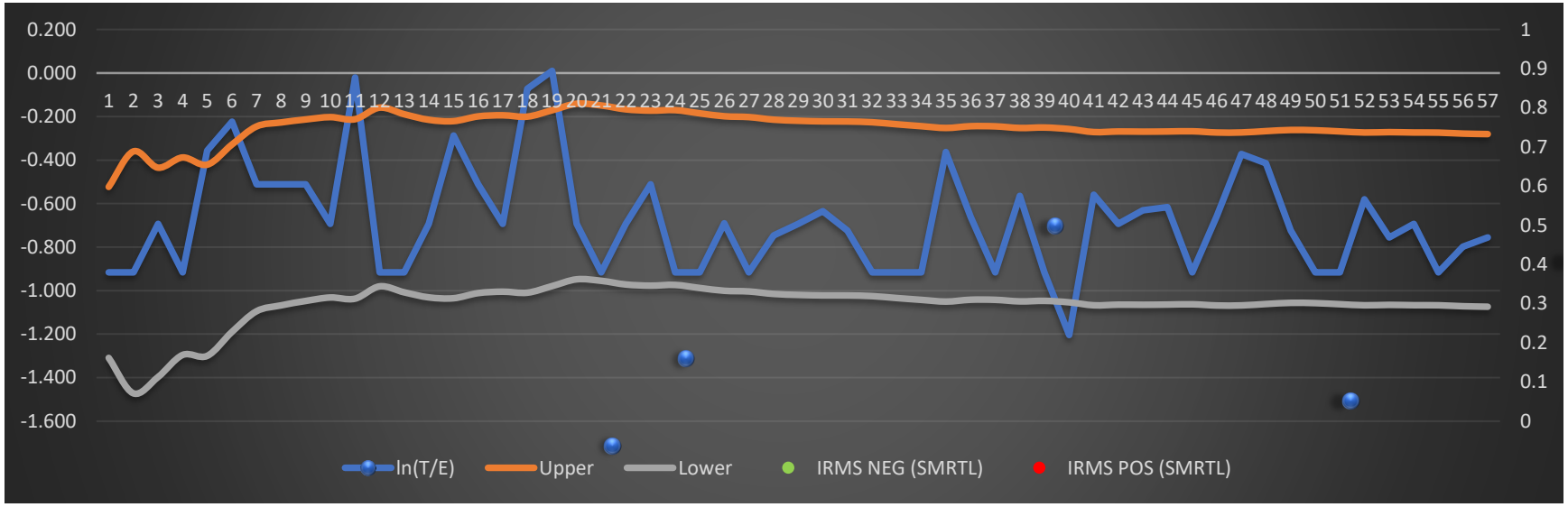
ERC – DHEA	6.2

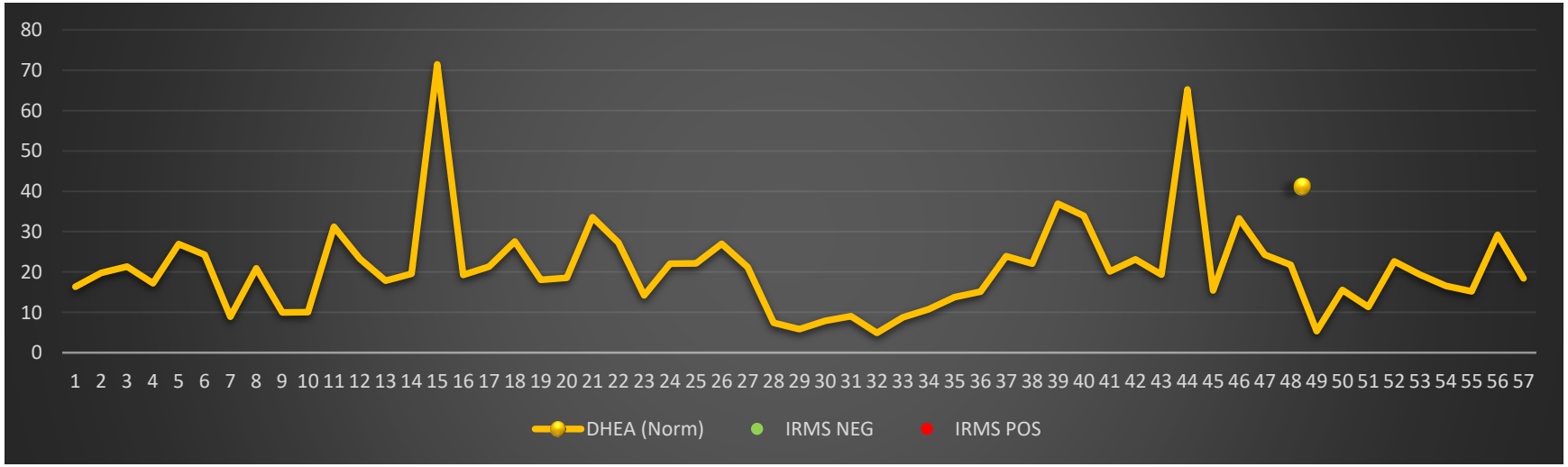
Case Study 2: DHEA?

Steroid profiles

	1	2	3	4
Collection	May 15	June 7	June 21	June 23
Andro	2000	6100	2200	900
Etio	1800	2000	1500	600
5α diol	27	94	27	16
5β diol	125	325	90	45
DHEA	33	58	36	24
T/E	0.54	0.52	0.68	0.66
IRMS Result	**AAF**	**N**	**ATF**	**DHEA only AAF?**

Case Study 2: DHEA?





Case Study 2: Product responsible

Perhaps also available in 0 X potency?

	Label claim	Lab results
DHEA	1 %	~ 1 %
T	5 %	N.D.

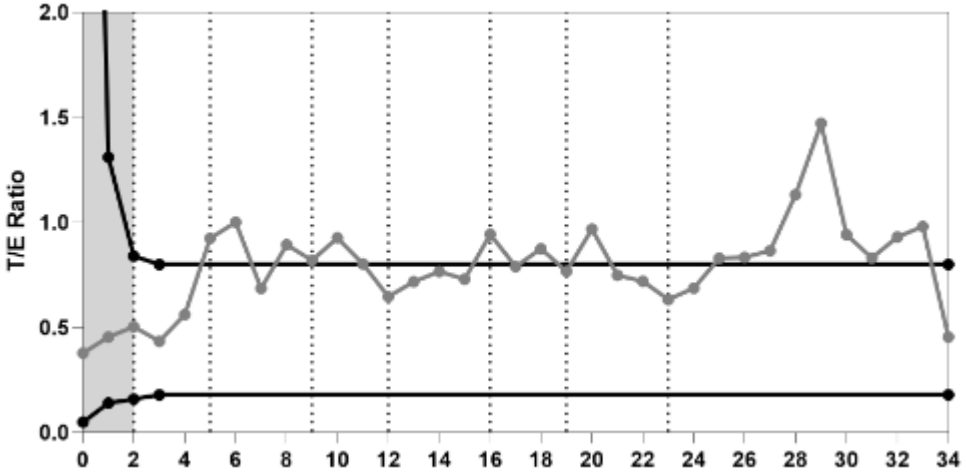
Label: 5% testosterone in 10X, 30X and 100X potencies.

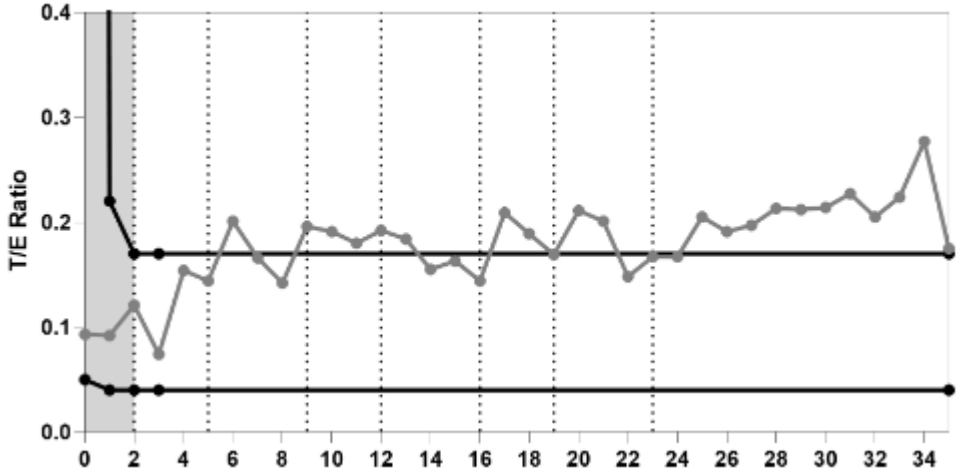
No isotopic discrimination between diols
T not affected

Hence, unlikely to contain T

Substances affecting longitudinal T/E

hCG administration – 250µg 7 times over 3 weeks

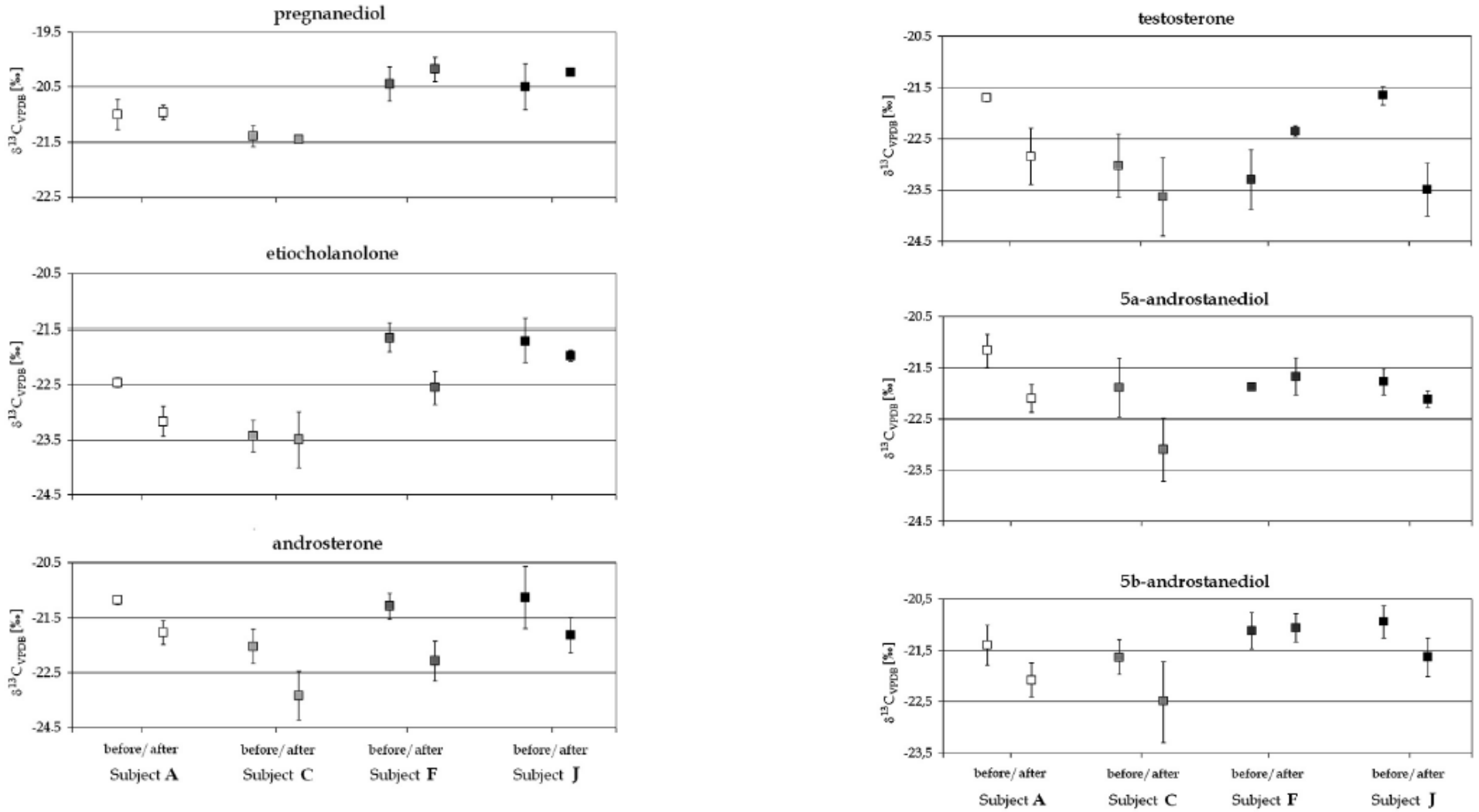




* Goodrum *et al* .Impact of Biotin Supplementation on Human Chorionic Gonadotropin Immunoassays Utilizing Biotin-Streptavidin Binding Methods in Urine

Does gonadal T production have a characteristic isotope signature?

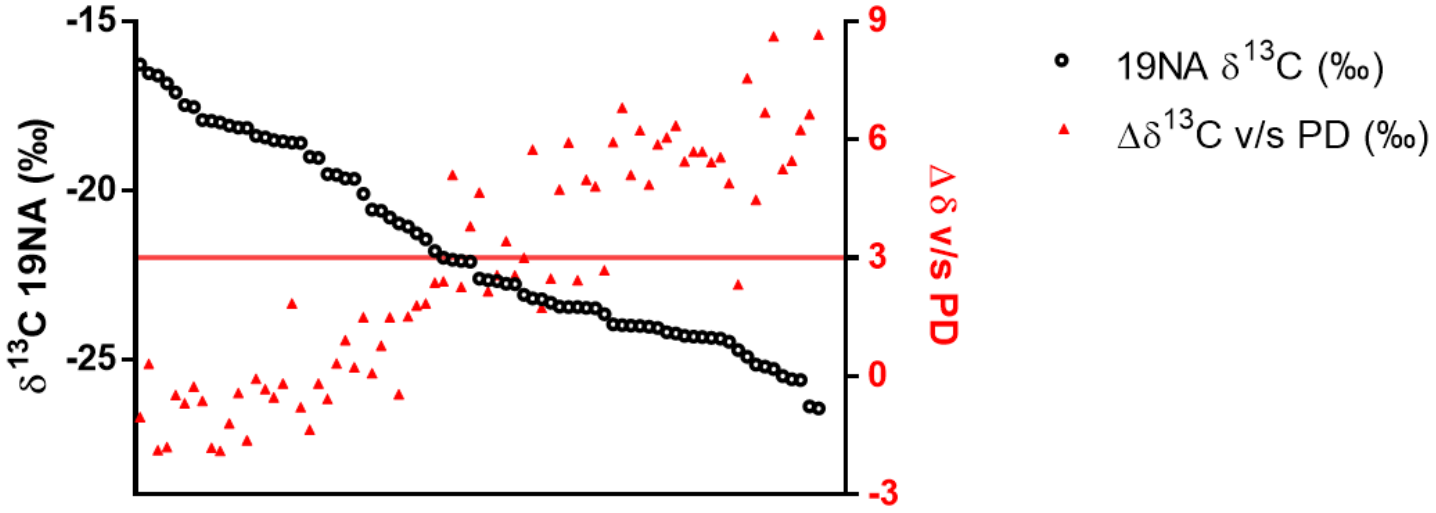
CIR of steroids before and after hCG administration



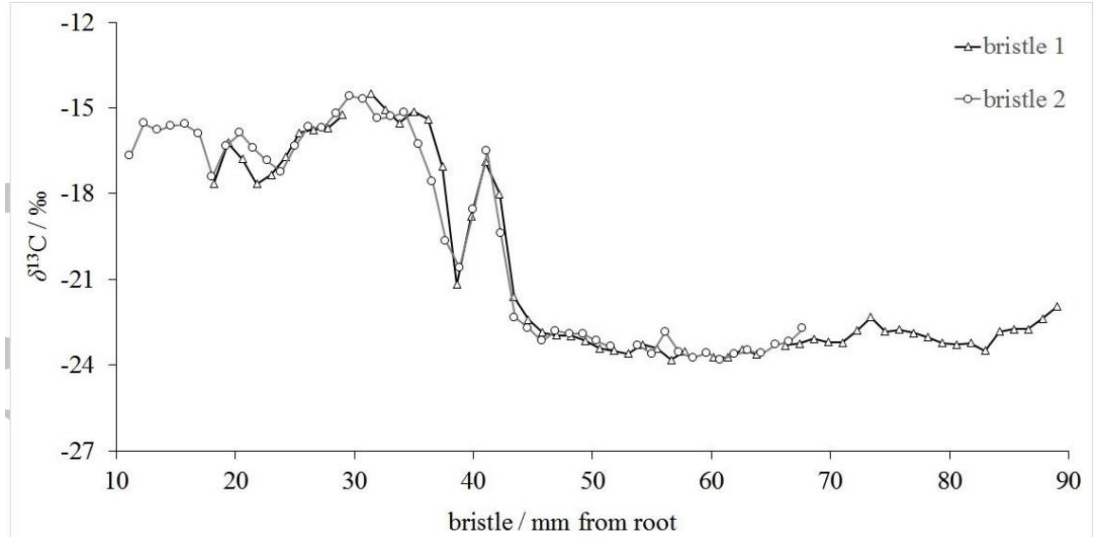
Piper *et al*: Influences of b-HCG administration on carbon isotope ratios of endogenous urinary steroids

19NA: Pseudoendogenous CIR signatures

Range of 19NA $\delta^{13}C$ values and $\Delta\delta^{13}C$ values v/s PD



Adventurous eating and potential AAFs



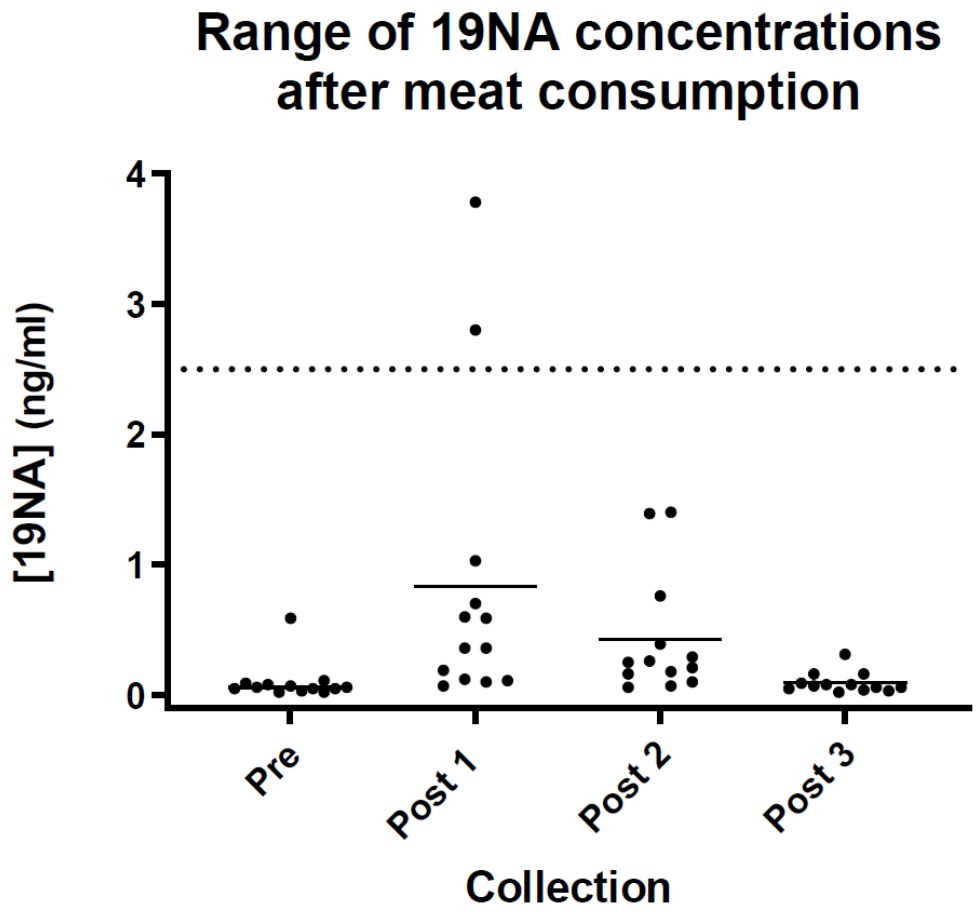
19NA	PD
-12.9	-23.3
-13.1	-23.4

Hulsemann *et al:* Case Study: atypical δ 13C values of urinary norandrosterone

The dodgy burrito defense

World Athletics vs Shelby Houlihan CAS 2021/O/7977

Results of the SMRTL culinary experiment



Source	C_{max} (ng/ml)	19NA $\delta^{13}C$ (‰)	PD $\delta^{13}C$ (‰)	16-en $\delta^{13}C$ (‰)	$\lvert\Delta\delta^{13}C\rvert$ vs PD (‰)	$\lvert\Delta\delta^{13}C\rvert$ vs 16-en (‰)
Boar, vol 1	2.8	−16.6	−20.3	-19.9	3.7	3.3
Boar, vol 13	3.8	−16.7	−20.2	-19.8	3.5	3.1

Making the most of your 4 year ban

- The beer mile
- 4 beers (at least 5% ABV), at least 12 oz
- One beer every quarter mile
- Shelby's time: 5m 43 s