Beat-to-beat stroke volume estimation from aortic pressure waveform in conscious rats: comparison of models

C. CERUTTI, M. P. GUSTIN, P. MOLINO, AND C. Z. PAULTRE
Faculty of Pharmacy, Department of Physiology and Clinical Pharmacology, Centre National de la Recherche Scientifique UMR 5014, 69373 Lyon Cedex 08, France

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Beat-to-beat stroke volume estimation from aortic pressure waveform in conscious rats: comparison of models. Am J Physiol Heart Circ Physiol 281: H1148–H1155, 2001.—Several methods for estimating stroke volume (SV) were tested in conscious, freely moving rats in which ascending aortic pressure and cardiac flow were simultaneously (beat-to-beat) recorded. We compared two pulse-contour models to two new statistical models including eight parameters extracted from the pressure waveform in a multiple linear regression. Global as well as individual statistical models gave higher correlation coefficients between estimated and measured SV (model 1, \( r = 0.97; \) model 2, \( r = 0.96 \)) than pulse-contour models (model 1, \( r = 0.83; \) model 2, \( r = 0.91 \)). The latter models as well as statistical model 1 used the pulsatile systolic area and thus could be applied to only 47 ± 17% of the cardiac beats. In contrast, statistical model 2 used the pressure-increase characteristics and was therefore established for all of the cardiac beats. The global statistical model 2 applied to data sets independent of those used to establish the model gave reliable SV estimates: \( r = 0.54 ± 0.07 \), a small bias between −8% to +10%, and a mean precision of 7%. This work demonstrated the limits of pulse-contour models to estimate SV in conscious, unrestrained rats. A multivariate statistical model using eight parameters easily extracted from the aortic waveform could be applied to all cardiac beats with good precision. Few old works have described methods that use pulse-wave analysis in dogs or in rats (5, 13, 14) via AP measured in the ascending aorta. Recently, Yang and Kuo (24) used a similar model in anesthetized rats via the femoral AP.

Most of the methods developed for humans are based on pulse-contour analysis, which relies on the windkessel arterial model, and several groups have proposed analytical models linking AP and cardiac blood flow (16, 21, 22). In the windkessel model, pulsatile systolic area and stroke volume (SV) are related by means of the characteristic impedance of the aorta (\( Z_{aort} \)). Several methods have been proposed for estimating \( Z_{aort} \) (2, 6, 8, 22). Many clinical applications have been made using aortic (22), radial (7, 19, 22), brachial (1), or finger (2, 17, 23) pressure waveforms. However, the validation of models in humans is quite difficult owing to the existing techniques for measuring cardiac output. Gold standard methods are thermodilution or dye-dilution methods, which cannot provide continuous measurement of cardiac output. In addition, in clinical research projects, data are generally recorded in controlled conditions during short periods of time. It appeared interesting to manage a project with rats because of the possibility of recording and processing beat-to-beat data over long periods of spontaneous activity and continuously measuring cardiac blood flow.

The aim of the present work was to test the validity of pulse-contour methods in conscious unrestrained rats for a beat-to-beat analysis using AP values that were measured in the ascending aorta and recorded during several hours. We compared two methods derived from human hemodynamic models that use pulse-contour analysis to two new methods that use multivariate statistical models including a large number of parameters extracted from the arterial pulse contour.

METHODS

Animals

Experiments were performed with male Sprague-Dawley rats (Iffa Credo; L’Arbresle, France) that weighed 300–400 g and were housed in controlled conditions (21 ± 1°C and a 50% humidity) by 10.220.33.6 on April 10, 2017 http://ajpheart.physiology.org/ Downloaded from
SV was defined by the integration of AP over the beat-to-beat SV values from the aortic waveform. In these cases, pulse-contour methods were used to estimate SV. However, sometimes critical in freely moving rats. These irregularities because the measuring conditions were described (11). After cannulation, rats were placed in large cylindrical recording cages with food and water ad libitum. After 48 h the animals had regained their initial body weight.

**Chronic Instrumentation**

An ultrasonic transit-time flow probe (model 2.5 SB; Transonic Systems; Ithaca, NY) was placed around the ascending aorta using our previously described technique (11). Rats were given 10–15 days to recover and to allow for the development of fibrosis around the probe. A polyethylene catheter was then inserted via the right common carotid artery into the ascending aorta as previously described (11). After cannulation, rats were placed in large cylindrical recording cages with food and water ad libitum. After 48 h the animals had regained their initial body weight.

**Signal Recording**

The aortic catheter was connected to a pressure transducer (Spectramed; Oxnard, CA) and AP signals were then amplified (model 13-4615-52; Gould; Cleveland, OH). The catheter was flushed (0.5 ml/h) with heparinized glucose (25 IU/ml) throughout the experiment to avoid blood diffusion and signal dampening. The cardiac flow-probe cable was connected via a spring-guarded cable to an ultrasonic transit-time flow-meter (model T-106; Transonic Systems). Analog-to-digital conversions of both signals were simultaneously performed on-line at 500 Hz with a personal computer equipped with an acquisition board (AT-MIO16H-9; National Instruments; Austin, TX) and software developed using LabView language (National Instruments).

The recording sessions began after stabilization periods of 15–30 min (when the animals were quiet and displayed normal activity) and lasted 2 h.

**Data Processing**

Off-line data processing was performed on a workstation (Ultra 5; Sun Microsystems; Mountain View, CA). For each cardiac cycle, cardiac output (defined as mean aortic flow) and SV were computed from the aortic flow signal. AP cycles were validated after testing pulse pressure (PP), heart rate (HR), and variation coefficients of mean AP (MAP), HR, and PP over 30-s periods. Adequate values were considered independent of those used to establish the computations were then performed on 10,000–27,000 validated cardiac cycles in each rat. Models were established using training data obtained from the first hour of recording in each rat. The models were then applied to data from the second hour of recording in each rat, which were considered independent of those used to establish the models.

**SV Estimation**

**Hemodynamic models.** Two different hemodynamic models relying on pulse-contour methods were used to estimate beat-to-beat SV values from the aortic waveform. In these methods, SV was defined by the integration of AP over the ejection phase divided by $Z_{aort}$.

$$SV = \frac{\int_{t_{ejection}} AP(t) dt}{Z_{aort}}$$

The end of the ejection phase could be determined from the AP curve for cardiac beats in which the characteristic incisura was distinct enough to be detected automatically (see Fig. 1). The AP integral (SysArea) was then defined as the area under the ejection portion of the aortic pressure curve above a horizontal line drawn from the diastolic point and bounded by a vertical line through the lowest point of the incisura. The previous equation then became

$$SV = \frac{\text{SysArea}}{Z_{aort}}$$

In our experimental conditions with rats, the end of the ejection phase was reliably detected in only 45% of all cardiac beats.

$Z_{aort}$ cannot be directly calculated, and several authors have proposed methods for estimating this parameter. First, we used the method proposed by Kouchokos and colleagues (8) for dogs and recently applied to rats by Yang and Kuo (24). In this method, $Z_{aort}$ was defined by the expression

$$Z_{aort} = k(1 + T_{sys}/T_{dias}) = kT_{dias}/T$$

where $k$ was a constant, $T_{sys}$ was the duration of systole, $T_{dias}$ was the duration of diastole (determined after automatic detection of the incisura), and $T$ was the heart period (see Fig. 1). This way, pulse-contour model 1 defined SV as

$$SV_{pulse-contour 1} = K \frac{\text{SysArea}}{TT_{dias}}$$

where $K$ was the parameter of the model.

We adapted a second method to rats that was previously proposed by Antonutto and colleagues (2) in which SV was estimated from Finapres AP signals in humans. The aortic impedance $Z_{aort}$ was defined from a multiple linear regression including HR, PP, and MAP by the expression

**Pulse Contour Models**

Fig. 1. Example of aortic pressure waveform showing variables included in pulse-contour models. $T_{sys}$, duration of systole; $T_{dias}$, duration of diastole.
Z_{sort} = a_4/(a_0 + a_1 \cdot HR + a_2 \cdot PP + a_3 \cdot MAP)

where \(a_0, a_1, a_2, a_3,\) and \(a_4\) are the theoretical parameters of the equation, and the next model, called pulse-contour model 2, then expresses SV as

\[ SV_{\text{pulse-contour} \ 2} = \text{SysArea}(k_0 + k_1 \cdot HR + k_2 \cdot PP + k_3 \cdot MAP) \]

where \(k_0, k_1, k_2,\) and \(k_3\) are the estimated parameters of the model.

Multivariate statistical models. In another way we proposed a new approach to estimate SV using classical multivariate statistical models. These empirical models do not make any hemodynamic hypothesis and rely on a multiple linear regression analysis including several variables extracted from the AP waveform.

In addition to the variables previously defined (SysArea, \(T_{\text{sys}}, T_{\text{dias}}, HR, PP,\) and MAP), we also determined the following values via beat-to-beat computations (see Fig. 2A): 1) systolic and diastolic AP (SAP and DAP, respectively); 2) three variables derived from the time derivative of AP, including the maximum derivative (\(dP/dt_{\text{max}}\)), and the time of its occurrence (\(T_{dP/dt_{\text{max}}}\)), and the pressure value at this time (\(AP_{dP/dt_{\text{max}}}\)); and 3) one feature of the diastolic relaxation, namely, the diastolic exponential decay time (\(\tau\)), which was identified using the method previously described (11).

These 12 variables were thus considered in the elaboration of statistical model 1: MAP, SAP, DAP, HR, PP, \(dP/dt_{\text{max}},\) \(AP_{dP/dt_{\text{max}}},\) SysArea, \(T_{\text{sys}}, T_{\text{dias}},\) and \(\tau\).

Because the end of the ejection phase was not always reliably detectable, we decided to propose a second model using the systolic peak instead of the lowest point of the incisura. As shown in Fig. 2B, we computed the integral PeakArea from the foot of the systolic ramp up to the systolic pressure peak (instead of SysArea) and we considered \(T_{\text{SAP}}\) and \(T - T_{\text{SAP}}\) (estimated from the systolic pressure peak) instead of \(T_{\text{sys}}\) and \(T_{\text{dias}}\). These 12 variables were considered in the elaboration of statistical model 2: MAP, SAP, DAP, HR, PP, \(dP/dt_{\text{max}}, T_{dP/dt_{\text{max}}}, AP_{dP/dt_{\text{max}}},\) PeakArea, \(T_{\text{SAP}}, T - T_{\text{SAP}},\) and \(\tau\).

Owing to the large number of variables first defined for both statistical models, variables containing redundant information were determined. The strength of the correlation between variables taken by two was evaluated in each rat. Figure 3 provides an example of a correlation matrix obtained for variables considered in the statistical models. DAP, SAP, and \(AP_{dP/dt_{\text{max}}}\) were highly correlated with MAP. The diastolic time, \(T_{\text{dias}},\) and the duration of the pressure decrease, \(T - T_{\text{SAP}},\) were highly inversely correlated with HR. In addition, SysArea and PeakArea were also well correlated. Although some differences appeared between the rats, the variables that exhibited the highest correlation coefficients were the same in all rats. Couples of variables with the most significant correlation were reduced to one of the variables. Couples of variables involving DAP, SAP, MAP, and \(AP_{dP/dt_{\text{max}}}\), and those involving \(T - T_{\text{SAP}}\) or \(T_{\text{dias}}\) and HR showed mean correlation coefficients \(>0.80,\) whereas the other mean correlation coefficients were all \(<0.68.\) These results prompted us to establish the correlation threshold at \(r = 0.80\) and to select eight variables: MAP, HR, PP, \(dP/dt_{\text{max}}, T_{dP/dt_{\text{max}}},\) SysArea, \(T_{\text{sys}},\) and \(\tau.\) Statistical model 1 was then defined as

\[ SV_{\text{statistical model} \ 1} = k_0 + k_1 \cdot \text{MAP} + k_2 \cdot \text{HR} + k_3 \cdot \text{PP} + k_4 \cdot dP/dt_{\text{max}} + k_5 \cdot T_{dP/dt_{\text{max}}} + k_6 \cdot \text{SysArea} + k_7 \cdot T_{\text{sys}} + k_8 \cdot \tau \]

Replacing SysArea and \(T_{\text{sys}}\) by PeakArea and \(T_{\text{SAP}},\) respectively, statistical model 2 was then defined as

\[ SV_{\text{statistical model} \ 2} = k_0 + k_1 \cdot \text{MAP} + k_2 \cdot \text{HR} + k_3 \cdot \text{PP} + k_4 \cdot dP/dt_{\text{max}} + k_5 \cdot T_{dP/dt_{\text{max}}} + k_6 \cdot \text{PeakArea} + k_7 \cdot T_{\text{SAP}} + k_8 \cdot \tau \]

Statistical Tools

The parameters (\(K, k_0, k_1, \ldots\)) of the four models were estimated with multiple linear regression analysis using Systat software (SPSS; Chicago, IL). Individual models were estimated for each rat using individual beat-to-beat data of the training data set. Global models were established with beat-to-beat training data for all rats pooled together thus allowing the creation of a unique file containing the entire training data set. The quality of the models was given by the correlation coefficients computed between estimated and measured SV values. Considering other individual data sets, the estimation of SV with the different models was compared.

The distributions of values for all variables were determined. All were close to normality except \(T_{dP/dt_{\text{max}}},\) which
exhibited a lognormal distribution, and a logarithmic transformation was applied to this variable. Pearson correlation analysis between variables taken by two was performed to eliminate highly correlated variables with $r > 0.80$. The models were then estimated using multiple linear regression with stepwise forward entries. The rank of entry of each variable was considered. The Bland-Altman method (3) was used to determine the bias and precision of SV values estimated with statistical model 2.

RESULTS

Mean values of all cardiovascular parameters obtained in the training data sets and other data sets used to test the models are given in Table 1. No statistical difference was observed between these two data sets.

Determination of Individual Models

The optimal parameters and correlation coefficients obtained for each rat in each model are given in Table 2. These parameters and correlation coefficients show interindividual variability. Correlation coefficients were specifically different between rats for pulse-contour model 1. Table 3 indicates that both the mean percentages of cardiac beats included in the individual models and the mean correlation coefficients were different between the models. Statistical model 2, which did not use the ejection time, was the only model that could be estimated with all of the cardiac beats. The other models used <50% of the cardiac beats. Pulse-contour model 2 as well as both statistical models gave similar correlation coefficients, which were higher than those obtained with pulse-contour model 1.

Determination of Global Models

As shown in Table 3, both statistical models yielded high correlation coefficients between estimated and measured SV values; however, pulse-contour model 1 was much worse than the other models. Differences between the correlation coefficients were highly significant due to the large amount of data (>60,000 values) used to establish the models. Table 4 shows the ranks of entry for pressure variables in the two statistical models established with the pooled data. In both models, PP was the most significant variable because it was entered first in the stepwise multiple regression, with $r^2 = 0.92$ for statistical model 2.

Table 1. Mean values of stroke volume and variables extracted from ascending aortic pressure waveform

<table>
<thead>
<tr>
<th>Selection</th>
<th>DAP</th>
<th>SAP</th>
<th>MAP</th>
<th>HR</th>
<th>PP</th>
<th>dP/dt max</th>
<th>Tsys</th>
<th>Tsys</th>
<th>Peak Area</th>
<th>Tsys</th>
<th>T-T sys</th>
<th>τ</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP</td>
<td>0.88</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>0.92</td>
<td>0.97</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>0.07-0.05-0.01</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>0.42</td>
<td>0.80</td>
<td>0.70</td>
<td>-0.19</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dt max</td>
<td>0.19</td>
<td>0.54</td>
<td>0.43</td>
<td>0.00</td>
<td>0.79</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsys</td>
<td>-0.01</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.48</td>
<td>0.12</td>
<td>-0.05</td>
<td>0.19</td>
<td>0.01</td>
<td>0.55</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsys</td>
<td>-0.06</td>
<td>0.03</td>
<td>-0.03</td>
<td>-0.81</td>
<td>0.13</td>
<td>0.02</td>
<td>-0.06</td>
<td>-0.07</td>
<td>0.14</td>
<td>-0.02</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Peak Area</td>
<td>0.29</td>
<td>0.57</td>
<td>0.54</td>
<td>-0.42</td>
<td>0.72</td>
<td>0.47</td>
<td>-0.11</td>
<td>0.31</td>
<td>0.62</td>
<td>0.37</td>
<td>0.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Tsys</td>
<td>0.14</td>
<td>0.17</td>
<td>0.21</td>
<td>-0.48</td>
<td>0.14</td>
<td>-0.16</td>
<td>0.17</td>
<td>0.13</td>
<td>0.30</td>
<td>0.49</td>
<td>0.26</td>
<td>0.74</td>
</tr>
<tr>
<td>T-T sys</td>
<td>-0.15</td>
<td>-0.03</td>
<td>-0.11</td>
<td>-0.86</td>
<td>0.13</td>
<td>0.09</td>
<td>-0.04</td>
<td>-0.14</td>
<td>0.25</td>
<td>0.27</td>
<td>0.78</td>
<td>0.05</td>
</tr>
<tr>
<td>τ</td>
<td>-0.32</td>
<td>-0.59</td>
<td>-0.50</td>
<td>-0.10</td>
<td>-0.72</td>
<td>-0.60</td>
<td>0.07</td>
<td>-0.37</td>
<td>-0.57</td>
<td>0.91</td>
<td>-0.11</td>
<td>-0.57</td>
</tr>
</tbody>
</table>
model 1 and $r = 0.86$ for statistical model 2. $dP/dt_{\text{max}}$, MAP, HR, $\tau$, SysArea, $T_{dP/dt_{\text{max}}}$, and $T_{\text{sys}}$ were then successively entered in statistical model 1, with $r$ increasing clearly for the first five steps and reaching 0.97. For the determination of statistical model 2, MAP, $dP/dt_{\text{max}}$, HR, $\tau$, PeakArea, $T_{\text{SAP}}$, and $T_{dP/dt_{\text{max}}}$ were successively entered in this order, with $r$ reaching 0.96 at step 7. The entry ranks of the variables were very close in the two models, and $r$ reached similar values. The regression coefficients of the statistical models and the corresponding standard errors are given in Table 4.

Application of Global Models to Other Individual Data

Quality of estimated SV values. To test the validity of the models, the different global models were applied to validated cardiac beats from the training data set and from the second hour of recording for each rat. The quality of the fits is given in Table 5 in terms of percentage of cardiac beats with SV estimate and correlation between estimated and measured SV values. Percentages of beats were similar for both data sets, with statistical model 2 allowing the estimation of SV in all of the validated cardiac beats. Although no significant difference was found between $r$ values obtained for the two conditions, those obtained for the second data set tended to be lower the training set values.

Bias and precision of statistical model 2. SV values estimated with statistical model 2 were compared with measured SV values using the Bland-Altman method. Bias and precision of the estimation were determined for each rat using all of the cardiac beats. Figure 4 provides an example of a scatterplot showing bias as a function of the average value of estimated and measured SV values. Precision, defined as the standard deviation of the bias, was 13.7 $\mu l$ (6.5%). Considering the five rats, mean bias was $7.2 \pm 7.6 \mu l$, which represents $2.2 \pm 3.4$% of the measured SV mean value, and precision was $6.6 \pm 0.3$%. The percentage of cardiac beats for which the absolute value of bias was <10% was computed for each rat; this value ranged between 47% and 87%, and the mean percentage computed for the five rats was $65 \pm 8$%.

DISCUSSION

This work aimed at demonstrating the feasibility of SV estimation from the arterial waveform analysis during long periods of spontaneous activity in conscious, unrestrained rats. Using aortic pressure mea-
measurements, we compared two hemodynamic models used for humans with two new models that are based on a multivariate statistical model. The results show that our statistical models were clearly better than the pulse-contour models. In contrast with the other models, statistical model 2 could be applied to all the cardiac beats. Thus a global model was estimated that yielded low bias and good precision for an indirect method.

Since the 1970s, several methods have been developed in humans for obtaining SV and cardiac output estimates from the pressure waveform (either aortic or peripheral) obtained either intraarterially or noninvasively using Finapres. These methods rely on knowledge models, which are associated to biophysical representations (6, 16, 21, 22). The methods provide analytical expressions of all of the variables included in a model, and the parameters of the models result from mechanical hypotheses. At the time of the development of these models, the computation means were weak; thus analytical expressions of the variables were essential. Surprisingly, such models have not often been tested and applied to rats for hemodynamic studies in physiology or pharmacology. Few studies using pulse-contour methods on anesthetized rats have been published; for instance, three studies were published 20 years ago (5, 13, 14) and only one recent work (24).

Table 4. Results of stepwise multiple regressions to estimate global statistical models: entry rank of each pressure variable, correlation coefficient of regression at each step, and final regression coefficients with SE

<table>
<thead>
<tr>
<th>PP</th>
<th>dP/dt_{max}</th>
<th>MAP</th>
<th>HR</th>
<th>( \tau )</th>
<th>SysArea</th>
<th>T_{dP/dt_{max}}</th>
<th>T_{sys}</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.92</td>
<td>0.94</td>
<td>0.96</td>
<td>0.96</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>4.02</td>
<td>-0.017</td>
<td>-1.84</td>
<td>-0.31</td>
<td>-0.07</td>
<td>37.6</td>
<td>7.71</td>
<td>-0.69</td>
</tr>
<tr>
<td>SE</td>
<td>0.08</td>
<td>0.000</td>
<td>0.02</td>
<td>0.01</td>
<td>0.00</td>
<td>1.4</td>
<td>0.38</td>
<td>0.04</td>
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</table>

<table>
<thead>
<tr>
<th>PP</th>
<th>MAP</th>
<th>dP/dt_{max}</th>
<th>HR</th>
<th>( \tau )</th>
<th>PeakArea</th>
<th>T_{SAP}</th>
<th>T_{dP/dt_{max}}</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.86</td>
<td>0.88</td>
<td>0.93</td>
<td>0.94</td>
<td>0.95</td>
<td>0.95</td>
<td>0.96</td>
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<tr>
<td>Regression coefficient</td>
<td>6.45</td>
<td>-0.92</td>
<td>-0.018</td>
<td>-0.60</td>
<td>-0.12</td>
<td>-130.3</td>
<td>2.06</td>
<td>0.78</td>
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<tr>
<td>SE</td>
<td>0.04</td>
<td>0.01</td>
<td>0.000</td>
<td>0.01</td>
<td>0.00</td>
<td>1.2</td>
<td>0.03</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table 5. Application of global models to individual data sets: percentages of cardiac beats with SV estimate and correlation coefficients between estimated and measured SV values

<table>
<thead>
<tr>
<th></th>
<th>Pulse-Contour Models</th>
<th>Statistical Models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>Percentage of beats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training data set</td>
<td>47±17</td>
<td>47±17</td>
</tr>
<tr>
<td>Other data set</td>
<td>50±17</td>
<td>51±17</td>
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<tr>
<td>Correlation coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training data set</td>
<td>0.49±0.10</td>
<td>0.57±0.08</td>
</tr>
<tr>
<td>Other data set</td>
<td>0.46±0.08</td>
<td>0.39±0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 5 \) rats. *\( P < 0.05 \) vs. pulse-contour models 1 and 2.

Fig. 4. Bias of stroke volume (SV) estimate obtained with global statistical model 2 in one rat during 1 h of recording expressed in absolute (A) and percentage values (B) and plotted against the average of measured and estimated SV values. Distribution of each variable is given along the corresponding axis; horizontal lines indicate mean bias and 95% confidence interval.
using pulse-contour model 1 for a pharmacological study was found in the literature. In the present work we have tested this latter model, which was first described by Kouchoukos and colleagues (8), and pulse-contour model 2, which was proposed by Antonutto and co-workers (2), for humans. This model appeared easy to transpose to rats because it did not include noncardiovascular variables.

These hemodynamic models (as well as others) include an operational approach to estimating $Z_{aort}$. A multiple linear regression involving MAP, HR, and PP was used in pulse-contour model 2, and a linear combination of MAP, HR, and age multiplied by an individual calibration factor was used by other authors (6, 21). These methods, therefore, combine a pure hemodynamic model and a statistical model to estimate some hemodynamic parameters. In the present work, we proposed two statistical models that did not rely on any hemodynamic model but were designed to be predictive. We did not aim to provide a new knowledge model of the cardiovascular system. We developed pure statistical models using multiple linear regression applied to a large training set of data. Whether computed from individual data or from the entire set of data, these models better fitted the data than did pulse-contour model 1, but results were close to those obtained with pulse-contour model 2.

Our statistical models take into account more information extracted from the pressure waveform than the pulse-contour models. In an additive manner, our models consider eight parameters extracted from the aortic waveform, which are included in a multivariate linear regression. The amount of information appears to be essential and compensates for lack of physical hypotheses. Among the eight parameters included in the multiple regression, MAP was chosen rather than SAP, $T_{dias}$, or $T - T_{SAP}$. The replacing of MAP or HR by one of the correlated variables led to similar models with similar $r$ values and entry ranks of the variables. In addition, the pulse-contour models as well as our statistical model 1 use the pulsatile systolic area SysArea. However, in many cardiac cycles there was no clear pressure incisura reflecting the aortic valve closing but only a slight change in the time derivative of the decreasing part of the pressure curve. Therefore, SysArea could reliably be determined in <50% of the recorded beats. Statistical model 2 did not use this variable, and thus it was the only model that could be applied to all of the cardiac beats.

In humans, comparing the results given by the models to direct cardiac flow measurements has validated the proposed models. However, standard dilution techniques (1, 7, 21, 22) as well as new noninvasive methods (2, 4, 17, 18) only yield discrete cardiac output values. Validation studies were carried out either in critically ill patients (19, 22) or in healthy subjects (2, 6) but at rest or during controlled conditions (17, 23). The models theoretically allowed beat-to-beat calculation of SV, but most often values were averaged over one or two respiratory cycles or several cardiac cycles. Study periods were generally very short (2, 21, 23), and comparisons with the reference method were necessarily made on a small number of direct measures. When long periods were studied, recalibration was performed at regular intervals (every 1 or 2 h) to recalculate $Z_{aort}$ (19). Because the techniques used for direct blood flow measurement did not allow for continuous measurements, all studies involving comparisons of methods used a restricted number of data selected in controlled hemodynamic conditions. This fact may partly explain the excellent correlations that have been found.

In rats, cardiac blood flow can be continuously measured with flow probes using ultrasonic or electromagnetic techniques that can be chronically implanted in rats (9, 10, 15). Nevertheless, these techniques are difficult to use in routine experiments and produce constraining conditions for the rat. Noninvasive Doppler echocardiographic methods have been adapted to rats (12, 20), but they are little used due to technical difficulties in signal processing and also because a restraining device is required for conscious rats. Therefore, we thought it particularly useful to validate the assessment of SV from the aortic waveform by means of the continuous measurement of cardiac blood flow in freely moving rats.

Considering the individual models, some differences appeared between the parameters of the models obtained in each of the rats, and some of these differences were rather large. These differences may be partly due to the variable quality of the individual models (mainly for pulse-contour model 1). In addition, in the statistical models including eight variables and thus eight parameters plus one constant, one can accept that the eight parameters are not independent and that little variation of one parameter may be associated with more or less large variations of the other parameters. Some of the individual parameters were also different from global parameters. This observation might be explained by the fact that the cardiovascular state slightly differed between rats although they were studied in similar experimental conditions. The global models took into account both interindividual and individual variability over time, because we had about 10,000 cardiac beats per rat. Therefore, the differences in the model parameters are likely to reflect individual characteristics that must be considered to establish a reliable global model. Of course, the application of the global model in one rat yields a less good estimation than the individual model, but this is the cost of the determination of a general model.

The two global statistical models exhibited similar quality, and the main difference comes from the fact that only statistical model 2 could be applied to all of the cardiac beats. In both models, PP was the most significant variable because it was entered first in the stepwise regressions with high correlation. The other variables were entered in a nearly identical order in both models. Although $T$ is not used in hemodynamic models, its rank of 5 in both models shows that periph-
eral vascular properties are of importance in the determination of SV.

The application of the four global models to data sets different from the one used to establish the models resulted in SV estimates that were logically slightly less good than SV estimates computed in the training data set. The Bland-Altman analysis was used to compare SV estimates obtained with global statistical model 2 to measured SV values. It disclosed the existence of a small bias between estimated and measured SV values, which expressed either over- or underestimation (<10%). For each rat the precision was <8%, which is quite good for an indirect method. Large differences in bias values were observed between rats (ranging between −8 and 10%), whereas precision was much more homogeneous. These observations suggest that information is lacking for the determination of the absolute SV level, whereas variability aspects are better estimated.

Further studies are thus needed to understand the differences between rats in the quality of the fit to individual and global models. More precisely, we plan to find an explanation of the bias by modeling it as a function of the pressure variables that we considered. In addition, because all of the rats were freely moving during the recording session, the physical activity is likely to be of importance in the definition of the model. Therefore, further studies will be designed to define models specific to each activity state. In addition, because the measurement of AP in the ascending aorta is rather critical, it appears also necessary to adapt the statistical models to the AP waveform obtained in the abdominal aorta.

In conclusion, this work presents rigorous comparisons of methods that were applied to large sets of data. We demonstrated the limits of hemodynamic models in estimating SV in a continuous way during long periods in conscious freely moving rats. A statistical model using eight parameters easily extracted from the aortic pressure waveform was designed to be applied to all cardiac beats, and it gave SV estimates with good global precision. The precise conditions of use for this model in freely moving rats remain to be clarified.

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