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## Original Research Article

# Influences of red blood cell and platelet counts on the distribution and elimination of crystalloid fluid

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## ABSTRACT

**Background and objective:** A high number of blood cells increases the viscosity of the blood. The present study explored whether variations in blood cell counts are relevant to the distribution and elimination of infused crystalloid fluid.

**Materials and methods:** On three different occasions, 10 healthy male volunteers received an intravenous infusion of 25 mL/kg of Ringer's acetate, Ringer's lactate, and isotonic saline over 30 min. Blood hemoglobin and urinary excretion were monitored for 4 h and used as input in a two-volume kinetic model, using nonlinear mixed effects software. The covariates used in the kinetic model were red blood cell and platelet counts, the total leukocyte count, the use of isotonic saline, and the arterial pressure.

**Results:** Red blood cell and platelet counts in the upper end of the normal range were associated with a decreased rate of distribution and redistribution of crystalloid fluid. Simulations showed that high counts were correlated with volume expansion of the peripheral (interstitial) fluid space, while the plasma volume was less affected. In contrast, the total leukocyte count had no influence on the distribution, redistribution, or elimination. The use of isotonic saline caused a transient reduction in the systolic arterial pressure ( $P < 0.05$ ) and doubled the half-life of infused fluid in the body when compared to the two Ringer solutions. Isotonic saline did not decrease the serum potassium concentration, despite the fact that saline is potassium-free.

**Conclusions:** High red blood cell and platelet counts are associated with peripheral accumulation of infused crystalloid fluid.

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## 1. Introduction

This viscosity is a key parameter in the assessment of blood flow and tissue perfusion in microcirculatory studies. In the isolated hind-limbs of dogs, the blood viscosity increases

exponentially when the hematocrit is raised, and the blood flow then requires a higher driving pressure [1]. However, the effects of variations in the red blood cell (RBC), platelet (Trc), and leukocyte counts within the normal range on the macrocirculation in healthy humans are poorly studied.

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Volume kinetics is a macroscopic method that is applicable to the study of these effects, if any [2]. The volume kinetic method is based on repeated measurement of the blood hemoglobin (Hb) concentration, which is the inverse of the blood water concentration [3]. Infusion fluids contain almost exclusively water; therefore, Hb changes are a useful index of the water volume that equilibrates with the circulating blood. This type of analysis can also be used for the simulation of the influences of combined differences in distribution and elimination of infused fluid on plasma volume expansion, peripheral edema, and urinary excretion [4].

The aim of the present study was to investigate the kinetics of a rapid fluid load of three different crystalloid fluids (Ringer's acetate, Ringer's lactate, and isotonic saline) in a randomized cross-over fashion in volunteers. The influence of variations in the RBC, Trc, and leukocyte counts on fluid kinetics was studied by applying these parameters as time-varying covariates in a population kinetic model for nonlinear mixed effects. The arterial blood pressure and the heart rate were also monitored because the hemodynamics may have competitive effects on the kinetics.

## 2. Materials and methods

The study was based on 30 strictly controlled infusion experiments performed in 10 male volunteers aged between 24 and 44 (mean, 32) years and with a body weight ranging between 72 and 95 (mean, 81) kg. All volunteers received an intravenous infusion of 25 mL/kg of Ringer's acetate, Ringer's lactate, and isotonic saline over 30 min, administered in a crossover fashion and in random order. The subjects gave their approval for participation after being informed of the study's purpose, which had been approved by the Ethics Committee of Huddinge University Hospital (Dnr 222/98, chairperson Lenart Kaijser). The project originally contained five infusions, and a two-step analysis of the hemodilution has been published [5]. Here, three of the infusions are studied in more detail.

The experiments were limited to only one experiment in one volunteer per day throughout the duration of the study. The wash-out period between the infusions was one week. On each occasion, the volunteer arrived at the Research Center at Söder Hospital in Stockholm at 8:30 AM. He had been allowed to ingest one glass (250 mL) of fluid and one sandwich 2 h before arrival. The volunteer rested for 30 min on a bed to reach a hemodynamic steady state. A cannula was placed in the cubital vein of each arm: one for blood sampling and the other for infusion of crystalloid fluid. Volunteers were covered in blankets to ensure good thermal comfort. The arm used for blood sampling was placed on a body-temperature heating pad.

The rate of infusion of crystalloid fluid was controlled using infusion pumps. During and after the infusions, venous blood (2–3 mL) was withdrawn to measure the Hb concentration, the hematocrit (Hct), RBC, Trc, and total leukocyte count on the Technicon H2 (Bayer, Tarrytown, NY, USA) instrument used for routine measurements in the Clinical Chemistry Laboratory of the Hospital. The samples were withdrawn in a standardized manner, using a discard sample to prevent admixture of

rinsing solution (isotonic saline), to ensure a coefficient of variation (CV) of approximately 1%. A volume of saline was then injected that corresponded to the sampled plasma volume. Sampling was performed every 5 min for 2 h and then every 10 min during the following 2 h, for a total of 37 samples over 4 h. The baseline sample was drawn in duplicate, and the mean of the two measurements was used in the calculations. The subjects voided just before the experiments, and this urine was discarded. The volume of the urine excreted during the study was recorded.

The ionic contents in mmol/L of the three crystalloid fluids (all manufactured by Baxter Healthcare) were as follows:

Ringer's acetate  $\text{Na}^+$  130,  $\text{K}^+$  4,  $\text{Ca}^{2+}$  2,  $\text{Cl}^-$  110, acetate 130 (273 mosmol/kg)

Ringer's lactate  $\text{Na}^+$  130,  $\text{K}^+$  4,  $\text{Ca}^{2+}$  2.7,  $\text{Cl}^-$  109, lactate 28 (273 mosmol/kg)

Isotonic saline  $\text{Na}^+$  154,  $\text{Cl}^-$  154 (308 mosmol/kg)

The systolic and diastolic arterial pressures and the heart rate were measured on a noninvasive hemodynamic monitor (Propaq, Protocol Systems Inc., Beaverton, OR, USA) and the results were recorded for the same time points as the blood samples were taken. The mean arterial pressure (MAP) was calculated as the diastolic pressure plus one third of the difference between the systolic and diastolic pressures.

The serum sodium and potassium concentrations and the serum osmolality were measured every 15–30 min by a direct potentiometry technique using an IL BGE analyzer (Instrumentation Laboratory, Milan, Italy) with a coefficient of variation of 1%–2%.

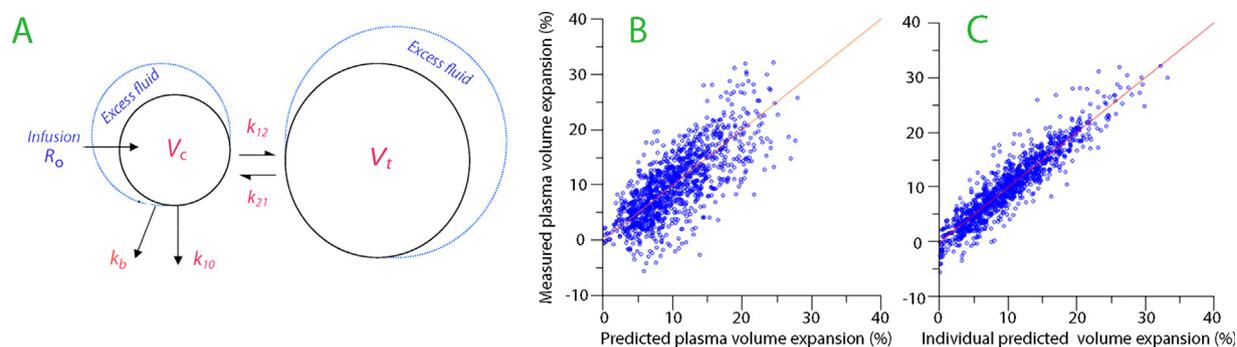
### 2.1. Kinetic model

A two-volume kinetic model with two routes of elimination was simultaneously fitted to the dependent variables (frequently measured plasma dilution and total urinary excretion) in all 30 experiments. The reason why two elimination functions are used is that not all of the fluid that is eliminated from the kinetic system can be recovered as urine. The influence of various covariates on the model parameters was then tested sequentially, as guided by a reduction of the residual error ( $-2 \text{ LL} = \log \text{ likelihood}$ ).

This kinetic model has been used previously [4] and agrees well with physiological data showing the isotonic infusion fluids distribute between two compartments, the plasma and the interstitial fluid space.

Fluid infused into the plasma ( $V_c$ , volume) is eliminated by urinary excretion ( $k_{10}$ , rate constant) and residual elimination ( $k_b$ , rate constant; previously called “ $k_{10}$  residual” [4]), as well as being distributed ( $k_{12}$ , rate constant) and re-distributed ( $k_{21}$ , rate constant) to the interstitial fluid space ( $V_b$ , volume). All flow rates are proportional to the volume expansion of the respective body fluid space. Several other models have been tested, including one- and two-compartment models and zero-order  $k_b$ , but the present one has found to be most appropriate [6].

A schematic drawing of the kinetic model is shown in Fig. 1A. In the model, fluid is infused at rate  $R_0$  and expands



**Fig. 1 – Kinetic model and goodness-of-fit. (A) Schematic drawing of the volume kinetic model. (B) Predicted vs. measured plasma dilution for all data points in the 30 volunteers according to the base model. (C) Same plot after correction for covariates. Random distribution around the solid line of unity indicates a good goodness-of-fit.**

the volume of a central body fluid space  $V_c$  to  $v_c$  (the plasma volume). The fluid distribution to the peripheral body fluid space  $V_t$  (the interstitial fluid volume) is governed by  $k_{12}$  and its return from  $v_t$  to  $v_c$  by rate constant  $k_{21}$ . The elimination was given as the sum of two flows: the urinary excretion was set to the product of the volume expansion of  $V_c$  and an elimination rate constant,  $k_{10}$ , and a residual elimination, which equaled the product of  $V_c$  and another rate constant,  $k_b$ .

The differential equations are:

$$\frac{dv_c}{dt} = R_o - k_{10}(v_c - V_c) - k_b(v_c - V_c) - k_{12}(v_c - V_c) + k_{21}(v_t - V_t)$$

$$\frac{dv_t}{dt} = k_{12}(v_c - V_c) - k_{21}(v_t - V_t)$$

The Hb-derived fractional plasma dilution was used to indicate the volume expansion of  $V_c$  resulting from the infusion, and thus served as dependent variable (input function) in the calculations. The fractional dilution provides a linear relationship between the added fluid volume and the concentration of a marker in an expandable fluid space [2]. Hence:

$$\frac{v_c - V_c}{V_c} = \frac{(Hb/hb) - 1}{1 - \text{baseline hematocrit}}$$

Symbols in capital letters denote baseline values. A minor correction was made for the effects of blood sampling on the plasma dilution [2].

The measured urinary excretion during 15-min intervals shows good linear correlation with the urinary excretion as predicted by a single exponential [7,8]. The relationships between urinary excretion,  $k_{10}$ , and the half-life of the infused fluid ( $T_{1/2}$ ) can then be written as follows (AUC = area under the curve);

$$k_{10} = \frac{\text{urinary excretion}}{\text{AUC for } (v_c - V_c)}$$

$$T_{1/2} = \frac{\ln 2}{k_{10}}$$

## 2.2. Population kinetic modeling

Population (mixed effects) kinetics include the traditional fixed model components used in pharmacokinetics but adds sub-models for the variations in these components that are individual-specific or occasion-specific. For example, the central volume of distribution ( $V_c$ ) in an individual is dependent both on the typical value (tv) for  $V_c$  in the population, but might also be dependent on the body weight (BW) of the individual (ind).

$$V_{c\text{ind}} = tv V_c + BW_{\text{ind}}$$

By considering the body weight, a more precise prediction of the dependent variable (here, the plasma dilution) can be made than in the traditional situation, where only the mean  $V_c$  for the population is used. BW is called a covariate, and its relationship to  $V_c$  can be described mathematically in many ways. In the present work, the exponential model error form was usually found to be the most appropriate one. Hence:

$$V_{c\text{ind}} = tv V_c \left( \frac{BW_{\text{ind}}}{\text{mean BW}} \right)^{\text{exp}}$$

The typical value of a fixed parameter for a population is derived from the THETA vector and the random effects from the ETA vector. The latter includes information about between-subject, within-subject, and residual variability [9].

## 2.3. Kinetic analysis

The fixed parameters in the structural model ( $V_c$ ,  $k_{12}$ ,  $k_{21}$ ,  $k_b$ , and  $k_{10}$ ) were estimated simultaneously using the Phoenix software for nonlinear mixed effects (NLME), version 1.3 (Pharsight, St. Louis, MO), while setting  $k_{10}$  equal to the total urinary excretion divided by the AUC of the volume-time curve during the entire experiment. The sandwich method was employed as the variance estimator, which is efficient when specifying covariance misspecification. This so-called *base model* was developed using the First Order Conditional Estimation search routine of Lindstrom-Bates (FOCE LB) and the additive model for the within-subject variability [9].

**Table 1 – Key features of the search protocol used to find the final population kinetic model.**

Optimization routine	Added covariate	Target parameter	LL	-2(LL)	AIC
Naive pooled	–	–	1498	-2996	-2947
FOCE LB	–	–	2071	-4142	-4119
–”–	Saline	$k_{10}$	2073	-4147	-4121
–”–	Platelets	$k_b$	2078	-4156	-4128
–”–	Platelets	$k_{21}$	2091	-4182	-4152
–”–	RBC	$k_{21}$	2100	-4200	-4168
–”–	RBC	$k_{12}$	2104	-4209	-4175
–”–	Full block model	All above	2120	-4240	-4186
–”–	Full block model	All + MAP	2125	-4250	-4194

FOCE LB, Lindstrom-Bates search routine; LL, log likelihood; AIC, Akaike Criterion; MAP, mean arterial pressure.

The base model was refined to become the *full model* by adding covariates in a sequential fashion. The decision to include a covariate was based on a reduction of the log likelihood (-2 LL) for the curve-fit by >3.8 points, which represents  $P < 0.05$  (>6.6 points represents  $P < 0.01$ ). The confidence interval for the covariate should also be statistically significant (not include 1.0) and the coefficient of variation, i.e. the inter-individual variability, should be <50%.

The covariate search was performed with the exponential error model for the between-subject variation, as suggested by random-effect plots. The following characteristics were evaluated as potential covariates in the model: the body weight and the use of Ringer's lactate, Ringer's acetate, or isotonic saline. The following parameters were evaluated as time-varying covariates in the model, which means that a new value was included for each point of measurement: the RBC count, the platelet count, the leukocyte count, and the systolic, diastolic, and mean arterial pressures, as well as the changes in all these parameters from baseline.

The goodness-of-fit of the model was illustrated by comparing the measured with the predicted fractional plasma volume expansion (using the base model parameters only) and the measured with the individual predicted plasma volume expansion (full model).

The performance of the model was studied by predictive checks based on 1000 simulations using a built-in function in

the Phoenix NLME software. Close agreement between the percentiles for the predictive check (predicted) and the original data (observed) is evidence of good model performance and indicates that the model is robust.

Computer simulations using Matlab R2013b (Math Works Inc., Natick, MA) were used to illustrate the influence of the key covariates on the distribution of fluid between central ( $V_c$ ) and peripheral body fluid space ( $V_p$ ). The best estimates of the model parameters and their covariates (if any) were entered into the solutions (solved by optODE) to the two differential equations describing the kinetic model.

Demographic data were reported as the mean (standard deviation) and the kinetic data are reported as the mean (95% confidence interval). Changes in hemodynamic parameters during the experiments were assessed by repeated-measures ANOVA and the paired *t* test.  $P < 0.05$  was considered statistically significant.

### 3. Results

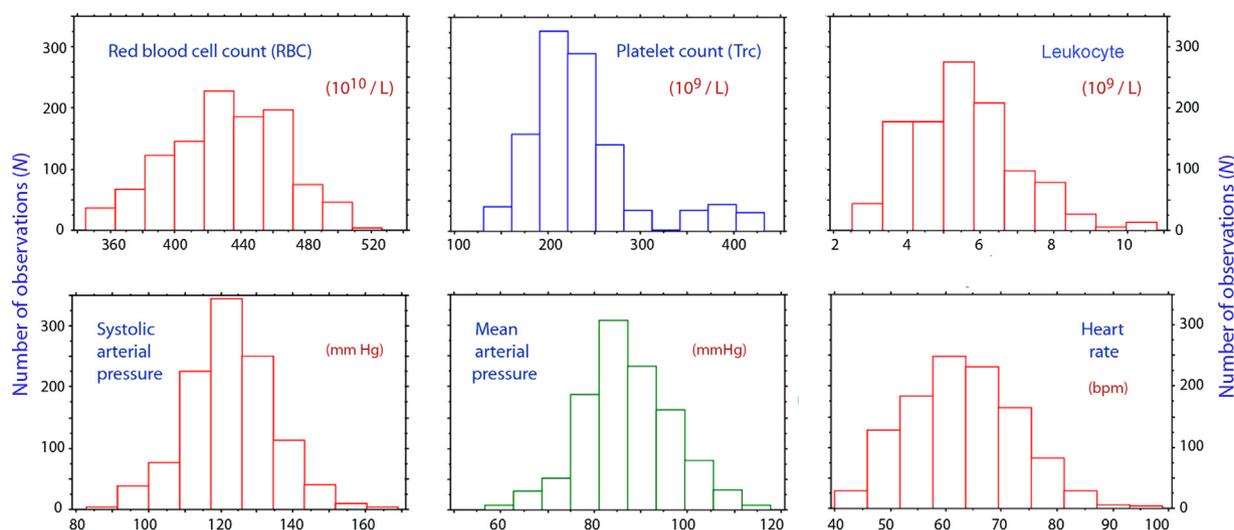
#### 3.1. Kinetic analysis

The kinetic analysis was based on 1108 data points. The key features of the development of the model are shown in Table 1. The fixed kinetic parameters and their covariates in the final model are shown in Table 2.

**Table 2 – Population kinetic parameters in the final model.**

	Covariate	Best estimate	2.5% CI	97.5% CI	CV%
<b>Kinetic parameter</b>					
tv $V_c$ (mL)	–	3835	3498	4172	4.5
tv $k_{12}$ ( $10^{-3} \text{ min}^{-1}$ )	–	102.4	79.3	125.6	9.3
tv $k_{21}$ ( $10^{-3} \text{ min}^{-1}$ )	–	48.7	36.1	61.2	13.1
tv $k_{10}$ ( $10^{-3} \text{ min}^{-1}$ )	–	15.4	11.3	18.0	9.3
tv $k_b$ ( $10^{-3} \text{ min}^{-1}$ )	–	2.5	1.5	3.6	21.7
<b>Covariate effect</b>					
tv $k_b$	Platelets	-3.5	-4.8	-2.2	-18.7
tv $k_{12}$	RBC	-4.5	-6.8	-2.1	-27.3
tv $k_{21}$	RBC	-6.6	-9.0	-4.2	-18.8
tv $k_{21}$	Platelets	-0.93	-1.48	-0.39	-29.8
tv $k_{10}$	Saline	-0.71	-1.25	-0.18	-37.9
tv $k_{10}$	MAP	2.8	0.97	4.61	33.3

tv, typical value; CI, confidence interval; CV, coefficient of variation; RBC, red blood cell count; MAP, mean arterial pressure.



**Fig. 2 – The distribution of the most important covariates. Each covariate was divided into 10 parts of similar width. Each of the 1108 data points contribute with one value.**

The covariate analysis showed that a high RBC count resulted in a decreased  $k_{12}$  and  $k_{21}$ , while a high Trc count was associated with lower  $k_{21}$  and  $k_b$ . The use of isotonic saline and a low MAP reduced  $k_{10}$ . The other hemodynamic parameters and the leukocyte count were not statistically significant covariates for any of the fixed parameters.

The distributions of the most important covariates are shown in Fig. 2.

The four fixed parameters affected by covariates had the following form, where the equations are constructed so that each significant covariate (such as RBC) at any of the 1108 data points is compared to the mean value of all data points in the population:

$$k_{12} = tv k_{12} \left[ \frac{RBC}{\text{mean RBC}} \right]^{-4.5}$$

$$k_{21} = tv k_{21} \left[ \frac{RBC}{\text{mean RBC}} \right]^{-6.6} \left[ \frac{Trc}{\text{mean Trc}} \right]^{-0.93}$$

$$k_{10} = tv k_{10} e^{-0.71(\text{Ringer} = 0, \text{Saline} = 1)} \left[ \frac{MAP}{\text{mean MAP}} \right]^{2.8}$$

$$k_b = tv k_{21} \left[ \frac{Trc}{\text{mean Trc}} \right]^{-3.5}$$

where the mean RBC was  $431 \times 10^{10}$ , mean Trc was  $236 \times 10^9$ , mean MAP was 87 mmHg, and “tv” is the typical value of the parameter.

The covariate effect of saline use of  $k_{10}$  only comes into play for saline, as the covariate effect on Ringer’s acetate and Ringer’s lactate becomes  $e^0 = 1$ . For these two fluids,  $k_{10}$  equals  $tv k_{10}$ . The half-life of the Ringer solutions is given by  $0.693 / tv k_{10}$ , which yields 45 min, while the half-life of the saline is  $0.693 / (tv k_{10} e^{-0.71})$ , or 92 min.

The influence of key covariates on the fixed parameters is illustrated in Fig. 3.

The goodness-of-fit and the performance of the model were adequate, as evaluated by residual plots (Figs. 1B, C) and predictive checks (Fig. 4A)

### 3.2. Simulations

Simulations based on the optimal estimates of the kinetic parameters (Table 1) illustrated that the use of isotonic saline retards the elimination when compared to Ringer solution use, and that the difference becomes smaller during arterial hypotension (Fig. 4B).

The accumulation of fluid in the peripheral fluid space ( $V_t$ ) increased when the RBC and Trc counts were high, whereas low counts had the opposite effect (Fig. 5).

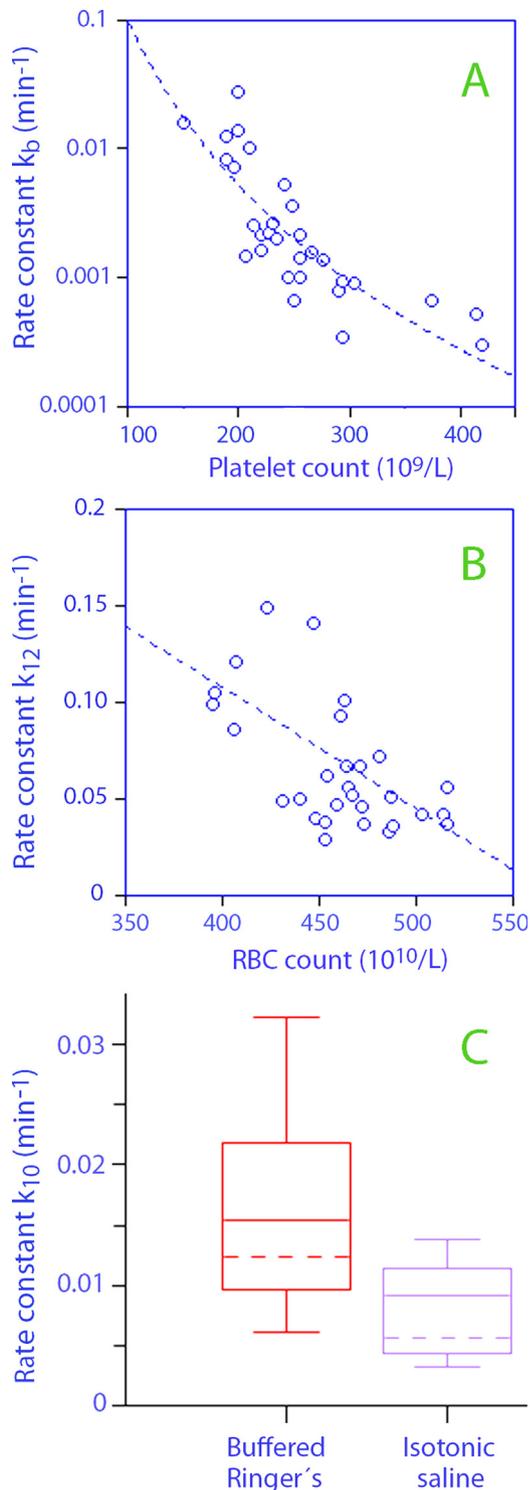
A low Trc count resulted in increased filtration via  $k_b$  (Fig. 3A), which partially explains the associated reduction in fluid volume in  $V_t$  (Fig. 5, lower row). In contrast, the RBC and platelet counts had a negligible effect on the amount of fluid eliminated by  $k_{10}$  (i.e., the urinary excretion).

### 3.3. Hemodynamics

No overall significant differences were noted in the hemodynamic parameters among the fluids during the three infusions (Table 3). However, the arterial pressure tended to decrease soon after the saline infusions were initiated (Fig. 6). Here, a reduction in the systolic arterial pressure was statistically significant after 15 min ( $P < 0.03$ ) and 20 min ( $P < 0.01$ ).

### 3.4. Serum electrolytes and osmolality

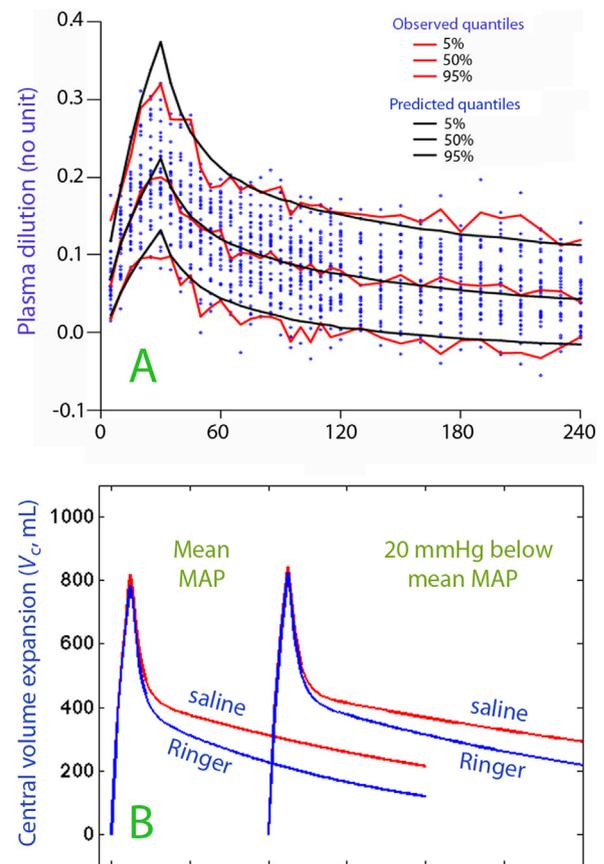
Only the use of saline raised serum sodium, and then only transiently (Fig. 7A). The two Ringer solutions increased the serum potassium concentration toward the concentration of the infusion (4.0 mmol/L) and slightly reduced serum osmolality (Fig. 7B and C). The use of isotonic saline also raised the serum potassium, even though the fluid is devoid of potassium and better maintained the serum osmolality (Fig. 7C). No statistical analysis was performed because of occasional missing data.



**Fig. 3 – Influence of covariates on the rate parameters.** Influence of the (A) platelet count on  $k_b$ , (B) red blood cell (RBC) count on  $k_{12}$ , and (C) the use of saline instead of the two buffered Ringer's solutions on  $k_{10}$ .

#### 4. Discussion

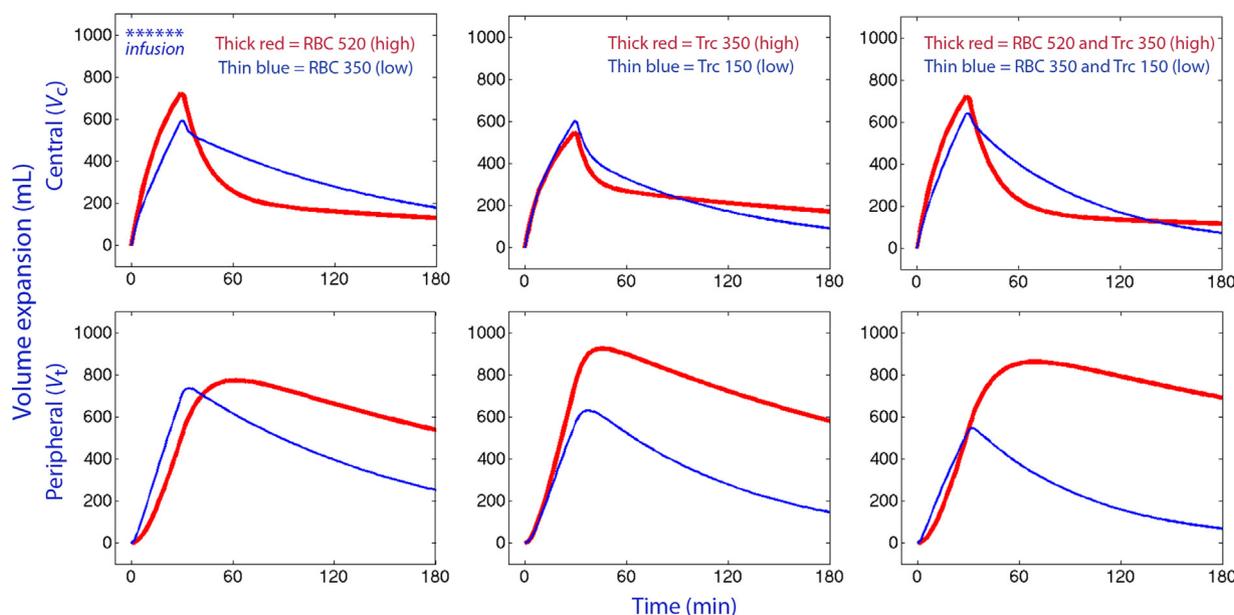
Several rate constants in the kinetic model showed lower values when the RBC and Trc counts were high. Both  $k_{12}$  and  $k_{21}$  were affected by the RBCs, while  $k_{21}$  and  $k_b$  became reduced



**Fig. 4 – (A) Predictive check.** The median and the 5% and 95% confidence limits for the observed and predicted plasma volume expansion during all 30 experiments. (B) Saline vs. Ringer solutions. Computer simulation of the volume expansion of  $V_c$  (the plasma) when 1.5 L of isotonic saline and any of the two Ringer solutions are infused over 15 min under the average MAP and when MAP is 20 mmHg below the average.

when the Trc count was high. Thus, both fluid distribution and re-distribution occurred more slowly when these cell counts were at the upper end of the normal range. Conversely, the equilibration of fluid between the body fluid compartments, as identified by the volume kinetic analysis, occurred faster when the RBC and Trc counts were at the lower end of the normal range.

A possible explanation for the reduced  $k_{12}$  that hemoconcentration changes the blood viscosity and blood flow rates so as to retain fluid in the bloodstream. The composite effect of the different cell counts, as illustrated by computer simulations, was still only a limited change in the volume expansion of the central space (the plasma volume) while fluid accumulation in the peripheral space (the interstitium) was markedly greater when the RBC and/or Trc counts were high. The greater retardation of re-distributional flow, as compared to distributional flow, promoted peripheral edema. This difference can also explain the poorer plasma volume expansion that was maintained after the infusions ended in the presence of high RBC and Trc counts, which is otherwise a paradoxical effect of retarded distribution (Fig. 5).



**Fig. 5 – Covariate effects on fluid distribution. Computer simulation of the volume expansion of  $V_c$  (the plasma; upper row) and  $V_t$  (the interstitium; lower row) when 1.5 L of Ringer solutions is infused over 30 min at average MAP. Red thick lines represent the 10% highest blood cell counts and thin blue lines the 10% lowest counts.**

Another factor that contributed to the variations in fluid distribution was a high Trc count was associated with lower  $k_b$  values (Fig. 3A). This covariance could possibly involve reduced lymphatic drainage, as  $k_b$  represents elimination from the kinetic model that cannot be recovered as urine. Platelets function to maintain the blood-lymph separation throughout life [10,11], may affect blood viscosity [12], and are present at high concentrations along the capillary wall, where they plug small leaks in the endothelium. The overall effect of a raised Trc count then seems to be to reduce fluid movements between compartments.

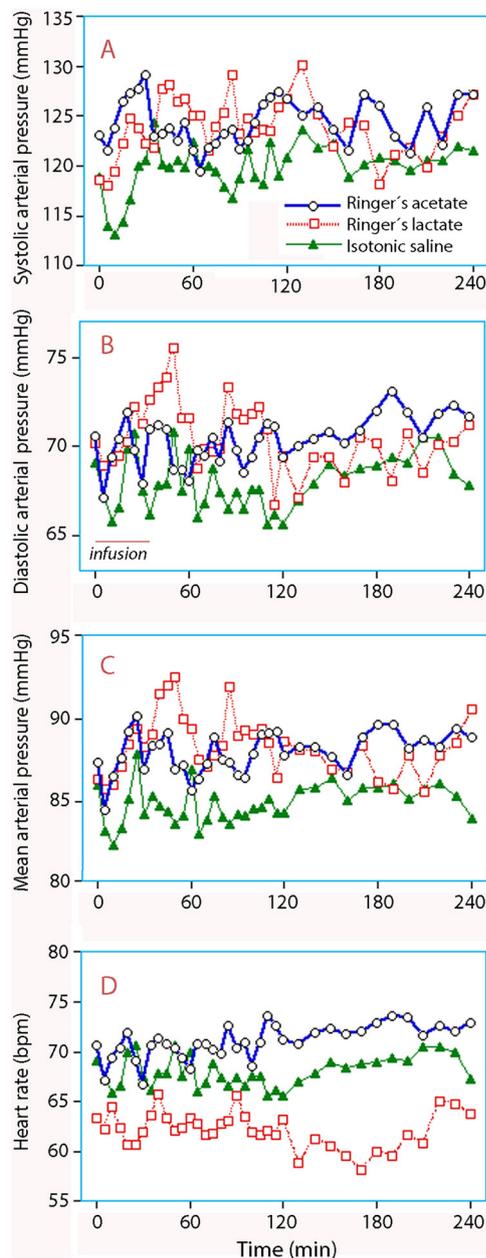
Other covariates are known from previous work. The use of isotonic saline instead of Ringer's acetate retards urinary

excretion [13,14], as shown by the statistically significant covariance between saline and  $k_{10}$  (Table 2; Fig. 4B). The half-life was then twice as long for isotonic saline as it was for the two Ringer solutions, assuming that the mean arterial pressures were the same. However, this difference is valid for males only. The half-life of infused crystalloid fluid seems to be shorter in females than in males [8]. A recent evaluation that used the same set-up and kinetic analysis showed that the half-life of Ringer's acetate was only 24 min in a cohort of females [15]. The half-life of isotonic saline in females does not seem to have been published.

The covariance effect of MAP on  $k_{10}$  was also statistically significant (Table 2) and implies that less urine is excreted

**Table 3 – Hemodynamic measurements during the three experiments.**

	Ringer's acetate	Ringer's lactate	Isotonic saline	ANOVA P value
<b>Systolic arterial pressure, mmHg</b>				
Before infusion	123.1 (11.7)	118.6 (7.0)	118.9 (12.8)	0.38
During infusion	125.6 (13.3)	121.3 (9.0)	116.8 (13.0)	0.17
After infusion	124.0 (12.5)	124.5 (11.2)	120.4 (11.7)	0.91
<b>Diastolic arterial pressure, mmHg</b>				
Before infusion	70.6 (12.9)	70.2 (10.7)	69.1 (12.9)	0.96
During infusion	69.3 (2.1)	70.2 (9.9)	68.1 (10.2)	0.90
After infusion	70.5 (9.3)	70.6 (10.9)	67.8 (11.3)	0.34
<b>Mean arterial pressure, mmHg</b>				
Before infusion	87.4 (12.2)	86.3 (8.6)	86.0 (12.1)	0.94
During infusion	87.5 (12.4)	87.3 (8.7)	84.6 (10.2)	0.66
After infusion	88.1 (9.3)	88.6 (9.8)	84.9 (10.9)	0.82
<b>Heart rate, bpm</b>				
Before infusion	70.6 (12.9)	63.4 (10.6)	69.1 (12.9)	0.47
During infusion	69.3 (12.1)	62.2 (9.7)	68.1 (10.2)	0.23
After infusion	71.3 (11.4)	62.4 (10.3)	68.0 (11.4)	0.22



**Fig. 6 – Hemodynamics. The mean values of the (A) systolic, (B) diastolic and (C) mean arterial pressures and (D) the heart rate during and after a 30-min infusion of 25 mL/kg of three crystalloid fluids over 30 min in 10 male volunteers.**

when MAP is low. However, the hemodynamics differed very little during and between the infusions, and the variability in arterial pressure was too small to explain the full strength of its influence on urinary excretion. A recent and more complete exploration of this issue can be found elsewhere [6]. The only significant change in response to fluid consisted of a transient reduction in the systolic pressure during the infusions of isotonic saline, which is probably due to the negative inotropic effect of chloride-induced acidosis (Fig. 6). The diastolic pressure seemed to increase after Ringer's lactate use, but the change was not statistically significant.

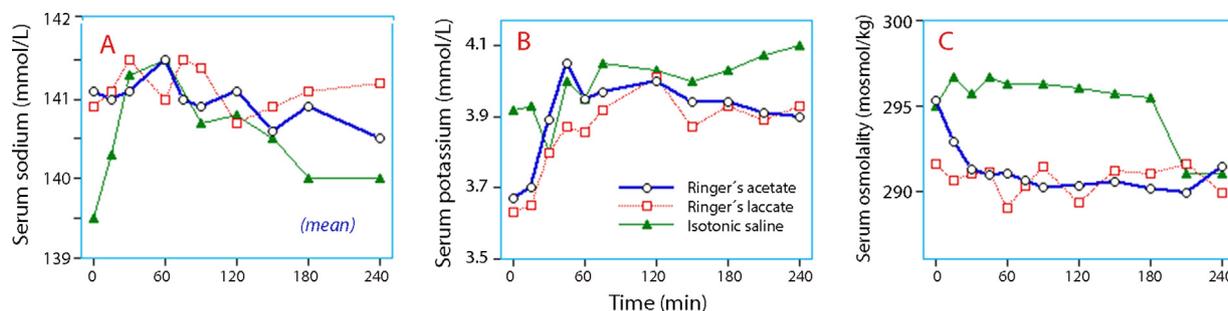
Variations in serum electrolytes and osmolality reflect the differences in ionic composition between the infusions and the extracellular fluid. An exception was that serum potassium did not decrease after infusion of isotonic saline, which confirms that saline is not a useful tool for decreasing serum potassium concentrations by dilution (Fig. 7B). The limited changes in serum osmolality ensured that no major shift between the intra- and extracellular fluid spaces occurred.

The volume kinetic model was built up by first estimating the five fixed parameters in the structural (base) model (Fig. 1A) which was then followed by sequential inclusion of the covariates that significantly reduced the in prediction error (cf. Fig. 1B and C). In the base model, the distributions of fluid between body compartments are determined by the rate constants  $k_{12}$ ,  $k_{21}$ ,  $k_{10}$ , and  $k_b$ . These are inversely proportional to the half-life of the fluid volume added to the respective body fluid space, and are therefore independent of the infused fluid volume and body weight.

By contrast, the size of the two expanded body fluid spaces,  $V_c$  and  $V_t$ , serve as scaling factors between plasma dilution, which is the input function, and the volume expansion, which is the output.  $V_c$  equals the plasma volume if the Hb molecules are, on average, evenly distributed in the blood volume during an experiment. In the present study, the kinetic analysis indicated an average volume of 3.8 L for  $V_c$ . The size of  $V_t$  is not obtained directly from the model, but an estimate based on the relationship  $V_t = V_c k_{12}/k_{21}$  yields 8.1 L. This equation is valid if fluid can pass freely to and from  $V_t$ . The volume 8.1 L is less than the physiological size of the interstitial fluid space because  $V_t$  only indicates parts that can be expanded by fluid [2]. These expandable spaces vary according to body size [4], but the body weights of the male volunteers who participated in the present experiments were too similar to challenge an influence on  $V_c$ .

Simulations of the plasma volume expansion over time show a marked distribution effect of crystalloid fluid. A sharp peak is reached at the end of each infusion, which levels off over a period of 30 min. Hence, during and shortly after an infusion, the plasma volume expansion is much greater than that observed at 30 min postinfusion, and it then reaches 50% of the infused volume (Fig. 4B). Both the magnitude and the timespan of the distribution effect are determined by  $k_{12}$  and  $k_{21}$ . The reason why equilibration requires a relatively long time for completion is probably that the jelly-like interstitial fluid matrix retards the flow of fluid (but not of nutrients) to resist the gravitational redistribution of body water that, to some degree, affects the circulating blood. The apparent distribution effect of crystalloid fluid has been demonstrated many times, but awareness of the phenomenon is still limited among those who practice fluid therapy [2]. After distribution is completed, further reduction in the volume expansion occurs by elimination, at a rate given by  $k_{10}$  and  $k_b$ . Evaporation contributes slightly to  $k_b$ , while losses of Hb molecules and fluid by the sampling is corrected by other measures.

One limitation of the present study is that the experiments induced hypervolemia, as the volunteers were reasonably normovolemic when the infusions were initiated. The results are also based on only 30 experiments, although more than one thousand data points were analyzed. The reported kinetic data are all statistically significant, but a larger number of



**Fig. 7 – Blood chemistry.** The mean values of the (A) serum sodium (B) serum potassium (C) concentrations, and the serum osmolality during and after a 30-min infusion of 25 mL/kg of three crystalloid fluids over 30 min in male volunteers.

experiments is needed to confirm the present findings. The retrospective nature of the evaluation must also be considered to represent a drawback.

### 5. Conclusions

The RBC and Trc counts at the upper end of the normal interval reduced movements of fluid between body compartments and promoted volume expansion of the interstitial fluid space.

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