

# Simulation of the effect of hyperhydration on urine levels of recombinant human erythropoietin from a doping control point of view



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## Introduction

Erythropoietin (EPO), a glycoprotein hormone mainly produced and secreted by the kidneys, is the main hormonal regulator of red blood cell (RBC) production (erythropoiesis). It increases the number of RBC in circulation and dramatically improves the blood oxygen-carrying capacity and therefore endurance performance. Since 1988, recombinant human erythropoietin (rHuEPO) has been available for the treatment of anemia associated with chronic renal failure, chemotherapy, HIV, and surgical procedures. The abuse of rHuEPO is prohibited by the World Anti-Doping Agency (WADA) [1], it is detected based on isoelectric-focusing analysis [2] and adverse analytical findings are reported according to WADA guidelines [3].

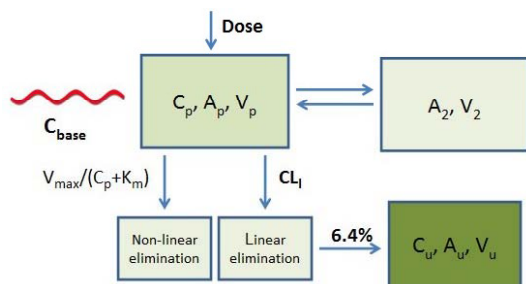
## Objectives

To study the effect of hyperhydration on the urine pharmacokinetic (PK) profile of rHuEPO and its possible role as a doping masking procedure used by athletes.

## Methods

### Simulations

1000 subjects administered single 1500, 3000 and 6000 IU subcutaneous doses of rHuEPO were simulated, using MatLab SimBiology toolbox, by a two-compartment popPK model [4] with a fast and a slow absorption rate and both first order and Michaelis-Menten elimination kinetics:



**Scheme 1:** Schematic representation of the pharmacokinetic model ( $C_p$ ,  $A_p$ ,  $V_p$ ) used in conjunction to the urine compartment ( $C_u$ ,  $A_u$ ,  $V_u$ ).

Endogenous EPO concentration ( $C_{base}$ ) varies diurnally and was modelled using the sum of two cosine functions with periods of 12 and 24 h [4].

In healthy persons, basic urinary excretion of EPO is less than 5% of the injected dose, corresponding to the 6.4% of the linear clearance [5].

Urine production was modelled based on the renal regulation of urine volume [6] and the conditions of water retention [7].

According to the circadian pattern of urine production and the normal water balance, 24 h were divided in three intervals simulating a urine sampling collection schedule in order to evaluate the effect of hyperhydration (Table 2).

Diurnal variation on urine output and rate production was modelled using data regarding the renal response to an acute water load [7].

**Table 1:** Calculation of urine rate production (ml/min) and water excretion (%) during 24 h.

| Variable                     | Time                     |                          |                          | Total |
|------------------------------|--------------------------|--------------------------|--------------------------|-------|
|                              | 07:00-10:00<br>(180 min) | 10:00-12:00<br>(120 min) | 12:00-15:00<br>(180 min) |       |
| Fluid Intake, ml             | 622                      | 415                      | 622                      | 1659  |
| Urine Output, ml             | 267                      | 178                      | 267                      | 713   |
| Urine rate, ml/min           | 1.49                     | 1.49                     | 1.49                     | -     |
| %(Urine output/Fluid intake) | 43%                      | 43%                      | 43%                      | -     |

| Variable                     | Time                     |                          |                          | Total |
|------------------------------|--------------------------|--------------------------|--------------------------|-------|
|                              | 15:00-18:00<br>(180 min) | 18:00-20:00<br>(120 min) | 20:00-23:00<br>(180 min) |       |
| Fluid Intake, ml             | 504                      | 336                      | 504                      | 1344  |
| Urine Output, ml             | 425                      | 283                      | 425                      | 1133  |
| Urine rate, ml/min           | 2.36                     | 2.36                     | 2.36                     | -     |
| %(Urine output/Fluid intake) | 84%                      | 84%                      | 84%                      | -     |

## Results

✓ rHuEPO urine concentration follows the circadian rhythm of urine production regardless of hydration state (Fig. 1A).

✓ A significant decrease on rHuEPO concentrations at early times after hyperhydration was observed compared to the normal profile (Table 2, Fig. 1A-D); the difference was more pronounced with hyperhydration administered twice daily and also as the hydration level increased (Table 2, Fig. 1A & E-G).

✓ Undetectable rHuEPO concentrations may also occur at early times after hyperhydration at all hydration levels since significant decrease of measured concentration at these time points is observed (Table 2).

✓ In addition, even in the normal PK profile (Fig. 1A), undetectable rHuEPO concentrations may also occur due to the circadian rhythm of urine production [8].

**Table 2:** rHuEPO concentration decrease (%) at early time points after hyperhydration at all hydration levels compared to normal urine profile at 3000 IU (given as median values). Similar results are obtained for the doses of 1500 and 6000 IU.

| Day                    | % rHuEPO Concentration Decrease        |                 |                 | % rHuEPO Concentration Decrease         |                 |                 |                 |                 |
|------------------------|----------------------------------------|-----------------|-----------------|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                        | Hyperhydration administered once daily |                 |                 | Hyperhydration administered twice daily |                 |                 |                 |                 |
| Day                    | 1 <sup>st</sup>                        | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 1 <sup>st</sup>                         | 2 <sup>nd</sup> | 3 <sup>rd</sup> | 1 <sup>st</sup> | 2 <sup>nd</sup> |
| Low Hydration Level    | -44.6                                  | -43.4           | -41.5           | -42.1                                   | -51.8           | -41.0           | -52.8           | -42.4           |
| Medium Hydration Level | -71.4                                  | -71.2           | -70.8           | -71.2                                   | -76.0           | -70.7           | -76.1           | -70.5           |
| High Hydration Level   | -81.0                                  | -80.4           | -79.6           | -80.7                                   | -83.9           | -80.4           | -84.1           | -80.8           |

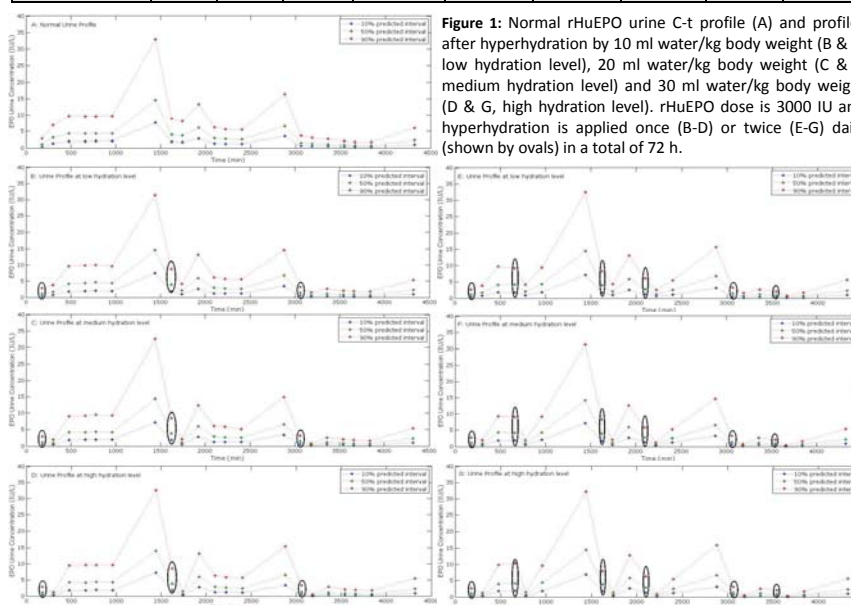


**Figure 1:** Normal rHuEPO urine C-t profile (A) and profiles after hyperhydration by 10 ml water/kg body weight (B & E, low hydration level), 20 ml water/kg body weight (C & F, medium hydration level) and 30 ml water/kg body weight (D & G, high hydration level). rHuEPO dose is 3000 IU and hyperhydration is applied once (B-D) or twice (E-G) daily (shown by ovals) in a total of 72 h.

## Conclusions

Clear effect of hyperhydration on rHuEPO urine profile was shown, supporting the reported cases of undetectable EPO urine levels. These findings may have practical implications on the right timing of urine collection during the anti-doping control sampling procedure in cases that hyperhydration is used by athletes as a masking procedure. To verify this hypothesis, a single dose rHuEPO PK study in healthy male athletes with or without hyperhydration is designed, based on the present simulation analysis.

## References

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