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The magnitude and course of exercise-induced stroke volume changes determine the exercise tolerance in heart transplant recipients with heart failure and normal ejection fraction

Original article

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Abstract

Objectives: There is a large variability of exercise-induced stroke volume behavior in healthy subjects. We sought to assess the course of exercise-induced changes in stroke volume index (SVI) and other functional parameters in post-heart transplant patients with heart failure and normal left ventricular ejection fraction (HFNEF).

Methods: Left ventricular function and systemic hemodynamics were assessed at 40 s intervals during the exercise in 39 patients using simultaneous right heart catheterization, bioimpedance, and echocardiography.

Results: Twenty-six patients had exercise tolerance ≥ 4.0 METs (Group A), while 13 patients exhibited severely limited exercise tolerance < 4 METs (Group B). Maximal SVI (maxSVI) achieved at any time during the exercise exceeded SVI at peak exercise (peakSVI) in 26 patients (67%). Both maxSVI and ΔmaxSVI (maxSVI minus SVI at rest) were significantly higher in Group A compared to Group B patients (59 ml/m^2 vs 41 ml/m^2, $p < 0.01$, and 21

ml/m² vs 6 ml/m², p < 0.01, respectively). With peakSVI, maxSVI, ΔpeakSVI, ΔmaxSVI and other variables evaluated, only ΔmaxSVI was independently associated with exercise tolerance.
Conclusion: When assessing exercise-induced SVI changes in HFNEF patients, SVI should be followed during the course of exercise and maximal SVI change from rest should always be determined.

Keywords: Heart failure; Stroke volume index; Exercise tolerance; Bioimpedance.

1. INTRODUCTION

Heart failure with normal left ventricular ejection fraction (HFNEF) is a frequent cause of dyspnea and/or fatigue and has a poor prognosis [1,2]. A significant proportion of patients with HFNEF have heart failure symptoms as well as hemodynamic and functional abnormalities only during exercise [3]; i.e. they suffer from the isolated, only exercise-induced HFNEF. In these patients, the recognition of exercise pathology is of utmost importance for understanding the pathophysiology of the underlying heart failure process, for the assessment of disease severity and prognosis, and maybe, in the future, for the more tailored therapy of this disease. However, limited data are available on the simultaneously obtained invasive and noninvasive exercise-induced hemodynamic and functional parameters in patients with HFNEF [4-6]. These studies analyzed small cohorts of patients, including maximally 20 HFNEF subjects [4]. Moreover, the majority of previous studies did not follow and stratify the course of exercise-induced parameter changes in individual patients but, instead of it, they presented the mean results in a group of HFNEF patients as a whole [3,7-12]. Another limitation for understanding the behavior of myocardial function during exercise is the fact that many projects measured and evaluated only the resting and submaximal or peak exercise data, but did not assess serial hemodynamic changes that occur during progressive levels of exercise [4,8-13]. In such an instance, many important parameter alterations at the early stages of exercise or during the mid-exercise period could be missed, and the peak exercise measures may not reflect the maximal parameter deviations achieved in the course of exercise. This fact can be especially important when assessing exercise stroke volume behavior that is known to be highly variable even in healthy subjects [14-16]. Continuous or short-term interval monitoring of exercise hemodynamics has many advantages and potential to offer more accurate and precise data providing the insight into the time relationship of parameter changes during the exercise [17]. The aim of our study was to assess the course of exercise-induced changes in stroke volume (SV), pulmonary capillary wedge pressure (PCWP), and other functional and hemodynamic parameters in patients with HFNEF, 2) to define various categories of exercise SV responses, and 3) to identify parameters accounting for a severely limited exercise tolerance in HFNEF patients.

2. METHODS

2.1 Patient population

Patients after orthotopic heart transplantation referred for post-transplant cardiac examination, who met study inclusion criteria, were prospectively enrolled. The inclusion criteria were as follows: 1) a time interval ≥ six months after heart transplantation, 2) a sinus rhythm on the electrocardiogram, 3) transthoracic echocardiography demonstrating left ventricular ejection fraction (LVEF) ≥ 50%, no significant pericardial effusion or valvular disease, and no mitral regurgitation other than trivial and, 4) no history of myocardial infarction or angina pectoris after heart transplantation. The study complies with the Declaration of Helsinki and was approved by the ethics committe at St. Anne's Hospital. All patients gave their written consent to the investigations.

2.2 Study protocol and measurement timing

Initially, conventional transthoracic echocardiography was performed to elucidate whether the patients meet echocardiographic inclusion criteria. On the following day, simultaneous echocardiography, right heart catheterization, thoracic bioimpedance cardiography, and continuous noninvasive blood pressure monitoring were implemented at rest and during the exercise. The study protocol started at the moment of achievement of the catheter balloon pulmonary capillary wedge position during right heart

catheterization. The protocol included a five min interval of rest with a patient in a supine position (resting period), a five min interval of patient's leg elevation (leg elevation period), as well as exercise and recovery periods. After obtaining the catheter pulmonary capillary wedge position (the onset of resting period), the acquisition of multi-lead electrocardiogram, heart sounds, noninvasive blood pressure, and thoracic bioimpedance signal started. Within the last two mins of a resting period, the echocardiographic apical four-chamber view for volumetric quantification was recorded, PCWP, heart rate, reference cuff brachial systolic (SBP) and diastolic (DBP) blood pressures were measured, and the catheterization cardiac output (CO) for obtaining SV reference value was determined. Subsequently, patients raised lower extremities into pedals and identical recordings and measurements (except for CO) were repeated within the last two mins of the leg elevation period. Following that, the exercise was initiated with heart rate and PCWP measurements at 40 s intervals, at peak exercise, and at one min post-exercise recovery intervals until PCWP normalization (achievement of the pre-exercise PCWP ± 2 mmHg, the end of protocol). At the exactly same time intervals, SV, SBP, and DBP values were obtained from continuous blood pressure and bioimpedance signal recordings during the post-protocol offline computer analysis. Exercise echocardiographic recordings of the apical four-chamber views were obtained at two min intervals during the exercise and at peak exercise; recovery recording was acquired at the time of PCWP normalization. On the day of catheterization, the morning medication was withdrawn.

2.3 Stress protocol

Supine bicycle ergometry was performed starting at 25 W for two mins. The load was then increased in increments of 25 W at two min intervals until the first occurrence of symptoms (dyspnea or fatigue). All exercise tests were performed on an ergometer Ergoline GmbH (type er900L, Bitz, Germany). The patients were lying and cycling with trunk in a horizontal position having legs slightly elevated (pedals were approximately 35 cm above the bench level). The maximal exercise tolerance was expressed as the number of metabolic equivalents (METs) according to the recommendation of the American College of Sports Medicine [18]. Fot the METs calculation, the maximal workload exerted for at least one min was used.

2.4 Right heart catheterization

A 7F Swan-Ganz thermodilution catheter (model 131HF7, Baxter Healthcare Corporation, Irvine, CA, USA) was advanced into the pulmonary capillary wedge position. The correct balloon position was verified by the presence of characteristic wedge pressure waveforms. PCWP was measured with a zero level at the mid-axillary line using a multi-parametric module Ultraview SL (TM) 91496 (Spacelabs Healthcare, Issaquah, WA, USA). PCWP was averaged over pressure waveform data obtained during a 12 s interval and expressed as a mean. Resting PCWP ≥ 15 mmHg and peak exercise PCWP ≥ 25 mmHg were defined as abnormal and suggestive of HFNEF in accordance with Borlaug et al. [3]. CO was measured by the thermodilution technique. Measurements were made at rest immediately after obtaining the baseline resting PCWP. To obtain final reference CO, at least three CO values were averaged. Baseline resting catheterization SV was automatically calculated by the software as the CO/heart rate ratio.

2.5 Continuous blood pressure monitoring and bioimpedance signal acquisition

The continuous noninvasive arterial SBP and DBP were measured on the middle finger of the left hand during the whole protocol by Finometer (Finapress Medical Systems B.V., Amsterdam, Netherlands) based on the Peňáz method [19,20]. To corroborate the accuracy of systolic and diastolic values, the intermittent measurements of the reference brachial arterial pressure by a cuff automated sphygmomanometer (SpaceLabs Healthcare, Issaquah, WA, USA) were acquired on the right arm during the protocol [21].

The methodology of SV measurements using thoracic bioimpedance cardiography (TIC) has already been described in detail [22-24]. In brief, the multi-lead electrocardiogram, heart sounds, and thoracic bioimpedance cardiography (TIC) (ISI ASCR, Brno, Czech Republic) were continually obtained during the protocol. All measured signals were digitized (16-bit A/D converter with a sampling

frequency of 500 Hz) by the AnnaLab acquisition system (AnnaLab MI2, St. Anne's University Hospital, Brno, Czech Republic) for a subsequent offline blind analysis in ScopeWin (ISI Brno, Czech Republic) and MATLAB environment. The thoracic impedance signal was filtered in pass band 0.6-18 Hz before the subsequent processing. TIC estimates SV from an impedance waveform measured at the thorax surface. Noninvasive measurement of absolute SV values calibrated in ml based on TIC is inaccurate. However, relative changes in SV can be reliably derived from the left ventricular ejection time (LVET) and from the peak velocity of blood flow in the aorta represented by the maximum amplitude of the negative first derivative of the impedance signal (-dZ/dtmax) [24,25]. These two parameters (Figures 1 and 2) provide information on hemodynamics and form results completely if only relative changes are analyzed. Thus, we defined the relative SV change in comparison with the resting state as follows: SVrel = SVe/SVrest, where SV = LVET x (-dZ/dtmax). SVe represents SV value during the various exercise stages and SVrest represents SV during resting period before the exercise. SV was calculated by averaging the negative first derivative of the thoracic impedance signal (-dZ/dt) from multiple heartbeats to eliminate noise, movement, respiratory effects, and other artifacts. During the resting conditions, the average signal from 60 beats was used. This relatively longer time interval was used to eliminate respiratory variations. During exercise, to detect exercise-induced SV changes, shorter time intervals comprising 20 heartbeats were utilized for the analysis. The second parameter – LVET was obtained from the heart sounds record filtered in pass band 20-120 Hz. The maximum of power envelope 20-60 Hz in the region of heart sound S1 and maximum of power envelope 45-120 Hz in the region of heart sound S2 were detected. The time distance between these two points represents LVET in ms (Figure 2). Because the crucial part of SV computation is the elimination and removal of artificial impedance signal, all intervals selected for impedance signal averaging were manually inspected and corrected. All areas with artificial beats were excluded from averaging. The minimal number of 20 beats was always kept.

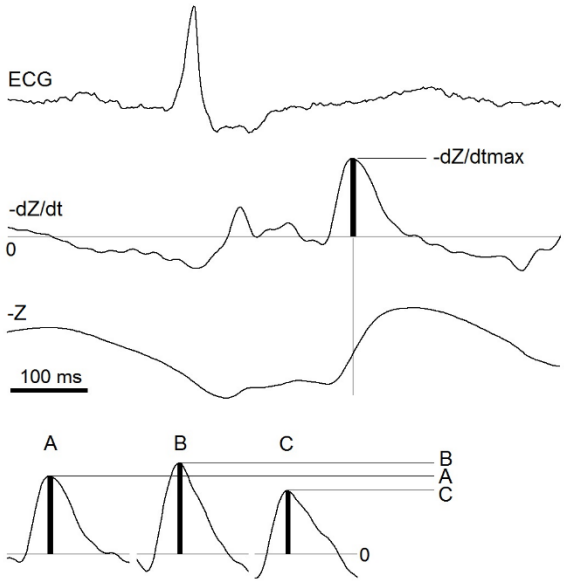

Figure 1. Determination of the maximum amplitude of the negative first derivative of the impedance signal (-dZ/dtmax) and demonstration of exercise-induced -dZ/dtmax changes in patient A.M.

From the top: electrocardiogram (ECG, lead II), derivative impedance signal -dZ/dt, impedance signal -Z and comparison of -dZ/dtmax at rest and during exercise in patient A.M. – curves A, B, and C. Black vertical bar determine individual -dZ/dtmax measured from the zero line to the maximum of derivative signal. Lower part of the Figure 1 represents -dZ/dtmax obtained at rest with patient´s leg elevation (curve A, calculated SVI of 50 ml/m^2), -dZ/dtmax acquired during exercise at the time interval of 280 s (curve B, calculated SVI rose up to 58 ml/m^2 = maximal SVI), and finally -dZ/dtmax obtained at peak exercise at 360 s (curve C, calculated peak SVI markedly dropped to 40 ml/m^2).

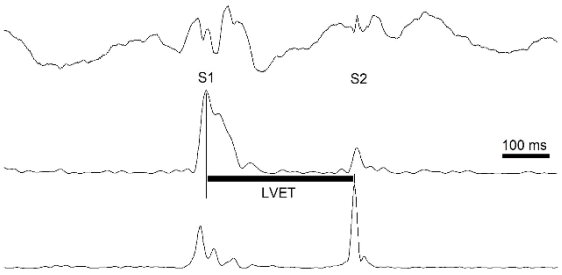

Figure 2. Determination of left ventricular ejection time (LVET).

From the top: phonocardiograph, power envelope 20-60 Hz, power envelope 45-120 Hz. S1 – LVET onset, S2 – LVET end. The time distance between these two points represents LVET in ms.

2.6 Echocardiography

Echocardiographic examinations were performed using Vivid E9 (GE Healthcare, Wauwatosa, WI) with a M5S transducer. Gray-scale two-dimensional images were recorded from the parasternal short axis views at the base, at the level of papillary muscles and at the apex, as well as from the apical two-, three-, and four-chamber views. Three to five consecutive cardiac cycles in each view were digitally stored. Aortic and transmitral flows were recorded using pulsed-wave Doppler echocardiography. All these recordings were made at five min intervals of rest and leg elevation periods. Resting two-dimensional and Doppler recordings were done during shallow respiration or end-expiratory apnea. During the exercise, multi-beat (eight to 12 heart cycles) two-dimensional apical four-chamber view recordings were made at two min intervals of exercise and at peak exercise.

2.7 Parameters analyzed

Echocardiographic measurements and analyses were performed according to recommendations of the American Society of Echocardiography [26]. The data were analyzed offline using EchoPAC PC version 108.1.5 (GE Vingmed Ultrasound A/S, Horten, Norway). Left ventricular (LV) mass was estimated using the Devereux formula [27] and indexed to body surface area. Pre-exercise and exercise LV volumes were derived from the apical four-chamber views. Volumes were calculated using the single-plane method of disks. From multi-beat exercise recordings, two to three consecutive cycles with the best endocardium delineation were selected for LV volumetric quantification and data were averaged. All echocardiographic analyses were performed offline by one experienced observer (J.M.) who was blinded to SV and PCWP values. For the determination of intra- and inter-observer variabilities of LV volume measurements, the resting and exercise end-diastolic volume, end-systolic volume, and LVEF of eight randomly selected patients were reassessed more than two months after the initial analysis by two observers (J.M. and H.P.). The reproducibility data are presented as the mean absolute difference of repeated measurements and expressed as a percentage of the mean of two absolute measurements.

SV relative changes were computed from the thoracic bioimpedance signal as described above. Real value of the initial resting bioimpedance-derived SV in ml was calibrated by the SV value determined by the thermodilution technique during right heart catheterization at the onset of the protocol. This initial SV indicated 100% and every subsequent percentual signal change was recalculated to express the absolute SV change.

All heart volumes were normalized to body surface area, yielding their respective indexes: left atrial volume index (LAVI), LV end-diastolic volume index (LVEDVI), LV end-systolic volume index (LVESVI), and stroke volume index (SVI). The following indexes and parameters were calculated [28-30]: effective arterial elastance index (EaI = end-systolic pressure/SVI); LV end-systolic elastance index (EesI = end-systolic pressure/LVESVI); ventricular-arterial coupling ratio (EaI/EesI = ESVI/SVI); and stroke work index (SWI = SVI x end-systolic pressure). End-systolic pressure (ESP) was calculated as SBP x 0.9.

2.8 Definition of heart failure with normal left ventricular ejection fraction

There were three obligatory conditions for the diagnosis of HFNEF: presence of signs or symptoms of heart failure, presence of normal or mildly abnormal LV systolic function (LVEF > 50%) without LV enlargement (LVEDVI < 97 ml/m^2), and the evidence of diastolic dysfunction [31]. Diastolic dysfunction was defined by the elevation of a mean PCWP at rest (≥ 15 mmHg) and/or at any time during exercise (PCWP ≥ 25 mmHg) [3]. Severe exercise intolerance was defined by METs < 4.0.

2.9 Statistical analysis

All parameters were tested prior to any statistical analysis whether or not they were normally distributed. Because the assumption of normal distribution was remarkably violated for most continuous parameters (Shapiro-Wilk test), nonparametric analyses were performed and appropriate descriptive statistics were used for the presentation of data. Continuous variables are presented as median (lower quartile-upper quartile) and categorical variables as number (%) of subjects. Comparison of resting (leg elevation) and exercise data and also comparison of individual consecutive

exercise protocol phases were performed by means of paired Wilcoxon signed-rank test. The Mann-Whitney test was applied to compare the results of patients with exercise tolerance < 4.0 METs versus those with exercise tolerance ≥ 4.0 METs. The Chi-square test was used to compare the groups in categorical data. The association between potential risk factors and level of exercise tolerance was analyzed via univariate and multivariate logistic regression. The parameters with potential predictive power (providing at least $p < 0.1$ in univariate logistic regression) were included into the multivariate logistic regression models. Correlations of exercise-induced parameter changes with exercise tolerance (METs) were evaluated via the calculation of Spearman correlation coefficients. The analyses were performed using Statistica 12. Results with p-value < 0.05 were considered statistically significant.

3. RESULTS

Out of the 57 initially screened patients, 55 subjects fulfilled the inclusion criteria and underwent simultaneous resting and exercise echocardiography, bioimpedance, and right heart catheterization. Interpretable exercise catheterization and bioimpedance results were obtained in 46 patients; one patient was excluded because of technical problems with the data acquisition during exercise, one subject had frequent premature ventricular ectopic beats during exercise, and seven patients had artifacts on bioimpedance recording due to a poor quality of exercise electrocardiogram. Thirty-nine patients met the criteria for the diagnosis of HFNEF and entered the final analysis. Their baseline clinical characteristics are demonstrated in Table 1.

Table 1. Baseline clinical characteristics in the entire patient population and in patients grouped according to exercise tolerance

Parameter	All patients (n = 39)	Patients with exercise tolerance ≥ 4.0 METs (n = 26)	Patients with exercise tolerance < 4.0 METs (n = 13)
Men (%)	36 (92.3)	24 (92.3)	12 (92.3)
Age of patients (years)	56 (46 - 63)	55.5 (43 - 62)	60 (55 - 64)
Allograft age (years)	39 (34 - 50)	37 (32 - 50)	40 (36 - 49)
Time since OHT (months)	36 (17 - 95)	36 (12 - 91)	35 (21 - 95)
Hypertension (%)	33 (84.6)	22 (84.6)	11 (84.6)
Diabetes mellitus (%)	21 (53.8)	11 (42.3)	10 (76.9) #
Body mass index (kg/m^2)	28 (26 - 30)	27 (24 - 30)	29 (27 - 31)
Supine bicycle ergometry			
Exercise tolerance (METs)	4.4 (3.8 - 4.6)	4.6 (4.4 - 4.8)	3.8 (3.5 - 3.8) ##
Medical therapy			
ACEi or AT II (%)	24 (61.5)	14 (53.8)	10 (76.9)
Beta blockers (%)	34 (87.2)	24 (92.3)	10 (76.9)
Diuretics (%)	15 (38.5)	10 (38.5)	5 (38.5)
Echocardiography			
LAVI (ml/m^2)	42 (35 - 50)	42 (36 - 49)	40 (32 - 50)
LVMI (g/m^2)	100 (82 - 115)	102.5 (86 - 115)	88 (82 - 101)
RWT	0.49 (0.43 - 0.57)	0.49 (0.43 - 0.57)	0.51 (0.46 - 0.52)
E (cm/s)	75 (64 - 85)	73 (64 - 84)	75 (62 - 113)
A (cm/s)	41 (34 - 46)	41.5 (31 - 46)	41 (34 - 44)
E/A	1.85 (1.54 - 2.20)	1.79 (1.51 - 2.10)	1.94 (1.79 - 2.57)

A, peak late diastolic transmitral velocity; ACEi, angiotensin-converting inhibitor; AT II, angiotensin II receptor blocker; E, peak early diastolic transmitral velocity; LAVI, left atrial volume index; LVMI, left ventricular mass index; OHT, orthotopic heart transplantation; RWT, relative wall thickness. ## $p < 0.01$ vs patients with exercise tolerance ≥ 4.0 METs. Data are presented as median (lower quartile-upper quartile) or number (%).

Patients exhibited a marked exercise intolerance, as indicated by METs median of 4.4 (3.8-4.6). In all patients, the exercise was limited by leg fatigue or dyspnea, mostly in combination. In addition to medication listed in Table 1, all patients were on immunosuppressive therapy. Myocardial biopsy was performed on 21 (54%) patients. According to the modified ISHLT histological classification of cellular rejection [32], 14 patients had grade 0, five patients grade 1A, and two patients grade 1B rejections. Thirty-one patients underwent coronary angiography during the post-transplant follow-up. Significant vasculopathy (luminal diameter narrowing ≥ 50% of at least one coronary artery) was present in two patients. HFNEF at rest was found in six patients. The remaining 33 patients had only isolated exercise-induced HFNEF (PCWP < 15 mmHg at rest, PCWP ≥ 25 mmHg during exercise).

3.1 Baseline and exercise hemodynamic and echocardiographic parameters in patients grouped according to exercise tolerance

Twenty-six patients were found to have exercise tolerance ≥ 4.0 METs (Group A), while the remaining 13 patients achieved exercise tolerance < 4.0 METs (Group B). Their baseline and exercise characteristics are demonstrated in Tables 1, 2, and 3. Patients with exercise tolerance < 4.0 METs suffered more frequently from diabetes mellitus. Otherwise, there were no significant differences between the groups in any of the baseline clinical variables. As the exercise test was a progressive one, the number of patients decreased towards the maximal level. The workload at 200 s was the highest stage of exercise achieved by all 39 patients. Table 2 demonstrates the course of exercise-induced parameter changes in patients with exercise tolerance ≥ 4.0 METs. Exercise durations of 240 s, 280 s, 320 s, and 360 s were accomplished by 26, 25, 23, and 16 subjects, respectively. Exercise results at 400 s, 440 s, and 480 s are not included because they were obtained only in three, one, and one patients, respectively. There was a progressive increase in the majority of parameters from rest to peak exercise. EaI/EesI, LVEDVI, and LVESVI decreased with exercise. EaI initially decreased and from 120 s plateaued. SVI increased up to 160 s and then plateaued.

Table 2. Hemodynamic and echocardiographic parameters at rest and during exercise in patients with exercise tolerance ≥ 4.0 METs

Parameter	Rest	Elev	Exercise									
			40 s	80 s	120 s	160 s	200 s	240 s	280 s	320 s	360 s	Peak
Hemodynamics												
Heart rate (bpm)	76	77	81††**	84††**	86.5††**	89.5††**	93††**	95.5††**	97††**	98††**	100.5††**	102††
ESP (mmHg)	122	126 **	129††**	135††**	135.5††**	137††**	140††**	141††**	141††	143.5††*	146††`*	148.5††
PCWP (mmHg)	10	16 **	21††**	21.5††	23††*	23.5††	24.5††**	26††**	27††**	26††	28††*	29††
SVI (ml/m²)	33.5	37.5 **	45††**	48††*	50††**	52.5††	52.5††	52††	54††	53††	51††	51††
EaI (mmHg/ml/m²)	3.41	3.45 **	3.04††**	2.85††	2.67††*	2.66††	2.61††	2.53††	2.67††	2.60††	2.65††	2.64††
EesI (mmHg/ml/m²)	7.9	8.1	-	-	10.9††	-	-	12.8††**	-	-	19.1††**	16.5††
EaI/EesI	0.45	0.40	-	-	0.23††	-	-	0.20††**	-	-	0.17††	0.18††
SWI (mmHg.ml.m^{-2})	3806	4597.5 **	5885.5††**	5902.5††**	6251††**	6795††*	6878††*	7355††*	7398††	7710††	8151††	7824††
Echocardiography												
LVEDVI (ml/m²)	44.5	47 **	-	-	47	-	-	44.5††**	-	-	39††	43††
LVESVI (ml/m²)	15	16	-	-	12††**	-	-	11††*	-	-	8††*	9.5††
LVEF (%)	65	69	-	-	74.5††**	-	-	76.5††	-	-	77.5††	78††

EaI, arterial elastance index; EesI, left ventricular end-systolic elastance index; Elev, leg elevation; ESP, end-systolic pressure; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume index; PCWP, pulmonary capillary wedge pressure; s, seconds; SVI, stroke volume index; SWI, stroke work index. * $p < 0.05$ vs value of preceding stage; ** $p < 0.01$ vs value of preceding stage, † $p < 0.05$ vs rest with leg elevation; †† $p < 0.01$ vs rest with leg elevation. Data are presented as median.

Table 3 shows resting and exercise parameters in patients with exercise tolerance < 4.0 METs. One patient terminated the exercise at 200 s; all other subjects accomplished exercise time of at least 240 s (six patients terminated the exercise at 240 s, six ones at 280 s). In addition to the course of exercise-induced parameter changes in Group B patients, Table 3 also provides the direct comparison of individual parameters between Group A and B patients at the exactly identical exercise time intervals of 40 s, 80 s, 120 s, 160 s, 200 s, and 240 s. At rest, there were no significant differences in hemodynamic and echocardiographic measures between Groups A and B. During the exercise, several differences between groups became apparent. Patients with exercise tolerance < 4.0 METs exhibited significantly lower SVI and higher EaI during the whole course of exercise. SWI was lower at the late phase and at peak exercise in Group B patients. Good quality exercise echocardiographic images for LV volumetric analyses were not obtained in 14 patients (eight Group A subjects and six Group B subjects).

Table 3. Hemodynamic and echocardiographic results in patients with exercise tolerance < 4.0 METs

Parameter	Rest	Elev	Exercise						
			40 s	80 s	120 s	160 s	200 s	240 s	Peak
Hemodynamics									
Heart rate (bpm)	80	81	88††**	91††**	93††*	98††**#	99††**	101††**	103††
ESP (mmHg)	114	125**	134†*	133††*	137††*	137††	142††*	144††	149††
PCWP (mmHg)	12	17**	20††**	22††	23††**	25††**#	26††	29.5††**	30††
SVI (ml/m²)	29	32**	35†#	36†#	35†##	37†#	41††##	42††#	39††##
EaI (mmHg/ml/m²)	4.38	3.92	3.47#	3.71#	3.81##	3.68#	3.90##	3.75#	4.15##
EesI (mmHg/ml/m²)	8.4	8.3	-	-	12.6†	-	-	15†*	22.4†
EaI/EesI	0.52	0.52	-	-	0.30†#	-	-	0.23†*	0.20†
SWI (mmHg.ml.m^{-2})	3556	4144**	5624†*	5688††*	5285††#	5508††	6493††	6109.5††**#	6681††##
Echocardiography									
LVEDVI (ml/m²)	44	46*	-	-	42	-	-	41†*	38†
LVESVI (ml/m²)	16	17	-	-	12	-	-	10†	7††
LVEF (%)	66	66	-	-	75†*	-	-	77†	75†

Abbreviations as in Table 2. * $p < 0.05$ vs value of preceding stage; ** $p < 0.01$ vs value of preceding stage; † $p < 0.05$ vs rest with leg elevation; †† $p < 0.01$ vs rest with leg elevation; # $p < 0.05$ vs corresponding value in patients with exercise tolerance ≥ 4 METs (Table 2); ## $p < 0.01$ vs corresponding value in patients with exercise tolerance ≥ 4 METs (Table 2). Data are presented as median.

3.2 Categorization and characterization of stroke volume index exercise responses

Due to a large variability in the course of exercise-induced SVI changes, the median SVI values demonstrated in Tables 2 and 3 do not provide information on the response of SVI to exercise in an individual patient and on the timing and magnitude of maximal exercise-induced SVI (maximal SVI at any time during the exercise, maxSVI). We found that 26 out of the 39 patients analyzed (67%) achieved their maxSVI before the peak of exercise. An example of a patient with a marked difference between maxSVI and SVI obtained at peak exercise (peakSVI) is demonstrated in Figure 1. Thus, for further analyses, both maxSVI and ΔmaxSVI (ΔmaxSVI = maxSVI minus SVI at rest with leg elevation) were also included. Table 4 demonstrates the exercise-induced parameter changes from rest to peak exercise (Δpeak) in Groups A and B complemented about ΔmaxSVI. Groups A and B significantly differed in ΔmaxSVI, ΔpeakSVI, ΔpeakEaI, and ΔpeakSWI. Of the parameter changes studied, the best correlation with exercise tolerance in METs was achieved for ΔmaxSVI ($r = 0.45$, $p = 0.004$), followed by ΔpeakSVI ($r = 0.385$, $p = 0.015$). Figure 3 shows the maximal and peak exercise SVI changes in Group A and B patients. Both ΔmaxSVI and ΔpeakSVI were significantly higher in patients with exercise tolerance ≥ 4.0 METs than in those with exercise tolerance < 4.0 METs (21 ml/m² vs 6 ml/m², $p < 0.01$, and 17.5 ml/m² vs 4 ml/m², $p < 0.01$,

respectively). The courses of exercise-induced SVI changes of individual patients were further analyzed in Table 5.

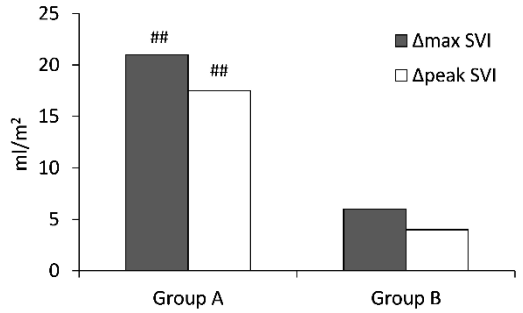

Figure 3. Maximal and peak exercise SVI changes in patients with exercise tolerance ≥ 4.0 METs (Group A) and those with exercise tolerance < 4.0 METs (Group B).
Both ΔmaxSVI and ΔpeakSVI were significantly higher in patients with exercise tolerance ≥ 4.0 METs than in those with exercise tolerance < 4.0 METs (21 ml/m² vs 6 ml/m², p < 0.01, and 17.5 ml/m² vs 4 ml/m², p < 0.01, respectively). ## p < 0.01 vs Group B.

To categorize SVI exercise responses, the total exercise time of every patient was divided in the early phase of exercise (0.1%-50.0% of the total exercise time) and in the late phase of exercise (50.1%-100% of the total exercise time). The changes in SVI ≥ 10% in the early phase of exercise versus baseline (patients at rest with leg elevation) and in the late phase of exercise versus early phase were considered significant for the categorization of exercise-induced SVI responses. Six types of SVI responses were looked for. Sixteen patients were found to have both the early and late exercise-induced SVI increase (type A response to exercise), while four subjects were found to have the early SVI increase followed by its late decrease (type B response). Ten patients exhibited the early exercise-induced SVI increase followed by SVI plateau at the late exercise (type C response). Five patients had no changes in SVI during the exercise (type D response). None of the patients had type E response characterized by no early change in SVI, but a decrease at the late exercise. Finally, four subjects exhibited no early change in SVI followed by an increase at the late exercise (type F response). The distribution of SVI exercise responses in patients with exercise tolerance ≥ 4.0 METs or < 4.0 METs is demonstrated in Table 5. The type A response occurred significantly more frequently in Group A patients indicating that progressive SVI increase during exercise is associated with better exercise tolerance. On the other hand, no significant change in SVI during exercise (type D response) was the most common response in Group B patients.

Table 4. Exercise-induced parameter changes in patients with exercise tolerance ≥ 4.0 METs and in those with exercise tolerance < 4.0 METs

Parameter	Patients with exercise tolerance ≥ 4.0 METs (n = 26)	Patients with exercise tolerance < 4.0 METs (n = 13)
ΔmaxSVI (ml/m²)	21 (15 - 28)	6 (4 - 17) ##
ΔpeakSVI (ml/m²)	17.5 (6 - 24)	4 (1 - 10) ##
ΔpeakPCWP (mmHg)	13 (10 - 16)	8 (6 - 16)
ΔpeakEaI (mmHg/ml/m²)	-0.5 (-1.2 - 0.0)	0.2 (0.0 - 0.4) ##
ΔpeakEesI (mmHg/ml/m²)	10.4 (4.8 - 13.1)	11.7 (5.9 - 15.9)
Δpeak(EaI/EesI)	-0.2 (-0.3 - (-0.2))	-0.3 (-0.3 - (-0.2))
ΔpeakSWI (mmHg.ml.m^{-2})	3170 (2316.5 - 4993)	1927 (1242 - 2953) ##
ΔpeakLVEDVI (ml/m²)	-7 (-13 - (-3))	-13 (-18 - (-8))
ΔpeakLVESVI (ml/m²)	-6 (-8 - (-5))	-7 (-10 - (-6))
ΔpeakLVEF (%)	10 (7 - 13)	7 (3 - 11)
ΔpeakHeart rate (bpm)	27 (22 - 33)	24 (20 - 28)
ΔpeakESP (mmHg)	22 (15.5 - 30.5)	21 (9 - 41)

Abbreviations as in Table 2. Δmax, maximal exercise value minus value at rest with leg elevation; Δpeak, value at peak exercise minus value at rest with leg elevation. ## p < 0.01 vs patients with exercise tolerance ≥ 4.0 METs. Data are presented as median (lower quartile-upper quartile).

Table 5. Categorization and characterization of stroke volume index responses to exercise

Parameter	Patients with exercise tolerance ≥ 4.0 METs (n = 26)	Patients with exercise tolerance < 4.0 METs (n = 13)
Exercise response type:		
A	14 (53.8)	2 (15.4) #
B	2 (7.7)	2 (15.4)
C	7 (26.9)	3 (23.1)
D	1 (3.8)	4 (30.8) #
E	-	-
F	2 (7.7)	2 (15.4)
maxSVI (ml/m²)	59 (51 - 78)	41 (35 - 57) ##
peakSVI (ml/m²)	51 (46 - 69)	39 (32 - 51) ##

maxSVI, maximal stroke volume index achieved during exercise; peakSVI, stroke volume index at peak exercise. # $p < 0.05$ vs patients with exercise tolerance ≥ 4.0 METs; ## $p < 0.01$ vs patients with exercise tolerance ≥ 4.0 METs. Data are presented as number (%) or median (lower quartile-upper quartile).

3.3 Parameters associated with a level of exercise tolerance

All baseline clinical characteristics, resting and peak exercise parameters evaluated, and variables listed in Tables 4 and 5 entered the univariate logistic regression analysis. Univariate logistic regression analysis revealed the potential association ($p < 0.1$) of PCWP, peakSVI, maxSVI, peakEaI (EaI at peak exercise), ΔpeakSVI, ΔmaxSVI, ΔpeakEaI, diabetes mellitus, and exercise responses of types A and D with the exercise tolerance. Of these parameters, only ΔmaxSVI was independently associated with a level of exercise tolerance (odds ratio 0.86, 95% confidence interval 0.77-0.96, $p = 0.005$).

3.4 Intra- and inter-observer variability

Mean variabilities of intra-observer repeated measurements (J.M.) for LVEDV, LVESV, and LVEF were 4%, 10%, and 6% for the resting values and 10%, 13%, and 6%, respectively, for the exercise values. Mean variabilities of inter-observer measurements (J.M. and H.P.) for LVEDV, LVESV, and LVEF were 7%, 10%, and 7% for the resting values and 7%, 12%, and 4%, respectively, for the exercise values.

4. DISCUSSION

Our study brings several important conclusions and has some priorities. To date, it is the largest study of patients with HFNEF evaluating hemodynamic and functional parameters in the course of exercise by simultaneous right heart catheterization, thoracic bioimpedance cardiography, and echocardiography. For the first time it analyzed the course of exercise-induced changes in SVI of individual patients at very short time intervals of 40 s to stratify their reactions and to categorize various types of SVI responses to exercise. We demonstrated the importance of this short-term interval monitoring of SVI to define the type of exertional SVI response and, mainly, to determine the maximal exercise-induced SVI value. We found that 67% of patients accomplished their maximal SVI (maxSVI) before the peak of exercise. Including peakSVI, maxSVI, ΔpeakSVI, ΔmaxSVI and other resting and exercise variables evaluated, only ΔmaxSVI was independently associated with exercise tolerance. Therefore, when assessing exercise-induced SVI changes in HFNEF patients, SVI should be followed during the course of exercise and maximal SVI change from rest should always be determined.

4.1 The course of exercise stroke volume changes

In healthy adults, several types of SV responses to exercise have been described. Probably the most common finding is a low-level exercise SV increase accompanied by no significant SV changes at a high-

level exercise [5,15-17,28,33-37]. The frequent response is also a continuous SV increase during the exercise, with larger SV increments at early loads [33,34,37]. Sometimes, initial exercise SV increase can be followed by a decrease at the end of exercise [7,15,16,34]. Asanoi et al. [16] studied both exertional hemodynamics and also course of SV and other parameter changes during supine exercise in individual healthy subjects. During aerobic exercise, SV substantially increased, largely mediated by an increase in LVEDV (through the Frank-Starling mechanism) and a reduction of Ea (a surrogate for afterload). During anaerobic exercise, a marked increase in Ees (reflecting an increase in myocardial contractility) mediated by enhanced sympathetic activity maintained SV, while LVEDV returned to the control level. Evaluating exercise responses in individual subjects revealed a concordant SV increase during aerobic exercise, followed by a large variability of SV responses at anaerobic exercise, including continuous SV increase, no SV change, or a decrease. Higginbotham et al. [15] analyzed hemodynamics of healthy subjects during upright bicycle exercise and reached similar results. During low levels of exercise, SV increased primarily as a consequence of an increase in LV filling pressure and LVEDV (the Frank-Starling mechanism) with a small contribution from a decreased LVESV. During high levels of exercise, LVEDV did not increase, despite a further increase in filling pressure. On the contrary, LVEDV mildly decreased, probably as a result of reduced filling at high heart rates. SV at high exercise levels was maintained through a progressive decrease in LVESV. With some simplification, one can conclude that SV is increased early during exercise through the Frank-Starling mechanism utilizing an increase in LVEDV and maintained at late exercise by an enhanced contractile state [15-17].

In patients with congestive heart failure and a normal LVEF (i.e. in patients with HFNEF), Kitzman et al. [5] found a flat SVI exercise response without an initial increase. As compared to healthy controls, SVI was reduced by 26% at peak exercise and SVI reduction was the primary factor responsible for reduced cardiac index (CI). Reduced CI was the primary factor responsible for the 48% reduction in peak oxygen consumption accounting for severe exercise intolerance. The main determinant of abnormal SVI response to exercise was a reduction of LVEDVI during submaximal and peak exercise. No significant changes in SVI from rest to peak exercise were also described by Maeder et al. [6] and Borlaugh et al. [4]. However, the course of exercise-induced SVI changes was not evaluated in these two studies. Haykowsky et al. [7] found a similar course of SV and LVEDV exercise changes in HFNEF patients and controls; both groups exhibited an initial SV and LVEDV increase, followed by a mild decrease at peak exercise. Ennezat et al. [13] showed a small SV decrease from rest to peak exercise (-4 ± 9 ml) in HFNEF patients. The majority of them exhibited minimal or no peak exercise change in SV, while some subjects were found to have ≥ 10 ml increase or decrease of peak exercise SV values. In our study of post-heart transplant patients with HFNEF, exercise monitoring of SVI was performed in short-term intervals allowing the detailed analysis of SVI exertional changes and the identification of maxSVI. We found a large variability in SVI exertional responses both at early and late stages of exercise. Patients with less limited exercise tolerance (METs ≥ 4.0) exhibited typically SVI increases in both early and late phase of exercise, while a flat SVI response was most frequently found in patients with severe exercise intolerance (< 4.0 METs).

4.2 The determinants of a low exercise tolerance in patients with HFNEF

Exercise intolerance is one of the basic symptoms in patients with HFNEF and significantly reduces their quality of life. Several mechanisms have been found to be responsible for the reduced exercise tolerance in HFNEF, including greater arterial stiffening [13,38], deterioration of global LV systolic performance during dynamic exercise [8,13], impaired chronotropic [7,9,39] and vasodilator [6,13,39] reserve, impaired LV relaxation [10] and increase in LV stiffness [5], as well as peripheral noncardiac factors accounting for the reduced arterial-venous oxygen content difference [7,35]. Thus, etiology of exercise intolerance in patients with HFNEF is multifactorial. Cardiac-related mechanisms result in an insufficient increase or a decrease in SV during exercise [5,6,13,38] and exercise-induced elevation of LV filling pressure [3,5], accounting completely or partially for exertional fatigue and/or dyspnea, respectively. Both exertional fatigue and dyspnea are characteristic symptoms of early stages of HFNEF. However,

limited data are available concerning the impact of individual mechanisms and parameters on the severity of exercise intolerance in HFNEF patients. Our study focused on this question. In view of a large variability of exertional SVI responses and our important finding that maxSVI measured anytime during exercise exceeded peakSVI in 67% patients, maxSVI and ΔmaxSVI were, in addition to peakSVI and ΔpeakSVI, included into the regression analyses. We hypothetized that maxSVI and ΔmaxSVI may be clinically more important than SVI values obtained at peak exercise and may better reflect SVI exertional reserve and its relationship to exercise tolerance. Actually, we found that of all resting and exercise parameters evaluated, including peakSVI and ΔpeakSVI, only ΔmaxSVI was independently associated with the level of exercise tolerance. This important finding, further supported by the impact of the type of SVI exertional response, underscores the need for continuous or short-term interval monitoring when assessing exertional SVI changes. The short-term interval exertional SVI monitoring can be especially important in patients with an initial exercise-induced increase in SVI followed by an exaggerated drop at peak exercise as demonstrated in Figure 1. In such a case, the evaluation of SVI reserve using ΔpeakSVI can be misleading and may result in incorrect clinical decision making. In view of an independent association of ΔmaxSVI with exercise tolerance described in this study and a previously published finding of a significant relationship of SV reserve to the development of future pulmonary edema [40] we recommend the assessment of maxSVI, in addition to peakSVI, in all HFNEF patients, where information on exercise SVI behavior is clinically helpful.

4.3 Study limitations

We analyzed a selected group of patients after orthotopic heart transplantation, in whom specific post-transplant left atrial and ventricular myocardial structural changes and vasculopathy may have contributed to the etiology of HFNEF. However, due to similar post-transplant characteristics listed in Table 1, the absence of a clinically significant myocardial rejection at the time of the study and minimal presence of a significant vasculopathy (only two patients), the transplanation-related factors are very unlikely to explain differences in exercise tolerance between the groups and to influence the main conclusion of our study, i.e. the need for short-term monitoring of SVI during exercise in order to determine ΔmaxSVI that is independently associated with exercise tolerance. We were unable to obtain a good quality of endocardial delineation during exercise in 36% of patients, who were excluded from LV volumetric quantification. Volumetric echocardiographic data were not acquired at so many time intervals during the exercise as the other functional and hemodynamic measures derived from bioimpedance and catheterization. However, 120 s time intervals still allowed us to follow the course of volumetric exercise-induced changes. Finally, we do not have information on skeletal muscle oxidative metabolism, the impairment of which was shown to significantly contribute to exercise intolerance in patients with HFNEF [7,35].

5. CONCLUSION

In post-heart transplant patients with HFNEF, there is a large variability in SVI response to exercise. In the majority of patients (67%), maximal SVI measured at anytime during exercise (maxSVI) exceeded SVI at peak exercise (peakSVI) and could only be determined by short-term interval monitoring of exercise-induced SVI changes. Of the commonly used functional and hemodynamic parameters, including peakSVI and ΔpeakSVI (peakSVI minus SVI at rest with leg elevation), only ΔmaxSVI (maxSVI minus SVI at rest with leg elevation) was independently associated with exercise tolerance. Therefore, when assessing exercise-induced SVI changes in HFNEF patients, SVI should be followed during the course of exercise and maximal SVI change from rest should always be determined.

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7. REFERENCES

1. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence

and outcome of heart failure with preserved ejection fraction. N Engl J Med 2006;355:251-9.
2. Bhatia RS, Tu JV, Lee DS, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006;355:260-9.
3. Borlaug BA, Nishimura RA, Sorajja P, Lam CSP, Redfield MM. Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. Circ Heart Fail 2010;3:588-95.
4. Borlaug BA, Jaber WA, Ommen SR, Lam CSP, Redfield MM, Nishimura RA. Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. Heart 2011;97:964-9.
5. Kitzman DW, Higginbotham MB, Cobb FR, Sheikh KH, Sullivan MJ. Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. J Am Coll Cardiol 1991;17:1065-72.
6. Maeder MT, Thompson BR, Brunner-La Rocca HP, Kaye DM. Hemodynamic basis of exercise limitation in patients with heart failure and normal ejection fraction. J Am Coll Cardiol 2010;56:855-63.
7. Haykowsky MJ, Brubaker PH, John JJ, Stewart KP, Morgan TM, Kitzman DW. Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction. J Am Coll Cardiol 2011;58:265-74.
8. Tan YT, Wenzelburger F, Lee E, et al. The pathophysiology of heart failure with normal ejection fraction. Exercise echocardiography reveals complex abnormalities of both systolic and diastolic ventricular function involving torsion, untwist, and longitudinal motion. J Am Coll Cardiol 2009;54:36-46.
9. Phan TT, Shivu GN, Abozguia K, et al. Impaired heart rate recovery and chronotropic incompetence in patients with heart failure with preserved ejection fraction. Circ Heart Fail 2010;3:29-34.
10. Donal E, Thebault Ch, Lund LH, et al. Heart failure with a preserved ejection fraction additive value of an exercise stress echocardiography. Eur Heart J Cardiovasc Imaging 2012;13:656-65.
11. Meluzín J, Sitar J, Křístek J, et al. The role of exercise echocardiography in the diagnostics of heart failure with normal left ventricular ejection fraction. Eur J Echocardiogr 2011;12:591-602.
12. Wenzelburger FWG, Tan YT, Choudhary FJ, Lee ESP, Leyva F, and Sanderson JE. Mitral annular plane systolic excursion on exercise: a simple diagnostic tool for heart failure with preserved ejection fraction. Eur J Heart Fail 2011;13:953-60.
13. Ennezat PV, Lefetz Y, Maréchaux S, et al. Left ventricular abnormal response during dynamic exercise in patients with heart failure and preserved left ventricular ejection fraction at rest. J Cardiac Fail 2008;14:475-80.
14. Ekelund LG, and Holmgren A. Central hemodynamics during exercise. Supplement I to Circulation Research 1967;20 and 21:I-33-I-43.
15. Higginbotham MB, Morris KG, Williams RS, McHale PA, Coleman RE, and Cobb FR. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. Circ Res 1986;58:281-91.
16. Asanoi H, Kameyama T, Ishizaka S, Miyagi K, and Sasayama S. Ventriculoarterial coupling during exercise in normal human subjects. Int J Cardiol 1992;36:177-86.
17. Plotnick GD, Becker LC, Fisher ML, et al. Use of the Frank-Starling mechanism during submaximal versus maximal upright exercise. Am J Physiol 1986;251:H1101-H1105.
18. Swain DP, Leutholtz BC. Exercise prescription. A case Study Approach to the ACSM Guidelines. 2nd edition, Human Kinetics, 2007, 208p.
19. Guelen I, Westerhof BE, Van der Sar GL, et al. Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. Blood Press Monit 2003;8:27-30.
20. Parati G, Ongaro G, Bilo G, et al. Non-invasive beat-to-beat blood pressure monitoring: new developments. Blood Press Monit 2003;8:31-36.
21. Zhang R, Claassen JAHR, Shibata S, et al. Arterial-cardiac baroreflex function: insight from repeated squat-stand maneuvers. Am J Physiol Regul Integr Comp Physiol 2009;297:R116-R123.
22. Kubicek WG, Karnegis JN, Patterson RP, Witsoe DA, Mattson RH. Development and evaluation of an impedance cardiac output system. Aerospd Med 1966;37:1208-12.

23. Sherwood A, Allen MT, Fahrenberg J, Kelsey RM, Lovallo WR, and Doornen LJP. Methodological guidelines for impedance cardiography. Psychophysiology 1990;27:1-23.
24. Bernstein DP, Lemmens HJM. Stroke volume equation for impedance cardiography. Medical & Biological Engineering & Computing 2005;43:443-50.
25. Bernstein DP. Impedance cardiography: Pulsatile bloodflow and the biophysical and electrodynamic basis for the stroke volume equations. J Electr Bioimp 2010;1:2-17.
26. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification. Eur J Echocardiogr 2006;7:79-108.
27. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450-8.
28. Chantler PD, Melenovsky V, Schulman SP, et al. The sex-specific impact of systolic hypertension and blood pressure on arterial-ventricular coupling at rest and during exercise. Am J Physiol Heart Circ Physiol 2008;295:H145-H153.
29. Najjar SS, Schulman SP, Gerstenblith G, et al. Age and gender affect ventricular-vascular coupling during aerobic exercise. J Am Coll Cardiol 2004;44:611-7.
30. Borlaug B and Kass DA. Ventricular-vascular interaction in heart failure. Heart Fail Clin 2008;4:23-36.
31. Paulus WJ, Tschope C, Sanderson JE, et al. How to diagnose diastolic heart failure: a 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. Eur Heart J 2007;28:2539-50.
32. Rodriguez ER. The pathology of heart transplant biopsy specimens: revisiting the 1990 ISHLT Working Formulation. J Heart Lung Transplant 2003;22:3-15.
33. Rodeheffer RJ, Gerstenblith G, Becker LC, Fleg JL, Weisfeldt ML, and Lakatta EG. Exercise cardiac output is maintained with advancing age in healthy human subjects: cardiac dilatation and increased stroke volume compensate for a diminished heart rate. Circulation 1984;69:203-13.
34. Granath A, Jonsson B, Strandell T. Circulation in healthy old men, studied by right heart catheterization at rest and during exercise in supine and sitting position. Acta Med Scand 1964;176:425-45.
35. Bhella PS, Prasad A, Heinicke K, et al. Abnormal heamodynamic response to exercise in heart failure with preserved ejection fraction. Eur J Heart Fail 2011;13:1296-1304.
36. Poliner LR, Dehmer GJ, Lewis SE, Parkey RW, Blomqvist CG, and Willerson JT. Left ventricular performance in normal subjects: a comparison of the responses to exercise in the upright and supine positions. Circulation 1980;62:528-34.
37. Julius S, Amery A, Whitlock LS, and Conway J. Influence of age on the hemodynamic response to exercise. Circulation 1967;36:222-30.
38. Tartiere-Kesri L, Tartiere JM, Logeart D, Beauvais F, Solal AC. Increased proximal arterial stiffness and cardiac response with moderate exercise in patients with heart failure and preserved ejection fraction. J Am Coll Cardiol 2012;59:455-61.
39. Borlaug BA, Melenovsky V, Russell SD, et al. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. Circulation 2006;114:2138-47.
40. Charoenpanichkit Ch, Little WC, Mandapaka S, et al. Impaired left ventricular stroke volume reserve during clinical dobutamine stress predicts future episodes of pulmonary edema. J Am Coll Cardiol 2011;57:839-48.