Do intravascular hypo- and hypervolemia result in changes in central blood volumes?

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Abstract

Background. Hypovolemia is generally believed to induce centralization of blood volume. Therefore, we evaluated to which extent hypo- and hypervolemia result in changes in central blood volumes (pulmonary blood volume (PBV), intrathoracic blood volume (ITBV)) and we explored the effects on the distribution between these central blood volumes and circulating blood volume ($V_{d\text{circ}}$).

Methods. Six anesthetized, spontaneously breathing dogs underwent blood volume alterations in a randomized order in steps of 150 ml (mild) to 450 ml (moderate) either by hemorrhage, retransfusion of blood, or infusion of colloids. PBV, ITBV and $V_{d\text{circ}}$ were measured using (transpulmonary) dye dilution technique. The PBV/$V_{d\text{circ}}$ ratio and the ITBV/$V_{d\text{circ}}$ ratio were used as an assessment of blood volume distribution.

Results. 68 alterations in blood volume resulted in changes in $V_{d\text{circ}}$ ranging from -33% to +31%. PBV and ITBV decreased during mild and moderate hemorrhage, while during retransfusion, PBV and ITBV increased during moderate hypervolemia only.

The PBV/$V_{d\text{circ}}$ ratio remained constant during all stages of hypo- and hypervolemia (mean values between 0.20 – 0.22). This was also true for the ITBV/$V_{d\text{circ}}$ ratio, which remained between 0.31 and 0.32, except for moderate hypervolemia, where it increased slightly to 0.33 (0.02), p<0.05.

Conclusions. Mild to moderate alterations of blood volume result in changes of $V_{d\text{circ}}$. PBV and ITBV. However, against the traditional belief of centralization we could show that the cardiovascular system maintains the distribution of blood between central and circulating blood volumes in anesthetized dogs.
Introduction

Circulation of blood is obligate for all mammals to maintain homeostasis. To accomplish this, the cardiovascular system is able to generate cardiac output (CO) by a complex interaction of multiple factors, of which venous return to the heart, the degree of vascular filling and cardiac performance are key factors. Especially the first two factors are influenced by the individual’s actual volume status. Whenever cardiac output is to be optimized, an adequate assessment of volume status is crucial to prevent excessive morbidity and/or mortality associated with either inadvertent hypo- or hypervolemia. While the traditionally used static pressure-based indicators of cardiac preload such as central venous pressure (CVP) or pulmonary capillary wedge pressure are relatively easy to measure, they do not provide a quantitative assessment of preload and actual volume status of an individual. This requires the use of indicator-dilution techniques, allowing determination of blood volumes after tracing of an intravenously administered indicator. Here, pulmonary blood volume (PBV), which is defined as the volume of blood between the pulmonary and the aortic valve, as well as intrathoracic blood volume (ITBV), defined as the volume of blood between the right atrium and the ascending aorta, can be calculated from the product of cardiac output (measured by an ultrasound flow probe) and the transpulmonary mean transit time of indocyanine green (ICG). Additionally, total circulating blood volume (\( V_{\text{circ}} \)), defined as the total amount of intravascular circulating (blood) volume, can be measured by tracing of ICG within the body over 30 minutes.

Acute hemorrhage and retransfusion resulting in hypo- or hypervolemia – i.e. an absolute change in \( V_{\text{circ}} \) might subsequently affect the intrathoracic compartment, i.e. PBV or ITBV. In addition, hypo- and hypervolemia might also influence the distribution of blood between the intra- and extrathoracic blood volume compartments. For instance, in case of hypovolemia, an individual is traditionally assumed to have a ‘centralized’ circulation with peripheral vasoconstriction in order to assure adequate blood flow to vital organs, which should be reflected by an altered distribution between \( V_{\text{circ}} \) and PBV or ITBV, in favor of the central blood volumes. Though, to the best of our knowledge, this assumption has not yet been demonstrated in previous research. Therefore, the aim of the present study was to determine \( V_{\text{circ}} \) and central blood volumes (PBV, ITBV) during alterations of blood volume resulting in hypo- or hypervolemia and subsequently calculating the relationship between circulating and central blood volumes. We hypothesized that the ratio between these central blood volumes and circulating blood volume is dependent on the actual volume status.

Methods

Animals and instrumentation
The dogs (six Foxhounds of both sexes, body weight between 28 and 35 kg, 29 ± 3 kg; all healthy, not splenectomized) were treated according to the principles of laboratory animal care (NIH publication nr 86-23, revised 1985). Furthermore, the study was approved by the local District Governmental Animal Investigation Committee.

Several weeks before the actual experiments were performed, both carotid arteries were exteriorized in skin loops and an ultrasound transit-time flow probe (16-20 mm S-series with silicone shielded U-reflector, Transonic Systems, NY, USA) was implanted around the pulmonary artery (figure 1) through a left-sided thoracotomy for continuous recording of pulmonary blood flow and subsequent continuous recording of CO, as described in detail before. Via the carotid arteries we introduced
two catheters into the ascending aorta, one for blood sampling and measurement of arterial blood pressure and the other for insertion of a fiberoptic thermistor probe (4F, Pulsiokath PV 2024, Pulsion Medical Systems, Munich, Germany) (figure 1). This probe continuously recorded intravascular indocyanine green concentration and blood temperature. In addition, a 7F fiberoptic thermodilution pulmonary artery catheter (Arrow International, Reading, MA) was introduced (figure 1) via a dog’s hindlimb under fluoroscopy prior to each experiment.

Figure 1: Illustration depicting the principles of the double indicator dilution technique. Indocyanine green (ICG) is injected into the right atrium. Two fiberoptic probes, placed in the pulmonary artery and ascending aorta, continuously measure ICG concentration. From the obtained dye dilution curves, mean transit times between injection site, pulmonary artery and aorta can be calculated to obtained ITBV and PBV.

Measurements
CO was continuously recorded using the Ultrasonic flow probe ("CO\textsuperscript{Transonic}"; ultrasonic transit-time flowmeter, T101, Transonic Systems, NY, USA) around the pulmonary artery as described previously. Blood volumes were determined as the product of CO\textsuperscript{Transonic} and mean transit times (mtt) of ICG, the latter derived by transpulmonary dye dilution. The indicator ICG (0.2 mg/kg, 5 ml) was injected via the proximal port of the pulmonary artery catheter into the right atrium and the resulting thermodye-dilution curves were recorded in the pulmonary artery and ascending aorta simultaneously with fiberoptic probes connected to an optoelectronic device (COLD-System Z021, Pulsion, Munich, Germany). The mtt of ICG from the pulmonary artery to the ascending aorta was determined by deconvolution of the dye dilution curves based on a pulmonary transport function as described in detail before. Subsequently, PBV was calculated as the product of CO\textsuperscript{Transonic} and mtt of ICG between pulmonary artery and ascending aorta.
ITBV was calculated as the product of CO\textsuperscript{Transonic} and mtt of ICG between injection site of ICG (right atrium) and ascending aorta.
As previously described, V\textsubscript{d\textsubscript{circ}} was calculated as the product of CO\textsuperscript{Transonic} and mtt of ICG through the circulation (mtt\textsubscript{circ}), which was obtained by fitting the aortic dye dilution curve to a recirculation model. Transport of a dye through circulating blood volume is best defined by a two-compartmental model. This model essentially consists of a fast and slowly perfused compartment and hence, mtt\textsubscript{circ} is the sum of the individual mtt through the fast (mtt\textsubscript{1}) and slow (mtt\textsubscript{2}) compartment, corrected by
associated correction factors ($R_1$ and $R_2$, respectively), to compensate for loss of tracer during passage (e.g. hepatic elimination). The product of $CO_{\text{transonic}}$ with $(\text{mtt}_1 \ast R_1)$ or $(\text{mtt}_2 \ast R_2)$ yields the respective volumes of distribution of the fast ($V_d$) and slow ($V_d$) compartment.

Each measurement was performed in duplicate of which mean values were used for subsequent calculation. In case the obtained measurement values showed substantial difference of more than 20%, a third measurement was performed.

Additionally, mean arterial blood pressure (MAP) and CVP (both measured using a Gould Statham pressure transducer P23 ID, Elk Grove, IL, USA, and adjusted to the level of the heart) were continuously recorded on an eight-channel polygraph (model RS 3800, Gould, Cleveland, OH) and simultaneously on a cassette data recorder (model XR-5000, TEAC®, Tokyo, Japan). To assess the influence of alterations in blood volume on the distribution of blood volumes, the ratios between PBV and $V_{d\text{circ}}$ (PBV/ $V_{d\text{circ}}$) as well as ITBV and $V_{d\text{circ}}$ (ITBV/ $V_{d\text{circ}}$) were calculated. In addition, the ratio between $V_{d1}$ and $V_{d2}$ was calculated as $(CO_{\text{transonic}} \ast \text{mtt}_1 \ast R_1) / (CO_{\text{transonic}} \ast \text{mtt}_2 \ast R_2)$, which is equal to $(\text{mtt}_1 \ast R_1) / (\text{mtt}_2 \ast R_2)$.

**Experimental program**

The animals were anaesthetized with pentobarbital (initial dose 20 mg/kg followed by 4 mg kg$^{-1}$ hr$^{-1}$). They were allowed to breathe spontaneously to exclude effects of mechanical ventilation with positive pressures on the distribution of blood volume. After introduction of the catheters into the pulmonary artery and ascending aorta we observed the dogs for 30 minutes to ensure stable hemodynamic conditions before we started data collection.

Blood volume was altered in both directions in a total of 68 steps of 150 ml to 450 ml by either hemorrhage and subsequent retransfusion of the shed blood or by infusion of colloid solution (hydroxyethyl starch (HES 200/0.5 6% solution, Fresenius Kabi GmbH, Bad Homburg, Germany)). The alterations in blood volume were performed in a randomly assigned fashion in order to reduce the possibility of systematic bias. Furthermore, all measurements were performed at the end of each intervention when all variables were in a steady state. Each dog was allowed to recover at least for one week between experiments.

**Statistical Analysis**

Statistical analysis was performed using Microsoft Excel 2010 (Microsoft, Redmond, USA) and SPSS Statistics version 19.0 (SPSS Inc., Chicago, USA). Normal distribution of continuous data was assessed using the Kolmogorov-Smirnov test. Results are given as mean (SD) or as median (range), when appropriate. If necessary, measured variables were normalized on body weight. The correlation between blood volumes was displayed in a scatterplot and the coefficients of determination ($R^2$ values) were calculated together with 95% confidence intervals.

Hemodynamic variables were analyzed in separate groups, based on the degree of alteration of blood volume (normovolemia, a decrease / increase of 0 – 150 ml ("mild"), or 150 – 450 ml ("moderate")). In order to account for between-subject variation and because the alterations of blood volume were performed in a randomly assigned order, a linear mixed model was used for analyzing the influence of blood volume alterations on the measured hemodynamic variables and blood volumes. Using this model, an overall effect on hemodynamics and blood volumes of hypo- and hypervolemia was assessed together with an assessment of differences between the defined groups (as described above). All performed tests were two-sided and statistical significance was set at a p-value < 0.05 after adjusting for multiple comparisons using the Bonferroni correction.
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Results

A total of 90 measurements were obtained in 22 experiments in 6 dogs. Changes in $V_{d\text{ circ}}$ ranged from -33% (hypovolemia, n=31) to +31% (hypervolemia, n=28).

Heart rate increased significantly during moderate hypovolemia, while CVP significantly decreased (table 1). The increase in heart rate from mild to moderate hypovolemia was also significant (table 1).

During mild hypervolemia (0-150 mL), none of the investigated hemodynamic variables were significantly different compared to normovolemia, while during moderate hypervolemia (150-450 mL), CO$_{\text{Transonic}}$, MAP and CVP significantly increased (table 1). MAP was also significantly higher during moderate than during mild hypervolemia (table 1).

Table 1: Hemodynamic changes in response to alterations in blood volume. Data are presented as mean (SD). $V_{d\text{ circ}}$: Circulating blood volume; MAP: Mean Arterial Pressure; CVP: Central Venous Pressure; CO$_{\text{Transonic}}$: Cardiac Output derived from Transonic Flow Probe. Data were analyzed separately for hypo- and hypervolemia using a linear mixed model analysis. 

Table 1: Hemodynamic changes in response to alterations in blood volume. Data are presented as mean (SD). $V_{d\text{ circ}}$: Circulating blood volume; MAP: Mean Arterial Pressure; CVP: Central Venous Pressure; CO$_{\text{Transonic}}$: Cardiac Output derived from Transonic Flow Probe. Data were analyzed separately for hypo- and hypervolemia using a linear mixed model analysis. 

<table>
<thead>
<tr>
<th>Relative volume status (ml kg$^{-1}$)</th>
<th>Normovolemia (n=31)</th>
<th>Hypovolemia</th>
<th>Hypervolemia</th>
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<tbody>
<tr>
<td>Change in $V_{d\text{ circ}}$ (%)</td>
<td>Mild (n=15)</td>
<td>Moderate (n=16)</td>
<td>Mild (n=10)</td>
</tr>
<tr>
<td>MAP (mm Hg)$^a$</td>
<td>86 (10)</td>
<td>83 (13)</td>
<td>81 (15)</td>
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<tr>
<td>Heart rate (bpm)$^b$</td>
<td>94 (17)</td>
<td>91 (15)</td>
<td>110 (13)$^*$</td>
</tr>
<tr>
<td>CVP (mm Hg)$^c$</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>CO$_{\text{Transonic}}$ (mL min$^{-1}$ kg$^{-1}$)$^d$</td>
<td>94 (16)</td>
<td>79 (15)</td>
<td>79 (12)</td>
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</table>

$^a$p<0.05 for the overall effect for hypovolemia as determined by the mixed model. $^b$p<0.05 for the overall effect for hypervolemia as determined by the mixed model. $^c$p<0.05 vs. corresponding value during normovolemia. $^d$p<0.05 for the comparison of moderate vs. corresponding value during mild hypo- or hypervolemia.

$V_{d\text{ circ}}$, PBV and ITBV during hypo- and hypervolemia

The scatterplot in figure 2 shows the correlation between changes in blood volume and values of $V_{d\text{ circ}}$, PBV and ITBV for all measured data points, together with the resultant slope of the linear regression curve. The correlation between these variables was significant (p<0.05) with associated R$^2$ values of 0.48 (95% Confidence Interval (CI) of 0.34-0.63) for $V_{d\text{ circ}}$, 0.31 (CI 0.16-0.46) for PBV, and 0.41 (CI 0.26-0.56) for ITBV. The correlation between $V_{d\text{ circ}}$ and PBV for data points during normo-, hypo- and hypervolemia is shown in figure 3A. Overall correlation was significant (p<0.05) with an R$^2$ value of 0.76 (CI: 0.67-0.85). In addition, the correlation between CBV and ITBV (figure 3B) for all data points was significant (p<0.05) with an associated R$^2$ value of 0.79 (CI 0.71-0.87). During mild hypovolemia, PBV and ITBV significantly decreased compared to normovolemia. $V_{d\text{ circ}}$ also tended to decrease, although statistical significance was not reached (p=0.055; table 2). During moderate hypovolemia, all blood volumes were significantly lower compared to both normovolemia and mild hypovolemia (table 2). No changes in blood volumes were observed during mild hypervolemia, while during moderate hypervolemia all blood volumes increased significantly compared to both normovolemia and mild hypovolemia (table 2).
Table 2: Changes in $V_{\text{d circ}}$, PBV and ITBV during periods of hypo- and hypervolemia. Data are presented as mean (SD). $V_{\text{d circ}}$: Circulating blood volume; PBV: Pulmonary Blood Volume; ITBV: Intrathoracic Blood Volume; Data were analyzed separately for hypo- and hypervolemia using a linear mixed model analysis. $^a$ $p<0.05$ for the overall effect for hypovolemia as determined by the mixed model. $^b$ $p<0.05$ for the overall effect for hypervolemia as determined by the mixed model. $^*$ $p<0.05$ vs. corresponding value during normovolemia. $^\dagger$ $p<0.05$ for the comparison of moderate vs. corresponding value during mild hypo- or hypervolemia.

<table>
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<tr>
<td></td>
<td>Mild (n=15)</td>
<td>Moderate (n=16)</td>
<td>Mild (n=10)</td>
</tr>
<tr>
<td>$V_{d circ}$ (ml kg$^{-1}$)</td>
<td>50.9 (8.1)</td>
<td>47.4 (6.2)</td>
<td>41.6 (3.9)</td>
</tr>
<tr>
<td>PBV (ml kg$^{-1}$)</td>
<td>10.4 (1.7)</td>
<td>9.5 (1.2)</td>
<td>9.1 (0.9)</td>
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<tr>
<td>ITBV (ml kg$^{-1}$)</td>
<td>16.0 (2.5)</td>
<td>14.4 (1.9)</td>
<td>13.3 (1.1)</td>
</tr>
<tr>
<td>PBV/ $V_{d circ}$ ratio</td>
<td>0.20 (0.02)</td>
<td>0.20 (0.02)</td>
<td>0.22 (0.02)</td>
</tr>
<tr>
<td>ITBV/ $V_{d circ}$ ratio</td>
<td>0.31 (0.02)</td>
<td>0.31 (0.04)</td>
<td>0.32 (0.02)</td>
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Figure 2: Scatterplot of change in blood volume versus corresponding values of $V_{d circ}$, PBV and ITBV. Also, the linear regression lines are shown, together with the respective coefficients of determination ($R^2$) are also shown.
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Figure 3A-B: Scatter plot of $V_d$ circ versus PBV (3a) and ITBV (3b). Data are shown for values during normovolemia (grey circles) and for values when blood volume was decreased (white circles) and increased (black circles). Also, the linear regression lines are shown for all data points, together with the coefficient of determination ($R^2$).
The ratio between PBV and $V_{d\text{circ}}$ (PBV/ $V_{d\text{circ}}$ ratio as an assessment of the (re)distribution of blood between intra- and extrathoracic compartments) remained constant for all gradations of both hypo- and hypervolemia. The same applies to the ratio between ITBV and $V_{d\text{circ}}$ (ITBV/ $V_{d\text{circ}}$ ratio), except for the data points obtained during moderate hypervolemia, where the ITBV/$V_{d\text{circ}}$ ratio was slightly increased. However, the correlation between blood volume changes and the PBV/$V_{d\text{circ}}$ and ITBV/$V_{d\text{circ}}$ ratio was not significant (figure 4), indicating a constant ratio. The ratio between the fast and slow compartment of circulating blood volume (the ($\text{mtt}_1 * R_1$)/($\text{mtt}_2 * R_2$) ratio) was not correlated with alterations of blood volume ($R=0.02; p=0.8$, data not shown). Finally, $V_{d\text{circ}}$ and the difference between ITBV and PBV correlated linearly with an $R^2$ value of 0.67 (95%CI 0.56 – 0.78; $p<0.05$) (figure 5).

**Figure 4**: Scatter plot of the relative volume status versus the PBV/$V_{d\text{circ}}$ and ITBV/$V_{d\text{circ}}$ ratio for all data points. Also, the linear regression line is shown together with the coefficient of determination ($R^2$).

**Figure 5**: Correlation between $V_{d\text{circ}}$ and the difference between ITBV and PBV. Also, the linear regression line is shown together with the coefficient of determination ($R^2$).
Discussion

In the current study we show that both hypo- and hypervolemia induce linear changes in the central blood volumes PBV and ITBV. In addition we show that alterations up to ± 20 ml kg\(^{-1}\) of \(V_{\text{d, circ}}\) did not influence the ratio between both central volumes and \(V_{\text{d, circ}}\), nor did it alter the ratio between the fast and slow compartment of circulating blood volume.

With this finding we have to reject our hypothesis and have to accept that even during periods of moderate hypo- and hypervolemia, the cardiovascular system maintains an equal distribution of blood between the central (intrathoracic) compartment and the peripheral (extrathoracic) compartment.

Critique of the methods:
At first, measurements were obtained in dogs and therefore data cannot be transposed to humans directly. Nevertheless, it is a very common procedure to investigate hemodynamic physiology in middle-large mammals and the use of such an animal model allowed us to alter blood volumes while keeping all other potential influencing factors standardized, a situation that would not be feasible if performed in humans. Furthermore, it allowed us to randomize the order of blood volume alterations to reduce the possibility of systemic bias when using a standardized experimental order.

At second, while the dogs were anesthetized during all experiments, they were allowed to breathe spontaneously, which less truly mimics a clinical situation of patients who receive general anesthesia and positive pressure mechanical ventilation. Here, maintaining spontaneous ventilation prevents reduction of central blood volumes associated with the application of positive pressure mechanical ventilation\(^{6,12}\), which would otherwise have influenced the distribution of blood between the intra- and extrathoracic compartments. Thus, while the current findings might be less directly applicable to ventilated patients, the findings might accurately reflect physiologic changes in patients not ventilated with absolute hypo- or hypervolemia in other settings, e.g. in trauma patients in the emergency department or in patients undergoing surgery under regional anesthesia.

In addition, to reduce the influence of spontaneous respiratory-induced variation in measured hemodynamics, injections of ice-cold ICG were spread equally over the respiratory cycle.\(^{13}\)

At third, all measurements were performed with the dogs being under general anesthesia, which renders the influence of the sympathetic nervous system negligible due to anesthesia-associated sympathicolysis.\(^{14}\) Additionally, in dogs, sympathetic induced vasoconstriction of splenic blood vessels could substantially alter blood volume distribution in case of hypo- or hypervolemia.\(^{15}\) However due to the anesthesia related sympathicolysis, the influence of the splenic blood on the observed blood volumes was rendered negligible.

Furthermore, calculated volumetric hemodynamic variables bear the risk of “mathematical coupling”. Whilst it was previously shown that such volumetric hemodynamic variables like ITBV are not mathematically coupled to CO\(^{16,17}\), we chose to derive CO values from a source independent of mtt-derived thermodilution, being the ultrasound flow probe which was calibrated a priori as described in detail previously and allow measurement of CO with a very high precision.\(^{10}\) The observed normovolemic CO was 94 mL min\(^{-1}\) kg\(^{-1}\), which is comparable to previously determined CO values in anesthetized dogs.\(^{10,11,18}\)

Finally, blood volume was altered to induce changes ± 20% of estimated \(V_{\text{d, circ}}\). Therefore, current data cannot be extrapolated to more severe hypo- or hypervolemic conditions. Especially for hypovolemia under these circumstances, it might well be that the distribution between central blood volumes
and circulating blood volume is altered due to additional compensatory cardiovascular control mechanisms aimed at maintaining cardiac preload. Since we did not intend to induce irreversible shock in our chronically instrumented dogs we restricted blood volume alterations up to ± 450ml. Yet, blood volume alterations up to 20% are relatively common in patients in anesthesia and critical care setting, reflecting the clinical importance of the current findings.

Reliability of measured blood volumes
We found a mean $V_{d\text{ circ}}$ of about 51 ml kg$^{-1}$ during normovolemia, which is only 60% of expected values of true total blood volume in dogs (80 ml kg$^{-1}$).$^{19}$ However, it has been demonstrated previously that $V_d\text{ circ}$ measurement using transpulmonary dye dilution technique provides reliable results compared to reference techniques (i.e. Evans blue dilution methods) although total blood volume is considerably underestimated up to about 40%.$^{5,9,11}$ Most probably, this underestimation results from incomplete mixing of the dye in slower body compartments due to the rather short measurement time (5 min) and therefore does not take the slowly perfused blood volume compartment into account. Yet, the observed changes in $V_{d\text{ circ}}$ correlated moderately with the induced alterations in blood volume, suggesting that the current technique permits accurate measurement of $V_{d\text{ circ}}$ both during hypo- and hypervolemia although this finding also demonstrates that not all volume alterations are solely reflected in corresponding $V_{d\text{ circ}}$ values (see further).

It has been already shown that central blood volumes, i.e. PBV and ITBV, can be measured accurately using the transpulmonary dye dilution technique both in humans$^{6,9}$ and in animals.$^{20}$ However, the ability to measure changes in these volumes during both hypo- and hypervolemia has not yet been demonstrated. The observed close correlation between $V_{d\text{ circ}}$ and either PBV or ITBV (figure 3) provides evidence to support that the transpulmonary dye dilution technique allows measuring changes in these variables during hypo- and hypervolemia. Also, the intra-individual variability of both PBV and ITBV was low with a value of 7%. In addition, we found no substantial difference in correlation between PBV and ITBV with respect to $V_{d\text{ circ}}$. From a clinical point of view, PBV measurement is less feasible as it requires pulmonary artery catheterization and therefore, ITBV obtained by transpulmonary dye dilution is clinically easier to obtain and therefore more frequently used.$^{8,9}$

Distribution of blood within the cardiovascular system
The goal of this study was to assess the distribution of blood volume between the intrathoracic and extrathoracic vascular compartments during periods of both hypo- and hypervolemia. From previous research it is well known that multiple factors other than blood volume alterations can influence the distribution of blood between intra- and extrathoracic compartments, e.g. induction of anesthesia or severe emphysema.$^{6,12,21,22}$ Though, despite extensive research$^{23}$, the effect of intravascular hypovolemia on the actual distribution of blood volume has not yet been fully explored, which is also true for the effect of intravascular hypervolemia. In hypovolemia, MAP remained constant during both mild and moderate hypovolemia, while heart rate increased only during the latter instance, CO markedly decreased already during mild hypovolemia. Furthermore, we found that both PBV and ITBV decreased during both mild and moderate hypovolemia, a finding that is similar to previous studies in swine$^{24}$ and humans.$^{25}$ Moreover and most importantly, we observed a constant ratio between $V_{d\text{ circ}}$ and the central blood volumes PBV and ITBV during all orders of hypovolemia. To the best of our knowledge, the ratio between $V_{d\text{ circ}}$ and PBV or ITBV has not been the subject of previous investigations. The constant ratio between $V_{d\text{ circ}}$ and PBV or ITBV suggests that the decrease in $V_{d\text{ circ}}$ was proportionate to the decrease in PBV and ITBV for both mild and moderate hypovolemia. In addition, we could not
demonstrate any effect at all of alterations in blood volume on the distribution of blood between the fast and slowly perfused compartments of circulating blood volume. Additionally, figure 6 shows the distribution between either \( R_1 \) vs. \( \text{mtt}_1 \) and \( R_2 \) vs. \( \text{mtt}_2 \), respectively, for data points obtained during normo-, hypo- and hypervolemia. In this figure, it can be seen that there is no substantial shift of the relationship \( R_1 \) vs \( \text{mtt}_1 \) or \( R_2 \) vs \( \text{mtt}_2 \) during any of the alterations in volume status. This additional plot adds further argumentation for the observation that both hypo- and hypervolemia, have no effect on the distribution between the fast and slowly perfused compartment.

**Figure 6:** Distribution-plot showing the relation between the correction factors \((R)\) and mean transit time \((\text{mtt})\) of both the fast and slowly perfused compartments \((R_1 \text{ vs. mtt}_1 \text{ and } R_2 \text{ vs. mtt}_2, \text{respectively})\) during normovolemia (black), hypovolemia (light grey) and hypervolemia (dark grey). Data are shown as error bars: mean ± SD.

Thus, even during mild to moderate hypovolemia due to hemorrhage, blood within the intrathoracic compartment also decreases and blood is lost from the circulation as a whole, not only from the non-central, extrathoracic compartments. This observation is in accordance with clinical findings that hypovolemia reduces cardiac preload, which is directly recruited from the central blood volumes but interestingly, it also suggests that “centralization” – i.e. rearranging blood flow from the slowly perfused compartment to the rapid perfused compartment, does not occur in the investigated range of blood volume alterations.

In the opposite direction – induced hypervolemia by colloid administration – we observed that the central blood volumes PBV and ITBV only increased significantly in case of moderate hypervolemia and not already during mild hypervolemia. Although one might argue that the resulting effect size of the observed differences between the two groups was not sufficient to demonstrate a significant increase in PBV and ITBV in mild hypervolemia, it might also be true that the cardiovascular system is able to adequately compensate for the increased filling during mild hypervolemia probably by prepulmonary venous dilatation. An argument for this hypothesis is the observation that for moderate hypervolemia, the ITBV/V_{d \text{ circ}} ratio increased slightly (table 2), which was not true for PBV.

Essentially, the difference between ITBV and PBV reflects the prepulmonary blood volume, i.e. the volumes of the right atrium and right ventricle. Although PBV and ITBV were well correlated, we found a slope between the difference of ITBV - PBV and V_{d \text{ circ}} (figure 5) which indicates that
Prepulmonary blood volume is influenced by intravascular volume shifts. As already discussed, V_d circ represents about 60% of true total blood volume. The observed slope of V_d circ for the changes in blood volume was 0.72 (figure 2). This means that if blood volume were decreased from normovolemia (V_d circ ± 50 ml kg⁻¹, total blood volume ± 80 ml kg⁻¹) by e.g. 20 ml kg⁻¹, V_d circ would be 0.72*20 = 36 ml kg⁻¹, which shows that the percentage change between V_d circ and total blood volume is constant, as a V_d circ value of 36 ml kg⁻¹ equals 60% of (80-20) ml kg⁻¹. The same calculation could also be applied to PBV or ITBV and demonstrates that, also when taking into account true total blood volume, there is no change between either the central compartment (PBV, ITBV), circulating compartment and total blood compartment.

In conclusion, we show in this study that in dogs during general anesthesia, the cardiovascular system maintains the distribution between central and circulating blood volume constant, even during alterations in total blood volume resulting either in moderate hypo- or hypervolemia.
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