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Echocardiographic reference ranges for normal left ventricular layer-specific strain: results from the EACVI NORRE study

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Aims To obtain the normal range for 2D echocardiographic (2DE) measurements of left ventricular (LV) layer-specific strain from a large group of healthy volunteers of both genders over a wide range of ages.

Methods and results A total of 287 (109 men, mean age: 46 ± 14 years) healthy subjects were enrolled at 22 collaborating institutions of the EACVI Normal Reference Ranges for Echocardiography (NORRE) study. Layer-specific strain was analysed from the apical two-, three-, and four-chamber views using 2DE software. The lowest values of layer-specific strain calculated as ±1.96 standard deviations from the mean were -15.0% in men and -15.6% in women for epicardial

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strain, -16.8% and -17.7% for mid-myocardial strain, and -18.7% and -19.9% for endocardial strain, respectively. Basal-epicardial and mid-myocardial strain decreased with age in women (epicardial; P = 0.008, mid-myocardial; P = 0.003) and correlated with age (epicardial; r = -0.20, P = 0.007, mid-myocardial; r = -0.21, P = 0.006, endocardial; r = -0.23, P = 0.002), whereas apical-epicardial, mid-myocardial strain increased with the age in women (epicardial; P = 0.006, mid-myocardial; P = 0.03) and correlated with age (epicardial; r = 0.16, P = 0.04). End/Epi ratio at the apex was higher than at the middle and basal levels of LV in men (apex; 1.6 ± 0.2, middle; 1.2 ± 0.1, base 1.1 ± 0.1) and women (apex; 1.6 ± 0.1, middle; 1.1 ± 0.1, base 1.2 ± 0.1).

Conclusion The NORRE study provides useful 2DE reference ranges for novel indices of layer-specific strain.

Keywords adult echocardiography • 2D echocardiography • deformation imaging • reference values

Introduction

Two-dimensional (2D) speckle tracking echocardiography (STE) enables quantitative evaluation of cardiac mechanics through image-based analysis of myocardial deformation.[1] Although left ventricular (LV) ejection fraction is the most commonly used parameter to assess LV mechanics, 2D-STE can detect latent LV dysfunction prior to a decline in LV ejection fraction by assessing mid-myocardial longitudinal strain.[2] Recently, technological advances in 2D-STE has enabled the assessment of layer-specific strain, thus allowing the measurement of epicardial, mid-myocardial, and endocardial longitudinal strain. The LV myocardium is divided into three myocardial layers consisting of circumferential fibres in the mid-myocardial layer and longitudinal fibres in the epicardial and endocardial layers.[3] In most heart diseases except some, such as sarcoidosis or hypertrophic cardiomyopathy, myocardial injury occurs predominantly in the endocardial fibres in the early stages of the disease.[4] Endocardial strain may have the potential to be more sensitive to assess myocardial function compared to epicardial or mid-myocardial strain in different cardiovascular diseases.[5–9] However, normal ranges for each type of layer-specific strain remain, to date, poorly defined.[10,11] The aim of this study was to establish the normal ranges of layer-specific strain from a large group of healthy volunteers of both genders over a wide range of ages.

The NORRE (Normal Reference Ranges for Echocardiography) study is the first European, large prospective, multicentre study performed in 22 laboratories accredited by the European Association of Cardiovascular Imaging (EACVI) and in one American laboratory, which has provided reference values for all 2D echocardiographic (2DE) measurements of all cardiac chambers,[12] Doppler parameters,[13] aortic dimensions,[14] 3D echocardiographic measurements of the LV volumes and strain,[15] 2DE measurements of LV strain,[16] 2D and 3D measurements of left atrial function,[17] and myocardial indices.[18] This study aimed to (i) establish normal reference limits for layer-specific strain in healthy adults and (ii) examine the influence of age and gender on these normal reference ranges.

Methods

Patient population

A total of 734 healthy European subjects constituted the final NORRE study population. The local ethics committees approved the study protocol. After the exclusion of patients that had incompatible image formats and/or poor image quality, the final study population consisted of 287 (39%) healthy subjects.

Echocardiographic examination

A comprehensive echocardiographic examination was performed using state-of-the-art echocardiographic ultrasound system (GE Vivid E9; Vingmed Ultrasound, Horten, Norway) following a recommended protocol approved by EACVI.[19,20] All echocardiographic images were recorded in a digital raw-data format (native DICOM format) and centralized for further analysis, after anonymization, at EACVI Central Core laboratory at the University of Liège, Belgium.

2D LV layer-specific strain

Quantification of layer-specific strain measurements were performed offline with dedicated software (EchoPAC V.203, GE). For measuring layer-specific strain, attention was taken to cover the entire myocardial wall thickness with the region of interest (ROI) of each segment and to avoid to include the pericardium. Calculation of transmural variation of longitudinal strain across the entire myocardium was based on the assumption of linear distribution. Endocardial and epicardial strain were measured on the endocardial and epicardial ROI border, respectively, whereas the mid (centre line) of the ROI represented the average values of the transmural wall thickness. The layer-specific strain values were obtained by averaging the peak longitudinal strain of 17 segments (*Figure 1*). The ratio of endocardial to epicardial was calculated using the End/Epi ratio for the assessment of the strain gradient.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD). The 95% confidence interval was calculated as ±1.96 SDs from the mean. Differences between groups were analysed for statistical significance with the unpaired t-test for normally distributed continuous variables. Comparison of continuous variables according to age groups was done with one-way analysis of variance test. When a significant difference was found, *post hoc* testing with Bonferroni comparisons to identify specific group differences was used. Correlation between continuous variables was performed using the Pearson correlation test. Multivariable linear regression analyses were performed to examine the independent correlates between layer-specific strain and baseline parameters. Intra-observer and inter-observer variability were assessed in 20 randomly selected subjects using Bland–Altman analysis. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using JMP 11.0 statistical software (SAS Institute, Cary, NC, USA).

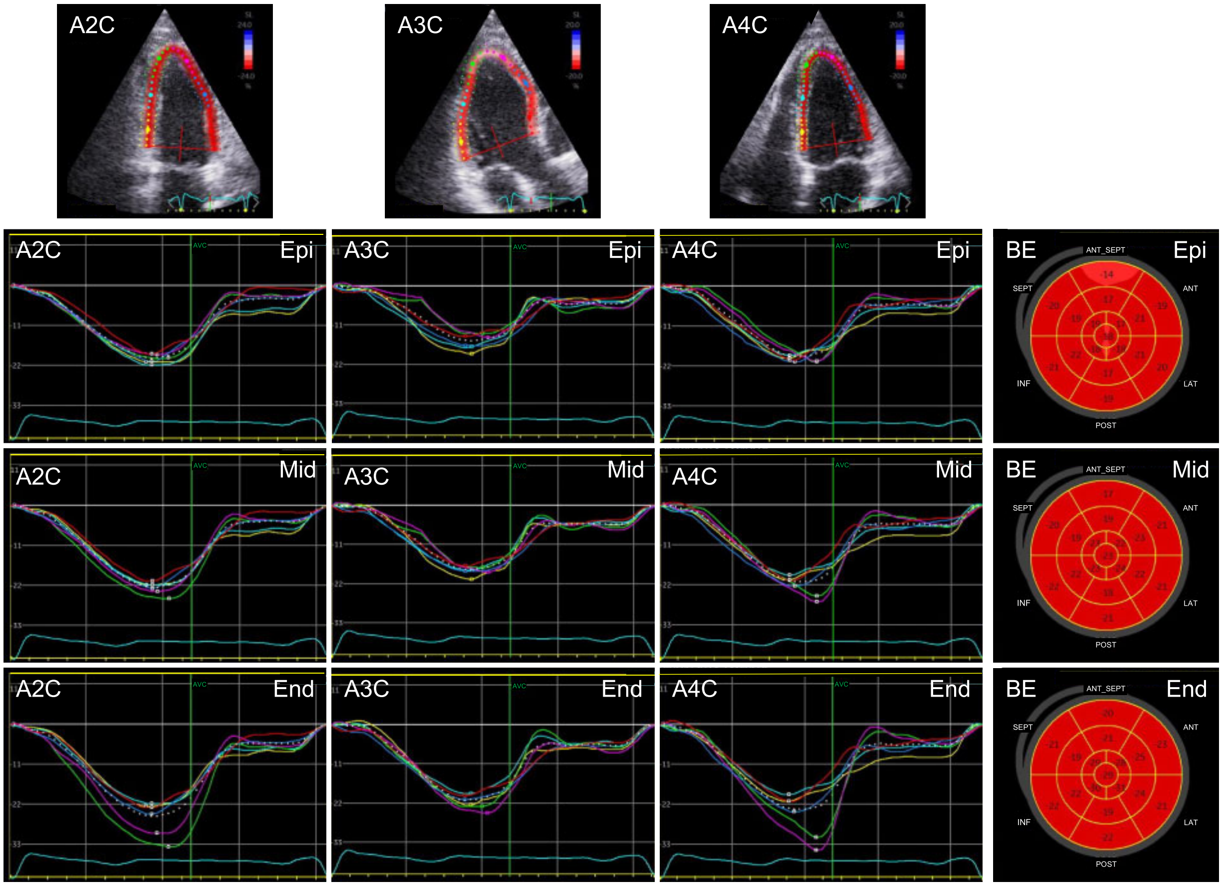

Figure 1 Layer-specific strain curves measurement by 2D speckle tracking echocardiography. A2C, apical two-chamber; A3C, apical three-chamber; A4C, apical four-chamber; Epi, epicardial strain; Mid, mid-myocardial strain; End, endocardial strain; BE, bull's eye of layer-specific strain.

Results

Demographic data

Table 1 summarizes the demographic data of the NORRE population analysed in the present study. A total of 109 men (mean age 46 ± 14 years) and 178 women (mean age 45 ± 14 years) were included. Systolic blood pressure was higher in men (mean age 123 ± 10 mmHg) than in women (116 ± 15 mmHg). Strain values may be affected by LV afterload. However, it remains to be clarified whether the strain values correlate with the LV afterload, and few studies have reported.[21] The mean frame rate was on the apical view were 63 ± 10/s (men 63 ± 11/s, women 64 ± 9/s, $P = 0.73$). Layer-specific strain results from the entire study population are depicted in *Table 2*. All average layer-specific strains were significantly higher in women than in men. The lowest values of layer-specific strains were -15.0% in men and -15.6% in women for epicardial strain, -16.8% and -17.7% for mid-myocardial strain, and -18.7% and -19.9% for endocardial strain, respectively. The highest values of layer-specific strain were -22.3% in men and -23.5% in women for epicardial strain, -25.1% and -26.0% for mid-myocardial strain, and -28.4% and -29.1% for endocardial strain, respectively.

Relationship between age, gender, and layer-specific strain

Relationships between gender and age with layer-specific strain in all apical views are shown in *Table 3* and *Figure 2*. No significant correlations were observed between age and layer-specific strains for all apical chamber views. In all age groups, layer-specific strain, including epicardial, mid-myocardial, and endocardial strain tended to be higher in women compared to men. In the age group between 20 and 40 years (epicardial, mid-myocardial, and endocardial strain) and in the age group >60 years, layer-specific epicardial and mid-myocardial strains were significantly higher in women than men.

Relationships between age and layer-specific strains in the apical, middle, and basal levels of the LV are shown in *Table 4* and *Figure 3*. No significant age dependency was observed with respect to layer-specific strain in all segments in men. However, the basal-epicardial and mid-myocardial strain decreased with age in women (epicardial; P = 0.008, mid-myocardial; P = 0.003) and correlated with age (epicardial; r = -0.20, P = 0.007, mid-myocardial; r = -0.21, P = 0.006, and endocardial; r = -0.23, P = 0.002). In contrast, theapical-epicardial and mid-myocardial strains increased with age in women (epicardial; P = 0.006 and mid-myocardial; P = 0.03) and correlated with age

Table 1 Characteristics of the population

Parameters	Total (n = 287)	Male (n = 109)	Female (n = 178)	P-value
Age (years)	46 ± 14	46 ± 14	45 ± 14	0.54
Height (cm)	170 ± 10	179 ± 8	165 ± 7	<0.001
Weight (kg)	69 ± 12	78 ± 10	63 ± 9	<0.001
Body surface area (m^2)	1.8 ± 0.2	2.0 ± 0.1	1.7 ± 0.1	<0.001
Systolic blood pressure (mmHg)	119 ± 14	123 ± 10	116 ± 15	<0.001
Diastolic blood pressure (mmHg)	74 ± 9	75 ± 8	73 ± 9	0.02
Glucose (mg/dL)	91 ± 11	95 ± 9	89 ± 11	<0.001
Cholesterol (mg/dL)	182 ± 30	186 ± 26	180 ± 32	0.17

Table 2 2DE parameters of layer-specific strain

	Total mean ± SD	Total 95% CI	Male mean ± SD	Male 95% CI	Female mean ± SD	Female 95% CI	P-value
Epicardial strain (%)							
Apical two-chamber	-19.8 ± 2.6	-14.7 to -24.8	-19.4 ± 2.4	-14.6 to -24.1	-20.0 ± 2.6	-14.9 to -25.1	0.03
Apical three-chamber	-18.8 ± 2.5	-13.8 to -23.7	-18.1 ± 2.3	-13.6 to -22.6	-19.2 ± 2.6	-14.1 to -24.3	<0.001
Apical four-chamber	-19.0 ± 2.4	-14.4 to -23.7	-18.5 ± 2.5	-13.6 to -23.4	-19.4 ± 2.3	-14.9 to -23.8	0.95
Average	-19.2 ± 2.0	-15.3 to -23.1	-18.7 ± 1.9	-15.0 to -22.3	-19.5 ± 2.0	-15.6 to -23.5	<0.001
Mid-myocardial strain (%)							
Apical two-chamber	-22.0 ± 2.7	-17.3 to -27.2	-21.6 ± 2.5	-16.6 to -26.5	-22.2 ± 2.8	-16.8 to -27.6	0.045
Apical three-chamber	-21.2 ± 2.8	-15.8 to -28.6	-20.5 ± 2.6	-15.5 to -25.6	-21.7 ± 2.8	-16.2 to -27.1	<0.001
Apical four-chamber	-21.1 ± 3.5	-14.2 to -28.1	-20.7 ± 2.7	-15.4 to -26.1	-21.3 ± 4.0	-13.6 to -29.1	0.11
Average	-21.5 ± 2.2	-17.3 to -25.7	-20.9 ± 2.1	-16.8 to -25.1	-21.8 ± 2.1	-17.7 to -26.0	<0.001
Endocardial strain (%)							
Apical two-chamber	-24.5 ± 3.0	-18.6 to -30.3	-24.1 ± 2.9	-18.5 to -29.7	-24.7 ± 3.0	-18.8 to -30.6	0.08
Apical three-chamber	-24.0 ± 4.2	-15.8 to -32.2	-23.4 ± 3.1	-17.3 to -29.5	-24.4 ± 4.7	-15.2 to -33.6	0.03
Apical four-chamber	-23.7 ± 2.9	-18.1 to -29.3	-23.2 ± 3.2	-17.0 to -29.4	-24.0 ± 2.6	-18.8 to -29.2	0.03
Average	-24.1 ± 2.4	-19.3 to -28.9	-23.6 ± 2.5	-18.7 to -28.4	-24.5 ± 2.3	-19.9 to -29.1	0.002
End/Epi ratio	1.3 ± 0.1	1.15 to 1.37	1.3 ± 0.1	1.15 to 1.38	1.3 ± 0.1	1.15 to 1.36	0.19

CI, confidence interval; SD, standard deviation.
P-value differences between genders.

(epicardial; $r = 0.16$, $P = 0.04$). Although all strain values tended to increase from the epicardium to the endocardium, this tendency was stronger at the apical compared to the basal LV. Therefore, End/Epi ratio at the apex was higher than at the middle or the basal LV levels in men (apex; 1.6 ± 0.2, middle; 1.2 ± 0.1, base 1.1 ± 0.1) and women (apex; 1.6 ± 0.1, middle; 1.1 ± 0.1, base 1.2 ± 0.1), and this relationship was preserved at all ages (*Table 4* and *Figure 3*).

Layer-specific strains determinants

Multivariable analysis for layer-specific strain showed that epicardial, mid-myocardial, and endocardial strain increased with body surface area (epicardial; β-coefficient = 0.32, $P = 0.009$, mid-myocardial; β-coefficient = 0.29, $P = 0.02$, endocardial; β-coefficient = 0.26, $P = 0.03$), whereas the End/Epi ratio was not related to body surface area. There was a significant increase in epicardial, mid-myocardial, and endocardial strain according to body surface area in univariable analysis but no association was observed after adjustment for confounders (*Table 5*).

Repeatability and reproducibility

Intra-observer and inter-observer variability for layer-specific strain are summarized in *Table 6*. Intra-observer and inter-observer analyses showed good repeatability and reproducibility in layer-specific strain (*Table 6* and *Figure 4*).

Discussion

The present prospective, EACVI multicentre study provides contemporary normal references values for 2DE measurements of layer-specific strain in a large cohort of healthy volunteers of both genders over a wide range of ages. Myocardial heterogeneity is characterized by higher deformation amplitude in the endocardial compared with

Table 3 Layer-specific strain at the apical two-chamber, apical three-chamber, and apical four-chamber according to gender and age

	Total ($n = 287$)		Age 20–40 ($n = 115$)		Age 40–60 ($n = 122$)		Age ≥60 ($n = 50$)		*P*-value		Male		Female	
	Male ($n = 110$), mean ± SD	Female ($n = 178$), mean ± SD	Male ($n = 39$), mean ± SD	Female ($n = 76$), mean ± SD	Male ($n = 50$), mean ± SD	Female ($n = 72$), mean ± SD	Male ($n = 20$), mean ± SD	Female ($n = 30$), mean ± SD	Male	Female	*R*	*P*-value	*R*	*P*-value
Epicardial longitudinal strain (%)														
Apical two-chamber	-19.4 ± 2.4	-20.0 ± 2.6[a]	-19.2 ± 2.5	-20.1 ± 2.6	-19.7 ± 2.5	-20.2 ± 2.6	-18.7 ± 2.0	-19.5 ± 2.6	0.23	0.59	-0.0002	1.00	0.08	0.28
Apical three-chamber	-18.1 ± 2.3	-19.2 ± 2.6[a]	-18.1 ± 2.1	-19.1 ± 2.8[a]	-18.5 ± 2.4	-19.4 ± 2.5	-17.3 ± 2.2	-18.9 ± 2.3[a]	0.14	0.38	0.06	0.53	0.06	0.45
Apical four-chamber	-18.5 ± 2.5	-19.4 ± 2.3[a]	-17.7 ± 2.2	-19.0 ± 2.5[a]	-19.3 ± 2.6	-19.9 ± 2.0	-18.2 ± 2.0	-19.1 ± 2.1	0.01	0.81	-0.17	0.08	-0.04	0.60
Average	-18.7 ± 1.9	-19.5 ± 2.0[a]	-18.3 ± 1.7	-19.4 ± 2.1[a]	-19.2 ± 2.1	-19.8 ± 1.9	-18.0 ± 1.5	-19.2 ± 1.8[a]	0.03	0.73	-0.05	0.63	0.05	0.48
Mid-myocardial longitudinal strain (%)														
Apical two-chamber	-21.6 ± 2.5	-22.2 ± 2.8[a]	-21.6 ± 2.5	-22.4 ± 2.7	-21.9 ± 2.6	-22.3 ± 2.8	-20.7 ± 2.2	-21.6 ± 2.9	0.19	0.32	0.06	0.53	0.12	0.12
Apical three-chamber	-20.5 ± 2.6	-21.7 ± 2.8	-20.6 ± 2.3	-21.6 ± 2.8	-20.8 ± 2.7	-21.9 ± 2.9[a]	-19.8 ± 2.6	-21.3 ± 2.6[a]	0.30	0.31	0.05	0.59	0.07	0.37
Apical four-chamber	-20.7 ± 2.7	-21.3 ± 4.0	-20.0 ± 2.4	-21.2 ± 2.7[a]	-21.4 ± 2.9	-21.5 ± 5.4	-20.4 ± 2.6	-21.3 ± 2.2	0.04	0.91	-0.14	0.14	0.02	0.78
Average	-20.9 ± 2.1	-21.8 ± 2.1[a]	-20.7 ± 1.8	-21.8 ± 2.2[a]	-21.4 ± 2.3	-22.1 ± 2.1	-20.3 ± 1.9	-21.4 ± 2.0[a]	0.11	0.57	-0.01	0.87	0.08	0.30
Endocardial longitudinal strain (%)														
Apical two-chamber	-24.1 ± 2.9	-24.7 ± 3.0	-24.4 ± 2.8	-25.0 ± 2.9	-24.3 ± 2.9	-24.6 ± 3.1	-22.9 ± 2.6	-24.0 ± 3.3	0.11	0.17	0.13	0.19	0.15	0.051
Apical three-chamber	-23.4 ± 3.1	-24.4 ± 4.7	-23.5 ± 2.8	-24.0 ± 6.1	-23.5 ± 3.3	-24.8 ± 3.4[a]	-22.7 ± 3.3	-24.3 ± 3.1	0.55	0.80	0.05	0.62	-0.0002	1.00
Apical four-chamber	-23.2 ± 3.2	-24.0 ± 2.6[a]	-22.6 ± 2.7	-23.5 ± 2.8	-23.9 ± 3.3	-24.5 ± 2.4	-22.9 ± 3.3	-23.9 ± 2.5	0.14	0.75	-0.11	0.24	-0.05	0.51
Average	-23.6 ± 2.5	-24.5 ± 2.3[a]	-23.5 ± 2.1	-24.4 ± 2.4[a]	-23.9 ± 2.8	-24.7 ± 2.3	-22.8 ± 2.4	-24.0 ± 2.3	0.25	0.45	0.02	0.83	0.09	0.21
End/Epi ratio	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	0.03	0.27	-0.15	0.12	-0.07	0.38

SD, standard deviation.
[a]$P < 0.05$ vs. male.

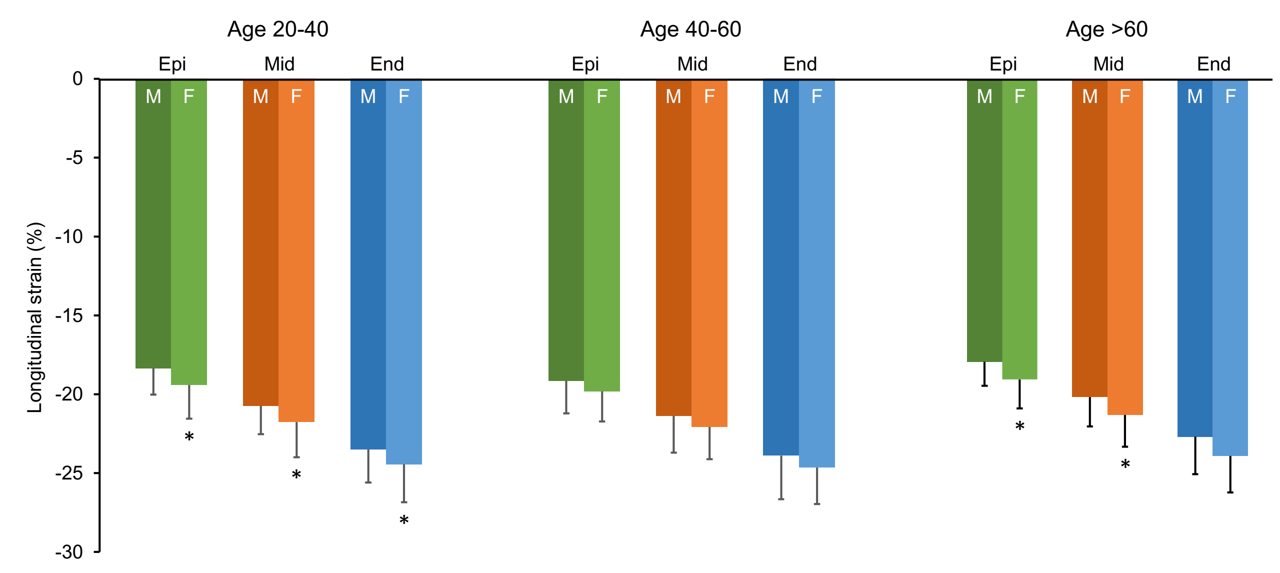

Figure 2 Bar graphs showing average layer-specific strain by 2D echocardiography analysis according to gender and age categories. End, endocardial strain; Epi, epicardial strain; Mid, mid-myocardial strain. *P-value differences between genders.

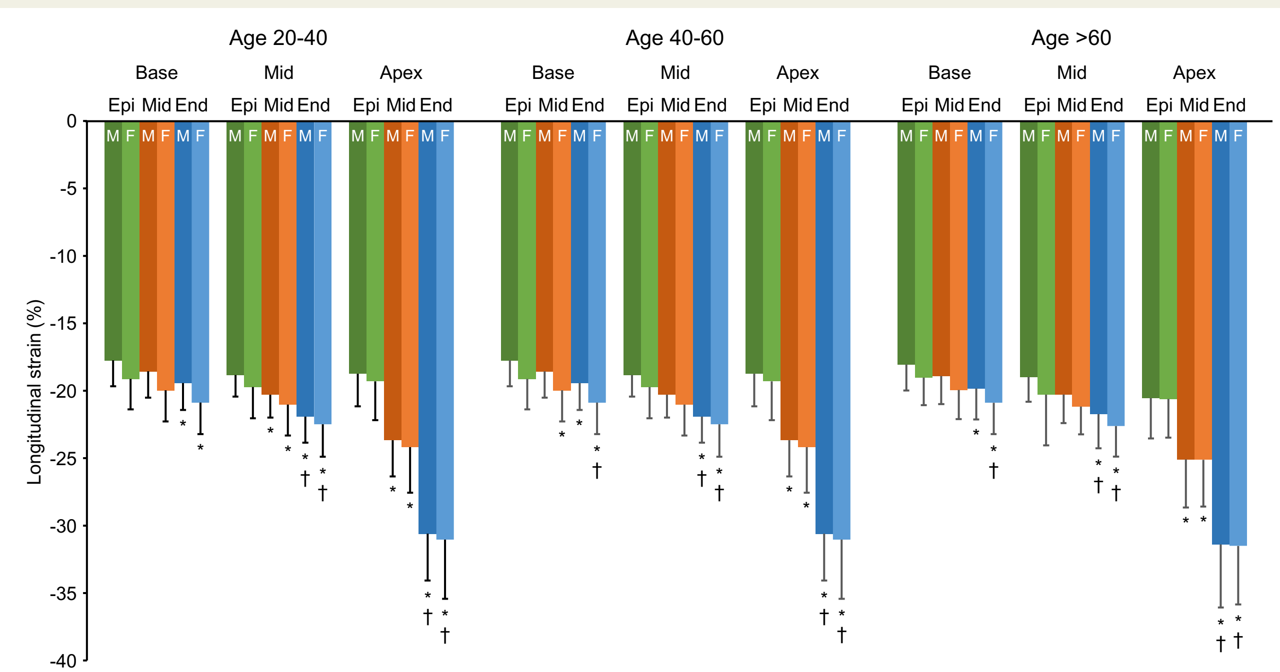

Figure 3 Bar graphs showing average layer-specific strain at the apical, the mid-ventricular, and the basal levels of the left ventricle by 2D echocardiography analysis according to gender and age categories. Epi, epicardial strain; Mid, mid-myocardial strain; End, endocardial strain. *$P < 0.05$ vs. epicardial strain. †$P < 0.05$ vs. mid-myocardial strain.

the epicardial layer.[22] Layer-specific strain is a novel method that is capable of assessing each layer of the myocardial function. Moreover, the absence of differences between vendors for layer-specific strain values makes this technique a useful tool for feasibility, accuracy, and reproducibility.[23]

Our results are consistent with previous studies showing good concordance with the absolute values of layer-specific strain and that all layer-specific strains in women were consistently higher than in men.[10,24,25] However, the relationship between layer-specific strain and age dependency is inconsistent. As reported by Nagata *et al.* and

Table 4 Layer-specific strain at the apical, mid-ventricular, and basal levels of left ventricle according to gender and age

	Total (n = 287)		Age 20–40 (n = 115)		Age 40–60 (n = 122)		Age ≥60 (n = 50)		P-value		Male		Female	
	Male (n = 109), mean ± SD	Female (n = 178), mean ± SD	Male (n = 39), mean ± SD	Female (n = 76), mean ± SD	Male (n = 50), mean ± SD	Female (n = 72), mean ± SD	Male (n = 20), mean ± SD	Female (n = 30), mean ± SD	Male	Female	R	P-value	R	P-value
Base														
Epi	17.8 ± 2.0	-18.9 ± 2.2[a]	-17.7 ± 1.9	-19.1 ± 2.3[a]	-18.1 ± 1.9	-19.0 ± 2.0[a]	-17.3 ± 2.0	-17.8 ± 1.8	0.32	0.008	-0.02	0.83	-0.20	0.007
Mid	18.6 ± 2.0	-19.7 ± 2.2[a]	-18.6 ± 2.0	-20.0 ± 2.3[a]	-18.9± 2.1	-19.9 ± 2.2[a]	-18.1 ± 2.0	-18.5 ± 1.8	0.30	0.003	-0.03	0.75	-0.21	0.006
End	-19.5 ± 2.2	-20.7 ± 2.6[a]	-19.4 ± 2.0	-20.9 ± 2.3[a]	-19.8 ± 2.3	-20.9 ± 2.3[a]	-18.9 ± 2.1	-19.7 ± 3.7	0.27	0.23	-0.04	0.67	-0.23	0.002
End/Epi ratio	1.1 ± 0.1	1.2 ± 0.1[a]	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.4	0.95	0.06	-0.05	0.61	0.13	0.09
Middle														
Epi	-18.8 ±1.8	-19.8 ± 3.0[a]	-18.8 ± 1.6	-19.7 ± 2.3[a]	-19.0 ± 1.8	-20.3 ± 3.8[a]	-18.1± 1.8	-18.9 ± 1.8[a]	0.14	0.09	-0.10	0.31	-0.12	0.12
Mid	-20.1 ± 2.0	-20.9 ± 2.1[a]	-20.3 ± 1.7	-21.0 ± 2.3	-20.3 ± 2.1	-21.2 ± 2.1[a]	-19.3 ± 2.1	-20.1 ± 1.8	0.12	0.06	-0.12	0.23	-0.16	0.04
End	-21.7 ± 2.3	-22.4 ± 2.3[a]	-21.9 ± 1.9	-22.5 ± 2.4	-21.7 ± 2.5	-22.6 ± 2.3[a]	-21.0 ± 2.5	-21.5 ± 2.3	<0.001	0.08	-0.09	0.37	-0.14	0.07
End/Epi ratio	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.19	0.61	-0.02	0.85	-0.008	0.92
Apex														
Epi	-19.6 ± 2.8	-20.1 ± 2.9	-18.7 ± 2.4	-19.3 ± 2.9	-20.5 ± 3.0	-20.6 ± 2.9	-19.1 ± 2.2	-20.9 ± 2.8[a]	0.007	0.006	0.16	0.10	0.16	0.04
Mid	-24.3 ± 3,2	-24.9 ± 3.5	-23.7 ± 2.8	-24.2 ± 3.4	-25.1 ± 3.6	-25.1 ± 3.5	-23.8± 2.6	-26.0 ± 3.6[a]	0.06	0.03	0.12	0.22	0.13	0.08
End	-30.9 ± 4.1	-31.6 ± 4.5	-30.6 ± 3.5	-31.0 ± 4.4	-31.4 ± 4.7	-31.5 ± 4.3	-30.1 ± 3.9	-33.0 ± 4.9[a]	0.41	0.10	0.04	0.71	0.10	0.19
End/Epi ratio	1.6 ± 0.2	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	0.01	0.002	-0.19	0.04	-0.05	0.61

SD, standard deviation.
[a]$P < 0.05$ vs. male.

Table 5 Univariable and multivariable analysis for layer-specific strain

Variables	Univariable analysis		Multivariable analysis	
	Coefficients	*P*-value	β-coefficients	*P*-value
Epicardial strain (%)				
Age (years)	0.02	0.70		
Male gender (=1)	0.21	<0.001		
Body mass index (kg/m²)	0.07	0.25		
Body surface area (m²)	0.24	<0.001	0.32	0.009
Systolic blood pressure (mmHg)	0.04	0.57		
Diastolic blood pressure (mmHg)	0.03	0.64		
Glycaemia (g/dL)	0.04	0.60		
Cholesterol (g/dL)	0.01	0.87		
Mid-myocardial strain (%)				
Age (years)	0.05	0.42		
Male gender (=1)	0.20	<0.001		
Body mass index (kg/m²)	0.07	0.21		
Body surface area (m²)	0.22	<0.001	0.29	0.02
Systolic blood pressure (mmHg)	0.04	0.58		
Diastolic blood pressure (mmHg)	0.06	0.32		
Glycaemia (g/dL)	0.07	0.33		
Cholesterol (g/dL)	0.04	0.57		
Endocardial strain (%)				
Age (years)	0.07	0.24		
Male gender (=1)	0.18	0.002		
Body mass index (kg/m²)	0.07	0.25		
Body surface area (m²)	0.19	<0.001	0.26	0.03
Systolic blood pressure (mmHg)	0.03	0.63		
Diastolic blood pressure (mmHg)	0.09	0.17	0.19	0.03
Glycaemia (g/dL)	0.10	0.19		
Cholesterol (g/dL)	0.07	0.38		
End/Epi ratio				
Age (years)	-0.12	0.04		
Male gender (=1)	0.08	0.19		
Body mass index (kg/m²)	-0.02	0.80		
Body surface area (m²)	0.10	0.08		
Systolic blood pressure (mmHg)	-0.0002	0.10		
Diastolic blood pressure (mmHg)	-0.14	0.03	-0.20	0.02
Glycaemia (g/dL)	-0.15	0.05		
Cholesterol (g/dL)	-0.12	0.11		

Shi *et al.*[10,24] no significant age dependency was observed concerning all layer-specific strains. In contrast, as reported by Alcidi *et al.* all layer-specific strains were progressively reduced with increasing age. The relationship between layer-specific strain and age dependence was inconsistent and different from the previous NORRE study of 2D strain.[16] This difference may be due to the smaller number of enrolled patients in this study than in previous NORRE study. Interestingly, the layer-specific strain gradient increased from the epicardial towards the endocardial layer. The mechanism underlying these findings remains unclear, but some considerations have been reported. The differences between epicardial and endocardial strain might be secondary to the ability of the endocardial fibres to stretch more potently compared to the epicardial fibres during end-diastole.[26] In addition, differences in coronary perfusion and metabolic demands between the epicardial and endocardial layers may also contribute to these differences.[27,28] In this context, the End/Epi ratio at the apex was higher than that at the middle or basal LV levels in both genders. (*Table 4* and *Figure 3*). The End/Epi ratio differs depending of the type of LV hypertrophic diseases, such as aortic stenosis[29] or hypertrophic cardiomyopathy,[30] and may have the potential to diagnose, not only these disease but also other forms of hypertrophic diseases. The hypertrophied myocardium may remodel differently in response to a variety of aetiologies, resulting in different epicardial and endocardial strains. Moreover, the results of our multivariable

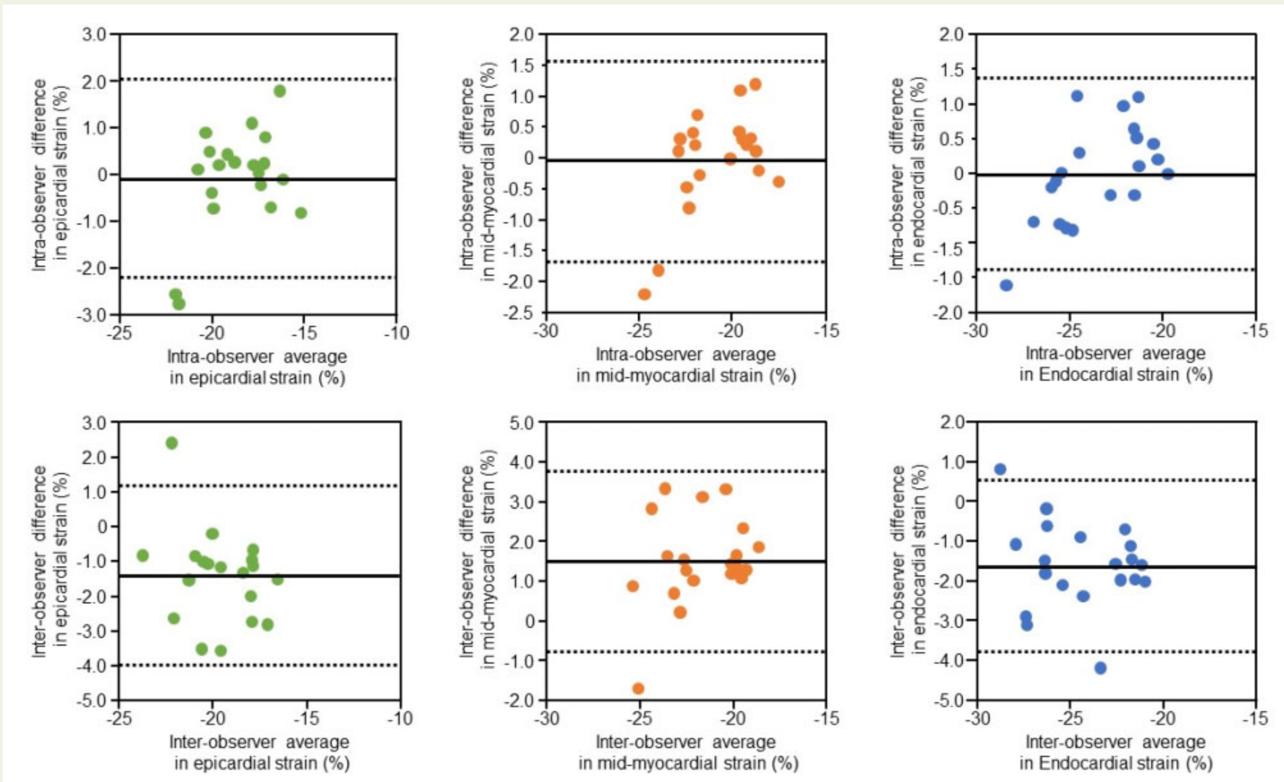

Figure 4 Bland–Altman analysis for assessing intra-observer and inter-observer variability of layer-specific strain. Dotted lines represent bias and 95% limits of agreement for measurements performed in 20 patients.

Table 6 Repeatability and reproducibility of 2D echocardiographic data

Variables	Mean ± SD	Mean ± SD	Bias	*P*-value	95% LOA
Intra-observer					
Average epicardial longitudinal strain (%)	-19.4 ± 1.9	-18.6 ± 1.6	-0.46	0.005	-19.7 to -19.1
Average mid-myocardial longitudinal strain (%)	-21.6 ± 2.1	-21.0 ± 2.0	-0.50	0.001	-22.1 to -21.5
Average endocardial longitudinal strain (%)	-24.4 ± 2.4	-23.5 ± 2.2	-0.60	0.003	-24.9 to -24.1
Inter-observer					
Average epicardial longitudinal strain (%)	-19.4 ± 1.9	-18.3 ± 1.7	1.05	<0.001	-18.2 to -17.4
Average mid-myocardial longitudinal strain (%)	-21.6 ± 2.1	-20.8 ± 1.9	0.82	0.001	-20.1 to -19.9
Average endocardial longitudinal strain (%)	-24.4 ± 2.4	-23.5 ± 2.2	0.90	<0.001	-23.6 to -23.1

LOA, lower limits of agreement; SD, standard deviation.

analysis (*Table 5*) suggest that the End/Epi ratio may have the potential to be a useful marker regardless of age, gender, or body surface area. Our data showed good reproducibility for the assessment of layer-specific strains, reinforcing the possibility of a promising application of this new advanced echocardiographic index in clinical practice.

Limitations

This study presents several limitations. First, only one-third of the patients included in the NORRE database could be analysed by the current available software. Second, since this study was conducted only on GE equipment, data on other equipment, such as Philips, is not available. However, in our previous study, we reported that no differences were noted between GE and Philips equipment with regard to longitudinal strain.[16] Third, the number of patients enrolled in this study was lower than in the previous NORRE study of LV 2D strain.[16] Therefore, the relationship between layer-specific strain and age dependency was inconsistent. The same tendency was observed for the basal and middle LV levels of all layer-specific strain. Fourth, whether the NORRE study results can be extrapolated to non-Caucasian European individuals is still unknown.

Conclusion

The NORRE study provides applicable 2DE reference ranges for layer-specific strain. Multivariable analysis did not show any significant association between layer-specific strain and age or gender.

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