ANESTHESIOLOGY

Population Kinetics of 0.9% Saline Distribution in Hemorrhaged Awake and Isoflurane-anesthetized Volunteers

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Volume kinetic modeling is an adaptation of pharmacokinetic modeling that characterizes the disposition of intravenously administered fluids using hemoglobin concentration as a natural tracer
- Population-based pharmacokinetic analysis enables assessment of variability between individuals and across populations and permits inclusion of covariates such as the presence or absence of anesthetization, body weight, and sex in the data analysis

What This Article Tells Us That Is New

- The distribution of infused fluid after hemorrhage (7 ml/kg during 20 min) in a randomized crossover study of 12 healthy volunteers while awake and while isoflurane-anesthetized was described by a two-fluid space model that included study arm, body weight, and sex as covariates
- Only sex had a statistically significant effect on the area under the plasma dilution curve and maximum plasma dilution, both of which were increased by a median of 17% in females (95% CIs, 1.08 to 1.38 and 1.07 to 1.39, respectively) compared with males

ABSTRACT

Background: Population-based, pharmacokinetic modeling can be used to describe variability in fluid distribution and dilution between individuals and across populations. The authors hypothesized that dilution produced by crystalloid infusion after hemorrhage would be larger in anesthetized than in awake subjects and that population kinetic modeling would identify differences in covariates.

Methods: Twelve healthy volunteers, seven females and five males, mean age 28 ± 4.3 yr, underwent a randomized crossover study. Each subject participated in two separate sessions, separated by four weeks, in which they were assigned to an awake or an anesthetized arm. After a baseline period, hemorrhage (7 ml/kg during 20 min) was induced, immediately followed by a 25 ml/kg infusion during 20 min of 0.9% saline. Hemoglobin concentrations, sampled every 5 min for 60 min then every 10 min for an additional 120 min, were used for population kinetic modeling. Covariates, including body weight, sex, and study arm (awake or anesthetized), were tested in the model building. The change in dilution was studied by analyzing area under the curve and maximum plasma dilution.

Results: Anesthetized subjects had larger plasma dilution than awake subjects. The analysis showed that females increased area under the curve and maximum plasma dilution by 17% (with 95% CI, 1.08 to 1.38 and 1.07 to 1.39) compared with men, and study arm (anesthetized increased area under the curve by 99% [0.88 to 2.45] and maximum plasma dilution by 35% [0.71 to 1.63]) impacted the plasma dilution whereas a 10-kg increase of body weight resulted in a small change (less than1% [0.93 to 1.20]) in area under the curve and maximum plasma dilution. Mean arterial pressure was lower in subjects while anesthetized ($P < 0.001$).

Conclusions: In awake and anesthetized subjects subjected to controlled hemorrhage, plasma dilution increased with anesthesia, female sex, and lower body weight. Neither study arm nor body weight impact on area under the curve or maximum plasma dilution were statistically significant and therefore no effect can be established.

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Fluid therapy is the most common therapeutic intervention during anesthesia and surgery.[1] The vascular system undergoes dynamic shifts attributable to volume demands, hormonal influences, vascular permeability, and other alterations in the blood-to-tissue transfer of fluids. However, the inability to correctly predict fluid shifts between tissues is a significant limitation of fluid therapy. Imprecise fluid substitution results in metabolic abnormalities, edema formation, and other morbidities. Efforts to use pharmacokinetic principles to predict the fate of fluids could enable more precise volume replacement therapy.[2–5] Volume kinetic modeling is an adaptation of pharmacokinetics that makes it possible to analyze, calculate, and simulate the distribution and elimination of infused fluids.[6,7] Volume kinetic modeling

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characterizes the anticipated distribution of infused fluids reasonably well, using a natural tracer, hemoglobin concentration, to determine fluid dilution and distribution.[2] Serial measurements of hemoglobin concentrations during rapid fluid loading (bolus) permit comparisons of plasma dilution data with computer-generated model data and thereby estimate parameters that dynamically describe the distribution and disposition of fluids and can be used to simulate fluid infusions. Most volume kinetic models use individual curve fitting and nonlinear regression analyses. However, these approaches have limitations such as inability to account for physiologic variation within populations.[8] On the other hand, population-based pharmacokinetics models, known as nonlinear mixed-effect models, enable assessment of variability between individuals and across populations and permit inclusion into the data analysis of covariates such as body weight, sex, and anesthetization.[8,9]

The aim of the present study was to compare the distribution of infused fluid after hemorrhage in awake and isoflurane-anesthetized humans. Our hypothesis was that dilution would be more pronounced in anesthetized than awake subjects and that we could distinguish differences related to covariates such as body weight and sex.

Materials and Methods

The study was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston, Texas (IRB#: 04-379) and conducted in the Institute for Translational Sciences – Clinical Research Center at University of Texas Medical Branch. Twelve (seven females and five males) American Society of Anesthesiologists (ASA) physical status I or II healthy volunteers, 21 to 35 yr old, were recruited. Exclusion criteria were age younger than 18 yr and pregnancy. Mean body weight was 70 ± 13 kg (mean ± SD). Subjects underwent a screening visit which included a medical history, physical examination, and laboratory screening. If the subjects met the inclusion criteria, the study procedures were explained and an informed consent form was presented and signed.

General Procedures

The study was designed as a randomized crossover study. Each subject participated in two separate experimental sessions, each separated by at least four weeks. The order of participation in the anesthetized or awake arm, respectively, was decided by a randomized schedule. The night before the experiment, the subjects were admitted to the Clinical Research Center and were given nothing to eat or drink after midnight. On the morning of the experiment, the subjects were weighed, given the opportunity to void, and placed in a hospital bed in the supine position. ASA physical status monitoring devices, including ECG leads, pulse oximetry, and a noninvasive blood pressure cuff were applied, and vital signs were recorded. A 16-gauge catheter was placed in a forearm vein for infusion of fluids. A 20-gauge catheter was placed in a radial artery under sterile technique after local anesthetic infiltration for purposes of blood withdrawal and blood sampling. If a subject appeared anxious, midazolam 2.0 mg iv was given. Subjects were then randomized to either the anesthetic arm or the awake arm. Both arms included hemorrhage and iv fluid resuscitation.

The two arms were designed as follows:

Arm 1 – Anesthetized, Blood Removal, Fluid Infusion. Anesthesia was induced by propofol 1.5 to 2.5 mg/kg body weight followed by insertion of a laryngeal mask airway device (ProSeal, Intavent Orthofix, United Kingdom). Maintenance of anesthesia was done by 1.0 to 1.5% of isoflurane in a mixture of air and oxygen, aiming for a target of 1.0 minimum alveolar concentration (MAC) for the entire anesthetic part of the experimental period. The subjects breathed spontaneously throughout the experiment.

Arm 2 – Conscious, Blood Removal, Fluid Infusion. Twenty minutes before the fluid infusion began, subjects in each arm underwent mild controlled hemorrhage (7 ml/kg: equivalent to a one-unit blood donation) during 20 min *via* the arterial catheter. Blood collection was accomplished using sterile technique and stored in accordance with the blood bank guidelines. We anticipated lower blood pressure in the anesthetized arm. If systolic blood pressure decreased to less than 80 mmHg, ephedrine 5.0 mg iv was given.

Hemodynamic Measurements, Fluids, Blood Sampling, and Urinary Scanning

Immediately after hemorrhage, subjects in each arm received 0.9% saline (Baxter, USA) 25 ml/kg iv during 20 min using tandem infusion pumps (IMED, USA). Saline was warmed to 41°C before infusion. The experimental protocol ended 180 min after the start of the saline infusion.

Arterial blood was sampled before the start of infusion, every 5 min for 60 min and every 10 min for an additional 120 min. Hematocrit and hemoglobin were measured in duplicate for kinetic analysis of the fluid distribution using 1.0 ml arterial blood samples. Analysis was done using a Sysmex 302 HST line on a Sysmex SE 9500 (Sysmex, USA). Hemoglobin was measured by a sodium lauryl sulphate method read at 540 nm and the duplicate measurements had a coefficient of variation of 0.5%, whereas the hematocrit was obtained by cumulative pulse height detection with a coefficient of variation of 0.7%. Before each sample withdrawal, 4 ml of blood was removed from the arterial catheter to avoid sample dilution. The withdrawn blood was subsequently reinfused and the catheter was flushed with 1 to 2 ml of heparinized saline. Heart rate, other noninvasive variables, and noninvasive blood pressure were measured every 5 min for the first 60 min and thereafter every 10 min for the next 120 min. After hemorrhage, fluid infusion, and collection of all hemoglobin samples, the shed blood was reinfused during at least 30 min.

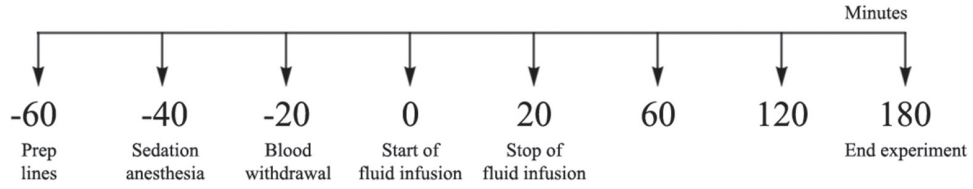

Fig. 1. Timeline for the experiment. Preparation (Prep) began at –60 (60 min before the start of the experiment). At –40 min, subjects were either anesthetized or remained awake (lightly sedated). From –20 min to 0 min, blood withdrawal progressed. From 0 min to 20 min, fluid was infused. Blood sampling started at 0 min with sampling every 5 min for the first 60 min, then every 10 min an additional 120 min until the 180-min time point.

A timeline of the experiment is shown in figure 1.

Urinary Measurements

Urinary bladder volumes were measured every 20 min using an ultrasound scanner (Bladderscan BVI 3000, Diagnostic Ultrasound, USA). In anesthetized subjects, if the bladder ultrasound indicated urinary volume was greater than 500 ml, the subject's bladder was catheterized to avoid overdistension.[10] In the awake protocol, the subjects were asked to void into a urine collection flask if bladder volume was greater than 500 ml or they felt uncomfortable. At study end, total urine was collected and weighed on a tared scale (in grams). We assumed each 1 g = 1 ml, based on density of water.

Kinetic Analysis

Parameters for fluid movement are generated from first-order differential equations. Fluid dilution was analyzed using hemoglobin and hematocrit values. Water is the primary constituent of both saline and blood plasma. The concentration of an infusion fluid is therefore expressed as dilution of hemoglobin with respect to time. The hemoglobin-derived fractional plasma dilution was used to indicate the volume expansion of the baseline central space, V_c. The fractional plasma dilution can be expressed as a linear relationship between added fluid volume and the concentration of an endogenous marker in an expanded fluid space. Values were corrected for blood sampling as presented earlier.[6]

Equation 1:

$$\frac{v_c - V_c}{V_c} = \frac{\left(Hb - hb\right)/hb}{\left(1 - Hct\right)}$$

In Equation 1, V_c is baseline value and v_c is the expanded fluid space. *Hb* is the baseline hemoglobin whereas *hb* is the time-dependent hemoglobin when expansion has occurred. *Hct* is the baseline hematocrit used to derive the plasma volume expansion.

A system of two differential equations describes a two-fluid space expansion of the central and peripheral spaces. The fluid distribution to the peripheral body fluid space V_t was governed by k_{12} and its return from v_t to v_c by a transfer rate constant k_{21} (fig. 2).

Equation 2:

$$\frac{dv_c}{dt} = R_0 - k_e\left(v_c - V_c\right) - k_{12}\left(v_c - V_c\right) + k_{21}\left(v_t - V_t\right)$$

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

where V_c is baseline central volume of distribution, V_t is volume of peripheral space, k_{12} is transfer rate constant between central and peripheral spaces, k_{21} is transfer rate constant between peripheral and central space, k_e is elimination rate constant from central space, and R_0 is infusion rate of incoming fluid, fixed to 1.25 ml · min^{-1} per kg of body weight.

Population Kinetics

In this report, the influence of demographic variables on model parameters was tested. Evaluated variables were awake *versus* anesthetized states (arm), body weight, and sex.

Nonlinear mixed effect modeling was used to estimate population parameters. Mixed effects are divided into either fixed or random effects. Fixed effects are population effects (*e.g.*, typical population trend [*e.g.*, mean or median] and variances in the population). Random effects are individual and sample-specific variations from the fixed effects. Random effects cannot be determined in advance but are instead estimated given the population parameters. Fluid elimination parameters are determined by volume kinetic analysis[6,8] and implemented in a population kinetic model (Equations 1 and 2, fig. 2). The population model is made up of structural and statistical parameters.

Equation 3:

$$y_{ij} = f(x_{ij}, \Theta_i) + \varepsilon_{ij}$$

where y_{ij} is the observed measurement or plasma dilution for subject *i* at time point *j*; $f(\boldsymbol{x}_{ij}, \boldsymbol{\Theta}_i)$ is a function that predicts within-individual behavior such as individual plasma dilution; $\boldsymbol{x}_{ij}$ is a vector with time-points and subject specific

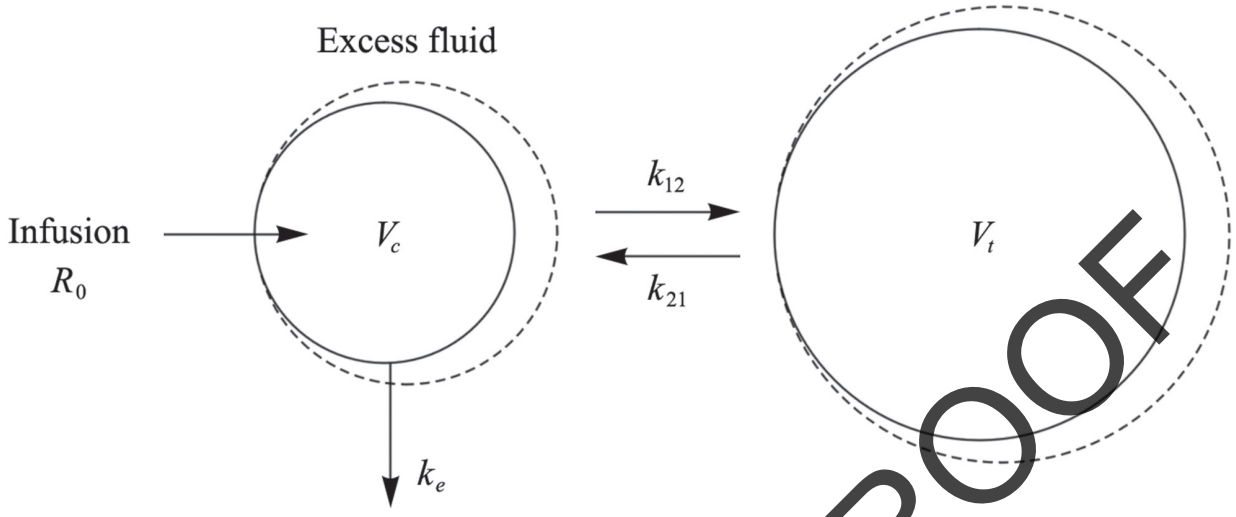

Fig. 2. Two-fluid space (central and peripheral) conceptual model. V_c, baseline central volume of distribution; V_t, baseline volume of peripheral space; k_{12}, transfer rate constant between central and peripheral space; k_{21}, transfer rate constant between peripheral and central space; k_e, elimination rate constant from central space; and R_0, infusion rate.

covariates; and Θ_i is a vector of volume kinetic parameters ($k_{c,i}$,$k_{12,i}$,$k_{21,i}$$V_{c,i}$) specific to subject i that describes the inter-individual variability where all parameters were assumed log-normally distributed.

Equation 4:

$$p_{ki} = \theta_k \exp(\eta_{ki})$$

where p_{ki} is the k^{th} parameter of subject i; θ_k is the typical value; and η_{ki} is the normally distributed discrepancy or random effect between the typical and individual value with a variance of ω_k^2.

Equation 5:

$$\varepsilon_{ij} = y_{ij} - f(x_{ij}, \Theta_i)$$

The intra-individual deviation ε_{ij}, which is also modeled as a normally distributed random effect, describes the discrepancy between the observed and predicted observation. The variance of ε_{ij} is denoted as σ^2.

The observed measurement or plasma dilution is a function of measurements associated with a certain response for a specific subject represented by the observed hemoglobin and hematocrit values (equations 1 and 2) and a vector of subject-specific volume kinetic parameters that describe inter-individual variability.

The nonlinear mixed effects estimation tool NONMEM 7.2 (Icon Development Solutions, USA),[11] using the first-order conditional estimation method with interaction, was used to develop the final model. In all steps of the model building goodness-of-fit plots were used to discriminate between models. Goodness-of-fit plots included factors such as observed concentrations *versus* individual predictions or population predictions, which should be randomly scattered around the line of identity, and individual residuals and conditional weighted residuals *versus* predictions and time after infusion, which should be randomly scattered around the horizontal line at zero. Another tool used for discriminating between models was the objective function value (–2 log likelihood) provided by NONMEM. The difference in objection function values between two hierarchical models is nominally χ^2 distributed where a difference of –3.84 between a larger model incorporating one more parameter than a smaller model corresponds approximately to a P value of less than 0.05 for the additional parameter in the larger model. Visual predictive checks[12] were also used as model diagnostics to evaluate the predictive performance of the model compared to the observed data. Data were simulated (*i.e.*, by sampling of individual parameters as well as residual errors from the estimated model) 1,000 times; the observed covariates and the observed 2.5th, 50th, and 97.5th percentiles were visually compared with the simulated median and the 95% prediction interval calculated with a 95% CI. Parameter uncertainty of the parameters in the final model was calculated using a bootstrap approach with 500 datasets.[13]

Once a final structural model was developed, a covariate analysis was performed based on a stepwise covariate modeling approach.[14] In stepwise covariate modeling a forward inclusion criterion was set to $P < 0.05$ whereas a more restrictive backward elimination P value was set to $P < 0.01$. The covariates tested were body weight, awake *versus* anesthetized states (study arm – conscious, bled, fluid-infused or anesthetized, bled, fluid-infused), and sex (male or female). A typical patient covariate effect was

studied by change in the area under the curve as well as maximum plasma dilution.

Statistical Analysis for Hemodynamic Data

Data are presented as mean ± SD. No formal statistical power calculation was conducted to guide sample size. Instead, the sample size was based on previous works.[8] Repeated measures of observations of hemodynamic parameters (anesthetized, bled, fluid-infused and conscious, bled, fluid-infused) and bladder volume were done. Separate models were estimated for the three outcome variables heart rate (HR), mean arterial pressure (MAP), and urine production. The following mixed model strategy was used to study differences between study arms and interactions between time and study arms:

1. Fixed effect with intercept of time, study arm, and interaction between time and study arm.
2. Random effects with intercept.

For descriptive statistics and the use of mixed models, a statistical package from SPSS was used (SPSS, Version 23, IBM). The population calculations have been described under the model building section. $P < 0.05$ was considered significant.

Results

General

The volunteers tolerated the two experimental procedures well. A total of 596 hemoglobin and hematocrit measurements from 12 subjects studied in both the conscious and anesthetized arms were included in the analysis.

An overview of the subjects and their baseline hemodynamic variables is provided in Supplemental Digital Content 1 (http://links.lww.com/ALN/B975).

Hemodynamic Results

Five of 12 anesthetized subjects received ephedrine 5.0 mg for hypotension. Four subjects received one dose and one subject received two doses.

Mixed-model analysis showed that there was a statistically significant interaction between time and study arm ($P = 0.044$); however, there was no statistically significant difference in HR between study arms ($P = 0.292$; fig. 3A).

MAP was higher in the conscious, bled, fluid-infused arm ($P < 0.0001$) without any study arm × time interaction ($P = 0.466$; fig. 3B).

There was a statistically significant different interaction between time and study arm ($P = 0.005$) for urinary bladder volume but the difference between study arms was not statistically significant ($P = 0.357$; fig. 3C).

Model Predictions. Figure 4 illustrates the overall model of central and peripheral volume expansion over time when 0.9% saline (25 ml/kg) was infused after hemorrhage (7.0 mg/kg) in subjects while conscious or anesthetized. In anesthetized subjects (right panel), central fluid space expansion is larger compared with conscious patients (left panel) and peripheral fluid space expansion is less. Less time is required to reach a steady-state expansion in anesthetized compared with conscious patients.

Figure 5 illustrates the overall influence on plasma dilution of sex and anesthetized state *versus* conscious state. The anesthetized and conscious arms generated similar plasma dilution profiles with a maximal increase of plasma dilution at the end of the infusion followed by stabilization at a level slightly above the baseline. In males, plasma dilution was less than in females, and in conscious subjects, plasma dilution was lower than in anesthetized subjects. This was further analyzed by changes in area under the curve and

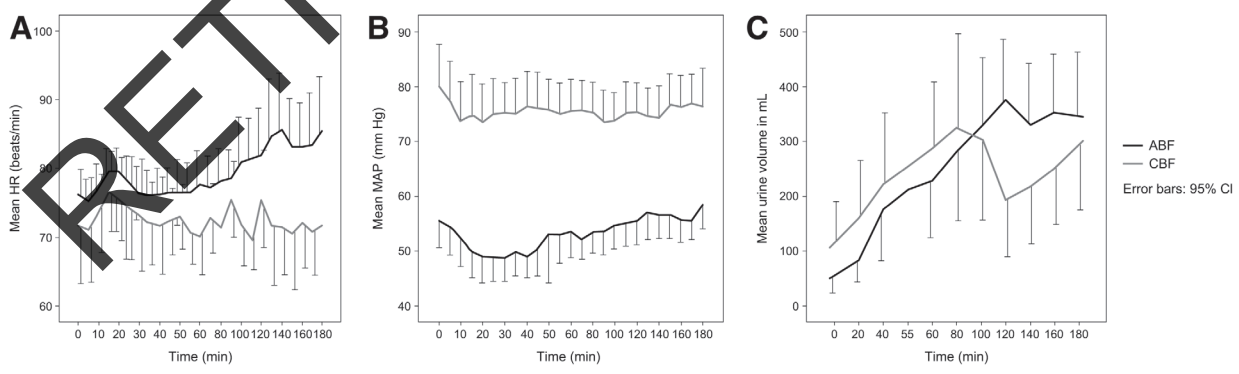

Fig. 3. (*A*) Heart rates (HR) for the anesthetized, bled, fluid-infused (ABF) arm and the conscious, bled, fluid-infused (CBF) subjects. There was a statistically significant interaction between time and study arm ($P = 0.044$) but no difference in HR between study arms ($P = 0.292$). Error bars show 95% CI. Only one directional bar is shown to improve the clarity of the figures. (*B*) Mean arterial pressure (MAP) for ABF and CBF subjects. MAP was higher in the CBF study arm ($P < 0.0001$) without any interaction with time ($P = 0.466$). (*C*) Urinary bladder volume by ultrasound scan for the ABF and CBF arms. There was a statistically significant interaction between time and arm ($P = 0.005$), but there was no difference in urinary bladder volumes between study arms ($P = 0.357$).

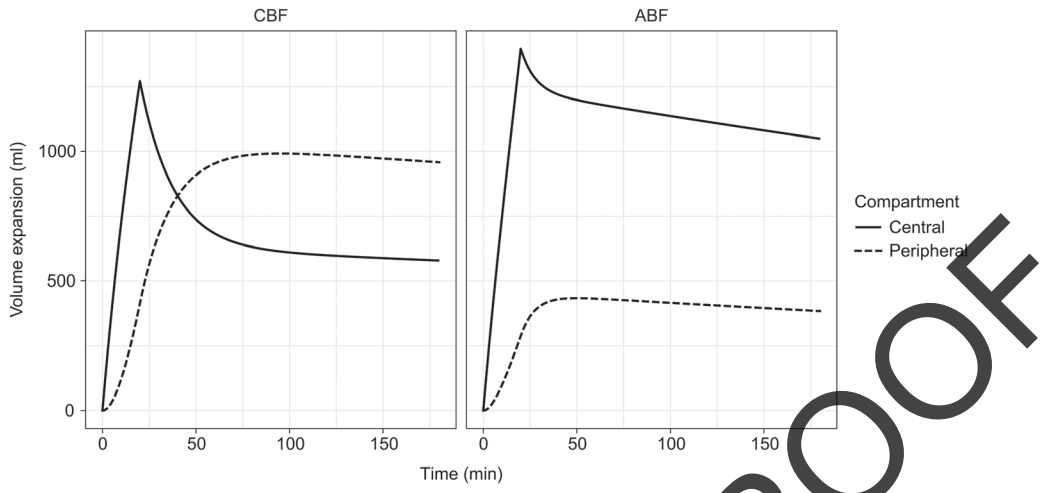

Fig. 4. Volume expansion for the two different fluid spaces *versus* time. The figure is stratified on conscious subject (*left*) and anesthetized subjects (*right*). ABF, anesthetized, bled, fluid-infused; CBF, conscious, bled, fluid-infused.

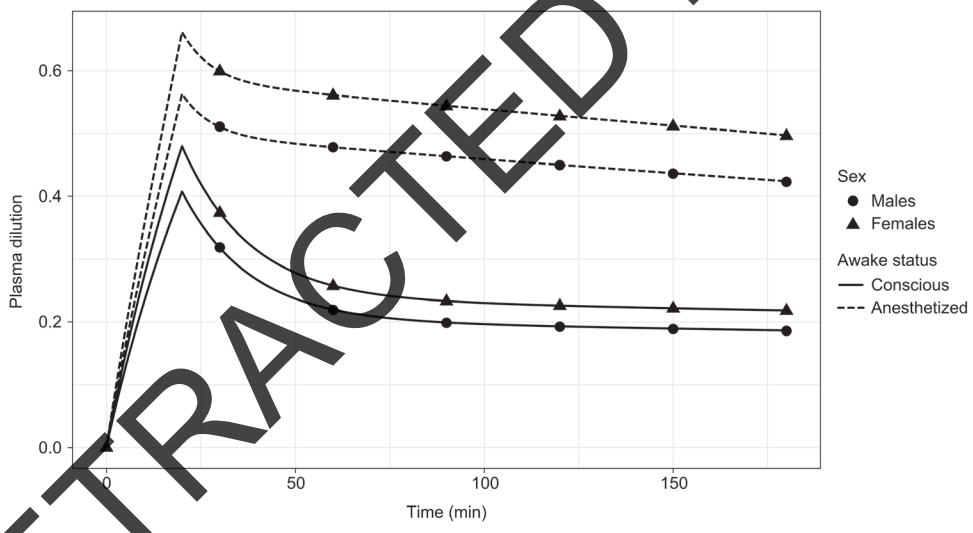

Fig. 5. Predicted plasma dilution *versus* time, stratified on awake *versus* anesthetized status and sex.

maximum plasma dilution attributable to covariate effects after the population modeling was done.

Population Volume Kinetic Analysis

The final model was a two-fluid space model with exponential inter-individual variability (on all parameters except k_{12}). The final model included the covariates: (1) study arm (V_c, k_{12}, k_{21}); (2) body weight (V_c); and (3) sex (V_c). Parameter estimations and uncertainties of the final model are presented in table 1. For an overview of the key NONMEM analyses examined during model development, see Supplemental Digital Content 2 (http://links.lww.com/ALN/B976). The conditional weighted residuals of the model and predictions together with individual's predictions *versus* observations are shown in figure 6. The model predicts the data well without any important model misspecifications over time.

The model simulation properties are visualized by a predictive comparison of observed inter-individual variability with model simulations (fig. 7, A and B). The percentiles (2.5th, 50th, and 97.5th) describe the distribution of 1,000 simulations. There is a tendency to overpredict the upper percentiles of the data, especially for the anesthetized subjects. This is not unexpected because of the low number of

Table 1. Parameter Estimates in Final Model

Parameter	Value [CI]	IIV (CV%) [CI]
k_e, min^{-1}	1.386·10^{-3} [2.7·10^{-4} to 4.2·10^{-3}]	123 [41 to 246]
V_c, ml	2,039 [1,570 to 2,323]	4.5 [0.0 to 9.9]
k_{12}, min^{-1}	3.46·10^{-2} [2.63·10^{-2} to 7.14·10^{-2}]	—
k_{21}, min^{-1}	2.13·10^{-2} [1.58·10^{-2} to 3.73·10^{-2}]	26.8 [1.6 to 15.1]
Males on V_c, %	17.2 [3.7 to 73.4]	—
BW on V_c, %*	1.29 [0.97 to 2.02]	—
ABF on V_c, %	–20.3 [–32.3 to 12.5]	—
ABF on k_{12}, %	174.0 [7.94 to 289.6]	—
ABF on k_{21}, %	56.5 [–10.6 to 111.0]	—
Proportional error, %	7.4 [1.5 to 10.6]	—
Additive error variance	7.40·10^{-4} [1.30·10^{-4} to 1.11·10^{-3}]	—

k_e, elimination rate from central compartment; V_c, baseline central volume of distribution; k_{12}, elimination rate between central and peripheral compartment; k_{21}, elimination rate between peripheral and central compartment; CI, 95% CI from bootstrap; IIV, inter individual variance; CV, coefficient of variation; Males on V_c, change in V_c for males compared with females; BW on V_c, change in V_c for body weight (BW) difference from mean BW (68.3 kg). ABF on V_c, Change in V_c for anesthetized subjects compared with conscious subjects. ABF on k_{12}, Change in k_{12} for anesthetized subjects compared with conscious subjects. ABF on k_{21}, Change in k_{21} for anesthetized subjects compared with conscious subjects.

*Approximate percentage using a first-order approximation of an exponential effect.

subjects and individual variability. Given these limitations, the model predicts the data reasonably well.

Figure 8 shows the typical patient covariate effects on the area under the plasma dilution curve as well as the maximum plasma dilution. It shows that sex (females increased area under the curve and maximum plasma dilution by 17% (95% CI, 1.08 to 1.38 and 1.07 to 1.39 compared with men) and study arm (anesthetized increased area under the curve by 99% [95% CI, 0.88 to 2.45]) and maximum plasma dilution by 35% (95% CI, 0.71 to 1.63 compared with awake) impact the plasma dilution while a 10 kg increase of body weight only resulted in a small increase of 0.5% (95% CI, 0.92 to 1.20) on area under the curve and 0.2% (95% CI, 0.93 to 1.19) on maximum plasma dilution. Note that both study arm and body weight impact on area under the curve or maximum plasma dilution were not statistically significant and the point estimates should therefore be regarded with caution.

Discussion

This population volume kinetic study in volunteers investigated the distribution of infused 0.9% saline after hemorrhage with and without general anesthesia. The aim was to define differences in fluid distribution based on covariates including anesthesia, body weight, and sex.

The main findings, when simulating using covariates, were that plasma dilution does not change with decreasing body weight (even after accounting for weight-adjusted input fluid), males showed reduced plasma dilution compared with females, and conscious subjects had lower plasma dilution compared with anesthetized subjects. Sex and study arm had a larger impact on plasma dilution than weight (assuming a 10-kg increase in weight from a typical weight of 68.2 kg).

Population kinetics is a tool to analyze drug pharmacokinetics for a population of subjects. There is a possibility to investigate covariates, which are factors that could impact drug plasma concentrations and thereby reduce interindividual variability. Very few efforts have been made to apply this to fluids.[8,9] The study model was an effort to mimic a surgical situation wherein patients are under the influence of anesthesia and hemorrhage. For obvious reasons, it was not possible to elicit a surgical trauma. In this work, and in accordance with previous work, MAP is consistently lower in anesthetized subjects.[8] The use of general anesthesia causes a decrease in blood pressure in all anesthetized subjects even before hemorrhage. Blood pressure in conscious subjects does not decrease significantly enough after similar hemorrhage as a result of preserved compensatory forces.[15] It appears that MAP was unaffected by the infused fluid, which further underscores that MAP is not a good indicator for when to infuse fluids (fig. 3B). In this study, MAP did not show differences over time other than by study arm (anesthetized, bled, fluid-infused *vs.* conscious, bled, fluid-infused). HR, however, showed a difference when both time and study arm were considered (fig. 3A).

There was no significant difference in urinary output between arms (fig. 3C). Urinary scans were unreliable because they were dependent on observer bias and hence not used in the population modeling. Bladder catheterization would have been even better for modeling purposes, but because of ethical restraints this was not an option. In a previous bleeding study in sheep, renal clearance was modeled as a logarithmic relationship to the fractional dilution of the central fluid space that could also describe diuresis when $v_c(t)$ decreased below V_c (fig. 2).[16] That model approach was also tested in the present study, but urinary data lacked the required precision which resulted in overparameterization.

The population kinetic approach, which is based on an underlying volume kinetic model, uses all data together. The major benefit from population modeling is the ability to evaluate covariates. Specifically, the model allows evaluating the impact of factors such as study arm, body weight, and sex on structural model parameters. The hemorrhage was done to elucidate the effects of bleeding on fluid distribution. It is known that bleeding causes transcapillary refill that has explicit impact on the distribution of fluid back into central spaces.[17] The hemorrhage was performed before the interventional fluid administration. Although this is not a perfect clinical model it still was an ambitious effort to mimic a clinical situation. Nevertheless, other factors in the systemic inflammatory response after surgical trauma might have additional impact on plasma dilution.

Volume kinetic modeling fits dilution data to an individual mathematical model. The models can be used for estimating parameters as well as simulations of experiments.[18,19] The method is, however, hampered by the requirement of

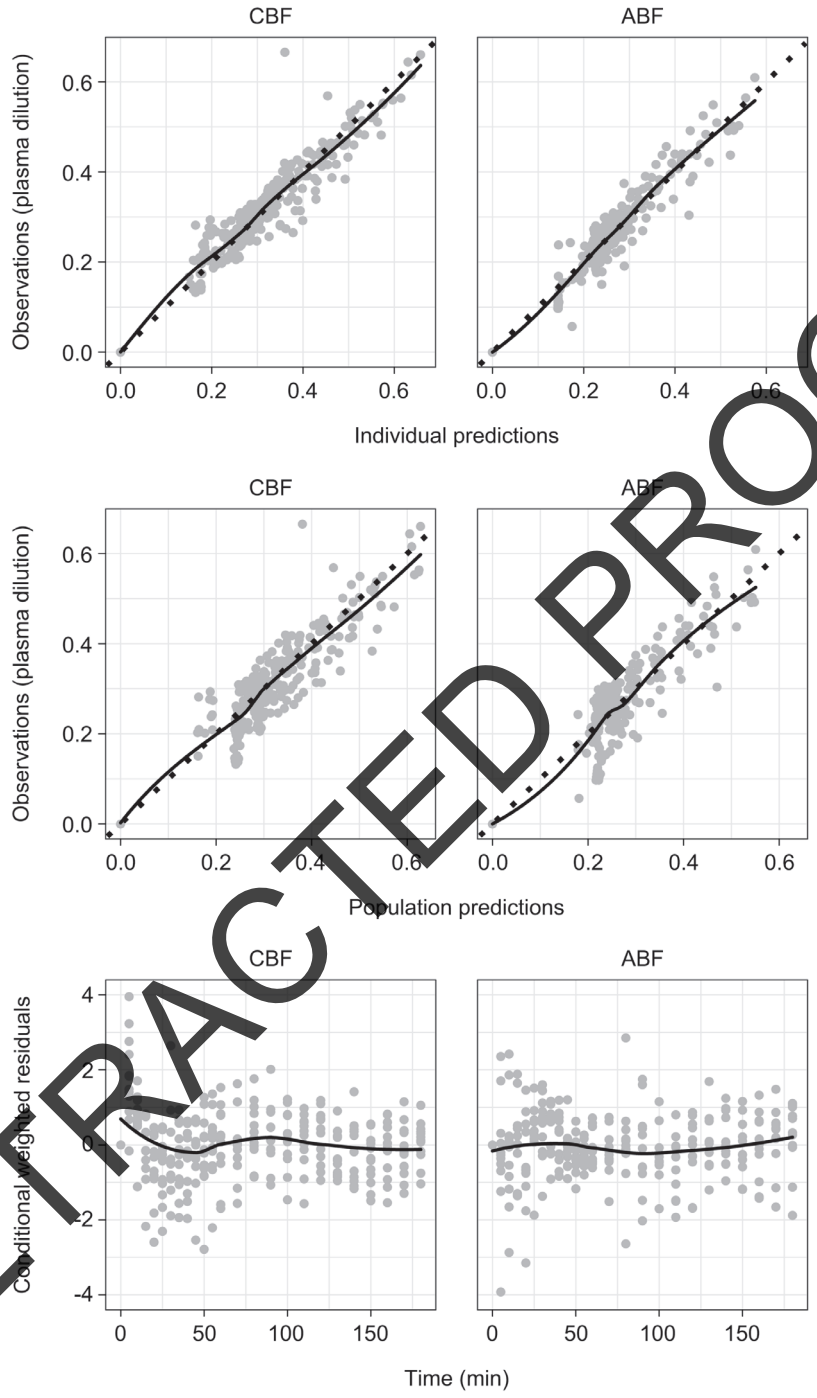

Fig. 6. Individual predictions (*upper*) and population predictions (*middle*) *versus* observations together with conditional weighted residuals *versus* time (*lower*) of the final model. The figure is stratified on conscious, bled, fluid-infused (CBF) subjects and anesthetized, bled, fluid-infused (ABF) subjects.

individual models, which is less efficient when it comes to estimating parameters with few subjects or few observations. The apparent solution is to put all data together in a population kinetic model and gain statistical power by pooling all information.

In a previous work, a population kinetic approach was applied to the data on plasma dilution and urinary excretion as a development of volume kinetics on distribution and elimination of fluid.[20] This was a study in sheep, and the anesthetic gas isoflurane was singled out as a significant

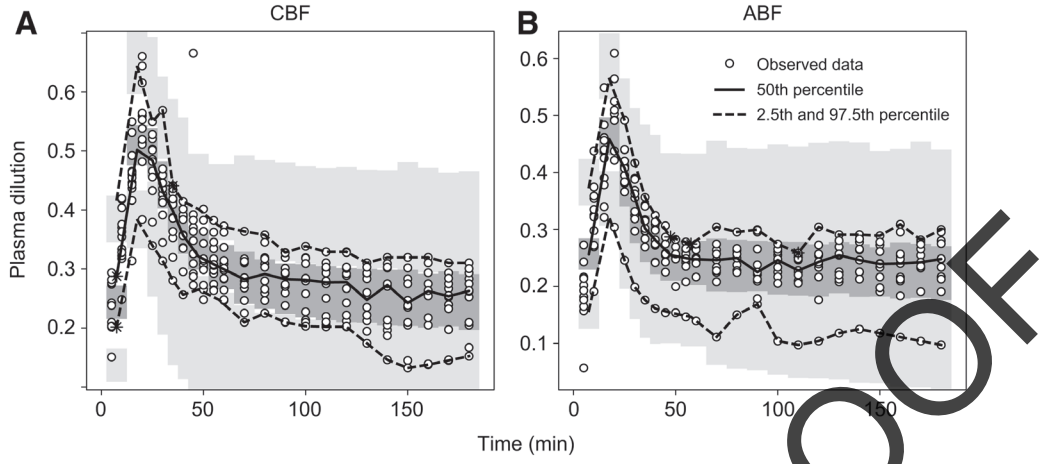

Fig. 7. Visual prediction check for the final model based on 1,000 simulated datasets, stratified on (*A*) conscious, bled, fluid-infused (CBF) and (*B*) anesthetized, bled, fluid-infused (ABF) subjects. The *dots* are the observed data. The *lines* are the 2.5th (*broken lower line*), 50th (*solid line*), and 97.5th (*broken upper line*) percentiles based on the observed data. The shaded areas are 95% CI for the 2.5th, 50th and 97.5th percentile prediction intervals based on the simulated data.

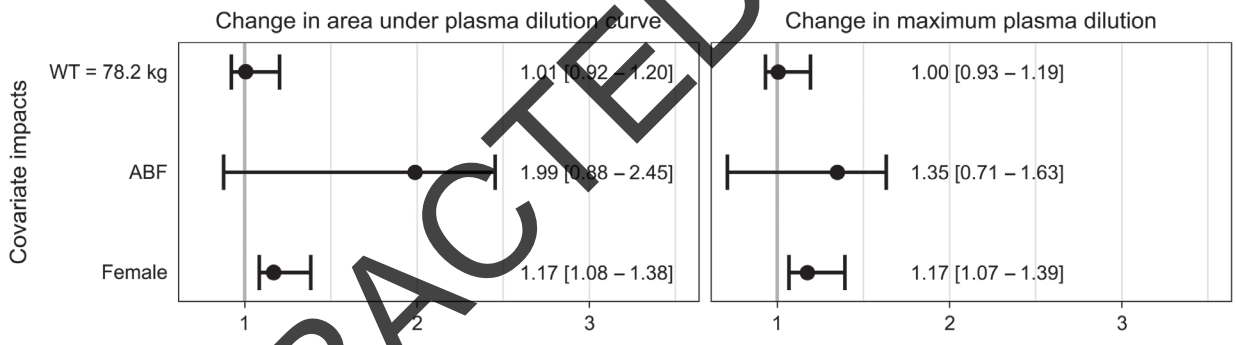

Fig. 8. The area under the plasma dilution curve (*left*) and maximum plasma dilution (*right*) difference to a typical individual (male, body weight of 68.2 kg in the conscious, bled, fluid-infused study arm) for the significant covariates. The median (marked as a dot) and 95% CI (the error bar) from a bootstrap of the final model are visualized for each covariate as well as their values. The typical value is marked as the *grey vertical line* for each.

factor that altered the disposition of infused fluid that was trapped in the peripheral compartment. Another finding in that study was that infused fluid did not increase MAP, which is interesting because a lowered MAP is still one of the major reasons for clinicians to administer fluids in the perioperative and intensive care settings.[21] Hundeshagen *et al.*[22] hypothesized in their resuscitation studies that the lowering of MAP despite fluid boluses was attributable to capillary shear stress and nitric oxide release, reduction of blood viscosity, and the release of atrial natriuretic peptide. Norberg *et al.*[8] further investigated the distribution of normal saline in a crossover study in awake *versus* anesthetized subjects without hemorrhage. The main finding was that fluid was retained in both arms. Clearance was reduced in the isoflurane arm, which rendered a small but significant increase in peripheral fluid accumulation. Our study is similar to the Norberg study except for that our subjects were hemorrhaged before the fluid infusion. Apparently, the bleeding had much more impact than isoflurane because there was no significant difference between elimination in the conscious, bled, fluid-infused and anesthetized, bled, fluid-infusedarms.

In this population model, all parameters are estimated with high uncertainty, which is not surprising because only 12 subjects were included in the study. Even though the additive error is estimated to a small value, including the additive error has a significant impact on the fit (a drop of objective function value of 16.91, corresponding to a P value of $P < 0.001$). This was likely to explain the difference of the predictions to the very flat steady-state like

profiles toward the end of the experiment. However, this small additive error is negligible when simulating using the model. As seen in the table 1, V_c is close to plasma volume for normal subjects, although it should not be regarded as an anatomical volume. The model also shows high interindividual variability in the elimination rate constant (k_e) of the plasma dilution, indicating that the variability between individuals is large. Nevertheless, accounting for individual covariates decreases the individual variability to a large degree, and the model still quantifies valuable information when giving fluid to patients with different sex and body weight. The volume kinetic model was not numerically stable to estimate a peripheral volume parameter together with estimating the other parameters because the data had a steady-state–like plateau at the end of the study, which pushed the estimate of the peripheral volume (V_t) to infinity. However, after model reparameterization to transfer rate constants, the model parameters were estimated with realistic estimates. Nevertheless, the peripheral volume is accounted for in the transfer rate constant ($k_{21} = Q/V_t$), which enables simulations of the model without estimating V_t separately. The modeling work did not include any renal data, which might have improved the fit of the model; despite this, the model predicted the data well (fig. 5).

An important difference of this work compared with other work is that it contains the same subjects in a crossover study where they have undergone the same experiment in both a conscious and anesthetized state. The plasma dilution seems to be different depending on whether the patient is awake or anesthetized, and this has the largest impact of all covariates. Moreover, sex and body weight (after accounting for sex differences) have some impact on the plasma dilution, but these effects are smaller compared with the study arm (awake or anesthetized). However, both study arm and body weight impact on area under the curve/maximum plasma dilution were not statistically significant and the point estimates should therefore be regarded with caution.

The study, however, has some limitations. The applicability of the volume kinetic model is hampered by use of repetitive invasive samples of hemoglobin, which could be difficult in a clinical setting. Also, the volume kinetic model requires a rapid and significant size fluid bolus, which could limit its use in patients with comorbidities or certain surgeries in which excess fluid can be problematic. Noninvasive sampling of hemoglobin is now possible to do, but noninvasive hemoglobin measurements are still too imprecise for kinetic modeling.[23] We acknowledge, because of our small sample size, that it is difficult to separate out weight and sex because female subjects weighed significantly less than males. A covariate such as age may have explained more if there would have been a larger spread of ages. Another aspect is the lack of a cardiac output measurement as sex, weight, and consciousness may all be surrogate covariates for cardiac output.

Conclusions

A population kinetic model was possible to apply to human crossover data with both anesthetized and awake subjects after controlled hemorrhage. The impact of covariates showed that plasma dilution does not change with decreasing body weight, males got lower plasma dilution than females, and conscious subjects had lower plasma dilution compared with anesthetized subjects. Conclusions are to be regarded with caution because the number of subjects was low and all effects except for sex were not statistically significant.

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Competing Interests

The authors declare no competing interests.

Reproducible Science

Full protocol available at: husli@utmb.edu. Raw data available at: husli@utmb.edu.

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