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Normal reference values of multilayer longitudinal strain according to age decades in a healthy population: A single-centre experience

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Aims Recent advancements in echocardiographic technology allow to analyse myocardial strain in multiple layers. Little is known about the impact of age on layer-specific longitudinal strain in healthy subjects. The aim of this study was to analyse the influence of age on multilayer longitudinal strain and establish normal reference values of layer-specific strain according to age decades in a healthy population referring to our echo laboratory using 2D speckle-tracking echocardiography with layer-specific software.

Methods and results Two-hundred sixty-six healthy, consecutive subjects (mean age = 39.2 ± 17.5 years, women/men = 137/129), free of cardiovascular risk factors, were enrolled. Subjects were divided according to six age decades: 10–19, 20–29, 30–39, 40–49, 50–59, >60 years. All subjects underwent a complete echo Doppler examination including quantitation of 2D global longitudinal strain (GLS). Subendocardial longitudinal strain (LSsubendo), subepicardial longitudinal strain (LSsubepi), and strain gradient (LSsubendo - LSsubepi) were also determined. GLS ($P < 0.001$), LSsubendo, and LSsubepi (both $P < 0.0001$) were all progressively reduced with increasing age decades, but *post hoc* intra-group analyses demonstrated that the decline of GLS, LSsubendo, and LSsubepi was significant in the decades 50–60 and ≥60 years. In separate multiple linear regression analyses, the effect of age on GLS, LSsubendo, and LSsubepi remained significant even after adjusting for clinical and echocardiographic confounders. Strain gradient remained unchanged in age decades.

Conclusion Ageing shows an independent effect on GLS, LSsubendo, and, particularly on, LSsubepi. Our data also provide normal reference values of layer-specific longitudinal strain for age decades.

Keywords global longitudinal strain • subepicardial strain • subendocardial strain • echocardiography • ageing

Introduction

Global longitudinal strain (GLS), a comprehensive parameter of left ventricular (LV) systolic function, obtainable by 2D speckle-tracking echocardiography, has gained growing importance in the clinical practice. GLS results impaired in early, subclinical stages of the majority of cardiac diseases, when LV ejection fraction (EF) is still normal.[1,2] GLS has also shown an important prognostic power in patients with heart failure, coronary artery disease, valvular heart disease, and cardiomyopathies.[3] Its use is even promoted to drive management of patients developing anticancer drug-related cardiotoxicity.[4]

Technological progression of 2D speckle-tracking software has recently enabled the estimation of layer-specific strain, thus allowing to differentiate subendocardial and subepicardial longitudinal strain

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(LSsubendo an LSsubepi, respectively). This differentiation has clinical implications as the longitudinal fibers of subendocardial layer could be firstly involved in the progression of myocardial dysfunction in the majority of cardiac pathologies. Accordingly, the clinical usefulness of the multilayer strain software has already been successfully tested in some clinical settings, including coronary artery disease, myocardial infarction, arterial hypertension, and heart failure.[5–9]

The purpose of using strain imaging in pathological conditions implies the need of considering normal reference values derived from the healthy population. This goal has been achieved for transmural GLS,[10,11] for which normal ranges have also been promoted in the 2016 American Society of Echocardiography (ASE)/European Association of Cardiovascular Imaging (EACVI) chamber quantification recommendations.[12] Conversely, little information is available on normal values of layer-specific strain.[13,14] This prospective study was designed to define normal values of both LSsubendo and LSsubepi and myocardial transmural gradient in a healthy population with a wide age range, in relation with demographic and echocardiographic variables and to propose reference values according to age decades.

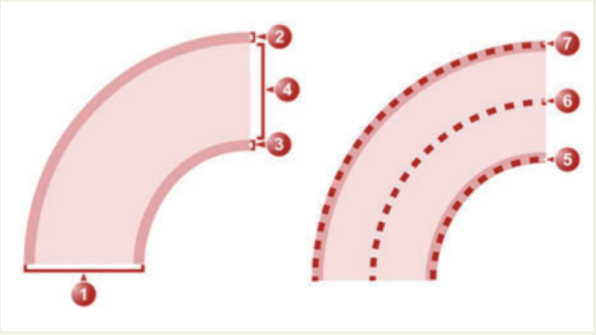

Figure 1 Methodology for obtaining layer-specific longitudinal strain. Left panel shows schematic representation of myocardial layers and the right panel shows depiction of ROI and position of the measured LSsubendo, mid, and subepi. 1, myocardial wall thickness; 2, epicardial layer; 3, endocardial layer; 4, myocardium; 5, inner tracked ROI line (subendocardial measurement); 6, mid wall (transmural measurement); 7, outer-tracked ROI line (subepicardial measurement).

Methods

Study population

We prospectively studied 266 consecutive healthy subjects (137 women and 129 men, mean age 39.2 ± 17.5 years, age range = 10–86 years) referring to our echo lab in the period between January 2017 and July 2017, who were recruited from the staff and relatives of our department in a screening of cardiovascular prevention. No subject had cardiovascular risk factors including arterial hypertension [blood pressure (BP) > 140/90 mmHg], diabetes mellitus (fasting glycaemia > 100 mg/dL), obesity (body mass index > 29.9 kg/m^2), dyslipidaemia (total cholesterol > 190 mg/dL and/or triglycerides > 150 mg/dL), and smoke habit. Other exclusion criteria were history of coronary artery disease and previous acute myocardial infarction, stroke, and transient ischaemic events, mild-to-severe valvular heart diseases, congestive heart failure, primary cardiomyopathies, congenital heart diseases, systemic diseases, pharmacological therapies, age < 10 years, any kind of resting electrocardiographic abnormalities and echocardiograms of poor imaging quality. All subjects gave their written informed consent.

Echocardiographic procedures

Standard Doppler echocardiography and speckle-tracking echocardiography were performed by Vivid E95 (GE Healthcare, Horten, Norway) machine. A 2.5-MHz phased array transducer was used according to standardized procedures of our laboratory.[15,16] At the end of the examination, a physician blinded to the examination estimated cuff BP (mean of three measurements).

LV quantitative analysis was performed in agreement with 2015 ASE/EACVI recommendations.[12] 2D LVEF was computed from LV end-diastolic and end-systolic volumes calculated according to the modified Simpson rule in apical four- and two-chamber views. Left atrial volume and LV mass were normalized for body surface area.[12] The cut-off points for diagnosing LV hypertrophy were 95 g/m^2 in women and 115 g/m^2 in men.[17] Transmitral pulsed Doppler and pulsed tissue Doppler of septal and lateral mitral annulus were recorded in apical four-chamber view and diastolic parameters determined and grades of LV diastolic dysfunction established according to the current ASE/EACVI recommendations.[18]

Speckle-tracking echocardiography procedures were performed according to standardized procedures of our laboratory[15,16] by an experienced operator blinded to the subject's clinical information. The acquisitions were performed in apical long-axis, four-chamber and two-chamber views, using a frame rate ranging between 70 and 90, as recommended.[1] Strain analysis was performed by a vendor-specific software (EchoPAC PC, version 201, GE Healthcare). Left ventricle was divided into six myocardial segments in each view, and GLS calculated as the average longitudinal strain (LS) at end systole. For measuring layer-specific strain, attention was taken to cover the entire myocardial wall thickness by the region of interest (ROI) of each segment. Calculation of transmural variation of LS across the entire myocardium was based on the assumption of a linear distribution. LSsubendo an LSsubepi were measured on the endocardial and epicardial ROI border, respectively, whereas the MID (centre line) of the ROI represents the average values of the transmural wall thickness (*Figure 1*). LS gradient was calculated as the difference between LSsubendo and LSsubepi.

Statistical analysis

Data were presented as mean value ± standard deviation. Normal distribution of data was checked using the Kolmogorov–Smirnov test. The study population was divided in six decades of age: 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, >60 years and intra-group differences analyzed by one-factor ANOVA. *Post hoc* test analyses (Bonferroni test) were also done to analyse inter-group differences. Least squares linear regression was used to evaluate univariable and multivariable correlates of strain measurements. For multiple linear regression models, multicollinearity was also examined by computation of in-model tolerance. Collinearity was considered acceptable for tolerance >0.70. Intra- and inter-observer variability of multilayer strain was assessed by calculating intra-class correlation coefficient (ICC) and 95% confidence intervals (CIs) of the strain components. The null hypothesis was rejected for $P \leq 0.05$.

Results

On a population of 281 normal subjects, originally evaluated for longitudinal strain analyses, 15 were excluded for inadequate imaging. The remaining 266 (94.7%) subjects represented our study population. *Table 1*

Table 1 Demographic and echocardiographic characteristics of the study population

Parameters	Mean ± SD	Range
Age (years)	39.2 ± 17.5	10–86
Height (cm)	168 ± 9.7	135–201
Weight (kg)	66.2 ± 11.5	30–95
BSA (m^2)	1.75 ± 0.19	1.14–2.23
BMI (kg/m^2)	23.2 ± 2.7	13.6–29.3
Systolic BP (mmHg)	119.5 ± 16.5	85–140
Diastolic BP (mmHg)	75.0 ± 8.5	50–90
Heart rate (bpm)	71.5 ± 10.9	46–116
LV mass index (g/m^2)	68.4 ± 16.3	26.9–114.7
Relative wall thickness	0.31 ± 0.05	0.15–0.40
LV EDV (mL)	88.2 ± 18.1	56–161
LV ESV (mL)	32.7 ± 8.6	23–65
LV EF (%)	63.3 ± 4.7	53–76
SV (mL)	60.2 ± 11.1	48–105
LAVi (mL/m^2)	24.1 ± 5.8	11–41
Transmitral *E/A* ratio	1.38 ± 0.45	0.54–2.92
E velocity DT (ms)	197.5 ± 33.3	107–266
E/e′ ratio	6.67 ± 1.8	2.0–13.8
Transmural GLS, average (%)	22.7 ± 1.8	18.3–28
Transmural GLS, 3-ch (%)	22.7 ± 2.0	18.1–27.1
Transmural GLS, 4-ch (%)	22.6 ± 1.8	20.2–27.4
Transmural GLS, 2-ch (%)	22.6 ± 1.9	19.7–28.1
LSsubendo, average (%)	25.4 ± 2.1	20.1–32.5
LSsubendo, 3-ch (%)	25.3 ± 2.0	21.1–31.3
LSsubendo, 4-ch (%)	25.4 ± 2.4	19.9–31.8
LSsubendo, 2-ch (%)	25.3 ± 2.1	21.9–32.5
LSsubepi, average (%)	21.1 ± 1.8	17.0–26.9
LSsubepi, 3-ch (%)	20.9 ± 2.1	16.7–26.6
LSsubepi, 4-ch (%)	21.3 ± 2.1	16.6–27.5
LSsubepi, 2-ch (%)	21.0 ± 1.9	17.8–21.0
LS gradient	4.3 ± 1.2	1.6–7.4

BMI, body mass index; BP, blood pressure; DT, deceleration time; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GLS, global longitudinal strain; LAVi, left atrial volume index; LS, longitudinal strain; LV, left ventricular; subendo, subendocardial; subepi, subepicardial; SV, stroke volume; 2-ch, apical 2-chamber view; 3-ch, apical 3-chamber view; 4-ch, apical 4-chamber view.

reports the clinical characteristics and the main echo Doppler measurements of the study population, including the strain analysis. Subjects had normal LV geometry and LVEF. No subject had LV hypertrophy. The prevalence of Grade I of LV diastolic dysfunction was 7.9% ($n = 21$) according to the ASE/EACVI criteria (transmitral *E/A* ratio $\leq 0.8 + E$ velocity < 50 cm/s), whereas no subjects had Grade II or III of diastolic dysfunction. Transmural GLS was 22.7 ± 1.7%. LSsubendo was higher than LSsubepi ($P < 0.0001$), whereas no significant difference of GLS, LSsubendo, and LSsubepi was found among the three different apical chambers (three-, two-, and four; data not shown in table).

By gender-specific analyses, no difference of GLS, LSsubendo, LSsubepi, and LS gradient was found between men and women ($P = 0.149$) in the pooled population. However, restricting the analysis to subjects with age ranging between 20 and 50 years ($n = 134$), GLS (23.2 ± 1.9 in women and 22.5 ± 1.6%, $P < 0.01$), LSsubendo (26.0 ± 2.r vs. 25.2 ± 1.8%, $P < 0.02$), and LSsubepi (21.6 ± 2.0 vs. 20.9 ± 1.6%, $P < 0.02$) were significantly higher in women.

Figure 2 depicts regional layer-specific and transmural LS as well as bull's eye of LSsubendo, LSsubepi, and transmural GLS in a young man. *Table 2* lists the results of GLS, LSsubendo, LSsubepi, and LS gradient according to the age decades. GLS ($P < 0.001$), LSsubendo, and LSSubepi (both $P < 0.0001$) were all significantly reduced with increasing age, whereas LS gradient remained unchanged in the different age decades. *Post hoc* analyses demonstrated a significant reduction of GLS in the group ≥60 years in comparison with the age decades 10–19 and 20–29 and in the decade 50–59 vs. decade 10–19, a significant reduction of LSsubendo in subjects ≥60 years vs. decades 10–19, 20–29, and 30–39 and of decade 50–59 vs. 10–19, and a significant reduction of LSsubepi in the group ≥60 years vs. decades 10–19 and 20–29 and of decade 50–59 vs. 10–19. LSsubendo was significantly higher than LSsubepi (LS gradient) in each of the age decades (all $P < 0.0001$), without significant changes among the different decades.

Figure 3 depicts univariable correlations of age with GLS, LSsubendo, and LSsubepi (all $P < 0.001$) in the pooled population. GLS, LSsubendo, and LSsubepi were all inversely related with age, but a discrete number of subjects were above the upper and the lower limits of the 95% CI of the normal relation in the age ranging 30–60 years. GLS, LSsubendo, and LSsubepi were also significantly related with body mass index ($r = -0.26$, $r = -0.28$, $r = -0.28$ respectively, all $P < 0.0001$), diastolic BP ($r = -0.19$, $P < 0.002$; $r = -0.18$, $P = 0.004$; $r = 0.13$, $P = 0.04$) and, among the echo variables, with LVEF ($r = 0.17$, $P = 0.007$; $r = 0.12$, $P < 0.05$; $r = 0.13$, $P = 0.03$), relative wall thickness ($r = -0$, 21, $P < 0.001$; $r = -0.15$, $P < 0.01$; $r = -0.17$, $P \ll 0.005$), LV mass index ($r = -0.14$, $P < 0.02$; $r = -0.15$, $P < 0.01$; and $r = -0.17$, $P < 0.005$), and *E/e′* ($r = -0.12$, $P = 0.04$; $r = -0.12$, $P = 0.04$; $r = -0.16$, $P < 0.01$). Heart rate was not related to GLS ($r = 0.03$, $P = 0.665$), LSsubendo ($r = 0.073$, $P = 0.238$), and LSsubepi ($r = 0.078$, $P = 0.205$). Also stroke volume was not significantly related with GLS, LSsubendo, and LSsubepi.

Separate multiple linear regression analyses were performed separately for the three strain components, adjusting for demographic and echocardiographic confounders (*Table 3*). By these analyses, age was independently associated with GLS ($P = 0.004$) and more significantly with LSsubendo and LSsubepi (both $P < 0.0001$). Among the other correlates, only LVEF was independently associated with GLS ($P = 0.006$), LSsubendo ($P = 0.039$), and LSsubepi ($P = 0.025$).

Reproducibility analyses performed on the same set of images (i.e. same apical three, four-, and two-chamber views) as well as on two different sets of images (i.e. on different apical views acquired during the echo examination) in 20 of our healthy subjects are summarized in *Table 4*. The intra-observer and the inter-observer variability of GLS, LSsubendo, and LSsubepi were excellent and also the intra-observer variability on two different set of images remained optimal for GLS, LSsubendo, and LSsubepi.

Discussion

This study demonstrates that in normal subjects (i) LSsubendo is always higher than LSsubepi, independent on the effect of age; (ii) ageing exerts an independent influence on transmural, subendocardial, and subepicardial longitudinal strain, but not on strain gradient, whereas the effect of gender appears to be restricted to the age ranging between 20 and 50 years; (iii) the effect of age is marginal between 30 and 50 years, whereas the decline of GLS, LSsubendo, and, in particular, LSsubepi appears to be clinically relevant after the age of 50 years.

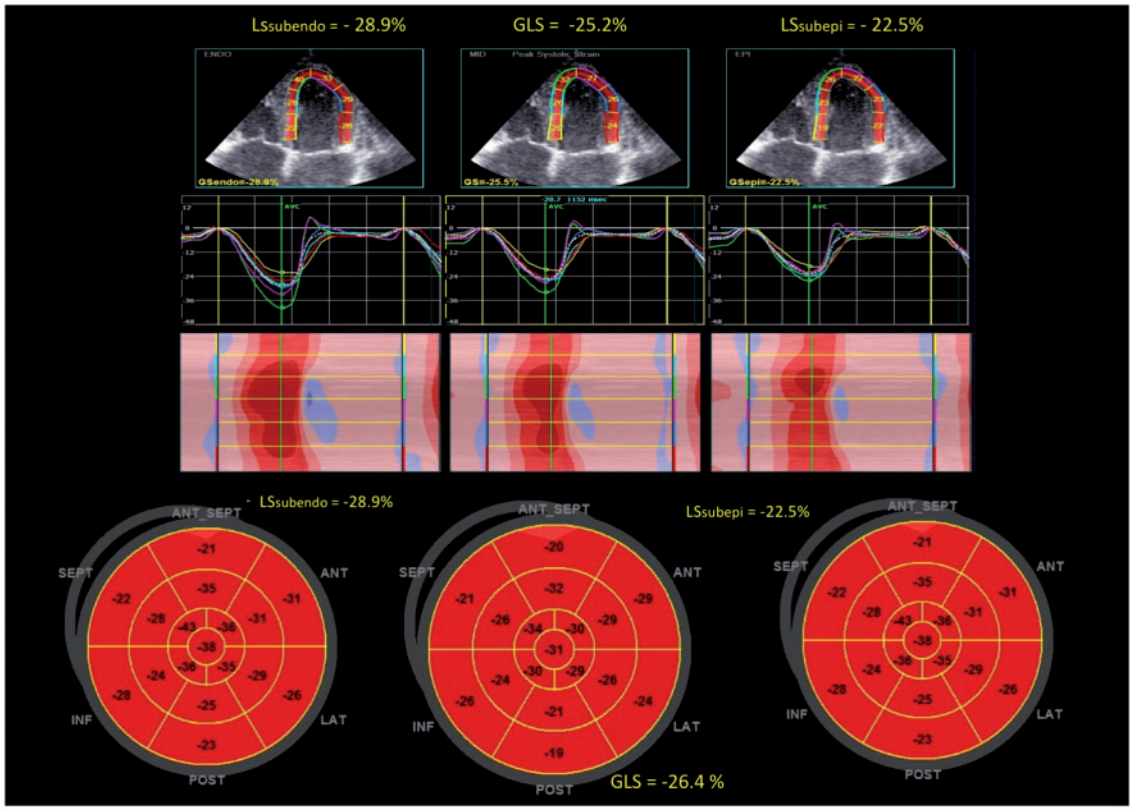

Figure 2 Sample of layer-specific LS in a 19-year-old man. Upper panel shows layer-specific and transmural LS (in the middle) of an apical four-chamber view, with the curves of regional strain values (dotted line corresponds to average strain) and qualitative colour M-mode strain. Lower panel shows bull's eye of regional LSsubendo, LSsubepi, and transmural GLS. GLS, global longitudinal strain; LSsubendo, subendocardial longitudinal strain; LS subepi, subepicardial longitudinal strain.

Table 2 Components of longitudinal strain according to age decades

Parameter	10–19 years ($n = 45$)	20–29 years ($n = 45$)	30–39 years ($n = 45$)	40–49 years ($n = 45$)	50–59 years ($n = 46$)	≥60 years ($n = 40$)	Cumulative *P* value
GLS (%)	23.5 ± 1.3 (23.1–23.9)	23.1 ± 1.8 (22.6–23.7)	22.6 ± 1.7 (22.1–23.1)	22.7 ± 1.7 (22.3–23.3)	22.3 ± 1.7** (21.8–22.8)	22.0 ± 1.8***,¶ (21.5–22.7)	<0.001
LSsubendo (%)	26.2 ± 1.5 (25.7–26.7)	25.9 ± 2.3 (25.2–26.6)	25.5 ± 1.9 (24.9–26.1)	25.3 ± 2.0 (24.7–25.9)	24.9 ± 2.2* (24.2–25.6)	24.2 ± 1.8****,¶¶¶,§ (23.6–24.8)	<0.0001
LSsubepi (%)	21.7 ± 1.4 (21.2–22.1)	21.5 ± 2.1 (20.9–22.2)	21.2 ± 1.7 (20.6–21.7)	21.0 ± 1.6 (20.6–21.6)	20.6 ± 1.6* (20.1–21.0)	20.2 ± 1.5***,¶¶¶ (19.7–20.7)	<0.0001
LS gradient	4.5 ± 0.8 (4.3–4.7)	4.4 ± 1.2 (4.0–4.8)	4.3 ± 1.4 (3.9–4.8)	4.3 ± 1.1 (3.9–4.6)	4.4 ± 1.3 (4.0–4.8)	4.0 ± 1.3 (3.6–4.5)	0.648

Values are expressed as mean ± standard deviation (95% confidence interval). LSsubendo is significantly higher than LSsubepi (LS gradient) in each of the age decades (all $P < 0.0001$).
Abbreviations as in Table 1. Values of GLS, LSsubendo and LSsubepi considered as 'positive' (sign +).
****$P < 0.0001$: vs. decade 10–19.
***$P < 0.001$: vs. decade 10–19.
**$P < 0.01$: vs. decade 10–19.
*$P < 0.05$: vs. decade 10–19.
¶¶¶$P < 0.001$: vs. decade 20–29.
¶$P < 0.05$: vs. decade 20–29.
§$P < 0.05$: vs. decade 30–39.

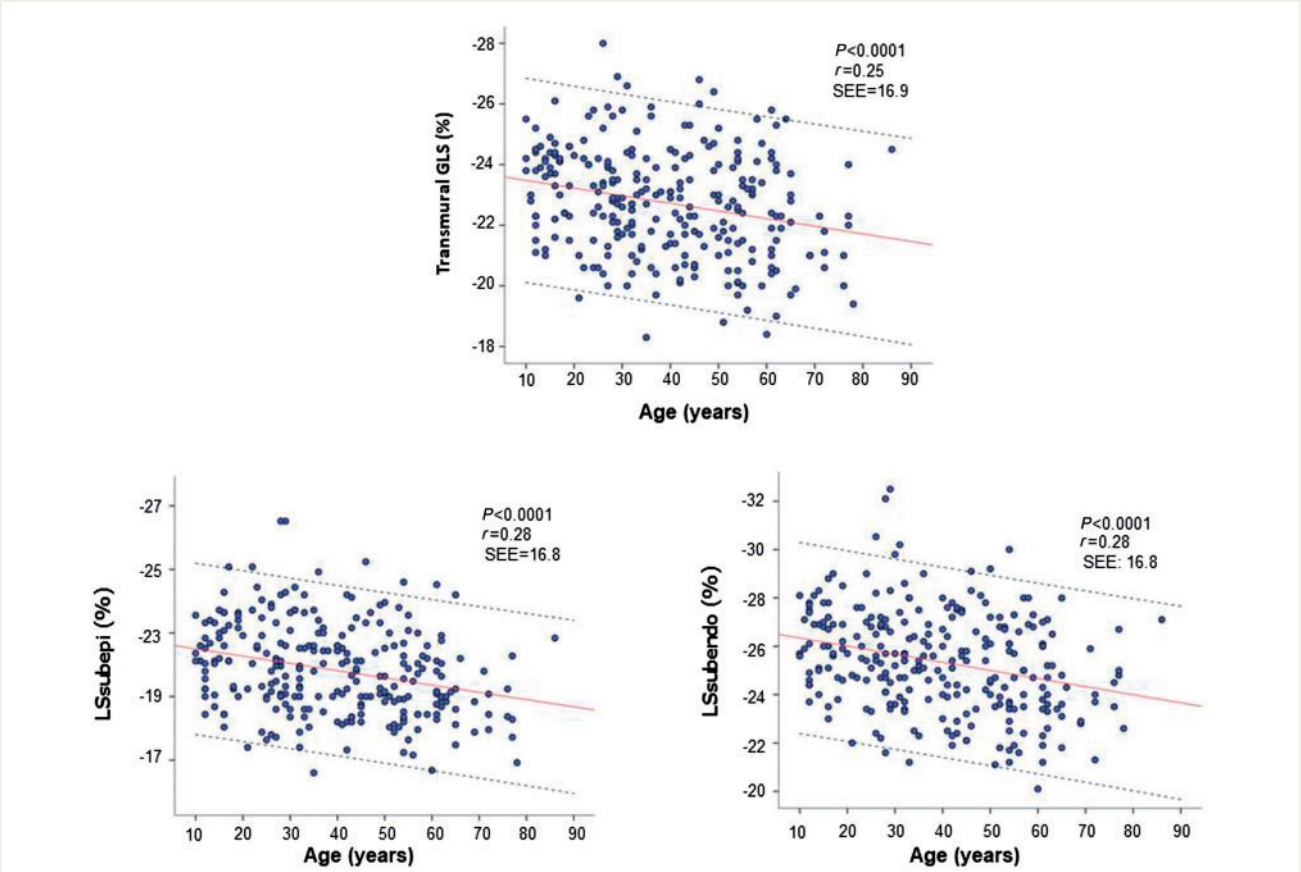

Figure 3 Relations of age (horizontal axis) with transmural GLS, LSsubendo, and LSsubepi (vertical axes) in the pooled population. Inverse relations are seen with all the strain components, with data points for a discrete number of subjects above the upper limit and the lower limit of the 95% confidence interval of the normal relation (parallel dotted lines). SEE, standard error estimate, other abbreviations as in *Figure 1*.

To the best of our knowledge, only two studies proposed normal reference values of layer-specific strain.[13,14] In the first study, prospectively performed on 119 healthy volunteers (age range = 22–76 years, 50% women), LSsubendo was substantially higher than LSsubepi, they being both influenced by gender, heart rate, and stroke volume but not by age.[13] In the second one, Nagata *et al.*[14] retrospectively collected data on 235 healthy subjects and presented normal reference values according to four age decades (from 20 years to 59 years). Layer-specific strain was not age, but gender dependent (GLS, LSsubendo, and LSsubepi were all higher in women), in agreement with the NORRE study in which GLS was higher in women than in men.[10] Our prospective investigation extends the results of these two studies, collecting data on 266 healthy subjects, consecutively recruited. The choice of analysing layer-specific strain according to six age decades (by including subjects between 10 and 19 and over than 60 years)—i.e. the greater dispersion of age values—is the likely reason of the age dependency which was not observed by Nagata *et al.* Conversely, we did not disclose a gender-specific difference of GLS and layer-specific strain in the overall population (age range = 10–86 years), but the subgroup with an age ranging between 20 and 50 years showed marginally higher values of GLS, LSsubendo, and LSsubepi in women. These results are only apparently discordant with the two previous studies dealing with this issue. In the NORRE study, GLS was higher in women than in men in a population that included mainly age groups 20–40 and 40–60, and only few subjects >60 years.[10] GLS and layer-specific strain of Nagata *et al.*[14] were higher in women in the age decades 20–29, 30–39, 40.49, and 50–59 but subjects <20 and ≥60 were not assessed. Hormonal effects in women of childbearing age could be postulated to explain our findings but this hypothesis needs further investigation. The absence of changes in LS gradient (we measured as the difference between LSsubendo and LSsubepi) with ageing confirms the data of Nagata *et al.* (who calculated LS gradient as the ratio of LSsubendo to LSsubepi).

In this study, additional insights were provided by the combination of univariable relations and multivariable models. The highly significant inverse linear relations of GLS, LSsubendo, and LSsubepi with age were substantially confirmed after adjusting for demographic and echocardiographic confounders. These variables were chosen on the grounds of univariable correlations, taking also into account inter-variable collinearity and their physiopathological value. Body mass index and diastolic BP are in fact raw indicators of preload and afterload respectively, both acting effectively on LS. Relative wall thickness is an accurate marker of LV concentric geometry, i.e. an important

Table 3 Multiple linear regression analyses in the pooled population

Dependent variable	Correlate	Standardized β coefficient	*P*-value	Collinearity tolerance
Transmural GLS	Male gender	0.025	0.688	0.925
	Age	-0.204	0.004	0.738
	BMI	-0.119	0.084	0.775
	Diastolic BP	-0.084	0.183	0.912
	Relative wall thickness	-0.099	0.785	0.922
	LV EF	0.167	0.006	0.976
Cumulative $R^2 = 0.15$, SEE = 1.62%, $P < 0.0001$				
LSsubendo	Male gender	0.083	0.189	0.925
	Age	-0.281	<0.0001	0.738
	BMI	-0.078	0.260	0.775
	Diastolic BP	-0.069	0.276	0.912
	Relative wall thickness	-0.904	0.904	0.922
	LV EF	0.128	0.039	0.976
Cumulative $R^2 = 0.14$, SEE = 1.94%, $P < 0.0001$				
LSsubepi	Male gender	0.110	0.083	0.925
	Age	-0.286	<0.0001	0.738
	BMI	-0.074	0.280	0.775
	Diastolic BP	-0.023	0.722	0.912
	Relative wall thickness	-0.042	0.510	0.922
	LV EF	0.139	0.025	0.976
Cumulative $R^2 = 0.14$, SEE = 1.67%, $P < 0.0001$				

Cumulative $R^2 = 0.14$, SEE = 1.67%, $P < 0.0001$.
SEE, standard error estimate. Other abbreviations as in *Table 1*.
Values of GLS, LSsubendo, and LSsubepi considered as 'positive' (sign +) to build the univariate relations to homogenize the results of analyses and strengthen their clinical meaning: the higher the values, the better the strain deformation independent on the plus/minus sign.

Table 4 Reproducibility of multilayer-specific longitudinal strain

Variables	Intra-class correlation (rho)	95% confidence interval	*P*-value
Intra-observer variability on the same set of images			
Transmural GLS, average (%)	0.978	0.954–0.990	<0.0001
LSsubendo, average (%)	0.977	0.951–0.989	<0.0001
LSsubepi GLS, average (%)	0.969	0.934–0.985	<0.0001
Inter-observer variability on the same set of images			
Transmural GLS, average (%)	0.958	0.912–0.980	<0.0001
LSsubendo, average (%)	0.938	0.870–0.980	<0.0001
LSsubepi GLS, average (%)	0.883	0.753–0.994	<0.0001
Intra-observer variability on two different sets of images			
Transmural GLS, average (%)	0.955	0.908–0.977	<0.0001
LSsubendo, average (%)	0.934	0.861–0.972	<0.0001
LSsubepi GLS, average (%)	0.877	0.750–0.989	<0.0001

determinant of GLS in the presence of normal LV systolic function.[19] LVEF is a recognized indicator of chamber systolic function.[12] However, by observing the 95% CI of the univariable relations (see *Figure 3*), it appears clear that a certain number of subjects were above the upper or the lower limits of the relations between age and GLS, LSsubendo, and LSsubepi, in the age range between 20 and 60 years. This finding reinforces the age decades analyses, where the reduction of GLS and layer-specific strain was significant in the decades 50–60 and ≥60. It is therefore conceivable that the age-dependent effect on longitudinal strain could became clinically

relevant in healthy subjects only in the old ages, starting from the 50th year, this being applicable to GLS and layer-specific strain as well.

Study limitations

Limitations of this study include the lack of validation of layer-specific strain against the gold standard, represented by cardiac magnetic resonance imaging,[20,21] and the application of single vendor-specific software. Although a good concordance of GLS between the two major vendors has been found in reference echocardiographic laboratories[22] and recently confirmed in the large sample size of the NORRE study,[10] the software dependence of 2D speckle-tracking echocardiography is recognized[23,24] and appears to be applicable also to layer-specific strain. Another limitation includes the lack of decades 70–79 and >80, which was shared with the Nagata *et al.*'s study, whereas the NORRE study combined 95 subjects of different ages in the group ≥60 years. The relatively small sample size of our Caucasian population, which was collected in a single centre, cannot be therefore generalized to the entire population. A large multicentre population could allow to deal more comprehensively with this issue. However, our healthy subjects were rigidly selected and represent therefore a reasonable sample in a single centre. The effort to create normal reference values of echo parameters, including strain, should be pursued in each laboratory.

Implications

The need for considering age-specific normal reference values is well established in the echocardiographic community when dealing with Doppler-derived measurements of LV diastolic function[18,25] and parameters obtainable by pulsed tissue Doppler of the mitral annulus.[18,26,27] This needs to be applied less restrictively to GLS and layer-specific longitudinal strain. The impact of ageing on GLS as well as on LSsubendo and LSsubepi should be in fact carefully taken into account only after the 50th year. These findings can have clinical implications when evaluating cardiac patients to differentiate with accuracy normalcy from pathology.

Conflict of interest: None declared.

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