# Sex- and Method-Specific Reference Values for Right Ventricular Strain by 2-Dimensional Speckle-Tracking Echocardiography

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***Background*****—**Despite the fact that assessment of right ventricular longitudinal strain (RVLS) carries important implications for patient diagnosis, prognosis, and treatment, its implementation in clinical settings has been hampered by the limited reference values and the lack of uniformity in software, method, and definition used for measuring RVLS. Accordingly, this study was designed to establish (1) the reference values for RVLS by 2-dimensional speckle-tracking echocardiography; and (2) their relationship with demographic, hemodynamic, and cardiac factors.

***Methods and Results*****—**In 276 healthy volunteers (55% women; age, 18–76 years), free wall and septum RVLS (6 segments) and free wall RVLS (3 segments) using both 6- and 3-segment regions of interest were obtained. Feasibility of 6-segment RVLS was 92%. Free wall RVLS from 3- versus 6-segment regions of interest had similar values, yet 6-segment region of interest was more feasible (86% versus 73%; $P<0.001$) and reproducible. Reference values (lower limits of normality) were as follows: 6-segment RVLS, −24.7±2.6% (−20.0%) for men and −26.7±3.1% (−20.3%) for women; 3-segment RVLS, −29.3±3.4% (−22.5%) for men and −31.6±4.0% (−23.3%) for women ($P<0.001$). Free wall RVLS was 5±2 strain units (%) larger in magnitude than 6-segment RVLS, 10±4% larger than septal RVLS, and 2±4% larger in women than in men ($P<0.001$). At multivariable analysis, age, sex, pulmonary systolic pressure, right atrial minimal volume, as well as right atrial and left ventricular longitudinal strain resulted as correlates of RVLS values.

***Conclusions*****—**This is the largest study providing sex- and method-specific reference values for RVLS. Our data may foster the implementation of 2-dimensional speckle-tracking echocardiography–derived RV analysis in clinical practice.
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**Key Words:** echocardiography ■ heart ventricle ■ normal values ■ reference values ■ right ventricle ■ sprains and strain ■ ventricular function, right



The assessment of right ventricular (RV) systolic function carries important implications for patient diagnosis, prognosis, and treatment in a variety of clinical settings. The quantification of RV function should be part of any routine echocardiographic workup, yet the complex geometry of the RV makes this task difficult in practice. Conventional echocardiographic indices of RV function are also limited by their load and angle dependency.[1] Two-dimensional speckle-tracking echocardiography (2DSTE) enables the quantification of the RV myocardial longitudinal strain (RVLS), which is less angle and load dependent than traditional RV function indices and less confounded by RV geometry and passive motion.[2] The analysis of RVLS offers important diagnostic and prognostic benefits in pulmonary hypertension,[3,4] arrhythmogenic cardiomyopathy,[5] congenital heart diseases,[6] and in candidates for left ventricular (LV) assist device implantation.[7] In late-stage heart failure, RVLS impairment correlates with the extent of myocardial fibrosis and functional capacity.[8] As a consequence, the latest guidelines on cardiac chamber quantitation by echocardiography have recommended RVLS for clinical use, as a sensitive and reproducible index of RV performance.[2] However, its implementation in daily clinical routine has been hampered by the limited reference values and the lack of uniformity on the software, method, and definition used for measuring and reporting RVLS in various studies.[9–12] This lack of standardization hampers the applicability of RVLS for ruling out RV dysfunction and the interpretation of serial changes in laboratories using different methods of measuring RVLS.

**See Clinical Perspective**

Accordingly, in this prospective study, we aimed to (1) assess the presence and extent of measurement variability in relation to the various methods and definitions of RVLS,

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to identify unifying standard(s) for its measurement (pilot study); (2) identify the reference ranges of global, regional, and segmental RVLS from a large population-based sample of healthy volunteers with a wide age range; (3) analyze the relationship of RVLS with demographic, hemodynamic, and cardiac factors.

## Methods

The present work is a substudy of a large research project (Padua 3D Echo Normal) designed to develop reference ranges for 2-dimensional (2D) and 3D echocardiography (3DE) for cardiac chamber quantification. Data from this project have been included in previous single- or multicenter publications and guidelines.[2,13–15] Subjects were prospectively recruited from October 2011 to July 2013 among hospital employees, fellows in training, their relatives, and people screened for driving or working licenses. Criteria of recruitment included age ≥18 years, no history or symptoms of cardiovascular or lung disease, normal physical examination, and ECG. Exclusion criteria were as follows: smoking, systolic blood pressure >140 mm Hg, diastolic blood pressure >80 mm Hg, history of drug-treated hypertension, diagnosis of diabetes mellitus, impaired fasting glucose >100 mg/dL, body mass index >30 kg/m$^2$, creatinine >1.3 mg/dL, estimated glomerular filtration rate < 60 mL/min per 1.73 m$^2$, history of dyslipidemia (total cholesterol >240 mg/dL, low-density lipoprotein cholesterol >130 mg/dL, and total triglycerides >150 mg/dL),[2] poor apical acoustic window, unknown silent pathology detected by echocardiography (wall motion abnormalities; valvular stenoses of any degree; more than mild valvular regurgitation by multiparametric quantitative assessment[16]), professional sport activity, pregnancy, frequent extrasystoles precluding echocardiographic protocol acquisitions. During enrollment, we aimed to include at least 20 men and 20 women per decade to achieve a fairly uniform age and sex distribution (especially for the group >60 years), yet the fulfillment of this condition for 1 decade did not represent an exclusion criterion. From the 338 subjects screened for eligibility, 62 (18%) were excluded because of the presence of at least one of our exclusion criteria. The sample size of the study was determined according to Altman,[17] who set 200 subjects as the minimum number to enroll in a study aiming to assess reference values for biological variables. Written informed consent was obtained from all volunteers, and the study was approved by the local Ethics Committee (protocol 2380P approved on 06/10/2011).

### Echocardiographic Acquisition

A complete standard M-mode, 2D, and Doppler examination were performed to rule out any pathology, and additional 2DE and 3DE acquisitions were obtained according to the study protocol. All echocardiographic studies were performed by 3 experienced researchers (D.M., L.P.B., and D.P.) using a Vivid E9 scanner (GE Vingmed, Horten, Norway) equipped with M5S and 4 V probes. The 2DE acquisition protocol included dedicated apical 4-chamber RV-focused views, on which all conventional and RVLS parameters were measured,[2] dedicated right atrial (RA) apical 4-chamber view for RA longitudinal strain (RALS), and 4-, 2-chamber view and long-axis for global LV longitudinal strain (LVLS) analysis. Image depth and sector size were adjusted to ensure an adequate temporal resolution (50–80 fps) for 2DSTE-derived strain quantification.[18] Three consecutive cardiac cycles were recorded. RV size was estimated by measuring RV midcavity diameter and areas. Fractional area change and tricuspid annular systolic excursion were also measured.[2] For 3DE analysis, 4- and 6-beat full-volume RV, LV, and RA data sets were

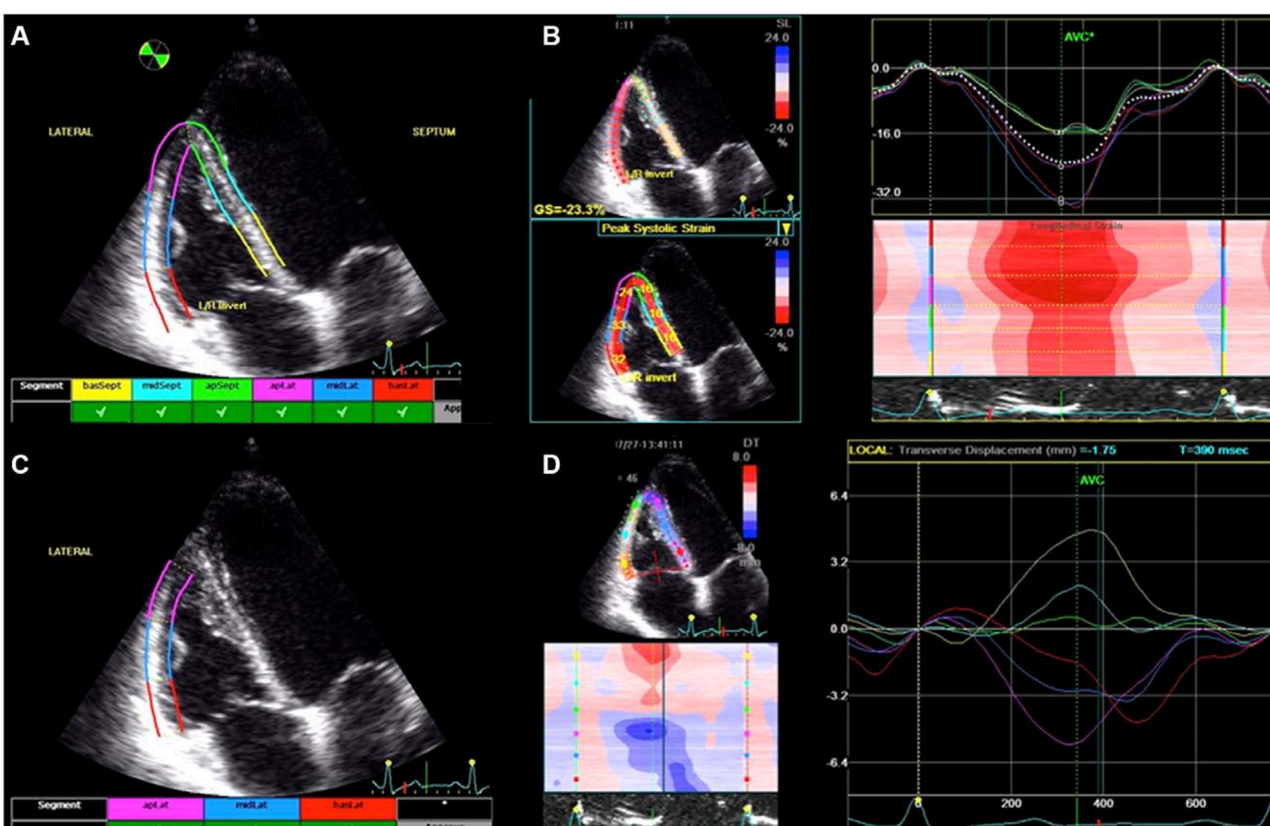


**Figure 1.** Example of 2-dimensional speckle-tracking analysis of the right ventricle (RV) from a dedicated apical focused view. **A**, The successful tracking of a 6-segment region of interest (ROI) from which segmental and global average strain values and time curves are obtained (**B**). The operator may either approve the tracing or manually exclude the septal segments by clicking on the respective green flags. **C**, The 3-segment ROI option. **D**, The analysis of RV transversal displacement.

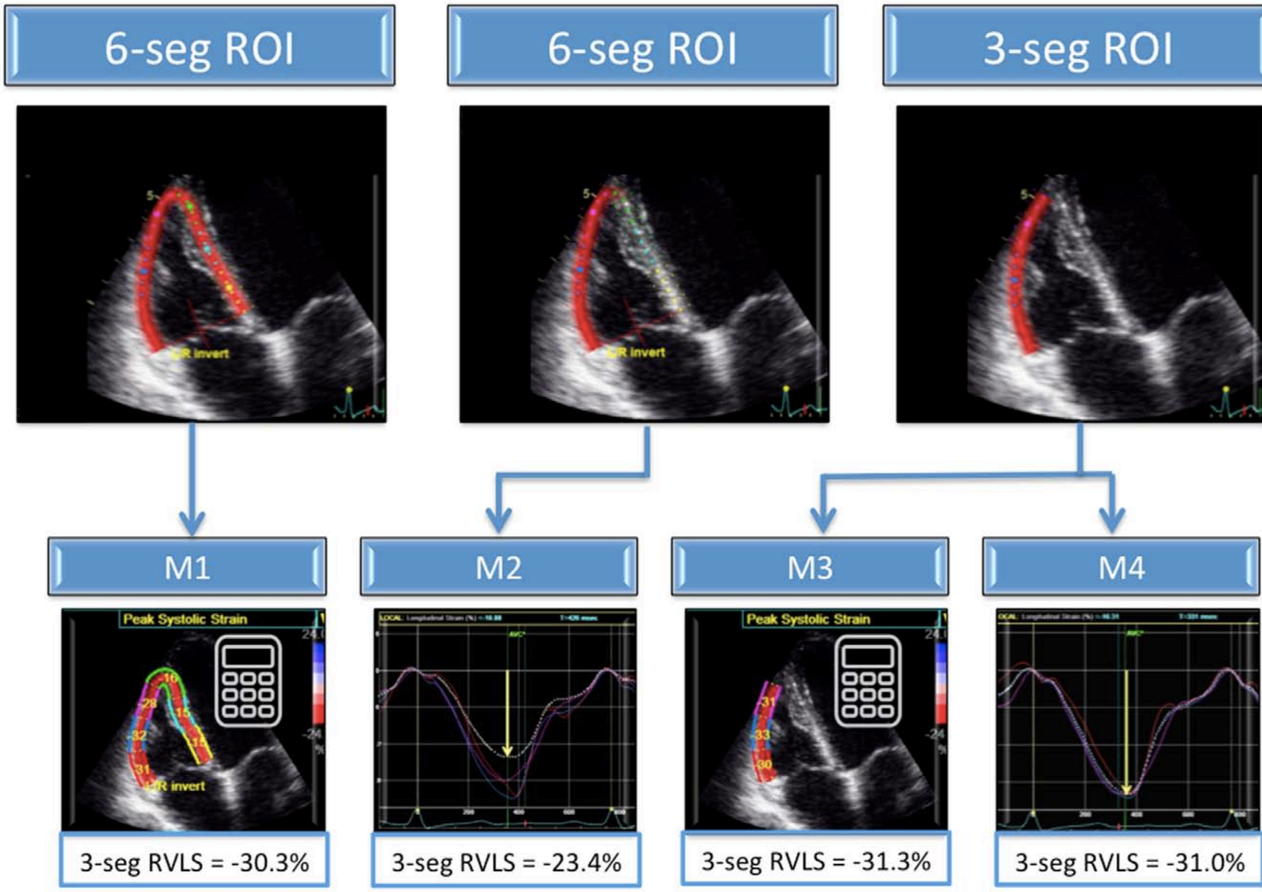


**Figure 2.** Four different methods (M1–M4) used in the pilot study for the computation of free wall right ventricular (RV) longitudinal strain using either a 6- or a 3-segment region of interest (ROI) and the corresponding measurements.



acquired during breath-hold from the apical approach, taking care to avoid any artifacts and include the entire structure in the acquisition.[14]

## Echocardiographic Analysis

Systolic pulmonary arterial pressure was estimated based on the tricuspid regurgitation peak gradient, to which the mean RA pressure (estimated from inferior vena cava size and respiratory excursion) was added. RV acceleration time was also recorded from the pulsed Doppler recording of pulmonary flow. RV and RA volumes, and RV ejection fraction (RVEF) were assessed offline using 4D RV Function 1.2 and LA Analysis software packages (TomTec Imaging Systems, Unterschleissheim, Germany) applied on dedicated 3D data sets. RA strain and LV analysis were done using EchoPac BT13 (GE Vingmed), according to a previously reported methodology.[15,19] RV, LV, and RA volumes were indexed for body surface area according to DuBois and DuBois formula.[20]

## RV Myocardial Deformation Parameters

Assessment of RV myocardial deformation parameters was performed by 2DSTE on the RV-focused apical 4-chamber view[2] using Q-Analysis package (EchoPAC BT13; GE Vingmed). Analysis was performed blinded to subject's demographic data. Briefly, after manual tracing, the end-systolic RV endocardial border, a region of interest (ROI) was automatically generated; its width and position were manually adjusted to include the entire myocardial wall and to exclude the pericardium (Figure 1). Pulmonary valve closure was identified on the pulse-wave Doppler tracing of the RV outflow tract. The software automatically divides the RV free wall and the interventricular septum in 3 segments each (basal, mid, and apical), resulting in a 6-segment model. The quality of the tracking was automatically validated by software and confirmed visually from the 2D images. Subjects in which >2 segments per ventricle showed persistent inadequate tracking despite attempts to readjust the ROI position and width were excluded from analysis.

### *Pilot Study*

In a sample of 20 random subjects, we compared the following measurements obtained by 5 different methods, used in previous studies:

1. Six-segment RVLS is the systolic peak of the average RVLS curve obtained from a 6-segment ROI (Figure 1A and 1B).
2. Free wall 3-segment RVLS (M1) is the arithmetic mean of the strain values in the 3 segments of RV free wall obtained from a 6-segment ROI (Figure 2).
3. Free wall 3-segment RVLS (M2) is the systolic peak of the average RVLS curve free wall obtained from a 6-segment ROI, after manually excluding the septal segments (Figure 2);
4. Free wall 3-segment RVLS (M3) is the arithmetic mean of the strain values in the 3 segments of RV free wall obtained from a 3-segment ROI (Figures 1C and 2).
5. Free wall 3-segment RVLS (M4) is the systolic peak of the average RVLS curve free wall obtained from a 3-segment ROI (Figure 2).

Based on the findings from the pilot study, we found that 6-segment RVLS and 3-segment RVLS (M1 and M4) were used for measuring the RVLS in the entire population. An inadequate tracking of any of the 3 segments of the RV free wall led to the exclusion of the respective subject from the analysis of free wall 3-segment RVLS.[21]

**Table 1. Demographic and Echocardiographic Characteristics of the Enrolled Population**

| | Overall (n=276) | Men (n=123) | Women (n=153) | *P* Value* |
|---|---|---|---|---|
| Age, y | 44 (32; 56) | 43 (32; 55) | 44 (32; 57) | 0.916 |
| Height, cm | 170 (164; 177) | 178 (173;182) | 165 (160; 170) | <0.001 |
| Weight, kg | 68 (59; 75) | 76 (72; 82) | 60 (55; 67) | <0.001 |
| Body surface area, m² | 1.78±0.18 | 1.93±0.13 | 1.66±0.12 | <0.0001 |
| Body mass index, kg/m² | 22.9 (21.2; 24.9) | 24.1 (22.3;25.7) | 22.1 (20.2; 24.2) | <0.001 |
| Systolic blood pressure, mm Hg | 120 (110; 130) | 130 (120; 140) | 115 (110; 125) | <0.001 |
| Diastolic blood pressure, mm Hg | 70 (70; 80) | 80 (70; 80) | 70 (70; 80) | <0.001 |
| Heart rate, bpm | 65 (60; 73) | 65 (60; 72) | 66 (60; 75) | 0.672 |
| Pulmonary artery systolic blood pressure, mm Hg | 21 (18; 26) | 21 (17; 26) | 23 (18; 26) | 0.183 |
| RV acceleration time, ms | 149 (134; 161) | 152 (134; 161) | 148 (134; 161) | 0.537 |
| RV mid diameter, mm | 28 (25; 32) | 30 (25; 34) | 27 (25; 31) | 0.002 |
| RV end-diastolic area, cm² | 17.6 (15.4; 21.4) | 21.5 (19.6; 23.7) | 16.1 (14.3; 18.0) | <0.001 |
| RV end-systolic area, cm² | 8.6 (7.5; 11.3) | 11.2 (9.8; 12.6) | 7.9 (6.9; 8.8) | <0.001 |
| Indexed RV mid diameter, mm/m² | 16.1±2.8 | 15.3±2.9 | 16.6±2.7 | 0.003 |
| RV end-diastolic volume, mL | 95 (81; 117) | 115 (99; 133) | 85 (75; 95) | <0.001 |
| Indexed RV end-diastolic volume, mL/m² | 54 (47; 62) | 61 (53; 67) | 51 (46; 59) | <0.001 |
| RV end-systolic volume, mL | 39 (33; 48) | 47 (41; 57) | 35 (29; 40) | <0.001 |
| Indexed RV end-systolic volume, mL/m² | 22 (19; 27) | 25 (21; 29) | 21 (17; 24) | <0.001 |
| RV ejection fraction, % | 59±6 | 58±6 | 60±6 | 0.008 |
| TAPSE, mm | 25 (23; 27) | 24 (23; 27) | 25 (24; 27) | 0.950 |
| RV fractional area change, % | 49±6 | 48±7 | 51±6 | 0.002 |
| RA area, cm² | 13.4 (11.7; 15.2) | 14.6 (12.9; 16.3) | 13.4 (11.8; 15.4) | <0.001 |
| RA volume, mL | 50 (38; 60) | 55 (48; 64) | 44 (35; 52) | <0.001 |
| Indexed RA volume, mL/m² | 28±7 | 29±7 | 27±8 | 0.1 |

RA indicates right atrium; RV, right ventricle; and TAPSE, tricuspid annular systolic excursion.
*Men vs women.



## Statistical Analysis

Normal distribution of variables was assessed by Kolmogorov–Smirnov test. Accordingly, continuous variables are summarized as mean±SD, if normally distributed, and as median (I and III quartiles) otherwise; categorical variables are reported as percentages. In all analyses, RV strain parameters were considered negative (lower strain values indicating better deformation). Lower limits of normality referring to RV strain magnitude were identified as the 97.5th percentile. Differences between values in men and women were assessed using unpaired *t* tests for normally distributed variables and Mann–Whitney *U* tests otherwise. Differences between reference values obtained using various methods of strain calculation were tested using Bland–Altman analysis. Comparison of RV strain values among various segments was done using repeated-measures ANOVA with Tukey multiple comparison test. Spearman rank correlation was used to analyze the relationships of RV strain parameters with demographic variables, RV parameter, and LV parameter. Multivariable stepwise logistic regression was performed to identify the correlates of 6- and 3-segment RVLS. The multivariate model included the following covariates: pulmonary arterial systolic pressure (PASP), RA and LV volumes, RAEF, RALS, LVEF, LVLS (having $P<0.10$ on bivariate analysis) plus age and sex with model selection using the backward likelihood ratio. Intra- and interobserver reproducibility of the different methods used to measure RVLS were assessed in 20 random subjects by a second investigator, as well as by the same primary investigator 2 months after the first analysis. Reproducibility was reported as bias±SD using Bland–Altman analysis, and the SDs of the different methods to compute RVLS were compared using the variance ratio test (*F*-test). All analyses were performed using SPSS 21.0 (SPSS Inc, Chicago, IL) and MedCalc software (Mariakerke, Belgium). $P<0.05$ were considered significant.

# Results

A total of 276 subjects fulfilled the enrollment criteria (Table 1). The age of subjects ranged from 18 to 76 years and >45 subjects per each age decade were included. Women were slightly more prevalent (55%). Men had larger body sizes and

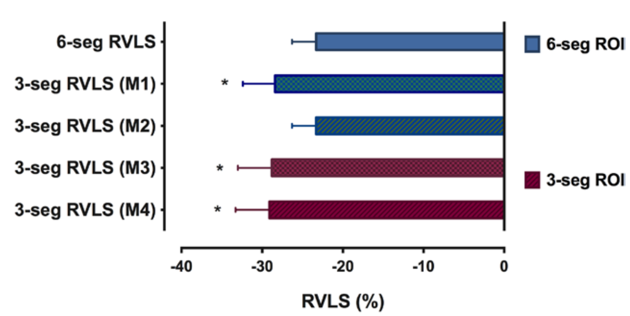


**Figure 3.** Comparison of the results obtained by different methods of calculating right ventricular longitudinal strain (RVLS) in the pilot study. Blue bars correspond to a 6-segment region of interest (ROI), and red bars to a 3-segment ROI. *$P<0.001$ for comparison with 6-segment RVLS.

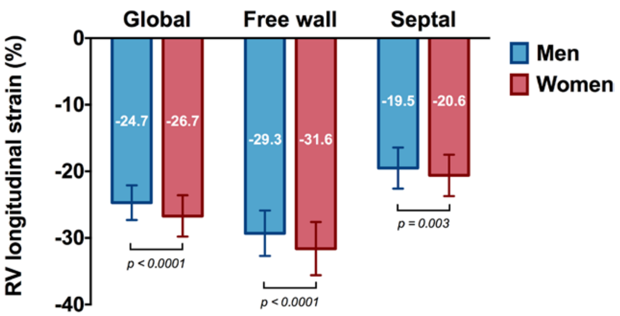


**Figure 4.** Bar graph showing the different magnitudes of right ventricular (RV) longitudinal strain parameters between men and women.

higher blood pressure. Also, men had larger RV size, lower fractional area change and RVEF, and similar tricuspid annular systolic excursion and systolic pulmonary arterial pressure in comparison with women. The frame rate of the 2D data sets was 74±17 frames/s.

## Pilot Study

The pilot study showed that the magnitude of 6-segment RVLS was significantly lower than that of free wall 3-segment RVLS by M1, M3, and M4 ($P$<0.0001); surprisingly, 6-segment RVLS was identical with 3-segment RVLS by M2 method (ie, manual exclusion of the septal segments from the 6-segment ROI by the user), counter to what one might expect from the strain curves aspect and from the septal ROI becoming transparent (Figure 2). Accordingly, as we have suspected this is because of an issue in the software display, we excluded M2 method from the study analysis protocol. Conversely, all the other approaches (M1, M3, and M4; Figure 3) to obtain free wall 3-segment RVLS average yielded similar values, irrespective of the use of a 3-segment or a 6-segment ROI or of the strain averaging method (M3: manually averaging of free wall segments by the operator from nonsimultaneous segmental values versus M4: measured as the systolic peak of the average strain curve; Figure 2). Because in healthy subjects the segmental peaks are almost simultaneous,[22] our pilot study was useful to confirm the assumption that M3 and M4 provide practically similar measurements. As both M1 and M4 were used interchangeably in previous articles, we aimed to compare them in the entire population not only in terms of reference values but also as feasibility and reproducibility and consequently to identify which one is more suitable for the routine clinical use. Accordingly, in the entire cohort, we have used 6-segment RVLS and 3-segment RVLS by M1 and M4.

## Reference Values of RV Strain

The overall feasibility of 2DSTE analysis of 6-segment RVLS in the entire population was 92% (n=250). Free wall 3-segment RVLS average showed larger magnitudes than 6-segment RVLS average, the latter including both free wall and septal segments (Figure 4). Men had lower magnitudes of RVLS than women, regardless of whether the 6- or 3-segment RVLS average was considered. Accordingly, sex- and method-specific reference values for RVLS parameters by 2DSTE are provided in Table 2. Overall and sex-specific limits of normality were −20% (−20.0% for men and −20.3% for women) for 6-segment RVLS and −23% (−22.5% for men and −23.3% for women) for free wall 3-segment RVLS.

According to the results of the pilot study, there was no significant difference between the M1 and M4 method for computing free wall 3-segment RVLS average in the entire cohort: −28.4±4% versus –29.1±4.2%, $P$=0.217. Yet, M1 approach enabled to analyze all 3 segments of the RV free wall in a significantly larger number of subjects in comparison with M4 (feasibility 86% versus 73%; $P$<0.0001).

## Regional Differences of RV Strain Values

Significant segmental differences in RVLS values were found for both the free wall (repeated-measures 1-way ANOVA, $F$=65; $P$<0.0001) and the septum ($F$=4.8; $P$=0.02; Table 2). The mid-segments showed larger LS magnitudes than their respective apical and basal segments. At the free wall level, basal segments had also larger strain magnitude than apical segments, whereas at the septal level, the basal and apical segments had similar strain values ($P$=0.91).

**Table 2. Reference Values of RVLS in the Overall Study Population and Separated by Sex**

| Strain Parameters | Overall | LLN | Men | LLN | Women | LLN | *P* Value* |
|---|---|---|---|---|---|---|---|
| 6-Segment RVLS, % | | | | | | | |
| Average | −25.8±3.0 | −20.2 | −24.7±2.6 | −20.0 | −26.7±3.1 | −20.3 | <0.0001 |
| Free wall 3-segment RVLS, % | | | | | | | |
| Average (M1) | −30.5±3.9 | −23.3 | −29.3±3.4 | −22.5 | −31.6±4.0 | −23.3 | <0.0001 |
| Basal | −30.0 (−26.0; −35.0)† | −20.0 | −28.0 (−25.0; −33.0)† | −18.0 | −31.0 (−27.0; −37.0)† | −21.0 | 0.002 |
| Mid | −34.0 (−30.0; −37.5) | −22.3 | −32.0 (−29.0; −35.0) | −20.9 | −35.0 (−32.0; −40.0) | −26.9 | 0.001 |
| Apical | −29.0 (−26.0; −32.0)† | −14.9 | −27.0 (−25.0; −30.0)† | −14.8 | −30.0 (−27.0; −33.0)† | −16.0 | <0.001 |
| Septal 3-segment RVLS, % | | | | | | | |
| Average | −20.1±3.2 | −14.0 | −19.5±3.1 | −13.7 | −20.6±3.1 | −13.9 | 0.003 |
| Basal | −20.0 (−18.0; −22.0)† | −13.0 | −20.0 (−18.0; −22.0) | −15.0 | −20.0 (−18.0; −23.0) | −13.0 | 0.002 |
| Mid | −20.0 (−19.0; −22.0) | −15.0 | −19.0 (−17.0; −22.0) | −14.0 | −21.0 (−19.0; −23.0) | −14.6 | 0.001 |
| Apical | −20.0 (−17.00; −23.0) | −11.0 | −19.0 (−15.0; −23.0) | −11.0 | −21.0 (−18.0; −24.0) | −13.0 | 0.017 |

LLN indicates lower limit of normality (97.5th percentile) referring to strain magnitude; and RVLS, right ventricular longitudinal strain.
*Men vs women.
†$P$<0.05 for mid vs basal or mid vs apex.

**Table 3. Relationship of 6-Segment RVLS and 3-Segment RVLS With Demographic and Cardiac Factors in Healthy Volunteers***

| | 6-Segment RVLS, % | | 3-Segment RVLS, % | |
|---|---|---|---|---|
| | ρ | *P* Value | ρ | *P* Value |
| Age, y | −0.07 | 0.30 | −0.03 | 0.96 |
| Weight, kg | −0.35 | <0.0001 | −0.36 | <0.0001 |
| Height, m | −0.23 | <0.0001 | −0.29 | <0.0001 |
| Body mass index, kg/m² | −0.27 | <0.0001 | −0.24 | <0.0001 |
| Body surface area, m² | −0.33 | <0.0001 | −0.35 | <0.0001 |
| Heart rate, bpm | 0.11 | 0.08 | 0.06 | 0.342 |
| Systolic blood pressure, mm Hg | −0.05 | 0.44 | −0.08 | 0.23 |
| Diastolic blood pressure, mm Hg | −0.05 | 0.48 | −0.1 | 0.145 |
| Pulmonary arterial systolic pressure, mm Hg | −0.15 | 0.063 | −0.13 | 0.144 |
| RV acceleration time, ms | −0.06 | 0.50 | −0.09 | 0.30 |
| RV fractional area change, % | 0.14 | 0.08 | 0.12 | 0.12 |
| TAPSE, mm | 0.03 | 0.70 | 0.09 | 0.27 |
| RV end-diastolic volume, mL | −0.23 | 0.001 | −0.22 | 0.002 |
| RV end-systolic volume, mL | −0.33 | <0.0001 | −0.32 | <0.0001 |
| RV ejection fraction, % | 0.27 | <0.0001 | 0.28 | <0.0001 |
| RA maximal volume, mL | −0.15 | 0.04 | −0.15 | 0.058 |
| RA minimal volume, mL | −0.31 | <0.0001 | −0.23 | 0.002 |
| RA total emptying fraction, mL | 0.28 | <0.0001 | 0.17 | 0.027 |
| RA longitudinal strain, % | 0.41 | <0.0001 | 0.30 | <0.0001 |
| LV end-diastolic volume, mL | −0.24 | 0.001 | −0.25 | <0.0001 |
| LV end-systolic volume, mL | −0.29 | <0.0001 | −0.28 | <0.0001 |
| LV ejection fraction, % | 0.25 | <0.0001 | 0.20 | 0.005 |
| LV mass, g | −0.28 | <0.0001 | −0.30 | <0.0001 |
| LV global longitudinal strain, % | 0.49 | <0.0001 | 0.36 | <0.0001 |

LV indicates left ventricular; RA, right atrial; RVLS, right ventricular longitudinal strain; and TAPSE, tricuspid annular systolic excursion.

*Strain values were considered as absolute values.

## Determinants of RV Strain Parameters

On bivariate analysis, several demographic and cardiac parameters showed weak, yet significant correlations with RVLS (Table 3). Multivariable linear regression analysis identified age, PASP, RA minimal volume, RALS, and LVLS as correlates of 6-segment RVLS of these factors accounting for 40% of the variability of 6-segment RVLS (Table 4). Sex and LVLS were selected as correlates of 3-segment RVLS, accounting for 40% of its variability.

## Reproducibility

RVLS parameters showed a low intra- and interobserver variability (Table 5). The reproducibilities of 6-segment RVLS and of 3-segment RVLS by M1 method were similar. Free wall 3-segment RVLS was more reproducible when assessed by M1 than by M4 method.

# Discussion

This is the largest prospective study to derive sex- and method-specific reference values for RVLS measured by 2DSTE from a population-based cohort of healthy subjects with a wide age range. The main results of our study can be summarized as follows: (1) free wall 3-segment RVLS was 2±4 strain units (%) larger in magnitude in women than in men, 5±2% larger than 6-segment RVLS, and 10±4% larger than septal RVLS ($P<0.001$); therefore, reference values were stratified according to sex and method of RVLS calculation; (2) values obtained from directly measuring free wall RVLS using a 3-segment ROI, or from manually averaging free wall segments using a 6-segment ROI are interchangeable, but the latter is more feasible and reproducible; (3) the manual exclusion of septal segments from the 6-segment ROI is not recommended for obtaining free wall 3-segment RVLS average; and (4) age, sex, and PASP, as well as RA minimal volume, RALS, and LVLS, are correlates of RVLS measurements in healthy subjects.

## Reference Values for RV Strain

Few studies including smaller cohorts of healthy subjects reported on the reference values of RVLS parameters. Our reference values are close to the data reported by Meris et al[22] from 100 subjects (free wall RVLS, −28.7%). In 142 multiethnic normal subjects, Chia et al[21] found a free wall RVLS value of −27.3%. In both studies, a different scanner (Vivid 7) and a presumably earlier EchoPac software version (not specified) were used, which may have accounted for minor interstudy differences in reference values, as observed from the studies reporting the reference values of LV 2DSTE strain.[15,23] Recently, Fine et al[9] published a meta-analysis of 10 studies including 486 subjects, in which reference values for free wall RVLS by 2DSTE were also lower in magnitude (−27±2%) than in our study. In this meta-analysis, of 10 studies, 7 were retrospective, 6 were

**Table 4. Correlates of 6-Segment RVLS and 3-Segment RVLS Identified by Multivariable Linear Regression Analysis**

| | 6-Segment RVLS, % | | 3-Segment RVLS, % | |
|---|---|---|---|---|
| | $R^2$ | *P* Value | $R^2$ | *P* Value |
| Model influence | 0.404 | <0.001 | 0.396 | <0.001 |
| Variables | β* | | β* | |
| Age, y | −0.174 | 0.016 | … | … |
| Sex (men) | … | … | −0.172 | 0.055 |
| RA longitudinal strain, % | 0.266 | 0.001 | … | … |
| LV global longitudinal strain, % | 0.34 | <0.0001 | 0.304 | 0.001 |
| PASP, mm Hg | −0.246 | 0.001 | … | … |
| RA minimal volume, mL | −0.14 | 0.056 | … | … |

*Standardized β value. Strain values were considered as absolute values. LV indicates left ventricular; PASP, pulmonary arterial systolic pressure; RA, right atrial; and RVLS, right ventricular longitudinal strain.

**Table 5. Results of RVLS Reproducibility Analysis**

| n=20 | 6-Segment RVLS | 3-Segment RVLS (M1) | 3-Segment RVLS (M4) |
|---|---|---|---|
| Intraobserver | 0.3±0.8 | 0.1±1.0 | 0.3±1.4* |
| Interobserver | 1.6±1.5 | 1.8±1.6 | 0.5±2.9*,† |

Data represent bias±SD. RVLS indicates right ventricular longitudinal strain.
*$P$<0.01 for M4 vs M1 3-segment RVLS.
†$P$<0.05 for M4 3-segment RVLS vs 6-segment RVLS.

part of a control group, and 4 included patients referred for clinically indicated evaluation and subsequently found to be without cardiopulmonary disease.[9] Seven studies included in this meta-analysis had small sample sizes (n=21–116) and used the EchoPac software, whereas only 1 larger study (n=186) used the VVI software.[24] As a consequence, the pooled reference values of RVLS were weighted by the significantly lower reference values of the latter (−21.7±4.2% for free wall RVLS by VVI software). Finally, several studies included in the meta-analysis used the conventional 4-chamber view for RVLS measurement and not the dedicated RV-focused view, the latter allowing a better definition of the RV free wall.[2] The limited normative data and the heterogeneity of the studies available to date for defining RVLS normalcy are also reflected by the lower limit of normality in the 2015 update of the chamber quantification guidelines, obtained from a meta-analysis of 18 small studies.[2] The larger spread around the pooled mean value resulted in a −20% abnormality threshold for free wall RVLS, which is lower than in our study (−23%).

## Technical Considerations on RV Strain Measurement

Previously, the term global RVLS has been used interchangeably for either the average of RV free wall and interventricular septal segments of RV in apical 4-chamber view[11,22] or the RV free wall segments alone[12,25] although the 2 approaches return significantly different results and evaluate RV inflow only. Many studies performed in different pathological conditions reported only free wall RVLS,[10] based on the consideration that interventricular septum is mainly a constituent part of the LV. However, also the interventricular septum contributes to RV systolic performance although with less magnitude.[26] Because the workload of the RV is significantly lower than that of the LV, the RV can benefit from the simultaneous LV systolic contraction. In healthy hearts, 20% to 40% of the RV pressure increase is because of LV contraction.[26] As such, LV dysfunction is likely to contribute to RV functional impairment through interventricular septum hypocontractility.[27] Because there is no consensus on which strain parameter is best to describe RV function, we provided reference values for RV strain with and without including the septum.

Another concern was whether the mean RVLS measured on the average strain curve using a 3-segment ROI leads to different strain values than the mean RVLS calculated by averaging the peak segmental values using a 6-segment ROI. We found no significant difference in measurements provided by the 2 approaches, but the latter was feasible in a substantially larger proportion of subjects, as the 3-segment ROI showed more frequently free wall segments that were inadequately tracked either at the base or at the apex. Therefore, we recommend to use a 6-segment ROI on the apical RV-focused view as a more robust analysis method, and to compute the free wall RVLS by averaging the peak segmental values displayed by software (Figure 2, M1).

## Segmental Variability of RV Strain

As previously reported by others,[9,22] significant variability between strain values in different segments of the RV was identified, with higher magnitude of strain at the free wall level than at the septal level. Furthermore, we also found the highest magnitude of strain at the midsegmental level of the RV free wall. Segmental RVLS analysis adds value over the conventional echocardiographic parameters in that it helps to understand whether reduced RV performance is because of a global failure or to a localized impaired contraction. Awareness of reference values of LS for each region and segment of the RV is important because specific patterns of strain impairment may provide valuable insights in RV pathology of different cause.[28,29]

## Relationship of RV Strain With Demographic, Hemodynamic, and Cardiac Parameters

We have found that both age and sex have a significant impact on the RVLS values. However, because the age-related differences were small and not clinically relevant, we have reported only sex-specific reference values. The relation of longitudinal strain with demographic factors has been previously described for RV, as well as for the other cardiac chambers.[15,19,24,30] This is the first study reporting the relationship between RVLS and 3D echocardiographic parameters of RV, LV, and RA size and function. Interestingly, both LV and RA longitudinal strain were identified as correlates of RVLS at multivariable analysis (RVLS increased in magnitude with the increase of both RALS and RVLS), suggesting the capability of 2DSTE to evaluate the functional coupling between RV–RA and RV–LV in the healthy heart. Finally, in our study, PASP, and not systemic blood pressure or heart rate, was inversely related with 6-segment RVLS, whereas 3-segment RVLS failed to show such relationship. This finding suggests that the assessment of the adaptation of RV mechanics to increased RV afterload might benefit of the inclusion of septal segments in the evaluation of RVLS.

We found a significant, but weak correlation between RVLS and RVEF in healthy subjects. In contrast with previous smaller sample studies,[9,22] we found no correlation between RVLS and tricuspid annular systolic excursion or fractional area change. It has been considered that the RV longitudinal shortening is the main contributor to the overall RVEF, generating 80% of the total RV stroke volume,[31] and therefore it can be assimilated with global RV function. We speculate that also RV radial shortening plays a role to the overall RVEF in healthy individuals (bellows effect), as the RV free wall has a larger surface in comparison with the tricuspid annular cross-sectional area. However, RV radial strain is difficult to measure by 2DSTE. Different echocardiographic parameters look at different aspects of RV function in different ways, and there is no “gold-standard” for assessing RV systolic function by echocardiography. Volume measurements appear more straightforward; however, the functional coupling between intrinsic myocardial mechanics and volume changes is more complicated, and several factors, such as aging, changes in geometry and load, ventricular interdependence through interventricular septum, and intrinsic myocardial damage may affect their balance. Advanced echocardiographic techniques, such as 2DSTE and 3D(ST)E, could provide new insights into the RV functional adaptation in the near future.[32]

## Clinical Implications

The implementation of our sex- and method-specific reference values is likely to contribute to an earlier detection of RV pathology by 2DSTE, in ventricles which otherwise would be classified as normal according to the limit of abnormality suggested in the current guidelines.[2] In addition, our study clarifies the implications of using different methods and definitions for RVLS computation used in previous researches, and proposes reference values pertaining to a unifying, guideline-supported methodology. We propose to avoid the term global when referring to free wall RVLS, in favor of a descriptive terminology (free wall or 3-segment longitudinal strain), whereas 6-segment terminology should be used for strain measurements including both free wall and septum. The availability of reference values of RVLS including the septum will hopefully clarify the importance of septal function for overall RV performance in biventricular or LV pathologies. Until normative data from larger multicenter, multiethnic, and multivendor studies will be produced, our study may foster the clinical implementation of 2DSTE and the standardization of RVLS across research and clinical laboratories equipped with the vendor-specific equipment used in this study.

## Limitations

This is a single-center study on a cohort of white subjects performed with a single vendor-specific software; therefore, our reference values may not apply to other populations and equipments. The application of 2DSTE to the RV free wall is more challenging than for the LV because of its smaller thickness, larger excursion, and lower quality of speckles, which accounted for a slightly lower feasibility (≈85%)[3,10] than for the 2DSTE-derived LVLS. Because there is no dedicated software for RVLS, we adapted the software designed for the LV for the purpose of our study. Although 2DSTE may be limited by through-plane motion, the analysis of RVLS by 3D speckle-tracking is technically difficult because of the complex geometry of the RV and the lack of software algorithms dedicated for RV.[32] The estimation of the PASP was possible only in 78% of this healthy cohort. As contrast agent administration for Doppler signal enhancement in otherwise healthy subjects without any clinical indication for contrast echocardiography would have been unethical, in the remaining 22%, we have assessed the RV acceleration time (>130 ms) to rule out a significant pulmonary hypertension.[33]

## Conclusions

This study provides sex- and method-specific reference values of RVLS obtained by 2DSTE from a large cohort of healthy volunteers. Age, pulmonary systolic pressure, as well as LV and RA volume and longitudinal strain have been identified as correlates of RV longitudinal strain. Our study may facilitate the implementation of quantitative RV myocardial mechanics in clinical practice.


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## Disclosures

Drs Muraru and Badano are on the advisory board of, and received research equipment and speakers' honoraria from, GE Vingmed Ultrasound (Horten, Norway). The other authors report no conflicts.

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## CLINICAL PERSPECTIVE

The advent of 2-dimensional (2D) speckle-tracking echocardiography allows for the accurate assessment of active myocardial contraction and has overcome the limitations of conventional 2-dimensional and Doppler parameters of right ventricular (RV) function. Moreover, evaluation of RV longitudinal strain (RVLS) offers important diagnostic and prognostic data in pulmonary hypertension, arrhythmogenic cardiomyopathy, congenital heart diseases, and in candidates for left ventricular assist device implantation. However, the implementation of RVLS analysis in daily clinical routine has been hampered by limited normative data and the lack of uniformity on the method and definition used for measuring and reporting in various studies. In 276 healthy volunteers (55% women; age, 18–76 years), free wall and septum RVLS (6 segments) and free wall RVLS (3 segments) using both 6-segment and 3-segment regions of interest were obtained. Reference values (lower limits of normality) were 6-segment RVLS, −24.7±2.6% (−20.0%) for men and −26.7±3.1% (−20.3%) for women; 3-segment RVLS, −29.3±3.4% (−22.5%) for men and −31.6±4.0% (−23.3%) for women ($P$<0.001). Age, sex, pulmonary systolic pressure, right atrial minimal volume and longitudinal strain, and left ventricular longitudinal strain resulted as correlates of RVLS values. Availability of reference values of RVLS including the septum will hopefully clarify the importance of septal function for overall RV performance in biventricular or left ventricular pathologies. Until normative data from larger multicenter, multiethnic, and multivendor studies are produced, our results may encourage the clinical implementation of 2D speckle-tracking echocardiography and the standardization of RVLS across research and clinical laboratories equipped with the platform used in this study.