

# Population Volume Kinetics Predicts Retention of 0.9% Saline Infused in Awake and Isoflurane-anesthetized Volunteers

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**Background:** In previous work, extravascular expansion was observed to be enhanced by isoflurane anesthesia in sheep when a crystalloid bolus was administered. The aim of the current study was to further elaborate these investigations to humans and to explore the use of population kinetics in the analysis of fluid shifts.

**Methods:** Eleven healthy volunteers participated in two experiments each, either awake or isoflurane anesthetized, during which they received 25 ml/kg saline, 0.9%, intravenously over 20 min. Plasma dilution data were derived from repeated sampling of hemoglobin concentration, and population pharmacokinetic analysis was conducted using the WinNonMix 2.0.1 software (Pharsight Corporation, Mountain View, CA). Plasma hormones were measured, and hemodynamic values were monitored.

**Results:** Fluid infusion during isoflurane anesthesia was followed by a higher cardiac output, lower arterial pressure, and lower urinary excretion as compared with the awake protocol ( $P < 0.05$ ). Albumin dilution was greater than hemoglobin concentration–derived plasma dilution, which indicates a transcapillary leak of albumin. A two-compartment model with an isoflurane-depressed, intercompartmental distribution parameter predicted that more than 50% of the infused volume was retained in the peripheral compartment at 180 min in both protocols. Isoflurane markedly increased the plasma levels of renin and aldosterone, whereas vasopressin was mostly unchanged.

**Conclusion:** Fluid retention after rapid infusion of 0.9% saline was prominent in both awake and isoflurane-anesthetized subjects. Altered kinetics of infused 0.9% saline during isoflurane anesthesia was expressed as reduced clearance and a slower distribution, resulting in a small but significant increase in fluid accumulation in the body fluid compartments. These changes may be due to the associated decreasing of mean arterial pressure and increased release of renin and aldosterone.

INTRAVENOUS administration of fluid is important during anesthesia and surgery. However, inability to correctly predict fluid shifts between tissues impedes precise matching of the rate of infusion with ongoing losses and volume demands. Volume kinetics has previously been applied to data from awake<sup>1</sup> and anesthetized<sup>2</sup> humans to study fluid shifts between different body fluid compartments. The current view is that fluid retention and postoperative tissue edema after surgery are promoted by trauma-induced stress<sup>3,4</sup> and by mechanical ventilation<sup>5</sup> rather than by the administration of an anesthetic *per se*. However, a study in sheep<sup>6</sup> demonstrated that the combination of isoflurane anesthesia and mechanical ventilation significantly alters the disposition of crystalloid fluid in comparison with conscious, spontaneously ventilating sheep. In a follow-up study in sheep, isoflurane was singled out as the cause for reduced urinary excretion and peripheral fluid accumulation.<sup>7</sup> The first aim of the current study was to investigate the hypothesis that isoflurane anesthesia promotes peripheral fluid retention and reduces urinary excretion in humans. To further develop kinetic modeling, a second aim was to apply population kinetic analysis to the plasma dilution data. With the development of a population kinetic model, it could be possible to attribute differences in fluid distribution to such parameters as body weight, age, and sex.

## Materials and Methods

Twelve healthy volunteers, 5 women and 7 men, aged 19–36 yr, were recruited for the study. Their mean ( $\pm$  SD) body weight was  $83.8 \pm 18.5$  kg, height was  $172 \pm 9$  cm, and body mass index was  $27.9 \pm 4.2$  kg/m<sup>2</sup>. Health status was assessed by history, physical examination, and laboratory screening. The study was approved by the Institutional Review Board and the General Clinical Research Center at the University of Texas Medical Branch at Galveston, Texas. Written informed consent was obtained from all volunteers.

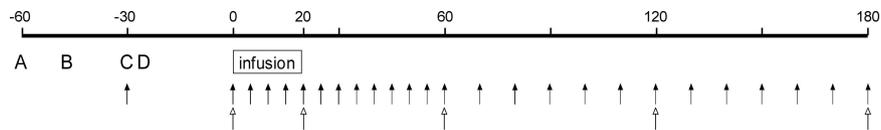
### Experimental Procedures

Each subject participated in two separate experimental sessions separated by at least 14 days. The volunteers arrived in the laboratory in the morning after an overnight fast. The timeline of the experimental procedures is presented in figure 1. Premedication consisted of 20

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**Fig. 1.** Timeline diagram of experimental procedures. *A* is premedication with 20 mg oral midazolam and application of topical lidocaine in the nose in the awake experiment, *B* is placement of arterial catheter for determination of plasma volume by indocyanine green and insertion of a venous line, *C* is induction of anesthesia, and *D* is placement of an esophageal Doppler probe. *Closed arrows* indicate blood sampling for hemoglobin concentration, and *open arrows* indicate blood sampling for hormones.

mg midazolam orally in both protocols. A 16-gauge catheter was placed in a forearm vein and a 20-gauge catheter was placed in a radial artery for crystalloid infusion and blood sampling, respectively. In the first session, the volunteers were anesthetized with isoflurane, and in the second session, they were awake, while in both experiments receiving an intravenous infusion of 25 ml/kg body-warmed 0.9% saline (Baxter, Irvine, CA) over 20 min with the aid of an infusion pump (FloGard 6201; San Diego, CA). The asleep experiments were performed first to minimize dropouts between experiments because of volunteer anxiety regarding the insertion of the esophageal Doppler probe in the awake state. The experimental protocol ended 180 min after the start of crystalloid infusion.

In the isoflurane experiment, anesthesia was induced with propofol, 1.5–2.5 mg/kg body weight, and a laryngeal mask airway (*ProSeal*<sup>™</sup>; Intavent Orthofix, Maidenhead, United Kingdom) was inserted.<sup>8</sup> Anesthesia was maintained with 1.0–1.5% of isoflurane in air:oxygen, corresponding to a target of 1.0 minimum alveolar concentration for the entire experimental period of 3 h. The subjects were breathing spontaneously throughout the experiment. We anticipated an end-tidal carbon dioxide of 30–35 mmHg. In both protocols, an esophageal Doppler probe (Cardio-Q; Deltex Medical, Branford, CT) was inserted for measurement of cardiac output (CO; actually descending aortic flow velocity converted flow based on aortic diameter calculated from a nomogram).<sup>9</sup> In the isoflurane experiment, the probe was inserted through the suction channel in the laryngeal mask. In the awake experiment, it was inserted nasally after administration of topical lidocaine and, if necessary for comfort ( $n = 9$ ), propofol at 1–1.5 mg/kg body weight. Baseline measurements were taken, and a stabilization period of approximately 30 min was permitted to elapse before starting the crystalloid infusion protocol.

During isoflurane anesthesia, we accepted a lower target of systolic arterial pressure of 80 mmHg. If a lower blood pressure was reached, a low-dose infusion of phenylephrine at  $0.07\text{--}0.15 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  was provided ( $n = 1$ ). Hypotension was to be expected in the absence of surgical stimulation, but the volunteers were well hydrated, spontaneously breathing, and well monitored by both continuous CO and end-tidal carbon dioxide registration. Furthermore, in the anesthetized state, urinary bladder volumes were measured every 20 min using ultrasound (Bladderscan BVI 3000; Diagnostic Ul-

trasound, Bothell, WA). If the scan indicated that the urinary volume exceeded 500 ml, the bladder was temporarily emptied by bladder catheterization to avoid overdistension.<sup>10</sup> In the awake session, the subjects voided when necessary. Urinary volumes were measured on a scale, assuming a density of 1.

### Measurements

Baseline plasma volume was measured using 5.0 mg indocyanine green dye (ICG) at the beginning of each session. The tracer was injected into a peripheral vein, arterial samples were taken at 1, 2, 3, 4, 5, and 6 min, and the volume of distribution was calculated by linear regression of the slope in a log concentration–time plot.<sup>11</sup> Standard decay curves were constructed for each subject from plasma collected before dye infusion.

Because the arterial catheter was used solely for blood sampling purposes, the systolic, diastolic, and mean arterial blood pressures were measured noninvasively by an inflated cuff every 5 min for the first 60 min and thereafter every 10 min. CO was measured every 20 s using the esophageal Doppler probe. The mean value of CO for each 5-min period was recorded in the experimental protocol.

Arterial blood was sampled before the start of infusion, every 5 min after the start of infusion until 60 min, and thereafter every 10 min until 180 min. Hematocrit, hemoglobin concentration, and mean corpuscular volume were measured in duplicate for mass-balance and volume kinetic analysis of the fluid distribution using 1.0-ml arterial blood samples using a Sysmex 302 HST line on the Sysmex SE 9500 (Sysmex, Mundelein, IL).<sup>12</sup> Hemoglobin concentration was measured by a sodium lauryl sulfate 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 sample withdrawal, 4 ml blood was removed from the arterial catheter to avoid sample dilution. The withdrawn blood was reinfused before the catheter was flushed with 1–2 ml heparinized saline. At the same time points, samples for total plasma protein and serum albumin were taken and analyzed by spectrophotometry (Vitros 51FS; Autoclinal Diagnostics, Rochester, NY).

Hormone concentrations were measured in arterial plasma at 0, 20, 60, 120, and 180 min from the start of crystalloid infusion. These samples were refrigerated at  $-70^{\circ}\text{C}$  and later transported on dry ice and analyzed in

the endocrine laboratories at the Karolinska University Hospital, Stockholm, Sweden. The renin concentration was analyzed immunochemically on the Nichols Advantage<sup>®</sup> chemiluminescence analyzer (Nichols Institute Diagnostics, Paris, France),<sup>13</sup> and radioimmunoassay kits were used to measure the plasma concentrations of aldosterone (Aldo-Riact; CIS Bio International, Yvette Cedex, France), arginine vasopressin (R&D Systems Inc., Minneapolis, MN), and brain natriuretic peptide (Shionoria BNP; Shionogi Ltd., Osaka, Japan).

#### *Volume Kinetic Model Development*

The plasma dilution values based on hemoglobin concentration were corrected for blood sampling as presented elsewhere.<sup>1</sup> A similar procedure was applied to dilutions of the plasma protein and albumin concentrations.<sup>12</sup>

Population pharmacokinetic analysis was performed using a nonlinear mixed effects regression program (WinNonMix 2.0.1; Pharsight Corporation, Mountain View, CA) bundled with Compaq Visual Fortran 6.6 (Hewlett-Packard, Palo Alto, CA). The first-order conditional estimation method was used for all modeling, and intersubject parameter variability was assumed to be log linear. A constant within-subject error variance was applied because plasma dilution, which represented the dilution of the central compartment, was in the narrow range of 0.92–1.59. Goodness of fit of the explored pharmacokinetic models was assessed by the objective function value, where a difference of  $-7.88$  is statistically significant at the  $P < 0.005$  level assuming a chi-square distribution, by examining the pattern of weighted population residuals, parameter precision, intersubject parameter variability, parameter correlations, and parameter estimability in terms of a positive Hessian matrix of the objective function.

Previously published two-compartment volume kinetic models<sup>1,2</sup> were tested separately for the anesthetized and awake subjects. To assess the effect of isoflurane anesthesia, each model parameter was compared for statistical differences between the awake and anesthetized states. In the absence of such a difference for a parameter between the two protocols, it was assumed that the parameter had the same value in both states. However, if a model parameter differed between sessions, it was treated as a baseline parameter for the awake state, with a multiplicative factor marking the effect of isoflurane anesthesia. Then, all experiments from both protocols were analyzed simultaneously.

The models have been presented in detail elsewhere<sup>1,2</sup> and contain the following model parameters:  $V_1$  and  $V_2$  are the baseline volumes of the central and peripheral compartments, respectively;  $k_t$  is the intercompartmental clearance factor that is governed by relative fractional dilutions (unitless) of the two fluid compartments; and  $k_r$  is the renal clearance, which is influenced by the frac-

tional dilution of the central compartment. The latter is either an estimated model parameter or calculated from the area under the time–dilution curve for the central compartment divided by the measured total urinary output. Finally,  $k_b$  is either a preset zero-order model constant describing insensible loss or, if  $k_r$  is modeled from urinary output, a zero-order rate parameter describing the loss of fluid volume from the central compartment to a deeper third compartment that could not be mobilized within the study time of 180 min.<sup>2</sup>

The influence of demographic parameters on model parameters was tested to determine whether any variability could be reduced. Evaluated parameters were awake *versus* anesthetized states, body weight, body mass index, sex, urinary output, baseline hemodynamic values, and baseline levels of plasma hormones.

#### *Cross-validation and Predictive Performance*

Cross-validation is an established method to estimate model performance even in the absence of a prospective test of the derived pharmacokinetic model.<sup>14,15</sup> The pharmacokinetic model was fitted to data while one subject was excluded from the analysis, and the obtained subset of model parameters was used to predict the plasma dilution–time data for the excluded subject. This procedure was repeated until each subject had been excluded once.

The bias and precision of the predictions were calculated from the median prediction error and absolute median prediction error, respectively.<sup>14,16</sup> Confidence intervals for the median were calculated as suggested by Campbell and Gardner.<sup>17</sup>

#### *Statistical Analysis*

Data are presented as mean  $\pm$  SD or otherwise as median (interquartile range) if significant according to the Shapiro-Wilk  $W$  test of normality. Study protocols were compared by Student paired  $t$  test or Wilcoxon signed rank test as appropriate.

Because no human data on the SDs of differences between the awake and anesthetized states were available, power analysis was based on previous sheep data.<sup>7</sup> The increase in fluid retention of the tissue compartment at 180 min found in anesthetized sheep was  $11.0 \pm 8.2$  ml/kg. With 7 subjects, it would be possible to detect such a difference (effect size 1.34) with a power of 80% by a two-sided paired  $t$  test. With 12 subjects, an effect size of 0.89 was detectable with 80% power.

Hemodynamic parameters and plasma dilution were compared at baseline before the start of anesthesia or Doppler probe insertion, respectively, immediately before the start of crystalloid infusion (0 min), the end of infusion (20 min), and the end of the experiment (180 min). CO was analyzed at 60 min because of missing data at 180 min. Analysis of variance for a two-factor experiment with repeated measures on protocol (isoflurane

**Table 1. Demographics and Fluid Mass Balance Data in Healthy Volunteers (n = 11) before and after Infusion of 25 ml/kg Saline, 0.9%, over 20 min in Isoflurane-anesthetized and Awake States**

	Isoflurane	Awake
Baseline measurements		
[Hb], g/dl	14.0 ± 1.7	13.7 ± 1.7
Plasma volume, l*	3.23 ± 0.85	3.15 ± 0.63
Hematocrit	0.402 ± 0.045	0.393 ± 0.046
Calculated blood volume, ml/kg	66.8 ± 18.3	61.8 ± 12.9
Plasma protein, g/dl	6.6 ± 0.3	6.7 ± 0.4
Plasma albumin, g/dl	3.6 ± 0.2	3.7 ± 0.3
Mean corpuscular volume, fl	86.6 ± 3.9	86.5 ± 4.0
Measurements at 20 min†		
Plasma volume dilution from [Hb], %	41.9 ± 9.3‡§	36.4 ± 8.4‡
Plasma protein dilution, %	38.6 ± 11.0‡	33.4 ± 8.1‡
Plasma albumin dilution, %	61.5 ± 13.3‡	60.3 ± 13.8‡
Measurements at 180 min†		
Plasma volume dilution from [Hb], %	7.2 ± 4.5‡	3.8 ± 4.3
Plasma protein dilution, %	8.4 ± 6.3‡§	1.9 ± 5.3
Plasma albumin dilution, %	18.8 ± 8.3‡§	9.4 ± 6.8‡
Mean corpuscular volume, fl	87.2 ± 3.8‡	86.7 ± 4.1‡
Cumulative urinary excretion, ml	458 ± 246	730 ± 255

Values are mean ± SD.

\* Baseline plasma volume measurement by dilution of indocyanine green.  
 † Minutes after start of crystalloid infusion. ‡  $P < 0.05$  compared with baseline values at 0 min. §  $P < 0.05$  for isoflurane-anesthetized vs. awake state by two-way analysis of variance on time and protocol followed by *post hoc* Tukey test for pairwise multiple comparisons. ||  $P < 0.05$  for anesthetized vs. awake state by Student paired *t* test.

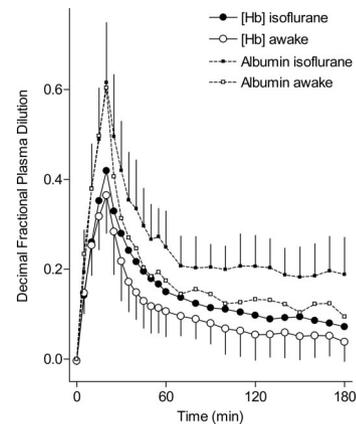
Hb = hemoglobin.

anesthetized vs. awake) and time with a *post hoc* Tukey test for pairwise multiple comparisons was used.

Changes over time in plasma hormones were analyzed by Friedman analysis of variance followed by Dunn test for multiple comparisons. Differences between protocols were analyzed by Wilcoxon signed rank test uncorrected for multiple testing. Data analysis was conducted using STATISTICA 5.1 (StatSoft Inc., Tulsa, OK) except for the Dunn test, where GraphPad Prism 4.02 was used (GraphPad Software Inc., San Diego, CA). Significance was accepted at  $P < 0.05$ .

## Results

The volunteers tolerated the two experimental procedures well. However, a few subjects tolerated the Doppler probe poorly in the awake state. Therefore, we removed the probe after 60 min in 3 subjects; only 4 subjects tolerated the probe for the entire study period while awake. In one female subject, we failed to insert a patent arterial line within our limit of three efforts. Therefore, she never participated in the awake protocol and was excluded from analysis. Baseline measurements for the other 11 subjects are presented in table 1.



**Fig. 2. Decimal fractional changes from baseline of the central compartment ( $V_1$ ), obtained from changes in hemoglobin (Hb) and albumin concentrations, versus time for awake and isoflurane-anesthetized volunteers (n = 11), after infusion of 0.9% saline, 25 ml/kg body weight, during 20 min with start of infusion at 0 min. Values are mean ± SD.**

### Plasma Dilution and Urinary Excretion

The anesthetized and awake sessions resulted in a similar pattern of plasma dilution profiles, calculated from dilution of hemoglobin, with a maximal increase of plasma dilution at the end of infusion followed by stabilization at a level slightly above the baseline (fig. 2). Plasma dilution calculated from dilution of total plasma protein was similar to that calculated from hemoglobin concentration but with a greater variability (table 1). In contrast, dilution of albumin was significantly greater (fig. 2). In the anesthetized state, crystalloid infusion caused a more pronounced plasma dilution than in the awake state (table 1). Cumulative urinary output was significantly lower in the anesthetized state ( $P < 0.05$  by *t* test) (table 2).

### Hemodynamic Effects

Cardiac output just before the start of the crystalloid bolus was higher in the anesthetized state,  $6.4 \pm 1.7$  l/min, compared with  $4.8 \pm 1.2$  l/min in the awake state ( $P < 0.01$ ). The infusion of saline further increased CO in both protocols by 35% on the average. Preinfusion values were retaken within the study period (fig. 3A).

Mean arterial pressure (MAP) did not differ between protocols at baseline before any interventions (fig. 3B). The induction of isoflurane anesthesia decreased MAP from 75 mmHg to 66 mmHg ( $P < 0.05$ ). The MAP was further decreased during isoflurane anesthesia despite the rapid fluid bolus, and this condition prevailed throughout the experiment.

Heart rate was also similar between protocols at baseline but was higher during isoflurane anesthesia than in the awake state (repeated-measures analysis of variance,  $P < 0.05$ ; fig. 3C). Heart rate was stable throughout the awake protocol.

**Table 2. Individual Fluid Balance Estimated by Applied Final Pharmacokinetic Model at 180 min after Bolus Infusion of 25 ml/kg Normal Saline in 11 Healthy Volunteers during Isoflurane-anesthetized and Awake States**

	Isoflurane	Awake	<i>P</i> †
Crystalloid infusion	2,147 ± 447	2,157 ± 471	0.43
Renal excretion (measured)	458 ± 246	730 ± 255	0.013
Expansion of the central compartment, $V_1$ *	311 ± 125	234 ± 112	0.023
Expansion of the tissue compartment, $V_2$ , explained by a isoflurane-dependent distribution parameter, $k_t$ †	1,374 ± 383	1,137 ± 477	0.016
Residual	3 ± 16	56 ± 146	0.30

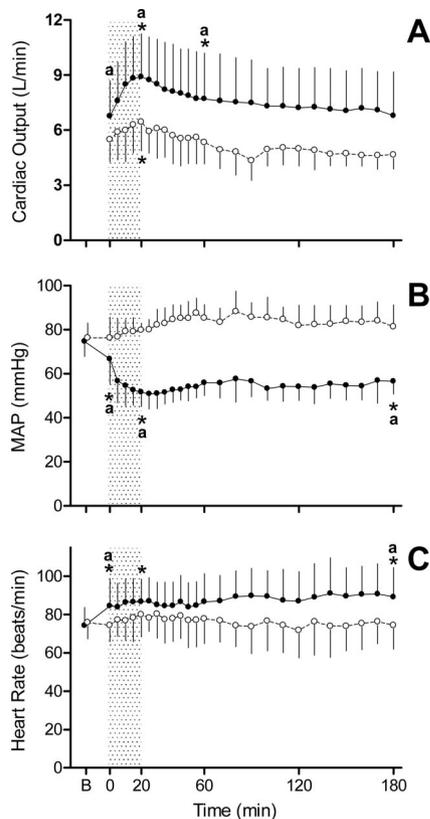
Values are mean ± SD, in milliliters.

\* Calculated as baseline volume of the central compartment  $V_1$  × predicted dilution at 180 min after start of infusion. † The fractional dilution of  $V_2$  was computed by WinNonLin 4.0.1 (Pharsight Corporation, Mountain View, CA) using the obtained individual parameters from the population pharmacokinetic analysis. The expansion of  $V_2$  was then calculated as baseline volume of the tissue compartment  $V_2$  × predicted dilution at 180 min. ‡ *P* values obtained from paired *t* test, uncorrected for multiple testing.

### Population Volume Kinetic Analysis

A total of 550 plasma dilution measurements, as calculated from hemoglobin concentration, from 11 subjects were included in the analysis. Previously used volume kinetic models were used as a base for further model development as presented in table 3.

The one-compartment model showed a wave-formed pat-



**Fig. 3.** Cardiac output (A), mean arterial pressure (MAP; B), and heart rate (C) in 11 volunteers in the awake state (open circles, broken line) or isoflurane-anesthetized state (closed circles, black line). A volume load of 0.9% saline, 25 ml/kg body weight, was given during 20 min (gray zone), with start of infusion at time 0. B marks the baseline measurement before start of induction of anesthesia. \* Significantly different from baseline for  $P < 0.05$  (only B, 0, 20, and 180 min were compared). <sup>a</sup> Significant different between protocols for  $P < 0.05$ . Data were analyzed by two-way analysis of variance followed by Tukey *post hoc* test for multiple comparisons. Values are mean ± SD.

tern of population residuals over time. Compared with the one-compartment model, the basic two-compartment model explained 12% of the variance and decreased objective function value by 706. By calculating  $k_t$  from the measured urine output and permitting  $k_t$  to be isoflurane dependent, the model could explain a further 37% of the variance, and the objective function value was reduced by 171. The model with  $k_b$  as a zero-rate model parameter also resulted in a decreased objective function value. However, parameter estimates became less precise, predictive performance was not improved, and the Hessian matrix of the objective function ceased to be positive definite, suggesting poor estimability. Therefore, the two-compartment model with isoflurane-dependent  $k_t$  was selected as the final model. The only one of the introduced patient covariates that improved the model fit significantly was isoflurane *versus* awake state, where isoflurane anesthesia reduced  $k_t$  by 25%.

In the final model, the individual model fit was significantly improved by introducing this covariate, whereas the parameter precision and interindividual variability were well preserved (table 4). Cross-validation of the final model was performed with  $k_t$  as a model constant calculated from the mean value of the other 10 subjects, and showed a median bias of  $-0.32\%$  (95% confidence interval of the median,  $-0.39$  to  $-0.12$ ) and a precision of 2.48% (2.29 to 2.66) (table 3), suggesting that the model would perform well in a truly prospective study.

The population estimate of  $V_1$  was 3.5 l—slightly larger than the measured mean plasma volume, and  $V_2$  was four times as large, 14.4 l. Renal clearance ( $k_r$ ) was lower in the anesthetized state,  $19.0 \pm 12.3$  ml/min, compared with the awake protocol,  $41.6 \pm 20.4$  ml/min ( $P < 0.01$ ; table 4).

A comparison of infused, excreted, and model-predicted preserved volumes at 180 min showed an expansion of  $V_2$  representing  $63 \pm 11$  and  $51 \pm 14\%$  of the crystalloid infusion in the isoflurane and awake protocols, respectively (table 2 and fig. 4).

### Hormone Levels

Plasma renin activity increased markedly during isoflurane anesthesia ( $P < 0.001$ , Friedman analysis of vari-

**Table 3. Model Development and Covariate Analysis**

Model	Parameters	Constants	OFV*	Comment	Bias, %†	Precision, %†
One-compartment	$V, k_r$	$k_b$	-1,568.8		1.32 (0.88 to 1.54)	3.80 (3.41 to 4.10)
Two-compartment, basic	$V_1, V_2, k_r, k_t$	—	-2,274.9	Two compartments improve model fit.	-2.35 (-2.71 to -2.01)	3.36 (3.08 to 3.69)
$k_r$ from measured diuresis	$V_1, V_2, k_t$	$k_{ra}, k_{riso}$	-2,373.0	$k_r$ is better determined as a model constant.	-0.50 (-0.68 to -0.36)	2.23 (2.13 to 2.35)
+ ( $k_{ta} \times f_{tiso}$ )‡	<b><math>V_1, V_2, k_{ta}, f_{tiso}</math></b>	<b><math>k_{ra}, k_{riso}</math></b>	<b>-2,435.4</b>	$k_t$ is isoflurane dependent.	<b>-0.29 (-0.38 to 0.01)</b>	<b>2.23 (2.15 to 2.46)</b>
+ $k_b = 0.5$	$V_1, V_2, k_{ta}, f_{tiso}$	$k_b, k_{ra}, k_{riso}$	-2,438.2	$k_b$ as a model constant does not improve model fit.	-0.30 (-0.39 to 0.06)	2.36 (2.25 to 2.48)
+ $k_b$ as model parameter	$V_1, V_2, k_{ta}, f_{tiso}, k_b$	$k_{ra}, k_{riso}$	-2,565.5	$k_b$ as a model parameter improves OFV but makes model parameters less estimable.	-0.69 (-1.04 to -0.52)	2.80 (2.43 to 2.97)
Cross-validation of final model ( <b>bold</b> )					-0.33 (-0.46 to -0.06)	2.44 (2.30 to 2.61)
Cross-validation with mean $k_r$ §					-0.32 (-0.39 to -0.12)	2.48 (2.29 to 2.66)

\* The change in objective function value (OFV) is identical to the change in -2 ML log likelihood. Assuming a chi-squared distribution, a change of -7.88 is statistically significant at the  $P < 0.005$  level. However, this does not necessarily mean that the model is superior by other means. † The bias and precision of the predictions were calculated from the median prediction error and absolute median prediction error, respectively.<sup>14,16</sup> Figures in parentheses represent the 95% confidence intervals for the median.<sup>17</sup> ‡  $f_{tiso}$  is 1 in the awake state. In the isoflurane-anesthetized state, it is a multiplicative factor explaining the effect of isoflurane on the rate of intercompartmental distribution. § During model development,  $k_r$  was calculated from the measured urine output in each experiment, but when validating the predictive performance of the model, it is more appropriate to use a population mean value of  $k_r$  because the individual value is unknown.  $f_{tiso}$  = multiplicative factor representing the effect of the isoflurane-anesthetized state on  $k_t$  (it is 1.0 in the awake state);  $k_b$  = insensible loss set to 0.5 ml/min if used as a model constant, else zero-order irreversible loss to a deeper third fluid space;  $k_t$  = intercompartmental distribution parameter;  $V$  = baseline volume of one-compartment model;  $k_r$  = renal clearance;  $V_1$  and  $V_2$  = baseline volumes of the central and peripheral compartments, respectively; subscripts *a* and *iso* refer to the awake and isoflurane-anesthetized states, respectively.

ance) and decreased slightly but significantly during fluid infusion in the awake state ( $P < 0.05$ ; fig. 5A). Baseline values before the start of the volume load but after induction of anesthesia were not significantly different between protocols. Likewise, plasma aldosterone was increased fourfold from baseline to 180 min in the anesthetized subjects ( $P < 0.001$ ) but decreased in the awake state ( $P < 0.001$ ; fig. 5B). Brain natriuretic peptide was transiently increased after the volume load in both awake and anesthetized subjects ( $P < 0.01$ ), reaching a maximum after 60 min (fig. 5C). Plasma vasopressin was

slightly increased in some cases, but the measurements only exceeded the limit of detection in 36 of 110 samples (fig. 5D).

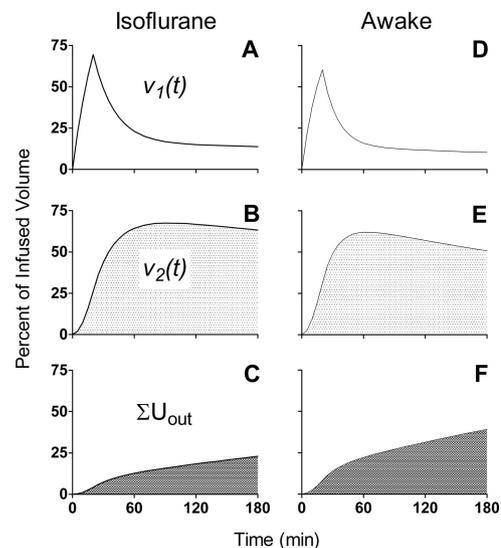
**Discussion**

This study was undertaken to investigate whether the anesthetic isoflurane promotes accumulation of infused

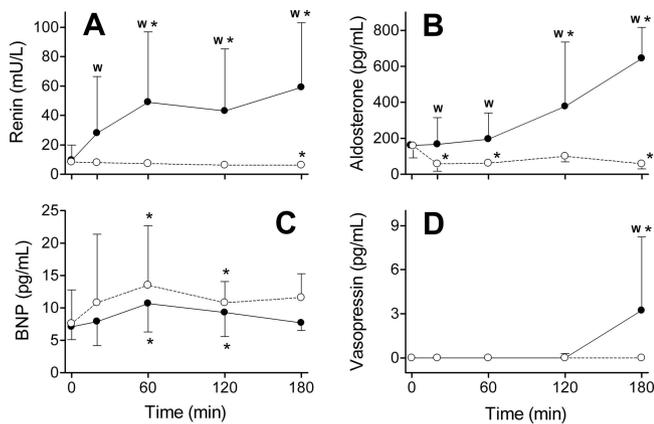
**Table 4. Population Estimates of Pharmacokinetic Parameters for the Final Model**

Model Parameter	Estimate	SE (CV%)	Population Variability*
$V_1, l$	3.53	0.26 (7)	28
$V_2, l$	14.4	2.0 (14)	23
$k_t, ml/min \times f_{tiso}$	$169 \times 0.746$	19 (11), 19 (46)	46
$k_{riso}, ml/min†$	$19.0 \pm 12.3$	—	—
$k_{rawake}, ml/min†$	$41.6 \pm 20.4$	—	—
Residual error‡	—	—	2.5

\* Expressed as coefficient of variation (CV) derived from the square root of the exponential intersubject variability. † Calculated from total renal excretion divided by the area under the time - fractional plasma dilution plot derived from hemoglobin concentration. ‡  $100 \times SE/median$  plasma dilution value.  $f_{tiso}$  = multiplicative factor in the isoflurane-anesthetized state that is 1 in the awake state;  $k_r$  = renal clearance (subscripts indicate protocol);  $k_t$  = intercompartmental distribution parameter in the awake state;  $V_1$  and  $V_2$  = baseline volumes of the central and peripheral compartments, respectively.



**Fig. 4. Model prediction of the fate of infused 0.9% saline, 25 ml/kg body weight, in volunteers in the isoflurane-anesthetized (A-C) and awake (D-F) states.**



**Fig. 5.** Renin (A), aldosterone (B), brain natriuretic peptide (BNP; C), and vasopressin (D) in 11 volunteers in the awake state (open circles, broken line) and isoflurane-anesthetized state (closed circles). A volume load of 0.9% saline, 25 ml/kg body weight, was given during 20 min, with start of infusion at time 0. \* Significant different from baseline for  $P < 0.05$  according to Friedman analysis of variance followed by Dunn *post hoc* test for multiple comparisons. \*\* Significant difference between protocols for  $P < 0.05$  for each time point according to Wilcoxon matched pair test uncorrected for multiple testing. Values are median  $\pm$  interquartile range.

fluid in a peripheral fluid compartment ( $V_2$ ), as has previously been shown in sheep.<sup>7</sup> These results in humans show that isoflurane anesthesia alone decreased the rate of excretion of a rapid infusion of 0.9% saline and caused a small but significant accumulation of fluid in the body compartments as compared with the awake state.

A population kinetic approach was applied to the data on plasma dilution and urinary excretion as a development of the more conventional volume kinetic analysis of the distribution and elimination of infused fluid.<sup>2,7</sup> This population kinetic analysis, albeit based on a limited number of subjects, showed that isoflurane alone was a significant factor that altered the disposition of fluid in volunteers in the absence of surgical stress. A further finding was that the decreased urinary excretion was accompanied by a marked stimulation of the release of renin and aldosterone, whereas antidiuretic hormone was largely unaffected despite a decrease in MAP.

The administered fluid bolus did not increase the lowered MAP in the anesthetized protocol, which is of interest because volume loading is often performed to combat anesthesia-induced hypotension. If the patient is well hydrated, vasopressors might be a better choice.

#### Model Development and Population Volume Kinetics

The current study is the first to investigate the kinetic performance of a fluid bolus in both awake and anesthetized volunteers to elucidate the effects of anesthesia *per se* without the potentially confounding effects of surgery. The population kinetic approach uses all data together and allows cross-validation of predictive model

performance and evaluation of the impact of anthropometric parameters such as body weight, age, sex, and protocol on model parameters.

The kinetic analysis demonstrated that most of the infused fluid is accumulated in the peripheral tissue compartment in both protocols. The expansion of the peripheral fluid space ( $V_2$ ) was more pronounced in the anesthetized state, but the difference (1,374 vs. 1,137 ml;  $P < 0.05$ ) was not as prominent as shown by Connolly *et al.*<sup>7</sup> in sheep (table 2). Isoflurane anesthesia was associated with a 25% decrease in  $k_t$ . Initially, this caused a greater expansion of the central compartment, but during redistribution, more fluid was retained in the peripheral compartment. This observation is in agreement with findings that a reduction of MAP during induction of general anesthesia initially promotes a preferential distribution of infused fluid to  $V_1$ , as evidenced by a lower  $k_t$ .<sup>18</sup>

Albumin dilution was more pronounced than hemoglobin dilution, and this difference was greater during isoflurane anesthesia (table 1 and fig. 2). We interpret these differences as extravasation of albumin that traps fluid in the peripheral compartment, a finding in accordance with previous findings that albumin is extravasated more easily during fluid loading in volunteers.<sup>1,19</sup> In surgical patients, however, there is an actual transfer of albumin back into the plasma space,<sup>2</sup> as is the case during hemorrhage.<sup>20</sup>

Thus, there are probably two opposing factors that change the balance of fluid distribution between  $V_1$  and  $V_2$ ; arterial hypotension promotes expansion of  $V_1$ , and transcapillary leakage of albumin acts to increase the expansion of  $V_2$ . The small but significant expansion of mean corpuscular volume (table 1) suggests that also the intracellular volume might be expanded and thus constitute some part of  $V_2$ .  $V_1$  probably consists of a central part of the plasma volume and some extracellular water in highly perfused organs.

The limited data regarding body composition, combined with a bolus dose related to body weight, caused a disappointing lack of relation between model parameters and body size. One way to improve population pharmacokinetics might be to measure body composition in some other way, such as total body water by isotope dilution or bioimpedance. Another speculation is to relate baseline hydration to a measurement of pre-experimental urine formation rate. Ideally, a larger population of subjects would be less sensitive to outliers.

#### Hemodynamics and Renal Output

The anticipated effect of isoflurane on renal excretion was small but statistically significant. This is somewhat different from the findings of the previous sheep experiments<sup>7</sup> in which urinary output was almost abolished during isoflurane anesthesia. There could be several reasons for this observation. Sheep are probably very differ-

ent from humans from the perspective that they have a more extended gastrointestinal system in which fluid can be sequestered. Admittedly, the placement of the esophageal probe in the volunteers required some facilitating propofol, which could have made the awake state more similar to the anesthetized state, although the total interval of sedation with propofol was brief.

Baseline MAP was lower after anesthesia induction compared with the awake state. Remarkably, MAP was unaffected by the fluid bolus in the anesthetized state (fig. 3B). Rather, MAP was further reduced and remained low during the whole study period. This was in contrast to the findings in sheep, where arterial blood pressure increased during isoflurane anesthesia.<sup>7</sup> This suggests that measurement of blood pressure as guidance for fluid therapy in humans could be a less useful tool during isoflurane anesthesia.

Although some anesthetics reduce urinary output directly by the action of nephrotoxic metabolites, this effect is clinically trivial with isoflurane.<sup>21</sup> Indirectly, the decrease in urinary output during anesthesia is attributed to systemic hypotension and renal vasoconstriction.<sup>22</sup> Isoflurane decreases sympathetic activity in man and reduces catecholamine release in a dose-dependent manner.<sup>23</sup> It decreases glomerular filtration by 30–50%, renal blood flow by 40–60%, and urinary flow rate to 34% of unanesthetized controls.<sup>21</sup> Because the current study shows reduced urinary output during anesthesia, together with peripheral accumulation of fluid, intraoperative urinary output may be an unreliable monitor as a guide to fluid therapy. Hence, two of the readily available instruments for fluid status assessment are doubtful and must be regarded with caution.

In this study, scanning of urinary bladder volume proved to be a poor estimate of urinary output, precluding use of these data for modeling. This possibly would have been improved by assigning one individual to perform all scanning. Bladder catheterization would have been even better for modeling purposes, but because of ethical restraints, we used a noninvasive scanning device. In a previous bleeding study in sheep,<sup>24</sup> renal clearance was modeled as a logarithmic relation to the fractional dilution of  $V_1$  that could also describe diuresis when  $v_1(t)$  decreased below  $V_1$ . That model was tested in the current study too, but urinary data lacked the required precision, which resulted in overparameterization.

Saline, 0.9%, seems to be followed by less brisk urinary excretion than the same quantities of buffered crystalloid solutions, which is probably due to the excess load of chloride ions. In one study in which 25 ml/kg was infused over 30 min in awake volunteers, 43% of the infused volume of 0.9% saline had been excreted 4 h later, whereas the corresponding fractions for lactated and acetated Ringer's solution were 60% and 50%, respectively.<sup>25</sup> Consequently, the renal clearance ( $k_r$ ) for

0.9% saline was lower in the current awake experiments than in previous studies of buffered solution, in which  $k_r$  is usually between 80 and 120 ml/min.<sup>1,26</sup>

#### *Plasma Volume Measured by ICG*

Mean plasma volume calculated from ICG dilution was close to the expected anthropometric values,<sup>27</sup> but the interindividual variation was large. In 7 of 11 subjects, there was a difference of more than 500 ml between experiments. After inspection of the time-log ICG concentration plots, we decided to omit the value of the first time point at 1 min because it clearly deviated from the regression line through the following values, and the peripheral injection of ICG is likely to cause a lag of complete mixing and interfere with definition of time zero for the back-extrapolation. Central venous injection has been suggested as necessary,<sup>28</sup> and even then, the reported time of complete mixing is 2.5 min.<sup>29,30</sup> For repeated plasma volume estimation by ICG dilution, baseline plasma volume assessments are necessary, but for volume kinetic calculations, they are not. However, if very different plasma volume values are applied to the same subject for different experiments, it will affect the area under the time-plasma dilution curve and thus  $k_r$ . Therefore, we used the mean of the two plasma volume estimates in each subject for both study days.

#### *Hormones*

Baseline hormone values were similar between isoflurane-anesthetized and awake sessions, suggesting small effects of anesthesia *per se* during the 30-min stabilization period preceding start of crystalloid infusion. However, as soon as the fluid bolus was started, there were considerable differences in the response between the isoflurane-anesthetized and awake states, reflecting that the protocols were sufficiently different to result in altered fluid kinetics.

In humans, renin and aldosterone increased in the anesthetized state, presumably because of decreased blood pressure, and these elevations probably contributed to the reduced urinary excretion during isoflurane anesthesia. Moreover, they raise the question of whether angiotensin-converting enzyme inhibitors, which are frequently prescribed cardiac drugs, could modulate fluid excretion during isoflurane anesthesia.

Interestingly, there was hardly any increase in vasopressin in several cases despite the isoflurane-induced decrease in arterial pressure. Maximum inhibition of water diuresis is obtained at 6 pg/ml in humans, and a decrease in arterial pressure due to hemorrhage or a vasovagal reaction increases the plasma level of this hormone to approximately 500 pg/ml.<sup>31</sup>

## **Conclusions**

The pronounced fluid retention of 0.9% saline in both awake and anesthetized subjects is an important finding.

Approximately 10% more of the fluid bolus was retained in the peripheral compartment in the anesthetized protocol, but this difference was considerably less than in sheep and may have limited clinical relevance. The altered disposition of fluid could be shown by both decreased urinary excretion and kinetic modeling. Despite a preferential distribution of infused fluid in favor of the central compartment initially, the combined effects of distribution and elimination finally resulted in slightly more pronounced expansion of the peripheral compartment in response to isoflurane. This was explained by a 25% decrease in the intercompartmental distribution parameter in the anesthetized state. Finally, a fluid bolus of 25 ml/kg during isoflurane anesthesia had no impact on decreased blood pressure, which suggests that blood pressure is a less reliable guiding tool for fluid therapy.

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