Cellular Basis of Wall Remodeling in Long-term Pressure Overload–Induced Right Ventricular Hypertrophy in Rats

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To determine the effects of long-term pressure overload on the structural mechanisms implicated in wall remodeling of the right ventricle, a mild pulmonary artery banding was applied to rats approximately 2 months old, and the animals were killed 150 days later. The surgical procedure resulted in a 60% reduction in the cross-sectional area of the constricted vessel and a 52% increase in the weight of the right ventricle. The hypertrophic myocardial response was associated with an elevation in right ventricular systolic pressure (from 33 ± 11 mm Hg to 71 ± 12 mm Hg), right ventricular end-diastolic pressure (from 3 ± 1 mm Hg to 10 ± 3 mm Hg), and central venous pressure (from 2 ± 0.2 mm Hg to 10 ± 3 mm Hg). The 76% increase in wall thickness after pulmonary artery stenosis was the result of a 24% lateral expansion of cardiac muscle cells and a 44% increase in the number of myocytes across the ventricular wall. The intermyocyte distance was also increased by 22%. These cellular adaptations occurred with no alterations in total myocyte length, average sarcomere length, and volume composition of the myocardium. Ventricular wall area was decreased by 14%, which suggests a small reduction in chamber volume. Myocyte growth was accompanied by proportional expansions of mitochondrial and myofibrillar components, so that the ratio of mitochondria to myofibrils in the cytoplasm remained essentially constant. In conclusion, ventricular remodeling in this model of chronic pressure hypertrophy is characterized by increases in cellular diameter and number that would both tend to decrease the magnitude of systolic and diastolic stresses on a per cell basis and thus improve the myocardial response to a prolonged and sustained mechanical load.

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Hypertrophy represents a response of the heart to a hemodynamic overload that tends to preserve normal cardiac function. Several experimental studies have demonstrated that as a result of a sudden and severe increase in afterload, the affected ventricle grows relatively rapidly,1,2 so that the expanded ventricular mass compensates for the enhanced work demand, after which no further hypertrophy occurs.1 In animal models of pressure hypertrophy, ventricular remodeling has been found to consist of an increase in wall thickness without chamber enlargement, brought about through an increase in myocyte diameter with little or no change in myocyte length.2,3 Thus, the lateral expansion of preexisting myocytes has been considered to be the only cellular process available for the augmentation of wall width, which tends to normalize the higher peak systolic wall stress.4 Investigations performed in humans, however, have indicated that when the heart is subjected to a prolonged and sustained load, the magnitude of cardiomegaly may progress to a greater degree, and the structural mechanisms implicated in the adaptive phenomena of the ventricular wall and chamber vary with the nature, duration, and severity of the mechanical stress.5–7 Hyperplasia of muscle cells has been suggested to represent an additional important component of the response of the myocardium at the cellular level of organization in humans.5–7 Myocyte proliferation appears to characterize the phase of transition from compensated physiological hypertrophy to cardiac dysfunction and overt failure.5

Because cardiac hypertrophy in humans, under a variety of pathological conditions, is a slowly evolving event that can maintain adequate ventricular

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performance for many years before functional deterioration becomes apparent, we used a model in which constriction of the pulmonary artery in 2-month-old rats was initially associated with no change in right ventricular intracavitary pressures or central venous pressure. Subsequently, as the animals grew, the constraint around the vessel was expected to produce an increase in pressure load of the ventricle in a slow but progressive fashion, which mimics the human situation. The hemodynamic changes and the structural alterations of the ventricular wall were examined 150 days after surgery. This interval was selected because it corresponds to approximately one fifth the life span of rats. The right ventricle was preferred to the left ventricle to avoid the influence of a rise in perfusion pressure of the coronary circulation on cardiac hypertrophy.

Materials and Methods

Animals

Seventeen male Wistar rats (Morini Breeding Laboratories, Reggio Emilia, Italy) were included in this study. Animals were housed in pairs in plastic cages and were allowed free access to tap water and standard rat pellet chow. All animals were exposed to cycles of 12 hours of darkness and 12 hours of light throughout the experimental period.

Study Design

Pulmonary artery banding (PAB) was performed at approximately 2 months of age in 10 rats weighing 250 ± 23 g. Surgery was conducted under a dual-view surgical microscope (Wild M600, Herrbrugg, Switzerland). Anesthesia consisted of fentanyl citrate (5 μg/kg) and droperidol (250 μg/kg i.p.; Leptofen, Farmitalia-Carlo Erba Laboratories, Milan, Italy) administered through the tail vein. Maintenance doses were subsequently given as required. The right external carotid artery was exposed and cannulated with a PE 50 catheter. After minimal dissection of the surrounding tissue, an identical catheter was inserted into the right jugular vein through the posterior facial vein. The total osmolarity of the fixative was 750 mosm. Fixation Procedure

Following anesthesia the right common carotid artery and the right jugular vein were cannulated for the measurements of mean arterial pressure and central venous pressure. The catheter in the jugular vein was then advanced into the right ventricular chamber for the estimation of intracavitary pressures.

Baseline values, a probe 1.7 mm in diameter was held in contact with the pulmonary artery. The entire vessel and the probe were tightly ligated, and then the probe was quickly removed in order to produce a constricted opening in the lumen equal to the diameter of the probe. A small, sterile catheter 1.6 mm in diameter was inserted in the pleural cavity between the seventh and eighth ribs on the right side of the thorax to reduce the pneumothorax and allow drainage of fluids. The chest was then closed, the catheters used for the recording of arterial and venous pressures were removed, the vessels used for vascular access were ligated, and the tracheostomy was repaired. The muscle layers and the skin were carefully reconstructed with synthetic absorbable sutures. To reduce postoperative pain, morphine sulfate (5 mg/kg) was administered subcutaneously. The intrathoracic catheter was left in place for 24 hours until the animals recovered completely and were able to move freely in the cage. Seven similarly treated rats, with the exception that the ligature around the pulmonary artery was not tied, served as sham-operated (SO) controls. The surgical procedure required an average of 45 minutes.

All animals were killed 150 days after surgery. Following anesthesia the right common carotid artery and the right jugular vein were cannulated for the measurements of mean arterial pressure and central venous pressure. The catheter in the jugular vein was then advanced into the right ventricular chamber for the estimation of intracavitary pressures.

Fixation Procedure

At the completion of the hemodynamic measurements, the abdomen was opened, and the abdominal aorta below the renal arteries was cannulated with a polyethylene catheter (PE 200) filled with phosphate buffer (0.2 M, pH 7.4) and heparin (100 IU/ml). The catheter was first sealed in place with a ligature and then connected to a perfusion apparatus. In rapid succession, the heart was arrested in diastole by an intravenous injection of approximately 1 ml KCl (1 meq/ml), the chest was opened, and the right atrial appendage and the coronary vasculature were perfused at a pressure equal to the measured mean arterial pressure. After perfusion with buffer for 3 minutes, the coronary bed was perfused for 15 minutes with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde. The total osmolarity of the fixative was 750 mosm.

Tissue Sampling

Following the fixation procedure, the heart and great vessels were excised. The main pulmonary artery was dissected free and cut transversely to expose the vessel lumen at the region of the ligation. The luminal diameter of the pulmonary artery in PAB and SO animals was measured with a dissecting microscope having an ocular micrometer accurate to 0.05 mm. Maximal and minimal internal
diameters were determined at a magnification of \( \times 25 \), and the geometric mean value was calculated to compute the average luminal cross-sectional area in each rat.\(^{10}\) Subsequently, the free wall of the right ventricle and the left ventricle including the septum were separated and their respective weights recorded. The volume of the right ventricular myocardium was determined by dividing its weight by the specific gravity of muscle tissue, 1.06 g/ml.\(^{11}\) The whole right ventricle was then serially sliced into approximately ten 1-mm thick sections, perpendicular to the axis of the heart from the apex to the base. Wall thickness was estimated by averaging four equally spaced measurements from each tissue slice made at a magnification of \( \times 25 \). The six middle slices in each ventricle were then cut radially to obtain 30 tissue blocks extending from the endocardial to the epicardial surface for electron microscopy. The specimens were postfixed in 1% OsO4, dehydrated in acetone, and infiltrated and embedded in Araldite.\(^9\) The remaining slices of the right ventricle were used for dry weight determination.

**Electron Microscopic Morphometry**

Sixteen plastic-embedded tissue blocks from each ventricle were sectioned at a thickness of 1 \( \mu \)m with an MT-1 Porter-Blum microtome. These sections were stained with methylene blue and safranin and viewed to obtain areas of myocardium in which the myofibers were oriented either in the longitudinal or transverse direction. Subsequently, 10 blocks from the right ventricle of each animal were trimmed for thin sectioning to obtain eight areas of tissue with transversely sectioned myofibers and two with longitudinally oriented cells. These areas were randomly chosen and no attempt was made to characterize morphometrically the different layers of the wall. Low power electron micrographs of transverse sections of myocardium, seven from each tissue block, were collected and printed at \( \times 5,300 \), calibrated with a superimposed grid consisting of 140 sampling points and 14 test-line segments each 150 mm long.\(^{12}\)

The volume fraction, \( V_v \), of myocardial components was measured in 952 of these low-power micrographs (SO rats, 392; PAB rats, 560) by counting the fraction of sampling points, \( P_p \), overlying myocytes, myocyte nuclei, and the interstitium:

\[
V_v = P_p
\]  
(1)

The number of myocyte profiles, \( N \), in the sampled area, \( A \), was counted following the criteria described by Gundersen\(^{13}\) to estimate their numerical density, \( N_A \),

\[
N_A = N/A
\]  
(2)

and average cross-sectional area, \( \overline{A} \),

\[
\overline{A} = V(m)/N_A \quad (3)
\]

where \( V(m) \) is the volume fraction of myocytes in the myocardium.

Length density (length per unit volume), \( L_v \), of myocytes is numerically equal to their measured numerical density (number per unit area) in transverse myocardial sections:

\[
L_v = N_A
\]  
(4)

Deviations from ideal orientation, however, result in an underestimation of \( N_A \) in myocardial sections. Correction factors have been obtained in skeletal muscle and range from 12% in immersed-fixed tissue\(^{14} \) to 3.4% in perfusion-fixed muscle.\(^{15}\) The effects of obliquity in cardiac muscle have been discussed recently with respect to numerical densities of structures and surface area measurements.\(^{16}\) It was concluded that to minimize the potential errors associated with oblique orientation of anisotropic structures, certain criteria have to be followed. In particular, micrographs of transversely sectioned myofibers can be used for quantitative analysis of these oriented cells only when the spacing between Z bands is at least greater than twice the true sarcomere length, measured in longitudinally oriented sections of myocytes.\(^{16}\) This approach was employed in the present study.

The mean center-to-center distance, \( d_{cc} \), between myocytes was calculated from the number of profiles counted per unit area of tissue, \( N_A \), in transverse myocardial sections by assuming the tendency for these roughly cylindrical cells to pack in a close hexagonal pattern\(^{17,18} \):

\[
d_{cc} = \sqrt{2 \over 3 N_A} = 1.0746 \sqrt{N_A} \quad (5)
\]

The preference for a hexagonal pattern in the distribution of myocytes in the myocardium was based on the morphometric measurements of cell cross section and myocyte surface-to-volume ratio.\(^{17}\) The same concept was used to estimate the average number of myocytes across the ventricular wall, that is, the number of myocytes that would be traversed by a thin transmural probe inserted perpendicular to the surface of the ventricle. In a hexagonal pattern, the spacing between planes of adjacent cells varies with the orientation of the array from a maximum of \( d_{cc} \) to a minimum of \( d_{cc} \sqrt{3/2} \) and has a mean value, \( \overline{d} \), representative of random orientation, given by:

\[
d = 3d_{cc} \sqrt{3 \over \pi} \int_0^{\pi/6} {da \over \cos \theta} = 0.9085 \overline{d}_{cc} \quad (6)
\]

Thus, the transmural number, \( N_{tm} \), of myocytes across a ventricular wall of thickness, \( W \), can be found from:

\[
N_{tm} = W/d = 1.0243 W \sqrt{N_A} \quad (7)
\]
The surface density of myocytes per unit volume of cell, \( S_{\text{myo}} \), was evaluated by counting the number of intersections between the sampling line, \( I_L \), and the myocyte membrane profiles per unit length of sampling line, according to the following equation:

\[
S_{\text{myo}} = \pi \frac{I_L}{L} V(m)
\]  

(8)

An additional sampling of five random fields representative of myocyte cytoplasm were collected from each of five blocks and similarly examined at a final magnification of \( \times 25,000 \). Measurements were made of the volume fractions of myofibrils, mitochondria, and other cytoplasmic components. A total of 320 micrographs (SO rats, 120; PAB rats, 200) were employed for this part of the study.

Five random fields, from longitudinally oriented sections of myocytes were collected from each of two tissue blocks and printed at \( \times 20,000 \). Mean sarcomere length in myofibrils was obtained from 100 measurements in each rat, using sections that had been cut perpendicularly to the fiber axis to avoid compression artifact. A total of 170 micrographs (SO rats, 70; PAB rats, 100) were used to achieve sarcomere length determinations.

Absolute volume, surface areas, and lengths of myocytes were evaluated from the products of total ventricular myocardial volume and their respective values per unit volume.\(^{18}\) The theoretical aspects and practical applications of the morphometric procedure briefly summarized above have recently been described in detail.\(^{19}\)

**Sampling Size**

The magnitude of sampling utilized in this investigation was selected on the basis of previous work performed in our laboratory\(^{19}\) and the principle of Poisson statistics.\(^{20}\) The latter can be used as a reasonable guideline for morphometric data collection since it provides a somewhat more conservative estimate of necessary counts than more specific formulations derived for point counts\(^{21}\) and profile counts.\(^{22}\) By assuming that biological variability among animals in a given experimental group is approximately 10%, counting errors in each animal should also be limited by the same order of magnitude for the least frequent structure.\(^{19}\) In the present study, the area of myocardium sampled yielded an average of 275 and 186 myocyte profile counts for each SO rat and PAB rat, respectively. Corresponding sampling errors for these values are 6.0% and 7.3%. The nested analysis of variance\(^{23}\) performed after the code was broken demonstrated that the number of blocks sampled, the number of micrographs collected from each block, and the numbers of sampling points, intersection counts, and profile counts were in excess of what would have been the minimum required for optimum efficiency.

**Data Collection and Analysis**

All tissue samples were coded and the code was broken at the end of the experiment. Standard morphometric relations and correction factors\(^{19}\) were applied in the computations of numerical density, size, and surface area of myocytes.

Results are presented as mean ± SD computed from the average measurements obtained from each rat. Statistical significance for comparisons between two measurements was determined using the unpaired two-tailed Student's \( t \) test. Values of \( p<0.05 \) were considered to be significant.

**Results**

Constriction of the pulmonary artery in 2-month-old rats, employing a probe of 1.7 mm in diameter, resulted in no change in right ventricular systolic pressure (RVSP), right ventricular end-diastolic pressure (RVEDP), central venous pressure, and systemic arterial pressure. The pressure in the right ventricle was elevated only during the short period of complete pulmonary occlusion involved in the surgical procedure, returning rapidly to control values after surgery (Figure 1). Changes in the opposite direction were observed in the arterial blood pressure, which also quickly returned to baseline levels. Thus, the initial constriction produced little or no narrowing of the pulmonary artery lumen since the hemodynamic parameters measured were not altered.

Figure 2 shows that at sacrifice, 150 days after surgery, a 60% reduction in the mean cross-sectional area of the pulmonary artery was measured in PAB rats with respect to SO animals (\( p<0.0001 \)). This magnitude of constriction provoked an elevation in RVSP from 33 ± 11 mm Hg to...
FIGURE 2. Changes of pulmonary artery cross-sectional area (PACSA), right ventricular systolic pressure (RVSP), right ventricular end-diastolic pressure (RVEDP), and central venous pressure (CVP) in pulmonary artery banded (PAB) rats with respect to sham-operated (SO) controls. Data are presented as mean±SD.

71 ± 12 mm Hg (p<0.0001), and in central venous pressure from 2 ± 0.2 mm Hg to 10 ± 3 mm Hg (p<0.0001) (Figure 2). Mean arterial pressure and heart rate were 103 ± 5 mm Hg and 400 ± 51 beats/min, respectively, in PAB rats. Similar values, 105 ± 5 mm Hg and 385 ± 47 beats/min, respectively, were seen in SO subjects.

Figure 3 illustrates that, in comparison with controls, PAB animals showed a 12% reduction in body weight at the time the animals were killed (p<0.05). There was also no evidence of ascites, pleural effusion, or edema. The modest increase in heart weight (6%) observed after pulmonary artery banding was not statistically significant. In contrast, the 52% increase in the weight of the free wall of the right ventricle (Figure 3) was found to be statistically significant (p<0.0001). As a consequence of this hypertrophic response, the ratios of right ventricular weight to body weight (not shown) and right ventricular weight to left ventricular weight (Figure 3) increased by 70% (p<0.0001) and 64% (p<0.01), respectively. The ratios of right ventricular tissue dry weight to tissue wet weight were 0.202 ± 0.023 in SO rats and 0.212 ± 0.029 in PAB animals (p<0.05).

Figure 4 demonstrates that right ventricular hypertrophy was not accompanied by alterations in the volume composition of myocytes and interstitium within the myocardium. Myocyte mean cross-sectional area, however, expanded by 53% (p<0.0001), which was comparable to the 52% augmentation in right ventricular tissue mass. The lateral expansion of cardiac muscle cells resulted in a 33% decrease in the numerical density of myocyte profiles per unit area of myocardium (p<0.0001) and a 37% decrease in the surface-to-volume ratio of these cells (p<0.0001) (Figure 4). The latter alteration indicated that myocyte hypertrophy was associated with a change in cell shape toward a relatively less elongated configuration.

Figure 5 shows the change in thickness of the right ventricular free wall produced by PAB. The progressive increase in the constriction of the pulmonary artery over a period of 150 days resulted in a 76% thickening of the wall of the affected ventricle, from a value of 0.95 ± 0.10 mm in control rats to a value of 1.77 ± 0.22 mm in PAB animals (p<0.0001). However, the magnitude of wall thickening was markedly greater than that of the increase in the average trans-
Figure 5. Changes in wall thickness, myocyte diameter, and number of myocyte profiles in the right ventricular wall 150 days after pulmonary artery banding. PAB, pulmonary artery banded rats; SO, sham-operated controls. Data are presented as mean±SD.

Because of the direct relation between changes in wall thickness and changes in myocyte diameter in pressure-overload hypertrophy,3 the lack of correlation in the present study could result from an increased center-to-center distance between myocytes, from an increased number of myocytes across the wall, or both. The variation in the spacing between myocytes closely reflects the changes in myocyte diameter when the volume composition of the myocardium is not markedly altered (Figure 4).

The intermyocyte distance, calculated according to Equation 5, was 16.25±2.01 μm and 19.80±3.06 μm in SO and PAB animals, respectively. As expected, this 22% difference (p<0.025) was almost identical to the 24% difference in myocyte transverse diameters (Figure 5).

Utilizing wall thickness measurements and the numerical concentration of myocyte profiles per unit area of myocardium, the average number of myocytes across the ventricular wall was computed according to Equation 7. The results obtained are illustrated in the bottom panel of Figure 5. The transmural number of myocytes increased 44%, from a mean value of 55±11 to a value of 79±14 after prolonged pulmonary artery banding (p<0.005). Thus, the 76% thickening of the right ventricular free wall in PAB rats was the result of the combined effects of the addition in parallel of 24 new myocytes within the wall and the 3.5-μm average increase in diameter of the muscle cells (Figure 5).

Figure 6 shows the volume fractions of mitochondria, myofibrils, and matrix in myocyte cytoplasm. The matrix compartment included glycogen, ribosomes, lipid, Golgi apparatus, smooth and rough endoplasmic reticulum, and amorphous regions. Pulmonary artery banding was not associated with changes in the volume composition of these cellular components, resulting in the preservation of the mitochondria-to-myofibrils volume ratio (Figure 6).

The availability of ventricular volume allows the quantitative estimation of tissue constituents and myocyte cytoplasmic structures in terms of absolute measurements per ventricle. These data, shown in Table 1, are the products of the relative measurements in Figures 4 and 6 multiplied by the corresponding values of ventricular volumes. It can be seen that the 52% right ventricular hypertrophy was the result of a 56% increase in myocyte volume that exceeded the 38% expansion of the interstitium. The growth response of myocytes occurred with no change in total myocyte surface, aggregate myocyte length, and average sarcomere length. The 56% mitochondrial volume increase was identical to myocyte volume enlargement but slightly less than myofibrillar volume hypertrophy (66%).

Discussion

The results of the current study indicate that slow and progressive constriction of the pulmonary artery
over a period of 150 days leads to a 60% reduction in the cross-sectional area of the vessel lumen and a 52% increase in the weight of the right ventricle. This hypertrophic response was associated with an elevation in RVSP, RVEDP, and central venous pressure. The absence of liver congestion, pleural effusion, ascites, and peripheral edema, and the fact that systemic arterial pressure was not decreased demonstrate that ventricular dysfunction was not present. Since the physiological parameters measured when the constriction of the pulmonary artery was initially placed were essentially normal, the functional adaptations had to occur sometime during the exposure of the right ventricle to the increasing pressure load, sustained by the continuous reduction in luminal diameter of the pulmonary artery with age and body growth. The 5-month interval chosen seems to correspond to a period of well-compensated hypertrophy with no evidence of ventricular deterioration.

Myocardial hypertrophy in the rat, induced by different experimental procedures, consists at the most of an increase in weight of the affected ventricle of nearly 80%,1,2,17,24 This growth response has been observed as early as 8 days after the imposition of an increased pressure load,2 and longer time intervals have not been found to produce greater magnitudes of cardiac hypertrophy.24–26 The fact that a 52% increase in right ventricular mass was obtained 5 months after PAB provides further evidence for the concept that the duration of the pressure load does not significantly affect the overall extent of ventricular growth in the rat heart. Clinical and animal studies appear to indicate that pressure overload-induced impairment of ventricular function is related more to the duration of the mechanical load than to the degree of the hypertrophic process.27–30 The current observations, however, suggest that ventricular performance was not altered. Thus, longer time periods will have to be evaluated to establish the relation between duration of load and magnitude of hypertrophy, on the one hand, and cardiac failure, on the other, in this animal model.

Increasing pressure loading of the heart induces concentric hypertrophy in which wall thickness increases without chamber enlargement.3,4 The gross morphological indexes of ventricular dimensions (i.e., wall thickness and chamber radius) are the expression of a combination of parameters that include the number of myocytes across the wall, the average myocyte cross-sectional diameter and length, and the proportion between myocytes and interstitium within the myocardium.3 In the present study, PAB produced 76% thickening of the right ventricular wall that exceeded the magnitude of hypertrophic growth in the whole ventricle (52%). The average diameter of the right ventricular chamber could not directly be measured because of the irregularity of the cavity on the right side of the heart due to the protrusion in it of the interventricular septum. A reasonable index of chamber size, however, can be obtained by the measurement of ventricular wall area.3,39 The calculation of ventricular wall area, which is derived by dividing wall volume by wall thickness,3,39 assumes that the ventricular wall may be treated as a thin sheet. Thus, changes in wall area imply corresponding changes in chamber volume. A 14% decrease in wall area was seen in the hypertrophied right ventricle, from a value of 347 ± 28 mm² in SO rats to a value of 298 ± 32 mm² in PAB animals (p < 0.01). This apparent reduction in chamber volume may account, at least in part, for the increase in RVEDP observed here and the previously noted decreased myocardial compliance following a prolonged and sustained pressure load on the ventricle.29–31

The morphometric approach employed for the evaluation of the number of myocyte profiles across the ventricular wall, their mean center-to-center distance and average cross-sectional diameter has allowed the interpretation and translation of the gross anatomical parameters of heart dimensions at the cellular level of organization. The 76% increase
in wall thickness associated with PAB was the result of a 24% lateral expansion of cardiac muscle cells and a 44% increase in the number of myocytes within the ventricular wall. The intermyocyte distance was expanded by 22%. These cellular changes occurred with no alterations in total myocyte length, average sarcomere length, and volume composition of the myocardium.

The pattern of myocyte growth summarized above is consistent with the concept of concentric hypertrophy in the intact ventricle. The adaptations of myocytes may be interpreted as a compensatory response of the myocardium at the cellular level that tends to minimize the effects of the increased pressure load on the heart. According to the law of LaPlace, the larger myocyte diameter and the greater number of cells across the wall would produce a proportional thickening of the wall that should offset the higher peak systolic wall stress resulting from the elevation in pressure. Because of the interaction of wall thickening, rise in systolic and diastolic pressures and reduction in chamber volume, the actual magnitude of wall stress is difficult to calculate. However, the lack of dilation, myocyte necrosis, and the preservation of the mitochondrial-to-myofibrillar volume ratio suggest that the increased pressure load was well compensated. Moreover, the increase of the interstitium was significantly less than the expansion of myocytes and ventricular volume, further demonstrating the absence of replacement scarring with myocyte loss and collagen accumulation.

The significance of cardiac size and shape in ventricular function, and their implications in terms of the law of LaPlace applied to the heart, have repeatedly been discussed in association with physiological growth of the myocardium from birth to adulthood, and cardiac hypertrophy produced by volume overload, pressure overload, and myocardial infarction. With the exception of early postnatal myocardial growth in which cellular hyperplasia and cellular hypertrophy both participate in the process of wall thickening, ventricular wall remodeling in the later stages of development and in induced cardiac hypertrophy has been found to be characterized by changes in myocyte diameter or length, or both, with no changes in myocyte number. The relatively young age of the animals used in the present study might have been an important factor in the type of cellular growth mechanisms observed. This possibility is in line with the recent findings that aging markedly affects the magnitude of ventricular hypertrophy produced by aortic banding in the rat model.

The 44% increase in the number of myocytes across the hypertrophied right ventricular wall was unexpected and surprising. The lateral addition of muscle cell profiles within the wall could have been the result of an increase in the number of branches at the myocyte ends, or in the number of cells in the ventricle, or both. Increases in myocyte branches, however, seem very unlikely because these processes would have produced an elevation in the surface-to-volume ratio of myocytes and a reduction in myocyte cross-sectional area, both at variance with the quantitative estimations obtained in the present study. In contrast, the magnitude of increase in the transmural number of cardiac muscle cells was found to be in close agreement with the 41% increase in the total number of myocyte nuclei previously measured in the same animal model. These independent morphometric results strongly suggest that myocyte cellular hyperplasia can occur in response to a prolonged and sustained mechanical stress on the ventricular myocardium, confirming the observations repeatedly reported in humans. Furthermore, the pattern of cellular hyperplasia documented here, in which new myocytes are added laterally within the wall, has the beneficial effect of decreasing systolic and diastolic stresses on a per cell basis, improving the myocardial response to long-standing pressure overload hypertrophy.

Reduction of the mitochondrial-to-myofibrillar volume ratio is a consistent subcellular alteration that occurs in myocytes after relatively short-term pressure overload hypertrophy. However, early in the hypertrophic response, mitochondrial growth exceeds myofibrillar growth, leading to a transitory elevation of the mitochondrial-to-myofibrillar volume ratio, that is associated with the prevailing synthesis of mitochondrial membranes. In the current report, the volume fractions of mitochondria and myofibrils remained nearly constant despite the prolonged mechanical load on the ventricle and the simultaneous occurrence of myocyte hyperplasia. This finding is consistent with previous observations showing that the ratio of mitochondria to myofibrils in myocyte cytoplasm can return to normal values in long-term hypertensive hypertrophy. Because the generation of ATP in the mitochondrial cristae represents the primary source of energy for myofibrils, the restoration of an optimum concentration of mitochondria and myofibrils in myocytes may provide adequate energy supply and prevent the precocious impairment of heart muscle function.

In conclusion, the major findings of the present study can be summarized as follows: 1) long-term pressure overload hypertrophy of the right ventricle leads to ventricular remodeling which is accomplished by increases in the lateral dimension of myocytes and in the number of myocytes across the ventricular wall; and 2) mitochondria and myofibrils in myocytes grow in proportion so that the mitochondria-to-myofibrillar volume ratio remains constant.

Caution should be taken in extrapolating these results to the entire heart because the right ventricle may behave differently from the left ventricle. The structural characteristics of the left and right ventricular myocardium are quantitatively different and their functional loads throughout life are not comparable. Differences also exist in the capacity of
the two ventricles to react and adapt to an increased work load.9,37 Because of these multiple variables, it remains to be determined whether the cellular mechanisms that implicated wall remodelling of the right side of the heart in long-term pressure overload are equally present in the left ventricle under the same experimental condition.

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