EFFECTS OF MODERATE HYPERTENSION ON CARDIAC FUNCTION AND METABOLISM IN THE RABBIT

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SUMMARY To study the early effects of hypertension on the heart, we examined isolated hearts from rabbits with slowly developing hypertension of up to 64 weeks in duration after unilateral nephrectomy and renal artery stenosis. Normotensive animals kept under identical conditions served as controls. Mean arterial blood pressure rose from 83 to 155 mm Hg in the hypertensive group of longest duration, but the ratio of left ventricular weight to body weight was not different between the experimental and control groups. Although left ventricular hypertrophy was not present, left ventricular peak systolic pressure of perfused hearts was significantly higher in hypertensive than in normotensive hearts. Furthermore, while in hypertensive hearts the left ventricular end-diastolic volume was increased, the peak systolic pressure did not respond to an increase in left ventricular end-diastolic volume. Functional changes were accompanied by metabolic changes in the left ventricle. Rates of glucose utilization were increased and rates of ketone body utilization were decreased in hypertensive hearts. Activities of key enzymes of carbohydrate metabolism (phosphorylase, hexokinase, phosphofructokinase, and lactate dehydrogenase) were increased, while those of ketone body metabolism (3-oxoacid-CoA transferase, acetoacetyl-CoA synthase) were decreased and those of the citric acid cycle (citrate synthase, 2-oxoglutarate dehydrogenase) were not different between groups. In summary, moderate hypertension for a period of more than 1 year resulted in functional and metabolic changes of the left ventricle in hypertensive animals that were already manifest at 8 weeks of hypertension. The mechanism for the structural and functional adaptations that characterize the response of the left ventricle to hypertension may rest in changes of enzyme activities and resultant changes in substrate supply of the heart. (Hypertension 11: 416-426, 1988)

KEY WORDS • renovascular hypertension • cardiac substrate metabolism • cardiac enzyme activities • left ventricular function

HYPERTENSION increases cardiac work by increasing plasma volume and peripheral vascular resistance. Sustained hypertension leads to changes in cardiac function and energy metabolism and, ultimately, to the development of cardiac hypertrophy. Adaptation of the heart to a high cardiac work load is important for survival of the organism as a whole and is governed by derepression of genetic information, leading to new synthesis of contractile proteins, mitochondria, and enzyme systems of myocardial energy production.

Acute pressure overload of the heart in experiments using banding of either the pulmonary artery or the aorta leads to rapid development of hypertrophy and a decrease in contractility in isolated papillary muscles, as measured by the rate of force development and the velocity of shortening by extrapolation to zero stress.1 More recent studies have reported decreased tension-dependent heat production and actin-activated myosin adenosine triphosphatase (ATPase) activity.2,3 Decreased contractility in pressure overloaded hearts has been ascribed to changes in the myosin isozyme pattern. In particular, the absolute predominance of myosin isozyme V3, the isozyme with the slowest mobility on pyrophosphate polyacrylamide gels, and alterations in the region of the myosin heavy chain molecule with the first class sulfhydryl group have been observed in pressure overloaded rabbit hearts.4,5 Most models of pressure overload–induced hypertrophy employ methods leading to acute overload and rapid onset of hypertrophy and failure, which is virtually never observed in human pathology, where pressure overload–induced hypertrophy is most commonly
of gradual onset and preceded by a long period of functional adaptation without evidence of hypertrophy. Although changes in the activity of energy-utilizing systems, such as myosin ATPase, have been noted early in the course of experimental hypertrophy, these changes have thus far not been documented in a consistent, well-controlled model of hypertension characterized by a more gradual onset in the increased cardiac work load.

A model of the temporal development of adaptation to an increased work load has been proposed by Meerson. Adaptation, irrespective of its stimulus, is characterized initially by an increase in the "intensity of function" per unit of heart muscle mass. This process activates the genetic apparatus, causing an increased synthesis of nucleic acids and proteins in the heart muscle cells. Consequently, heart muscle increases the machinery for energy production by increasing the size and number of mitochondria before it increases both contractile proteins and its cell volume. As a result, the "intensity of function" first increases and then returns to its initial level, and since the cell mass has increased, adaptation enters a phase of chronic compensation in the form of hypertrophy. Only in the late, decompensated state do mitochondria and contractile proteins degenerate, giving way to the emergence of "unspecified cytoplasm." A requisite for the present study was the need to establish a model of gradually evolving hypertension. Having attained this criterion in the one-kidney, one clip (1K1C) model, we sought to characterize relationships between moderate hypertension and the left ventricular myocardium, to examine intrinsic contractile performance of the heart, and to define the interaction of energy-providing substrates during sustained hypertension. In this model, the moderate hypertension and duration did not induce hypertrophy but led to altered function of the left ventricle. These functional alterations were accompanied by changes in substrate metabolism favoring glucose as fuel for respiration in the hypertensive heart.

Materials and Methods

Experimental Protocol

Pathogen-free, male New Zealand white rabbits (Dutchland Rabbitry, Denver, PA, USA) weighing 2.0 to 2.5 kg were housed in separate cages in a temperature-controlled room (27–29°C) and maintained on regular rabbit chow diet (100 g/day). Rabbits initially underwent a 2-week conditioning period that consisted of daily handling. Blood pressure measurements were made once a week. After conditioning, each rabbit was assigned to one of 10 groups. The designation and the number of animals in each group at the end of the study are given in Table 1. Groups 1A to E were normotensive, Groups 2A to E were hypertensive. One animal died of pyelonephritis and renal failure, one animal was killed because of severe glaucoma, and one animal died during handling. All data from these animals were excluded from the final data analysis. During the subsequent weeks, blood pressure measurements were made once per week and body weights were recorded once per month. During the fourth or fifth week, a clip was placed over the left renal artery of Groups 2A to E.

Blood Pressure Measurements

A modified Grant-Rothschild (Scientific Instrument Machine Shop, University of Texas Medical School at Houston) pressure capsule was used to measure systolic and diastolic blood pressures from the median artery of the right ear. Values obtained with a similar capsule were shown to be in close agreement (10–15%) with the diastolic and systolic pressures obtained directly from the aorta.

Blood pressure measurements were obtained from the proximal third of the ear. Measurements were made independently in triplicate from each rabbit once a week by two observers. The three systolic-diastolic values obtained for each animal each week were averaged for each animal and for each group.

Renal Hypertension

The 1K1C Goldblatt hypertension model was created by placing a silver clip (inside diameter, 0.90 mm) over the left renal artery. Clipping was followed by contralateral nephrectomy 2 weeks later. The surgical procedures were performed with the animals under a combination anesthesia previously described.

Determination of Weights

To determine body weights, each animal was weighed once per month and at the time of death. To determine heart and kidney weights, the left and right ventricles were weighed separately at the time of the perfusions or before enzyme extractions. An aliquot of each was dried to constant weight, and the dry weight of both the left and the right ventricle was calculated from the wet weight/dry weight ratio. The left kidneys were weighed at necropsy and after they had been dried to constant weight.

Isolated Heart Perfusion

At the end of the period designated for each group, the animals received an intravenous heparin (200 units) infusion and were killed within 1 minute there-
Tissue Extraction
described earlier. A portion of the powder was used for wet
removal and metabolite production were determined as
to precipitate proteins. Samples were stored on ice
butyrate (10 mM) and acetoacetate (1 mM).

Determination of Enzyme Activities
The conditions for assays of enzyme activity were
were described by Crabtree et al. Specifically,
and dehydrogenase, 23 3-oxoacid-CoA transferase, 23 and acetoacetyl-CoA
thiolase. 22 Enzyme activities are expressed as micro-
moles of substrate transformed per minute per gram of
weight/dry weight determinations, and the remaining
powder was suspended in 5 volumes (ml/g) of cold 6%
perchloric acid and homogenized with a motor-driven
Teflon pestle in a polycarbonate tube. The homogenate
was centrifuged, and the supernatant fraction was neu-
tralized with 20% KOH.

Analysis of Metabolites
The following metabolites in the perfusate were ana-
yzed: D-glucose, L(-)-lactate, acetoacetate, and
3-hydroxybutyrate. Tissue samples were assayed for
adenosine 5'-triphosphate (ATP), adenosine
5'-diphosphate, adenosine 5'-monophosphate, phos-
phocreatine, L(-)-lactate, pyruvate, 2-oxoglutarate,
alanine, aspartate, malate, and oxaloacetate. All ass-
ays were performed spectrophotometrically by stand-
ard enzymatic methods.

Determination of Rates
Aliquots of the perfusate (1 ml) were removed every
10 minutes. Each sample was immediately mixed with
0.1 ml of 60% (weight/vol) HClO₄ to destroy trace
enzymes activities that might have leaked from the heart
and to precipitate proteins. Samples were stored on ice
until neutralization with 20% KOH. Rates of substrate
removal and metabolite production were determined as
described earlier.

Tissue Extraction
Frozen hearts were crushed in a mortar containing
liquid N₂. A portion of the powder was used for wet
after by cervical dislocation. Hearts were removed
through a left-sided lateral thoracotomy and immedi-
ately immersed in ice-cold Krebs-Henseleit solution.
The aorta was cannulated and perfused retrogradely in
a modified Langendorff apparatus. Briefly, the per-
fusion apparatus included a multibulb oxygenator
(P.A. Brooks, Whitney, England) and a Millipore filter
system (Bedford, MA, USA), which permitted re-
circulation of the medium. The perfusion medium was
Krebs-Henseleit bicarbonate saline oxygenated with a
mixture of O₂ (95%) and CO₂ (5%) and maintained at
39°C. A left atriotomy was performed, and a left ven-
tricular apical vent was made. All hearts were paced at
200 beats/min by an electrode, connected to a Grass
SD9 stimulator (Quincy, MA, USA), and sutured to
the right atrium.

To assess left ventricular function, a high compli-
ance polyurethane balloon was introduced into the left
ventricle and secured by a pursestring suture around
the mitral valve. The balloon was filled with distilled
water (39°C) and connected to a Millar Mikro-Tip 5F catheter pressure transducer (Houston, TX, USA).
The transducer signal was amplified by a Millar TCB 100 transducer control unit connected to a physiologi-
ical recording system (Gould Electronics, Cleveland,
OH, USA). Peak left ventricular pressure and left ven-
tricular end-diastolic pressure (LVEDP) were recorded
continuously.

Hearts were initially perfused for 5 minutes by a
single pass to remove remaining blood and to allow for
the resumption of forceful contractions. For recircu-
lation, the heart was placed inside a temperature-
controlled perfusion chamber connected to a closed
perfusion circuit of the perfusion apparatus. The bicar-
carbonate saline in the recirculating system (200 ml) con-
tained glucose (5 mM) plus a mixture of 3-hydroxy-
butyrate (10 mM) and acetoacetate (1 mM).

During the initial 15 minutes, left ventricular per-
fusion was performed by inflating the balloon and
raising the LVEDP from 1 to 20 mm Hg in 2 mm Hg
increments. The hearts were then perfused for another
90 minutes, the first 45 minutes at a high work load
(LVEDP = 16 mm Hg) and the last 45 minutes at a low
work load (LVEDP = 4 mm Hg). At the end of perfu-
sion, the hearts were frozen between aluminum clamps
cooled in liquid N₂ before determination of dry weight
and extraction.

Determination of Rates
Aliquots of the perfusate (1 ml) were removed every
10 minutes. Each sample was immediately mixed with
0.1 ml of 60% (weight/vol) HClO₄ to destroy trace
enzyme activities that might have leaked from the heart
and to precipitate proteins. Samples were stored on ice
until neutralization with 20% KOH. Rates of substrate
removal and metabolite production were determined as
described earlier.

Growth and Body Weight
The mean body weights for the animals in each
group are shown in Figure 1. The average weight at the
time of entry into the study ranged from 2.15 to 2.92
kg. There was a continuous increase in body weight as
the animals aged; no significant difference in weight
was observed between any of the groups after the sec-
dom month.

Results
The mean body weights for the animals in each
group are shown in Figure 1. The average weight at the
time of entry into the study ranged from 2.15 to 2.92
kg. There was a continuous increase in body weight as
the animals aged; no significant difference in weight
was observed between any of the groups after the sec-
dom month.
Arterial Blood Pressures

Arterial systolic and diastolic blood pressures increased significantly in the hypertensive groups (Groups 2A–E) compared with those of the respective control groups (Groups 1A–E; Figure 2). There were differences in the blood pressure response to the renal artery constriction between groups. Since experimental variables were controlled, these differences must be ascribed to variability of the model.

The hypertensive groups demonstrated relatively steady increases in arterial systolic and diastolic blood pressure; blood pressure values were significantly greater than preoperative values 5 weeks after operation. The mean arterial blood pressure ranged from 83 ± 5 mm Hg before operation to 155 ± 7 mm Hg after operation at the end of the observation period (Group 2E).

Organ Weights

The results of heart and kidney weights are given in Table 2. The left ventricular dry weight ranged from 0.637 (Group 1A) to 1.176 g (Group 2D). In spite of this range, the ratios of left ventricle to body weight or left ventricle to right ventricle, or both, showed little variation between normotensive and hypertensive animals. Thus, sustained moderate hypertension of up to 64 weeks caused no interest in left ventricular mass. In contrast, the kidneys of animals that had undergone renal artery clipping and nephrectomy showed a significant increase in weight (about 60%).

Intrinsic Left Ventricular Performance

After 10 minutes of preliminary perfusion and before perfusion at high and low work loads, hearts were subjected to functional assessment. This assessment was done by recording the developed peak systolic pressure as a function of increased preload at a constant afterload of 90 mm Hg (Frank-Starling curve) and by determining the balloon volume required to attain a predetermined left ventricular filling pressure (from 2 to 20 mm Hg). The results given in Figure 3 show a representative example of left ventricular function curves for hearts from normotensive (Group 1B) and hypertensive animals (Group 2B). Taken collectively, an increase of left ventricular filling pressure from 4 to 20 mm Hg resulted in a rise in peak systolic pressure from 70 (minimum) to 142 mm Hg (maximum) in the normotensive group. In contrast, peak systolic pressure was elevated up to 144 mm Hg and did not rise further with an increase in preload in the hypertensive group. The Frank-Starling curve was flat and was not due to decreased left ventricular compliance. To the contrary, Figure 3 shows a markedly increased left ventricular volume for a given LVEDP in hypertensive hearts compared with controls.

Substrate Competition

Earlier work by Wittels and Spann26 and Bishop and Atschuld27 suggests that glucose becomes the preferred fuel for respiration in hypertrophied heart muscle. The present experiments tested the hypothesis that a change
FIGURE 2. Systolic and diastolic blood pressures. Pressures were recorded weekly as described in Materials and Methods. Each point represents the blood pressure (mean ± SD) of the animals listed for each group (see Table 1). Groups 1A to E (normotensive controls) are in Panel A; Groups 2A to E (hypertensive animals) are in Panel B. An asterisk denotes the first blood pressure value significantly greater than the preoperative value (p<0.05). All subsequent values were also significantly greater than control values, but the asterisks have been omitted for clarity.
Table 2. Organ Weights

<table>
<thead>
<tr>
<th>Group</th>
<th>LV (g dry wt)</th>
<th>LV/body wt (g dry wt/kg)</th>
<th>LV/RV</th>
<th>Kidney/body wt (g dry wt/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.637 ± 0.183</td>
<td>0.21 ± 0.06</td>
<td>2.5 ± 0.5</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td>B</td>
<td>0.791 ± 0.087</td>
<td>0.23 ± 0.03</td>
<td>3.2 ± 0.4</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>C</td>
<td>0.997 ± 0.113</td>
<td>0.26 ± 0.03</td>
<td>2.9 ± 0.6</td>
<td>0.50 ± 0.06</td>
</tr>
<tr>
<td>D</td>
<td>1.011 ± 0.109</td>
<td>0.26 ± 0.05</td>
<td>3.1 ± 0.5</td>
<td>0.48 ± 0.06</td>
</tr>
<tr>
<td>E</td>
<td>0.923 ± 0.070</td>
<td>0.26 ± 0.01</td>
<td>3.0 ± 0.9</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.804 ± 0.085</td>
<td>0.27 ± 0.03</td>
<td>2.8 ± 0.7</td>
<td>0.70 ± 0.06*</td>
</tr>
<tr>
<td>B</td>
<td>0.947 ± 0.371</td>
<td>0.24 ± 0.09</td>
<td>2.6 ± 0.8</td>
<td>0.75 ± 0.07*</td>
</tr>
<tr>
<td>C</td>
<td>1.088 ± 0.250</td>
<td>0.29 ± 0.06</td>
<td>3.2 ± 0.5</td>
<td>0.80 ± 0.16*</td>
</tr>
<tr>
<td>D</td>
<td>1.176 ± 0.100</td>
<td>0.32 ± 0.03</td>
<td>2.8 ± 0.3</td>
<td>0.71 ± 0.05*</td>
</tr>
<tr>
<td>E</td>
<td>1.114 ± 0.223</td>
<td>0.31 ± 0.05</td>
<td>3.5 ± 0.7</td>
<td>0.64 ± 0.06†</td>
</tr>
</tbody>
</table>

Values are means ± SD. Groups are defined in Table 1. LV = left ventricle, RV = right ventricle.

*p < 0.01, †p < 0.05, compared with Group 1 (normotensive animals) values.

in substrate selection occurs in hearts of hypertensive animals (i.e., in hearts showing no evidence of hypertrophy). A mixture of glucose and ketone bodies as substrates was chosen because ketone bodies are oxidized by heart muscle without the need for a special transport system at the inner mitochondrial membrane. These substrate concentrations were within a physiological range for both glucose (e.g., in the postprandial state) and ketone bodies (e.g., with fasting), considering that these concentrations are the highest starting concentrations. Concentrations fell continuously with recirculation of medium during the course of the experiment, while at the same time metabolites of glucose metabolism (lactate) and of 3-hydroxybutyrate metabolism (acetoacetate) were released into the perfusate.

Data on substrate utilization and metabolite production at high and low work loads by normotensive and hypertensive hearts are summarized in Table 3. Glucose utilization increased in hearts after 8, 24, 28, and 64 weeks of hypertension. Since enhanced rates of glucose utilization could not be accounted for by enhanced rates of lactate production and since activation of glycogen synthesis is a relatively slow process, the enhancement of glucose utilization probably reflects increased rates of glucose oxidation. In contrast to glucose utilization, utilization of 3-hydroxybutyrate was decreased in the hearts from hypertensive animals. As in the hearts from normotensive animals, glucose and 3-hydroxybutyrate both contributed to the fuel of respiration, but in four of five groups of hypertensive hearts, the contribution of glucose to the fuel of respiration was greater than the contribution of 3-hydroxybutyrate.

Tissue Metabolites

There was no significant difference in the key metabolites of energy metabolism between hearts from normotensive and hypertensive animals. Specifically, there was no difference in phosphocreatine (e.g., 24.7 ± 5.4 vs 23.2 ± 9.1 μmol/g dry weight in Groups 1E and 2E, respectively) and in adenine nucleotides (data not presented). The lactate/pyruvate concentration ratio ranged from 8.7 ± 4.1 (in Group 1A) to 19.5 ± 6.8 (in Group 2D), but there was no difference between normotensive and hypertensive hearts. Citrate levels were elevated (up to 3.2 ± 0.8 μmol/g dry weight) as expected in hearts perfused with a medium containing ketone bodies,16 but there was no difference in citrate, 2-oxoglutarate, glutamate, malate, and aspartate levels between normotensive and hypertensive groups.

Enzyme Activities

Because of the preferential utilization of glucose in hearts from hypertensive animals (Groups 2A, 2B, C, and E), we examined the activities of cytosolic enzymes of the glycolytic pathway, of mitochondrial enzymes of ketone body metabolism, and of the citric acid cycle. Table 4 summarizes the activities of key enzymes of carbohydrate metabolism. The activities of hexokinase, phosphofructokinase, and phosphorylase of hearts from normotensive control rabbits were simi-
TABLE 3. Rates of Substrate Removal and Metabolic Production

<table>
<thead>
<tr>
<th>Group</th>
<th>Work load</th>
<th>Glucose removed (µmol/hr/g dry wt)</th>
<th>Lactate produced (µmol/hr/g dry wt)</th>
<th>3-Hydroxybutyrate removed (µmol/hr/g dry wt)</th>
<th>Acetoacetate produced (µmol/hr/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Low</td>
<td>-114 ± 76</td>
<td>103 ± 68</td>
<td>-427 ± 94</td>
<td>72 ± 37</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-270 ± 102</td>
<td>301 ± 101</td>
<td>-230 ± 117</td>
<td>189 ± 34</td>
</tr>
<tr>
<td>B</td>
<td>Low</td>
<td>-175 ± 21</td>
<td>132 ± 22</td>
<td>-332 ± 113</td>
<td>44 ± 10</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-365 ± 44</td>
<td>155 ± 47</td>
<td>-270 ± 64</td>
<td>27 ± 9</td>
</tr>
<tr>
<td>C</td>
<td>Low</td>
<td>-109 ± 64</td>
<td>77 ± 31</td>
<td>-263 ± 120</td>
<td>188 ± 78</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-204 ± 61</td>
<td>91 ± 37</td>
<td>-263 ± 112</td>
<td>65 ± 31</td>
</tr>
<tr>
<td>D</td>
<td>Low</td>
<td>-242 ± 101</td>
<td>189 ± 12</td>
<td>-126 ± 21</td>
<td>43 ± 7</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-225 ± 140</td>
<td>82 ± 29</td>
<td>-193 ± 54</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>E</td>
<td>Low</td>
<td>-236 ± 65</td>
<td>146 ± 29</td>
<td>-198 ± 40</td>
<td>36 ± 4</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>-219 ± 38</td>
<td>250 ± 22</td>
<td>-150 ± 15</td>
<td>69 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SD of four hearts in each group except for groups IB, IE, and 2D, where there were only three hearts. Work loads are defined in Materials and Methods.

*p < 0.05, **p < 0.001, ***p < 0.001, compared with the corresponding value in Group 1.

for those published for rat heart by Opie and Newsholme and Crabtree and Newsholme. Lactate dehydrogenase activity was substantially lower in rabbit than in rat heart. Activities of hexokinase, phosphofructokinase, and phosphorylase were increased in the left ventricles from hypertensive animals. This finding corresponds to the increased rate of glucose utilization by hearts from hypertensive rabbits.

The table summarizes the activities of the mitochondrial enzymes of ketone body metabolism. Control data of 3-hydroxybutyrate dehydrogenase, 3-oxoacid-CoA transferase, and acetoacetyl-CoA synthase were similar to those found in rat hearts by Williamson et al. There was no change in enzyme activities in the normotensive groups. However, activities of 3-oxoacid-CoA transferase and acetoacetyl-CoA synthase were all decreased in the left, but not the right, ventricle of hypertensive rabbits.

Table 6 summarizes the activities of two key enzymes of the citric acid cycle, citrate synthase and 2-oxoglutarate dehydrogenase. Both enzymes have been implicated in the regulation of citric acid cycle activity in heart muscle. There was no change in activities of these enzymes between normotensive and hypertensive groups. However, there was a tendency toward increased activity of 2-oxoglutarate dehydrogenase in the left ventricles. This enzyme has been found to control flux through the citric acid cycle in rat heart.
Although the exact mechanism of its regulation is not fully understood.

**Time Course of the Change in Enzyme Activities, Mechanical Function, and Rates of Substrate Removal**

With increased duration of the observation period, we observed either an increase or a decrease in the activity of some, but not all, enzymes in the left ventricle. We observed either an increase or a decrease in the activity of some, but not all, enzymes in the left ventricle. Although the exact mechanism of its regulation is not fully understood.

**Discussion**

Although left ventricular hypertrophy has long been recognized as a consequence of systemic hypertension, studies of the natural history of hypertension-induced metabolic changes in left ventricular myocardi

**Table 5. Enzyme Activities at 25°C: Mitochondrial Enzymes**

<table>
<thead>
<tr>
<th>Enzyme Activities at 25°C: Mitochondrial Enzymes</th>
<th>Group</th>
<th>RV</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hydroxybutyrate dehydrogenase (μmol/min/g wet wt)</td>
<td>A</td>
<td>11±4</td>
<td>15±8</td>
</tr>
<tr>
<td>3-Oxoadic-CoA synthase (μmol/min/g wet wt)</td>
<td>B</td>
<td>14±5</td>
<td>14±5</td>
</tr>
<tr>
<td>Acetoacetyl-CoA synthase (μmol/min/g wet wt)</td>
<td>C</td>
<td>13±1</td>
<td>14±1</td>
</tr>
<tr>
<td>2-Aminobutyrate dehydrogenase (μmol/min/g wet wt)</td>
<td>D</td>
<td>8±4</td>
<td>16±9</td>
</tr>
<tr>
<td>2-oxoglutarate dehydrogenase (μmol/min/g wet wt)</td>
<td>E</td>
<td>16±4</td>
<td>17±3</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of three hearts. RV = right ventricle; LV = left ventricle; ND = not determined. *p<0.05, tp<0.01, $p<0.001, compared with the corresponding value in Group 1.

**Table 6. Enzyme Activities at 25°C: Citric Acid Cycle Enzymes**

<table>
<thead>
<tr>
<th>Enzyme Activities at 25°C: Citric Acid Cycle Enzymes</th>
<th>Group</th>
<th>RV</th>
<th>LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase (μmol/min/g wet wt)</td>
<td>A</td>
<td>13±9</td>
<td>15±7</td>
</tr>
<tr>
<td>2-Oxoglutarate dehydrogenase (μmol/min/g wet wt)</td>
<td>B</td>
<td>149±17</td>
<td>112±16</td>
</tr>
<tr>
<td>2-Aminobutyrate dehydrogenase (μmol/min/g wet wt)</td>
<td>C</td>
<td>151±40</td>
<td>127±28</td>
</tr>
<tr>
<td>2-oxoglutarate dehydrogenase (μmol/min/g wet wt)</td>
<td>D</td>
<td>89±22</td>
<td>141±18</td>
</tr>
<tr>
<td>3-oxoacid-CoA transferase (μmol/min/g wet wt)</td>
<td>E</td>
<td>138±34</td>
<td>145±16</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of three hearts. RV = right ventricle; LV = left ventricle; ND = not determined.
trophy, and although even mild forms of hypertension lead to an elevated tension-time index, we did not observe hypertrophy even after 64 weeks of moderate sustained hypertension. Instead, we observed an increase in intra-left ventricular volume and pressure development in the isolated heart. There is much evidence that increased afterload is not the sole factor that leads to hypertrophy. Indeed, a number of clinical studies have shown a surprisingly poor correlation between left ventricular mass and arterial blood pressure in children and adults. We conclude from the present data that, even though hypertension does not necessarily lead to hypertrophy, it produces changes in left ventricular function and energy metabolism that may be observed after only 18 weeks of moderate hypertension.

The earliest functional changes we observed related to both systolic and diastolic function of the left ventricle. At low LVEDPs, pressure development in hearts from hypertensive animals was about twice the pressure development of normotensive control animals (see Figure 3). In contrast to normotensive animals, pressure did not rise with an increase in filling pressure of the left ventricle, suggesting maximal left ventricular systolic function. Although increased resting stiffness of the left ventricle is well described in hypertrophied myocardium, no such changes have been described in hearts from hypertensive animals before the onset of hypertrophy. We use the term myocardial stiffness to differentiate those changes in the stiffness of the myocardium itself from those changes seen with increased muscle mass alone. Our results seem to confirm the commonly held notion that diastolic function does not constrain cardiac performance in normal hearts subjected to an increased work load.

The flat Frank-Starling curve could also be the result of an artifact. One important consideration is that the coronary perfusion pressure may have been too low for the hypertensive heart. Pressure development is affected by perfusion pressure when it is less than 60 mm Hg. Although a 90 mm Hg afterload would be enough for the control heart (peak systolic pressures, 70–103 mm Hg), there is a possibility that the coronary flow was too low for hypertensive hearts that exhibited more than 140 mm Hg pressure. Such relative ischemia would be reflected in increased release of lactate (which was not the case in the present study). Furthermore, since the hypertensive heart exhibited a high pressure against a relatively low afterload, either the rigid aortic cannula (inside diameter, 3.8 mm), or a prolapse of the intraventricular balloon into the aortic outflow tract may also have been causes for the flat Frank-Starling curve.

Aside from a mechanical artifact, increased stiffness of the left ventricle due to interstitial fibrosis, subendocardial fibrosis, infiltration, or scar tissue could be implicated for the altered intrinsic performance. It is unlikely, however, that any of these pathological changes were present in our model, which demonstrated an increase in diastolic compliance. Furthermore, although fibrosis has been shown in hypertrophied hearts by direct and indirect methods, quantitative ultrastructural analysis of biopsy samples of left ventricular myocardium from Groups 1E and 2E did not demonstrate any interstitial changes (J. Schaper, personal communication, 1987).

Alterations in energy metabolism underlying changes in diastolic left ventricular function are not well defined. Morphometric analyses in early hypertrophy by Page et al., Rabinowitz and Zak, and more recently, Matlib et al. have demonstrated an increase in mitochondrial mass before there is an increase in myofibrillar mass, suggesting an increase in the capacity for oxidative phosphorylation while cardiac function is well compensated. Our data on the activity of the mitochondrial enzymes citrate synthase, 2-oxoglutarate dehydrogenase and 3-hydroxybutyrate dehydrogenase do not suggest an increase in mitochondrial enzyme activity with sustained hypertension. Instead, the data on metabolite removal show a preferential use of glucose when compared with ketone bodies as competing substrates for the fuel of respiration. At the same time the activities of key glycolytic enzymes increased, while those of two of the three enzymes involved in ketone body metabolism decreased. These findings suggest that changes in enzyme activities control the flux through the two different metabolic pathways. Others have shown an increase in hexokinase and a decrease in 3-hydroxybutyrate dehydrogenase activity in hypertrophied left ventricles of hypertensive rats.

Little is known about the effects of hypertension on the fuel selection of the heart, although the increased work load must profoundly affect myocardial energy metabolism. Studies in heart muscle homogenates suggest that there is decreased oxidation of fatty acids and increased glycolysis in the advanced stage of hypertrophy. More recently, Yonekura et al. investigated regional myocardial substrate uptake by hearts in hypertensive rats with positron emission tomography and reported decreased fatty acid uptake and increased glucose uptake in severe hypertension.

The present study confirms these observations and suggests that a change in substrate selection is a hallmark of metabolism by the hypertensive heart. Preferred glucose utilization may be due to either increased activity of glycolytic enzymes or increased synthesis of a glucose transporter protein. The actual mechanism has not yet been elucidated. In isolated normotensive rat hearts in vitro, we have reported that, at high work load, fatty acids not only suppress glucose utilization, but that, vice versa, glucose also suppresses fatty acid oxidation. Glucose seems to have an important advantage over fatty acids as metabolic fuel, when the supply of O2 is stressed, as may be the case in the heart in hypertension; glucose oxidation provides more ATP per mole of O2 than do fatty acids.

In conclusion, our results suggest that changes in myocardial function and energy metabolism are associated with moderate, sustained hypertension. Thus, the earliest changes of the heart are probably found in adaptation of its energy metabolism rather than in a
quantitative increase in contractile proteins or cellular mass.

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References
22. Tanase H, Yamori Y, Hansen CT, Lovenberg W. Heart size in inbred strains of rats: II. Cardiovascular DNA and RNA content during the development of cardiac enlargement in rats. Hypertension 1982;4:872–880
23. Frohlich ED, Tanzi RC, Bumpus FM. Is arterial pressure the sole factor responsible for hypertensive cardiac hypertrophy? Am J Cardiol 1979;44:959–963
24. Drayer JT, Weber MA, DeYoung JL. Blood pressure as a...
determinant of left ventricular mass. Arch Intern Med 1983; 143:90–92
Effects of moderate hypertension on cardiac function and metabolism in the rabbit.
H Taegtmeyer and M L Overturf

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