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Remodeling of Myocyte Dimensions in Hypertrophic and Atrophic Rat Hearts

Scott E. Campbell, Borivoj Korecky, and Karel Rakusan

Changes in hemodynamic load cause alterations in cardiac myocyte size, with regional variations in myocyte size distribution possible within the ventricular wall. We studied regional changes in cellular dimensions and their distribution in two models of cardiac hypertrophy and in cardiac atrophy in the rat. Combined volume–pressure overload was produced by 3,3',5-triiodo-L-thyronine (T<sub>3</sub>) treatment; atrophy was produced by heterotopic isointransplantation. Our previous data from long-term pressure overload after aortic constriction were used for comparison. Isolated ventricular myocytes were obtained after in vitro coronary perfusion with collagenase. Cell volume and its distribution were determined; cell length was directly measured by image analysis, and cross-sectional area was estimated from the cell volume/cell length ratio, assuming a cylindrical model. Myocyte hypertrophy resulting from hyperthyroidism and aortic constriction was primarily due to increased cross-sectional area. In both cases, the relative response was greater in the right ventricle than in the left ventricle. Within the left ventricle, epicardial myocytes enlarged the most. Aortic constriction and T<sub>3</sub> treatment predominantly increased the size of smaller myocytes. Heterogeneity in myocyte size increased after constriction but remained relatively unaffected after T<sub>3</sub> treatment. Atrophy of left ventricular myocytes was due to a proportional decrease in cell length and cross-sectional area, with the greatest decrease in the left ventricular endomyocardium. Atrophy predominantly affected larger myocytes, resulting in a more homogeneous overall population of smaller myocytes. We conclude that various alterations in load lead to diverse remodeling in the myocyte population throughout the ventricular wall. In general, smaller myocytes show the highest growth potential, whereas larger myocytes exhibit the highest potential to atrophy. (Circulation Research 1991;68:984–996)

S tructural remodeling of cardiac myocytes is a major component in both cardiac hypertrophy and atrophy brought about by increased and decreased work loads, respectively. Volume overload is generally associated with eccentric hypertrophy, with increased cell length being the predominant feature. Pressure overload in the heart generally results in concentric hypertrophy, characterized by increased myocyte cross-sectional area. It appears that a combined volume and pressure overload can be produced by administration of thyroid hormone, with increased cell length and cross-sectional area present. Conversely, structural remodeling of cardiac myocytes leading to decreased cell volume can be elicited by a reduction in afterload.

These modifications in cellular geometry brought about by changes in preload and afterload can vary not only between ventricles but within different layers of the ventricular wall. Anversa et al. have shown a greater right ventricular response, compared with the left ventricle, in volume overload resulting from moderate and strenuous exercise. A similar disparity in response has been reported in rats with chronic thyroid treatment. Varying degrees of hypertrophy have been reported within the left ventricle with pressure and volume overload. Greater hypertrophy of the endomyocardium compared with the midwall has been documented with aorticaval fistulas. Tomanek has demonstrated greater hypertrophy of the endomyocardium during the early phase of pressure overload in spontaneously hypertensive rats and greater hypertrophy of the epimyocardium in the later phase. A preferential hypertrophic response in the epimyocardium has been reported in rats after renal artery constriction, and experimental hyperthyroidism. Korecky and Rakusan indicated a different response between the endomyocardium and midmyocardium in cardiac at-

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trophy. Thus, these regional variations in structural remodeling indicate fundamental differences in the cardiac myocyte response based on type and duration of stimulus and location of the myocytes within the wall.

The present study was undertaken in an effort to understand better the effects of loading state of the myocardium on cardiac myocyte size and shape. Changes in cellular dimensions and their distribution resulting from cardiac hypertrophy, elicited by two different mechanisms, and cardiac atrophy were determined. Hypertrophy resulted from long-term pressure overload due to aortic constriction or a combined volume-pressure overload brought about by thyroid administration. Atrophy occurred after reduction of load in heterotopically isografted hearts.

Materials and Methods

Thyroid Treatment Protocol

Adult female (150 g body wt) Sprague-Dawley rats were obtained from Charles River (Montreal, Quebec). The rats were initially divided into control and thyroid-treated groups. Thyroid-treated rats received subcutaneous injections (20 µg/100 g body wt) of 3,3',5-triiodo-L-thyronine (T3) (Sigma Chemical Co., St. Louis) three times a week for 5 weeks. Control rats received the same treatment regimen using vehicle only (distilled H2O; pH 9). After 5 weeks of treatment, half of the control and thyroid-treated rats were killed, and their hearts were recovered and weighed. Hearts from the remaining rats were recovered and weighed 5 weeks after cessation of treatment.

Aortic Constriction Protocol

To provide a more comprehensive comparison between two types of cardiac hypertrophy on one hand and cardiac atrophy on the other, we have included a portion of the data from a recently published report from our laboratory. In that study, a detailed description of the aortic constriction protocol can be found. Briefly, an abdominal aortic constriction was introduced by tying a ligature around the aorta and a calibrated template in 5-day-old Sprague-Dawley rats of both sexes. Sham-operated littermates served as controls. At 3 months of age, the rats were killed, and their hearts were recovered and weighed.

Heart Transplantation Protocol

Adult male (350–400 g body wt) Lewis inbred rats obtained from Charles River were used. Details of the transplantation technique and long-term survival of the transplants are described in Korecky and Rakusan. Briefly, a donor heart was transplanted into the abdominal cavity of a recipient rat of similar body weight. The aortic and pulmonary arterial stumps of the donor heart were connected to the recipient rat’s abdominal aorta and inferior vena cava, respectively, via end-to-side anastomoses. Transplanted hearts were beating and perfused through the coronary circulation. At 1 month after transplantation, both recipient and transplanted hearts were removed and weighed. The in situ recipient hearts served as controls since previous results from our laboratory indicated that they do not differ from hearts of normal or sham-operated animals of corresponding body weight.

Cell Isolation Procedure

Cardiac myocytes were isolated from control and experimental whole hearts that were recovered from rats in all of the above-mentioned protocols. In view of the fact that the heart could not be dissected into its components before perfusion, only total heart weight could be determined. The isolation procedure used has been previously described. Retrograde coronary perfusion with calcium-free Joklik media containing 0.1 mM EGTA was followed by perfusion with Joklik media containing collagenase. After perfusion, hearts were initially divided into right ventricular free wall and left ventricle (plus septum). The left ventricle was further divided into outer (epimyocardium), middle (midmyocardium), and inner (endomyocardium) thirds. The tissue collected was minced in calcium-free, EGTA-containing Joklik media and poured through 250 µm nylon mesh. Freshly isolated cells were immediately fixed in 1.5% glutaraldehyde in 0.08 M phosphate buffer. Isolated myocytes were subsequently centrifuged through 4% Ficoll in 0.15 M phosphate buffer to effectively remove most endothelial cells, blood cells, and other unwanted debris without altering the undamaged myocyte population. Each region of all preparations characteristically contained more than 70% undamaged cells.

Volume of isolated myocytes was determined using a Coulter Channelizer (model C1000, Coulter Corp., Hialeah, Fla.) linked to a Coulter Counter (model ZM). The Coulter system determines cell volume by measuring the change in electrical resistance across an aperture resulting from displacement of electrolyte as cells move through the aperture. Since volume distribution curves were often skewed to the right, median cell volume values were used. Assessment of the population distribution based on cell volume was accomplished using the Coulter Electronics ACCUCOMP software package.

Quantitation of cell volume using the Coulter Channelizer system has been previously validated. Comparison of measured myocyte volume obtained by the Coulter Channelizer or by morphometric determination using fixed isolated myocytes and perfusion-fixed whole tissue sections indicated no differences. We use a shape factor that is appropriate for the rod-shaped myocytes passing through the aperture of the Coulter Counter. Although we routinely use isolated cell suspensions containing at least 70% rod-shaped myocytes, the inclusion of some rounded myocytes does not alter the calculated cell volume. Data recently reported by Burres and Cass further support the accuracy of the Coulter system in determining cell
volumes of several different cell types of irregular shape. We prefer to use isolated myocytes and the Coulter system for determination of cell volume because this method is more expeditious and objective and because it samples from a much larger portion of the myocyte population than methods using morphometric analysis of fixed cardiac tissue sections.

Cell length, defined as the longest length parallel to the longitudinal axis of the myocyte, was directly measured using the Bioquant BQ System IV (R&M Biometrics, Inc., Nashville, Tenn.). Myocyte cross-sectional area was estimated from the cell volume/cell length ratio. A minimum of 50 cells from each region of each rat was measured. Nucleation of cardiac myocytes was determined from 100 hematoxylin and eosin-stained cells.

Statistics

Student’s t test for unmatched pairs was used to compare body weight, heart weight, and the heart weight/body weight ratio. To examine the differences in cell volume, cell length, and cross-sectional area as a function of group and region, a two-way analysis of variance (ANOVA) was done. ANOVA was also used to examine differences in nuclearity between left ventricles of each group. If significant differences were indicated with the ANOVA, multiple comparisons were made using a Bonferroni t test.

Results

Cardiac Mass

Changes in body weight and heart weight resulting from the three experimental protocols described above are shown in Table 1. Both thyroid treatment and aortic constriction resulted in significant increases in heart weight and in the heart weight/body weight ratio, although the extent of cardiomegaly in hearts of constricted rats was greater. In thyroid-treated rats, 5 weeks after discontinuation of treatment, mean heart weight and the heart weight/body weight ratio had decreased but were still significantly greater than corresponding control values. In rats with aortic constriction, the extent of the cardiomegaly was greater in females compared with males. Transplanted hearts were significantly smaller than corresponding control recipient hearts. By using the heart weight/body weight ratio determined in control rats (2.44 ± 0.22 mg/g) and the mean body weight of all rats at the time of transplantation (355 ± 10 g), an average expected heart weight for donor and recipient groups (865 ± 24 mg) was calculated. According to these estimates, the in situ heart weight of recipient rats increased by 17% over the 1-month experimental period, whereas the nonworking, transplanted hearts considered as a single group decreased in mass by 7%. The transplanted hearts could be divided into two subgroups, mildly and severely atrophic, primarily based on isolated myocyte volume. Compared with control hearts at the time of recovery, the mildly atrophic hearts were not significantly different in heart weight, but the difference was highly significant (p < 0.001) in severely atrophic hearts.

Myocyte Dimensions

Thyroid treatment. Dimensions of cardiac myocytes for the right ventricle and three regions of the left ventricle are shown for thyroid-treated rats in Table 2. Right ventricular myocytes were smaller than myocytes from all regions of the left ventricle. Within the normal left ventricle, a transmural gradient in the size of myocytes was apparent: epimyocardial myocytes were the smallest, and endomyocardial myocytes were the largest. The gradient was maintained in hearts from rats treated for 5 weeks with T3. The hypertrophic response in T3-treated rats, based on cell volume, was greatest in myocytes of the right ventricle and epimyocardial region of the left ventricle. Although cell lengths were slightly increased in T3-treated rats, the increased cell volume was primarily due to significant increases in cross-sectional area. Five weeks after discontinuation of T3 treatment, cardiac myocyte dimensions had returned to normal size.

Aortic constriction. Regional changes in cardiac myocyte dimensions in aortic-constricted rats are shown in Table 3. Once again, right ventricular myocytes were smaller than those in the left ventricle, and a transmural gradient in myocyte size was evident in the left ventricle. In both males and females,
**Table 2. Dimensions of Cardiac Myocytes After Thyroid Treatment**

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell volume (μm³×10³)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁</td>
<td>11.1±1.9</td>
<td>13.3±0.7</td>
<td>20.4±2.8*</td>
<td>21.3±2.7*</td>
</tr>
<tr>
<td>T</td>
<td>18.7±3.2†</td>
<td>21.3±3.9†</td>
<td>30.0±4.6†</td>
<td>32.3±4.4†</td>
</tr>
<tr>
<td>C₂</td>
<td>12.5±2.0</td>
<td>14.0±1.8</td>
<td>22.7±4.4*</td>
<td>23.0±4.2*</td>
</tr>
<tr>
<td>T₀</td>
<td>11.1±1.5</td>
<td>14.2±2.4</td>
<td>21.1±4.8*</td>
<td>23.0±2.9*</td>
</tr>
<tr>
<td><strong>Cell length (µm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁</td>
<td>106.8±6.1</td>
<td>115.2±7.0</td>
<td>122.1±3.4‡</td>
<td>113.0±9.5</td>
</tr>
<tr>
<td>T</td>
<td>112.1±5.6</td>
<td>118.4±10.6</td>
<td>128.2±9.3‡</td>
<td>116.6±9.9</td>
</tr>
<tr>
<td>C₂</td>
<td>110.3±6.9</td>
<td>118.9±4.1</td>
<td>125.0±4.7‡</td>
<td>117.0±4.4</td>
</tr>
<tr>
<td>T₀</td>
<td>107.0±6.3</td>
<td>109.0±9.2§</td>
<td>123.0±5.8¶</td>
<td>110.1±11.9</td>
</tr>
<tr>
<td><strong>Cross-sectional area (µm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁</td>
<td>104±17</td>
<td>116±9</td>
<td>167±22*</td>
<td>189±22*</td>
</tr>
<tr>
<td>T</td>
<td>167±29†</td>
<td>181±33†</td>
<td>234±31‡</td>
<td>277±25§†</td>
</tr>
<tr>
<td>C₂</td>
<td>114±16</td>
<td>117±12</td>
<td>181±32*</td>
<td>197±38*</td>
</tr>
<tr>
<td>T₀</td>
<td>103±13</td>
<td>130±19</td>
<td>172±39*</td>
<td>211±32§¶</td>
</tr>
</tbody>
</table>

Values are mean±SD. RV, right ventricle; LV, left ventricle; EPI, epimyocardium; MID, midmyocardium; ENDO, endomyocardium; C₁, vehicle-treated controls for T group (rats killed after 5 weeks of thyroid treatment); C₂, vehicle-treated controls for T₀ group (rats killed 5 weeks after cessation of 5-week thyroid treatment).

*Significantly greater than RV and EPI at p<0.01.
†Significantly greater than C₁, C₂, and T₀ at p<0.01.
‡Significantly greater than RV at p<0.01.
§Significantly less than C₂ at p<0.05.
¶Significantly greater than RV, EPI, and END at p<0.01.

The hypertrophic response was greatest in myocytes from the right ventricle and epimyocardial portion of the left ventricle. The extent of the response resulted in the abolishment of the transmural size gradient observed in the left ventricle of control hearts. Significant increases were seen in cell length, but most of the significant increase in cell volume was because of the significantly increased cross-sectional area.

**Table 3. Dimensions of Cardiac Myocytes After Aortic Constriction**

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell volume (μm³×10³)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>14.3±4.5</td>
<td>18.1±5.0</td>
<td>23.9±3.5</td>
<td>24.7±3.4*</td>
</tr>
<tr>
<td>CSF</td>
<td>47.7±10.3†</td>
<td>57.4±5.7†</td>
<td>55.6±6.7†</td>
<td>65.5±6.5†‡</td>
</tr>
<tr>
<td>CM</td>
<td>29.1±2.5</td>
<td>31.8±2.1</td>
<td>36.4±1.9</td>
<td>40.9±2.1*</td>
</tr>
<tr>
<td>CSM</td>
<td>53.9±17.6†</td>
<td>65.6±6.3†</td>
<td>60.4±6.5†</td>
<td>67.9±6.8*†</td>
</tr>
<tr>
<td><strong>Cell length (µm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>104.8±6.1</td>
<td>112.7±5.7</td>
<td>113.2±4.0</td>
<td>110.7±4.7</td>
</tr>
<tr>
<td>CSF</td>
<td>137.1±9.6†</td>
<td>138.4±11.3†</td>
<td>136.2±10.8†</td>
<td>136.9±12.0†</td>
</tr>
<tr>
<td>CM</td>
<td>120.6±4.1</td>
<td>125.8±4.8</td>
<td>127.1±4.8</td>
<td>125.4±3.5</td>
</tr>
<tr>
<td>CSM</td>
<td>138.9±10.2†</td>
<td>143.6±2.3†</td>
<td>140.3±4.5†</td>
<td>143.2±2.6†</td>
</tr>
<tr>
<td><strong>Cross-sectional area (µm²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>135±37</td>
<td>161±46</td>
<td>211±30</td>
<td>223±36*</td>
</tr>
<tr>
<td>CSF</td>
<td>348±72†</td>
<td>417±56†</td>
<td>410±53†</td>
<td>483±74‡†</td>
</tr>
<tr>
<td>CM</td>
<td>241±20</td>
<td>253±19</td>
<td>287±12</td>
<td>327±17§§</td>
</tr>
<tr>
<td>CSM</td>
<td>384±109†</td>
<td>457±44†</td>
<td>431±50†</td>
<td>471±41†‡</td>
</tr>
</tbody>
</table>

Values are mean±SD; data are from Campbell et al.15 RV, right ventricle; LV, left ventricle; EPI, epimyocardium; MID, midmyocardium; ENDO, endomyocardium; CF, control female; CSF, constricted female; CM, control male; CSM, constricted male.

*Significantly greater than RV at p<0.05.
†Significantly greater than control at p<0.01.
‡Significantly greater than RV at p<0.01.
§Significantly greater than EPI at p<0.01.
A greater decrease in cross-sectional area, as compared with cell length, was responsible for the decrease in cell volume. In mildly atrophic hearts, the greatest decrease in cell size among left ventricular regions was seen in the endomyocardium. Although a similar trend was seen in severely atrophic hearts, the disparity in the atrophic response between left ventricular regions was not as great. Right ventricular myocytes in mildly atrophic hearts were moderately hypertrophied, largely due to increased cross-sectional area. A moderate degree of atrophy was seen in right ventricular myocytes from severely atrophic hearts.

Myocyte Nucleation

Table 5 presents a comparison of myocyte nucleation for groups in each experimental protocol. There were no significant differences in myocyte nucleation associated with T3 treatment. Hypertrophied cardiac myocytes in aortic-constricted rats of both sexes showed no significant differences in nuclearity compared with corresponding controls. However, male rats exhibited a significantly higher \((p<0.01)\) percentage of binucleated myocytes and a significantly lower \((p<0.01)\) percentage of mononucleated myocytes in the left ventricle compared with female rats. Cardiac myocyte atrophy did not significantly alter nuclearity compared with recipient controls, although there was a significant interaction \((p<0.01)\) between values for transplanted and recipient hearts. There was a significantly higher \((p<0.05)\) percentage of mononucleated and multinucleated

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**Table 4. Dimensions of Cardiac Myocytes After Heterotopic Transplantation**

<table>
<thead>
<tr>
<th></th>
<th>RV</th>
<th>EPI</th>
<th>MID</th>
<th>ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell volume ((\mu m^3\times 10^3))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>16.8±4.7</td>
<td>21.7±5.2</td>
<td>27.3±4.1*</td>
<td>26.0±3.3*</td>
</tr>
<tr>
<td>TR</td>
<td>16.3±7.7</td>
<td>8.8±3.9†</td>
<td>9.8±3.6†</td>
<td>8.5±2.7†</td>
</tr>
<tr>
<td>TR-m</td>
<td>20.3±4.2</td>
<td>12.4±2.2†</td>
<td>12.7±2.6†</td>
<td>11.1±1.0†</td>
</tr>
<tr>
<td>TR-s</td>
<td>10.4±9.2</td>
<td>6.1±2.1†</td>
<td>7.6±2.6†</td>
<td>6.5±1.5†</td>
</tr>
<tr>
<td><strong>Cell length ((\mu m))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>110.8±6.5</td>
<td>119.3±7.1</td>
<td>122.9±5.2§</td>
<td>119.3±4.5</td>
</tr>
<tr>
<td>TR</td>
<td>82.7±14.4†</td>
<td>77.9±11.7†</td>
<td>83.4±9.3†</td>
<td>82.5±12.7†</td>
</tr>
<tr>
<td>TR-m</td>
<td>89.5±5.6†</td>
<td>84.7±7.5†</td>
<td>88.6±6.2†</td>
<td>89.7±4.0†</td>
</tr>
<tr>
<td>TR-s</td>
<td>72.6±20.5†</td>
<td>72.9±12.4†</td>
<td>79.5±10.0†</td>
<td>77.1±14.8†</td>
</tr>
<tr>
<td><strong>Cross-sectional area ((\mu m^2))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>151±41</td>
<td>181±38</td>
<td>221±30*</td>
<td>217±23*</td>
</tr>
<tr>
<td>TR</td>
<td>189±78</td>
<td>110±37†</td>
<td>115±33†</td>
<td>101±23†</td>
</tr>
<tr>
<td>TR-m</td>
<td>228±50§</td>
<td>146±18‡</td>
<td>143±21‡</td>
<td>124±12∥</td>
</tr>
<tr>
<td>TR-s</td>
<td>130±90</td>
<td>83±16‡</td>
<td>94±25</td>
<td>85±9</td>
</tr>
</tbody>
</table>

Values are mean±SD. RV, right ventricle; LV, left ventricle; EPI, epimyocardium; MID, midmyocardium; ENDO, endomyocardium; R, recipient; TR, transplant; TR-m, transplant subgroup with mild atrophy; TR-s, transplant subgroup with severe atrophy.

*Significantly greater than RV at \(p<0.01\).
†Significantly less than recipient at \(p<0.01\).
‡Significantly less than RV at \(p<0.05\).
§Significantly greater than RV at \(p<0.05\).
∥Significantly less than RV at \(p<0.01\).
#Significantly greater than TR-s at \(p<0.05\).

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Comparison of male and female rats indicated that the extent of the response was approximately two times greater in myocytes from all regions in female hearts as compared with male hearts.

**Heterotopic isotransplantation.** Table 4 presents regional myocyte dimensions from heterotopically transplanted hearts. Similar to Sprague-Dawley control rats, right ventricular myocytes from Lewis control rats were smaller than left ventricular myocytes, and endomyocardial myocytes were larger than those in the epimyocardium of the left ventricle. In transplanted hearts considered as a single group, cell volume was significantly decreased in left ventricular myocytes; no change was seen in right ventricular myocytes. Significant decreases in both cell length and cross-sectional area in all regions of the left ventricle were responsible for the decreased cell volume, with cross-sectional area contributing slightly more to the change in cell volume than cell length. Atrophy was greatest in the midmyocardial and endomyocardial myocytes. Although no significant decrease in size was seen in right ventricular myocytes based on cell volume, it appears that some structural remodeling did occur. A significant decrease in cell length was seen, but this was compensated by an increase in cross-sectional area.

Transplanted hearts could be subdivided into two groups, mildly atrophic and severely atrophic (see Table 4). Within the left ventricle, cardiac myocyte cell volume decreased by approximately 50% in mildly atrophic and 75% in severely atrophic hearts.
myocytes and a significantly lower ($p<0.05$) percentage of binucleated myocytes in right ventricles compared with left ventricles in Lewis rats.

**Regression Analysis**

Figure 1 shows the correlation between cell volume and heart weight for all groups. Although the correlation was highest in rats with aortic constriction, all groups showed a significant correlation between heart weight and cell volume in both right and left ventricles. Ideally, right and left ventricular cell volume should be compared with right and left ventricular weight, respectively. However, our cell isolation procedure does not allow us to weigh individual ventricles. Therefore, we compared cell volumes of the right and left ventricle with whole heart weight. Considering that the left ventricle constitutes a greater proportion of total heart weight, the regression line determined for the left ventricle should be more accurate, have higher correlation coefficients, and come closer to the theoretical $y$ intercept of zero. This was generally the case. Although correlation coefficients were significant in the right ventricles, $y$ intercepts were further from the ideal value of zero, indicating greater accuracy in the calculated regression lines based on the use of whole heart weights instead of right ventricular weights. Although a single regression line was sufficient to describe the right ventricular response in all groups, it was apparent that within the left ventricle of transplanted hearts two regression lines were necessary. The slopes of regression lines were similar in all groups except for those of atrophic left ventricles. These results indicate that changes in cardiac mass may be primarily explained by alterations in the volume of the individual myocytes, whereas it seems likely that the total number of cardiac myocytes remained the same during both hypertrophic or atrophic responses.

**Myocyte Population Distribution**

Figure 2 describes the changes seen in distribution of myocytes for right and left ventricles of control and experimental hearts. In both right and left ventricles of thyroid-treated rats, the width of the distribution curves remained the same, but individual percentages for specific myocyte sizes differed. The distributions for control rats and those for thyroid-treated rats after discontinuation of treatment for 5 weeks were virtually identical. In control rats in the aortic constriction study, distribution curves for myocytes from the smaller hearts of female rats, compared with hearts of males, were narrower and positioned at lower cell volume values for both ventricles. As a result of the greater hypertrophic response in females as compared with males, the distribution curve for myocytes from females was shifted to relatively higher cell volumes, resulting in identical curves for constricted male and female rats for both ventricles. In atrophic hearts considered as one group, no difference was seen in the distribution of right ventricular myocytes, whereas the left ventricular myocyte distribution curve of atrophic hearts was shifted to lower values and narrower range compared with that of controls. If atrophic population distributions were analyzed using two subgroups, mildly and severely atrophic, the interpretation was slightly different. In the right ventricle, the distribution for mildly atrophic hearts was virtually identical to control, whereas that for severely atrophic hearts was narrower and more homogeneous. Within the left ventricle, a narrowing of the distribution curve and shift to lower values was seen in both subgroups, and the magnitude of the response was greater in severely atrophic hearts.

**Discussion**

This study demonstrates that changes in hemodynamic load of myocardium from normal physiological levels can substantially alter cardiac myocyte size and shape. The remodeling that occurs varies not only between ventricles but also within specific regions of the ventricular wall. These myocyte structural adaptations to chronically altered loads are indicative of the type and duration of the stimulus. Furthermore, they may provide some insight into the processes governing these alterations.

**Hemodynamic and Functional Alterations**

The onset of pressure overload was relatively gradual with the aortic constriction procedure used for this investigation. At 3 months of age, both systolic and diastolic blood pressure were significantly in-
creased, although heart rate was not changed compared with the control value. In the rat, chronic pressure overload during the compensatory phase is characterized by a slowing of contractile velocity. The decrease in maximum unloaded shortening velocity is accompanied by slowing of action potential duration, intracellular calcium transience, and relaxation velocity. Possible explanations for these reductions may include a major shift in isogene expression of myosin from the isoenzyme with highest specific ATPase activity (V₁) to that with the lowest activity (V₃), a reduction in the density of Ca²⁺ ATPase molecules, and perhaps decreased β₁-adrenergic receptor density.

Hemodynamic and functional alterations associated with T₃ administration at an identical dose to that used in the present investigation have been documented. Heart rate and left ventricular dP/dtₑₓₑ are increased very rapidly, most probably due to greater sensitivity and numbers of β-adrenergic binding sites. Increased left ventricular systolic pressure and cardiac output follow shortly after these changes and are subsequently maintained. Right ventricular systolic pressure and dP/dtₑₓₑ are also increased with T₃ treatment. In fact, during development of cardiac hypertrophy, the percent increase in right ventricular systolic pressure is greater than that of left ventricular systolic pressure. T₃ treatment supports the predominance of the fast V₁ isomyosin in the rat. The hemodynamic data confirm that the T₃ treatment model used in this study produces a combined pressure and volume overload.

The transplanted heart performs no external work, and the heart continues to beat, although at a slower rate, with ventricular chambers virtually empty. The degree of reduced afterload in the left ventricle depends on the competency of the aortic valve. Coronary flow drains into the right ventricle, primarily via thebesian veins, and the chamber volume and intracavitary pressure depend on the size of the opening at the anastomosis of the pulmonary artery and the recipient animal's inferior vena cava. Measurements in transplants in situ indicate some variability in intracavitary pressure in the right ventricle (B. Korecky, unpublished results, 1989). Despite considerable atrophy of the transplanted heart due to decreased load, it appears that the intrinsic contrac-

**FIGURE 1.** Graphs showing relation between cell volume and heart weight in thyroid-treated (panel A), aortic-constricted (panel B), and transplanted (panel C) rat hearts. T₃, 3,3',5-triiodo-l-thyronine.
tile mechanisms (e.g., maximum developed tension, maximum rate of development, and time to maximum tension) of the myocardium, represented by papillary muscles, remain normal. This contrasts with the model of unloading involving in vivo transection of papillary muscle chordae tendineae, in which a decrease in both velocity and extent of shortening of the papillary muscle occurred.

**Cardiac Mass**

*Altered load.* In accordance with the gradual onset of pressure overload with aortic constriction in this study, increase in cardiac mass was due to myocyte hypertrophy and not hyperplasia. This is in contrast to our previous study, in which induction of abrupt pressure overload in neonates with aortic constriction resulted in both hypertrophy and hyperplasia. Although production of pressure overload was gradual, the relatively early onset and subsequent long duration allowed us to achieve a greater level of hypertrophy than that seen with aortic constriction in adult rats. Comparison of the hypertrophic response in males and females showed a relatively greater increase in female heart weight compared with that in male rats.

**T₃ treatment.** Cardiac mass was significantly increased with T₃ treatment in this study, but the extent of the response was mild as expected from the relatively moderate pressure overload and duration of treatment associated with this chronic model. The percent increase in heart weight with hyperthyroidism in our study compares well with a study using a similar treatment regimen of shorter duration. However, longer duration of thyroid treatment can elicit a slightly greater hypertrophic response. Zimmer et al and Lortet et al have reported a greater proportional increase in right ventricular weight compared with left ventricular weight in response to the relatively greater increase in right ventricular systolic pressure than left ventricular systolic pressure with T₃ treatment.

**Heterotopic isotransplantation.** Analysis of atrophic events associated with unloading in normal hearts was done using heterotopic isotransplantation. In mildly atrophic transplants, total mass of the heart increased slightly compared with recipient rat hearts, most likely due to hypertrophy of the right ventricle, as noted visually at the time of death. Total mass significantly decreased in severely atrophic transplants compared with recipient hearts. The extent of the decrease in size of severely atrophic transplants

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**Figure 2.** Graphs showing changes in cardiac myocyte population distribution curves with cardiac hypertrophy and atrophy resulting from thyroid treatment (panel A), aortic constriction (panel B) (from Campbell et al), and heterotopic isotransplantation (panel C). T₃, 3,3',5-triiodo-L-thyronine.
comparates well with that reported by Klein and Hong and Korecky and Rakusan.

**Myocyte Dimensional Changes**

**Control.** In Sprague-Dawley rats, a transmural gradient in myocyte size existed in the left ventricle. These findings compare favorably with previous reports for Sprague-Dawley rats and other rat strains. Although cell volume of endomycocardial myocytes was larger than that of the epimycocardial myocytes of the left ventricle in Lewis rats, due to increased cross-sectional area, no transmural gradient was seen. Larger endomycocardial cell size compared with that of epimycocardial myocytes may be associated with higher developed wall tension and work load under normal circumstances in the endomycocardium in some species. However, because there is no consistent pattern of regional myocyte size differences in normal mammalian hearts, factors other than wall tension (e.g., genetics) may be more important in determining normal regional myocyte size.

Differences in estimated myocyte size for the left ventricle, as well as right ventricle, of adult control rats of the same age and different sex are primarily associated with heart weight differences that are due to variations in body weight. Our determination of myocyte nuclearity indicated an increased percentage of mononucleated myocytes in the left ventricle of female rats compared with males. A higher percentage of smaller mononucleated myocytes would result in a lower mean average of myocyte size in female rats. It has been suggested that the development of binucleation may indeed depend more on heart weight than on the age of the animal. It is also possible that sex may play a role, perhaps mediated by hormonal differences, in determining cell size in the myocyte population.

Similar regional patterns for myocyte size were seen in control Sprague-Dawley and Lewis rats. Right ventricular cell dimensions were smaller than comparable values in the left ventricle for both strains. Smaller right ventricular myocyte size compared with left ventricular myocyte size is most probably due to normally smaller afterload in the right ventricle. Previous reports have shown no difference in the percentage of binucleation of cardiac myocytes between right and left ventricles in Sprague-Dawley rats. However, our comparison of nuclearity between right and left ventricles of Lewis rats indicates a significantly higher proportion of mononucleated myocytes in the right ventricle. A similar variation in myocyte nuclearity between right and left ventricles has been reported in humans.

Considering that mononucleated myocytes are smaller in volume than binucleated myocytes, a larger population of mononucleated myocytes could contribute to differences in the average size of myocytes between the two ventricles. This variation could be characteristic for the Lewis strain of rats.

**Altered load.** The changes in myocardial load brought about by aortic constriction and T3 treatment resulted in a hypertrophic response in the cardiac myocytes of a similar nature, albeit a different extent. The greatest increase in size of left ventricular myocytes was seen in the epimycocardium with both protocols. The milder increase in load with T3 treatment caused myocyte hypertrophy with maintenance of the transmural gradient in cell size, whereas the gradient was abolished with the relatively greater response elicited by the aortic constriction protocol. However, it has been shown that thyroid treatment for a longer duration can also disrupt the transmural size gradient in the left ventricle. The greatest relative increase in the size of myocytes was in the right ventricle, and the majority of the hypertrophic response was due to increased thickness. This would theoretically implicate an increased pressure load on the right ventricle. Increased right ventricular pressure has been reported in hyperthyroid humans, rabbits, and rats. In principle, aortic constriction should primarily affect the left ventricle by increased systemic pressure. However, several investigators have shown an association between right and left ventricular hypertrophy with systemic hypertension. Considering the significant increase in cell length as well with our aortic constriction protocol, at 3 months after constriction some level of compensated heart failure leading to increased end-diastolic volume and volume overload may have been present. Therefore, the similarity in response with the two protocols producing cardiac hypertrophy could reflect the presence of some level of volume overload at 3 months after constriction, approximating the combined pressure-volume overload with T3 treatment or perhaps indicating the predominance of pressure overload as the primary factor affecting myocyte hypertrophy in these models.

Experimental hyperthyroidism did not change the width of the population distribution curve for either of the ventricles. This fact in concert with changes in myocyte size indicated that most of the changes occurred in the population of smaller myocytes; no appreciable response was seen in the population of larger myocytes. The changes observed in hearts from aortic-constricted rats, as expected, were more pronounced. The distribution curves for myocyte size from both right and left ventricles were shifted to higher cell volumes and widened, indicating a greater heterogeneity. The smaller myocytes hypertrophied to a greater extent, and the population distribution curves corroborate this fact by showing a relatively large percentage of the small myocyte population shifting to higher values. Contrary to hyperthyroid hearts, a small percentage of larger-than-normal myocytes were also involved in the restructuring of the myocyte population. This indicated the presence of growth potential in at least a portion of the larger myocyte population. Considering that the relative hypertrophic response was two times greater in females compared with that in males, the change in the
population distribution curve for females was greater, resulting in no apparent differences in distribution curves for males and females at 3 months.

Discontinuation of T₃ treatment initiated an unloading of the hypertrophied heart, leading to regression of the hypertrophy. In the present study, myocyte size reverted to normal, indicating the plasticity of the myocytes with the reestablishment of a normal load. However, heart weight remained significantly increased. The extent of the incomplete reversal in heart weight is identical to that reported after discontinuation of thyroid treatment of shorter and longer duration than that in the present study. Edema does not appear to be associated with this residual increase in heart weight, considering that thyroid treatment does not increase the wet weight/dry weight ratio. It has been shown that the right ventricle is more resistant to regression in size than the left ventricle and maintains a higher degree of hypertrophy. We previously reported that heart rate and systolic blood pressure reverted to normal within 1 week after discontinuation of a relatively longer duration of thyroid treatment. Lortet et al have shown that heart rate, cardiac output, and total peripheral resistance return to normal after 2 weeks of regression, but mean aortic pressure, left ventricular systolic pressure, and pressure-volume performance of both ventricles remain elevated. Normal myocyte size and residual increase in heart weight suggest that some extracellular factor may have contributed to the persistence of increased heart weight. A disproportionate increase in connective tissue in the right ventricle of pressure-overloaded cats has been reported. Gerdes et al have shown a preferential right ventricular fibrosis in hyperthyroid rats. It is possible that a relatively early fibrotic response was elicited in the right ventricle in the present study and that this response contributed to the residual increase in heart weight.

In transplanted hearts, significant decreases in cell volume were seen in all regions of the left ventricle. These changes were due to significant decreases in both cross-sectional area and cell length, indicating decreased preload and afterload. In mild atrophy, the decrease in cell size in the endomyocardium was greater than that in the midmyocardium, which was in turn greater than the decrease in the epicardium. In severe atrophy, the greatest decrease in size was in endomyocardial myocytes, but the extent of the difference between the endomyocardium and the other two left ventricular regions had lessened. These results agree with those shown by Korecky and Rakusan for transplanted hearts, in which the subendocardium responded to the reduction of load earlier than the midwall layer. They proposed that this response was due to a greater relative decrease in wall stress in the endomyocardium.

Although the difference in response in the left ventricle in mild and severe atrophic transplants is a matter of degree, there appears to be a disparity in type of response in the right ventricle. Considered as one group, no change in cell size was seen in the right ventricle after transplantation, which agrees with previously reported data. Although no change was evident based on cell volume alone, it appears that some remodeling did occur, with cell length decreasing and cross-sectional area increasing. Considered as mildly and severely atrophic groups, a moderate increase in the size of myocytes occurred in the right ventricle of mildly atrophic hearts, and a moderate cellular atrophy was seen in severely atrophic hearts. The increase in size was primarily due to increased cross-sectional area, indicating some degree of increased right ventricular pressure. Several possibilities exist that may explain this apparent contradiction in mildly atrophic hearts. In hearts with right ventricular hypertrophy, the opening at the end-to-side anastomosis between pulmonary artery and inferior vena cava may be relatively smaller than that in severely atrophic hearts, resulting in higher right ventricular pressure. This could result from variations in the surgical procedure or from clot formation at the opening. It is not uncommon for clot formation to also occur within the ventricular chambers of transplanted hearts, and this may also contribute to increased ventricular pressure and myocyte hypertrophy.

The size distribution for myocytes from the right ventricle in mildly atrophic hearts was virtually identical to that of the control, but a shift to lower cell volume values was apparent in severely atrophic hearts. It appears that a greater portion of the larger myocytes atrophied as compared with smaller myocytes. Similar changes in distribution curves for the left ventricle of mildly and severely atrophic hearts were noted, with the extent of the change larger in severe atrophy. A greater percentage of larger myocytes were affected, resulting in a shift to lower volumes and a narrowing of the distribution curves, indicating a more homogeneous population.

Correlation of Changes in Heart Weight and Myocyte Volume

The percent changes in heart weight and myocyte volume after alterations in normal load should be similar if no significant changes occur in myocyte number or myocyte volume composition of the heart. In all three protocols comprising this investigation, a disparity was evident in these comparisons. A part of these differences could be due to naturally occurring animal variability. It has been documented that significant morphological differences can occur in normal hearts from rats of the same strain that are obtained from different suppliers. Furthermore, it has been noted that differences in myocyte size and number can be present in different shipments of a given strain of rats from the same facility (A.M. Gerdes, personal communication, 1990). Considering the relatively modest difference in the percent change in heart weight and cell volume in T₃-treated rats, this variability could account for the disparity.

In aortic-constricted rats, there is good agreement between changes in heart weight and cell volume in
males, but there is a large difference in these changes in females. A much larger percentage increase was seen in myocyte volume than in heart weight. We have determined that the estimated myocyte number was not significantly different in the left ventricle of these rats, although it was slightly decreased, and that there was evidence for a possible reduction in cell number in the right ventricle. It is possible that some level of myocyte cell loss occurred in female rats with aortic constriction and that this could explain the inconsistency in the comparison. Anversa et al have reported myocyte cell loss with aging in rats, and similarities in the hypertrophic response between experimental pressure overload and aging have been reported.

In atrophic hearts, the degree of myocyte atrophy was considerably larger than the loss in heart weight. An increased percentage of connective tissue could account for this difference. It appears that the collagen volume fraction in atrophic hearts is increased (B. Korecky, unpublished results, 1989). Cooper and Tomanek have found an increased hydroxyproline content in unloaded papillary muscles, indicating connective tissue proliferation. Whether the increase is an active process or reactive to myocyte loss remains questionable. Korecky and Rakusan have concluded that the decrease in myocardial mass in atrophic hearts results from a loss of volume in existing myocytes and not from a change in total number of myocytes. However, Tomanek and Cooper found decreased myocyte number with long-term unloading of papillary muscles.

Potential Triggers and Mechanisms for Structural Changes

The mechanical alterations brought about by changes in load are certainly involved in the cascade of events leading to the remodeling of the cardiac myocyte. The extent of the stretch of the ventricular wall can influence the rate of protein synthesis. Peterson and Lesch were among the first to show that an increase in passive and active tension in myocardium (papillary muscle) can stimulate protein synthesis. Proteolysis may become the dominant factor below a certain level of loading. Disruption of normal fiber orientation, disorientation of contractile filaments, and loss of Z line substance have been shown to result from unloading of normal myocardium. These changes may alter normal tension in cardiac myocytes and lead to atrophy by protein degradation. Increase in collagen content with both pressure overload and unloading is known to occur and can lead to an increase in active and passive stiffness of the myocardium, potentially resulting in cardiac myocyte atrophy. Modification of two of the cytoskeletal elements of the myocyte (i.e., microtubules and desmin) has been reported during the initial stage of cardiac overload. This cytoskeletal rearrangement may be the first component of the sequence of events transmitting mechanical information from the sarcolemma to the myocyte nucleus, resulting in myofibrilogenesis. However, a definite link between mechanical changes and protein synthesis has not as yet been found.

The difference in the response between the right ventricle and left ventricle with pressure overload, as seen in our investigation, may be related to normal differences in anatomy and function. Cooper has remarked that the right ventricle operates as a thin-walled volume pump and that the left ventricle is a thick-walled pressure pump; therefore, pressure overload is a qualitative change for the right ventricle and a quantitative difference for the left ventricle. Variations in regional ventricular wall tension resulting from changes in load may, in part, account for different responses in the myocytes across the wall as found in our investigation. Regional variations in the extent of fibrosis could affect the level of myocyte response. Additionally, inherent regional genetic differences under normal circumstances may also be involved in this variable response.

There may also be a correlation between myocyte size and extent of myocyte hypertrophy. The regions with the smallest myocytes under normal physiological conditions (i.e., the right ventricle and the epicardium of the left ventricle) increased the most in relative size in our study. Additionally, the smaller myocytes of hearts from female rats compared with those from male rats of the same age hypertrophy to a greater extent with aortic constriction. Cardiac hypertrophy is an adaptation to chronic overload that operates at both the cellular level, by multiplying the number of contractile units, and the physiological level, by lowering wall stress. Swynghedauw points out that there are biological limitations to this adaptational process in that the myocyte cannot continually hypertrophy, a given DNA/cytoplasm ratio must be maintained, and adult cardiac myocytes cannot undergo mitosis. The maximum cell volume possible may vary with species and type of hypertrophy. Alternatively, the extent of muscle growth may depend on the rapidity with which the growth is induced. Young male rats grow more rapidly than female rats, and with the same level of constriction, as induced in our study, the aorta may become constricted relatively faster in male rats. It has been shown that the extent of left ventricular hypertrophy produced in male rats with more rapid onset of hypertrophy resulting from aortic constriction was less than that of females over the same time period, but with a more gradual induction of hypertrophy.

Proto-oncogenes (cellular oncogenes) may act as signals for DNA synthesis leading to the biochemical and metabolic changes required for structural remodeling associated with cardiac hypertrophy. The c-sis oncogene has been implicated as regulating fibrosis associated with left ventricular hypertrophy, while the c-erb A product may be involved in the effect of thyroid hormone. T3 is known to increase the RNA/DNA ratio, enhance adenine nucleotide synthesis, and increase protein synthesis and degradation. Klein et al have recently documented decreased protein syn-
thesis and increased protein degradation in hearts after heterotopic transplantation.

Conclusions

Cardiac hypertrophy, initiated by aortic constriction and T3 treatment in the present investigation, is characterized by cellular enlargement in the population of smaller myocytes within the ventricular walls. More extensive hypertrophy results in a greater heterogeneity in myocyte size within the myocyte population. Experimental data indicate that there may be a practical upper limit to myocyte size, although a portion of the larger myocyte population may maintain some growth potential. In contrast, atrophy predominantly affects larger myocytes, resulting in a more homogeneous overall population of smaller myocytes. Various alterations in load lead to diverse remodeling in the myocyte population within the heart, with smaller myocytes possessing the highest growth potential and larger myocytes exhibiting the greatest potential to atrophy.

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