Published in final edited form as:

J Clin Monit Comput. 2017 February ; 31(1): 167–175. doi:10.1007/s10877-015-9813-5.

Heart period and blood pressure characteristics in splanchnic arterial occlusion shock-induced collapse

Federico Aletti^{1,2}, Nicolò Gambarotta¹, Alexander H. Penn², Manuela Ferrario¹, and Geert W. Schmid-Schönbein²

¹Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Piazza Leonardo da Vinci, 32, 20133 Milan, Italy

²Department of Bioengineering, University of California San Diego, 9500 Gilman Drive MC 0412, La Jolla, CA 92093-0412, USA

Abstract

The nature of hemodynamic instability typical of circulatory shock is not well understood, but an improved interpretation of its dynamic features could help in the management of critically ill patients. The objective of this work was to introduce new metrics for the analysis of arterial blood pressure (ABP) in order to characterize the risk of catastrophic outcome in splanchnic arterial occlusion (SAO) shock. Continuous ABP ($f_s = 1$ kHz) was measured in rats during experimental SAO shock, which induced a fatal pressure drop (FPD) in ABP. The FPD could either be slow (SFPD) or fast (FFPD), with the latter causing cardiovascular collapse. Time series of mean arterial pressure, systolic blood pressure and heart period were derived from ABP. The sample asymmetry-based algorithm *Heart Rate Characteristics* was adapted to compute the *Heart Period Characteristics* (HPC) and the *Blood Pressure Characteristics* (BPC). Baroreflex sensitivity (BRS) was assessed by means of a bivariate model. The approach to FPD of the animals who collapsed (FFPD) was characterized by higher BRS in the low frequency band versus SFPD animals (0.36 ± 0.15 vs. 0.19 ± 0.12 ms/mmHg, p value = 0.0196), bradycardia as indicated by the HPC (0.76 ± 0.57 vs. 1.94 ± 1.27 , p value = 0.0179) and higher but unstable blood pressure as indicated by BPC (3.02 ± 2.87 vs. 1.47 ± 1.29 , p value = 0.0773). The HPC and BPC indices demonstrated promise as potential clinical markers of hemodynamic instability and impending cardiovascular collapse, and this animal study suggests their test in data from intensive care patients.

Keywords

Heart period characteristics; Blood pressure characteristics; Shock; Cardiovascular variability; Baroreflex; Splanchnic arterial occlusion

Correspondence to: Federico Aletti.

Compliance with ethical standards: **Conflict of interest** Geert W. Schmid-Schönbein owns stock in Inflammagen Inc., a company that develops new shock treatments.

1 Introduction

One of the most challenging problems in the intensive care unit (ICU) is the identification of patients with life-threatening hemodynamic instability, characterized by significant swings in arterial blood pressure (ABP), hypotensive crises and irregular heart beat, which can anticipate sudden cardiovascular (CV) collapse. The combined use of fluids, vasopressors, inotropic and chronotropic drugs within complex paradigms, such as the *Early Goal Directed Therapy* [1, 2] target the maintenance of blood pressure in order to ensure adequate tissue perfusion in circulatory shock patients. Still, the importance of promptly identifying patients at higher risk for hemodynamic instability reaffirms the need for quantitative tools to enhance the extraction of useful information from the hemodynamic measurements available in the ICU, with the goals of assisting and improving the timeliness and efficacy of therapy, and monitoring progression of the disease and the response to the treatment more accurately.

The dynamics of heart rate (HR) and ABP convey important information on autonomic regulation [3–9], whose role in the presence of CV dysfunction due to shock is still unclear. The mathematical analysis of CV signals, which are routinely available in the ICU, can shed light on the impairment of the autonomic status of a patient, and contribute to the detection of hemodynamic instability and hypotension. However, the patho-physiological interpretation of quantitative indices related to the performance of the autonomic nervous system (ANS), which are estimated from CV recordings, remains an important issue. An approach such as the *Heart Rate Characteristics*, introduced by Moorman et al. [10, 11], proved able to discriminate between patients at higher risk for sepsis in the neonatal ICU [10–13]. Still, several hypotheses were discussed to explain the mechanisms underlying the risk of sepsis predicted by this index [11], demonstrating that assessment of the autonomic status in a critically ill patient remains difficult to address. The potential of such methods could therefore benefit from integration with classical methods for the analysis of ANS regulation of circulation, such as models of baroreflex function.

We present here the study of the dynamic features of heart period and mean arterial pressure collected from rats during splanchnic arterial occlusion (SAO) shock experiments by readapting the *Heart Rate Characteristics* algorithm in order to derive the *Heart Period Characteristics* (HPC) and *Blood Pressure Characteristics* (BPC), and to test them as potentially useful indices in the retrospective characterization of the risk of sudden death after cardiovascular collapse. We integrated this approach with the analysis of baroreflex control of heart rate, in order to describe the possible adaptations of ANS regulation in this form of shock.

SAO is an experimental model of circulatory shock [14–17] that results in massive injury to the gut and subsequent cardiovascular instability and death. Penn and Schmid-Schönbein [17] demonstrated that prolonged intestinal ischemia in rats causes a steady decline in arterial pressure, with the possibility that sudden death by cardiovascular collapse may be a consequence of a vagally mediated mechanism. A subset of animals from [17] was analyzed in the present study with the objective of characterizing the dynamics of arterial blood pressure and heart period during SAO shock.

2 Methods

2.1 Experimental protocol

The experimental SAO shock protocol was approved by the University of California San Diego Animal Subjects committee, and the experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. A detailed description of the protocol is reported in [17]. Briefly, male Wistar rats were anesthetized with sodium pentobarbital (50 mg/kg). A fluid filled arterial catheter connected to a pressure transducer was placed in the left femoral artery for continuous recording of ABP. A venous line was inserted in the left femoral vein for medication and supplemental anesthesia. The animals received different treatments either via intramuscular (i.m.) injection or intravenous (i.v.) infusion or enteral injections into the intestinal lumen (i.l.) following a laparotomy and exposition of the guts (Table 1):

- A. (n = 4): i.l. glucose (100 mg/ml) after anesthesia
- B. (n = 7): i.l. saline (100 mg/ml) after anesthesia
- C. (n = 6): pre-anesthesia i.m. xylazine (4 mg/kg) + i.l. glucose (100 mg/ml) after anesthesia
- D. (n = 7): pre-anesthesia i.m. xylazine (4 mg/kg) and i.m. glycopyrrolate (0.5 mg/kg) + i.l. glucose (10.0 mg/ml) after anesthesia + i.v. glycopyrrolate (0.5 mg/kg)

Xylazine was injected before anesthesia as a tranquilizer (groups C and D), in order to attenuate sympathetic control and stimulate reflex parasympathetic activity. Enteral glucose in saline (0.9 % NS) was provided as a source of energy during the interruption of intestinal blood flow, with enteral saline alone as a control. Glycopyrrolate was used as an anticholinergic, and its administration (group D) provided to counteract the xylazine-induced vagal stimulation.

SAO was achieved by ligation of the superior mesenteric and celiac arteries after administration of the described treatments and following laparotomy and exposure of the bowel. No further intervention was performed after arterial occlusion and the animals were either left undisturbed until spontaneous death or euthanized (120 mg/kg i.v. sodium pentobarbital) at 4 h after SAO.

Seventy-seven rats were divided into seven different groups [17]. Only a subset of that database was included in our analysis since the sampling frequency of the ABP recordings (100 Hz) in fifty-one animals out of the original seventy-seven was not sufficient for analysis. A review by Bathia et al. [18] on the minimum sampling rate for the analysis of cardiovascular dynamics in rodents explains in detail that ABP waveforms require higher fidelity for accurate mathematical processing, due to the fast heart rate of these species. Hence, only the rats from the four groups described above (ABP fs = 1 kHz), were considered for analysis (Table 1).

The experiment displayed two outcomes. In the aftermath of initiation of SAO and clamping of the arteries, ABP transiently rose in all animals, and typically rebounded rapidly. Then, a

variable-duration progressive ABP decay followed until the fatal pressure drop (FPD), which evolved according to two patterns: (1) gradually over ~1–3 h (slow fatal pressure drop, SFPD) or (2) rapidly (often by more than 80 mmHg) over a period of 1–6 min (fast fatal pressure drop, FFPD) (Fig. 1).

2.2 Arterial blood pressure preprocessing and analysis

ABP waveforms were processed to search for systolic and diastolic values within each cardiac cycle. Beat-by-beat series of systolic blood pressure (SBP) and mean arterial pressure (MAP) calculated as the average of ABP over a cardiac cycle, and heart period (HP; i.e. the equivalent of the RR interval assessed as the time interval between two consecutive diastolic values), were derived, filtered to eliminate ectopic beats and artifacts, and resampled in the time domain by an antialiasing filter. The sampling frequency of the time series was set to 5 Hz, a suitable frequency to capture the whole spectral band of CV signals in rats [16, 19–21].

Two windows of data were considered separately in the analysis:

- a. baseline before SAO to 30 min after initiation of SAO, for characterization of the initial cardiovascular response to the strong mechanical disturbance to hemodynamics caused by arterial ligation. The choice of the duration of this period was justified by previous observations of the impact of SAO on intestinal damage and on the ensuing onset of shock in a similar animal model [22];
- b. 30 min after initiation of SAO until the FPD, for the examination of the ABP decay and the approach to cardiovascular collapse, by grouping the animals according to the outcome (FFPD vs. SFPD).

2.3 Heart period characteristics and blood pressure characteristics

Moorman and coworkers [10–13] introduced the *Heart Rate Characteristics* (HRC) index, i.e. the Sample Asymmetry (SA) of the distribution of the differences between the duration of RR intervals and the median of the RR series, and applied it successfully to predict outcomes in acutely ill neonates. We adapted this algorithm to compute the *Heart Period Characteristics* and *Blood Pressure Characteristics* indices from the HP and MAP series, respectively.

Given n samples of a series $\{x_i\}$, the quadratic function r is computed as:

$$r(x_i) = (x_i - m)^2 \quad (1)$$

where m is the median of the series. The amplitudes of the two branches of the quadratic function r are characterized by two parameters (R_1 and R_2 , see Eqs. 2, 3), which quantify the deviation from the median and which are used to calculate the sample asymmetry:

$$R_1 = \frac{1}{n} \sum r^2(x_i), \quad \text{with } r(x_i) = 0 \text{ for } x_i < m \quad (2)$$

$$R_2 = \frac{1}{n} \sum r^2(x_i), \quad \text{with } r(x_i) = 0 \text{ for } x_i > m \quad (3)$$

$$SA = R_1 / R_2 \quad (4)$$

An evenly distributed function r is characterized by a value of SA close to 1. A skewed distribution of the function r is characterized by values of SA either <1 or >1 . In the former case, the technique detects the prevalence of increases in the heart beat duration (for HPC) or reductions in MAP (for BPC), i.e. of bradycardia and hypotension, respectively. In the latter case, it detects the prevalence of tachycardia and hypertension, respectively.

The *Heart Period Characteristics* and *Blood Pressure Characteristics* were calculated for the SFPD versus FFPD comparison: (a) over the entire duration of the interval from 30 min after SAO initiation until FPD; and (b) over 3 min long, 50 % overlapped running windows along the entire interval.

2.4 Baroreflex sensitivity

The relationship between HP and SBP was assessed by means of a closed loop model [23, 24] accounting for both the feedback (FB) control of HP by baroreflex (i.e. SBP as the input, HP as the output) and the feed-forward (FF) mechanism (i.e. HP as the input, SBP as the output) due to the mechanical coupling between the duration of the heart cycle and blood pressure.

An autoregressive bivariate model of order $p = 8$ (5), the coefficients (6) and the gain of the FB transfer function were computed. BRS was determined as the gain $G_{SBP \rightarrow HP}$ (7):

$$Y[n] = \sum_{k=1}^p A[k] Y[n-k] + W[n] \quad (5)$$

$$\begin{aligned} A[k] &= \begin{bmatrix} a_{11}[k] & a_{12}[k] \\ a_{21}[k] & a_{22}[k] \end{bmatrix}, & Y[n] &= \begin{bmatrix} \text{HP}[n] \\ \text{SBP}[n] \end{bmatrix}, \\ W[n] &= \begin{bmatrix} W_{\text{HP}}[n] \\ W_{\text{SBP}}[n] \end{bmatrix}; & A_{ij}(f) &= \sum_{k=1}^p a_{ij}[k] e^{-j2\pi f k} \end{aligned} \quad (6)$$

$$G_{\text{SBP} \rightarrow \text{HP}}(f) = \frac{A_{12}(f)}{1 - A_{11}(f)} \quad (7)$$

The coherence function between the HP and SBP series was calculated and a value >0.5 was required for BRS assessment in the low frequency (LF, $0.25 \text{ Hz} < f < 0.75 \text{ Hz}$) band. High frequency (HF, $0.75 \text{ Hz} < f < 2.5 \text{ Hz}$) BRS is affected by respiration, a confounding factor of the relationship between HP and ABP fluctuations. The lack of respiratory measurements from the experiments suggested the limitation of the analysis to the LF band.

Baseline values of BRS were computed in a 3-min stationary window before initiation of SAO. Stationarity verification implied that the BRS was not computed in correspondence of any sudden increase or decrease in ABP. The BRS was then tracked from 30 min after SAO initiation until FPD by considering 3 min long, 50 % overlapped windows. The first three and last three of these windows were averaged to obtain the values relevant to the periods named respectively “30 min after SAO” and “before FPD”.

2.5 Statistical analysis

The determination of the effects of arterial clamping in each group (A, B, C, D, see Table 1) in the period from baseline until 30 min after the initiation of SAO were performed by means of One-way repeated measures ANOVA, with the Tukey–Kramer test for the post hoc comparisons.

For the second period, both intra-group comparisons and SFPD versus FFPD comparisons were carried out. In particular: (a) paired Student's t tests were applied for the comparison between the two time points at 30 min after SAO and immediately before FPD within the same group (either SFPD or FFPD); (b) the non-parametric Wilcoxon rank-sum test was applied, after logarithmic transformation of the data, to the unpaired SFPD versus FFPD comparison at the same time point (either 30 min after SAO or immediately before FPD).

In the following, all results are presented as mean $\pm$ standard deviation.

3 Results

Before SAO (baseline), the animals treated with xylazine had significantly lower MAP (Table 2) and HP (Table 3) compared to non-xylazine treated animals, as expected. Artery ligation induced a spike in ABP (Fig. 1), followed by a rapid rebound in pressure such that post-SAO maximum and minimum values could be identified (Tables 2, 3). MAP reached the same maximum value after SAO in all four groups, and the distinct effects of the initial treatment were not recognizable at 30 min after initiation of SAO.

The approach to FPD was analyzed to gain insight into features that may be determinants of the outcome of SAO shock (i.e. FFPD vs. SFPD), once the initial effects of arterial clamping (SAO) vanished. In the window from 30 min after initiation of SAO until FPD (Table 4) MAP decreased progressively in both groups, but less markedly in FFPD than in SFPD. The heart rhythm decelerated in the FFPD group and accelerated in the SFPD group.

Simple visual inspection of the ABP tracings during the progression towards collapse in the FFPD animals revealed the presence of several dips of different amplitude (Fig. 2), which were promptly compensated, although the pressure level before the dip was never fully recovered. In the following, we will refer to these events as *dip and recovery episodes*.

In synthesis, the main findings were that:

- a. BPC showed ability to track sudden variations in MAP (Fig. 2) when *dip and recovery episodes* occurred in FFPD rats, consistently with the BPC result at point b above. The HPC index did not capture variations in HP in concert with the *dip and recovery episodes*, and for this reason only the evolution over time of BPC is shown in Fig. 2.
- b. HPC was capable of detecting (Fig. 3) the prevalence of bradycardia in FFPD rats, and of tachycardia in SFPD rats (FFPD HPC: 0.76 ± 0.57 vs. SFPD HPC: 1.94 ± 1.27 , p value = 0.018);
- c. BPC was capable of detecting (Fig. 3) the higher frequency of pressure drops in FFPD rats than in SFPD rats (FFPD BPC: 3.02 ± 2.87 vs. SFPD BPC: 1.47 ± 1.29 , p value = 0.077);

The BRS significantly increased in FFPD during intestinal ischemia, and decreased in the SFPD group (Table 4).

4 Discussion

4.1 Heart period characteristics, blood pressure characteristics, and baroreflex sensitivity

The main objective of this study was to introduce and test the *Heart Period Characteristics* and *Blood Pressure Characteristics* as metrics capable of identifying distinctive dynamic features of ABP and HP during the approach to sudden death by cardiovascular collapse in SAO shock. The HPC and BPC indices detected opposite behaviors in the FFPD and SFPD groups, which were characterized respectively (Fig. 3) by recurring bradycardia and hypotension (FFPD), and by prevalence of tachycardia and more pronounced hypotension but more stable arterial pressure (SFPD). These differences may be related to a possible dysfunction of autonomic control, as suggested by the results of the BRS analysis.

The baseline hypotension and bradycardia in the xylazine rats (groups C and D, Tables 2, 3) were consistent with the sympatholytic action of xylazine [25–29]. Enteral saline loading was the control to glucose loading, which enhances vagal activity [30, 31], but the direct impact of enteral fluid loading on circulatory control is unclear.

The steep raise in ABP after SAO to the same level in all four groups was likely caused by the sudden increase of resistance, of mechanical origin (ligation of the arteries) with subsequent shunting of cardiac output away from the splanchnic circulation, possibly

modulated by sympathetic vasoconstriction, as intestinal ischemia activates sympathetic afferents [32].

The ABP course in the first 30 min after arterial ligation, a time at which the onset of SAO-induced shock symptoms have been reported to occur [22], was not affected by SAO, but was rather characterized by hypotension in all the animals, as is typical in shock.

The effectiveness of the HRC algorithm in detecting the risk of sepsis in critically ill neonates [10–13] suggested the adaptation of this index to the available data (i.e. ABP waveforms) in order to carry out a retrospective analysis of our database by computing the HPC and BPC indices.

As regards HPC, the results appear to support the hypothesis of a contribution of the parasympathetic system [17] to sudden collapse (FFPD), since a value of $R1/R2 < 1$ during FFP indicates the prevalence of decelerations of heart rate. The opposite was found with the SFPD group. The trend of BRS at LF in FFPD rats also showed an enhanced baroreflex control of heart rate during ischemia, which is also related to vagal activity [33].

As to BPC, $R1/R2$ in both groups was >1 , and greater in the FFPD group. This result may also entail the involvement of the sympathetic system, caused by intestinal ischemia [32] and aimed at maintaining blood pressure during shock. In the case of the FFPD group, a mainly vagally mediated control of the heart beat in the presence of repeated *dip and recovery episodes*, prompts speculation that a sudden shift of the sympatho-vagal balance may cause cardiovascular collapse.

Moorman and colleagues discussed several hypotheses [11] to explain the decelerations of the heart beat obtained by the *Heart Rate Characteristics* algorithm in neonates at higher risk of sepsis, and the reduced heart rate variability. In particular, two of their observations are consistent with our data and their interpretation: (1) the criticism of the interpretation that reduced heart rate variability is associated to diminished parasympathetic activity; this stance is supported by our findings, obtained using an approach directly derived from the *Heart Rate Characteristics*, and is in line with Penn's hypothesis of the involvement of the vagal system in sudden death in SAO shock [17]; (2) the role of cytokines and inflammation typical of acute disease, which could also entail the activation of the sympathetic system.

Along the same lines, the analysis of baroreflex control of heart rate provides another element of discussion. In addition to the presumption of enhanced vagal activity in FFPD animals as revealed by higher values of BRS, the lower but more stable ABP in SFPD animals during the progression of FPD points to a better maintenance of autonomic control, which could explain the absence of *dip and recovery episodes* and the longer survival of SFPD subjects.

4.2 Limitations

The lack of a physiological interpretation of the mechanisms, which could explain the observed trends in BRS as well as HPC and BPC, is an acknowledged limitation of the statistical methods utilized this study. However, the consistency between the possible interpretations of the sample asymmetry results obtained both by Moorman and colleagues

[10–13] and by our analyses suggests the inclusion of measurements of ANS function in future animal protocols aimed at clarifying the mechanisms of hemodynamic instability in shock.

Another possible limit is the lack of spontaneous (i.e. conscious animals) recordings. We cannot ignore the depressant effect of pentobarbital anesthesia on reflexes, but at the same time it is important to take into account that the values of the BRS estimates are in agreement with the literature [34, 35], albeit in unconscious animals. Nonetheless, previous reports from our and other laboratories have already shown the robustness of the application of BRS models to data collected from patients under anesthesia, as well as the importance of introducing such indices to characterize the ANS performance in the surgical or ICU setting [24, 36, 37].

A final limitation to list is the marginal significance of the BPC result for the FFPD versus SFPD comparison.

5 Conclusions

Despite the large body of work on SAO shock, to our knowledge this is the first work which addresses cardiovascular dynamics in this experimental model of circulatory shock.

The consistency of our findings with several observations by Moorman's laboratory [10–13], where the *Heart Rate Characteristics* was developed, also supports the potential usefulness of the indices presented in this manuscript. The *Heart Period Characteristics* and *Blood Pressure Characteristics* were able to retrospectively identify differences in the dynamics of ABP and HP of FFPD and SFPD rats, but in the context of this study no predictive estimate could be derived. Therefore, the predictive potential of the proposed approach needs to be validated. These algorithms could improve the description of blood pressure dynamics of critically ill patients, allowing further testing of their translational potential in bedside applications. In particular, we plan to apply the *Heart Rate Characteristics*, the *Heart Period Characteristics* and the *Blood Pressure Characteristics* to high fidelity CV data recorded in circulatory shock patients during a multiple day stay in the ICU, in order to confirm the value of these indices as metrics capable of identifying the instability of patients at higher risk, as well as describing the responsiveness to standard therapies.

Lastly, our findings also emphasized the need for ever more robust combinations of data analysis approaches, where standard physical models and statistical approaches that are blind to the fundamental physiological mechanisms, mesh and enhance the reliability of patient risk prediction.

Acknowledgments

This research was supported by the “ShockO-mics” Grant #602706 of the European Union, by the “CeSys Shock” Marie Curie International Outgoing Fellowship PIOF-GA-2012-328796 of the European Union in support of the corresponding author, and by National Institutes of Health Grants HL 67825 and GM85072.

References

1. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M, Early Goal-Directed Therapy Collaborative Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med*. 2001; 345:1368–77. [PubMed: 11794169]
2. Rivers EP, Katranji M, Jaehne KA, Brown S, Dagher GA, Cannon C, Coba V. Early interventions in severe sepsis and septic shock: a review of the evidence one decade later. *Minerva Anesthesiol*. 2012; 78(6):712–24. [PubMed: 22447123]
3. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*. 1981; 213:220–2. [PubMed: 6166045]
4. Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol*. 1985; 249:H867–75. [PubMed: 4051021]
5. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res*. 1986; 59:178–93. [PubMed: 2874900]
6. Saul JP, Berger RD, Chen MH, Cohen RJ. Transfer function analysis of autonomic regulation. II. Respiratory sinus arrhythmia. *Am J Physiol Heart Circ Physiol*. 1989; 256:H153–61.
7. Saul JP, Berger RD, Albrecht P, Stein SP, Chen MH, Cohen RJ. Transfer function analysis of the circulation: unique insights into cardio-vascular regulation. *Am J Physiol Heart Circ Physiol*. 1991; 261:H1231–45.
8. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation*. 1991; 84:1482–92.
9. Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation*. 1998; 98:2640–3. [PubMed: 9843477]
10. Kovatchev BP, Farhy LS, Cao H, Griffin MP, Lake DE, Moorman JR. Sample asymmetry analysis of heart rate characteristics with application to neonatal sepsis and systemic inflammatory response syndrome. *Pediatr Res*. 2003; 54(6):892–8. [PubMed: 12930915]
11. Moorman JR, Lake DG, Griffin MP. Heart rate characteristics monitoring for neonatal sepsis. *IEEE Trans Biomed Eng*. 2006; 53:126–32. [PubMed: 16402612]
12. Fairchild KD, Schelonka RL, Kaufman DA, Carlo WA, Kattwinkel J, Porcelli PJ, Navarrete CT, Bancalari E, Aschner JL, Walker MW, Perez JA, Palmer C, Lake DE, O'Shea TM, Moorman JR. Septicemia mortality reduction in neonates in a heart rate characteristics monitoring trial. *Pediatr Res*. 2013; 74(5):570–5. [PubMed: 23942558]
13. Lake DE, Fairchild KD, Moorman JR. Complex signals bioinformatics: evaluation of heart rate characteristics monitoring as a novel risk marker for neonatal sepsis. *J Clin Monit Comput*. 2014; 28(4):329–39. [PubMed: 24248424]
14. Kistler EB, Lefer AM, Hugli TE, Schmid-Schönbein GW. Plasma activation during splanchnic arterial occlusion shock. *Shock*. 2000; 14(1):30–4. [PubMed: 10909890]
15. Ishimaru K, Mitsuoka H, Unno N, Inuzuka K, Nakamura S, Schmid-Schönbein GW. Pancreatic proteases and inflammatory mediators in peritoneal fluid during splanchnic arterial occlusion and reperfusion. *Shock*. 2004; 22:467–71. [PubMed: 15489640]
16. Kozar RA, Holcomb JB, Hassoun HT, Macaitis J, DeSoignie R, Moore FA. Superior mesenteric artery occlusion models shock-induced gut ischemia-reperfusion. *J Surg Res*. 2004; 116(1):145–50. [PubMed: 14732361]
17. Penn AH, Schmid-Schönbein GW. Severe intestinal ischemia can trigger cardiovascular collapse and sudden death via a parasympathetic mechanism. *Shock*. 2011; 36:251–62. [PubMed: 21617580]
18. Bathia V, Rarick KR, Stauss HM. Effect of the data sampling rate on accuracy of indices for heart rate and blood pressure variability and baroreflex function in resting rats and mice. *Physiol Meas*. 2010; 31:1185–201. [PubMed: 20664161]

19. Japundzic N, Grichois ML, Zitoun P, Laude D, Elghozi JL. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J Auton Nerv Syst.* 1990; 30:91–100. [PubMed: 1973426]
20. Cerutti C, Gustin MP, Paultre CZ, Lo M, Julien C, Vincent M, Sassard J. Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach. *Am J Physiol Heart Circ Physiol.* 1991; 261:H1292–9.
21. Julien C, Zhang ZQ, Cerutti C, Barrés C. Hemodynamic analysis of arterial pressure oscillations in conscious rats. *J Auton Nerv Syst.* 1995; 50:239–52. [PubMed: 7714320]
22. Chang M, Alsaigh T, Kistler EB, Schmid-Schönbein GW. Breakdown of mucin as barrier to digestive enzymes in the ischemic rat small intestine. *PLoS ONE.* 2012; 7(6):e40087. [PubMed: 22768227]
23. Wyller VB, Barbieri R, Saul PJ. Blood pressure variability and closed-loop baroreflex assessment in adolescent chronic fatigue syndrome during supine rest and orthostatic stress. *Eur J Appl Phys.* 2011; 111(3):497–507.
24. Dorantes Mendez G, Aletti F, Toschi N, Canichella A, Dauri M, Coniglione F, Guerrisi M, Signorini MG, Cerutti S, Ferrario M. Baroreflex sensitivity variations in response to propofol anesthesia: comparison between normotensive and hypertensive patients. *J Clin Monit Comput.* 2013; 27(4):417–26. [PubMed: 23314842]
25. Antonaccio MJ, Robson RD, Kerwin L. Evidence for increased vagal tone and enhancement of baroreceptor reflex activity after xylazine (2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazine) in anesthetized dogs. *Eur J Pharmacol.* 1973; 23:311–6. [PubMed: 4746747]
26. Klide AM, Calderwood HW, Soma LR. Cardiopulmonary effects of xylazine in dogs. *Am J Vet Res.* 1975; 36(7):931–5. [PubMed: 1147358]
27. Sanford TD, Colby ED. Effect of xylazine and ketamine on blood pressure, heart rate and respiratory rate in rabbits. *Lab Anim Sci.* 1980; 30(3):519–23. [PubMed: 7431851]
28. Hsu WH, Lu ZX, Hembrough FB. Effect of xylazine on heart rate and arterial blood pressure in conscious dogs, as influenced by atropine, 4-aminopyridine, doxapram, and yohimbine. *J Am Vet Med Assoc.* 1985; 186:153–6. [PubMed: 2857705]
29. Paddleford RR, Harvey RC. Alpha 2 agonists and antagonists. *Vet Clin N Am Small Anim Pract.* 1999; 29:737–45.
30. Fujita S, Donovan CM. Celiac-superior mesenteric ganglionectomy, but not vagotomy, suppresses the sympathoadrenal response to insulin-induced hypoglycemia. *Diabetes.* 2005; 54:3258–64. [PubMed: 16249453]
31. Thorens B. Glucose sensing and the pathogenesis of obesity and type 2 diabetes. *Int J Obes (Lond).* 2008; 6:S62–71. [PubMed: 19079282]
32. Longhurst JC, Rotto DM, Kaufman MP, Stahl GL. Ischemically sensitive abdominal visceral afferents: response to cyclooxygenase blockade. *Am J Physiol.* 1991; 261(6):H2075–81. [PubMed: 1750553]
33. van de Vooren H, Gademan MG, Swenne CA, TenVoorde BJ, Schalijs MJ, Van der Wall EE. Baroreflex sensitivity, blood pressure buffering, and resonance: what are the links? Computer simulation of healthy subjects and heart failure patients. *J Appl Physiol.* 2007; 102(4):1348–56. [PubMed: 17185500]
34. Su DF, Miao CY. Arterial baroreflex function in conscious rats. *Acta Pharmacol Sin.* 2002; 23:673–9. [PubMed: 12147187]
35. Yoshino K, Hayawaka M, Niki E, Matsuoka K. Closed-loop analysis of cardiovascular variability in rats under restraint stress. *Auton Neurosci.* 2005; 119:61–6. [PubMed: 15893709]
36. Valenza G, Akeju O, Pavone KJ, Citi L, Hartnack KE, Sampson A, Purdon PL, Brown EN, Barbieri R. Instantaneous monitoring of heart beat dynamics during anesthesia and sedation. *J Comput Surg.* 2014; 1:13.
37. Chen Z, Purdon PL, Harrell G, Pierce ET, Walsh J, Brown EN, Barbieri R. Dynamic assessment of baroreflex control of heart rate during induction of propofol anesthesia using a point process method. *Ann Biomed Eng.* 2011; 39(1):260–76. [PubMed: 20945159]

Fig. 1.

Example of mean arterial pressure tracings during an experiment of splanchnic arterial occlusion shock ending with a fast fatal pressure drop and sudden cardiovascular collapse (**a**), and during an experiment of splanchnic arterial occlusion shock characterized by a slow fatal pressure drop and ending with sacrifice of the animal after 4 h (**b**), with the main events and periods considered in the analysis

Fig. 2.

Trends of mean arterial pressure (*solid line*) and sample asymmetry (*dashed line*), i.e. *Blood Pressure Characteristics* (BPC) during the steady decrease towards fatal pressure drop in a typical FFPD rat. The *dip and recovery episodes* were identified by sudden increases in BPC

Fig. 3.

Sample asymmetry distributions of heart period (*Heart Period Characteristics*, HPC) and mean arterial pressure (*Blood Pressure Characteristics*, BPC) in one FFPD rat (*left*), and one SFPD rat (*right*) for the period from 30 min after SAO until collapse (FFPD) or sacrifice (SFPD). FFPD HPC: 0.76 ± 0.57 versus SFPD HPC: 1.94 ± 1.27 , p value = 0.018; FFPD BPC: 3.02 ± 2.87 versus SFPD BPC: 1.47 ± 1.29 , p value = 0.077

Table 1**Experimental groups**

Experimental group	#rats	#FFPD	#SFPD
A: Glucose	4	1	3
B: Saline	7	3	4
C: Xylazine glucose	6	2	4
D: Xylazine glycopyrrolate glucose	7	0	7

Mean arterial pressure (mmHg) before and after ligation of the superior mesenteric and celiac arteries

Table 2

	Before SAO *	Maximum value after SAO	Minimum value after SAO	30' after SAO *	One-way ANOVA for repeated measurement
A: Glucose	111 ± 9	145 ± 8 ^a	121 ± 23 ^b	134 ± 8 ^a	<i>p</i> < 0.01
B: Saline	122 ± 4	145 ± 9 ^a	126 ± 6 ^b	129 ± 7 ^b	<i>p</i> < 0.01
C: Xylazine glucose	76 ± 15 ^{#,§}	145 ± 9 ^a	106 ± 12 ^{a,b}	107 ± 13 ^{#,§,a,b}	<i>p</i> < 0.01
D: Xylazine glycopyrolate glucose	79 ± 14 ^{#,§}	155 ± 8 ^a	117 ± 15 ^{a,b}	120 ± 10 ^{a,b}	<i>p</i> < 0.01

Comparisons among different groups at each time point:

* one-way repeated measurements ANOVA *p* value < 0.01,

post hoc comparisons *p* value < 0.05 versus group A,

§ post hoc comparisons *p* value < 0.05 versus group B

Comparisons among different time points:

^a post hoc comparisons *p* value < 0.05 versus before SAO,

^b post hoc comparison *p* value < 0.05 versus maximum value

Table 3
Heart period (ms) before and after ligation of the superior mesenteric and celiac arteries

	Before SAO *	Minimum value after SAO *	Maximum value after SAO *	30' after SAO *	One-way ANOVA for repeated measurement
A: Glucose	176 ± 15	166 ± 10	190 ± 21 ^b	186 ± 16	$p < 0.05$
B: Saline	171 ± 29	163 ± 24	187 ± 28 ^{a,b}	186 ± 27 ^{a,b}	$p < 0.01$
C: Xylazine glucose	287 ± 30 ^{#,§}	239 ± 20 ^{#,§,a}	287 ± 21 ^{#,§,b}	276 ± 23 ^{#,§,b}	$p < 0.01$
D: Xylazine glycopyrrolate glucose	249 ± 22 ^{#,§}	204 ± 28 ^{#,§,a}	270 ± 20 ^{#,§,b}	263 ± 30 ^{#,§,b}	$p < 0.01$

Comparisons among different groups at each time point:

* one-way repeated measurements ANOVA p value < 0.01 ,

post hoc comparisons p value < 0.05 versus group A,

§ post hoc comparisons p value < 0.05 versus group B

Comparisons among different time points:

^a post hoc comparisons p value < 0.05 versus before SAO,

^b post hoc comparisons p value < 0.05 versus minimum value

Table 4
Mean arterial pressure (MAP, mmHg), heart period (HP, ms) and baroreflex gain estimated in LF band (BRS_{LF}, ms/mmHg) during the progression towards the fatal pressure drop

	30' after SAO	Pre FPD	= PreFPD—30' after SAO
FFPD MAP (mmHg)	123 ± 12	105 ± 23 ^b	−19 ± 15
SFPD MAP (mmHg)	118 ± 17	91 ± 17 ^a	−27 ± 9
FFPD HP (ms)	220 ± 47	238 ± 58	18 ± 27 [*]
SFPD HP (ms)	230 ± 54	211 ± 50 ^a	−19 ± 27 ^{**}
FFPD BRS _{LF} (ms/mmHg)	0.22 ± 0.11	0.36 ± 0.15 ^{*a}	0.142 ± 0.072 ^{**}
SFPD BRS _{LF} (ms/mmHg)	0.25 ± 0.21	0.19 ± 0.12 ^{*a}	−0.053 ± 0.157 ^{**}

Data are presented as mean ± SD

Comparison between periods within the same group: paired *t* test (Pre FPD vs. 30' after SAO),

^a *p* value <0.01,

^b *p* value <0.05. Comparisons FFPD versus SFPD at the same time point:

^{*} Wilcoxon rank-sum test *p* value = 0.0196,

^{**} Wilcoxon rank-sum test *p* value <0.05