

# Right atrial size and function assessed with three-dimensional and speckle-tracking echocardiography in 200 healthy volunteers

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## Aims

Right atrial (RA) size predicts the outcome in some pathological conditions but reference values for RA volumes and myocardial function remain to be defined. Thus, we used two-dimensional speckle-tracking echocardiography (2D-STE) and three-dimensional echocardiography (3DE) to define normative reference values of RA volumes and function.

## Methods and results

Two hundreds healthy volunteers (43 ± 15 years, range 18–75; 44% men) underwent two-dimensional echocardiography (2DE) to obtain RA volumes and longitudinal strain (LS) of RA wall using 2D-STE, and 3DE to measure maximal ( $V_{\max}$ ), minimal, and preA volumes to calculate total, passive, and active emptying volumes (TotEV, PassEV, and ActEV) and emptying fractions (TotEF, PassEF, and ActEF). Three-dimensional echocardiography volumes ( $V_{\max}$ , 52 ± 15 mL vs. 41 ± 14 mL), EVs (TotEV, 33 ± 10 mL vs. 24 ± 9 mL), and EFs (TotEF, 63 ± 9 vs. 58 ± 9%) were larger than 2DE ones (all  $P < 0.0001$ ). Indexed 3D volumes were significantly larger in men than in women. RA TotEF correlated with total LS ( $r = 0.24$ ,  $P = 0.025$ ) and PassEF with positive LS (LSpos;  $r = 0.34$ ,  $P < 0.0001$ ). Ageing was associated with a decrease in passive (LSpos,  $r = -0.41$ ; PassEV,  $r = -0.26$ ; PassEF,  $r = -0.38$ ; all  $P < 0.0001$ ) and an increase in active RA function (negative LS,  $r = 0.34$ ; ActEV,  $r = 0.25$ ; all  $P < 0.0001$ ; and ActEF,  $r = 0.15$ ;  $P = 0.035$ ) in order to maintain TotEV ( $r = -0.14$ ,  $P = 0.05$ ).

## Conclusion

Our study provides normative values for RA volumes and function measured by 3DE and 2D-STE in a relatively large cohort of healthy subjects with a wide age range. These data will help clinicians to identify RA remodelling and dysfunction.

## Keywords

Right atrium • Three-dimensional echocardiography • Speckle-tracking echocardiography • Reference values • Longitudinal strain

## Introduction

The right atrium (RA) plays an integral role in cardiac performance by modulating right ventricular function with its reservoir, conduit, and contractile functions.<sup>1</sup> Recent reports have documented that the RA size enlarges in patients with atrial arrhythmias, chronic heart failure, pulmonary hypertension, and congenital heart diseases.<sup>2–4</sup> Although data on clinical significance of RA enlargement are sparse, two-dimensional echocardiography (2DE) RA area has been demonstrated to be a predictor of the outcome in primary pulmonary hypertension and chronic heart failure.<sup>3,5,6</sup>

Conventional assessment of RA size includes the measurement of RA diameters and area on 2DE four-chamber apical view.<sup>7,8</sup> RA volumetric assessment is not currently recommended.<sup>7,8</sup> In addition, 2DE calculation of atrial volume is limited by view dependency and geometric assumptions about atrial shape. However, atrial remodelling as a consequence of disease processes is often asymmetrical, rendering these assumptions inadequate. Three-dimensional echocardiography (3DE) overcomes these limitations enabling accurate atrial volume measurement.<sup>9,10</sup> Moreover, 3DE enables the assessment of atrial phasic volume changes, permitting to describe atrial function over its size.<sup>11</sup>

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In addition to RA chamber function, RA myocardial mechanics is needed to detect subclinical dysfunction and differentiate among several pathophysiological situations.<sup>12</sup> Two-dimensional speckle-tracking echocardiography (2D-STE) has been shown to be feasible to investigate RA myocardial mechanics.<sup>13</sup>

Knowledge of reference echocardiographic values of RA volumes and myocardial function is a prerequisite for the introduction of RA quantitation in the clinical routine. At present, only very few studies reported the normal values of 3DE RA maximal ( $V_{\max}$ ) and minimal ( $V_{\min}$ ) volumes, but they did not report phasic RA function parameters.<sup>14,15</sup> Padeletti *et al.*<sup>13</sup> reported normal values of RA myocardial function with STE, but they studied a limited number of subjects and the timing of events was set according to the ventricular cycle (i.e. R–R on ECG tracing). Finally, there is no report studying the relationship between RA volumetric function assessed by 3DE and RA myocardial wall mechanics measured with STE.

Accordingly, we have designed this prospective, observational study to (i) determine the normative reference values for RA geometry and function in a large cohort of healthy subjects using 3DE and 2D-STE, (ii) analyse the effects of age and gender on these parameters, (iii) compare the parameters measured by 3DE with the same values calculated using 2DE, and (iv) compare RA volumetric function parameters with RA myocardial mechanics assessed with 2D-STE.

## Methods

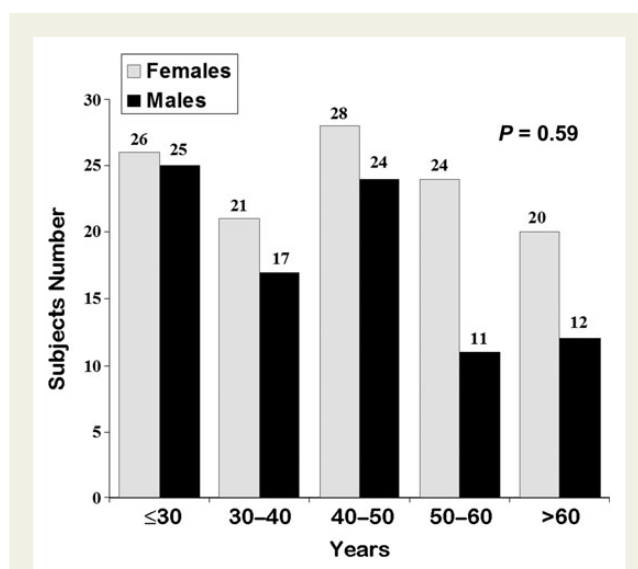
### Study population

Between October 2011 and May 2012, 210 healthy volunteers with a wide age range (Figure 1) were prospectively recruited among hospital employees, fellows in training, their parents, and people who underwent medical visits for driving or working license and met the inclusion criteria. Prospective criteria for recruitment included: age >17 years, no previous history of cardiovascular or lung disease, no symptoms, absence of cardiovascular risk factors (i.e. systemic arterial hypertension, smoking, diabetes, hypercholesterolaemia, and familial history of cardiovascular disease), normal electrocardiogram, physical examination, and no cardio-active treatment. Exclusion criteria included trained athletes, pregnancy, and body mass index >30 kg/m<sup>2</sup>.

The study was approved by the University of Padua Ethics Committee (protocol # 2380 P, approved on 6 October 2011) and all volunteers signed an informed consent before undergoing physical examination, blood pressure and anthropometric measurement, and echocardiography. Body surface area (BSA) was calculated according to the formula by DuBois and DuBois.<sup>16</sup>

### Image acquisition

Study subjects underwent standard transthoracic echocardiography using a commercially available Vivid E9 ultrasound machine (GE Healthcare, Horten, Norway) equipped with M5S probe. Two-dimensional echocardiography studies included an apical four-chamber view optimized for RA.<sup>8</sup> Three consecutive heart cycles were recorded during breath-hold with stable ECG tracing, in order to minimize respiratory movements and obtain images suitable for RA size quantitation<sup>8</sup> and 2D-STE analysis.<sup>17</sup> All patients were examined in the left lateral decubitus position using greyscale second-harmonic 2D imaging technique, with the adjustment of image contrast, frequency, depth, and sector size for adequate frame rate and optimal RA border visualization.



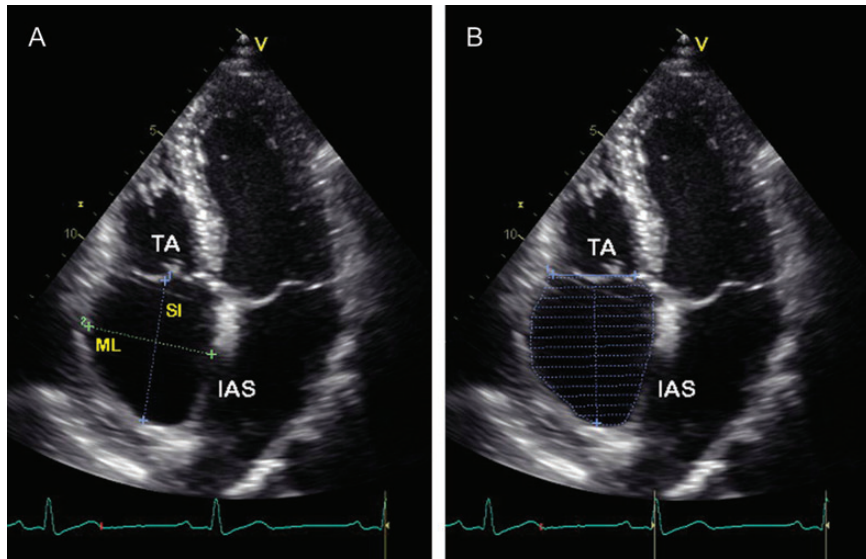
**Figure 1.** Study population age distribution according to gender. Age distribution of the study population, divided by gender.

At the end of the 2DE examination, consecutive four-beat ECG-gated subvolumes were acquired during breath-hold to generate the full-volume 3DE data set of the RA using a 4V matrix-array transducer (GE Healthcare, Horten, Norway) from the apical approach, taking care to encompass the entire RA cavity in the data set. Data sets were stored digitally and exported to a separate workstation for offline analysis.

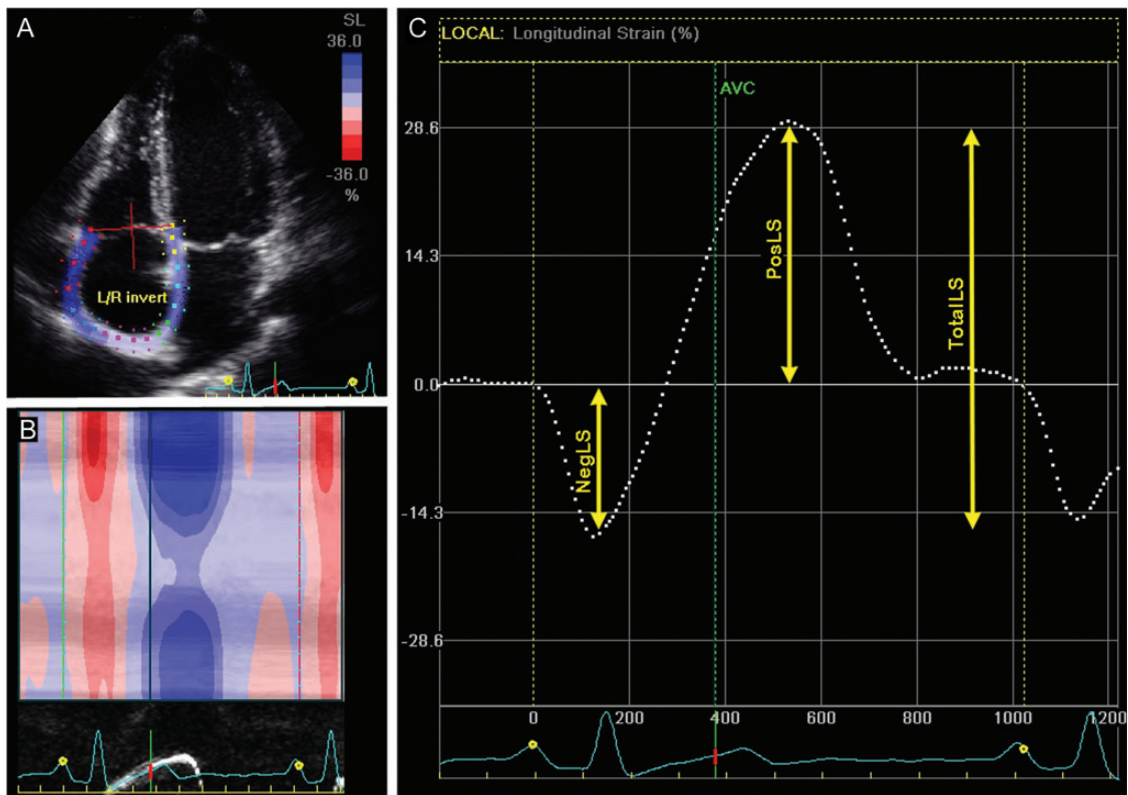
### Image analysis

Two-dimensional echocardiography images were analysed using EchoPAC v110.1.3 (GE-Healthcare, Horten, Norway). The right ventricular end-systolic frame corresponding to the largest RA area, just before the tricuspid valve opening, was identified in the dedicated apical four-chamber view. Supero-inferior and medio-lateral diameters were measured according to current guidelines.<sup>8</sup> Then endocardial border was manually traced in the same frame paying attention to exclude the area between the tricuspid leaflets and annulus to obtain RA area (Figure 2). RA  $V_{\max}$  was automatically calculated by the software using the area-length method.<sup>7</sup> Using the same procedure, we also measured the RA  $V_{\min}$  as the smallest RA cavity during the cardiac cycle, just before tricuspid valve closure, and pre-A volume ( $V_{\text{preA}}$ ) as the RA volume corresponding at P-wave peak on the ECG tracing.

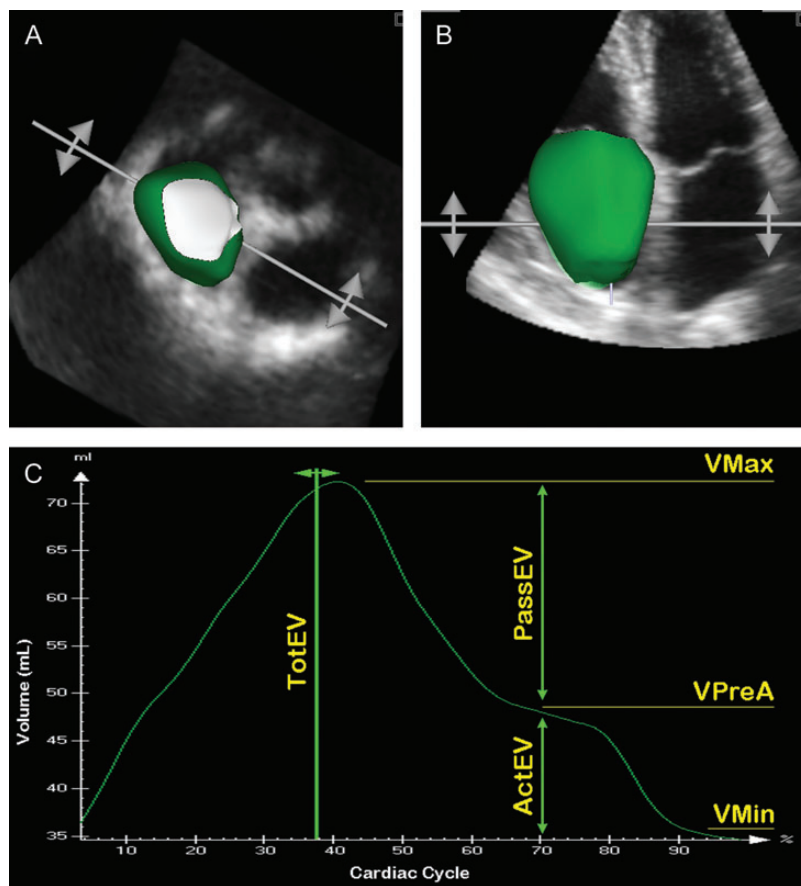
To assess the RA myocardial function with 2D-STE, the RA endocardium was manually traced when the RA was at its minimum volume after contraction. Then the software automatically generated a 15-mm wide region of interest. Shape and width of the region of interest were manually adjusted. A cine loop preview feature allowed visual confirmation that the internal line followed the RA endocardium movements throughout the cardiac cycle. If tracking of the RA endocardium was unsatisfactory, manual adjustments of the region of interest size were performed to ensure optimal tracking. The analysis was then approved and an average longitudinal strain (LS) curve was automatically generated, which included a negative deflection (LSneg), representing RA active contraction, followed by a positive one (LSpos) during RA filling (Figure 3).<sup>12</sup> LSneg, LSpos, and their summation (LStot) were recorded.



**Figure 2.** Two-dimensional echocardiography assessment of right atrial (RA) size. (A) RA supero-inferior (SI) and medio-lateral (ML) diameters measured on a dedicated four-chamber view. (B) RA area tracing. IAS, interatrial septum; TA, tricuspid annulus.



**Figure 3.** Speckle-tracking echocardiography of the right atrium (RA). (A) Apical four-chamber view with the 2D STE region of interest encompassing the RA myocardium. (B) Curved colour M-mode of RA longitudinal strain LS(%) of all walls throughout the cardiac cycle. (C) Average LS (%) time curve from which negative (NegLS), positive (PosLS), and total LS are measured.



**Figure 4.** Three-dimensional echocardiography assessment of the right atrial (RA) size and function. Transversal (A) and longitudinal (B) visualization of a 3D RA surface superimposed on grey scale 3DE data set, and time–volume curve depicting RA volume changes during the cardiac cycle (C). From this time–volume curve, the values for  $V_{\max}$ ,  $V_{\min}$ , and  $V_{\text{preA}}$  RA volumes can be obtained, from which passive (PassEV), active (ActEV), and total (TotEV) emptying volumes has been calculated.

Three-dimensional echocardiography RA analysis was performed using a dedicated software designed for volumetric analysis of the left atrium (LA analysis, Tomtec Imaging Systems, Unterschleissheim, Germany) and recently validated against cardiac magnetic resonance (CMR).<sup>9</sup> At the beginning of the 3D analysis workflow, RA data set was automatically sliced four-, two-chamber, and long-axis apical planes and a short-axis plane. Rapid manual data set alignment was performed by translating and rotating the four-chamber plane in order to obtain orthogonal non-foreshortened planes of the RA in all three apical views. In each apical view, the RA blood–tissue interface was manually initialized on two frames identifying RA  $V_{\max}$  and  $V_{\min}$ . These initialized RA endocardial boundaries were used to reconstruct the RA endocardial surface. A 3D surface of the RA volume was then generated for each frame throughout the cardiac cycle resulting in a dynamic cast of the RA cavity (Figure 4, see Supplementary data online, Video S1). For each consecutive frame, the voxel count inside the 3D RA surface was used to measure RA volume, resulting in a smooth interpolated time–volume curve from which  $V_{\max}$ ,  $V_{\min}$ , and  $V_{\text{preA}}$  can be easily obtained (Figure 5). From RA volumes, we calculated total emptying volume (TotEV), which represents the RA reservoir function, as the difference between  $V_{\max}$  and  $V_{\min}$ ; passive EV (PassEV), which represents conduit function, as the difference between  $V_{\max}$  and  $V_{\text{preA}}$ ; and active EV (ActEV), which corresponds

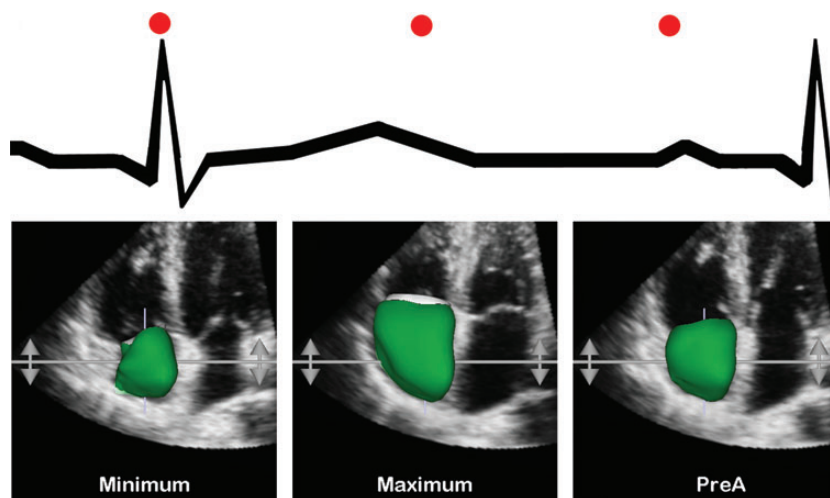
to RA booster function, as the difference between  $V_{\text{preA}}$  and  $V_{\min}$ .<sup>1,12</sup> Accordingly, total emptying fraction (TotEF) was  $\text{TotEV}/V_{\max}$ , passive EF (PassEF) was  $\text{PassEV}/V_{\max}$ , and active EF (ActEF) was  $\text{ActEV}/V_{\text{preA}}$ .

### Statistical analysis

The normal distribution of study variables was checked using the Kolmogorov–Smirnov test. Continuous variables were summarized as mean  $\pm$  SD if normally distributed. Variables were compared between men and women using the unpaired t-test. Three-dimensional echocardiography and 2DE measurements obtained from the same subject were compared using the paired t-test. Pearson correlation was used to analyse the relationships between RA size and function parameters and age, and between volumetric and 2D-STE function parameters.

Intra- and inter-observer variability for 2DE and 3DE RA volumes and 2D-STE function parameters was analysed in 15 random subjects using Bland–Altman method. To obtain intra-observer variability, one observer evaluated the same studies on a separate occasion. For the inter-observer variability assessment, two independent observers performed the analysis.

All analyses were performed using SPSS 19.0 (SPSS, Inc., Chicago, IL, USA) and MedCalc (version 10.0.1 MedCalc Software, Mariakerke,



**Figure 5.** 3DE dynamic quantitation of right atrial (RA) volumes and function. The 3D RA surface is displayed at three different phases of the cardiac cycle, namely the  $V_{\min}$ ,  $V_{\max}$ , and  $V_{\text{preA}}$  RA, along with the electrocardiogram to show temporization of RA volume changes.

**Table 1.** Demographic characteristics of the study population

	Overall (n = 200)	Men (n = 87)	Women (n = 113)	P-value*
Age (years)	43 ± 15	41 ± 14	45 ± 14	0.078
Height (cm)	170 ± 10	177 ± 7	164 ± 8	< 0.0001
Weight (kg)	67 ± 11	76 ± 9	61 ± 9	< 0.0001
Body mass index (kg/m <sup>2</sup> )	23 ± 3	24 ± 2	23 ± 3	0.001
BSA (m <sup>2</sup> )	1.78 ± 0.19	1.93 ± 0.13	1.67 ± 0.15	< 0.0001
Heart rate (bpm)	68 ± 11	67 ± 12	69 ± 11	0.15
Systolic blood pressure (mmHg)	122 ± 14	127 ± 11	118 ± 14	< 0.0001
Diastolic blood pressure (mmHg)	74 ± 8	76 ± 7	71 ± 8	< 0.0001

\*P-values refer to Gender differences.

Belgium). Differences among variables were considered significant at  $P < 0.05$ . Upper and lower limits of normality were computed as the mean value plus or minus 2 SD.

## Results

Six volunteers met all criteria for inclusion but were excluded from enrolment because their acoustic window was inadequate for 2DE and 3DE analysis. Among the remaining 204 subjects, 9 could not be evaluated by 2D-STE and 4 by 3DE due to poor image quality. Thus, in our study population, feasibility of RA LS by 2D-STE was 92.8%, while the feasibility of 3DE volume measurements was 95.2%. Temporal resolution of 2DE images for STE was 73 frames/s, and that of 3DE data sets was  $34 \pm 9$  volumes/s.

Demographic characteristics of the study population are summarized in Table 1. Fifty-six per cent of study subjects were women. There were no differences in age and heart rate between men and women (Table 1). Women showed significantly smaller body size and lower blood pressure compared with men (Table 1).

RA size and function parameters obtained with 2DE, 3DE, and 2D-STE are summarized in Table 2. Irrespective on the echo technique used, parameters of RA size were significantly larger in men than in women, even after normalization for BSA. Upper limits for 3DE RA  $V_{\max}$ ,  $V_{\min}$ , and  $V_{\text{preA}}$  were 49, 20, and 27 mL/m<sup>2</sup> in men and 38, 16, and 22 mL/m<sup>2</sup> in women. Three-dimensional echocardiography RA TotEV, PassEV, and ActEV were higher in men, but these differences disappeared after indexing for BSA (Table 2). Lower limits of normality for 3DE RA indexed TotEV, PassEV, and ActEV were 11, 6, and 2 mL/m<sup>2</sup>. Three-dimensional RA TotEF, PassEF, and ActEF were higher in women (Table 2). Lower limits of reference values for 3DE RA TotEF, RA PassEF, and RA ActEF were 44, 27, and 18% in men and 50, 22, and 19% in women, respectively. RA total and LSpos were higher in women, while LSneg was similar in men and women (Table 2).

Table 3 summarizes the relationships between age, 3DE RA volumes and function parameters, and 2D-STE LS. TotEV did not change significantly with age, but PassEV decreased and ActEV increased with age in both genders. Similarly, PassEF decreased

**Table 2.** Right atrial size and function by 2DE and 3DE echocardiography and right atrial myocardial wall function by 2D-STE in healthy subjects

	Overall (n = 200)	Men (n = 87)	Women (n = 113)	P-value*
RA max diameter (mm)	46 ± 5	49 ± 5	44 ± 4	<0.0001
RA min diameter (mm)	38 ± 6	41 ± 6	36 ± 5	<0.0001
RA area (cm <sup>2</sup> )	15 ± 3	17 ± 3	13 ± 2	<0.0001
RA area/BSA (cm <sup>2</sup> /m <sup>2</sup> )	8.4 ± 1.5	8.9 ± 1.6	8.1 ± 1.3	<0.0001
2D RA V <sub>max</sub> (mL)	41 ± 14	50 ± 15	35 ± 10	<0.0001
2D RA V <sub>max</sub> /BSA (mL/m <sup>2</sup> )	23 ± 7	26 ± 7	21 ± 6	<0.0001
2D RA V <sub>min</sub> (mL)	17 ± 7	21 ± 7	14 ± 5	<0.0001
2D RA V <sub>min</sub> /BSA (mL/m <sup>2</sup> )	10 ± 4	11 ± 4	8 ± 3	<0.0001
2D RA V <sub>preA</sub> (mL)	27 ± 11	33 ± 11	22 ± 8	<0.0001
2D RA V <sub>preA</sub> /BSA (mL/m <sup>2</sup> )	15 ± 5	17 ± 5	13 ± 4	<0.0001
3D RA V <sub>max</sub> (mL)	52 ± 15	60 ± 16	45 ± 11	<0.0001
3D RA V <sub>max</sub> /BSA (mL/m <sup>2</sup> )	29 ± 7	31 ± 8	27 ± 6	<0.0001
3D RA V <sub>min</sub> (mL)	19 ± 8	24 ± 8	16 ± 5	<0.0001
3D RA V <sub>min</sub> /BSA (mL/m <sup>2</sup> )	11 ± 4	12 ± 4	9 ± 3	<0.0001
3D RA V <sub>preA</sub> (mL)	28 ± 10	34 ± 10	24 ± 7	<0.0001
3D RA V <sub>preA</sub> /BSA (mL/m <sup>2</sup> )	16 ± 5	18 ± 5	14 ± 4	<0.0001
3D RA TotEV (mL)	33 ± 10	37 ± 11	30 ± 8	<0.0001
3D RA TotEV/BSA (mL/m <sup>2</sup> )	18 ± 5	19 ± 5	18 ± 5	0.22
3D PassEV (mL)	24 ± 9	27 ± 10	22 ± 8	<0.0001
3D PassEV/BSA (mL/m <sup>2</sup> )	13 ± 5	14 ± 5	13 ± 4	0.34
3D RA ActEV (mL)	9 ± 4	10 ± 4	8 ± 4	0.001
3D RA ActEV/BSA (mL/m <sup>2</sup> )	5 ± 2	5 ± 2	5 ± 2	0.37
3D RA TotEF (%)	63 ± 9	61 ± 8	65 ± 8	<0.0001
3D RA PassEF (%)	46 ± 11	44 ± 10	48 ± 12	0.013
3D RA ActEF (%)	31 ± 8	29 ± 7	33 ± 9	0.002
	(n = 195)	(n = 85)	(n = 110)	
2D RA LSpos (%)	27 ± 9	25 ± 9	28 ± 10	0.02
2D RA LSneg (%)	-17 ± 4	-17 ± 4	-17 ± 4	0.62
2D RA total LS (%)	44 ± 10	42 ± 9	45 ± 10	0.014

3DE, three-dimensional echocardiography; 2D, two-dimensional; EF, emptying fraction; EV, emptying volume; LS, longitudinal strain; RA, right atrium; V<sub>max</sub>, maximum volume; V<sub>min</sub>, minimum volume; and V<sub>preA</sub>, preA volume.

\*P-values refer to Gender differences.

with age and ActEF increased with a mild decrease of TotEF in both genders (Table 3). A similar relationship with age was found for parameters describing RA myocardial mechanics with 2D-STE, LSpos decreased with age associated with an increase of LSneg and a mild decrease of LStot (Table 3).

RA volumes calculated on 2DE images were significantly smaller than those measured using 3DE (Table 4 and Figure 6). TotEV, PassEV, TotEF, and PassEF obtained with 2DE were lower than those obtained by 3DE (Table 4).

LS parameters were significantly correlated with the volumetric function parameters measured with 3DE. Total LS was positively correlated with TotEV ( $r = 0.22$ ,  $P = 0.04$ ) and TotEF ( $r = 0.24$ ,  $P = 0.025$ ). LSpos was negatively correlated with V<sub>preA</sub> ( $r = -0.20$ ,  $P = 0.005$ ) and positively correlated with PassEV ( $r = 0.23$ ,  $P = 0.002$ ) and EF ( $r = 0.34$ ,  $P < 0.0001$ ). LSneg was positively correlated with ActEF ( $r = 0.21$ ,  $P = 0.003$ ).

Intra- and inter-observer variability of 2DE and 3DE RA volumes and LS at 2D-STE are shown in Table 5. All three techniques

showed very good intra- and inter-observer reproducibility. However, inter-observer reproducibility of 3DE RA volumes was significantly better than that of 2DE RA volumes.

## Discussion

The present study provides the normative reference values for RA size and function measured by 3DE and 2D-STE in a relatively large cohort of healthy volunteers with a wide age range. We found that: (i) RA assessment with 3DE and 2D-STE is feasible and reproducible, (ii) Three-dimensional echocardiography allows accessing not only RA volumes, but also its phasic function parameters, (iii) RA volume values obtained with 3DE cannot be used interchangeably with 2DE-derived volumes, (iv) reference values of RA volumes should be gender-specific while reference values for RA function should be age-specific, and (v) RA myocardial mechanics parameters obtained with 2DE-STE correlate with 3DE phasic volumetric parameters.

**Table 3** Relationship between age and right atrial volumes and function parameters assessed by 3DE and 2D-STE

	All (n = 200)		Men (n = 87)		Women (n = 113)	
	r	P-value	r	P-value	r	P-value
3D RA V <sub>max</sub> (mL)	-0.05	0.52	0.016	0.88	0.031	0.75
3D RA V <sub>min</sub> (mL)	0.10	0.14	0.17	0.12	0.26	0.005
3D RA V <sub>preA</sub> (mL)	0.16	0.026	0.21	0.05	0.32	<0.0001
3D RA TotEV (mL)	-0.14	0.05	-0.10	0.34	-0.10	0.26
3D RA PassEV (mL)	-0.26	<0.0001	-0.23	0.04	-0.25	0.008
3D RA ActEV (mL)	0.25	<0.0001	0.31	0.003	0.27	0.004
3D RA TotEF (%)	-0.24	0.001	-0.26	0.015	-0.31	0.001
3D RA PassEF (%)	-0.38	<0.0001	-0.43	<0.0001	-0.40	<0.0001
3D RA ActEF (%)	0.15	0.035	0.04	0.71	0.17	0.06
	n = 195		n = 85		n = 110	
2D RA LS pos	-0.41	<0.0001	-0.46	<0.0001	-0.43	<0.0001
2D RA LS neg	-0.335	<0.0001	-0.39	<0.0001	-0.28	<0.0001
2D RA LS tot	-0.271	<0.0001	-0.27	0.012	-0.31	0.001

3DE, three-dimensional echocardiography; 2D, two-dimensional; EF, emptying fraction; EV, emptying volume; LS, longitudinal strain; RA, right atrium; V<sub>max</sub>, maximum volume; V<sub>min</sub>, minimum volume; and V<sub>preA</sub>, preA volume.

**Table 4** Comparison between 3DE and 2DE parameters of RA size and function

	3DE	2DE	P-value	Limit 3D	Limit 2D
V <sub>max</sub> (mL)	52 ± 15	41 ± 14	<0.0001	78 <sup>a</sup>	69 <sup>a</sup>
V <sub>min</sub> (mL)	19 ± 8	17 ± 7	<0.0001	36 <sup>a</sup>	33 <sup>a</sup>
V <sub>preA</sub> (mL)	28 ± 10	27 ± 11	<0.0001	49 <sup>a</sup>	49 <sup>a</sup>
Total SV (mL)	33 ± 10	24 ± 9	<0.0001	17 <sup>b</sup>	11 <sup>b</sup>
Passive SV (mL)	24 ± 9	14 ± 7	<0.0001	10 <sup>b</sup>	4 <sup>b</sup>
True SV (mL)	9 ± 4	10 ± 5	0.017	4 <sup>b</sup>	3 <sup>b</sup>
TotEF (%)	63 ± 9	58 ± 9	<0.0001	49 <sup>b</sup>	42 <sup>b</sup>
PassEF (%)	46 ± 11	34 ± 12	<0.0001	24 <sup>b</sup>	14 <sup>b</sup>
True EF (%)	31 ± 8	35 ± 11	<0.0001	18 <sup>b</sup>	17 <sup>b</sup>

3DE, three-dimensional echocardiography; 2DE, two-dimensional echocardiography; EF, ejection fraction; RA, right atrium; SV, stroke volume; V<sub>max</sub>, maximal volume; V<sub>min</sub>, minimal volume; and V<sub>preA</sub>, preA volume.

<sup>a</sup>upper limit.

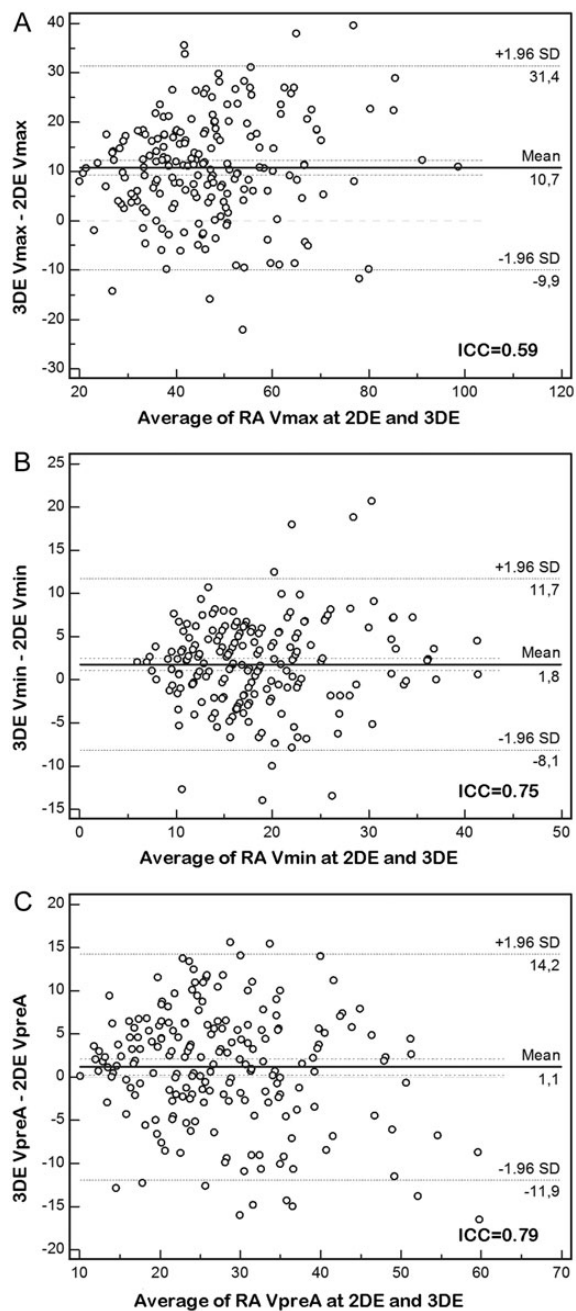
<sup>b</sup>lower limit.

There is an increasing evidence that RA enlargement is an outcome predictor in pathologic conditions such as primary pulmonary hypertension and chronic heart failure.<sup>3-5</sup> However, before introducing RA size and function assessment in the routine of the echo laboratories, the reference values for these parameters should be identified. At present, diameters and area measured in a 2DE apical four-chamber view are the only recommended methods to assess RA size, while RA volume computation is not included in routine echocardiography because of the lack of reference data.<sup>7,8</sup> In the present study, we used 3DE to obtain reference values of RA volume and function because of recognized superiority of 3DE over 2DE in heart chamber measurement.<sup>18</sup>

In particular, a previous study by Keller et al.<sup>10</sup> demonstrated that the 3DE measurement of RA volume is comparable with CMR and it is superior to current 2DE techniques. In addition, the software we have used to quantitate 3DE RA datasets has recently been validated against CMR in a multicentre validation study of 3DE left atrial volumes.<sup>9</sup> Finally, our results about RA V<sub>max</sub> (upper limit 49 vs. 50 mL/m<sup>2</sup>, and 38 vs. 41 mL/m<sup>2</sup>, for men and women, respectively) and TotEF (lower limit 49 vs. 46%) are very similar to those previously reported by Aune et al.<sup>14</sup> Therefore, we are confident that our study has provided robust data about the reference values of RA size and function. There is only another study that provides RA volume. However, it must be noticed that data provided by Quraini et al.<sup>15</sup> were calculated on multiplane 2DE images extrapolated by 3DE data sets obtained from a limited number of healthy subjects and they provided only maximal volumes.

Compared with the study performed by Aune et al.,<sup>14</sup> we were able to measure the RA V<sub>preA</sub> which allowed us to assess also the conduit and booster functions of the RA, providing a comprehensive assessment of RA phasic functions. Finally, 2D-STE analysis of RA myocardium LS showed significant correlations with most of RA volumetric phasic function parameters.

Assessment of RA phasic function parameters is clinically important, because the RA is a dynamic structure whose role is to assist filling the right ventricle. The three components of RA function are: (i) reservoir function, storing blood when the tricuspid valve is closed, (ii) conduit function, passive blood transfer directly from the coronary and systemic veins to the right ventricle when the tricuspid valve is open, and (iii) booster pump function, atrial contraction in late diastole to complete ventricular filling.<sup>1</sup> RA is able to modulate the relationship between reservoir and conduit function in a dynamic manner. The RA conduit-to-reservoir ratio is directly related to the right ventricular pressure-RA pressure gradient at the time of RA V<sub>max</sub>, with increased ventricular



**Figure 6.** Comparison of RA volumes between 3DE and 2DE. Bland–Altman plots comparing RA  $V_{max}$ ,  $V_{min}$ , and  $V_{preA}$  volumes obtained with 3DE and 2DE.

pressures favouring conduit function, but it is inversely related to cardiac output, with an increase in the reservoir contribution favouring improved cardiac output.<sup>1</sup> Since pathological conditions could affect and modify RA phasic functions, there is a need to know reference values of each phase to be able to understand the pathophysiology of RA function in various cardiac diseases.

In comparison to the number of studies published about the LA, few investigators have looked at RA geometry and function. In

**Table 5** Intra- and inter-observer variability of two- and three-dimensional right atrial volumes and LS

	Intra-observer variability		Inter-observer variability			
	Bias	95% CI	ICC	Bias	95% CI	ICC
2DE $V_{max}$ (mL)	-1.2	-14.3; 11.9	0.91	-1.3	-19.2; 16.6	0.85
2DE $V_{min}$ (mL)	-0.2	-4.9; 4.5	0.95	-5.3	-15; 4.3	0.56
2DE $V_{preA}$ (mL)	-1.7	-8.3; 4.9	0.94	-5.3	-21.3; 10.8	0.69
3DE $V_{max}$ (mL)	0	-9.6; 9.7	0.94	3.7	-9.1; 16.5	0.86
3DE $V_{min}$ (mL)	1	-3.4; 5.5	0.95	-1.5	-7.3; 4.4	0.91
3DE $V_{preA}$ (mL)	-0.2	-10.6; 10.2	0.9	-1	-9.3; 7.2	0.92
LSpos (%)	1.3	-6.5; 9.1	0.9	-3	-10.5; 4.6	0.87
LSneg (%)	-0.8	-7.9; 6.4	0.45	0.5	-4.7; 5.8	0.52

3DE, three-dimensional; 2DE, two-dimensional; LS, longitudinal strain;  $V_{max}$ , maximum volume;  $V_{min}$ , minimum volume; and  $V_{preA}$ , preA volume.

addition to RA phasic function parameters, we have also assessed RA myocardium mechanics by using 2DE-STE to obtain LS. When applying STE to measure left or RA LS, it is important to consider the different timing of atrial mechanics in comparison with ventricular one. In our study, we timed our strain analysis to the P-wave on the ECG (atrial contraction); therefore, active RA contraction is shown as an early ‘negative’ strain (myocardial shortening), which is followed by a large positive wave describing RA myocardial lengthening during passive conduit function. Overall RA reservoir function is represented by ‘total’ strain, the sum of all strain components when the RA fills from the  $V_{min}$  to  $V_{max}$ .<sup>19</sup>

Our results showed significantly different RA volumes between men and women even after indexing for BSA, suggesting the need for gender-specific reference values. These findings are consistent with data reported by Aune *et al.*<sup>14</sup> RA EF was also higher in women than in men.

In our study, there was no relation between age and  $V_{max}$  and  $V_{min}$ , confirming previous findings.<sup>14</sup> Conversely,  $V_{preA}$  increased significantly with age. This finding parallels the age-related changes in RA function with a significant decrease in passive RA function parameters (LSpos, passEV, and passEF) associated with an increase in active RA function (LSneg, ActEV, and ActEF) in order to maintain constant TotEV. These results are in agreement with the documented age-related decrease of the tricuspid valve E/A ratio<sup>8</sup> and with previous reports showing an age-related increase in active left atrial contraction in response to increased left ventricular stiffness.<sup>20</sup>

### Study limitations

Two-dimensional speckle-tracking echocardiography and 3DE dataset acquisitions and quantifications have been performed using a single vendor platform, which may have implications for



the applicability of these reference values to datasets that are acquired with other vendor platforms. However, we used a vendor-independent software for 3DE RA volume measurements, which may ensure generalizability of our results.

This study did not include a CMR validation of RA volume measurements. However, several studies comparing 3DE and CMR to assess cardiac chamber volumes have documented the accuracy of 3DE and consistently reported smaller volumes by 3DE than by CMR in normal subjects as well as in a variety of cardiac diseases.<sup>18</sup> There is now enough evidence that the two techniques are different and we need different reference values specific for the technique which will be used in clinical routine. Furthermore, the analysis algorithm used in this study has recently been validated against CMR.<sup>9</sup> Finally, limited availability and costs of CMR, as well as ethical reasons, prevented us to perform CMR in healthy subjects without clinical indication.

The number of men and women was not equal, especially in the middle and advanced age decades of life (Figure 1), raising the possibility that some gender differences may have been amplified or not adequately detected. In addition, the number of subjects older than 60 years was limited. However, the enrolment of truly healthy subjects at this age is difficult.

Finally, despite all subjects were asymptomatic with normal physical examination, normal electrocardiogram, no wall motion abnormality at 2DE, and no cardiovascular risk factor, we cannot exclude the possibility of subclinical coronary artery disease particularly in older subjects.

## Conclusions

Our study provides reference values for RA geometry and function obtained with 3DE and 2DE-STE in a relatively large population of healthy subjects. Availability of reference values and age- and gender-specific cut-off values to identify RA remodelling and dysfunction should facilitate the implementation of 3DE and STE to assess RA geometry and function in both clinical routine and research.

## Supplementary data

Supplementary data are available at *European Heart Journal – Cardiovascular Imaging* online.

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## References

1. Gaynor SL, Maniar HS, Prasad SM, Steendijk P, Moon MR. Reservoir and conduit function of right atrium: impact on right ventricular filling and cardiac output. *Am J Physiol Heart Circ Physiol* 2005;**288**:H2140–5.
2. Do DH, Therrien J, Marelli A, Martucci G, Afilalo J, Sebag IA. Right atrial size relates to right ventricular end-diastolic pressure in an adult population with congenital heart disease. *Echocardiography* 2011;**28**:109–16.
3. Sallach JA, Tang WH, Borowski AG, Tong W, Porter T, Martin MG et al. Right atrial volume index in chronic systolic heart failure and prognosis. *JACC Cardiovasc Imaging* 2009;**2**:527–34.
4. Cioffi G, de Simone G, Mureddu G, Tarantini L, Stefanelli C. Right atrial size and function in patients with pulmonary hypertension associated with disorders of respiratory system or hypoxemia. *Eur J Echocardiogr* 2007;**8**:322–31.
5. Raymond RJ, Hinderliter AL, Willis PW, Ralph D, Caldwell EJ, Williams W et al. Echocardiographic predictors of adverse outcomes in primary pulmonary hypertension. *J Am Coll Cardiol* 2002;**39**:1214–9.
6. Bustamante-Labarta M, Perrone S, De La Fuente RL, Stutzbach P, de la Hoz RP, Torino A et al. Right atrial size and tricuspid regurgitation severity predict mortality or transplantation in primary pulmonary hypertension. *J Am Soc Echocardiogr* 2002;**15**:1160–4.
7. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA et al. American Society of Echocardiography's Nomenclature and Standards Committee; Task Force on Chamber Quantification; American College of Cardiology Echocardiography Committee; American Heart Association; European Association of Echocardiography, European Society of Cardiology. Recommendations for chamber quantification. *Eur J Echocardiogr* 2006;**7**:79–108.
8. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography. *J Am Soc Echocardiogr* 2010;**23**:685–713.
9. Mor-Avi V, Yodanis C, Jenkins C, Kühl H, Nesser HJ, Marwick TH et al. Real-time 3D echocardiographic quantification of left atrial volume: multicenter study for validation with CMR. *JACC Cardiovasc Imaging* 2012;**5**:769–77.
10. Keller AM, Gopal AS, King DL. Left and right atrial volume by freehand three-dimensional echocardiography: in vivo validation using magnetic resonance imaging. *Eur J Echocardiogr* 2000;**1**:55–65.
11. Murata M, Iwanaga S, Tamura Y, Kondo M, Kouyama K, Murata M et al. Real-time three-dimensional echocardiographic quantitative analysis of left ventricular diastolic dysfunction. *Am J Cardiol* 2008;**8**:1097–102.
12. To AC, Flamm SD, Marwick TH, Klein AL. Clinical utility of multimodality LA imaging: assessment of size, function, and structure. *JACC Cardiovasc Imaging* 2011;**4**:788–98.
13. Padeletti M, Cameli M, Lisi M, Malandrino A, Zacà V, Mondillo S. Reference values of right atrial longitudinal strain imaging by two-dimensional speckle tracking. *Echocardiography* 2012;**29**:147–52.
14. Aune E, Baekkevar M, Roislien J, Rodevand O, Otterstad JE. Normal reference ranges for left and right atrial volume indexes and ejection fractions obtained with real-time three-dimensional echocardiography. *Eur J Echocardiogr* 2009;**10**:738–44.
15. Quraini D, Pandian NG, Patel AR. Three-dimensional echocardiographic analysis of right atrial volume in normal and abnormal hearts: comparison of biplane and multiplane methods. *Echocardiography* 2012;**29**:608–13.
16. Dubois D, Dubois EF. Clinical calorimetry. A formula to estimate the approximate surface area if height and weight are known. *Arch Intern Med* 1916;**17**:863–71.
17. Vianna-Pinton R, Moreno CA, Baxter CM, Lee KS, Tsang TSM, Appleton CP. Two-dimensional speckle tracking echocardiography of the left atrium: feasibility and regional contraction and relaxation differences in normal subjects. *J Am Soc Echocardiogr* 2009;**22**:299–305.
18. Badano LP, Boccia F, Muraru D, Bianco LD, Peluso D, Bellu R et al. Current clinical applications of transthoracic three-dimensional echocardiography. *J Cardiovasc Ultrasound* 2012;**20**:1–22.
19. Saraiva RM, Demirkol S, Buakhamsri A, Greenberg N, Popović ZB, Thomas JD et al. Left atrial strain measured by two-dimensional speckle tracking represent a new tool to evaluate left atrial function. *J Am Soc Echocardiogr* 2010;**23**:172–80.
20. Spencer KT, Mor-Avi V, Gorcsan J III, DeMaria AN, Kimball TR, Monaghan MJ et al. Effect of aging on left atrial reservoir, conduit and booster pump function: a multi-institution acoustic quantification study. *Heart* 2001;**85**:272–7.