Higher Antioxidative Capacity During a Chronic Stable Heart Hypertrophy

Madhu Gupta and Pawan K. Singal

Changes in oxygen radical mechanisms during 6–48 weeks of heart hypertrophy in rats subjected to a narrowing of the subdiaphragmatic aorta were examined. During this period, hypertrophied hearts demonstrated a stable hyperfunction, as indicated by an elevated but stable left ventricular systolic pressure, dP/dt, and aortic pressure and no change in left ventricular end diastolic pressure. Experimental animals showed increased heart-to-body weight ratios; however, the conventional signs of heart failure such as increased wet-to-dry weight ratios of liver and lung, ascites, or pleural effusion were absent. Hearts were examined for superoxide dismutase, glutathione peroxidase, and lipid peroxide activities. The superoxide dismutase activity was significantly higher in hypertrophied hearts at 6 and 12 weeks as compared with sham-operated rats (sham controls), while no difference was seen at 24 and 48 weeks due to a marked increase in the superoxide dismutase activity of sham control hearts in these age groups. During the period studied, glutathione peroxidase activity remained unchanged in controls but was significantly elevated in hypertrophied hearts. Lipid peroxide activity as indicated by the malondialdehyde content was significantly lower in hypertrophied hearts. Perfusion of isolated control and hypertrophy hearts with xanthine-xanthine oxidase, an exogenous source of oxygen radicals, resulted in contractile failure and rise in resting tension. In hypertrophied hearts, however, the contractile force was better maintained and there was a lesser rise in resting tension after exposure to xanthine-xanthine oxidase. The study suggests the development of a higher antioxidative capacity during the stable phase of hypertrophy due to a chronic pressure overload. (Circulation Research 1989;64:398–406)

Single electron reduction of oxygen in vivo can result in the formation of highly reactive oxygen species, for example, superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These partially reduced forms of oxygen can cause lipid peroxidation and have been shown to play a role in producing myocardial damage in a variety of conditions such as ischemia-reperfusion, catecholamine-induced arrhythmias and cardiomyopathy, adriamycin-induced cardiomyopathy, and atherosclerosis. Although these radical species are also produced under conditions of normal metabolism, their toxic effects are prevented by scavenging enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase. Furthermore, in vivo use of the above enzymes has been shown to prevent oxygen radical–induced damage. Since the concentration of catalase in the heart is very low, the major endogenous antioxidant enzymatic defense mechanism in myocytes may involve SOD and GSHPx. Although heart failure due to cardiac hypertrophy remains a major clinical problem, little research effort has been directed at identification of changes in specific antioxidant enzymes and related events during the process of cardiac hypertrophy. There are, however, reasons to believe that oxygen radical mechanisms do change during heart hypertrophy. In this regard, mitochondria isolated from hypertrophied rabbit heart were demonstrated to produce increased superoxide radical. Earlier, we showed an increased SOD activity and greater resistance to exogenous oxygen radical–induced damage in rat hearts during an early phase of hypertrophy. In this study, we examined myocardial SOD, GSHPx, and lipid peroxide activities as the hypertrophy duration is prolonged into a chronic stable hyperfunction phase. To further identify differences in control and hypertrophic hearts with...
respect to oxygen radical mechanisms, contractile 
force in the isolated hearts was studied in the pres-
ence of an exogenous source of oxygen radicals.

Materials and Methods

Animals and Hypertrophy Procedure

Male Sprague-Dawley rats weighing 120±20 g 
were kept in individual cages and were provided 
with food and water ad libitum. Pressure overload 
was produced by narrowing of the abdominal aorta 
in anesthetized (Nembutal, 35 mg/kg i.p.) rats. 
Access to the aorta was gained through a midline 
abdominal incision, and a 0.5 cm segment of this 
vessel in the subdiaphragmatic region was cleared 
from the adhering tissue. A blunt steel wire (1.15 
mm diameter) was placed along the aorta, and a 3-0 
silk ligature was tied snugly around the wire and 
aorta. The wire was then withdrawn from the li-
gature and the abdominal incision was closed. In 
this way, a gradual and progressive pressure overload 
was imposed by allowing young rats to grow into an 
initially nonconstricting band to cause 24±2% con-
striction at 6 weeks and 45±1.9% constriction at 48 
weeks of postoperative duration. Sham-operated 
rats (sham controls) were handled in an identical 
manner except that the aortic narrowing was not 
induced.

After surgery, hypertrophic and sham-operated 
rats were randomly divided into four groups: 6, 12, 
24, and 48 weeks. After a hemodynamic assessment 
at these time intervals, the rats were killed and their 
hearts removed for further studies.

Hemodynamic Measurement

Animals were anesthetized with Nembutal (35 
mg/kg i.p.), and a miniature pressure transducer 
catheter (model PR 249, Millar Instruments, Hou-
ton, Texas) was inserted into the right carotid artery 
catheter (model PR 249, Millar Instruments, Hous-
ton, Texas) was inserted into the right carotid artery 
and then quickly advanced into the left ventricle. 
Heart rate, left ventricular systolic pressure (LVSP), 
left ventricular end-diastolic pressure (LVEDP), 
maximal rate of rise and fall of left ventricular 
pressure (±dP/dt), and aortic pressures were 
recorded on a precalibrated multichannel dyno-
graph (Beckman Instruments, Fullerton, Califor-
nia). Measurements were taken 15 minutes after 
catheterization of the left ventricle when a steady 
state had been reached.

Tissue Weights

After decapitation, the heart, lung, and liver 
were immediately removed, freed from adhering 
tissues, and weighed. The left ventricle (including 
interventricular septum) was dissected and 
weighed. Dry weights for liver and lung were 
obtained by placing these organs in an oven (65°C) 
for 48 hours, and wet-to-dry weight ratios for 
these tissues were calculated.

Determination of Total RNA

RNA synthesis during cardiac hypertrophy was 
examined by a method described elsewhere. Briefly, 
the left ventricle was homogenized in 9 volumes of 
sodium phosphate buffer (pH 7.4), and 5 volumes of 
0.6 M perchloric acid was added to precipitate 
protein and nucleic acids. After cooling on ice for 10 
minutes, samples were centrifuged at 8,500g for 20 
minutes in Sorval RC2B centrifuge; the pellets were 
washed twice with 10 volumes of 0.2 M perchloric 
acid, resuspended in 4 volumes of 0.3N KOH, and 
icubated at 37°C for 1 hour to hydrolyze RNA. 
After protein and DNA (2.5 ml of 1.2 M perchloric 
acid) were precipitated out, the RNA content of the 
supernatant was determined at 260 nm. The data was 
calculated with standard RNA as milligrams RNA 
per gram left ventricular weight.

Superoxide Dismutase Assay

Cytosolic concentration of SOD was determined 
by a procedure characterized by Heikkila and 
Cabbat. Left ventricular tissue was homogenized 
in 9 volumes of 0.05 M sodium phosphate buffer 
(pH 7.4) and centrifuged at 30,000g for 45 minutes. 
A fresh 6-OH dopamine hydrobromide (Sigma Chem-
ical, St. Louis, Missouri) solution (10⁻³ M) was 
prepared in distilled water bubbled with nitrogen. 
Autooxidation of dopamine in the control test tubes 
was followed by addition of 50 μl of the above 
solution (final concentration, 1.66×10⁻⁵ M) to the 
buffer (final volume, 3 ml) and increase in absor-
bance was recorded for 5 minutes at 490 nm with a 
Spectronic 2000 spectrophotometer. The effects 
of different concentrations (100–2,000 units) of 
standard SOD (Sigma) as well as different concen-
trations of tissue protein on this autooxidation 
were examined to assess heart SOD activity in 
different groups. It is pointed out that autooxida-
tion of 6-hydroxydopamine yields high absorb-
bance increases per unit of protein at a physiolog-
ical pH 7.4 as opposed to the epinephrine-
adrenochrome assay and pyrogallol assay systems 
carried out at pH 10.2 and 7.9, respectively.

Glutathione Peroxidase Assay

GSHPx activity was measured by the method 
of Paglia and Valentine, and the activity was 
expressed as micromoles reduced nicotinamide ade-
nine dinucleotide phosphate (NADPH) oxidized to 
nicotinamide adenine dinucleotide phosphate 
(NADP) per minute per gram wet tissue, with a 
molar extinction coefficient for NADPH at 340 nm 
of 6.22×10⁶. Cytosolic GSHPx was assayed in a 
3-ml cuvette containing 2.0 ml of 75 mM phosphate 
buffer pH 7.0. The following solutions were then 
added: 50 μl of 60 mM glutathione, 0.10 ml glu-
tathione reductase solution (30 units/ml), 50 μl of 
0.12 M NaN₃, 0.1 ml of 15 mM N₃, EDTA, 0.1 ml of 
3.0 mM NADPH, and aliquot of cytosolic fraction 
(100 μl). Water was added to make a total volume
TABLE 1. Hemodynamic Studies on Sham Control and Hypertrophied Hearts Subjected to Pressure Overload for Different Durations

<table>
<thead>
<tr>
<th>Groups</th>
<th>ASP (mm Hg)</th>
<th>ADP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>+dP/dt (mm Hg sec⁻¹)</th>
<th>−dP/dt (mm Hg sec⁻¹)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 WS</td>
<td>136.4 ± 2.9</td>
<td>113.4 ± 3.1</td>
<td>138.0 ± 2.9</td>
<td>3.91 ± 0.30</td>
<td>4,945 ± 575</td>
<td>4,558 ± 483</td>
<td>340 ± 79</td>
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<tr>
<td>6 WH</td>
<td>170.8 ± 8.9*</td>
<td>131.6 ± 4.7*</td>
<td>170.8 ± 8.9*</td>
<td>3.74 ± 0.89</td>
<td>5,629 ± 175</td>
<td>5,514 ± 170</td>
<td>360 ± 16</td>
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<tr>
<td>12 WS</td>
<td>134.6 ± 4.0</td>
<td>111.0 ± 3.2</td>
<td>133.5 ± 4.6</td>
<td>2.75 ± 0.20</td>
<td>5,207 ± 272</td>
<td>5,221 ± 268</td>
<td>360 ± 10</td>
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<tr>
<td>12 WH</td>
<td>168.0 ± 5.2t</td>
<td>130.8 ± 5.0*</td>
<td>170.8 ± 4.6t</td>
<td>2.56 ± 0.45</td>
<td>5,871 ± 268</td>
<td>6,143 ± 420</td>
<td>397 ± 13</td>
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<tr>
<td>24 WS</td>
<td>138.0 ± 6.8</td>
<td>107.0 ± 5.3</td>
<td>135.0 ± 5.5</td>
<td>3.26 ± 0.59</td>
<td>4,541 ± 202</td>
<td>4,133 ± 180</td>
<td>323 ± 34</td>
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<tr>
<td>24 WH</td>
<td>175.5 ± 4.6f</td>
<td>134.5 ± 5.7*</td>
<td>177.5 ± 3.8f</td>
<td>3.42 ± 0.48</td>
<td>6,335 ± 257t</td>
<td>5,735 ± 316t</td>
<td>356 ± 17</td>
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<tr>
<td>48 WS</td>
<td>130.0 ± 6.2</td>
<td>115.0 ± 2.6</td>
<td>138.0 ± 2.8</td>
<td>3.82 ± 0.80</td>
<td>5,067 ± 130</td>
<td>4,922 ± 310</td>
<td>341 ± 15</td>
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<tr>
<td>48 WH</td>
<td>168.0 ± 3.2t</td>
<td>138.0 ± 5.2*</td>
<td>177.0 ± 4.8t</td>
<td>3.75 ± 0.90</td>
<td>5,810 ± 300*</td>
<td>5,910 ± 300*</td>
<td>348 ± 12</td>
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</table>

Data are expressed as mean±SEM with number of experiments in parenthesis. S, sham control; H, hypertrophy; W, weeks postoperation. Hypertrophied groups are compared with their respective sham control (ANOVA; unpaired t test). ASP, aortic systolic pressure; ADP, aortic diastolic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt, rate of change of left ventricular pressure.

*p<0.05,
tp<0.001.

2.9 ml. The reaction was started by the addition of 0.1 ml of 7.5 mM H2O2 and the conversion of NADPH to NADP was monitored by a continuous recording of the change in absorbance of the system at 340 nm every minute for 5 minutes.

Malondialdehyde Assay

Lipid peroxide formation was determined by the thiobarbituric acid method for the estimation of malondialdehyde content as described.20,21 Heart homogenate (10% wt/vol) was prepared in 0.2 M Tris-0.16 M KCl buffer, pH 7.4 and incubated for 1 hour at 37° C in a water bath. A 1-ml aliquot was withdrawn from the incubation mixture and was then centrifuged at 800 g for 15 minutes. The supernatant was used for the determination of thiobarbituric acid content. Mixed for 90 minutes. All hearts were allowed to stand for 20 minutes and were then centrifuged at 800 g for 15 minutes. The color was read at 532 nm. Commercially available malondialdehyde was used as a standard.

Heart Perfusion

In these experiments, animals were killed by decapitation, and their hearts were rapidly excised and placed in an ice-cold oxygenated buffer solution. The hearts were mounted on a steel cannula and perfused in a retrograde fashion through the coronary arteries at a constant flow rate of 8 ml/min according to the Langendorff method. The perfusion medium was a modified Krebs-Henseleit (KH) solution containing (mM) NaCl 120, NaHCO3 25.4, KCl 4.8, KH2PO4 1.2, MgSO4 0.86, CaCl2 1.25, and glucose 11.0 (pH 7.4). This solution was continuously gassed with a mixture of 95% O2-5% CO2. The temperature of the entire perfusion system was maintained at 37° C. The hearts were trimmed of all atrial material and electrically paced with bipolar platinum electrodes attached to the ventricular muscle. Supramaximal stimuli of 1.5 msec duration were delivered at the rate of 300 pulses/min. Myocardial contractile force and rate of change of developed tension (dF/dt) were recorded on a Beckman recorder via a force displacement transducer (FT 03). A resting tension of 2 g was applied to the hearts.

To generate partially reduced forms of oxygen, xanthine (2 mM at 40° C) and xanthine oxidase (10 units/l) were dissolved in the above buffer solution and mixed for 90 minutes. All hearts were allowed to equilibrate for 15 minutes with normal oxygenated KH buffer before switching to the KH medium containing xanthine-xanthine oxidase.

Protein Estimation and Statistics

For determination of protein concentration of tissue homogenate bovine serum albumin (fraction V, Sigma) was used as a standard.22 The results are expressed as mean±SEM. A one-way analysis of variance was carried out to test for any differences between the mean values of all groups. Individual means were compared with unpaired Student's t test. Values of p<0.05 were considered significant.

Results

Heart Function and Hypertrophy

Following 6, 12, 24, and 48 weeks of pressure overload, rats were examined for left ventricular...
Gupta and Singal

Antioxidants in Stable Hypertrophy

Comparison of systolic and diastolic left ventricular pressures (LVP) and their rate of rise (+dP/dt) and fall (−dP/dt), aortic pressures (AP) and electrocardiographic recordings in anaesthetised sham control (C), 6 (6WH) and 48 (48WH) week hypertrophy rats.

function (LVSP, LVEDP, and ±dP/dt), aortic pressures, and heart rate. Changes in these parameters were compared with their respective sham controls (Table 1). Pressure overload for different durations resulted in 24–30% increase in LVSP, and a corresponding increase in +dP/dt and −dP/dt was also seen. There was no change in the LVEDP in any of the rats. Both aortic systolic and diastolic pressures were significantly higher in the hypertrophied groups. The heart rate of hypertrophied rats was not significantly different from controls in any of the groups. There was no difference in these parameters when different sham controls were compared with each other. Typical recordings of these parameters in sham control, 6-week hypertrophic, and 48-week hypertrophic rats are shown in Figure 1.

Table 2. Changes in Left Ventricular Weight, Body Weight, and Myocardial RNA Content in Different Groups of Rats With Aortic Constriction or Sham Operation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Left ventricle weight (mg)</th>
<th>Body weight (g)</th>
<th>Left ventricle/body weight (mg/g)</th>
<th>RNA content (mg/g left ventricle wet weight)</th>
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</thead>
<tbody>
<tr>
<td>6 WS</td>
<td>804±18.5</td>
<td>437±7.9</td>
<td>1.83±0.04</td>
<td>2.23±0.09</td>
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<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
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<td>(6)</td>
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<tr>
<td>6 WH</td>
<td>1,010±23.4**</td>
<td>445±6.9</td>
<td>2.27±0.04**</td>
<td>3.06±0.08**</td>
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<td>(12)</td>
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<tr>
<td>12 WS</td>
<td>904±29.7</td>
<td>533±9.04</td>
<td>1.68±0.04</td>
<td>2.02±0.12</td>
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<td>(7)</td>
<td>(11)</td>
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<td>(6)</td>
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<tr>
<td>12 WH</td>
<td>1,112±39.5**</td>
<td>525±12.0</td>
<td>2.16±0.08**</td>
<td>2.21±0.08</td>
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<td>(11)</td>
<td>(11)</td>
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<td>(6)</td>
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<tr>
<td>24 WS</td>
<td>1,010±46.5</td>
<td>568±33.2</td>
<td>1.76±0.06</td>
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<td>(5)</td>
</tr>
<tr>
<td>24 WH</td>
<td>1,328±53.0*</td>
<td>579±15.3</td>
<td>2.16±0.06*</td>
<td>1.98±0.11</td>
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<td>(6)</td>
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<td>(5)</td>
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<tr>
<td>48 WS</td>
<td>1,026±38.4</td>
<td>607±23.6</td>
<td>1.68±0.03</td>
<td>2.11±0.09</td>
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<td>(7)</td>
<td>(7)</td>
<td></td>
<td>(5)</td>
</tr>
<tr>
<td>48 WH</td>
<td>1,262±32.1**</td>
<td>580±28.2</td>
<td>2.17±0.04**</td>
<td>2.00±0.16</td>
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<td>(7)</td>
<td>(7)</td>
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<td>(5)</td>
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</tbody>
</table>

Data are expressed as mean±SEM with number of experiments in parenthesis. Each hypertrophied group is compared with its respective sham control (ANOVA; unpaired t test). W, weeks after surgery; S, sham; H, hypertrophy.

*p<0.01.

**p<0.001.
Antioxidative Enzymes

Superoxide dismutase. Autooxidation of 6-hydroxydopamine in a test tube gives rise to oxidation products with an increase in absorbance at 490 nm. This reaction is promoted by superoxide anion and inhibition of this reaction has been used to assess SOD activity.\textsuperscript{18} Dopamine autooxidation, thus color intensity, increased with time and reached a saturation level at 4 to 5 minutes (Figure 2a). Addition of standard solution of SOD containing 100 to 2,000 units showed a dose-dependent reduction in this autooxidation; that is, 100 units of SOD had no effect and 2,000 units of SOD had a maximum effect. Addition of 400 µg of homogenate protein had a significant effect on autooxidation. To examine the linearity of this homogenate effect, studies were done with different concentrations (200 to 1,200 µg) of protein (Figure 2b). The reaction was linear up to 600 µg of protein. Therefore, in

| Table 3. Comparison of Wet Weight, Dry Weight and Wet/Dry Weight Ratios of Liver and Lung of Sham Operated and Aortic Constricted Rats at Different Durations of Surgical Procedure |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Liver           | Wet wt (g)      | Dry wt (g)      | Wet wt (g)      | Dry wt (g)      |
| Wet wt-to-dry wt ratio | Liver | Lung | Liver | Lung |
| 6 WS            | 12.64 ± 0.50    | 3.95 ± 0.13     | 1.77 ± 0.06     | 0.31 ± 0.01     | 3.20 ± 0.32     | 5.71 ± 0.18     |
| 6 WH            | 12.81 ± 0.20    | 3.80 ± 0.14     | 1.80 ± 0.05     | 0.33 ± 0.02     | 3.36 ± 0.40     | 5.40 ± 0.20     |
| 12 WS           | 15.80 ± 0.30    | 4.52 ± 0.20     | 2.12 ± 0.03     | 0.42 ± 0.03     | 3.49 ± 0.03     | 5.00 ± 0.16     |
| 12 WH           | 15.70 ± 0.40    | 4.60 ± 0.16     | 2.20 ± 0.02     | 0.43 ± 0.02     | 3.41 ± 0.04     | 5.11 ± 0.12     |
| 24 WS           | 17.90 ± 1.02    | 5.45 ± 0.35     | 2.33 ± 0.24     | 0.48 ± 0.04     | 3.28 ± 0.03     | 4.77 ± 0.16     |
| 24 WH           | 19.89 ± 1.28    | 6.04 ± 0.39     | 2.80 ± 0.10     | 0.60 ± 0.03     | 3.29 ± 0.02     | 4.68 ± 0.17     |
| 48 WS           | 16.20 ± 1.60    | 4.89 ± 0.50     | 3.94 ± 0.35     | 0.86 ± 0.10     | 3.20 ± 0.04     | 4.60 ± 0.17     |
| 48 WH           | 18.37 ± 0.90    | 5.51 ± 0.22     | 3.89 ± 0.19     | 0.87 ± 0.05     | 3.32 ± 0.06     | 4.49 ± 0.18     |

Data are expressed as mean ± SEM of six experiments. W, weeks after surgery; S, sham; H, hypertrophy.
further studies on the SOD activity in the heart homogenates, 400 μg of protein was used.

Heart homogenates from different hypertrophic groups showed a greater degree of inhibition (range, 56–70%) of autooxidation of dopamine over 5 minutes (Figure 3B) as compared with the degree of inhibition (range, 35–68%) observed with homogenates from different sham controls (Figure 3A). There was an age-related variation in the inhibition of dopamine autooxidation; that is, 6-week sham controls showed a 35% inhibition (less SOD) compared with 68% inhibition (more SOD) in 48-week sham controls. When individual hypertrophied groups were compared with their respective sham controls, the greatest stimulation, more than 170%, of SOD activity was observed at 6 weeks, with 130% at 12 weeks, and no significant difference in 24- and 48-week hypertrophied hearts (Figure 3C).

Glutathione peroxidase. The GSHPx activity was measured in different groups of heart homogenates by following the rate of glutathione oxidation by H$_2$O$_2$ at 240 nm (Figure 4). There was an increase (19.7±1.2%) in GSHPx activity in the 6-week hypertrophied heart compared with its sham control, and this elevated level of activity was maintained in 12-, 24-, and 48-week hypertrophied hearts. All the increases were statistically significant (p<0.05). There was no significant difference in GSHPx activity when different sham control groups were compared with each other.

Lipid Peroxidation

Different groups of hypertrophied and sham control hearts were examined for malondialdehyde content, a relatively stable end product and a good indicator of lipid peroxide activity and the results are shown in Table 4. Hypertrophied hearts at each postoperative duration showed a smaller

<table>
<thead>
<tr>
<th>Postoperative duration (weeks)</th>
<th>Malondialdehyde (nmol/g wet weight)</th>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
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<tr>
<td>6</td>
<td>29.04±2.24 (6)</td>
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<tr>
<td>12</td>
<td>27.07±1.54 (7)</td>
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<tr>
<td>24</td>
<td>27.51±1.43 (6)</td>
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<tr>
<td>48</td>
<td>26.54±0.94 (7)</td>
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</table>

Data are expressed as mean±SEM with number of experiments in parenthesis.

*Significantly different (p<0.05) when compared with sham control.
TABLE 5. Xanthine-xanthine Oxidase-induced Changes in Developed Tension in Different Groups of Isolated Perfused Hearts

<table>
<thead>
<tr>
<th>Group</th>
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<th>16</th>
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<th>24</th>
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<tr>
<td>6 WS</td>
<td>65.0±2.8</td>
<td>40.8±6.8</td>
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<td>3.8±2.8</td>
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<td>0</td>
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<tr>
<td>6 WH</td>
<td>80.5±8.2*</td>
<td>60.5±3.9*</td>
<td>52.8±9.8*</td>
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<td>12 WS</td>
<td>77.2±3.5</td>
<td>52.3±9.4</td>
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<tr>
<td>12 WH</td>
<td>90.2±4.4*</td>
<td>69.3±2.1*</td>
<td>43.3±3.7</td>
<td>17.6±2.5</td>
<td>4.2±2.0</td>
<td>0</td>
</tr>
<tr>
<td>24 WS</td>
<td>70.4±3.1</td>
<td>49.7±5.8</td>
<td>34.9±4.5</td>
<td>16.9±3.8</td>
<td>3.6±2.5</td>
<td>0</td>
</tr>
<tr>
<td>24 WH</td>
<td>93.0±4.6*</td>
<td>78.2±10.1*</td>
<td>44.9±9.6</td>
<td>19.1±8.2</td>
<td>2.9±2.8</td>
<td>0</td>
</tr>
<tr>
<td>48 WS</td>
<td>73.0±4.8</td>
<td>49.6±6.8</td>
<td>38.6±3.8</td>
<td>15.3±3.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48 WH</td>
<td>84.6±3.0*</td>
<td>70.8±5.6*</td>
<td>41.8±6.4</td>
<td>22.0±4.5</td>
<td>0</td>
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</tr>
</tbody>
</table>

Values are mean±SEM of five to six experiments, expressed as percentage of zero time control data. W, weeks after surgery, H, hypertrophy.

*Significantly (p<0.05) different compared to respective sham controls (S).

Xanthine-xanthine oxidase perfusion. Similarly, following exposure to xanthine-xanthine oxidase, both +dF/dt and −dF/dt showed a relatively lesser drop in all of the hypertrophied hearts as compared with sham controls (data not shown). The rise in resting tension was significantly less in different groups of hypertrophied hearts at the majority of the time points (Figure 5). The mean value for rise in resting tension for the four hypertrophy groups at 28 minutes of xanthine-xanthine oxidase perfusion was 327±19% as compared with the sham control groups mean value of 480±30%.

Discussion

Cardiac hypertrophy is an adaptive response to a chronic increase in workload on the heart, which can progress into a state of exhaustion or heart failure. This transition from a compensated hypertrophy state to heart failure is determined by different factors such as the severity, the duration, and the type of workload. In the present study, we used a chronic model of hypertrophy in which a gradual and progressive constriction of the abdominal aorta resulted in a sustained heart hypertrophy (23–28%) and a stable cardiac hyperfunction. Increased RNA content at 6 weeks indicated greater protein synthesis probably during an early growth phase and has also been observed in cardiac growth induced by a variety of experimental procedures. Since there

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Comparison of effect of xanthine-xanthine oxidase perfusion on rise in resting tension in different groups of sham control (SHAM) and hypertrophied (HYPERTROPHY) hearts. W, weeks of post-operative duration. Each value represents mean±SEM of six experiments.
were no signs of heart failure as indicated by 1) lack of ascites or pleural effusions, 2) lack of change in wet-to-dry weight ratio of liver and lungs for up to 48 weeks of postoperative duration, and 3) maintained end-diastolic pressure, we suggest it to be a "stable hypertrophy" state.

Our data show that a mild chronic pressure overload results in a significantly increased SOD activity for 12 weeks after surgery. As the hypertrophy duration is prolonged further, this increase does not remain significant because of an age-related increase in the SOD activity of controls. GSHPx activity, on the other hand, remained elevated throughout the study. An increase in GSHPx in response to increased pressure load for 60 days has recently been reported in rabbits. Our data show that both SOD and GSHPx respond to pressure overload and that the change in SOD activity may not remain as important as GSHPx when the duration of hypertrophy is prolonged. Decreased malondialdehyde formation, even at the time when increase in SOD was no longer apparent, suggests that GSHPx may play an important role in maintenance of low lipid peroxides in hypertrophied hearts. In this regard, GSHPx has been shown to be a significant factor in the stabilization of polyunsaturated membrane lipids. At any rate, an increase in antioxidative capacity with a stable hypertrophy is strongly suggested by the present study.

Xanthine-xanthine oxidase as an exogenous source of oxygen radicals has been used by a number of investigators. Some of the active oxygen species identified in the myocardial effects of xanthine-xanthine oxidase are superoxide, hydrogen peroxide, and hydroxyl radicals. We have shown that these oxygen species induce contractile failure, which is associated with increased lipid peroxidation, loss of high energy phosphates and structural damage. A relatively higher resistance of hypertrophied hearts to this exogenous oxidative stress in the present study may be partially due to the increased antioxidative capacity afforded by SOD and GSHPx along with a drop in lipid peroxidation. However, it is pointed out that contribution of other factors, such as increased contractile units and changes in the permeability of coronary vessels so that the free radical injury cannot extend into the hypertrophied myocardium with the same speed and intensity as in hearts, cannot be ignored. In spite of their higher antioxidative capacity, the contractile failure time in 12-, 24-, and 48-week hypertrophied hearts due to xanthine-xanthine oxidase was comparable to sham controls, and this may be related to the overwhelming effect of the high dose of xanthine-xanthine oxidase used in this study.

Rabbit hypertrophied heart has been found to be more sensitive to adriamycin cardiotoxicity as well as to ischemia-reperfusion injury. Both conditions are considered to involve oxygen radicals in producing myocardial dysfunction and as such, these observations may appear to be contrary to the increased resistance of the hypertrophied hearts to oxygen radical injury seen in the present study. These differences may be due to the difference in the animal model and/or variations in the degree or stage of hypertrophy. It is well established that the contractile force development and metabolic functions in the myocardium vary according to the degree of hypertrophy, namely mild, moderate, or severe. In the present study, an apparent increase in the SOD activity at 6 and 12 weeks was dissipated at 24 and 48 weeks because of the age-dependent increase in SOD activity in controls, which clearly documents a perceived variation even within the stable state of hypertrophy.

The exact stimulus for the increased activity of these enzymes is not known; however, one possibility can be that increased radical formation itself during increased metabolic activity due to pressure overload may be the signal. This is supported by the fact that GSHPx activity increased in intestinal mucosa and liver after oral administration or peroxidized lipids and in lung after peroxidation caused by ozone or 90% oxygen for two weeks. In this regard, increased formation of oxygen radicals has been demonstrated in the mitochondria isolated from a hypertrophied heart. Furthermore, a number of studies have reported changes in antioxidative enzymes under a wide range of physiological and pathological conditions such as age, exercise, and beta-thalassaemia minor. In this study, an age-related increase in SOD activity in sham control hearts was also apparent.

In conclusion, our data show that myocardial adaptation to increased pressure load is accompanied by higher activity of radical scavenging enzymes. This greater antioxidative capacity of the heart reduces lipid peroxidation and probably helps to maintain the heart function against higher oxidative stress caused by increased metabolism. This study emphasizes that antioxidant status in the heart is a dynamic function adjusting to the physiological and/or pathophysiological conditions imposed.

References

6. Singal PK, Beamish RE, Dhalla NS: Potential oxidative pathways of catecholamines in the formation of lipid perox-
36. Chow CK, Tappel AL: An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. LBD 1972;7:518–524

Key Words • cardiac hypertrophy • superoxide dismutase • glutathione peroxidase • lipid peroxidation • antioxidant activity
Higher antioxidative capacity during a chronic stable heart hypertrophy.
M Gupta and P K Singal

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