Spectral transfer function analysis of respiratory hemodynamic fluctuations predicts end-diastolic stiffness in preserved ejection fraction heart failure

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Running Head: Spectral transfer function analysis in experimental HFpEF

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Abstract

Preserved ejection fraction heart failure (HFpEF) diagnosis remains controversial and invasive left ventricular (LV) hemodynamic evaluation and/or exercise testing is advocated by many. The stiffer HFpEF myocardium may show impaired stroke volume (SV) variation induced by fluctuating LV filling pressure during ventilation. Our aim was to investigate spectral transfer function (STF) gain from end-diastolic pressure (EDP) to indexed (SVi) in experimental HFpEF. Eighteen-week-old Wistar-Kyoto (WKY), ZSF1 lean (ZSF1 Ln) and obese rats (ZSF1 Ob) randomly underwent LV open-chest (OC, n=8 each group) or closed-chest hemodynamic evaluation (CC, n=6 each group) under halogenate anesthesia and positive-pressure ventilation at constant inspiratory pressure. Beat-to-beat fluctuations in hemodynamic parameters during ventilation were assessed by STF. End-diastolic stiffness (βi) and end-systolic elastance (Ees,i) for indexed volumes were obtained by inferior vena cava occlusion in OC (multi-beat) or single-beat method estimates in CC. ZSF1 Ob showed higher EDP spectrum (P<0.001), higher STF gain between end-diastolic volume and EDP and impaired STF gain between EDP and SVi when compared with both hypertensive ZSF1 Ln and normotensive WKY controls (P<0.001). Likewise βi was only higher in ZSF1 Ob whilst Ees,i was raised in both ZSF1 groups. On multivariate analysis βi and not Ees,i correlated with impaired STF gain from EDP to SVi (P<0.001) and receiver-operating characteristics analysis showed an area under curve of 0.89 for higher βi prediction (P<0.001). Results support further clinical testing of STF analysis from right heart catheterization-derived EDP surrogates to non-invasively determined SV as screening/diagnostic tool to assess myocardial stiffness in HFpEF.

Keywords: Heart failure with preserved ejection fraction; spectral analysis; spectral transfer function; myocardial stiffness
New and Noteworthy

We demonstrate that spectral transfer function analysis of gain between end-diastolic pressure and stroke volume is impaired in experimental preserved ejection fraction heart failure (HFpEF) validating previous exploratory clinical data. We further demonstrate a clear relation to end-diastolic stiffness which suggest it may be used as screening/diagnostic tool in HFpEF.
Glossary

AUC, area under curve
BW, body weight
CC, closed-chest
CO, cardiac output
E, peak Doppler velocity of early filling wave (E-wave) in transmitral flow
E’, peak mitral annulus tissue Doppler velocity in early diastole
EDP, end-diastolic pressure
EDPVR, end-diastolic pressure-volume relationship
EDV and EDVi, end-diastolic volume and indexed end-diastolic volume
Ees and EesVi, end-systolic elastance and end-systolic elastance for indexed volumes
EF, ejection fraction
ESPVR, end-systolic pressure-volume relationship
GLZ, generalized linear models
HF, heart failure
HFpEF, heart failure with preserved ejection fraction
HR, heart rate
IVC, inferior vena cava
LV, left ventricle or left ventricular
OC, open-chest
PAD, pulmonary artery diastolic pressure
PEEP, positive end-expiratory pressure
Pmax, maximum developed pressure
PSD, power spectral density
RHC, right heart catheterization
ROC, receiver-operating characteristics
RR, respiratory rate
STF, spectral transfer function
SV and SVi, stroke volume and indexed stroke volume
βi, left ventricular chamber stiffness constant for indexed volumes
\( \tau \), time constant of isovolumetric relaxation

\( \tau_{\text{exp}} \), \( \tau \) derived by monoexponential method

\( \tau_{\text{log}} \), \( \tau \) derived by logistic method
Introduction

Heart failure (HF) with preserved ejection fraction (HFP EF) accounts for a rising proportion of over 50% of HF cases (25) and remains one of the unmet cardiovascular research challenges. Though it may constitute a heterogeneous disorder with complex determinants (32), most experts believe abnormalities in myocardial relaxation and compliance that develop under the influence of aging and comorbidities are the main underlying pathophysiological mechanisms (12). Diagnosis remains challenging and controversial. Biomarkers and non-invasive echocardiographic indexes would be desirable (26) but many question their accuracy and sensitivity based on invasively derived left ventricular (LV) hemodynamic evaluation and exercise testing in asymptomatic patients (27). In a small proof-of-concept study carried out in HFP EF patients and age-matched controls using a non-invasive surrogate of stroke volume (SV) and pulmonary artery diastolic pressures (PAD) obtained from right heart catheterization (RHC) as surrogate of LV filling pressure Shibata et al. demonstrated by spectral transfer function (STF) analysis that beat-to-beat fluctuations during quiet ventilation could yield an overall index of myocardial stiffness. HFP EF patients showed larger shifts in filling pressure and comparably lower changes in SV and therefore a lower gain in STF that was attributed to both high end-systolic elastance (Ees) and end-diastolic stiffness (33). We hypothesize that such an index might help screening or diagnosis of myocardial stiffness in HFP EF.

Our aim was to reproduce Shibata’s findings under tightly controlled experimental settings using gold-standard invasive hemodynamic evaluation in experimental HFP EF. For this purpose we performed spectral and STF analysis of beat-to-beat hemodynamic parameters during ventilation in both open-chest (OC) and closed-chest conditions (CC) in the ZSF1 obese rat model of metabolic syndrome and cardiometabolic-risk related HFP EF, a model that we newly characterized (12, 22). Finally we tested the possibility that the gain of STF between end-diastolic pressure (EDP) and SV was mainly related with end-diastolic stiffness and could be used as a screening or diagnostic tool.
Methods

Animal model

ZSF1 obese (ZSF1 Ob, n=14), ZSF1 lean (ZSF1 Ln, n=14) and Wistar-Kyoto rats (WKY, n=14; Charles River Laboratories, Barcelona, Spain) were fed with standard diet (Purina diet 5008) to achieve metabolic syndrome and HFpEF, systemic arterial hypertension or absence of any cardiovascular risk factor, respectively, as previously reported. Animals were kept in individually ventilated chambers, in groups of 2-per cage under controlled environment with a 12-h-light-dark cycle at 22°C room temperature. All animals received humane care. Experimental procedures were approved by the ethical committee of the Faculty of Medicine of Porto and were performed in accordance with Portuguese law on animal welfare, EU Directive 2010/63/EU for animal experiments and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 2011).

Hemodynamic evaluation

At their 18th week of life animals were block-randomized to undergo two distinct hemodynamic evaluation protocols: closed-chest evaluation (CC, n=6 per group) and open-chest evaluation (OC, n=8 per group). Upon sedation and analgesia with 100 µg.kg⁻¹ and 5 mg.kg⁻¹ intraperitoneal fentanyl and midazolam, respectively, and anesthesia by sevoflurane inhalation (8% and 2.5-3% for induction and maintenance, respectively) animals were endotracheally intubated (14G) and monitored with electrocardiogram (Animal Bio Amp, FE136, ADInstruments), peripheral oximetry (MouseSTAT™ - Pulse Oximeter & Heart Rate Monitor, Physiosuite, Kent Scientific), capnography, respiratory rate (RR), minute ventilation (CapnoScan™ - End-Tidal CO2 Monitor, Physiosuite, Kent Scientific) and central temperature by a rectal probe. Animal temperature was automatically controlled on a heating pad (RightTemp™ - Temperature Monitor & Homeothermic Controller, Physiosuite, Kent Scientific). Fluid replacement with 32mL.Kg⁻¹.h⁻¹ warmed Ringer’s lactate (NE-1000, New Era Pump Systems) was instituted by the right dorsal foot vein (24G). Additional fluid replacement was
instituted at a ratio of 4:1 based on estimated blood loss. Mechanical ventilation was instituted with 100% O₂ and end-expiratory pressure held at 5 cmH₂O (PEEP). Ventilation was instituted in pressure support ventilation mode with RR determined by the animal’s inspiratory effort in CC whereas a controlled pressure ventilation mode with RR manually adjusted by the researcher in each animal until a stable RR was achieved that could maintain normocapnia (end-tidal carbon dioxide target was 30 mmHg in capnography) was adopted in OC. In both inspiratory pressure was set at 12 cm H₂O above PEEP and inspiration to expiration ratio was 1:2. Experimental preparation for CC consisted of neck dissection under surgical microscopy and insertion of a pressure volume catheter (SPR-847 Millar Instruments) through the right common carotid artery into the LV under echocardiography guidance by a 15MHz probe and an echocardiography system (Siemens Acuson Sequoia C512) whilst preparation for OC consisted of a left thoracotomy with pericardial removal, flow-probe placement in the ascending aorta (2.5PS, Transonic), 3-0 silk lace positioning around the inferior vena cava (IVC) and pressure-volume catheter insertion through the apex. Parallel conductance was determined by 40 μL 10% hypertonic saline injection and slope factor α was derived by simultaneous measurement of cardiac output (CO) by either the flowmeter (TS420, Transonic) in OC or echocardiography in CC. The latter was obtained by pulsed-wave Doppler aortic flow velocity curve integration in the apical 5-chamber view, aortic root dimension estimation on long axis parasternal M-mode and heart rate (HR) assessment by the electrocardiogram. Signals were continuously acquired (MPVS 300, Millar Instruments) and digitized at 1000 Hz (ML880 PowerLab 16/30, ADinstruments). CO and volumes were indexed to body surface area as defined by 9.1*body weight (BW)²/₃ (21) to account for large differences in BW and heart size which are genetically mediated in ZSF1 rats (12). Recordings were obtained after a stabilization period of 30 mins without interrupting ventilation. In the OC preparation transient IVC occlusion recordings with ventilation suspended at end-expiration were also obtained.
Data analysis

In the OC preparation, load independent indexes of cardiac performance were derived from the linear (End-systolic pressure=\(E_{es} \cdot \text{indexed end-systolic volume} + V_0\)) and exponential fits (\(\text{EDP}=\alpha \cdot e^{\beta \cdot \text{indexed end-diastolic volume (EDV)}}\)) of the multi-beat end-systolic and end-diastolic pressure-volume relationships (ESPVR and EDPVR) obtained during transient IVC occlusion, respectively, whereas in the CC preparation surrogate single-beat methods were used. ESPVR was established by the actual end-systolic and the predicted isovolumic pressure volume point. The latter was estimated by fitting a 5th-order degree polynomial function to LV pressure curve data from end-diastole to maximum rate of pressure rise and from maximum rate of pressure fall to 90% pressure decay, as described by ten Brinke et al. (38). As for EDPVR we applied the single-beat method first proposed by Zile et al. which consists of deriving a corrected LV pressure excluding the active component of pressure decay due to relaxation as predicted by monoexponential fitting of time constant of isovolumic relaxation \(\tau\) (41). Although we and others (16) were unable to obtain suitable exponential fits from the full-corrected pressure tracing, a simplified robust approach based on 3 pressure-volume points (minimum pressure, end-diastole and a point half-way between the first two) yielded consistent results.

Joint respiratory fluctuations of EDV, EDP and SV, were modelled as a linear time-invariant system by STF analysis as originally performed by Shibata et al. (34). Time invariance is assured by study design but although SV, EDV and EDP are quasi-linearly related within the physiological range as expected by Frank-Starling’s law (37) essentially every nature process is nonlinear in nature so this model is clearly an approximation to reality. Indeed, several new highly-demanding computational-based methods have expanded the assessment of the correlation between input and output variables in the cardiovascular system to non-linear relationships by various approaches (30). Still, model reduction is usually feasible because the local linear approximations are robust enough when only minor fluctuations around an equilibrium point are analyzed. Transfer functions capture most system properties and enable extensive analysis of a system for which there is no comprehensive
analog in non-linear system analysis thus transfer function analysis remains a valuable tool even in non-linear systems. The amount of linear coupling between two signals in the frequency domain can be expressed by means of squared coherence, a measure comparable to the correlation of determination in regression analysis that is computed for each frequency region in the time domain. At frequencies where coherence approaches unity the output depends linearly on the input. Where the coherence falls, output fluctuations reflect the influence of system nonlinearities, loss of time invariance, other system input signals, or measurement noise (4). None of our recordings was excluded because they all showed coherence values > 0.5. Simplistically, a transfer function analyses the frequency response of a system when both input and output are periodic and can be decomposed in a sum of sets of sine and cosine functions by spectral analysis. For spectral analysis, stored signals of continuous 192 sec recordings were processed for beat-to-beat detection of end-diastole with the aid of software (MATLAB, Mathworks, Inc.). Beat-to-beat values were aligned using linear interpolation to obtain an equidistant time-series at a new sampling frequency of 8 Hz. A 3rd-order polynomial fit was implemented for trend removal while preserving fluctuations due to breathing. Power spectral density (PSD) was calculated using Welch’s method (40) by dividing the detrended signals into 512-point segments overlapping by 50%. Then, fast Fourier transforms were executed on each hanning-windowed segment to obtain the frequency components (spectrum) of the time series, averaged, and squared to compute autospectral function of input (x), $S_{xx}(f)$, or cross-multiplied to compute cross-spectral function between input and output (y), $S_{xy}(f)$, to obtain periodograms with a minimum resolution of 0.015625 Hz. Complex STF function, $H(f)$, between x and y was calculated as:

$$H(f) = \frac{S_{xy}(f)}{S_{xx}(f)}$$

The real, $H_R(f)$, and imaginary, $H_I(f)$, parts of the STF were then used for evaluating the gain, $|H(f)|$, and phase between signals as follows:
\[ |H(f)| = \left\{ [H_R(f)]^2 + [H_i(f)]^2 \right\}^{1/2} \]

\[ \text{Phase} = \tan^{-1} \left[ \frac{H_i(f)}{H_R(f)} \right] \]

Gain reflects the level to which changes in output can be attributed to input at a certain frequency whereas phase indicates the synchronicity between signals, with best synchrony at 0.

For each STF, coherence between input and output signals, which ranges from 0 to 1 with perfect linearity at 1, was estimated as:

\[ C_{xy}(f) = \frac{|S_{xy}(f)|^2}{S_{xx}(f)S_{yy}(f)} \]

To calculate the PSD or variance of the time series at the RR, the corresponding autospectra were integrated at the RR range in each of the animals (± 0.045 Hz with slight adjustments for wider peaks). At this same RR frequency range values of STF gain, phase and coherence were averaged for each animal (4, 34). Overall mean coherence and peak coherence were high (0.79 and 0.92, respectively) ensuring the safety of approximation of the linear model and phase was close to 0 indicating minimum lag between input and output.

**Statistical analysis**

As expected by their mathematical derivation methods, PSD and STF gain data as well as their residuals were non-normally distributed in general linear models as assessed by Shapiro-Wilk’s test. Homogeneity of variances was also badly violated as assessed by Levene’s test even after log transformation therefore we used generalized linear models (GLZ). Several distributions of the exponential family (Weibull, lognormal, binomial and gamma) and link functions (log, identity and power) were assessed for goodness-of-fit. Gamma distribution with log-link function best described the data according to the lower Akaike’s information-theoretic criterion values (1) compared with other models using alternative distributions and link functions. Indeed, the gamma distribution is
particularly well tailored for skewed positive only distributions with heterogeneous variance. Adequacy of the model was also checked by visual inspection of residuals and of the relationship between standardized residuals and standardized predicted values. Main effects and interaction were assessed by maximum likelihood estimation with type III log-likelihood ratios and type III sum of squares. End-systolic and end-diastolic stiffness were analyzed by covariance methods to account for the influence of $V_0$ and $\alpha$, respectively, as proposed by Burkhoff et al. (6). Correlations between the gain of STF and $E_{es}$ and LV chamber stiffness were assessed by simple and multiple regression. An $R$ measure for continuous variables in GLZ was obtained by Kullback-Leibler divergence according to the method proposed by Cameron & Windmeijer (7). Correlations were tested for the influence of group and experimental preparation by homogeneity of slopes models. To assess for potential diagnostic accuracy in predicting end-diastolic myocardial stiffness of STF gain between EDP and SV a receiver-operating characteristics (ROC) curve analysis was also performed following the non-parametric method of De Long et al. (8). Normally distributed variables complying with homogeneity of variances and normal distribution of residuals were analyzed with general linear models and least-squares methods. Non-normally distributed variables are presented as median [range] whereas normally distributed variables are presented as mean±standard error of mean. Statistical significance was set at two-tailed $P<0.05$. 

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Results

On hemodynamic evaluation both ZSF1 groups showed high maximum LV pressure ($P_{max}$) denoting systemic arterial hypertension compared with WKY but ZSF1 Ob also showed higher $P_{max}$ than ZSF1 Ln (Table 1). Both ZSF1 groups also presented high $E_{esi}$ compared with WKY (Table 1), as denoted by the steeper slope of the ESPVR (Figure 1), whilst only ZSF1 Ob showed prolonged time constant of isovolumic relaxation $\tau$ indicating delayed relaxation, elevated EDP and upward-shifted EDPVR (Figure 1) as assessed by chamber stiffness constant $\beta_i$, implying higher diastolic stiffness (Table 1). Time constant $\tau$ was derived by both monoexponential ($\tau_{exp}$) and logistic fitting ($\tau_{log}$). Between group comparisons in CC were discrepant from OC for $\tau_{exp}$ but not for $\tau_{log}$ which is less load and heart rate dependent (31). Regarding EDPVR, analysis of covariance taking in account the effect of the other parameter in exponential fitting ($\alpha$) did not alter the overall results. Likewise, the effect of the intercept ($V_0$) as covariate in linear fitting of the ESPVR did not affect the results, therefore only $\beta_i$ and $E_{esi}$ were used for subsequent analyses. No differences between groups were found for EDVi, ejection fraction (EF) or cardiac index (Table 1) indicating preserved EF in ZSF1 Ob, but all of these were higher in CC compared with OC (Table 1). We must underscore however that without volume indexation to BSA ZSF1 Ob and Ln would appear to have higher CO compared with WKY (134 ± 20, 121 ± 17 and 99 ± 17 mL.min$^{-1}$, respectively) as well as higher SV (406 ± 66, 338 ± 52 and 307 ± 46 $\mu$L, respectively) and LV dilation, as assessed by raw EDV (600 ± 74, 490 ± 52 and 446 ± 46 $\mu$L, respectively).

As for the analysis of the PSD periodograms, the largest peak frequency coincided with the simultaneously recorded RR for every animal, with minimal dispersion signifying that respiratory variability was accurately tracked. Representative tracings are given in Figure 2. Overall, RR was higher in OC compared with CC. ZSF1 Ob showed lower RR in OC and higher RR in CC compared with the other groups (Table 1). PSD was remarkably higher in CC than in OC for EDP, EDVi and indexed SV (SVi) indicating higher fluctuations with ventilation. While no differences between groups were
observed for EDVi and SVi. ZSF1 Ob showed higher EDP PSD and thus increased variation of EDP with ventilation compared with the normotensive WKY and hypertensive ZSF1 Ln control groups, which was significantly higher in the OC preparation (Table 1, Figure 1). Taking EDP spectrum as an input and SVi spectrum as output the STF gain at the RR frequency was lower for ZSF1 Ob compared with both hypertensive and normotensive ZSF1 Ln and WKY controls, respectively, regardless of experimental preparation suggesting that changes in EDP during ventilation in ZSF1 Ob prompted lower magnitude changes in SVi (Figure 3, panel A). For a clearer visualization of the physiological meaning of the STF analysis, a representative tracing of joint variations of EDP, EDVi, and SVi is superimposed with airway pressure (AwP) in WKY and ZSF1 Ob CC preparation (Figure 4). Notice that despite similar fluctuations in AwP ZSF1 Ob shows more remarkable changes in EDP with minor variations in EDVi, and SVi compared with WKY which translates into higher EDP PSD and lower STF gain. Reciprocally, taking EDVi as input and EDP as output at the same frequency the STF gain was higher in ZSF1 Ob (Figure 3, panel C). On the other hand, while no differences were found between groups regarding STF from EDVi to SVi, CC showed higher gain compared with OC at the RR frequency (Figure 3, panel B). No differences were found between groups or experimental preparation for phase or coherence in STF.

Since both STF gain impairment between EDP and SVi and STF gain enhancement between EDVi and EDP denote increased myocardial stiffness we assayed correlations with simplified surrogates of end-systolic and end-diastolic stiffness, \( E_{es} \) and \( \beta_i \), respectively, to understand whether STF gain could specifically track diastolic stiffness. Although significant correlations were also found between STF gain relating EDVi and EDP and \( \beta_i \) (\( P=0.025 \)) these were stronger for the STF between EDP and SVi (Figure 5). Though heterogeneity of slopes was observed (ZSF1 Ob differed from WKY), correlations held significant in homogeneity of slopes models taking in account both experimental preparation and group in the case of \( \beta_i \) (\( P=0.017 \)) but not in the case of \( E_{es} \). Also, in multiple regression including both \( \beta_i \) and \( E_{es} \) as predictors of STF gain, experimental preparation and group (recoded as dummy variables) between EDP and SVi, only \( \beta_i \) and not \( E_{es} \) remained as significant independent predictor.
\(P<0.019\) providing evidence for a strong relationship between the gain of STF relating EDP and SV, and end-diastolic myocardial stiffness, but not Ees.

Finally, adopting a cutoff of 5 \(\mu\text{L} \cdot \text{cm}^{-2}\) for \(\beta\), which was able to discriminate all of the ZSF1 Ob animals in both CC and OC preparations a ROC curve analysis showed an area under curve (AUC) of 0.89 \((P<0.001)\) for STF gain from EDP to SVi (Figure 6).
We applied PSD and STF analysis to beat-to-beat fluctuations induced by ventilation in EDP, EDVᵢ, and SVᵢ in the robust ZSF1 obese rat model of metabolic syndrome and HFpEF that we recently described (12, 22) documenting elevation of EDP PSD, impaired STF gain between EDP and SVᵢ, and enhanced STF gain between EDVᵢ and EDP in experimental HFpEF but not in hypertensive or healthy controls. STF gain impairment from EDP to SVᵢ was independently correlated with end-diastolic stiffness as assessed by the EDPVR and showed good diagnostic performance in discriminating elevated end-diastolic stiffness supporting the use of STF gain analysis of respiratory fluctuations in LV filling pressure and SV or any non-invasive surrogate of these as a means to screen or diagnose HFpEF.

Building upon the concept that blood pressure variability may be induced not only by autonomic nervous system regulation of vessel tone and HR but also by fluctuations in SV derived from changing LV filling patterns and dynamic ventricular-arterial coupling during respiration (11, 28) Shibata et al. proposed that dynamic ventricular-arterial coupling was modulated by changes in LV compliance associated with altered preload during spontaneous ventilation (35) and coined the term dynamic Starling mechanism to address this physiological mechanism. Moreover, applying STF analysis to beat-to-beat fluctuations induced by spontaneous ventilation in PAD as a surrogate of LV EDP and SVᵢ as derived indirectly from pulse contour analysis of finger arterial pulse photoplethysmography after calibration for CO measurements obtained from the acetylene rebreathing method Shibata et al. also showed significantly impaired gain in elderly sedentary individuals compared with younger individuals or elderly Masters athletes (34) and further impairment in HFpEF patients compared with healthy age-matched controls which was accompanied by higher EDP beat-to-beat variation and PSD at the RR (33). Shibata’s works suggest that impaired dynamic Starling mechanism is an essential pathophysiological feature of HFpEF due to progressive increases in both LV diastolic and arterial stiffness and that it may play an important role in lung congestion during exercise because of increased respiratory effort and venous return. Two
important limitations of these works have been acknowledged by the authors themselves. First, the small subsets of patients enrolled and the exclusion of atrial fibrillation cases precludes generalization to the large population of patients with HFpEF which is highly heterogeneous (32). Second, despite the widespread validation of pulse contour analysis and the strong correlation between pulmonary capillary wedge-pressure and PAD, both are indirect measurements of SV and EDP, respectively. The later problem can hardly be dealt with in clinical research given the invasiveness required for hemodynamic evaluation in heart failure or healthy outpatients and therefore warrants validation in animal models. Our main objective was to validate the conceptual framework laid out by Shibata et al. in tightly controlled experimental conditions using an animal model of HFpEF in which we could invasively measure respiratory fluctuations of SV and EDP by gold standard methodology. We have previously described the ZSF1 obese rat metabolic syndrome model as a good model of HFpEF. Indeed, ZSF1 obese rats show myocardial hypertrophy, elevated LV filling pressures, upward-shifted EDPVR, increased myocardial stiffness and myofilament derangements involved in the pathophysiology of HFpEF (12), along with lung congestion, effort intolerance and impaired maximum oxygen consumption ($\dot{V}O_2$max) thereby closely mimicking HFpEF (22). Standard measures of contractility are preserved or even suggest a hypercontractile phenotype. Indeed, $E_{es}$ is increased in ZSF1 animals because of concentric remodeling and ventricular stiffening (3) though other indexes which are less dependent on chamber geometry such as preload-recruitable stroke work may be within normal range (5). Additionally, this model has convenient lean hypertensive reference controls as well as healthy normotensive Wistar-Kyoto controls. Resource to animal models however raises important questions. PSD analysis of respiratory fluctuations is usually performed under spontaneous negative-pressure ventilation. This was unfeasible in our experimental setup. Animals had to be anesthetized and given strong analgesics in order to carry out invasive evaluations and surgical procedures. To prevent hypoventilation we chose assisted ventilation in CC whereas for OC controlled ventilation was mandatory. Still, although the determinants of dynamic changes in hemodynamics during ventilation are probably reversed in
positive-pressure ventilation, since intra-thoracic pressure rises during inspiration contrarily to spontaneous ventilation, similar principles may be applied (28). Indeed, SV variation is usually used to assess fluid responsiveness in the critically ill mechanically ventilated patient (24). Regarding positive pressure ventilation, although partial ventilation support (assisted ventilation) and full support (controlled ventilation) elicit similar hemodynamic responses for matched ventilator parameters (36), inspiratory pressure level which is the main determinant of LV function changes through intra-thoracic pressure (9) has a strong impact on SV variation (19) therefore we established constant inspiratory pressure and PEEP levels in all groups and experimental preparations to minimize the confounding influence of varying intra-thoracic pressures. This however demanded RR adjustment according to the minute ventilation required by the animals to achieve normocapnia in the controlled ventilation OC preparation. Not surprisingly, RR varied significantly particularly in the heavier obese ZSF1 rats. Varying RR however does not obviate STF analysis. Constant RR is mostly used for convenience in analysis but the underlying concepts do not rely on the assumption of constant RR. We recorded RR throughout the experiments and analyses were performed at each animal’s own RR. Moreover, large variations in SV are known to persist despite increasing RR (11).

Despite the clear deviation from physiology, in an OC preparation using gold standard hemodynamic evaluation conditions with direct CO measurement by transit time flowmeter and multiple beat ESPVR and EDPVR acquisition we reproduced the findings of Shibata et al. in HFpEF patients (33). ZSF1 Ob showed higher PSD of EDP and impaired EDP to SVt STF gain because their stiffer myocardium imposes higher fluctuations in EDP with ventilator excursions without a normal increase in SVt. This is depicted in Figure 7 and clearly supports the concept of impaired dynamic Frank-Starling mechanism first proposed by Shibata et al. in HFpEF (33, 34). Nevertheless, the influence of positive-pressure ventilation on LV EDP and SVt are less remarkable in OC when compared with the CC condition (9) thus we tried to further validate results in a less invasive CC preparation which better reflects physiology and the clinical setting. In CC with assisted ventilation direct measurement of CO and IVC occlusions were not performed though. We relied on CO measurement and volume...
calibration (slope factor $\alpha$) by echocardiography and on single-beat methodologies for assessment of
the ESPVR and EDPVR. Though the agreement between SV measurement by echocardiography and
transit time flow measurement was found to be high in healthy mice (17) in our experience and
routine clinical practice (15) it is prone to variability and overestimation. Likewise, single-beat
methods used in CC for both ESPVR and EDPVR assessment are less reliable than multiple beat
methods (16). Nevertheless, higher EDP PSD and impaired STF gain from EDP to SV, were also
observed for HFpEF ZSF1 obese rats in CC and we found good overall coherence between OC and CC
preparation results. Indeed, main findings and correlations remained unaltered by experimental
preparation thus data were pooled for correlation and ROC curve analysis. This further supports the
validity of the application of STF gain between surrogates of LV filling pressure and SV in estimation
of LV stiffness regardless of ventilation and experimental preparation settings.

Though our work was not designed or particularly tailored to compare the OC and CC preparations,
for which case the ideal setting would have been to perform CC and OC recordings in the same
animal with CO determination and volume calibration by the same method and multiple-beat
methodology for pressure-volume relationship analysis in both circumstances. We must point out
that compared with CC EDV$_i$ and CI were lower in the OC preparation, as expected by impaired
venous return (10) and previously reported in healthy mice (23). Contrarily to previous reports (14)
however we did not observe decreased EDP in the OC preparation when compared to CC which
could be due to a more adequate fluid replacement and blood loss compensation or distinct
respirator settings. In fact, despite pericardial opening chamber stiffness constant was higher in the
OC preparation supporting trends previously observed by Lips et al. (23). A potential explanation
could by ventilation-perfusion uncoupling, increased pulmonary vascular resistance, right ventricular
load and ventricular interdependence (20). Most importantly, compared with the CC setting, OC
ZSF1 Ob showed more hypertension as assessed by $P_{\text{max}}$ and further prolongation of relaxation which
could be explained by ventricular-vascular mismatch and increased surgical stress in OC settings (23).
Prolongation of relaxation has been previously reported under OC compared with CC conditions
An important contribution from this study to the conceptual framework raised by Shibata et al. is the pinpointing of end-diastolic and not end-systolic stiffness as the best correlate of impaired dynamic Starling mechanism. This is supported by our data firstly because hypertensive ZSF1 Ln animals that showed similar elevation in $E_{esi}$ but no significant change in LV end-diastolic chamber stiffness $\beta_i$ compared with ZSF1 Ob also showed preserved gain in EDP to SV, STF which only became impaired in ZSF1 Ob with HFpEF, and lastly because a stronger and independent correlation of the gain of EDP to SV STF was found for $\beta_i$ and not for $E_{esi}$. As a consequence the gain of STF between EDP and SV, during either spontaneous or controlled ventilation or any surrogate may be used to assess LV end-diastolic stiffness in HFpEF as a screening or diagnostic tool. In our experimental work we found good diagnostic performance as assessed by the AUC in ROC curve analysis supporting future studies where this approach is tested in the clinical setting. Diagnostic criteria for HFpEF are still controversial (39) and non-invasive diagnosis remains elusive (2). An approach based on the analysis of STF between surrogate estimators of LV filling pressure and SV such as PAD and pulse pressure or SV variation obtained by pulse contour analysis methodologies, respectively, during ventilation may provide a feasible and robust alternative to invasive LV pressure-volume catheterization, or exercise testing. The clinical usefulness of STF might be expanded if respiratory fluctuations in non-invasive parameters derived from echocardiography can be demonstrated but this would require prolonged continuous acquisitions of ventricular volumes and surrogates of ventricular stiffness (18) or continuous strain analysis by speckle-tracking, which is currently unfeasible. Nevertheless, since PSD of EDP is also increased in ZSF1 Ob a simple assessment of respiratory variation of a surrogate of EDP such as the ratio of peak Doppler velocity of early filling wave (E-wave) in transmitral flow (E) to peak mitral annulus tissue Doppler velocity in early diastole ($E'$) may also aid screening or diagnosis of HFpEF patients. In reality, respiratory fluctuations of E already help diagnosis in constrictive pericarditis (13).

Other original contributions are the documentation of no impairment in STF between EDV$_i$ and SV$_i$, as would be expected in preserved EF, and concomitant enhancement of STF gain between EDV$_i$ and
EDP as expected for a stiffer myocardium. Also, to our knowledge no previous study as addressed the issue of the dynamic influence of positive pressure ventilation on beat-to-beat hemodynamics in HFpEF, mechanical ventilation or the OC condition thus we provide an experimental background for future clinical studies. Nevertheless, we must highlight that our results were obtained under anesthesia and therefore cannot be linearly extrapolated to the conscious unanesthetized scenario. Our findings should be validated in conscious animal models using telemetrics. We must also reinforce that a linear system approximation was employed for STF analysis.

In conclusion, we have demonstrated the validity of STF analysis between EDP and SV, beat-to-beat fluctuations with ventilation originally proposed in a proof-of-concept study carried out in a small subset of HFpEF patients by surrogate hemodynamic measurements in a robust experimental model under carefully controlled experimental settings and gold standard hemodynamic evaluation methodologies. Results were validated in both OC and CC preparations. We further documented a close correlation between STF gain and end-diastolic stiffness which may enable its potential application as a screening or diagnostic tool in clinical evaluation of HFpEF since it requires only data from joint standard RHC and SV measurement or non-invasive surrogates.

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**Disclosures**

The authors have no conflict of interest to disclose.
References


consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. European heart journal 28: 2539-2550, 2007.


Figure Legends

Figure 1. End-diastolic and end-systolic pressure volume relationships obtained by multiple beat method in open-chest (OC, panel A) and single beat method in closed-chest preparations (CC; panel B). Notice the upward-shift in end-diastolic pressure-volume relationships in ZSF1 obese (ZSF1 Ob, dark gray symbols and dashed lines) compared with both ZSF1 Lean (ZSF1 Ln, black symbols and dash-dotted lines) and Wistar-Kyoto (WKY, white symbols light gray solid lines) control rats and the steeper slope of the end-systolic pressure-volume relationship in both ZSF1 groups compared with WKY in both OC (circles, n=8 each group) and CC preparations (triangles, n=6 each group). For results and statistical analysis see table 1. For details on single beat methods please refer to the methods section.

Figure 2. Representative power spectra of end-diastolic pressure (EDP, panels A and D), indexed end-diastolic volume (EDVi, panels B and E) and stroke volume (SVi, panels C and F) in Wistar-Kyoto (WKY, light-gray solid lines), ZSF1 lean (ZSF1 Ln, black dash-dotted lines) and ZSF1 obese (ZSF1 Ob, dark-gray dashed lines) in open-chest (panels A-C) and closed-chest preparations (panels D-F). DC component was filtered out with a digital high-pass filter. Boundaries for area under curve estimation are represented by thin vertical lines. Overall these boundaries were defined by peak frequency±0.045 Hz with slight adjustments for broader peaks. For results and statistical analysis see table 2.

Figure 3. Box plots of transfer function gain between (panel A) end-diastolic pressure (EDP) and indexed stroke volume (SV), (panel B) indexed end-diastolic volume (EDV) and SV, and (panel C) EDV and EDP in open-chest (OC) and closed-chest preparations (CC) for Wistar-Kyoto (WKY), ZSF1 lean (ZSF1 Ln), and ZSF1 obese rats (ZSF1 Ob). Median, interquartile range and individual values outside this range are represented; n=8 for each group in OC and n=6 for each group in CC; *P<0.05 vs WKY, †P<0.001 vs ZSF1 Ln (main effects), ‡P<0.05 vs OC (main effects).
Figure 4. Representative time series from a Wistar-Kyoto (WKY, left panel) and ZSF1 obese (ZSF1 Ob, right panel) showing cyclic variations of end-diastolic pressure (EDP, black symbols and medium dashed line), indexed end-diastolic volume (EDV, gray symbols and dark gray dash-dotted line) and indexed stroke volume (SV, white symbols and long dash light-gray line) with ventilation in a closed-chest preparation. Symbols represent beat-to-beat values. Airway pressure (AwP, thick solid black lines) is plotted at the bottom. A short 6 sec time frame was chosen to improve visualization, the closed-chest preparation was chosen because it better reflects physiology. Notice that fluctuations of EDP are more pronounced in ZSF1 Ob whereas fluctuations in EDV, and SV, are more conspicuous in WKY.

Figure 5. Correlations between both left ventricular chamber stiffness constant $\beta$ (panel A) and end-systolic elastance ($E_{es}$) and gain of the transfer function from end-diastolic pressure (EDP) to indexed stroke volume (SV) by log-fitting (panel B). Regression line and 95% prediction intervals are plotted along with symbols for individual observations from Wistar-Kyoto (WKY, white), ZSF1 lean (ZSF1 Ln, black), and ZSF1 obese (ZSF1 Ob, gray) in the open-chest (OC, circles, n= 8 each group) and closed-chest preparations (CC, triangles, n=6 each group). R and $P$ values are also reported.

Figure 6. Receiver-operating characteristics curve analysis for gain of the transfer function from end-diastolic pressure to indexed stroke volume in detecting high left ventricular chamber stiffness as assessed by a $\beta$ cutoff of $5 \mu L^{-1} cm^{-2}$. Area under curve (AUC) and $P$ value are reported.

Figure 7. Schematic representation of translation of findings to preserved ejection fraction heart failure (HFpEF) as represented by pressure-volume loops (panel A) and signal processing and analysis to obtain spectral transfer function (STF) gain (panel B). Steeper slopes of the end-diastolic pressure volume relationship which denote increased stiffness of HFpEF left ventricular myocardium translate into marked respiratory oscillations (bidirectional arrows) of end-diastolic pressure (EDP) without a proportional increase in stroke volume (SV) variation. When beat-to-beat fluctuations of input (EDP) and output (SV) with respiratory oscillations within the same time interval ($t_0$-$t_1$) are
processed by STF analysis (which can simplistically be viewed as a black box) lower gain is obtained for HFP EF compared with healthy myocardium.
AUC=0.89
P<0.001
A

Pressure (mmHg)

Volume (mL)

HFpEF

Healthy

Respiratory oscillation

B

Input (EDP)  Output (SV)

mmHg

HFpEF

Healthy

Gain

HFpEF

Low

Healthy

Normal

mmHg

ml

Time (s)
### Table 1. Hemodynamics.

<table>
<thead>
<tr>
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<th>Main effects</th>
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<td>WKY</td>
<td>ZSF1 Ln</td>
<td>ZSF1 Ob</td>
<td>WKY</td>
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<td>ZSF1 Ob</td>
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<tr>
<td>$P_{\text{max}}$, mmHg</td>
<td>105±4</td>
<td>143±7</td>
<td>169±8$^{*,**,‡}$</td>
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<td>HR, min$^{-1}$</td>
<td>315±7</td>
<td>382±13$^{*}$</td>
<td>308±6$^{†}$</td>
<td>318±18</td>
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<td>320±9$^{†}$</td>
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<td>EF, %</td>
<td>57±3</td>
<td>55±4</td>
<td>61±3</td>
<td>80±3</td>
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<td>$\tau_{\text{exp}}$, ms</td>
<td>8.4±0.5</td>
<td>6.5±0.4</td>
<td>12.6±1.4$^{*,**,‡}$</td>
<td>10.7±0.8</td>
<td>9.9±0.5</td>
<td>10.4±0.3$^{*,‡}$</td>
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<tr>
<td>EDVi, μL.cm$^{-2}$</td>
<td>0.86±0.08</td>
<td>0.73±0.10</td>
<td>0.73±0.12</td>
<td>1.13±0.19</td>
<td>1.18±0.08</td>
<td>1.23±0.15</td>
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<td>$E_{\text{esi}}$, mmHg.μL$^{-1}$.cm$^{-2}$</td>
<td>107±21</td>
<td>276±47$^{*}$</td>
<td>299±24$^{*}$</td>
<td>193±39</td>
<td>219±42$^{*}$</td>
<td>287±76$^{*}$</td>
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Left ventricular (LV) hemodynamic parameters in Wistar-Kyoto (WKY), ZSF1 lean (ZSF1 Ln) and ZSF1 obese (ZSF1 Ob) rat groups (G) in open-chest (OC, n=8 each groups) and closed-chest (CC, n=6 each group) experimental preparations (P). Main effects of G and P and interaction (G * P) are presented in the rightmost columns. $P_{\text{max}}$, maximum pressure; CI, cardiac index; HR, heart rate; EF, ejection fraction; $\tau_{\text{exp}}$, time constant of isovolumic relaxation derived by monoexponential fitting; EDP, end-diastolic pressure; EDVi, indexed end-diastolic volume; $\beta_i$, LV chamber stiffness constant obtained from the exponential fitting of the end-diastolic pressure volume relationship with indexed volumes; $E_{\text{esi}}$, end-systolic elastance obtained by linear fitting of the end-systolic pressure-volume relationship with indexed volumes. Data are mean±standard error of mean or median [range]: *$P<0.01$ vs WKY; †$P<0.01$ vs ZSF1 Ln; ‡$P<0.01$ vs CC.
<table>
<thead>
<tr>
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<th>OC</th>
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<th>Main Effects</th>
<th>Interaction</th>
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<tr>
<td>RR, Hz</td>
<td></td>
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<td></td>
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<td>WKY</td>
<td>2.44 [1.28-3.22]</td>
<td>2.95 [1.28-3.23]</td>
<td>1.59 [1.28-2.23]</td>
<td>0.68 [0.59-0.80]</td>
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<td>ZSF1 Ln</td>
<td>0.78 [0.15-2.17]</td>
<td>0.51 [0.09-1.72]</td>
<td>7.16 [2.11-19.3]</td>
<td>156 [37-201]</td>
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<td>ZSF1 Ob</td>
<td>0.20 [0.03-1.3]</td>
<td>0.50 [0.08-1.4]</td>
<td>0.42 [0.01-6.26]</td>
<td>47 [6-88]</td>
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<td></td>
<td>0.31 [0.05-1.03]</td>
<td>0.21 [0.04-1.22]</td>
<td>0.41 [0.05-0.93]</td>
<td>41 [14-118]</td>
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</table>

Areas under curve of the power spectrum are given for Wistar-Kyoto (WKY), ZSF1 lean (ZSF1 Ln) and ZSF1 obese (ZSF1 Ob) groups (G) in both OC (n=8 each group) and CC (n=6 each group) preparations (P). Units were adjusted to facilitate comparison between OC and CC data. Main effects of G and P and interaction (G*P) are presented in the rightmost columns. RR, respiratory rate; EDP, end-diastolic pressure; EDVi, indexed end-diastolic volume; SVi, indexed stroke volume. Data are median [range]: *P<0.001 vs WKY; †P<0.001 vs ZSF1 Ln; ‡P<0.001 vs behavior in CC preparation.