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Urinary profiles of corticosteroids after intra-articular and related administrations

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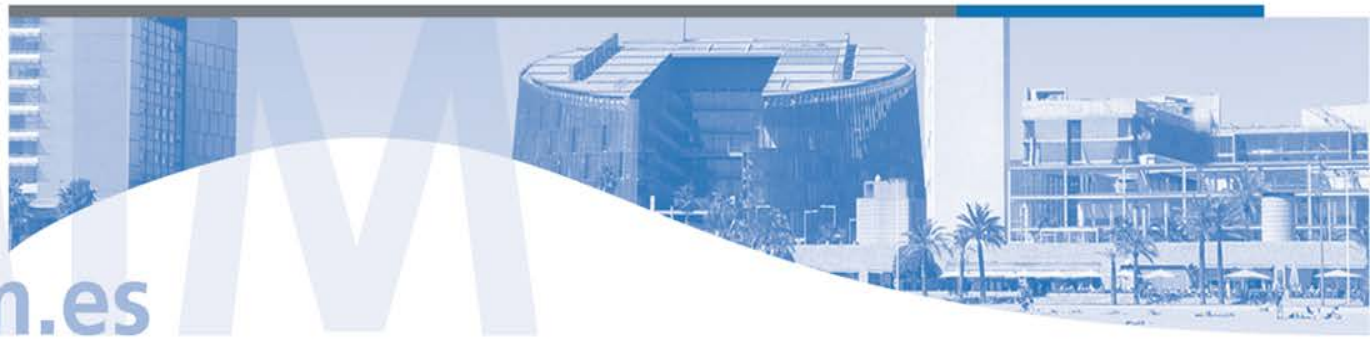
33rd Cologne Workshop on Dope Analysis, March 2015



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

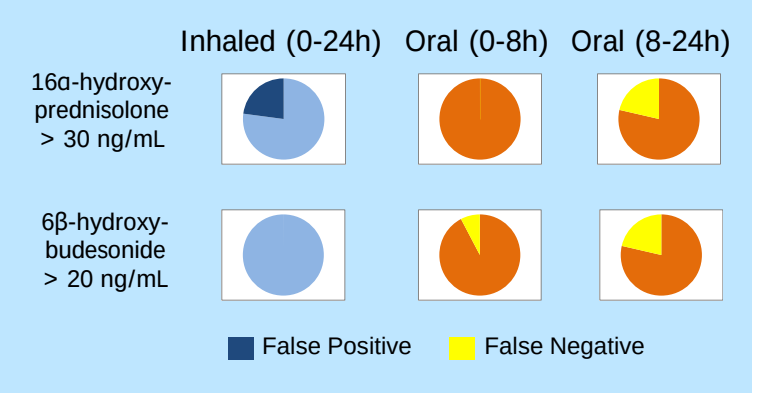
Matabosch X, Pozo OJ, Pérez-Mañá C, Farré M, Marcos J, Segura J, Ventura R.
Discrimination of prohibited oral use from authorized inhaled treatment of budesonide in sports.
Ther Drug Monit 2013;35(1):118-218

Glucocorticoids
forbidden / allowed administrations

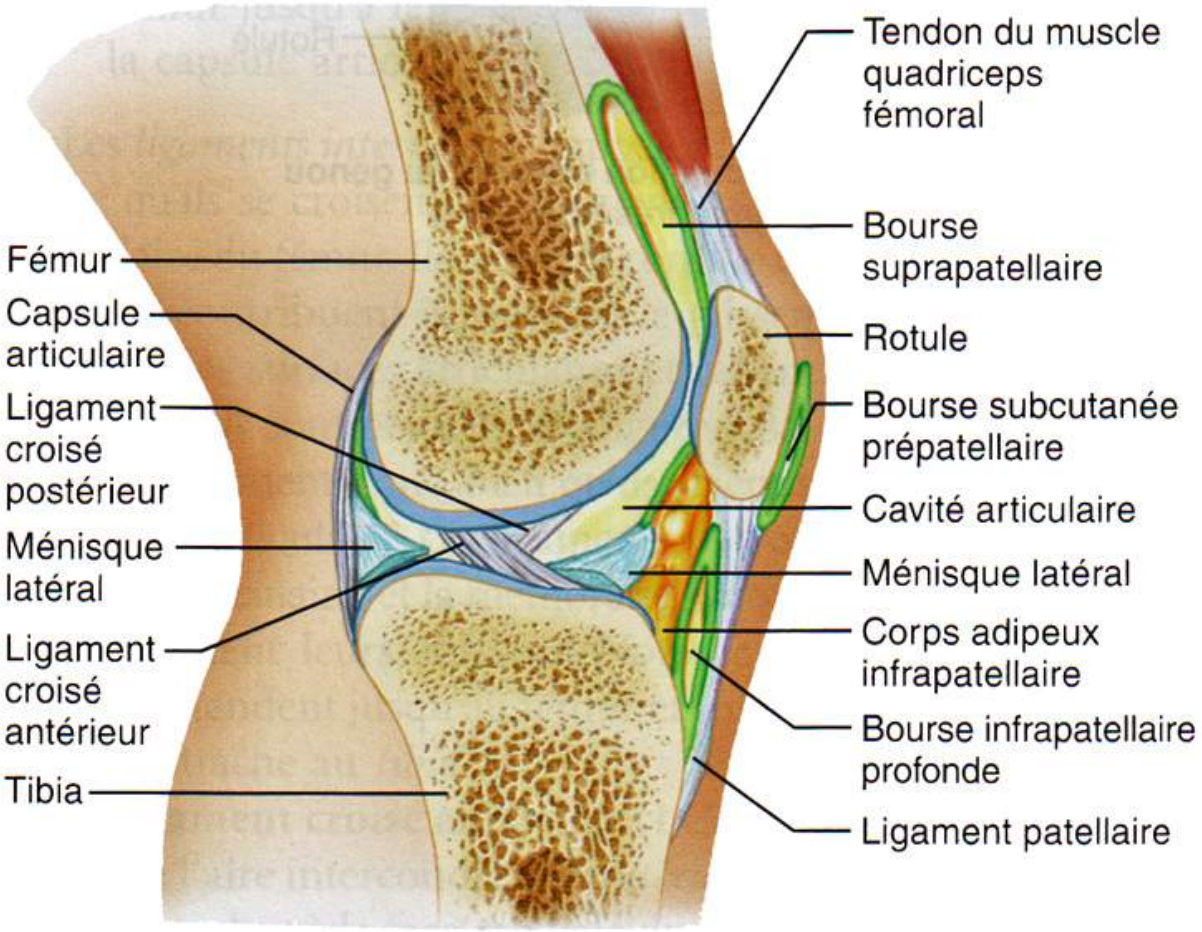
- Classification of routes of administration
- Discrimination between routes of administration:
  - To detect cheating athletes
  - To protect innocent athletes

BUDESONIDE: inhaled vs. oral

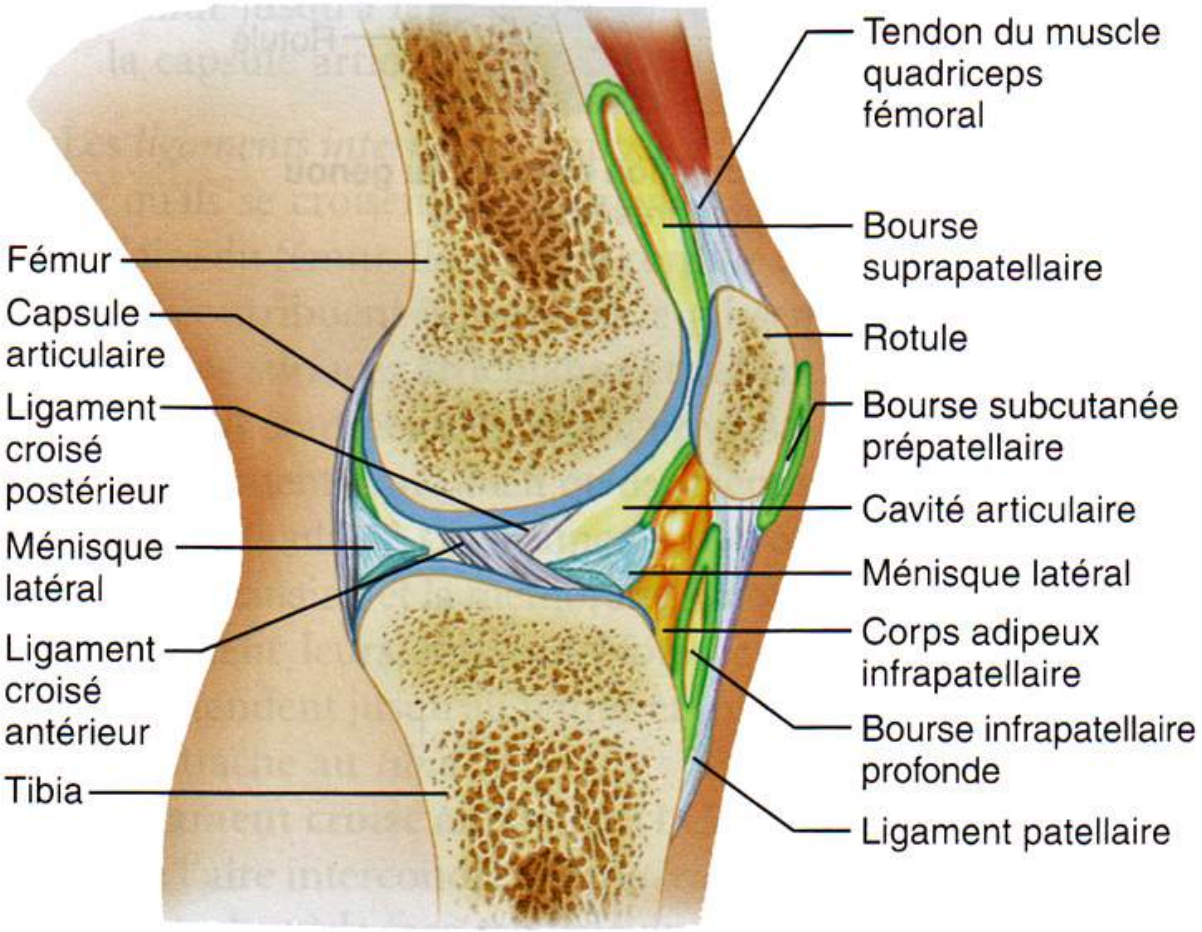
- 16α-hydroxy-prednisolone was not a good marker !!!!!!
- Best marker: **6β-hydroxy-budesonide**



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

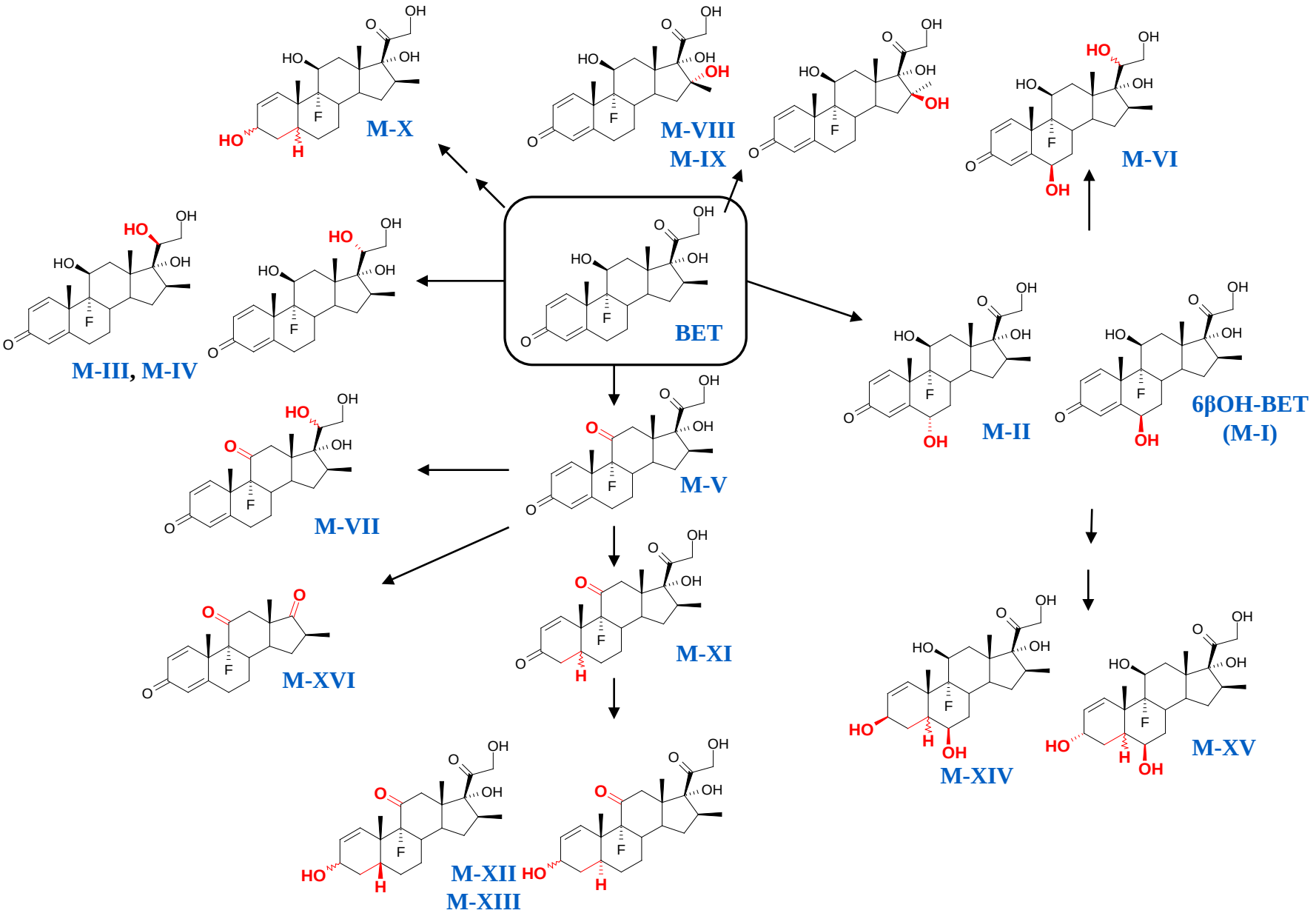
- Matabosch X, Pozo OJ, Monfort N, Pérez-Mañá C, Farré M, Segura J, Ventura R. Drug Test Anal 2014, doi: 10.1002/dta.1770.
- Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. J Steroid Biochem Mol Biol 2014;145:94-102.

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	

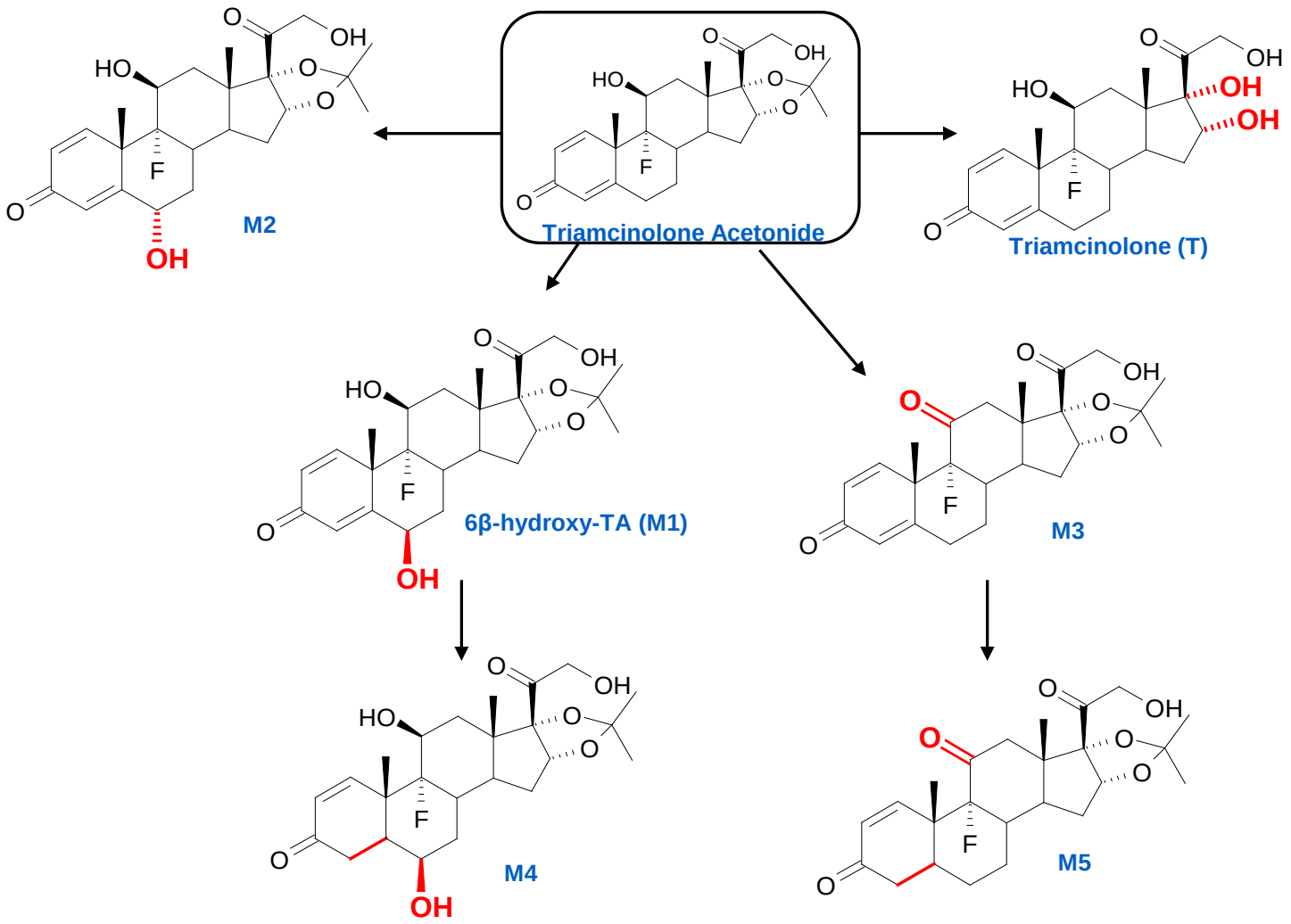
Collision gas:	Ar
Ionization mode:	ESI, positive/negative
Source T:	120ºC
Desolvation T:	450ºC
Detection mode:	SRM

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

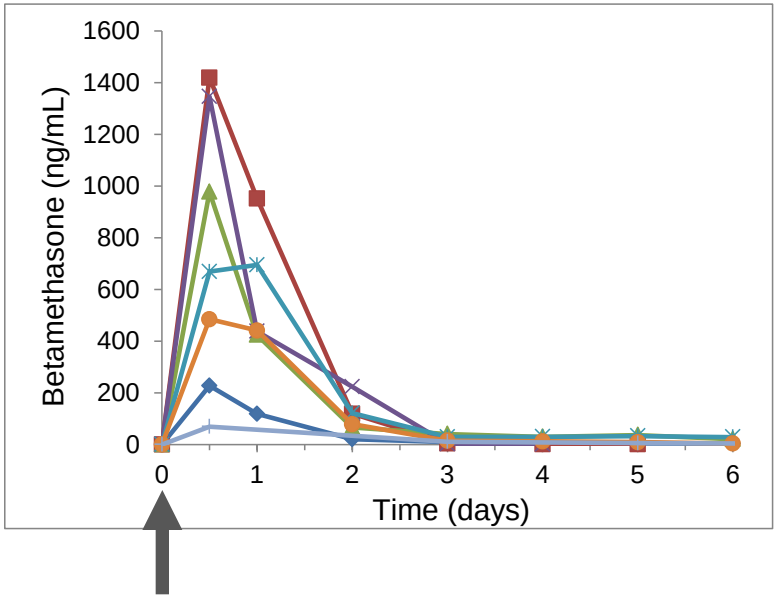


Matabosch X, Pozo OJ, Papaseit E, Farré M, Marcos J, Segura J, Ventura R.
Rapid Commun Mass Spectrom 2014;28(16):1829-39.

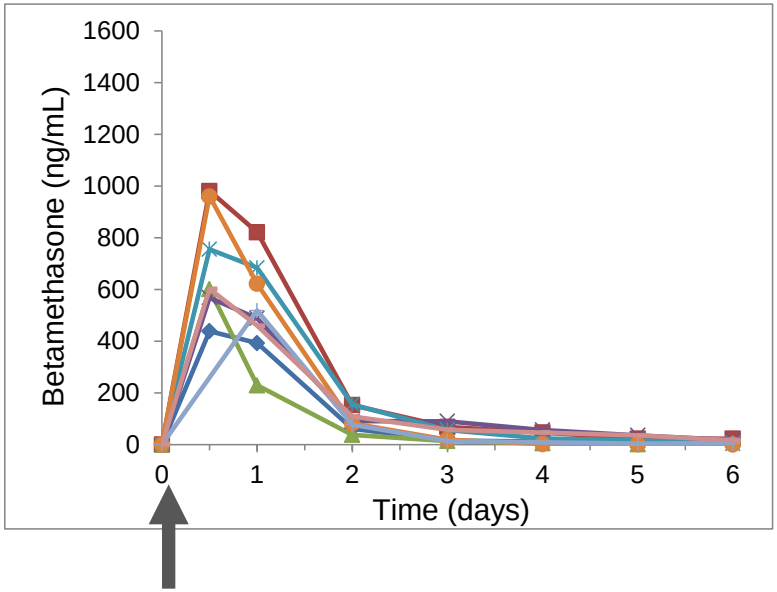
EXCRETION PROFILES

BETAMETHASONE

IA (3-12 mg)



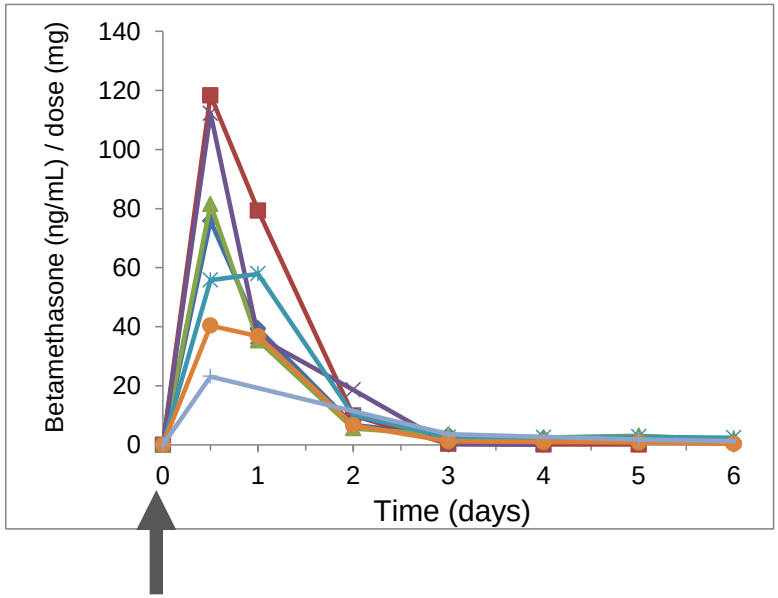
Soft-tissue (6-12 mg)



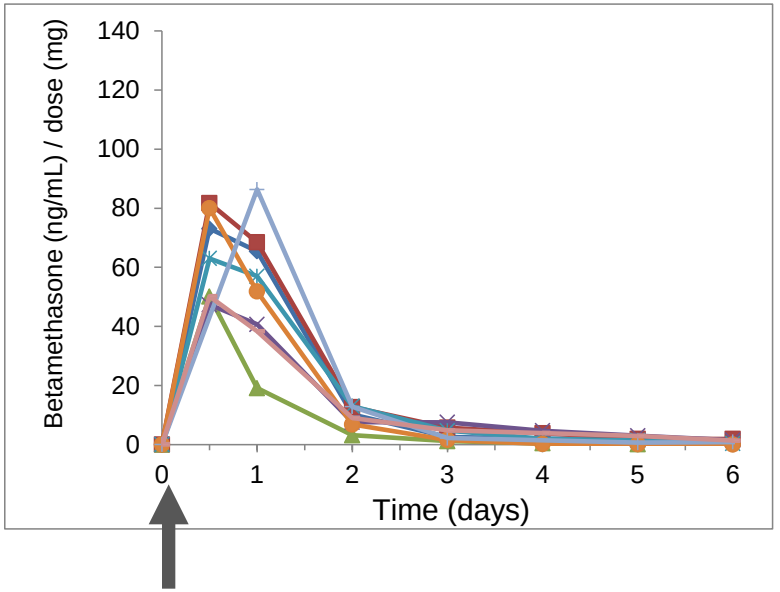
EXCRETION PROFILES

BETAMETHASONE

IA (3-12 mg)



Soft-tissue (6-12 mg)

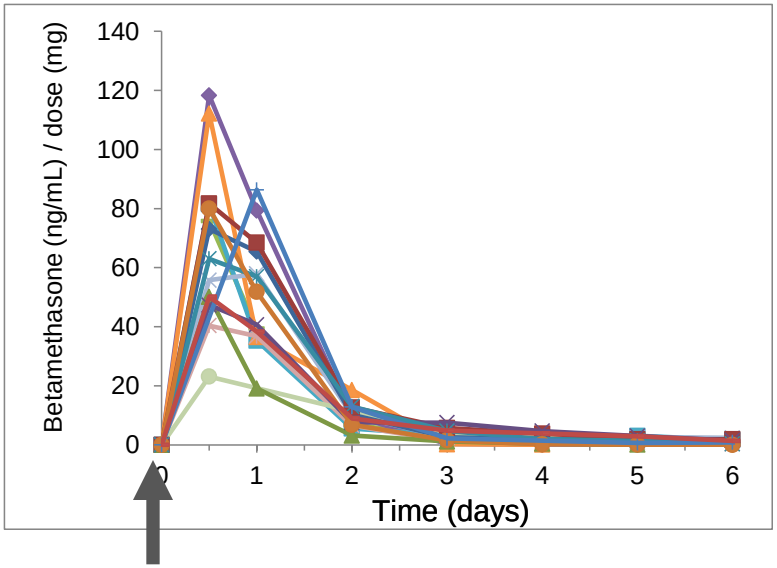


EXCRETION PROFILES

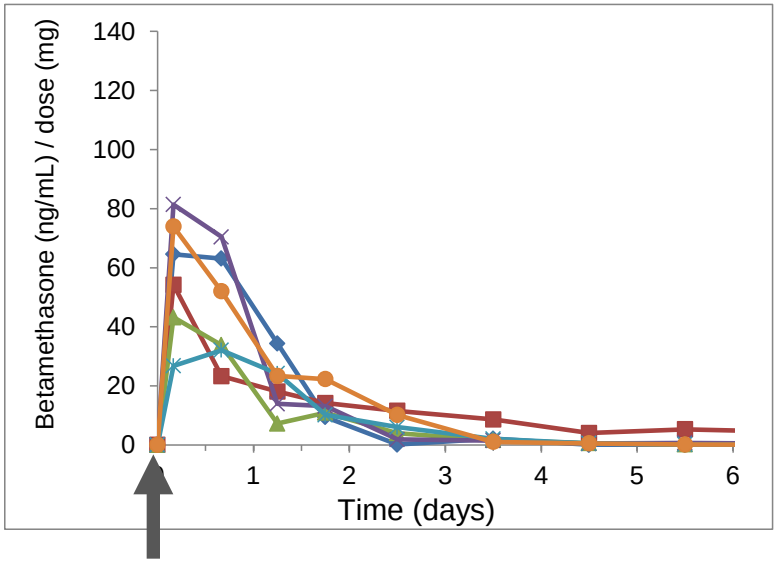
BETAMETHASONE

IA, soft-tissue *vs.* IM

IA and soft-tissue



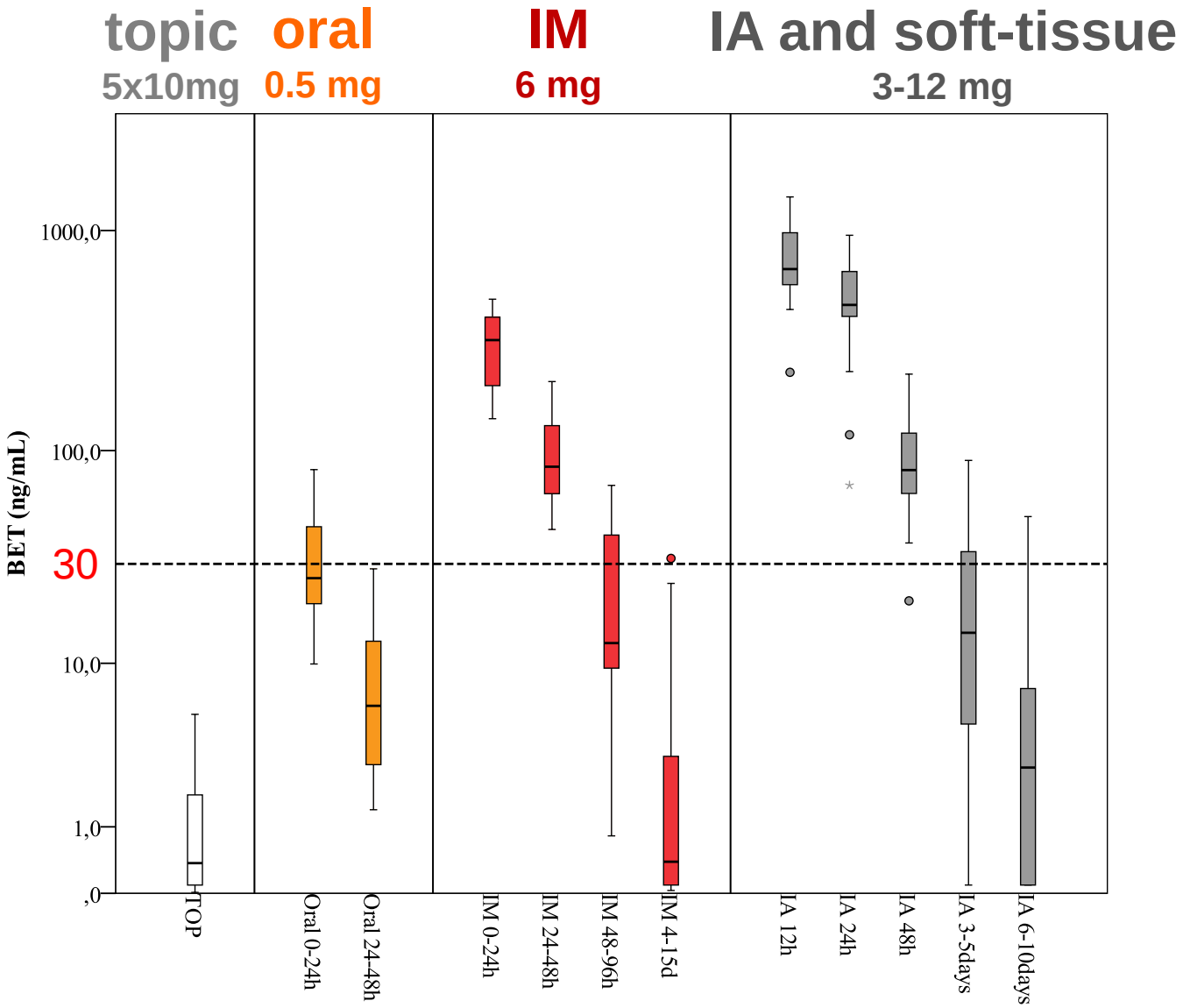
IM



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

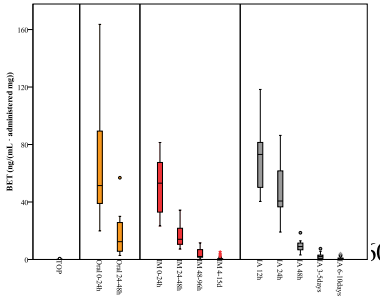
EXCRETION PROFILES

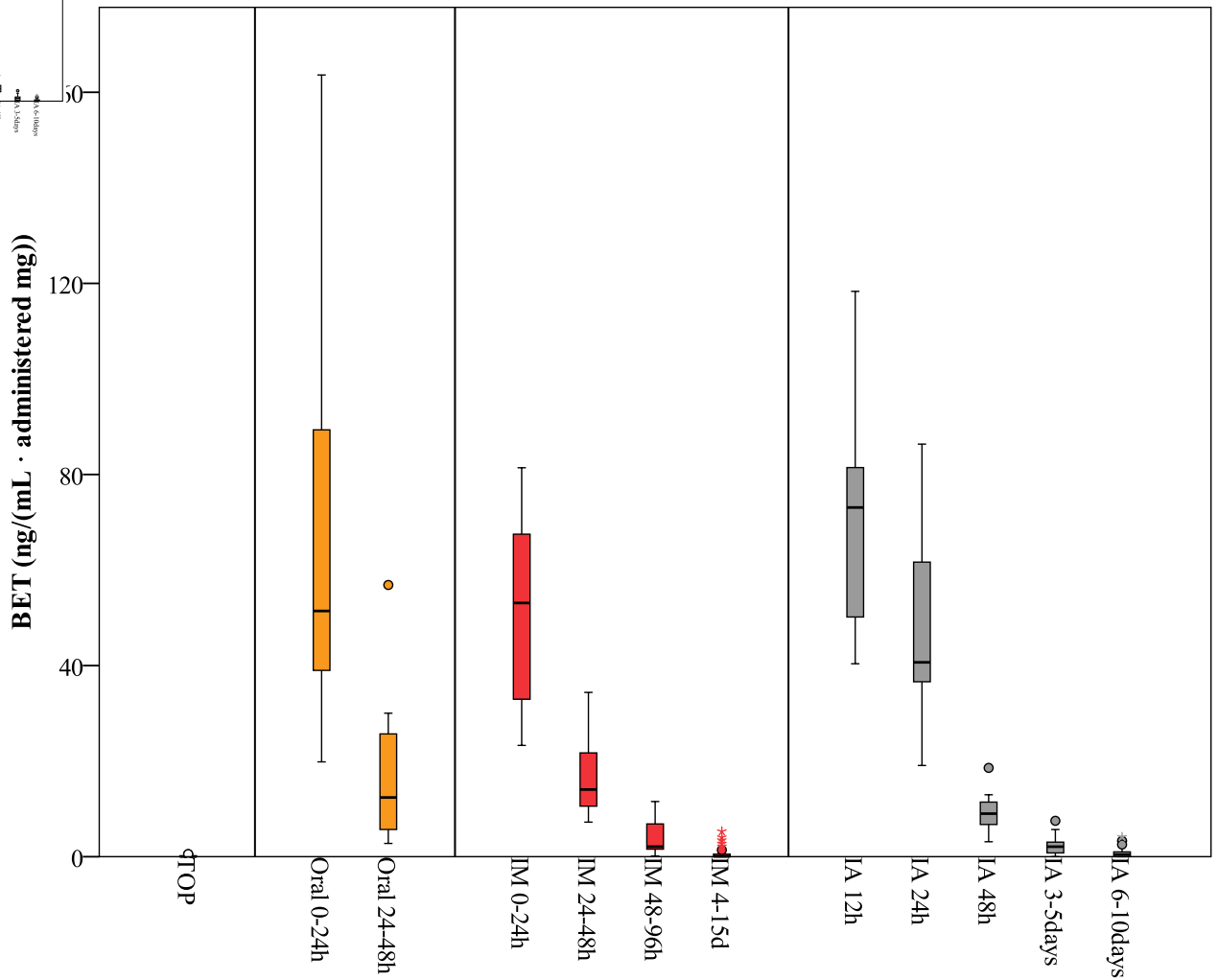
BETAMETHASONE



Excretion studies:

topical, oral, IM, IA and soft-tissue





Excretion studies:

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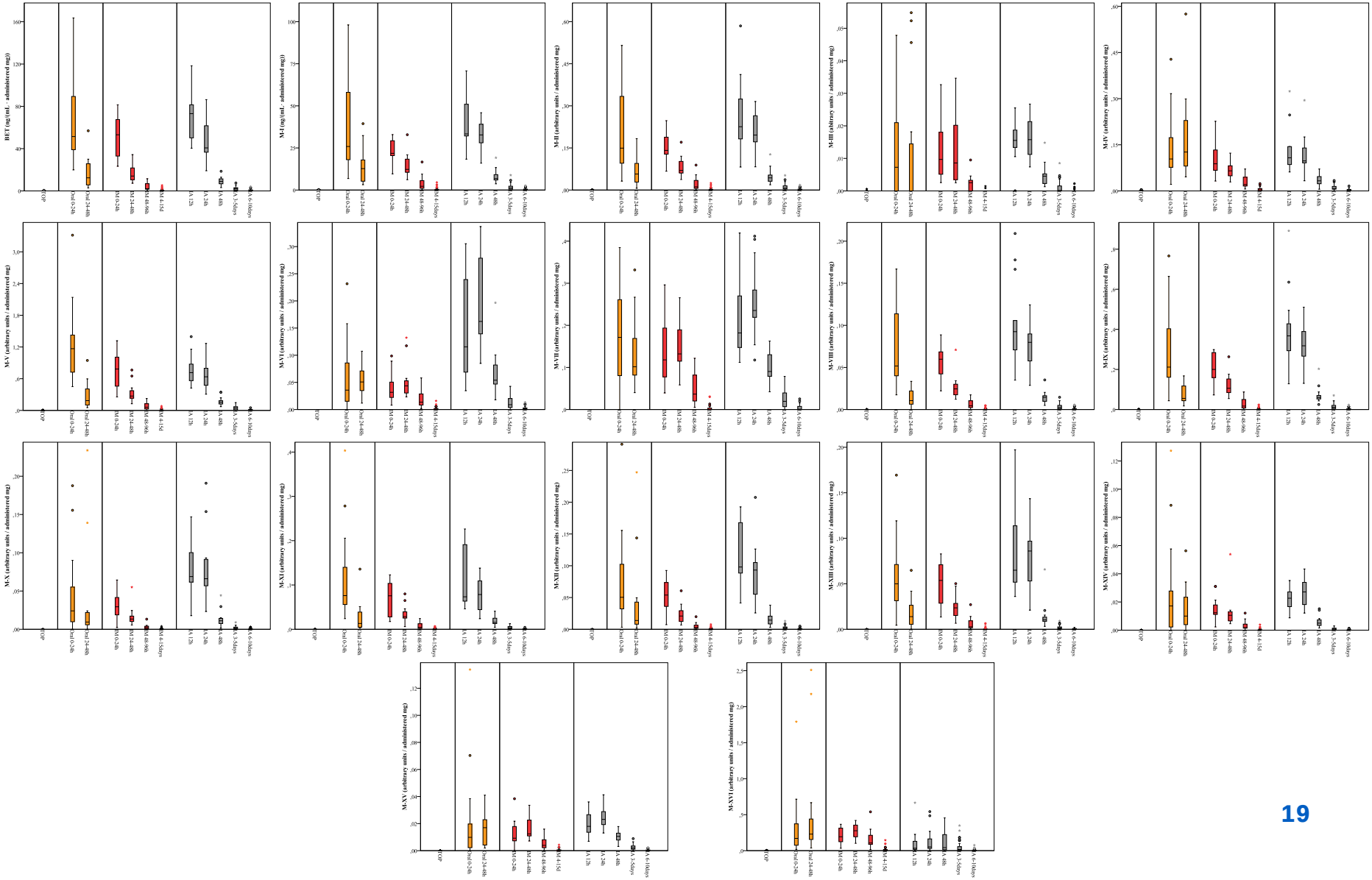
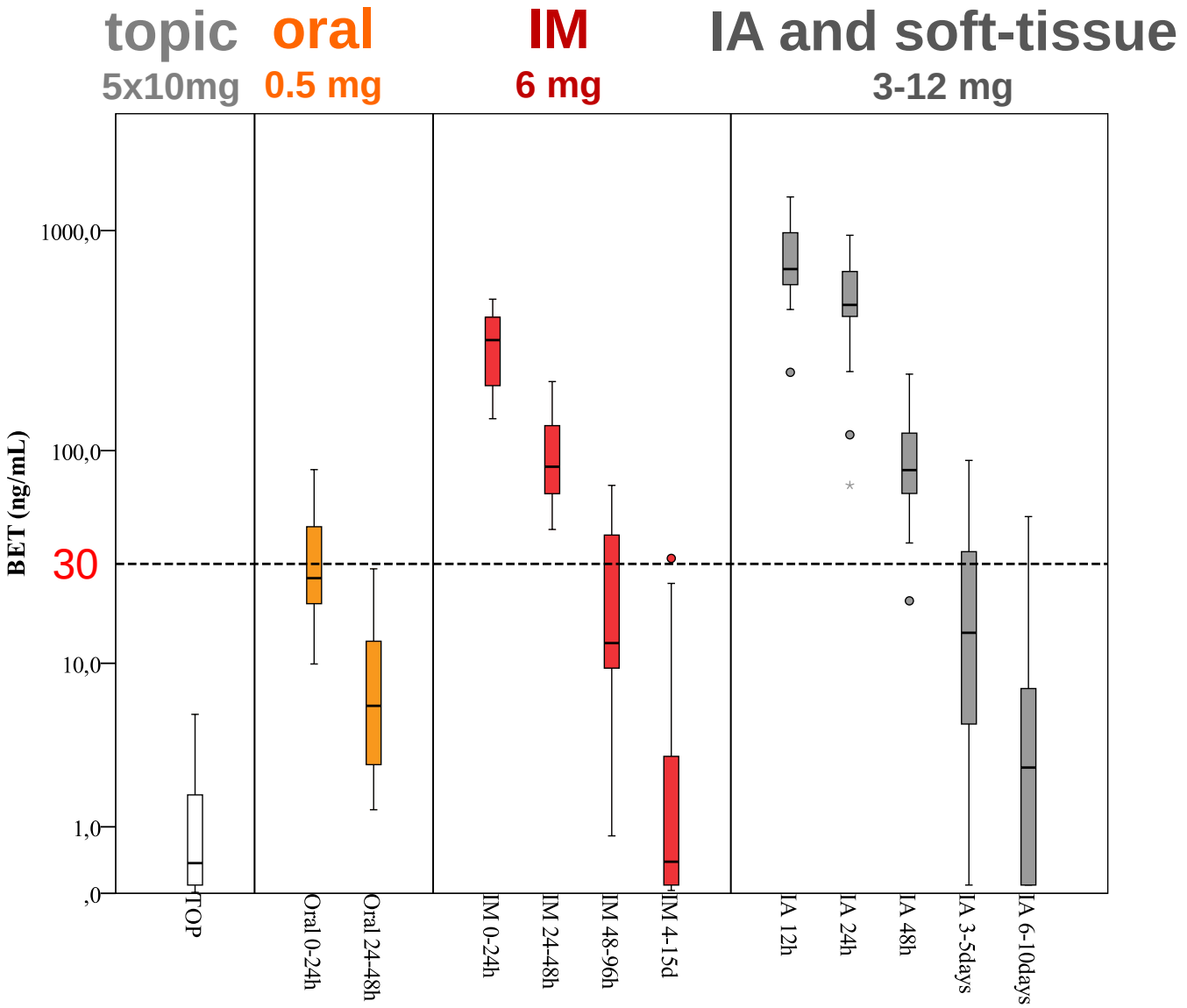


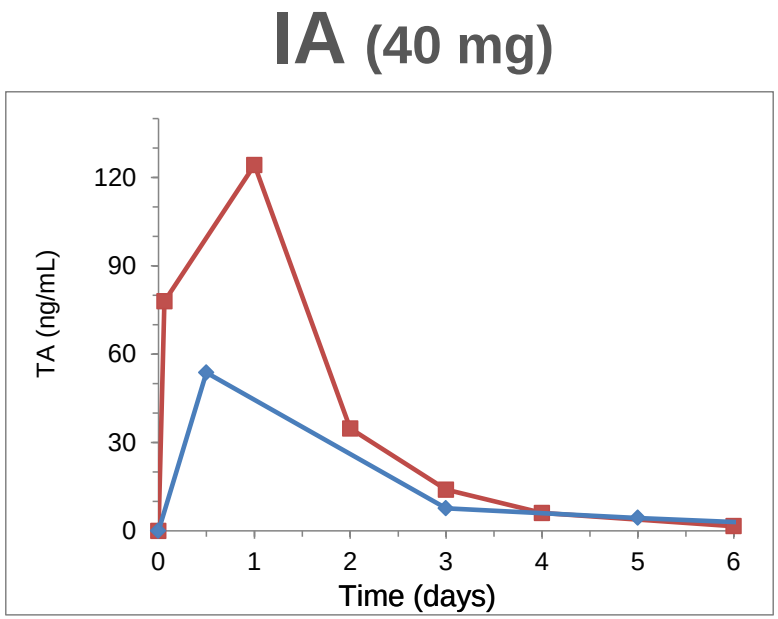
EXCRETION PROFILES

BETAMETHASONE



EXCRETION PROFILES

TRIAMCINOLONE ACETONIDE

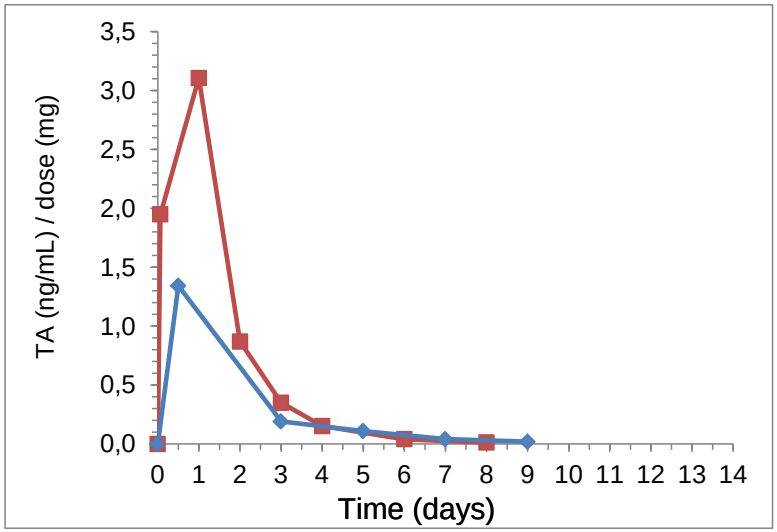


EXCRETION PROFILES

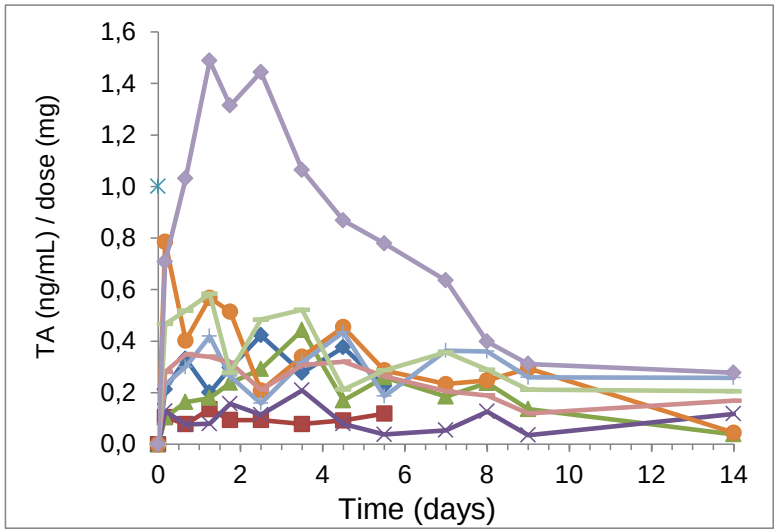
TRIAMCINOLONE ACETONIDE

IA *vs.* IM

IA (40 mg)



IM (20 mg)

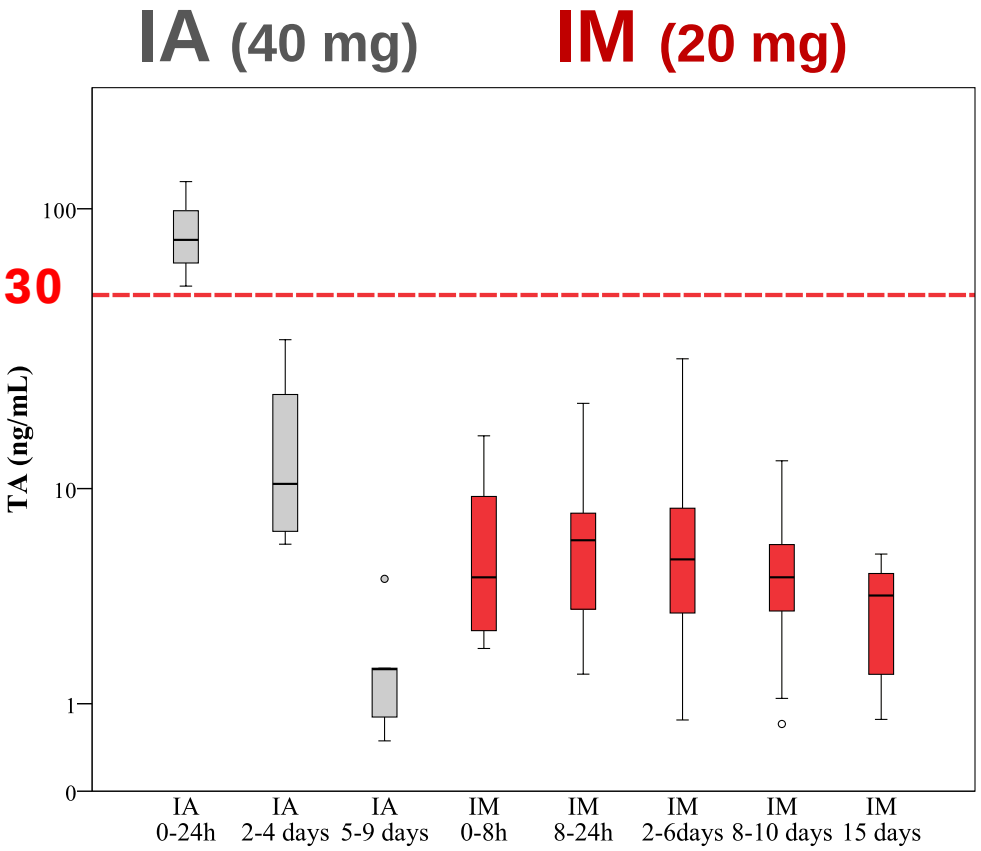


Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. *Evaluation of the reporting level to detect triamcinolone acetonide misuse in sports.* J Steroid Biochem Mol Biol 2014;145:94-102.

EXCRETION PROFILES

TRIAMCINOLONE ACETONIDE

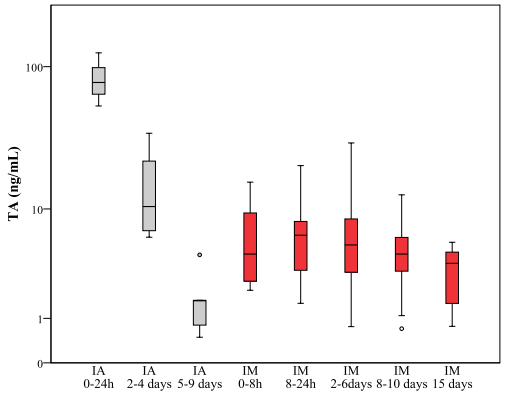
IA *vs.* IM

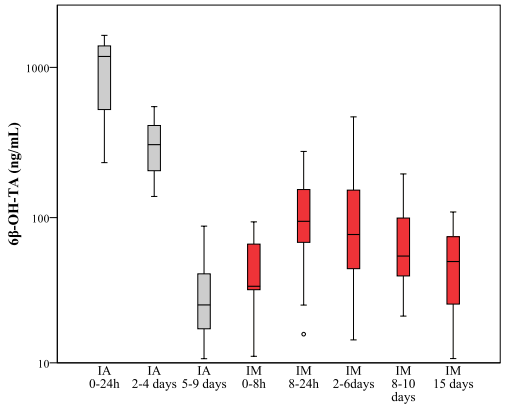


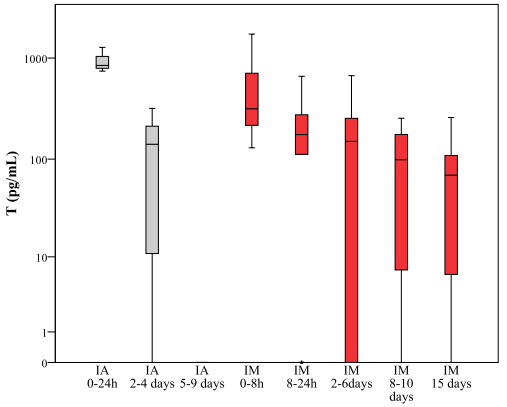
Matabosch X, Pozo OJ, Pérez-Mañá C, Papaseit E, Marcos J, Segura J, Ventura R. *Evaluation of the reporting level to detect triamcinolone acetonide misuse in sports.* J Steroid Biochem Mol Biol 2014;145:94-102.

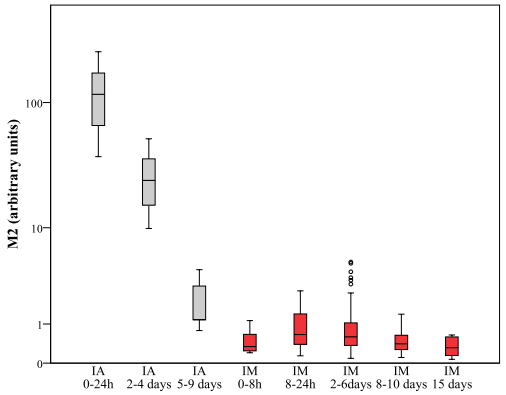
EXCRETION PROFILES

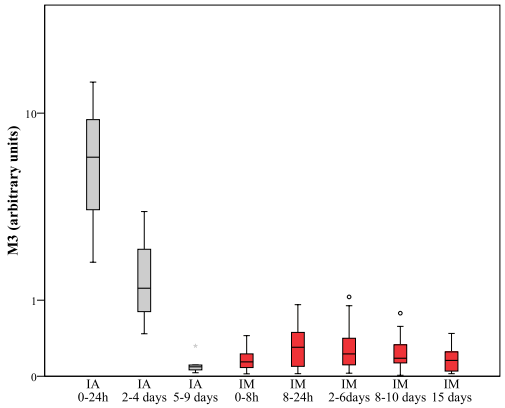
TRIAMCINOLONE ACETONIDE, IA *vs.* IM

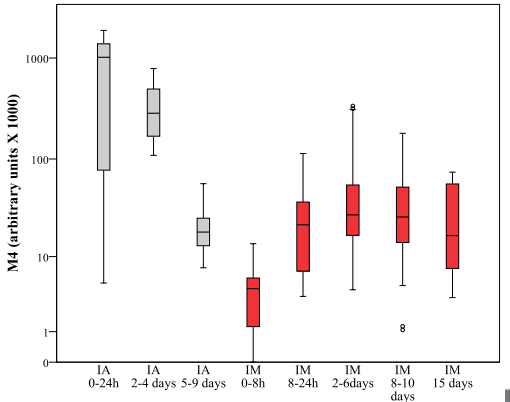


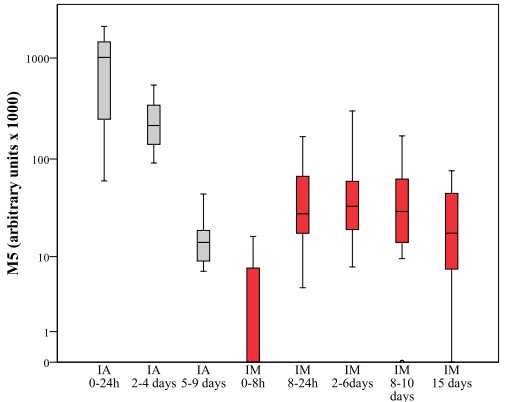












Have IA and soft-tissue administrations
the same systemic effects
as IM use ?

BETAMETHASONE PLASMA CONCENTRATIONS

IA *vs.* IM

IA (7 mg)

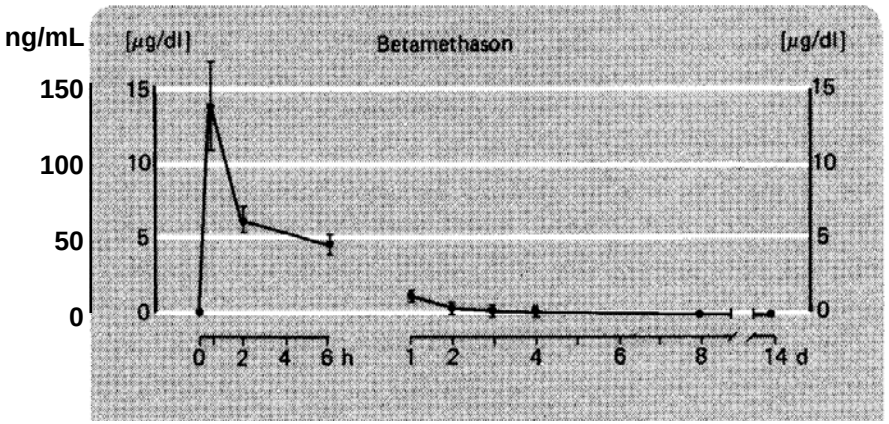
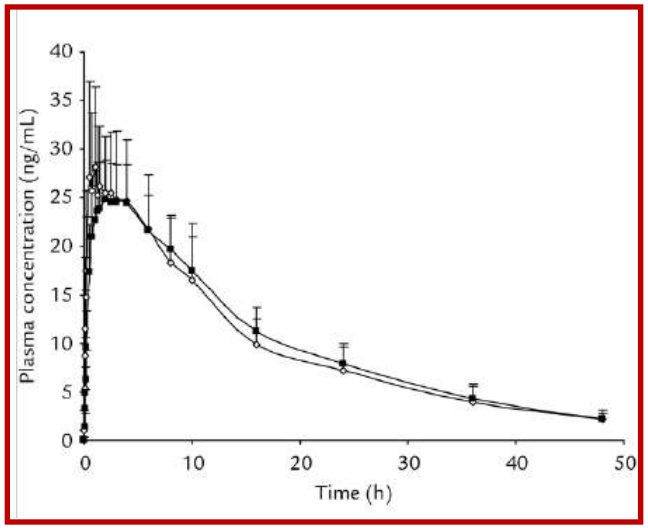


Abb. 1. Plasmakonzentrationen von Betamethason, Cortisol und Corticosteron nach intraartikulärer Injektion von Betamethason bei 31 Patienten mit Gelenkerkrankungen unterschiedlicher Ätiologie. Mittelwerte und Standardfehler des arithmetischen Mittels.

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

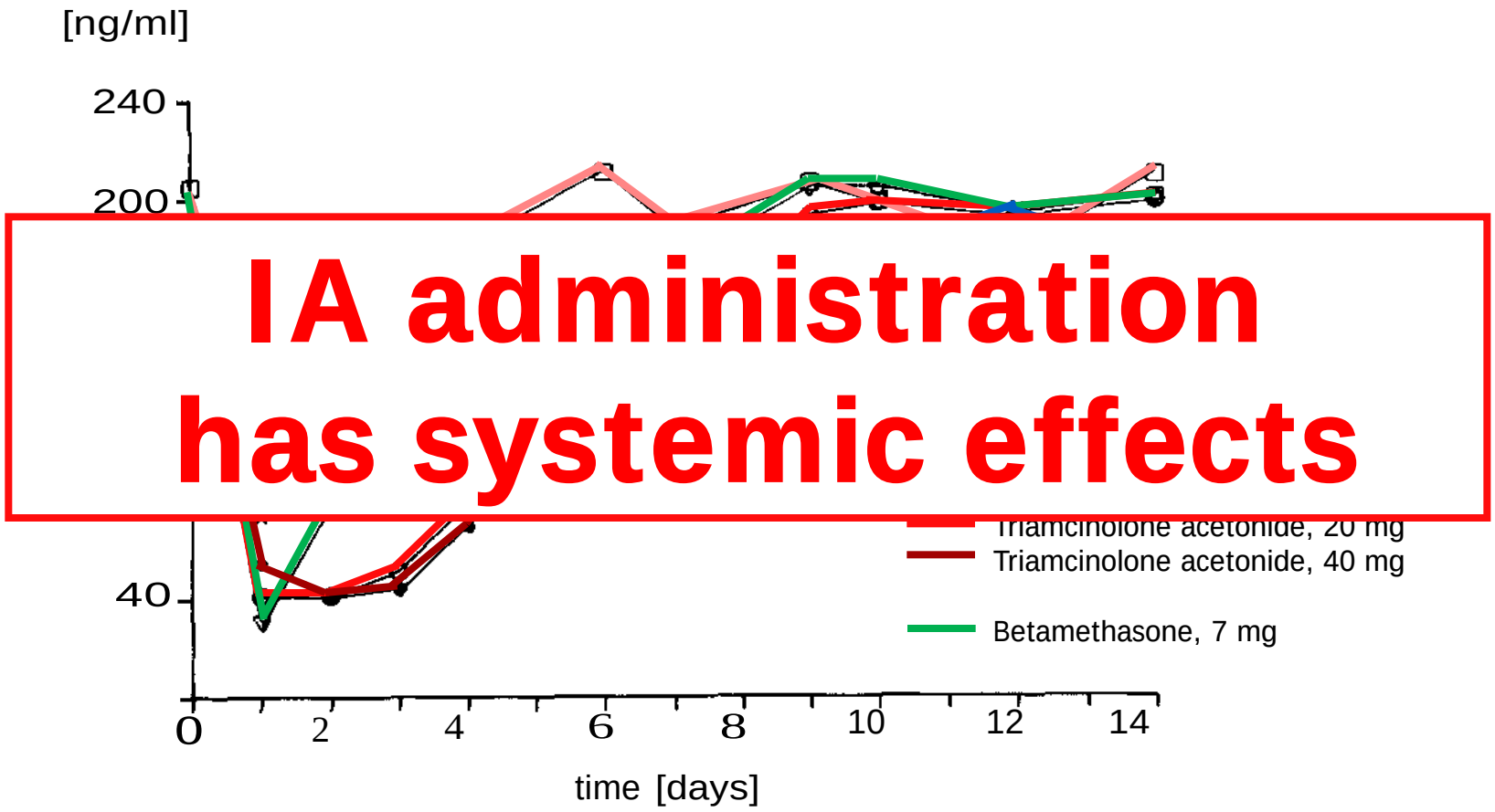
IM (6 mg)



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