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### **Research Article**

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## Physiologic Cardiac Hypertrophy Corrects Contractile Protein Abnormalities Associated with Pathologic Hypertrophy in Rats

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**ABSTRACT** To evaluate the combined effects of cardiac overload imposed by hypertension and by chronic exercise, male and female rats were made hypertensive by unilateral renal artery stenoses and made to exercise in an 8-10-wk swimming program. Sedentary normotensive animals, sedentary hypertensive animals and normotensive animals exposed to the swimming program were also studied. Hypertension was associated with the development of cardiac hypertrophy, and this was exaggerated in hypertensive swimmers. Actomyosin,  $\text{Ca}^{2+}$ -myosin, and actin-activated  $\text{Mg}^{2+}$ -myosin ATPase activities were enhanced in normotensive swimmers, depressed in hypertensives and were normal or increased in hypertensive swimmers. Myosin isoenzyme analysis showed a predominant  $V_1$  pattern in normals; an increase in percent  $V_1$  isoenzyme in swimmers; a predominant  $V_3$  pattern in hypertensives; and a return to the predominant  $V_1$  pattern in hypertensive swimmers. These findings suggest that the hypertrophy imposed by hypertension and hypertrophy imposed by physical training using a chronic swimming program are distinctly different biological phenomena. Physical training by swimming prevents the changes in cardiac myosin induced by hypertension despite the exaggeration of hypertrophy.

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### INTRODUCTION

Numerous studies have demonstrated that cardiac hypertrophy resulting from systolic overload results in depressed contractile function, diminished contractile protein adenosine triphosphatase (ATPase)<sup>1</sup> activity (1), and in the rat a shift of the myosin heavy-chain isoenzyme  $\text{HC}_\alpha$  homodimer with high ATPase activity ( $V_1$ ) to  $\text{HC}_\beta$  form with lower ATPase activity ( $V_3$ ) (2). On the other hand, it has been observed in the rat that physiologic loads such as chronic exercise, which result in cardiac hypertrophy in the rat, may increase contractile performance (1), contractile protein ATPase activity (1), and a shift to even a greater predominance of the  $V_1$  myosin isoenzyme (3).

The hypothesis for the current investigation was that cardiac hypertrophy is a continuum and that the superimposition of a physiologic load upon the pathologic load induced by renal hypertension in rats would increase the total load on the heart, exaggerate both the cardiac hypertrophy and the alterations in contractile proteins associated with the pathologic cardiac hypertrophy.

### METHODS

Female rats aged 10 wk and weighing ~175 g at the beginning of the experiment, and male rats ~7 wk of age weighing 175 g at the beginning of the experiment were made hypertensive by placing renal artery clips 0.18-0.25 mm around the left renal artery (4). Male and female rats were used because although hypertensive hypertrophy occurs in both sexes with consequent depressed contractile protein ATPase levels, physical training by swimming is associated with physiologic hypertrophy only in the females (5). Male

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<sup>1</sup> Abbreviation used in this paper: ATPase, adenosine triphosphatase.

and female sham animals were also prepared from the same shipment as the experimental animals and had the same surgical procedure except for the placement of the renal artery clips. After 3 wk blood pressure was measured by the tail-cuff pressure method under light ether anesthesia (4). When the blood pressure exceeded 150 mm Hg the animals were considered to be hypertensive. Half of the animals in the control and hypertensive animal groups were subjected to a swimming program and the other half allowed to remain sedentary. The exercise program, described previously (5, 6), consisted of swimming 5 d a week, 75 min twice a day for 8 wk in males and 10 wk in females. At the end of that time, animals were anesthetized with ether, their hearts were removed, washed in saline, homogenized and prepared for actomyosin or myosin analysis (6, 7).

For preparation and purification of myosin, 1–2 hearts were pooled and myosin was obtained that was shown by sodium dodecyl sulfate (SDS) gel electrophoresis to be free of actin, troponin, and tropomyosin and without evidence of proteolytic degradation of myosin (6, 7).

Ca<sup>2+</sup>-ATPase activities were assayed at 30°C in 0.3 M KCl, 50 mM Tris-Cl (pH 7.6), 10 mM CaCl<sub>2</sub>, 5 mM ATP, and 5 mM sodium azide (6, 7). Results are expressed as micromoles of inorganic phosphate (Pi) liberated per milligram protein per minute at 30°C.

Actin activated Mg<sup>2+</sup>-ATPase activity was measured at varying concentrations of actin at 25°C (7).

Myosin isoenzymes were analyzed by electrophoresis from crude myosin extracts (2) and from purified myosins on polyacrylamide gels using nondissociating conditions at 2–3°C as reported by Hoh (8) and modified by d'Albis et al. (9). Densitometric scans were recorded at 550 nm and semi-quantitative estimates of each isoenzyme were calculated from the height of each peak.

For statistical comparisons the four groups within each sex were evaluated by a two-factor analysis of variance to determine whether any statistically significant differences occurred among the groups, and, if such differences were present, individual groups were compared using the Newman-Keuls multiple-comparison test (10).

## RESULTS

Table I shows that tail-cuff blood pressure was elevated in both female and male hypertensive animals to approximately the same degree and remained quite constant throughout the experimental period. Blood pressure was not altered by swimming in either the normotensive or the hypertensive group.

Table II shows that there was no difference among the groups in body weight for females, but male swimmers, male hypertensives, and hypertensive swimmers all weighed less than male sedentary controls. There was no significant difference in body weight between hypertensive swimmers and hypertensive sedentary animals.

Among females the hearts of swimmers were 30% heavier than sedentary controls, and the hearts of hypertensives were 46% heavier than sedentary controls. The hearts of hypertensive swimmers were 19% heavier than hypertensive sedentary animals. Thus, swimming and hypertension both cause significant cardiac hy-

TABLE I  
Blood Pressure Course

Females		Start	5 wk	10 wk
	n			
C	5	101±5	100±3	95±6
SW	5	98±7	95±6	99±6
H	5	163±4*	157±5*	158±5*
H-SW	5	163±4*	164±7*	160±5*
Males		8 wk		
	n			
C	9	105±6		99±4
SW	9	103±5		106±6
H	7	165±11*		171±8*
H-SW	7	161±12*		167±9*

Results are mean±SE in millimeters Hg. C, control; SW, swimmer; H, hypertensive; H-SW, hypertensive swimmer.

\*  $P < 0.001$  for H vs. C or H-SW vs. SW.

Other notations are the same as in Table II.

pertrophy in the female rats and this was exaggerated by the combination of hypertension and swimming. In males there was no effect of swimming on heart weight in normotensive animals, but hypertension caused a significant 14% increase in heart weight. The heart weight of hypertensive swimmers was 12% greater than that of hypertensive sedentary animals.

The ratio of left ventricular to total heart weight also increased in both female and male hypertensives, and hypertensive swimmers, but not in swimmers. Left ventricular to total heart weight ratio declined in female swimmers. The heart to body weight ratios were greater than control in all experimental groups.

Table III shows that in both groups swimming was associated with a significant increase in actomyosin ATPase activity, hypertension with a significant decrease in actomyosin ATPase activity and hypertensive swimmers with an ATPase activity that was similar to the control value. The same relationships existed for calcium ATPase activity of pure myosin in the different groups. The K<sup>+</sup>-EDTA ATPase was not significantly different among the groups.

Fig. 1 shows that actin-activated Mg<sup>2+</sup>-ATPase activity was significantly greater in myosin from swimmers than controls, was significantly lower than controls in the preparations from hypertensive animals and was significantly higher than controls in hypertensive swimmers.

Fig. 2 shows representative pyrophosphate gel patterns and densitometric gel scans of myosin from the four groups. The myosin from control hearts and hearts from swimmers showed normal predominant V<sub>1</sub> isoenzyme patterns. There was a reversal of the normal

TABLE II  
Heart and Body Weight Relationships

	n	BW	DHW	DLV/DHW	DHW/BW
		g	mg		mg/g
Females					
C	5	275±4	139±6	0.828±0.004	0.505±0.014
SW	5	275±11	181±8*	0.811±0.006*	0.658±0.014‡
H	5	269±4	203±4§ <sup>  </sup>	0.861±0.004¶	0.753±0.015**
H-SW	5	274±4	241±10‡* <sup>†</sup>	0.854±0.008§§	0.892±0.040‡‡‡
Males					
C	9	460±14	229±7	0.812±0.007	0.498±0.009
SW	9	379±8‡	234±8	0.801±0.006	0.616±0.013‡
H	7	320±17§	261±10¶	0.857±0.006§	0.823±0.029§
H-SW	7	334±9‡‡	293±11* <sup>†</sup>	0.843±0.008§§	0.875±0.022‡‡

Results are mean±SE.

n, number of hearts; BW, body weight; DHW, dry heart weight; DLV, dry left ventricular weight.

\*  $P < 0.05$  for C vs. SW or H vs. H-SW.

‡  $P < 0.001$  for C vs. SW or H vs. H-SW.

§  $P < 0.001$  for C vs. H.

<sup>||</sup>  $P < 0.06$  for H vs. SW.

¶  $P < 0.05$  for C vs. H.

\*\*  $P < 0.05$  H vs. SW.

‡‡  $P < 0.001$  for C vs. H-SW.

§§  $P < 0.05$  for C vs. H-SW.

pattern in the hearts of hypertensive animals so that  $V_3$  predominated and  $V_1$  was the smallest peak, but this reverted to the normal isoenzyme pattern in the myosin from hypertensive swimmers.

Fig. 3 shows the mean percent peak height for the

different isoenzymes for four samples from each female experimental group. The percent content of myosin isoenzyme contributed by the  $V_1$  peak was significantly higher than control in the myosin preparations from the hearts of swimmers, was significantly

TABLE III  
Contractile Protein ATPase Activities

	Control	Swimmer	Hypertensive	Hypertensive-Swimmer
Actomyosin ATPase				
Females	0.60±0.03 (7)	0.76±0.08 (8)*	0.52±0.05 (6)‡	0.60±0.05 (8)*§
Males	0.55±0.08 (5)	0.76±0.08 (5) <sup>  </sup>	0.43±0.09 (6)‡	0.61±0.06 (6)*¶
Ca-ATPase of myosin				
Females	1.08±0.03 (5)	1.33±0.08 (5) <sup>  </sup>	0.86±0.04 (5)**	1.10±0.06 (5)§ <sup>  </sup>
Males	1.03±0.02 (5)	1.19±0.02 (5)*	0.76±0.04 (5)**	1.01±0.04 (5)¶ <sup>  </sup>
K <sup>+</sup> -EDTA ATPase of myosin				
Females	1.23±0.09 (5)	1.25±0.04 (5)	1.50±0.07 (5)	1.40±0.09 (5)
Males	1.24±0.04 (4)	1.19±0.02 (4)	1.47±0.04 (4)‡	1.31±0.06 (4)*¶

Results are mean±SE in micromoles Pi/mg protein · min<sup>-1</sup>.

Numbers in parentheses are numbers of preparations.

\*  $P < 0.05$  C vs. SW or H vs. H-SW.

‡  $P < 0.05$  C vs. H.

§  $P < 0.001$  H-SW vs. SW.

<sup>||</sup>  $P < 0.001$  C vs. SW or H vs. H-SW.

¶  $P < 0.05$  H-SW vs. SW.

\*\*  $P < 0.001$  C vs. H.

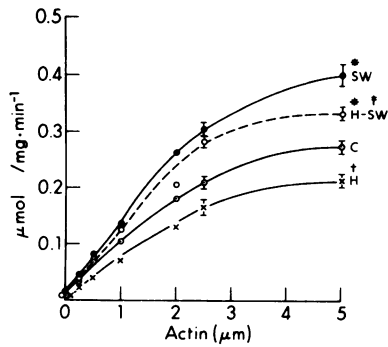


FIGURE 1 Actin-activated  $Mg^{2+}$ -myosin ATPase activity as a function of actin concentration. Results are mean  $\pm$ SE of four samples. C, control; SW, swimmer; H, hypertensive; H-SW, hypertensive swimmer.

\* $P < 0.001$  comparing SW with C or H-SW with H.

† $P < 0.05$  for H vs C.

‡ $P < 0.05$  for H-SW vs C.

lower than control in the myosin from hearts of hypertensives, and similar to control in the hearts of hypertensive swimmers. The  $V_2$  isoenzyme content was slightly depressed in myosin from the hearts of swim-

mers but was the same as control in the hearts of hypertensive swimmers. The  $V_3$  isoenzyme was similar to control in the hearts of swimmers and hypertensive swimmers but was markedly increased in the hearts of hypertensive animals.

## DISCUSSION

Many workers have demonstrated that pathologic systolic overload resulting in cardiac hypertrophy is associated with depressed cardiac contractile protein ATPase activity (1). We have repeatedly shown that physical training by swimming results in elevated actomyosin and myosin ATPase activity (1, 6). The present studies confirm these previous findings. Female rats subjected to a swimming program develop cardiac hypertrophy, although male rats do not (5, 6). Therefore both sexes were used in these studies. We had assumed, however, that by making hypertensive animals swim an additional burden would be placed on the heart that would exaggerate both the pathologic hypertrophy and the associated biochemical changes. Although hemodynamics during exercise were not measured in the current experiments, it seems likely

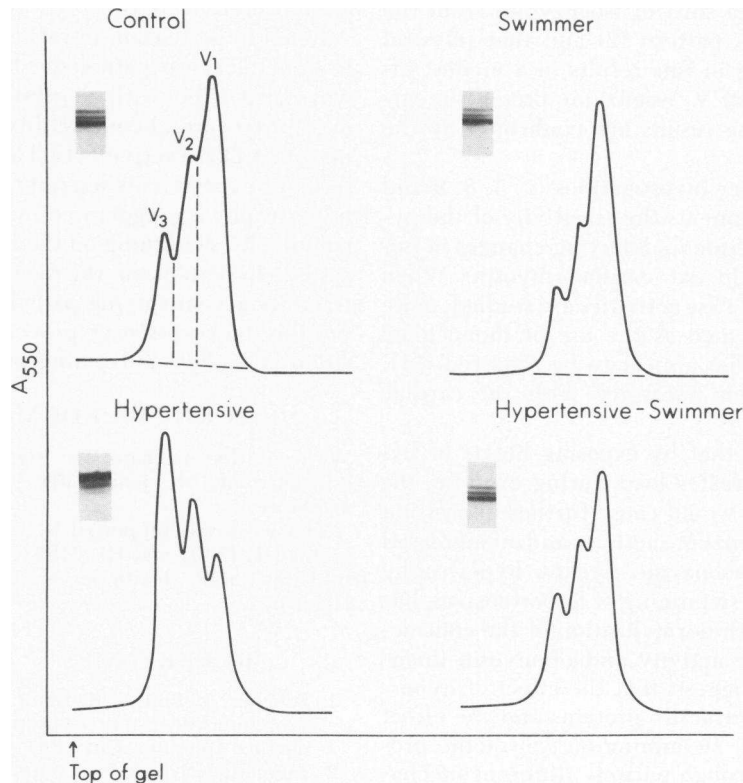


FIGURE 2 Representative pyrophosphate gel patterns (inserts) and representative densitometric scans of isoenzyme gels for the four groups.

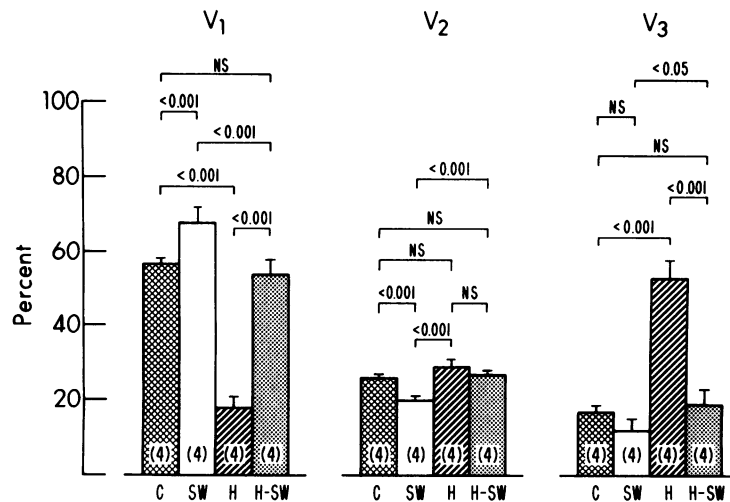


FIGURE 3 Percent peak height for myosin isoenzymes. The results are mean  $\pm$ SE for four samples.

that the above supposition of increased load is correct, because both male and female hypertensive swimming groups had greater hypertrophy than the hypertensive groups alone.

It has also been previously reported that hypertension in rats results in a shift of isoenzymes from the predominant V<sub>1</sub> to V<sub>3</sub> pattern (2) and that physical training by swimming in rats results in a modest increase in the percent of V<sub>1</sub> isoenzyme present in cardiac myosin (3). Those results are confirmed by the present experiment.

As in numerous other investigations (2, 3, 8, 9 and others) this study documents the sensitivity of the pyrophosphate gel technique in detecting changes in isoenzyme distribution in rat cardiac myosin. When myosins with lower ATPase activities are studied, more sensitive approaches, such as the use of monoclonal antiisoenzyme antibodies appear to be required (11), but this has not been necessary with rat cardiac myosin.<sup>2</sup>

We had postulated that by exposing hearts of hypertensive rats to a greater load during exercise, the resultant hypertrophy would cause further depression of contractile protein enzyme activity and an increased proportion of the V<sub>3</sub> isoenzyme. Greater hypertrophy was observed with the swimming of hypertensives, but this was associated with normalization of the contractile protein enzymatic activity and of myosin isoenzyme patterns. This suggests that the effect of hypertension on cardiac contractile proteins and the effect of physical training by swimming on contractile proteins are mediated through entirely different mechanisms, and that the stimulus induced by repeated

swimming can prevent or nullify that induced by hypertension. Whether the present results can be extrapolated to physical training in general is unsure, since the effects on contractile proteins of training by swimming and by running in rats appear to be quantitatively different (5, 6).

Renal hypertension in rats (4) and systolic overload in general cause pathologic hypertrophy, almost always associated with depressed contractile function (1, 4). Myocardial contractility generally parallels the myosin ATPase activity (1). The findings in the current series of experiments suggest that despite the fact that hypertrophy is exaggerated by superimposing physical training by swimming on the hypertrophy induced by systolic hypertension, the decrease in myocardial contractility accompanying pathologic hypertrophy might possibly be reversed or prevented by the superimposition of a physical training program.

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<sup>2</sup> Zak, R. Personal communication.

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