Excess Aldosterone Is Associated With Alterations of Myocardial Texture in Primary Aldosteronism

Gian Paolo Rossi, Vitantonio Di Bello, Chiara Ganzaroli, Alfredo Sacchetto, Maurizio Cesari, Alessio Bertini, Davide Giorgi, Roldano Scognamiglio, Mario Mariani, Achille C. Pessina

Abstract—Hyperaldosteronism has been causally linked to myocardial interstitial fibrosis experimentally, but it remains unclear if this link also applies to humans. Thus, we investigated the effects of excess aldosterone due to primary aldosteronism (PA) on collagen deposition in the heart. We used echocardiography to estimate left ventricular (LV) wall thickness and dimensions and for videodensitometric analysis of myocardial texture in 17 consecutive patients with PA and 10 patients with primary (essential) hypertension who were matched for demographics, casual blood pressure, and known duration of hypertension. The groups differed in serum K⁺, ECG PQ interval duration, plasma renin activity, and aldosterone levels (all P<0.002) but not for casual blood pressure values, demographics, and duration of hypertension. Compared with hypertensive patients, PA patients showed a higher LV mass index (53.7±1.8 versus 45.5±2.0 g/m²; P=0.008) and lower values of the cyclic variation index of the myocardial mean gray level of septum (CVIₛ; −12.02±5.84% versus 6.06±3.08%; P=0.012) and posterior wall (−11.13±6.42% versus 8.63±9.62%; P=0.012). A regression analysis showed that CVIₛ was predicted by the PQ duration, supine plasma renin activity, plasma aldosterone, and age, which collectively accounted for ≈36% of CVIₛ variance. PA is associated with alterations of myocardial textures that suggest increased collagen deposition and that can explain both the dependence of LV diastolic filling from presystole and the prolongation of the PQ interval. (Hypertension. 2002;40:23-27.)

Key Words: hypertension, endocrine ▪ aldosterone ▪ myocardial ▪ hypertrophy ▪ fibrosis ▪ echocardiography

Left ventricular hypertrophy (LVH) is commonly associated with arterial hypertension and represents an important independent predictor of cardiovascular events, including congestive heart failure. Extracellular matrix and collagen deposition are invariably findings of LVH and lead to cardiac fibrosis (CF), which occurs particularly in the perivascular areas and correlates directly with the severity of LVH. CF is a major cause of cardiac dysfunction because an excessive deposition of collagen may be responsible for abnormal tissue stiffness and diastolic dysfunction. The latter is an early marker of heart involvement in hypertension (for review, see Agabiti-Rosei and Muiesan) and is associated with CF more closely than with LVH.

Fibroblasts constitute the vast majority (>90%) of non-myocyte cells in the heart; they can increase the production of extracellular matrix on exposure to a variety of injuries, including pressure overload. The latter seems to be only one of the determinants of CF, because it was experimentally shown, both in vitro and in vivo, that CF in both ventricles was linked to activation of the renin-angiotensin-aldosterone system and that it could be prevented by antihypertensive dosages of spironolactone.

Thus, angiotensin II and aldosterone play important roles in the heart (for review, see Swynghedauw). Angiotensin II induces cardiomyocyte hypertrophy in both ventricles, stimulates collagen synthesis by fibroblasts, and regulates collagen degradation by blunting the activity of matrix metalloproteinase-1, the key enzyme of collagen degradation. Aldosterone is extracted through the human heart through a spironolactone-sensitive pathway and promotes CF by acting through different pathogenic mechanisms. It increases types I and III procollagen mRNA in both ventricles, although it does not seem to influence matrix metalloproteinase-1 activity in cultured cardiac fibroblast preparations. Aldosterone may also act on the cardiac angiotensin II receptor, because its administration, along with a high-salt diet, increased angiotensin II type 1 (AT-1) receptor density in the left ventricle of rats; this increase was prevented by both spironolactone and losartan. Thus, aldosterone might cause extracellular matrix deposition by enhancing the transcription of collagen type I and III genes and by augmenting the effects of angiotensin II on AT-1 receptors.

Primary aldosteronism (PA) offers a unique opportunity for investigating the role of excess aldosterone in humans, independent of that of angiotensin II, which is suppressed. We previously reported that in white PA patients there is an
excess of LVH\(^{15}\); this finding was thereafter confirmed in Japanese PA patients.\(^{16}\) Doppler flow velocity indexes of early diastolic transmural flow are decreased compared with those from patients with primary (essential) hypertension (EH). These alterations were more marked in patients with a Conn’s adenoma, in whom aldosterone excess and renin suppression were also more prominent, and were corrected by adrenalectomy,\(^{17}\) thus indicating a causative role of excess aldosterone in CF. To prove this link conclusively, histological analysis of myocardial biopsies would be necessary, but this is unfeasible for ethical reasons. However, newer noninvasive technologies, such as the videodensitometric analysis of myocardial texture that have proven to be accurate for assessing CF,\(^{18,19}\) provide a tool to reexamine this question. Therefore, we sought to compare myocardial texture indexes between consecutive PA and EH patients.

**Methods**

**Patients**

We studied 27 white hypertensive patients; 17 were consecutive cases of PA, which was caused by a Conn’s adenoma in 15 patients and by idiopathic hyperaldosteronism in 2 patients.\(^{20}\) In all cases, Conn’s adenoma was identified with adrenal vein sampling\(^{21}\) and confirmed at surgery, by histological analysis, and by correction of PA at follow-up. Ten patients (2 female and 8 male) in whom EH was diagnosed after the exclusion of all possible causes of secondary hypertension were studied as controls.

**Echocardiography**

All patients were in sinus rhythm, and none had any valvular or ischemic heart disease. Conn’s adenoma patients were studied before adrenalectomy. M-mode and 2D echocardiograms and Doppler analysis were performed with a commercially available apparatus (Hewlett-Packard Sonos 2500). The measurement of left ventricular (LV) diameters and posterior wall and septum thickness and the calculation of LV mass and relative wall thickness were performed as described previously.\(^{22}\) LV mass was normalized for height\(^{23}\) to obtain the LV mass index (LVMI). Criteria for concentric and eccentric LVH and LV remodeling have been described previously.\(^{17}\)

A single reader (A.S.) blindly performed the following measurements on a pulsed Doppler transmural flow velocity profile: early diastolic (E wave) peak flow velocity (PFVE), diastolic peak flow velocity at atrial contraction (A wave, PFVA), their ratio (PFVE/PFVA), E wave integral (Ei), A wave integral (Ai), their ratio (Ei/Ai), and the atrial contribution to LV filling (ACLVF), as reported previously.\(^{17}\)

Blood pressure was measured while the patients were off medications using a mercury sphygmomanometer with phase V of Korotkoff for diastolic pressure, before and after echocardiography.

**Videodensitometry**

To achieve a precise and reproducible sampling of textural parameters, the gain settings and compensation profiles were adjusted for all subjects to obtain uniform myocardial brightness throughout the echocardiogram. The gray scale transfer function was adjusted to be linear for the entire video signal range; no reject, enhancement, or dynamic ranges were used; and a 25 to 30 dB amplification at a depth of 18 cm was set. The optimal echocardiographic images were transferred to a calibrated video digitization system and converted into 256×256 pixels of 256 gray levels, each with a real-time videodigitizer (Tomtec Imaging Systems).\(^{19,22}\) Each cardiac cycle was digitized into 12 frames independently of heart rate. The images corresponding to the end-diastolic and end-systolic phases, all in long-axis projection, were selected with an optimal visualization of both the interventricular septum and the LV posterior wall.

The regions of interest for texture analysis were chosen by consensus of 2 observers who were blinded to the diagnosis group, as described previously.\(^{22}\) The regions of interest, which were always the same size (32×42 pixels), were placed in the same location in the septum (midseptum) and in the posterior wall (midposterior) in both end-systolic and end-diastolic frames. We also considered the delay or phase-shift of the cyclic variation in all septum and posterior wall samplings; a time delay of 1.0 corresponded to a peak near end-diastole, and a nadir near end-systole was found in mean gray level cyclic variation.\(^{19,22}\) A histogram of the gray level distribution was generated for each region of interest.

The mean gray level of each cavity region (background signal) was subtracted from the absolute mean gray level obtained for each region of interest. A quantitative analysis of the shape of each distribution was also performed using skewness and kurtosis. The cyclic variation index (CVI) of the myocardial gray level amplitude was calculated as described previously.\(^{22}\) Measurements were averages of at least 5 consecutive cardiac cycles. Reproducibility was estimated by analyzing, in a blinded fashion, all recordings on 2 separate occasions by the same (intraobserver variability) or different (interobserver variability) investigators. Intraobserver and interobserver coefficients of variation averaged 7.5% and 10.2%, respectively. The intraclass correlation coefficient for septum mean gray level was 0.92 for diastolic and 0.90 for systolic samples; for posterior wall mean gray level, it was 0.89 for diastolic and 0.91 for systolic samples.

**Statistical Analysis**

Results are presented as mean±SD (or SEM or range); comparison between groups was performed with Student’s t test for unpaired data or the Mann-Whitney test. The relationship between individual variables was examined by scatter plot analysis and correlation matrix followed by a stepwise linear regression analysis on the CVI of the septum (CVI) of the variables that were of interest in the scatter plot analysis.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Results**

**Clinical Features and Echocardiographic and Doppler Indexes of the Patients**

After matching, the groups were demographically similar and had superimposable heart rates, blood pressure values, and known durations of hypertension (Table 1). They differed in serum potassium, supine baseline and captopril-stimulated plasma renin activity (PRA; which were significantly lower), and plasma aldosterone, which was higher in PA than in EH patients. A longer PQ ECG interval was also noticed in PA patients. Table 2 shows the results of the measurements of LV wall thickness and dimensions. LVMI was significantly higher in PA than in EH patients, whereas relative wall thickness did not differ between groups. LVH was present in 30% of the 17 PA and none of the EH patients; LV concentric remodeling was seen in 17% of PA and 10% of EH patients (P<0.05). Doppler flow velocity recordings were qualitatively adequate in all patients (Table 2). In the PA group, PFVE/PFVA was lower than in EH patients and showed E/A inversion, whereas E/A was close to unity in the EH group. Similarly, A, was higher and E/A, was lower in PA than in EH patients; the ACLVF was significantly increased in the PA patients compared with EH patients. A correlation matrix showed that LVMI correlated with age (r=0.420, P=0.015), PQ duration (r=0.689, P<0.0001), CVI, (r=−0.418,
TABLE 1. Clinical Features of the Patients With PA and EH

<table>
<thead>
<tr>
<th>Variable</th>
<th>PA (n=17)</th>
<th>P</th>
<th>EH (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female:male), %</td>
<td>35/65</td>
<td>NS</td>
<td>20/80</td>
</tr>
<tr>
<td>Age, y</td>
<td>52±13</td>
<td>NS</td>
<td>48±14</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±2</td>
<td>NS</td>
<td>25±3</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.9±0.18</td>
<td>NS</td>
<td>1.9±0.10</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>3.46±0.10</td>
<td>0.002</td>
<td>4.01±0.07</td>
</tr>
<tr>
<td>Known duration of hypertension, mo</td>
<td>92±74</td>
<td>NS</td>
<td>94±60</td>
</tr>
<tr>
<td>ECG PQ interval, ms</td>
<td>175±6</td>
<td>0.002</td>
<td>132±4</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>283±34</td>
<td>&lt;0.001</td>
<td>134±6</td>
</tr>
<tr>
<td>Supine PRA, ng of angiotensin I·mL⁻¹·h⁻¹</td>
<td>0.48±0.22</td>
<td>&lt;0.001</td>
<td>0.95±0.35</td>
</tr>
<tr>
<td>Captopril-stimulated PRA, ng of angiotensin I·mL⁻¹·h⁻¹</td>
<td>0.68±0.77</td>
<td>NS</td>
<td>1.45±1.42</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>159±15</td>
<td>NS</td>
<td>165±11</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>100±8</td>
<td>NS</td>
<td>101±3.27</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>120±10</td>
<td>NS</td>
<td>123±5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>76±10</td>
<td>NS</td>
<td>71±4</td>
</tr>
<tr>
<td>Tumor size, mm²</td>
<td>14.1±1.4</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are mean±SD. SBP, DBP, and MBP indicate systolic, diastolic, and mean blood pressure, respectively.

*Measured in the 15 PA patients with a Conn’s adenoma.

P=0.015, baseline supine PRA (r=-0.376, P=0.029), PFVA (r=0.411, P=0.017), and ACLVF (r=0.376, P=0.046).

Videodensitometric Analysis of the Left Ventricule Myocardial Texture

The Figure shows the results of the videodensitometric measurements of the interventricular septum (CVIs), LV posterior wall (CVIpw), and the mean of the 2 indexes (CVIm). Compared with EH patients, PA patients had lower values of both CVIs and CVIpw, whereas the difference of CVIm was of borderline statistical significance, mainly because of an inferior reproducibility of this measurement compared with that of measurements of the septum. CVI, correlated with CVIpw (r=0.528, P=0.02), PQ duration (r=−0.0451, P=0.009), baseline supine PRA (r=0.435, P=0.013), LVMI, and the Doppler-derived index of LV filling PFVA (r=−0.340, P=0.041), whereas no significant correlation with plasma aldosterone levels was detected. However, aldosterone entered in the regression analysis on CVI, (β=0.464, P=0.042), as did PQ duration (β=0.563, P=0.022), baseline supine PRA (β=0.503, P=0.033), and age (β=0.338, P=0.135). This model significantly (P=0.026) predicted CVI, and accounted for ≈36% (adjusted R²=0.357) of its variance.

Discussion

We compared echocardiographically-derived indexes, which estimate myocardial texture and were shown to correlate with extracellular matrix and collagen deposition in humans, between consecutive hypertensive PA and EH patients. These patients were carefully matched for demographics, casual blood pressure values, and duration of hypertension to minimize potential biases due to an unbalanced distribution of variables between groups. Accordingly, the groups differed only for PRA, aldosterone, and serum potassium levels, because of the different causes of hypertension. Nonetheless, PA patients exhibited notable differences in LV mass, filling, and myocardial textures, including a higher LVMI, which

TABLE 2. Echocardiographic Features and Transmitral Flow Velocity Doppler-Derived Indexes of Diastolic Function of the Patients With PA and EH

<table>
<thead>
<tr>
<th>Variable</th>
<th>PA (n=17)</th>
<th>P</th>
<th>EH (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDD, mm</td>
<td>5.32±0.14</td>
<td>NS</td>
<td>5.25±0.09</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>3.51±0.14</td>
<td>NS</td>
<td>3.30±0.07</td>
</tr>
<tr>
<td>NSd, mm</td>
<td>1.07±0.03</td>
<td>NS</td>
<td>0.98±0.03</td>
</tr>
<tr>
<td>PWD, mm</td>
<td>1.05±0.03</td>
<td>NS</td>
<td>1.02±0.03</td>
</tr>
<tr>
<td>RWT, mm</td>
<td>0.40±0.02</td>
<td>NS</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>LVMII, g/m²</td>
<td>53.7±1.8</td>
<td>0.008</td>
<td>45.5±2.0</td>
</tr>
<tr>
<td>LAD, mm</td>
<td>38.3±0.3</td>
<td>NS</td>
<td>37.4±0.3</td>
</tr>
<tr>
<td>AoD, mm</td>
<td>35.9±0.4</td>
<td>NS</td>
<td>33.8±0.3</td>
</tr>
<tr>
<td>LAD/AoD</td>
<td>1.08±0.04</td>
<td>NS</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>FS, %</td>
<td>34.2±1.3</td>
<td>NS</td>
<td>37.2±0.9</td>
</tr>
<tr>
<td>LVEDV, mL</td>
<td>138.7±8.8</td>
<td>NS</td>
<td>132.9±4.9</td>
</tr>
<tr>
<td>LVESV, mL</td>
<td>53.1±5.8</td>
<td>NS</td>
<td>44.4±2.17</td>
</tr>
<tr>
<td>EF, %</td>
<td>62.5±1.8</td>
<td>NS</td>
<td>66.6±1.2</td>
</tr>
<tr>
<td>SV, mL</td>
<td>85.6±4.5</td>
<td>NS</td>
<td>88.4±3.6</td>
</tr>
<tr>
<td>CO, L/min</td>
<td>6.4±0.3</td>
<td>NS</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>PFVE, mm/s</td>
<td>56.5±2.0</td>
<td>NS</td>
<td>58.5±3.8</td>
</tr>
<tr>
<td>PFVA, mm/s</td>
<td>71.9±3.9</td>
<td>0.012</td>
<td>55.4±4.2</td>
</tr>
<tr>
<td>PFVE/PFVA</td>
<td>0.83±0.06</td>
<td>0.011</td>
<td>1.09±0.08</td>
</tr>
<tr>
<td>Eₛ, mm</td>
<td>102.8±6.3</td>
<td>NS</td>
<td>102.4±5.3</td>
</tr>
<tr>
<td>Aₛ, mm</td>
<td>84.4±5.3</td>
<td>0.003</td>
<td>52.4±7.4</td>
</tr>
<tr>
<td>E/Aₛ</td>
<td>1.26±0.09</td>
<td>&lt;0.001</td>
<td>2.02±0.15</td>
</tr>
<tr>
<td>ACLVF, %</td>
<td>45.1±1.6</td>
<td>&lt;0.001</td>
<td>33.6±1.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. ACLVF indicates atrial contribution to LV filling; AoD, aortic root diameter; CO, cardiac output; EF, ejection fraction; Eₛ, E (early diastolic LV filling) wave integral; Aₛ, A (late diastolic LV filling) wave integral; FS, fraction shortening; LAD, left atrial dimension; LVDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; NSd, end-diastolic interventricular septum thickness; LVMI, left ventricular mass index; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; PFVE, early diastolic peak flow velocity; PFVA, late diastolic peak flow velocity; PWD, end-diastolic LV posterior wall thickness; RWT, relative wall thickness; SV, stroke volume.

The bar graph shows the mean values of CVIs, CVIpw, and CVIm. Patients with PA had significantly lower values for both indexes compared with EH patients, thus suggesting the presence of CF.
translated into a higher prevalence of LVH and of LV remodeling, and significant changes in Doppler-derived indexes of LV filling (Table 2) in accord with previous results. Thus, although confirming that excess aldosterone affects cardiac mass independent of blood pressure, our findings indicate that the left ventricle is more dependent on the atrial contraction for its filling in PA patients. No conclusions on the role of CF could be made because LV filling was assessed indirectly by transmitral Doppler flow velocity measurements with no concomitant pressure gradient measurements, analysis of pulmonic venous flow, and/or of isovolumetric relaxation time.

The present results provide an important novel piece of information, which is relevant for the understanding of all the aforementioned changes in LV mass and filling. Despite the modest increase of LV mass and the lack of overt clinical signs of diastolic dysfunction, the PA patients exhibited greater alterations of videodensitometric indexes of LV myocardial texture, compared with demographically and hemodynamically similar EH patients. The changes of myocardial texture involved the interventricular septum and the LV posterior wall and were evident in PA patients with quite small Conn’s adenoma, suggesting that these changes occur early in the course of the disease.

We could identify only a small series of suitable EH patients to match our PA patients within the time span of this study. However, these EH patients showed values of CVI, and CVI∞, that were almost identical to those found in a larger series of EH patients with normal LV mass and concentric remodeling, thus making a selection bias unlikely. Furthermore, in another series of EH patients who had a marked concentric LVH, CVI values were quite similar to those seen in the PA patients, who had a much lower LV mass index. Thus, excess aldosterone can act synergistically with the pressure overload in altering myocardial texture. The alterations in the acoustic properties of myocardium might be explained on several grounds. It is possible that an increased deposition of extracellular matrix and collagen, occurring as a result of the excess aldosterone in PA, could create increased scattering in systole. One of the mechanisms that explains the loss of acoustic myocardial reflectivity seen in normal subjects in systole is the shortening of myocardial fibers during contraction. Therefore, an augmented collagen content, which was found in postmortem specimens of hearts from a few patients with autopsy-proven adrenal adenoma, could decrease the normal cyclic variation of scattering by impairing the intrinsic contractile properties of the heart. Another mechanism relates to the pressure-volume overload which, by stretching the myocardium, could change the orientation, structure, or geometry of both the muscle fibers and the collagen network, thereby influencing the acoustic properties of the myocardium. Whatever the mechanisms, our results indicate that videodensitometric analysis of myocardial texture could be a sensitive test, along with indexes of LV mass and LV filling changes, for the early identification of cardiac involvement in patients with PA. To gain further mechanistic insight, we examined the relationships between individual variables and videodensitometric indexes of CF. We found a direct relationship of CVI, with PRA, indicating that lower PRA values are associated with higher collagen deposition in the heart. A significant inverse relationship with duration of PQ interval, LVMI, and PFVA was also found, thus showing that increased collagen deposition is associated with prolongation of the PQ interval, which accords well with previous findings, and with increasing LV mass and peak flow velocity rate during presystole. A regression analysis also identified PQ duration, baseline supine PRA, plasma aldosterone, and age as predictors of CVI. Because the ECG PQ interval reflects conduction time from the sinoatrial node through the atria, the atrioventricular node, and the Purkinje fibers to the left ventricle, the highly significant (P=0.009) correlation of CVI, with PQ duration suggests that CF can also be an important determinant of the latter.

Conclusions

PA patients exhibit significant changes of myocardial texture compared with demographically and hemodynamically similar EH patients, which may be due to CF and occur before the development of overt LVH and diastolic dysfunction. These changes correlated with a prolongation of the PQ interval, a decrease of LV early filling, and an increase of ACLVF, thus indicating that LV filling occurs predominantly during atrial contraction in PA patients. Collectively, these results support the hypothesis that aldosterone excess affects LV anatomy and function by increasing LV mass through increased deposition of extracellular matrix and collagen and by changing LV filling, in part through a prolongation of atrioventricular conduction time. The greater dependence of LV diastolic filling from presystole can explain the nefarious effects of atrial fibrillation in PA patients; the prolongation of the PQ interval changes along with hypokalemia might account for their predisposition to atrioventricular block.

Perspectives

The present results might be relevant for understanding the LV changes of patients with secondary aldosteronism, such as those with congestive heart failure in whom blockade of the mineralocorticoid receptor with spironolactone strikingly improved outcome, decreased LV mass index and LV end-diastolic diameter and volume, and lowered biochemical markers of myocardial fibrosis and hypertrophy. The newer technologies, such as backscatter analysis and MRI, that are being developed to assess myocardial texture and extracellular matrix might eventually be useful to confirm the detrimental role of excess aldosterone on the heart found in this study in congestive heart failure patients with hyperaldosteronism.

Acknowledgments

This study was supported by grants from the Italian Cabinet of University and Scientific Research (MURST) to A.C.P. (9906193152_001/06) and from Regione Veneto to G.P.R. (863/01/98).

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_Hypertension_. 2002;40:23-27; originally published online June 3, 2002;
doi: 10.1161/01.HYP.0000023182.68420.EB

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Echocardiography

M-mode and 2D echocardiograms and Doppler analysis were performed in all subjects by means of a commercially available apparatus (Hewlett-Packard Mod. Sonos 2500, USA equipped with a 2.5 or 3.5 MHz transducer), as previously reported in detail.\textsuperscript{1,2} In brief, the M-mode echocardiograms were recorded under 2-D inspection using a (Hewlett-Packard Mod. Sonos 2500, USA) with a 2.5 MHz probe. The measurement of left ventricular diameters, posterior wall and septal thickness was performed at the levels of the tip of the mitral valve leaflets, according to the criteria of the American Society of Echocardiography (ASE),\textsuperscript{3,4} using a table digitizer (Summasketch Plus, Summagraphics Co, Fairfield, CT, USA) interfaced to a personal computer and calculating the average of at least 3 cardiac cycles. Left ventricular mass and relative wall thickness (RWT) were calculated as previously reported with the method of Devereux.\textsuperscript{1,4} LV mass was normalized for height\textsuperscript{2,7} to obtain left ventricular mass index (LVMI), since there was no obese patient.\textsuperscript{5}

Left ventricular hypertrophy (LVH), defined as an LVMI $\geq 110$ g/m$^2$ in women and $\geq 134$ g/m$^2$ in men, was classified as concentric in the presence of a RWT $\geq 0.45$ and eccentric with a RWT $< 0.45$. Left ventricular concentric remodeling was diagnosed in the presence of a RWT $\geq 0.45$ and of a normal LVMI.\textsuperscript{1,2} Left ventricular meridional end systolic stress (ESS) and peak systolic stress (PSS) were calculated according to Wilson and Coll.\textsuperscript{6}

Blood pressure was measured with mercury sphygmomanometer and auscultatory method using the phase V of Korotkoff for diastolic before and after echocardiography, using the mean of 3 measurements taken in the supine position at least 3 minutes apart one from another.

A pulsed Doppler transmitral flow velocity profile was obtained from the apical four-chamber view, and the sample volume was positioned just below the mitral valve leaflets, as reported.\textsuperscript{2} On the Doppler recording, the following parameters were measured: early diastolic (E wave) peak flow velocity (PFVE), diastolic peak flow velocity at atrial contraction (A wave, PFVA), their ratio PFVE/PFVA, E wave acceleration (Acc.T.) and deceleration time (Dec.T.), E wave integral
(Ei), a wave integral (Ai), their ratio Ei/Ai, and the atrial contribution to left ventricular filling (ACLVF), i.e. the percent atrial contribution to total diastolic filling.7,8 All measurements were performed by the same reader (A.S.), who was blinded to the etiology of hypertension, using the table digitizer set to a spatial resolution of 0.1 mm. The measurements of all indexes were carried out on at least 3 different cardiac cycles and the average value was used for the analysis. Mean intra-observer variability (variation coefficients) of selected transmitral flow velocity indexes was: E wave area 3.8 %, peak E wave velocity 3.4 %, and E wave duration 4.5 %.

**Videodensitometry (echo image digitization):** To achieve a precise and reproducible sampling of textural parameters, the gain settings and compensation profiles were adjusted for all study subjects to obtain apparently uniform myocardial brightness throughout the echocardiogram. The gray scale transfer function was adjusted to be linear for the entire video signal range, and no reject, enhancement, or dynamic range was used, with a 25-30 dB amplification at a depth of 18 cm. The optimal echocardiographic images were directly transferred from the echocardiograph to a calibrated video digitization system. These images were converted into 256x256 pixels of 256 gray levels each (0 = black, 255 = white), with 8 bits of intensity range, by using a commercial real-time video digitizer (Tomtec Imaging Systems, Inc., Boulder, CO, USA).9,10 One cardiac cycle (RR wave) was automatically divided into 12 frames independently of heart rate. The images corresponding to the end-diastolic and end-systolic phases, all in long-axis projection, were selected with an optimal visualization of both the interventricular septum and the LV posterior wall. The regions of interest for texture analysis were chosen by consensus of two observers, who were blinded to the results of conventional echocardiography, by using an interactive computer program. Particular care was taken that the sonic beam angle of incidence be at approximately 90° to the area of interventricular septum or to the LV posterior wall when scanning the parasternal left ventricular long axis. The region of interest, always of the same size (32x42 pixels), was placed in the same location in the septum (mid-septum) and in the posterior wall (mid-posterior) both at end-systolic and end-diastolic
frames. We also considered the delay or phase-shift of the cyclic variation in all septum and posterior wall samplings (left parasternal long-axis view); a time delay of 1.0 corresponded to a peak near end-diastole and a nadir near end-systole was found in both mean gray level cyclic variation. Only the myocardium was included, whereas the endocardial and epicardial specular echoes were excluded to avoid areas of echo dropouts and obvious artifacts. A histogram of the echocardiographic gray level distribution was generated for each region of interest. The mean gray level (MGL) of each cavity region (background signal) was subtracted from the absolute mean gray level obtained for each region of interest. A quantitative analysis of the shape of each distribution was also performed using skewness and kurtosis. The cyclic variation index of the gray level amplitude was calculated according to the formula (MGL_{ED} - MGL_{ES})/MGL_{ES}, and expressed in percent. Measurements were averages of at least 5 consecutive cardiac cycles.

Reproducibility was estimated by analyzing all recordings on two separate occasions (intra-observer variability); to assess inter-observer variability, investigators were blinded. Intra- and inter-observer coefficients of variation averaged 7.5% and 10.2%, respectively. As expected, the reproducibility of measurements of the posterior wall was inferior to that of measurements of the septum. Intra-class correlation coefficients were calculated according to Bland and Altman's procedure by one-way analysis of variance (ANOVA) for repeated measurements. The correlation coefficient for septum MGL was 0.92 for diastolic and 0.90 for systolic sample; for posterior wall MGL, it was 0.89 for diastolic and 0.91 for systolic sample.

References


