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# Urinary profiles of corticosteroids after intra-articular and related administrations

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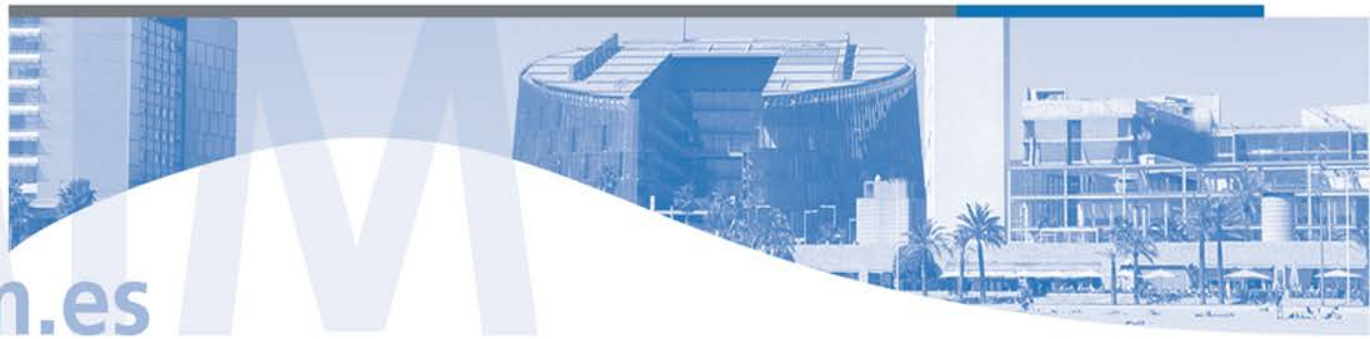
33rd Cologne Workshop on Dope Analysis, March 2015



# Glucocorticoids

- Synthetic analogs of cortisol.

- Used for their anti-inflammatory and immunosuppressive properties in the treatment of many different pathologies (rheumatologic, hormonal, allergic, and respiratory disorders, among others).

- Widely used in sports for the treatment of conditions such as asthma and acute injuries:
  - 24.6% of AAF in Tour of France in 2002 [1],
  - 36% of cyclists declared glucocorticoid use in samples analyzed in 2005 by DoCoLab [2],
  - 15.8% cyclists received glucocorticoid therapy in a survey performed by French Cycling Federation [3].


1. Duclos M. Use and abuse of anabolic steroids and glucocorticoids in sport. Ann Endocrinol (Paris) 2007;68(4):308-14.
2. Thuyne WV, Delbeke FT. Declared used of medication in sports. Clin J Sport Med 2008;18(2):143-7.
3. Guinot M, Duclos M, Idres N, Souberbielle JC, Megret A, Le Bouc Y. Value of basal serum cortisol to detect corticosteroid-induced adrenal insufficiency in elite cyclists. Eur J Appl Physiol 2007;99(3):205-216.

# Glucocorticoids

## Effects on sport performance:

- Evidences of positive effects on exercise performance

Duclos M. Evidence or ergogenic action of glucocorticosteroids as a doing agent risk. The physsician and sports medicine 2010;38(3):121-7

## Health risks associated with glucocorticoid therapy:

- Derived from inhibition of the hypothalamic-pituitary-adrenal axis
➔ adrenal insuficiency

# THE 2015 PROHIBITED LIST

## S9. GLUCOCORTICOIDS

All glucocorticoids are prohibited when administered by oral, intravenous, intramuscular or rectal routes.

## WADA Technical Documents

TD2010MRPL, TD2013MRPL, TD2014MRPL

**Table 1. MRPLs for detection of Non-Threshold *Prohibited Substances* in human urine**

| Prohibited Class | Specific Examples / Exceptions | MRPL[(a)] |
|---|---|---|
| S9. Glucocorticosteroids | | 30 ng/mL |
| | Budesonide (6β-hydroxy-budesonide)[(d)] | 30 ng/mL |

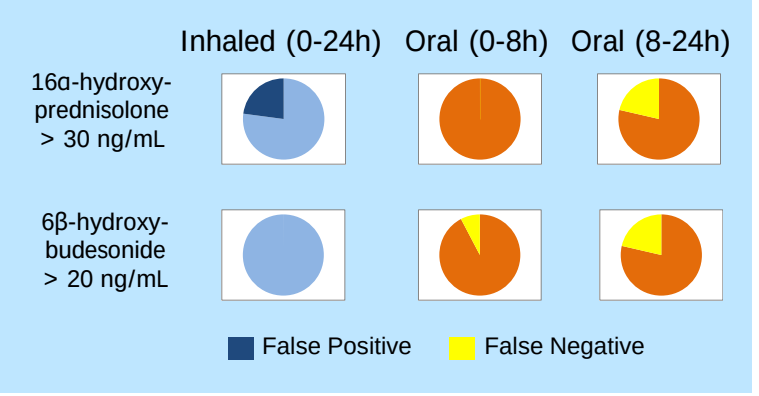
Matabosch X, Pozo OJ, Pérez-Mañá C, Farré M, Marcos J, Segura J, Ventura R.
*Discrimination of prohibited oral use from authorized inhaled treatment of budesonide in sports*.
Ther Drug Monit 2013;35(1):118-218

# Glucocorticoids
## forbidden / allowed administrations

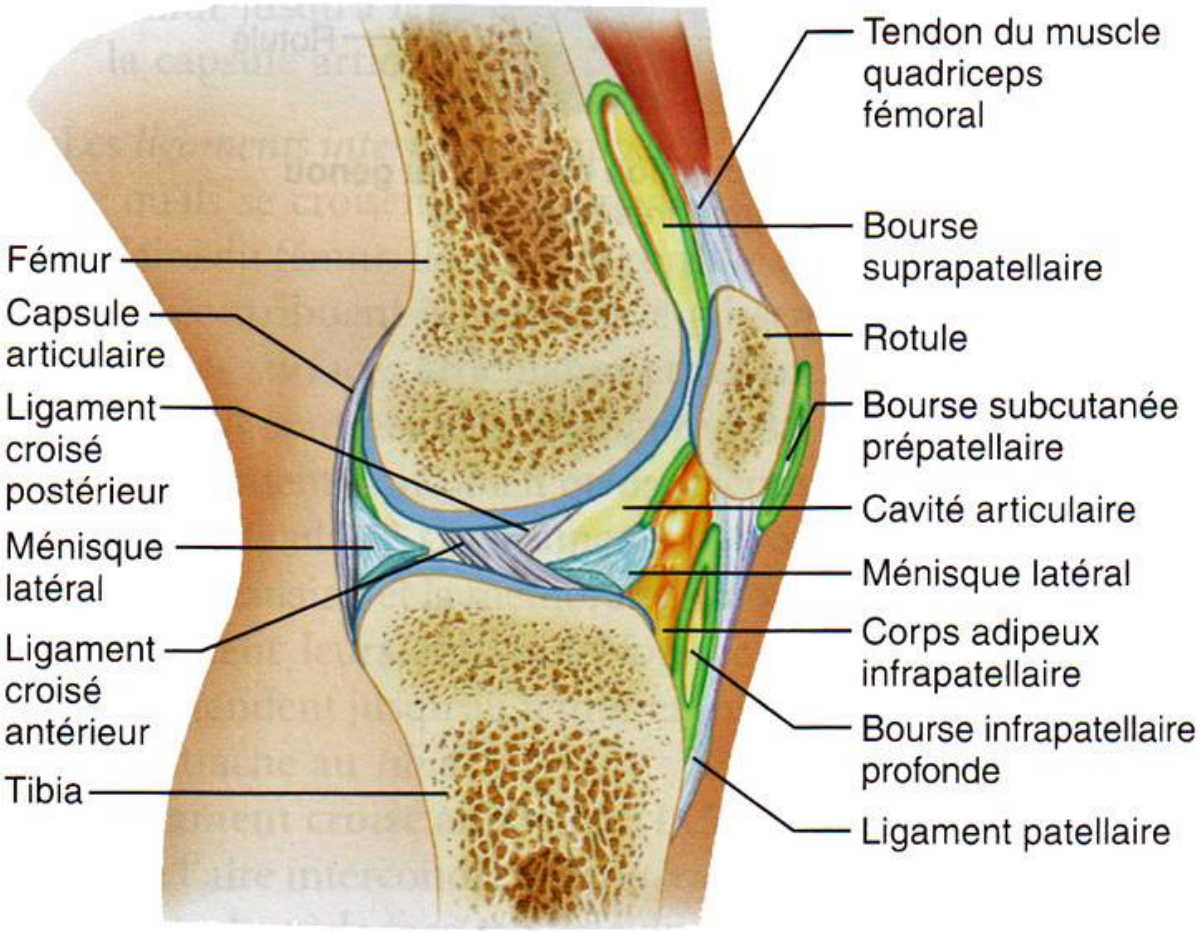
- Classification of routes of administration
- Discrimination between routes of administration:
  - To detect cheating athletes
  - To protect innocent athletes

**BUDESONIDE: inhaled vs. oral**

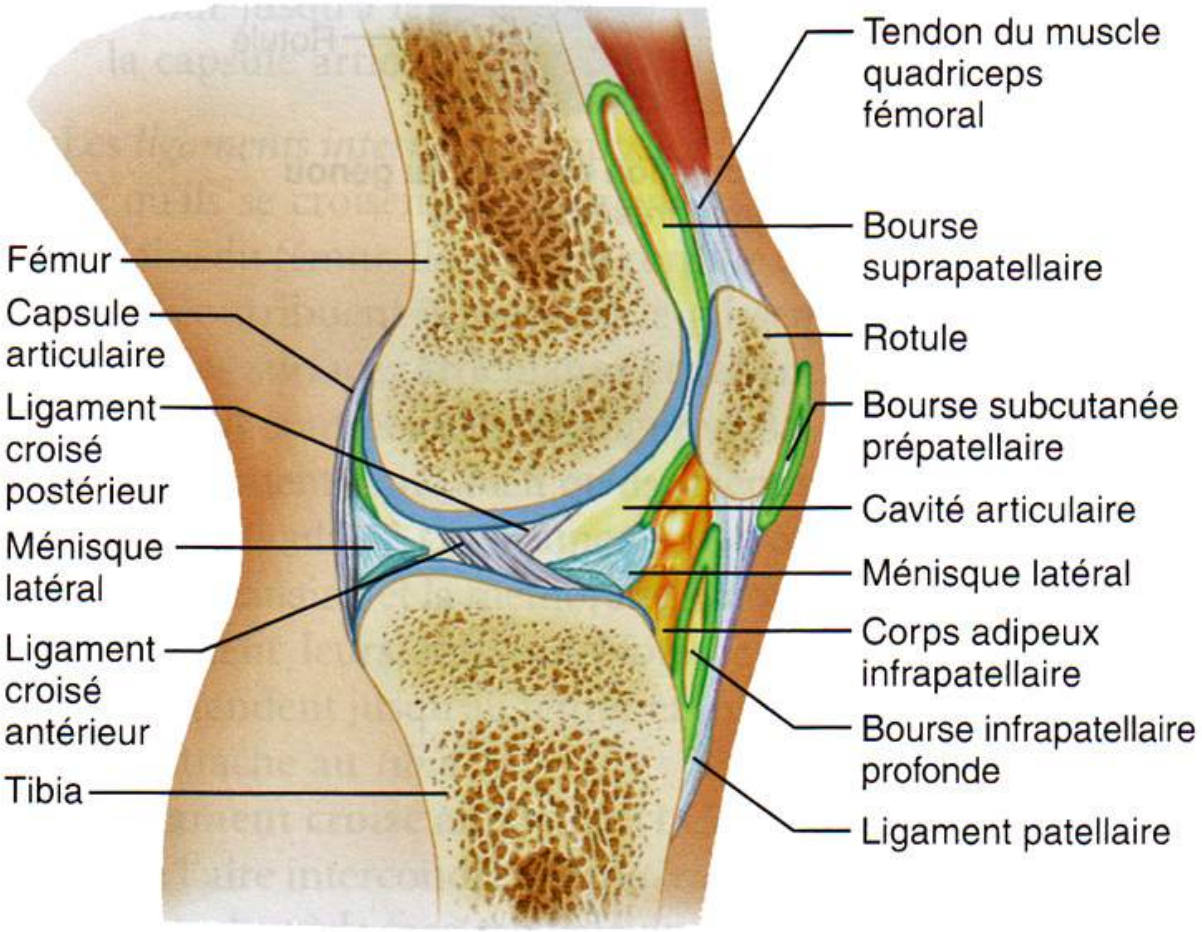
- 16α-hydroxy-prednisolone was not a good marker !!!!!!
- Best marker: **6β-hydroxy-budesonide**



# INTRA-ARTICULAR administration



# PERI-ARTICULAR (SOFT-TISSUE) administration



# OBJECTIVE

Study urinary profiles of **betamethasone** and **triamcinolone acetonide** after

- intra-articular administration
- soft-tissue administration

# CLINICAL STUDIES

**Betamethasone:**

## 1. Intra-articular administration

- Subjects: 7 (caucasian patients subjected to treatment)
- Treatment: single dose, 3-12 mg (n=2, 3 mg; n=1, 6 mg; n=4, 12 mg)
  Knee joint, foot
- Urine collection: spot urines up to day 10

## 2. Soft-tissue administration

- Subjects: 8 (caucasian patients subjected to treatment)
- Treatment: single dose, 6-12 mg (n=1, 6mg; n=7, 12 mg)
  Trochanteric bursitis, shoulder, knee joint
- Urine collection: spot urines up to day 10

**Triamcinolone acetonide:**

## 1. Intra-articular administration

- Subjects: 2 (caucasian patients subjected to treatment)
- Treatment: single dose, 40 mg
  shoulder
- Urine collection: spot urines up to day 10

# SAMPLE PREPARATION

**URINE (2-5 mL)**

+ deuterated ISTDs
+ 1 M phosphate buffer pH 7
+ β-glucuronidase *E. coli*

Incubation (1h 55ºC)

+ 25% $K_2CO_3$
+ Ethyl acetate

Shaking, Centrifugation
Evaporation organic layer

+ 150 µL $H_2O$:$CH_3CN$ (75:25, v/v)

**UPLC-MS/MS**

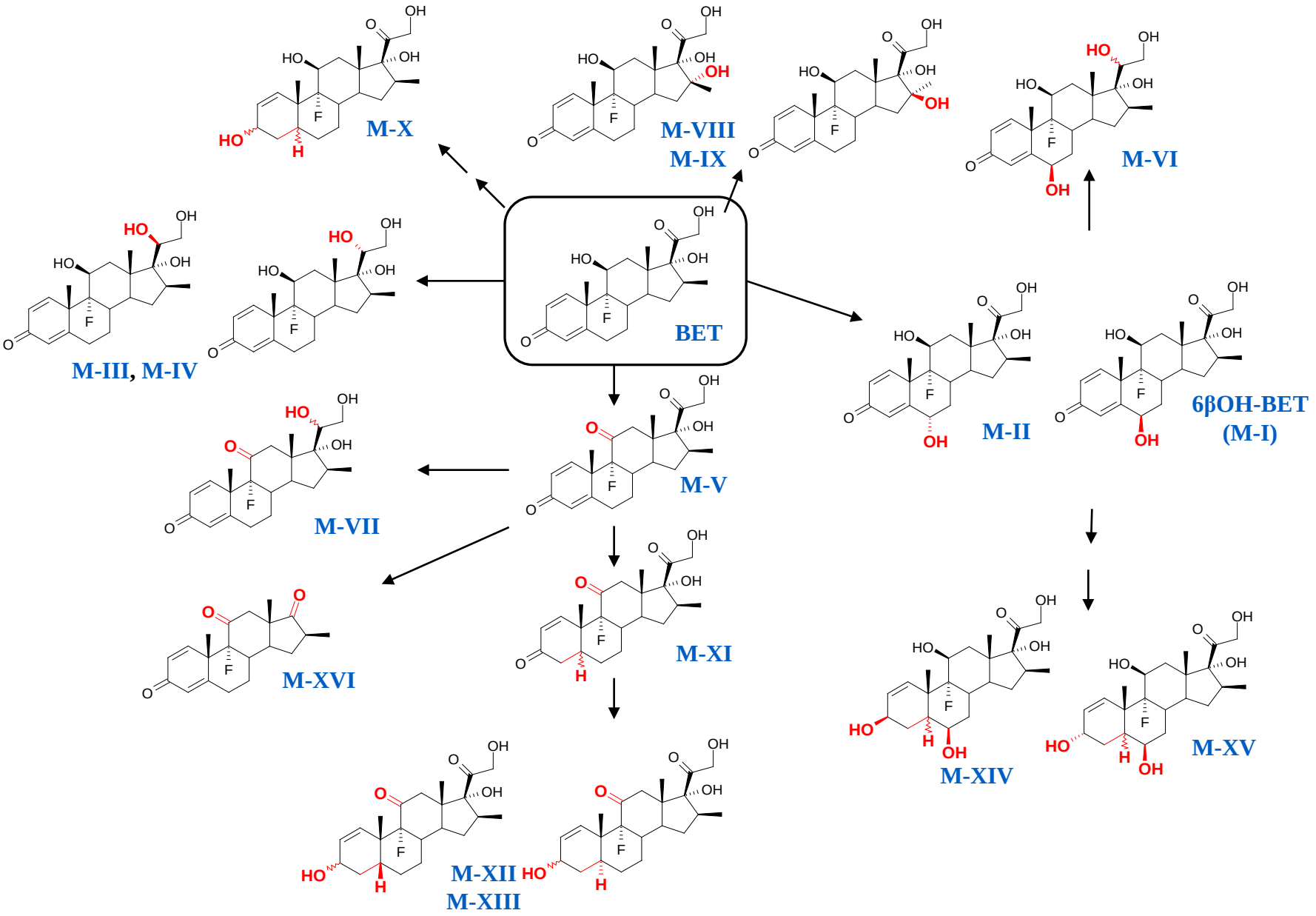

- Matabosch X, Pozo OJ, Monfort N, Pérez-Mañá C, Farré M, Segura J, Ventura R. Drug Test Anal 2014, doi: 10.1002/dta.1770.
- Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. J Steroid Biochem Mol Biol 2014;145:94-102.

# INSTRUMENTAL ANALYSIS

| | | |
|---|---|---|
| Instrument: | Acquity UPLC™ – Xevo TQMS (Waters) | |
| Column: | Column BEH $C_{18}$ (100 mm x 2.1 mm i.d., 1.7 µm) | |
| Column T: | 45ºC | |
| Mobile phase: | BET | A: $H_2O$ (HCOOH, 0.01%) |
| | | B: $CH_3CN$ (HCOOH, 0.01%) |
| | TA | A: 1 mM $HCOONH_4$ (HCOOH, 0.01%) |
| | | B: $CH_3OH$ with 1 mM $HCOONH_4$ (HCOOH, 0.01%) |
| Gradient elution | | |
| Flow rate: | 0.4 mL/min-0.3 mL/min | |
| Injection volume: | 10 µL | |

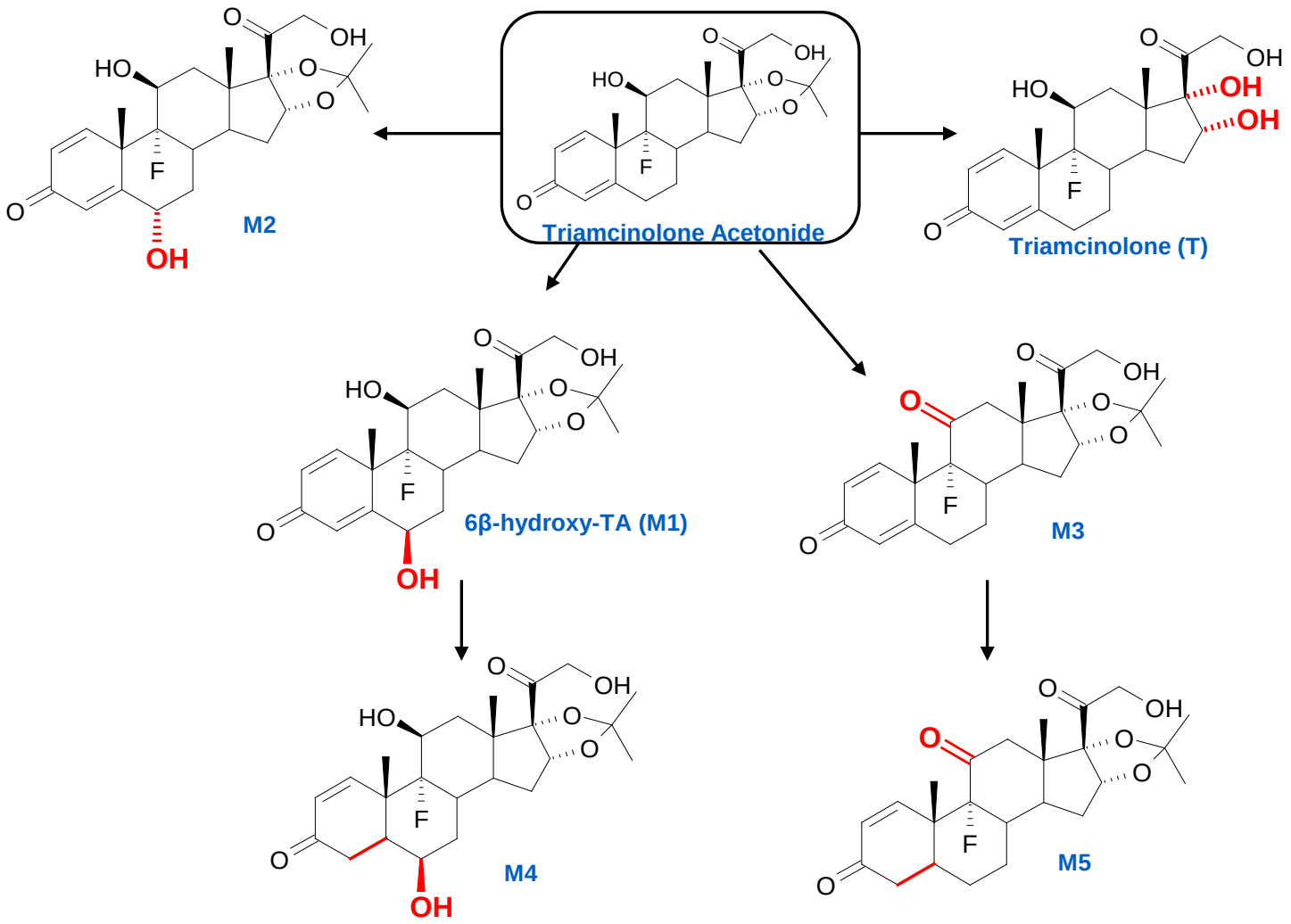
| | |
|---|---|
| Collision gas: | Ar |
| Ionization mode: | ESI, positive/negative |
| Source T: | 120ºC |
| Desolvation T: | 450ºC |
| Detection mode: | SRM |

# BETAMETHASONE and metabolites




Matabosch X, Pozo OJ, Monfort N, Pérez-Mañá C, Farré M, Segura J, Ventura R.
Drug Test Anal 2014, doi: 10.1002/dta.1770.

# TRIAMCINOLONE ACETONIDE and metabolites

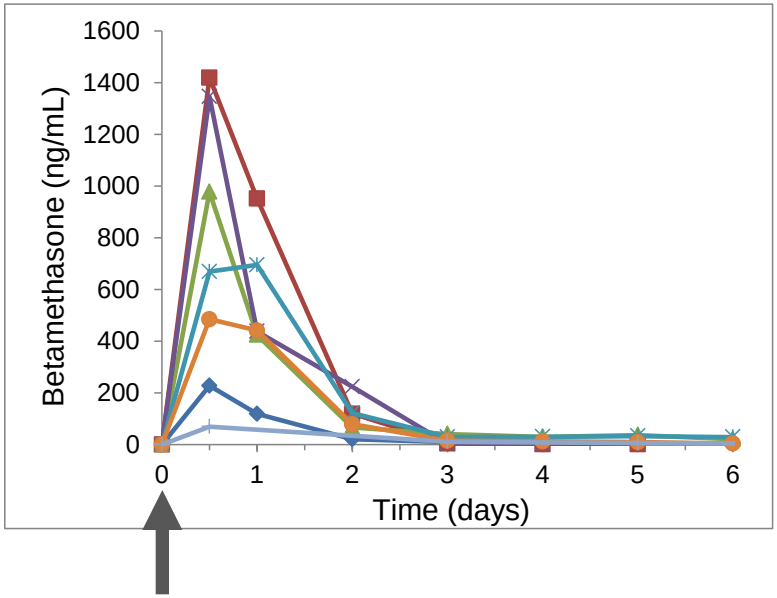



Matabosch X, Pozo OJ, Papaseit E, Farré M, Marcos J, Segura J, Ventura R.
Rapid Commun Mass Spectrom 2014;28(16):1829-39.

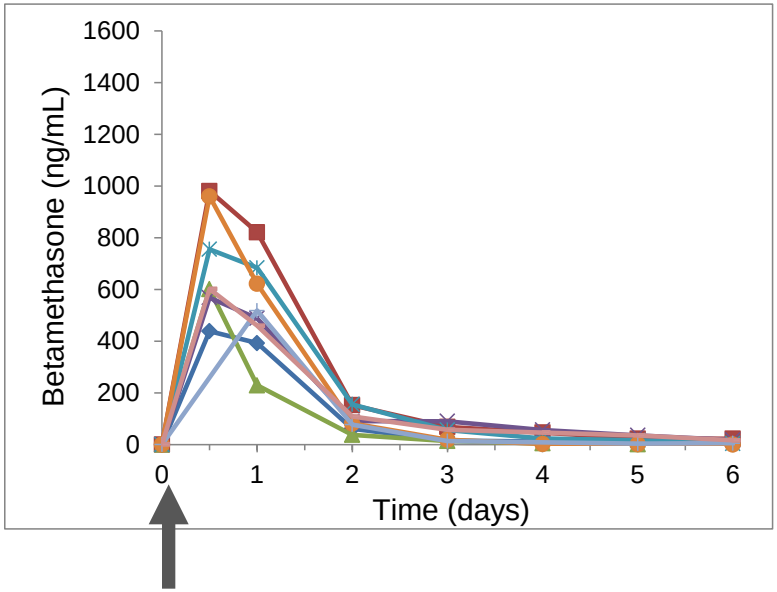
# EXCRETION PROFILES

## BETAMETHASONE

### IA (3-12 mg)



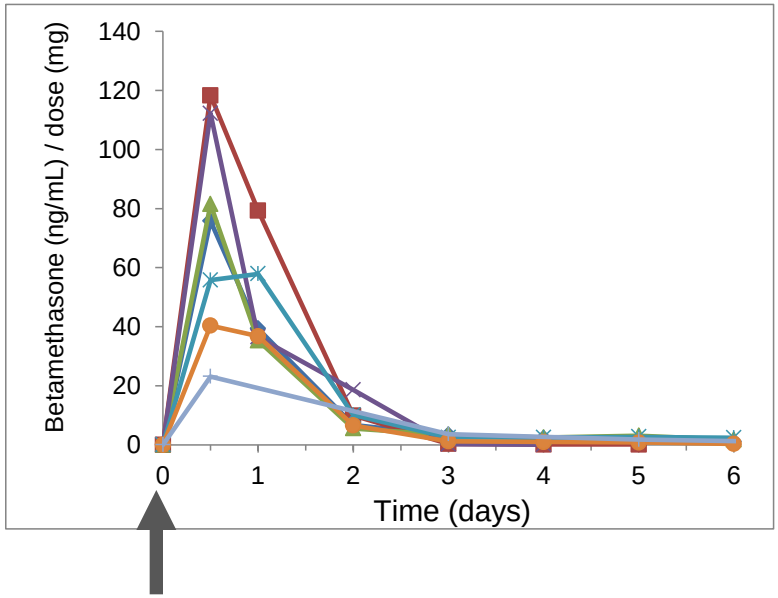

### Soft-tissue (6-12 mg)



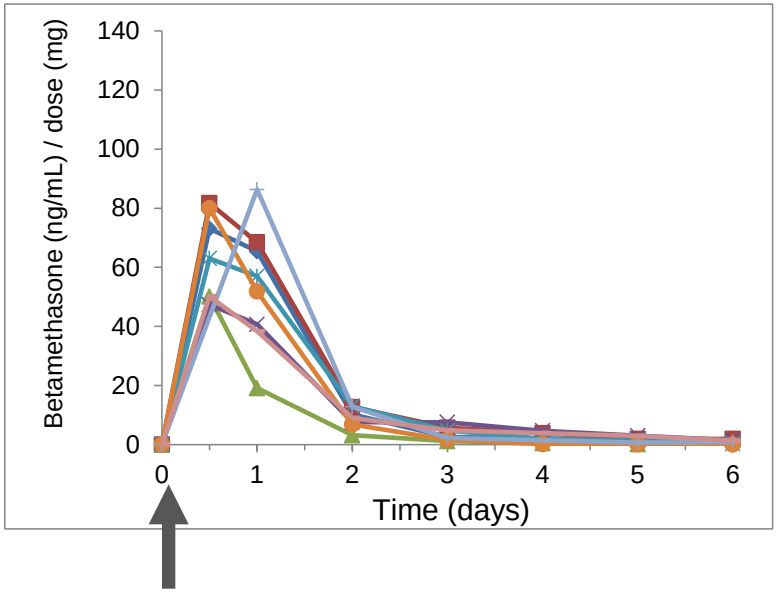
# EXCRETION PROFILES

## BETAMETHASONE

### IA (3-12 mg)




### Soft-tissue (6-12 mg)

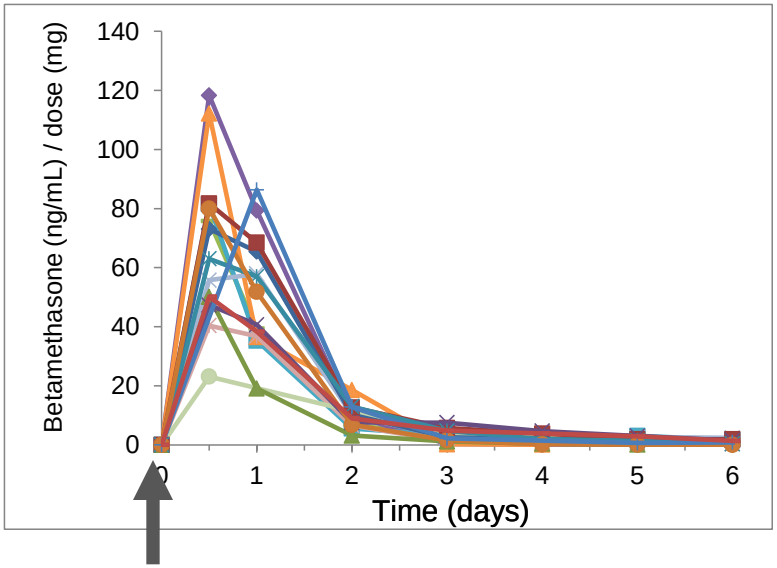


# EXCRETION PROFILES

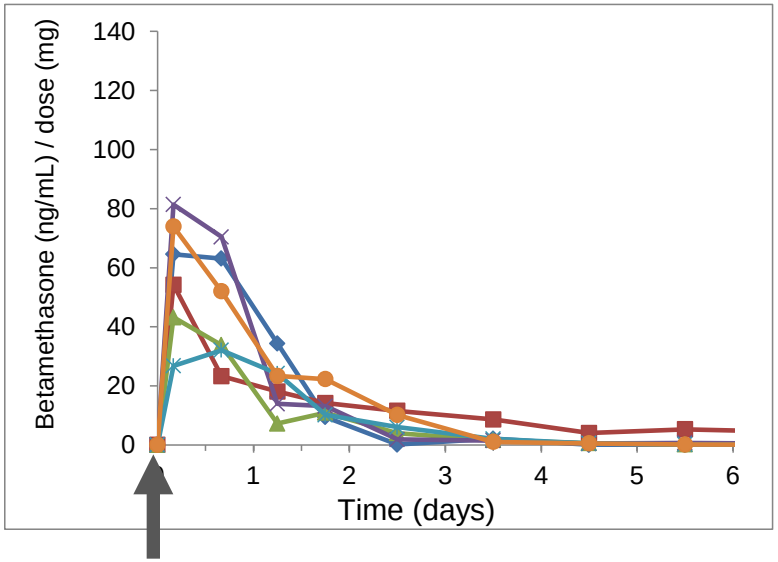
## BETAMETHASONE

## IA, soft-tissue *vs.* IM

### IA and soft-tissue



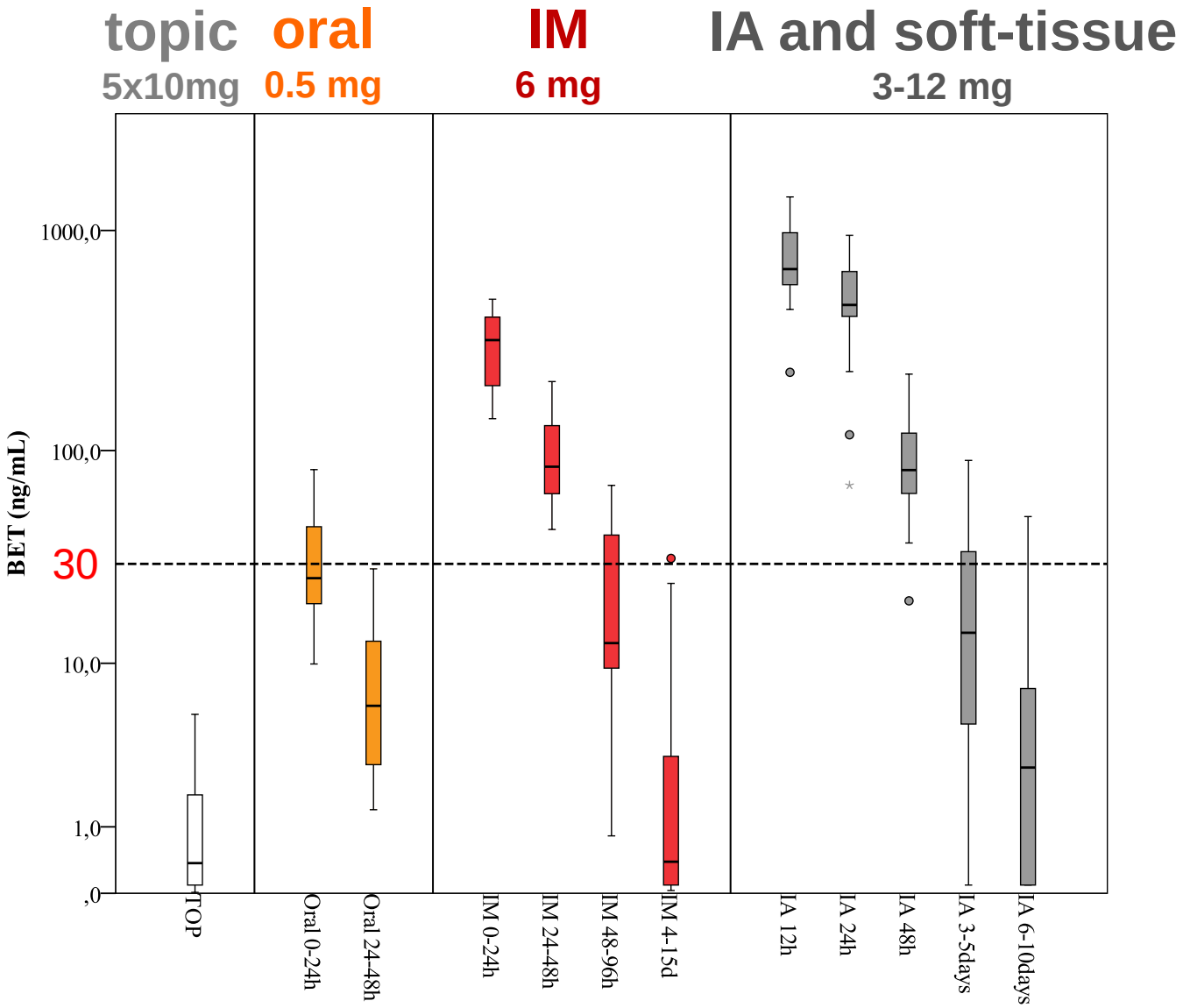

### IM




Matabosch X, Monfort N, Pozo OJ, Ferrés M, Pérez-Mañá C, Monfort J, Llorente-Onaindia J, Farré M, Segura J, Ventura R
*Urinary profiles of betamethasone metabolites after different administration routes.*
Presented at the 32nd Cologne Workshop on Dope Analysis, 2014

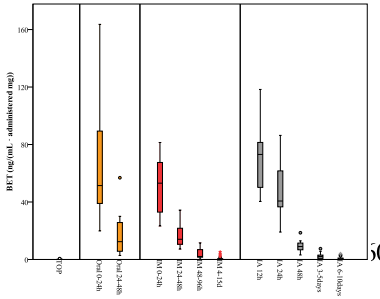
# EXCRETION PROFILES

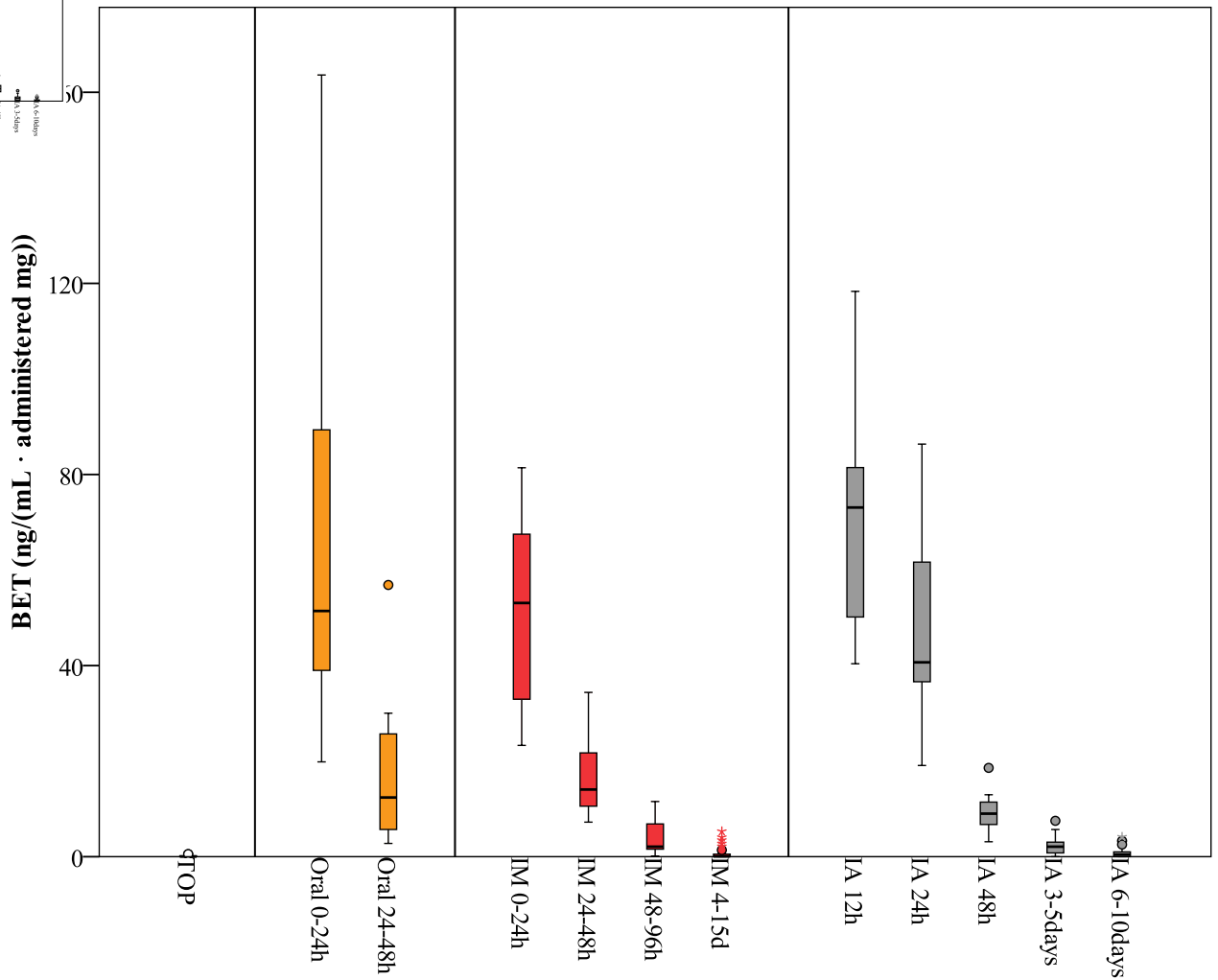
## BETAMETHASONE



# Excretion studies:

## topical, oral, IM, IA and soft-tissue





# Excretion studies:

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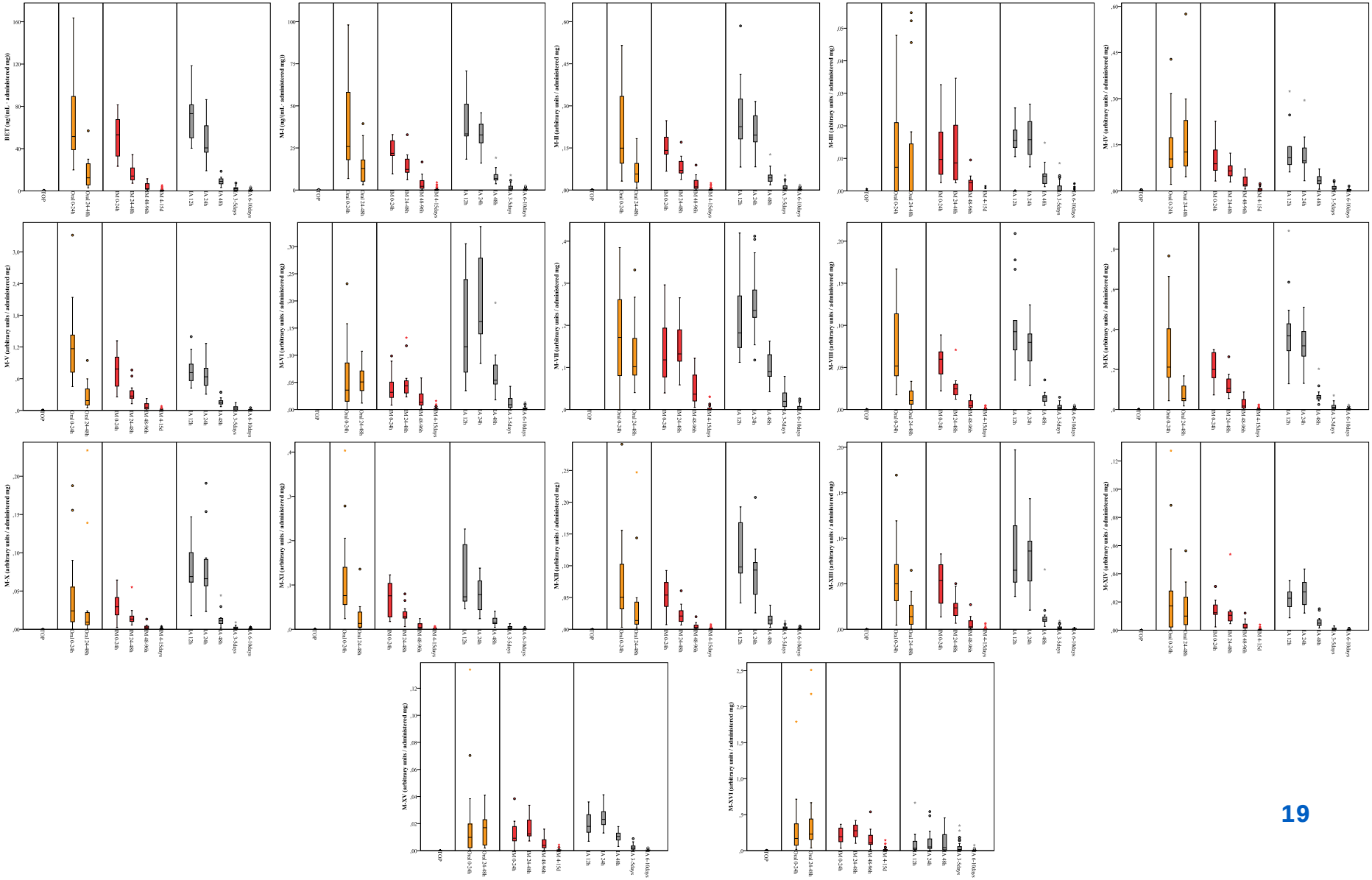
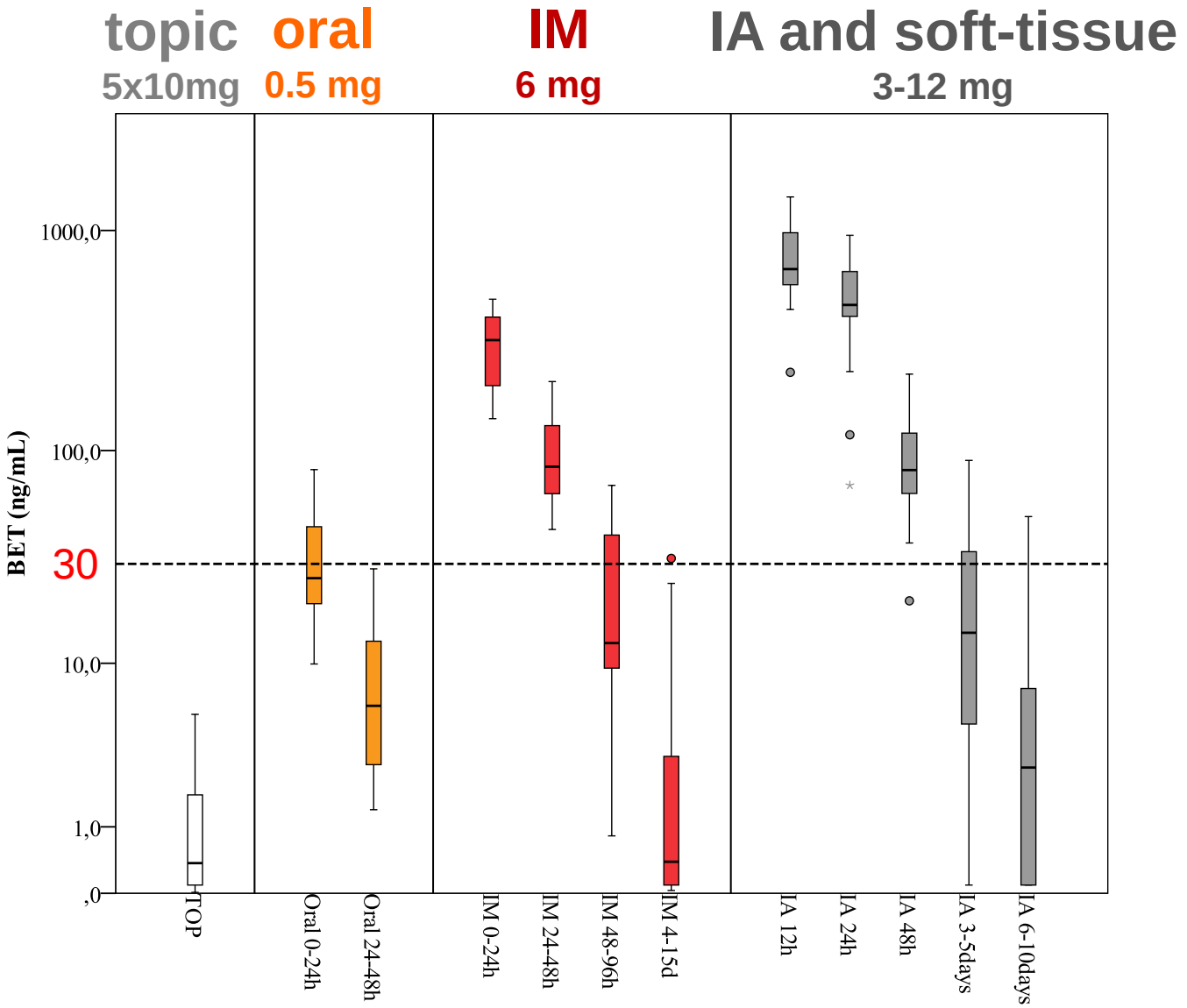


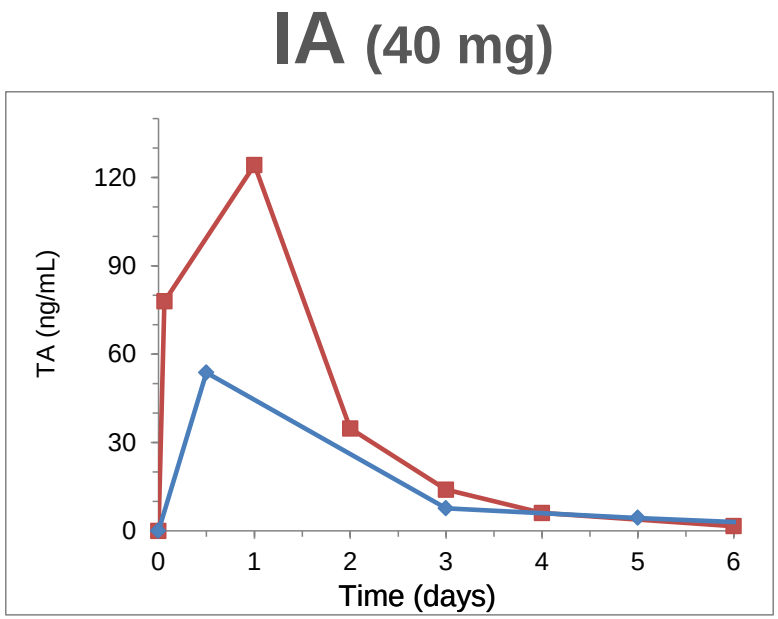
# EXCRETION PROFILES

## BETAMETHASONE



# EXCRETION PROFILES

## TRIAMCINOLONE ACETONIDE

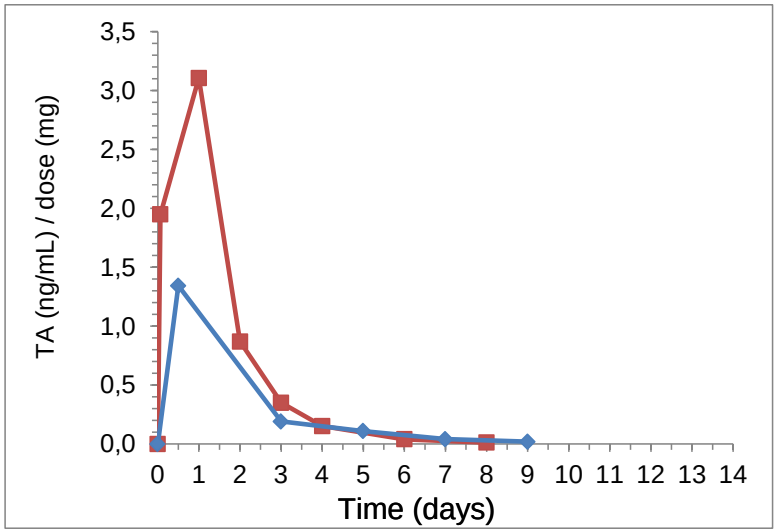


# EXCRETION PROFILES

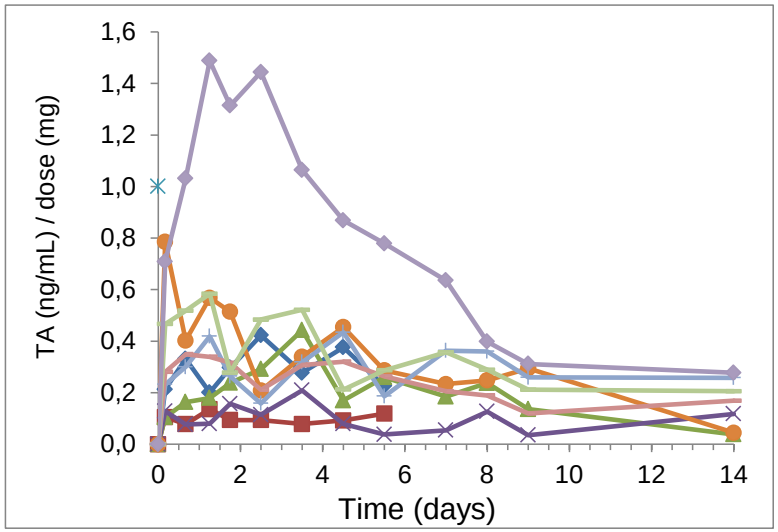
## TRIAMCINOLONE ACETONIDE

## IA *vs.* IM

### IA (40 mg)




### IM (20 mg)

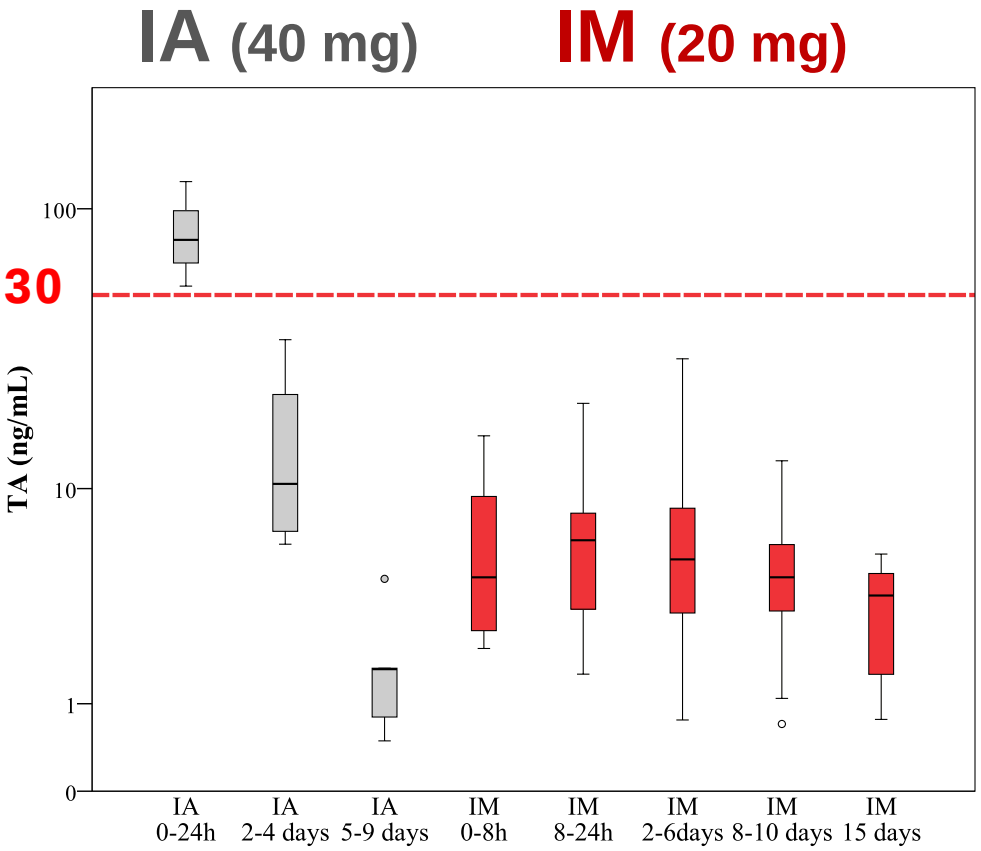



Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. *Evaluation of the reporting level to detect triamcinolone acetonide misuse in sports.* J Steroid Biochem Mol Biol 2014;145:94-102.

# EXCRETION PROFILES

## TRIAMCINOLONE ACETONIDE

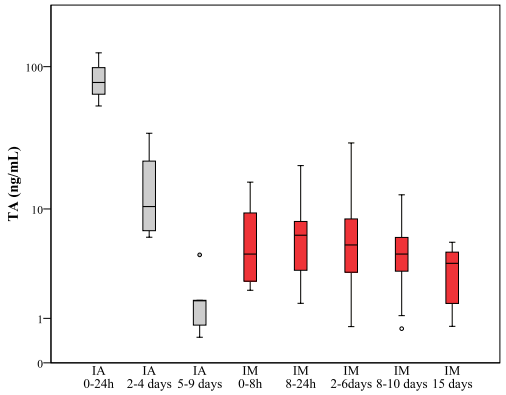
## IA *vs.* IM

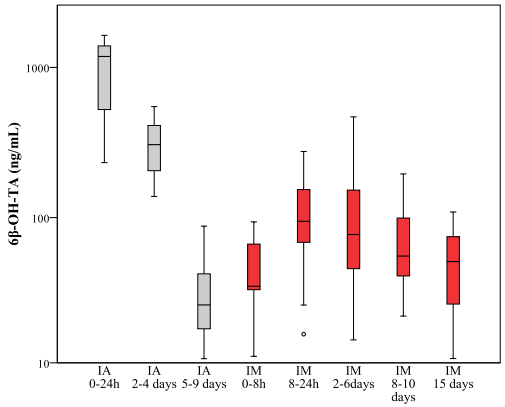


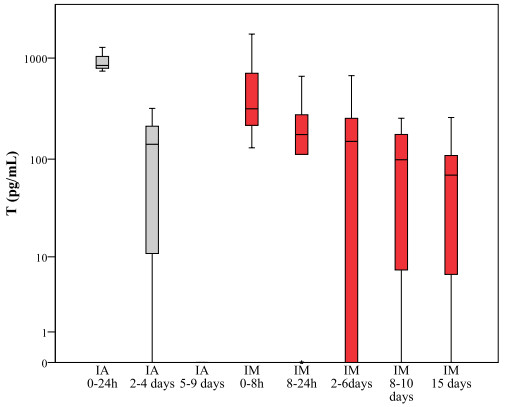

Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. *Evaluation of the reporting level to detect triamcinolone acetonide misuse in sports.* J Steroid Biochem Mol Biol 2014;145:94-102.

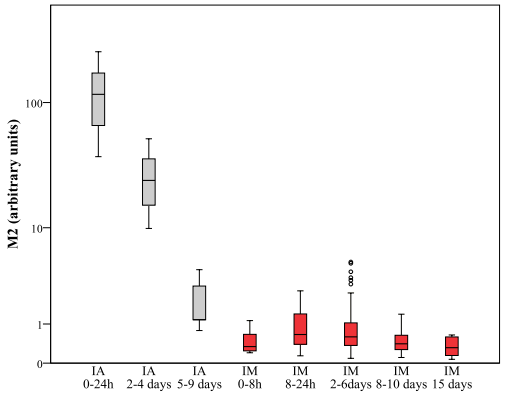
# EXCRETION PROFILES

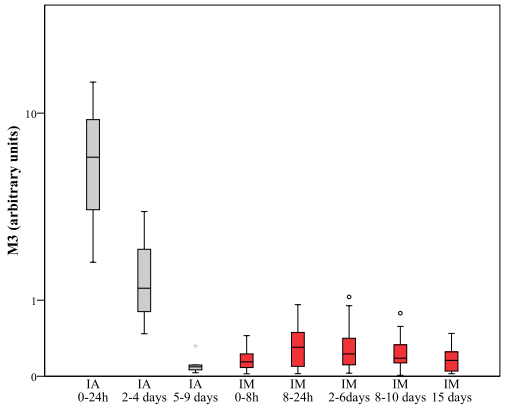
## TRIAMCINOLONE ACETONIDE, IA *vs.* IM

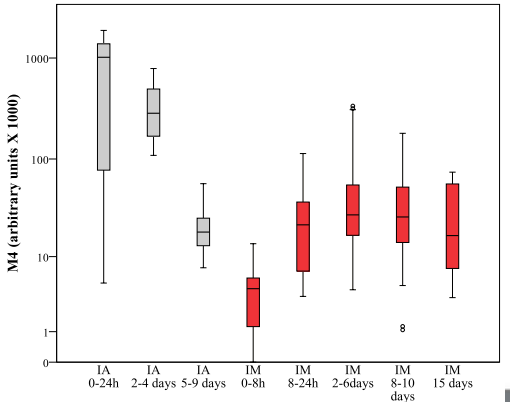


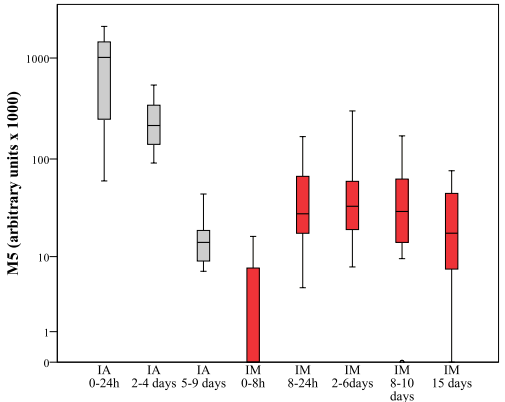



Have IA and soft-tissue administrations
the same systemic effects
as IM use ?

# BETAMETHASONE PLASMA CONCENTRATIONS

## IA *vs.* IM

### IA (7 mg)

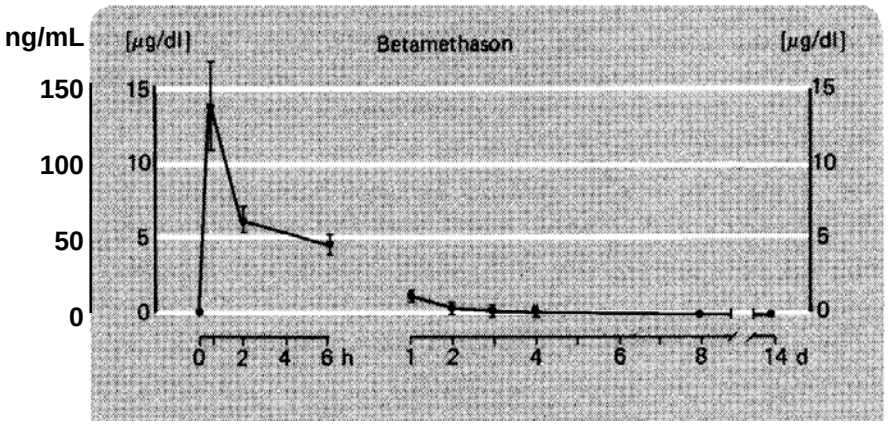
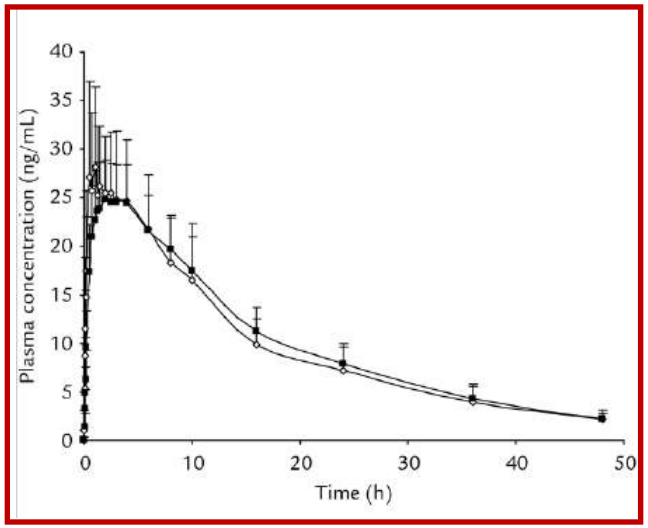



Abb. 1. Plasmakonzentrationen von Betamethason, Cortisol und Corticosteron nach intraartikulärer Injektion von Betamethason bei 31 Patienten mit Gelenkerkrankungen unterschiedlicher Ätiologie. Mittelwerte und Standardfehler des arithmetischen Mittels.

Gless et al.
*Plasmakonzentration und systemische wirkung von betamethason nach intraartikulärer injektion.*
Deutsche Medizinische Wochenschrift 1981;106(22):704-7.

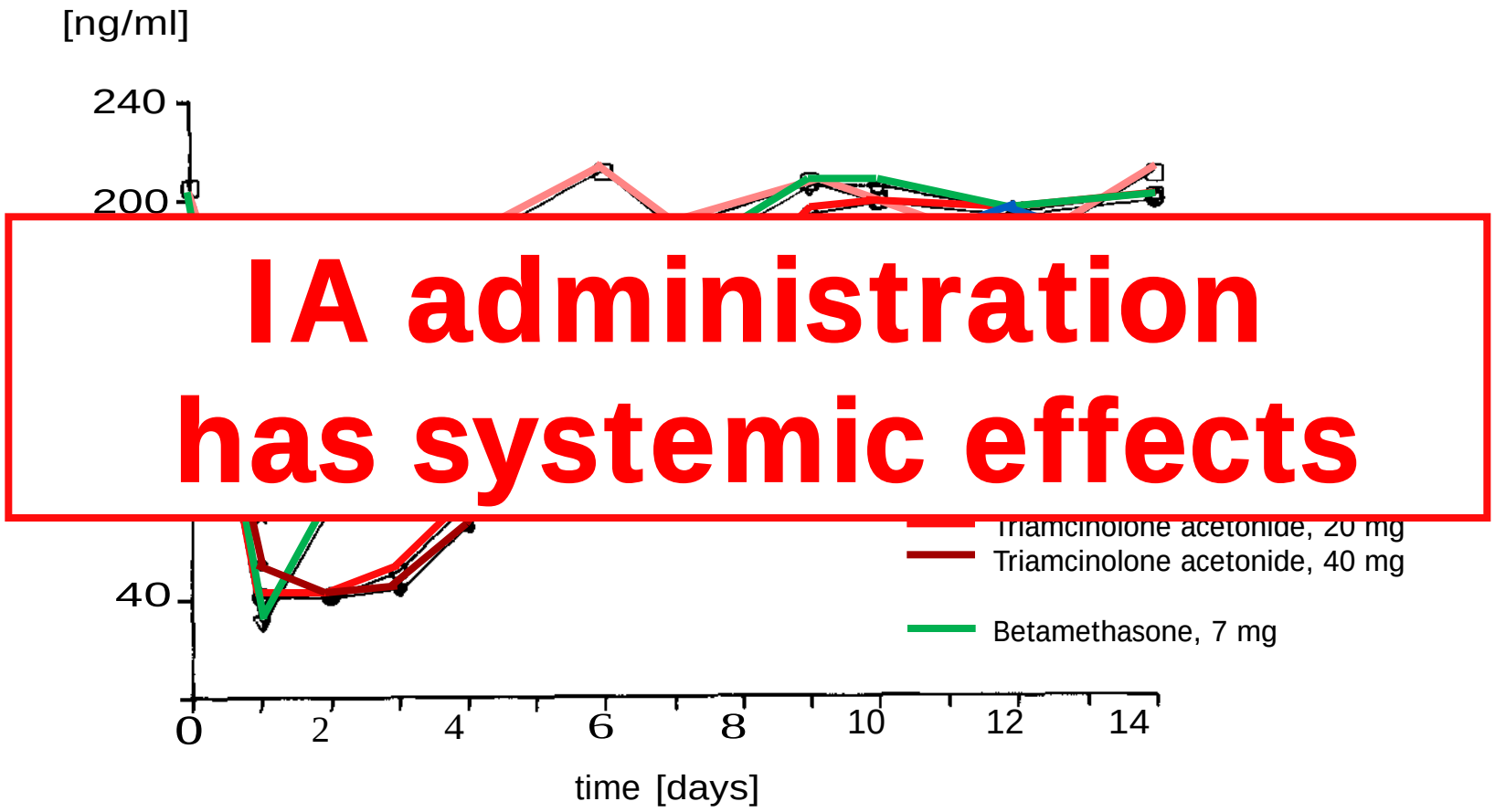
### IM (6 mg)




Salem and Najib
*Pharmacokinetcs of betamethasone after single-dose intramuscular administration of betamethasone phosphate and betamethasone acetate to healthu volunteers.*
Clinical Therapeutics 2012;34(1):214-20.

**IA use has the same systemic effects as IM**

# INTRA-ARTICULAR ADIMINISTRATION
## Cortisol in plasma




**IA administration has systemic effects**

Derendorf H, Mollmann H, Grilner A, Haack D, Gyselby G.
*Pharmacokinetics and pharmacodynamics of glucocorticoid suspensions after intraarticular administration.*
Clin Pharmacol Ther 1986; 39:313-7

# CONCLUSIONS

- Urinary concentrations of BET and TA and their metabolites after IA and soft-tissue administrations have been measured.
- Urine elimination kinetics after IA and soft-tissue administrations are not equal to those after IM administration for all glucocorticoids.
- After IA and soft-tissue administrations:
  - Concentrations of BET were greater than 30 ng/ml during 48 h
  - Concentrations of TA were greater than 30 ng/ml during 24 h

  resulting in “false positive” results according to the current rules.
- The status of IA in the prohibited list should be reviewed due to demonstrated systemic effect.
- Although according to data available, a similar systemic effect between soft-tissue and IA administrations can be predicted, the systemic effect of soft-tissue administration needs to be proved.

# Acknowledgements


- **World Anti-Doping Agency**
  grant 13D22RV

- **Ministerio de Economía y Competitividad**
  grant DEP2009-11454

- **Consell Català de l'Esport, Generalitat de Catalunya**


**Thank you for your attention**

*Gràcies per la vostra atenció*