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Running performance and cardiovascular capacity are not impaired in creatine-depleted rats

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Adams, Gregory R., Paul W. Bodell, and Kenneth M. Baldwin. Running performance and cardiovascular capacity are not impaired in creatine-depleted rats. *J. Appl. Physiol.* 79(3): 1002–1007, 1995.—Several published reports have indicated that derangement of the phosphocreatine/creatine (Cr) energy-buffering system via Cr analogue feeding results in cardiomyopathy when cardiac performance is assessed in vitro. The present study was designed to examine indexes of cardiac performance in rats that have been chronically Cr depleted. Adult (180 ± 4 g) rats were assigned to a normal diet (ND) ($n = 8$) or a Cr-depletion diet (CD) group ($n = 10$). After 61 ± 1 days of ad libitum feeding, measurements of steady-state exercise O_2 consumption were made. Hemodynamic indexes were then assessed during incremental running to peak sustained levels. Rats were then killed and the left ventricle was excised. In the CD group Cr was depleted 82% and V_1 isomyosin decreased while V_2 increased. O_2 consumption during steady-state running was not different in CD rats. The respiratory exchange ratios of CD rats reflected a bias toward fat utilization during the latter stages of prolonged exercise. The exercise heart rates and peak systolic blood pressures of CD rats were slightly lower than those of ND rats. Both negative and positive rates of left ventricular pressure development were significantly reduced at all running speeds in the CD rats. CD rats were capable of exercise performance equal to that of ND animals. The hemodynamic and metabolic data suggest that the adaptations seen in the CD animals may be similar to those reported after endurance training. These results indicate that chronic Cr depletion does not impair either the circulatory or exercise capacity of rodents.

cardiac contractility; heart rate; blood pressure; exercise; β -guanidinopropionic acid; creatine analogue; myosin; skeletal muscle.

CREATINE (Cr) and phosphocreatine (PCr) are thought to be critical components of an intracellular energy transport system in cardiac and skeletal muscles (for review see Ref. 25). An essential role for Cr and PCr suggests that a reduction of these metabolites to low levels should have a negative impact on measurements of contractile performance during periods of maximal or near-maximal activity. In apparent support of this hypothesis, several studies have reported that chronic Cr depletion, which reduces the available PCr to low levels, results in decreased cardiac performance or compromised heart function when studied in vitro (14, 15, 19, 26). However, it has also been reported that chronic depletion of Cr or acute depletion of PCr have little or no effect on a range of cardiac performance criteria in similar in vitro models (11, 24). Several studies have also reported that Cr depletion can increase fatigue resistance of skeletal muscles (20, 22). Dorgan et al. (4) reported significant increases in run time to exhaustion of CD compared with control rats. Clearly, either unchanged or increased exercise endurance in CD ani-

mals appears to be at odds with the notion that hearts of CD rats have impaired functional capacity (14, 15).

The present study was designed to fill the gap between reports of unchanged or improved whole animal exercise performance and in vitro studies indicating some level of cardiac impairment both resulting from the CD model. The present studies examined indexes of cardiac performance in intact rats that have been chronically Cr depleted via a dietary intervention. The hypothesis tested was that the cardiac performance of the CD animals would not be adequate to meet the demands imposed by an incremental exercise protocol that was designed to acutely exceed the maximal steady-state exercise capacity of animals with normal Cr levels. The findings reported herein clearly suggest that peak exercise capacity and cardiac performance is not compromised in CD rats.

METHODS

Experimental design. β -Guanidinopropionic acid (β -GPA), a slowly metabolized analogue of Cr, was synthesized from β -alanine and cyanamide (Sigma Chemical, St. Louis, MO) (23). β -GPA was added (1% wt/wt) to standard rat chow, which was then formed into food pellets.

Eighteen female Sprague-Dawley rats were purchased as adults (initial weight 180 ± 4 g) and randomly assigned to either a normal diet (ND) ($n = 8$) or a Cr-depletion diet (CD) group ($n = 10$). All animals were housed in standard vivarium cages and allowed food and water ad libitum.

At the end of 61 ± 1 days, rats from both groups were instrumented for collection of hemodynamic data (see below). Approximately 1 wk before collection of hemodynamic data, rats were familiarized with the treadmill exercise via two 5-min bouts of treadmill running at 0.5 mph.

O_2 consumption ($\dot{V}O_2$) measurement. Several days before the hemodynamic measurements were performed, $\dot{V}O_2$ ($\text{ml } O_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) was measured using a metabolic chamber designed to fit in one stall of a rodent treadmill (6). $\dot{V}O_2$ was determined both at rest and during 2-min time blocks of a 22-min run at 0.75 mph, a running speed expected to produce $\sim 70\%$ of maximal $\dot{V}O_2$ (8). The respiratory exchange ratio (RER) was calculated for each time block. The purpose of these measurements was to determine whether the metabolic profiles of control and CD rats were different as reflected by changes in $\dot{V}O_2$ or RER during a steady-state period of increased metabolic load.

Hemodynamic measurement. High-fidelity myocardial functional measurements were collected 24 h after the implantation of a model SPR-249 solid-state ultra miniature pressure transducer (Millar Instruments, Houston, TX) with a nominal frequency response of 35 kHz. The rats were anesthetized with ketamine HCl/acepromazine (80/2 mg/kg), and the tracheal and scapular areas were shaved and aseptically prepared. A midline incision was made at the tracheal region, and blunt dissection techniques were used to isolate the right carotid artery. A Millar catheter was inserted into the artery and advanced through the arterial lumen into the left ventric-

ular chamber. Pressure signals were monitored during this procedure so that entry of the catheter tip into the left ventricular chamber could be observed (see below). The catheter was secured with silk ligatures, tunneled under the skin, and exteriorized at the scapular region. The animal was placed in a backpack where the distal portion of the catheter could be placed and thereby be protected from damage during surgical recovery.

The blood pressure monitoring system consisted of a SensorMedics Dynagraph (model R511A, SensorMedics, Anaheim, CA) connected to the Millar catheter via a transducer control unit (model TC-510, Millar Instruments). A PC computer was interfaced with the dynagraph for data acquisition and processing. Heart rate (HR) and peak left ventricular blood pressure (PLVP) were acquired on-line using blood pressure analysis software (Gould Scientific, Valley View, OH). The rate of left ventricular pressure development (dP/dt) was calculated using data analysis software (Gould Scientific) from the left ventricular pressure trace data set.

The testing protocol consisted of blocks of treadmill running starting at 0.5 mph and increasing in 0.25 mph increments every 3 min. Hemodynamic measurements were collected during each 3-min block. Treadmill speed was increased until the animal could not maintain the running speed for the full 3-min period.

Tissue collection. After the exercise bout, each animal was killed with an overdose of pentobarbital sodium and the heart and several skeletal muscles were excised. The left (LV) and right ventricles were separated and weighed. One portion of the LV was freeze-clamped (liquid N_2) and stored at $-70^\circ C$ for Cr determination. The second portion of the LV was placed in ice cold glycerol and stored at $-20^\circ C$ for subsequent detection of myosin isoform.

The medial gastrocnemius (MG) and vastus lateralis (VL) muscles from one leg were excised, and a tissue sample was collected from the superficial "white" and deep "red" regions of each muscle. Muscle samples were placed in ice cold glycerol and stored at $-20^\circ C$ for subsequent detection of myosin heavy chain (MHC) isoform distribution.

Myofibril preparation. Muscle samples were homogenized in ~ 20 volumes of an ice cold solution containing (in mM) 250 sucrose, 100 KCl, and 5 EDTA. The homogenate was washed successively in three solutions: 1) 250 mM sucrose, 100 mM KCl, and 5 mM EDTA; 2) 0.5% Triton X and 175 mM KCl; and 3) 150 mM KCl. The final pellet was resuspended in 1 ml of 150 mM KCl. The protein concentration of this solution was determined using the biuret method (10). An aliquot of myofibril suspension was added to a solution containing 50% vol/vol glycerol, 100 mM $Na_4P_2O_7$, and 5 mM EDTA at a concentration of 1 mg/ml and stored at $-20^\circ C$.

Native myosin analysis. The left ventricular isomyosin distribution was determined by nondenaturing polyacrylamide gel electrophoresis (6). Myofibrillar protein was loaded onto 6-cm polyacrylamide tube gels (4% total, 2.6% cross-link). Gels were run cold for 20 h with running buffer recirculation. Gels were stained with brilliant blue G dye (Sigma Chemical) and scanned on a laser densitometer (Molecular Dynamics, Sunnyvale, CA). The relative densities of the V_1 , V_2 , and V_3 myosin bands are expressed as percentages of total myosin density.

MHC analysis. The myofibril fraction was analyzed to determine relative content of types I, IIa, IIx, and IIb MHCs in the skeletal muscle samples. Five-microliter aliquots of stored myofibril solution were added to 35 μ l of denaturing buffer (62.5 mM tris(hydroxymethyl)aminomethane, 20% glycerol, 1% β -mercaptoethanol, 2.3% sodium dodecyl sulfate (SDS), and 0.05% bromophenol blue) and heated at $100^\circ C$ for 2 min. After this treatment, a 16- μ l aliquot (2 μ g) was

TABLE 1. Effect of CD on body weight, muscle-to-body weight ratio, and creatine content in rats

| | ND | CD |
|-------------------------------|-----------------|-----------------|
| Body wt, g | 248 \pm 6.8 | 243 \pm 6.4 |
| Muscle/body wt, mg/g | | |
| Right ventricle | 0.50 \pm 0.01 | 0.52 \pm 0.01 |
| Left ventricle | 2.16 \pm 0.05 | 2.16 \pm 0.06 |
| Creatine content, μ mol/g | | |
| Left ventricle | 12.0 \pm 1.0 | 2.1 \pm 0.3* |

Values are means \pm SE. ND, normal diet (control); CD, creatine-depleted (β -guanidinopropionic acid) diet. * Significantly different compared with ND value, $P < 0.05$.

withdrawn for analysis by SDS-polyacrylamide electrophoresis. Samples were analyzed via SDS-polyacrylamide electrophoresis as previously described (2). Separating gels contained 30% vol/vol glycerol, 8% wt/vol acrylamide (2% cross-link), 1% wt/vol glycine, and 0.4% wt/vol SDS. Stacking gels contained 40% vol/vol glycerol and 4% wt/vol total acrylamide (2.7% total). Gels were run at 275 V for ~ 22 h. Gels were stained for 1 h with brilliant blue G 250 dye (Sigma Chemical) and then destained with 25% methanol and 5% acetic acid. MHC bands were scanned using a laser densitometer (Molecular Dynamics). The peaks of interest were identified in the digitized densitometric data sets and the area of each peak was determined by integration. Individual MHC isoforms are expressed as a percentage of the total MHC pool.

Cr analysis. The quick frozen LV samples were pulverized in liquid N_2 and extracted in perchloric acid. Cr was assayed using standard enzymatic techniques (17).

Statistical analysis. All values are reported as means \pm SE. Treatment effects were determined by Student t -test using the Instat software package (Graphpad Software, San Diego, CA). Analysis of within-group HR values was accomplished using an analysis of variance with post hoc testing. For all statistical tests $P < 0.05$ was accepted for statistical significance. Analyses of all percent data were performed on arcsine transformed values to correct for nonnormal distribution. Percent data presented in tables and figures represent the nontransformed values.

RESULTS

Cr depletion. β -GPA feeding resulted in an 82% reduction in the Cr content of the LV compared with age-matched control animals (Table 1). There were no differences in body weight or the ratio of LV weight to body weight between the CD and control animals.

Running performance. Most animals in both groups failed to complete 1 min of running at 1.75 mph. Thus the highest running speed reported was 1.5 mph. One ND and 2 CD rats completed 3 min of running at 1.75 mph (data not shown).

Hemodynamic indexes. The exercise HR of CD rats tended to be lower at all running speeds and was significantly lower than that of ND animals at 1.0 mph (Fig. 1). Within groups, the peak HRs recorded at running speeds of 1.25 and 1.5 mph were not significantly different, suggesting that this value had reached a plateau.

PLVP was significantly lower than that of controls at the three highest running speeds (Fig. 2). Positive dP/dt was significantly lower and negative dP/dt was higher at rest and at all running speeds in CD rats

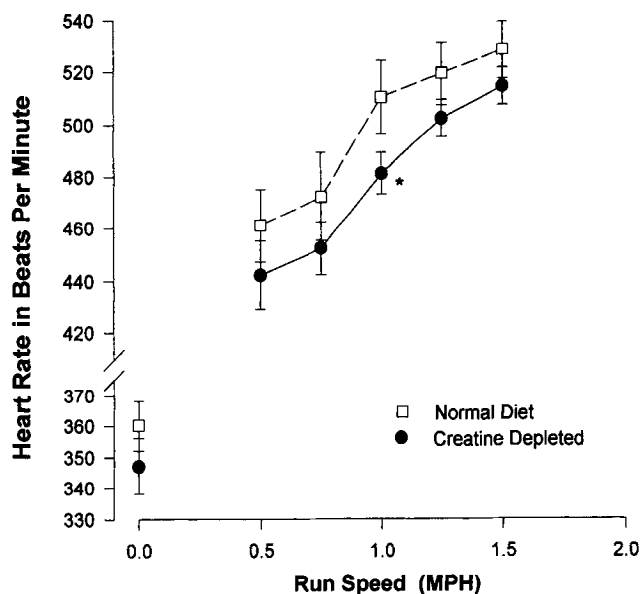


FIG. 1. Heart rate response for normal diet (ND) and creatine-depleted (CD) rats during incremental treadmill running. * Significantly different compared with ND value, $P < 0.05$.

(Fig. 3). This result indicates that the Cr-depleted hearts had a lower contractility compared with those from ND animals. The end-diastolic pressure was not significantly different between the ND and CD groups (data not shown). The lower blood pressures during exercise and unchanged diastolic pressures suggest that the apparent change in contractility was not the result of changes in afterload or preload. When $+dP/dt$ is plotted as a function of HR, CD animals exhibit a similar response to exercise compared with control animals (Fig. 4). Regression analysis of this data suggests a slight but nonsignificant shift to the right for the CD group with similar slopes for each regression line (ND: $m = 25$; CD: $m = 24$).

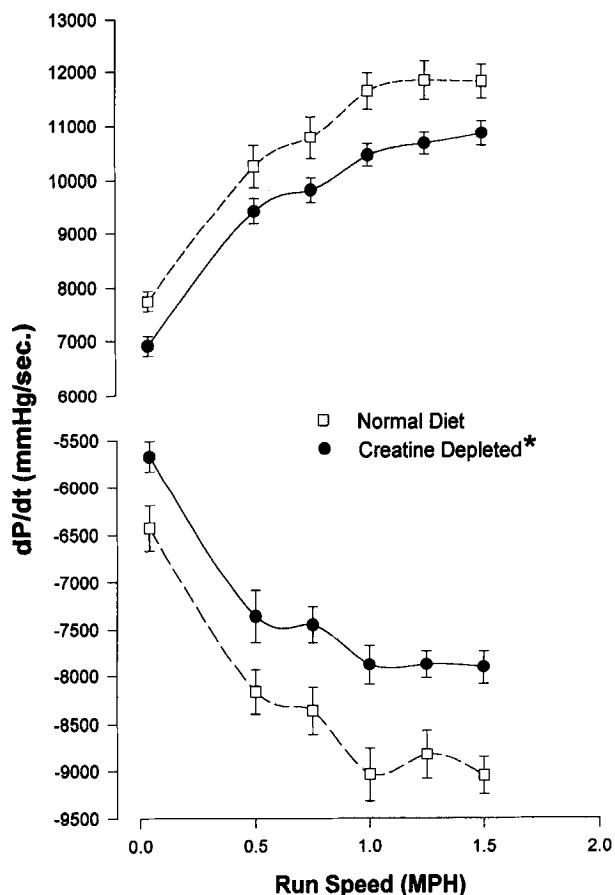


FIG. 3. Rate of left ventricular pressure development and relaxation for ND and CD rats during incremental treadmill running. * Significantly different compared with ND values at all running speeds, $P < 0.05$.

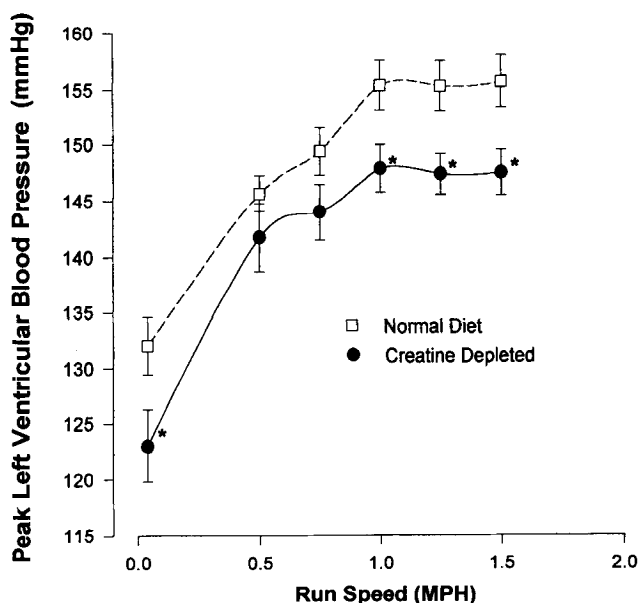


FIG. 2. Peak left ventricular blood pressure for ND and CD rats during incremental treadmill running. * Significantly different compared with ND values, $P < 0.05$.

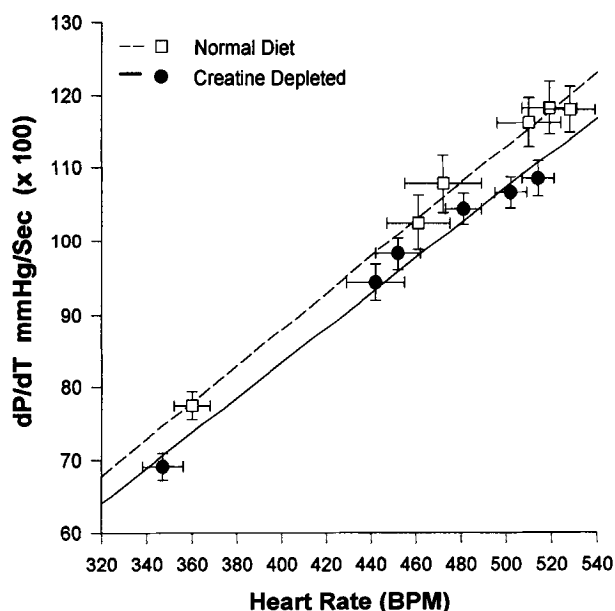


FIG. 4. Rate of left ventricular pressure development as function of heart rate for ND and CD rats during incremental treadmill running. Slope for regression lines was 25 for ND ($r^2 = 0.99$) and 24 for CD ($r^2 = 0.98$) groups. BPM, beats/min.

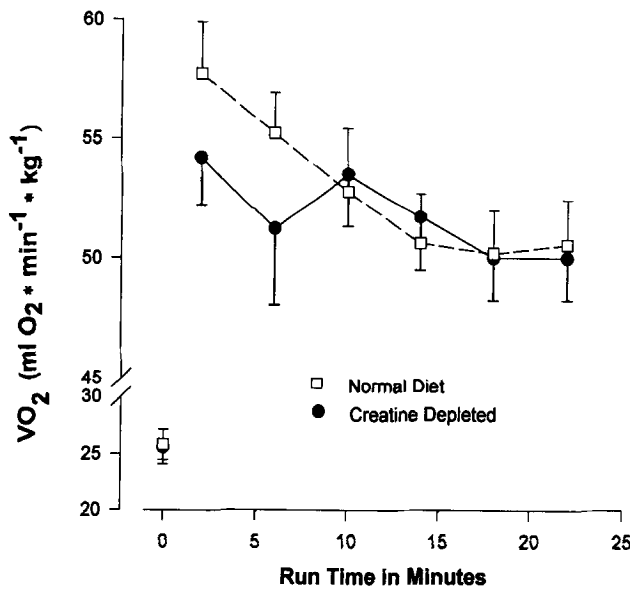


FIG. 5. O₂ consumption ($\dot{V}O_2$) of ND and CD rats during steady-state running at 0.75 mph. All rats were naive runners.

Exercise metabolism. $\dot{V}O_2$ both at rest and while running at 0.75 mph for 22 min was not different in CD rats compared with control animals (Fig. 5). RER of the CD rats tended to be lower for much of the exercise period and was significantly lower at the 16-min time point (Fig. 6). By using equations provided by Hutter et al. (13), the hemodynamic parameters of HR, PLVP, and dP/dt were used to estimate cardiac $\dot{V}O_2$. Estimated cardiac $\dot{V}O_2$ was significantly lower at rest and at the three highest running speeds in CD vs. ND rats (Fig. 7).

LV myosin isoforms. The percentage of V₁ isomyosin decreased from 79 to 69% in the CD group while that of V₂ increased from 14 to 21% (Fig. 8). There also appeared to be a trend toward an increase in V₃ myosin (6 vs. 10%) in the CD group.

Skeletal muscle MHC. Cr depletion resulted in a shift

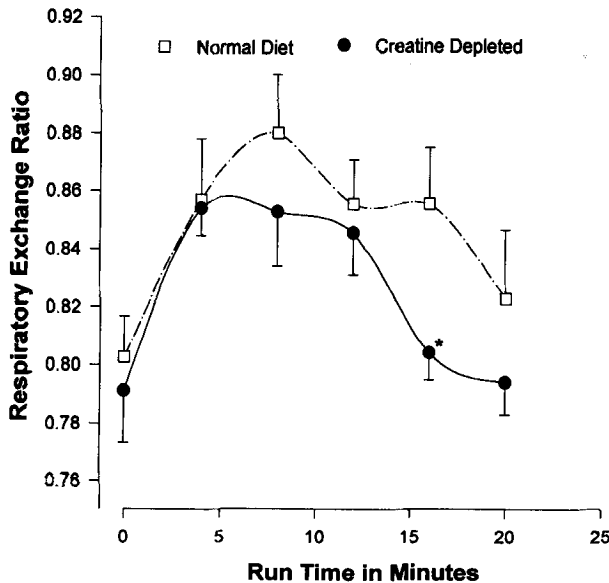


FIG. 6. Respiratory exchange ratios of ND and CD rats during steady-state running at 0.75 mph. * Significantly different compared with ND value, $P < 0.05$.

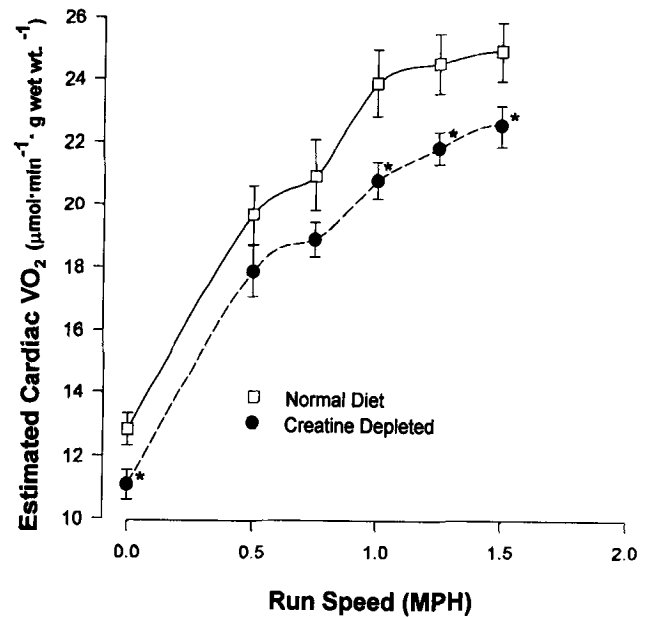


FIG. 7. Cardiac $\dot{V}O_2$ estimated using hemodynamic parameters of heart rate, peak left ventricular blood pressure, and rate of left ventricular pressure development recorded during incremental treadmill running. * Significantly different compared with ND values, $P < 0.05$.

toward expression of slower MHC isoforms in the deep red region of MG and VL (Table 2). The white superficial region of VL also exhibited a shift toward expression of slower MHC types, whereas the superficial region of MG was unchanged.

DISCUSSION

Several studies have reported that chronic Cr depletion results in decreased cardiac performance or compromised rat heart function when measured in vitro (i.e., isolated perfused hearts) (14, 15, 19, 26). As an

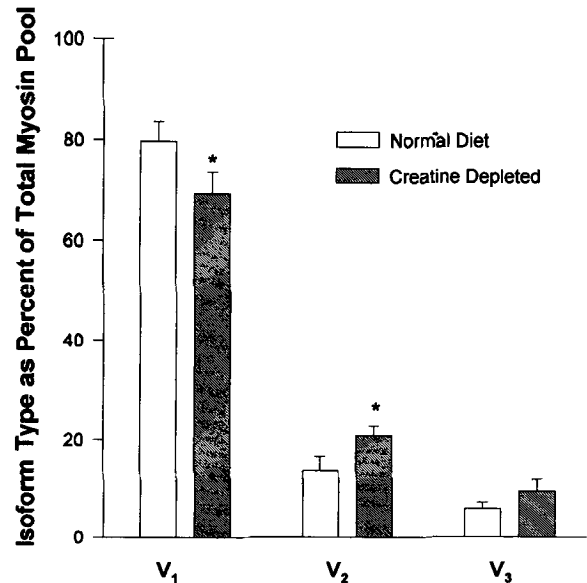


FIG. 8. Native myosin isoform distribution of left ventricles of ND and CD rats. * Significantly different compared with ND values, $P < 0.05$.

TABLE 2. Effect of CD on skeletal muscle MHC distribution

| | I | IIa | IIx | IIb |
|----------|----------|-----------|-----------|-----------|
| Red-MG | | | | |
| ND | 21.6±2.2 | 14.7±1.6 | 47.0±1.9 | 17.0±2.9 |
| CD | 22.0±1.2 | 19.3±1.4* | 40.0±0.8* | 19.1±2.3 |
| Red-VL | | | | |
| ND | 7.0±2.2 | 15.1±1.8 | 33.7±1.4 | 44.3±4.4 |
| CD | 6.7±1.4 | 21.0±1.5* | 38.0±1.1* | 34.3±2.7* |
| White-MG | | | | |
| ND | | | 11.8±1.0 | 88.2±1.0 |
| CD | | | 12.4±1.0 | 87.6±1.0 |
| White-VL | | | | |
| ND | | | 4.9±1.0 | 95.1±1.0 |
| CD | | 1.0±0.5 | 6.9±2.7* | 92.2±0.8* |

Values are means ± SE where indicated; $n = 8$ for both ND and CD. Myosin heavy chain (MHC) isoforms expressed as percentage of total myosin heavy chain pool. MG, medial gastrocnemius; VL, vastus lateralis. Red and white refer to deep and superficial regions, respectively. * Significantly different compared with ND values, $P < 0.05$.

example, Kapelko et al. (15) reported that maximal work capacity was 40% less in hearts isolated from CD animals compared with control animals. In contrast, using a similar in vitro preparation, Shoubbridge et al. (24) found no significant differences in cardiac performance criteria in hearts from CD rats. Kapelko et al. (15) suggested that the differences between the two studies resulted from the fact that the highest rate × pressure value achieved by hearts in the study of Shoubbridge et al. (24) was 33.5 mmHg/min ($\times 10^{-3}$), whereas that same index was 40 in their study.

The results from both the submaximal steady-state and incremental exercise tests conducted in the present study suggest that rats that have significant Cr depletion in the LV (e.g., 82%) do not exhibit impaired running or cardiac performance. When instrumented for hemodynamic analysis, both control and CD rats were able to reach and maintain running speeds of 1.5 mph. This running speed would be expected to impose metabolic energy demands above the maximum sustainable by oxidative pathways for animals of this size (8). For comparison to the work published by Kapelko et al. (14), the mean rate-pressure product was 41.1 ± 1.2 and 41.3 ± 1.0 for ND and CD groups, respectively, when rats were running at 1.5 mph in the present study. The observed shift in RER toward greater utilization of lipids suggests an adaptation, whereas impairment might be expected to produce an increased dependence on carbohydrate as the demands placed on the system were increased.

CD rats exhibited several changes in hemodynamic indexes compared with control animals. These changes, decreased HR, lower systolic pressure, and decreased contractile intensity, appear similar to those seen after endurance training (6, 21). For example, Fitzsimons et al. (6) reported that both $+dP/dt$ and $-dP/dt$ were altered as seen in the present study (e.g., at 1.25 mph dP/dt was decreased 14% by training and 10% by Cr depletion) (6). Fitzsimons et al. also reported that normalizing the dP/dt to HR produced a relationship that was similar for both trained and untrained rats, a result similar to that in the present study. (Fig. 4).

The changes in the functional, hemodynamic, and metabolic indicators determined in the present study were paralleled by alteration of key protein determinants of contractile characteristics. In skeletal muscles, analyses of the MHC distribution indicated a shift toward isoforms that are thought to be energetically more economical than relatively faster isoforms (9). In this regard, Cr depletion resulted in adaptations that in some ways mimic those seen after endurance exercise training (2, 7). The observed shifts toward expression of slower MHC isoforms in Cr-depleted skeletal muscles were similar to those reported previously (1, 2, 18). In LV, there was a similar shift in myosin distribution toward isoforms that are putatively more economical with regard to energy usage (3). Again, this adaptation, a decrease in V_1 and increase in V_2 (and possibly V_3), was quantitatively and qualitatively similar to that seen after 70 days of endurance training (6).

In agreement with the present results, Mekhfi et al. (19) reported isomyosin shifts that were qualitatively similar to those reported herein. However, when Mekhfi et al. and other research groups collected functional measurements from hearts that had been isolated and perfused with crystalloid solutions, they concluded that Cr-depleted hearts were impaired because they were unable to maintain a maximal force that was similar to that seen in control hearts (14, 15, 19).

It is interesting to note that Mekhfi et al. (19) reported much greater changes in isomyosin distribution than seen in the present study (e.g., a 32% decline in V_1 vs. the 13% decline seen in the present study). Although the Cr analogue feeding duration was similar in the two studies, the developmental stage of the animals was quite different. In the study of Mekhfi et al., the animals were placed on the CD diet at an age of 21 days (body wt 60–74 g), whereas the animals used in the present study were young adults (54 days old, 180 g). We have recently reported that Cr depletion has a more extensive effect on the MHC distribution of skeletal muscles when the dietary intervention is imposed from a young age (28 days) as opposed to Cr depletion starting in adulthood (1). It seems possible that a similar age-related difference in responsiveness may be operating in the heart as well.

The data presented in this paper are in general agreement with several studies using the isolated perfused heart model. For example, Kapelko and co-workers (14, 15) reported a decline in contractility in isolated externally paced Cr-depleted hearts. In a similar preparation, Mekhfi et al. (19) reported shifts toward expression of “slower” myosin isoforms. In light of these findings, the decreased contractility and shift in myosin isoform expression found in the present study suggest that Cr depletion results in an alteration in the contractile properties of the heart. This alteration in contractile properties spans a wide range of exercise intensity and HR. The differences in conclusion between this and other studies (14, 15, 19) arise from the fact that data collected using isolated heart models cannot be interpreted in the context of the intact animal and therefore would not take into account coordinate somatic function. The present results, indicating adequate hemody-

namic function during intense exercise in the intact rat, do not provide any evidence of cardiovascular impairment. Rather, the present findings, from both cardiac and skeletal muscle, suggest that chronic Cr depletion acts as a stimulus similar in nature to that of endurance-type training, leading to a coordinate adaptation of both the demand tissues (skeletal muscle) as well as the organ system responsible for O₂ and nutrient supply (cardiovascular). Chronic Cr depletion via feeding of a Cr analogue is a systemic intervention that has the potential to affect many body systems (e.g., 5, 12). Thus the adaptation of multiple systems (e.g., muscular and cardiovascular) must be considered to evaluate the cardiac response to various levels of work imposed in the intact CD animal.

Summary. In agreement with several isolated heart studies, the hearts of CD rats in the present study did in fact exhibit altered cardiac performance parameters (i.e., decreased HR and contractility). However, similar results have been reported after endurance training in rats (6). When interpreted in the context of the intact animal, changes in contractile parameters such as lower HR and contractility appear to be well matched to the demands imposed by high levels of exercise. The results of the present study suggest that chronic Cr depletion results in cardiac adaptations that appear to qualitatively mimic some adaptations resulting from endurance-type exercise training.

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