Metabolic Abnormalities and Differential Responses to Stress Associated with Hamster Cardiomyopathy (44315)

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Abstract. **Metabolic differences between cardiomyopathic hamsters (CMHs), as they progress through various physiologic phases before reaching end-stage heart failure (HF), and healthy hamsters (HHs) are often difficult to demonstrate. We suggest that metabolic differences, magnified by application of chronic stress (S: cold immobilization 2 hr/day for 5 days) followed by acute stress (AS: 55 min global ischemia /30 min reperfusion), can be used to characterize different stages in this cardiomyopathic process. High performance liquid chromatography (HPLC) and ^{31}P NMR methods were used to monitor the effects of acute stress applied to nonstressed (NS) and previously stressed CMHs (NS-2.5-month NS-5-month; S-2.5-month, S-5-month) and HHs (NS-HH, S-HH).**

Cardiac tissue extracts from nonstressed and stressed hamsters were analyzed for ATP and PCr at baseline and after completion of ischemia/reperfusion (AS) using HPLC. In nonstressed hamsters, ATP and PCr were 12% lower in CMHs (both NS-2.5- and NS-5-month) than in NS-HHs. After exposure to stress, ATP was 26% lower in CMHs (S-2.5- and S-5-month) compared to S-HHs, whereas there were minimal differences in PCr between the groups.

^{31}P NMR monitoring of metabolism in the perfused beating heart during application of acute stress produced similar changes (%) in ATP and PCr in all groups (NS and S), whereas P_i increase was less in NS-5-month (118%) compared to NS-2.5-month (179%) and NS-HHs (306.8%), $P < 0.05$; and in S-5-month (148%) compared to S-2.5-month (216%) and S-HHs (222%). The changes in myocardial pH were inversely related to changes in P_i: NS-5-month (−13.5%); NS-2.5-month (−9.7%); NS-HH (−17.7%). pH changes in stressed cardiomyopathic hamsters were similar to those of S-HHs. The postischemic recovery of ATP and P_i return closer to baseline values in cardiomyopathic hamsters (both NS and S) compared to healthy hamsters.

The data suggest that cardiomyopathic hamsters have baseline metabolic abnormalities, and their responses to chronic cold immobilization stress, acute ischemia, and chronic cold immobilization stress plus acute ischemia are different from those in HHs. These responses may help to characterize specific stages of disease.

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The hamster model of cardiomyopathy (CMH), which results from an inherited autosomal dominant polymyopathy whose cardiac manifestations eventually result in heart failure (HF), is a reasonable model to use to study the various stages of heart failure, comparable to those that occur in human ischemic cardiomyopathy. The cardiomyopathic disease process appears to have five histological phases associated with representative clinical phases; prenecrotic, necrotic, quiescent, hypertrophic, and terminal (1). Spasm of coronary microvessels (2), myocardial hypoperfusion (3), and coronary hyperactivity to arginine vasopressin (AVP) (4) are all found in cardiomyopathic hamsters during the necrotic phase, suggesting a global coronary vasculopathy. Although low oxygen uptake, associated with decreased coronary flow, was found in the early necrotic

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phase, bioenergetic processes have not been found to be defective until the end of the necrotic phase (5).

Because of our interest in the role of stress in the pathogenesis of ischemic heart disease, we decided to use the cardiomyopathic hamster as an experimental model to study these potentially interactive processes. In earlier work, we found that cardiomyopathic hamsters were at a higher risk of succumbing to stress during the vasospastic, lesion-forming period (2–3 months of age) compared to the more stable hypertrophic phase (5–6 months of age) (6). To explain this, we hypothesized that stress exacerbated the underlying coronary pathophysiology (i.e., the pre-existing vasospastic process). In support of this hypothesis, in previous work done in our laboratory using isolated mitochondria from both HHs and CMHs, we showed that 2.5-month-old CMHs (in the necrotic phase of disease), were more sensitive to stress-induced changes of oxidative phosphorylation than 5-month-old CMHs (14). This was expressed as an increase in mitochondrial oxygen use in the presence of adequate substrate but without added ADP (State 4 respiration), and decrease in the ratio of State 3/State 4 respiration (RCI), where State 3 was determined by addition of 320 nmol ADP to the mitochondria.

In the present study, to determine whether the pathophysiology of the cardiomyopathic process can be characterized by changes in the end products of oxidative phosphorylation, adenosine triphosphate (ATP) and phosphocreatine (PCr) were monitored with HPLC and ^{31}P NMR spectroscopy. Our hypothesis was that changes in bioenergetic function are the basis of the underlying cardiomyopathic process, and that exposure to chronic stress followed by acute stress would uncover an underlying hypoxic/ischemic state (i.e., by exacerbation of an existing vasculospastic process).

Bioenergetic function was measured in perfused beating hearts of healthy hamsters (HHs) and cardiomyopathic hamsters in necrotic (2.5-month) and hypertrophic (5-month) phases, who were previously exposed to cold immobilization stress (S) (S-HH, S-2.5-month, S-5-month) and compared to similar groups not exposed to stress (NS-HH, NS-2.5-month, NS-5-month). In order to evaluate the resistance of cardiomyopathic hamsters to changes in energy metabolism during hypoxia, both nonstressed and stressed animals were exposured to an episode of acute ischemia/reperfusion. ^{31}P NMR was used to estimate the bioenergetic metabolites: ATP, PCr, inorganic phosphate (P_i), and pH at baseline, and during acute ischemia and reperfusion in all groups of hamsters.

While we found that there were baseline abnormalities in ATP and PCr content, possibly related to chronic exposure to ischemia of the vasculopathic process, we also found that prior exposure to this vasculopathic process and/or exposure to chronic exogenous stress appeared to increase the resistance of the heart to acute ischemia.

Materials and Methods

Animal Model. The study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania, and conforms with the PHS *Guide for the Care and Use of Laboratory Animals* and the *U.S. Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.* Cardiomyopathic hamsters (Bio 14.6 strain) and FIB healthy hamsters were obtained from Canadian Hybrid Farms (Nova Scotia, Canada). The animals were housed individually in shoe-box cages with freely available food and water for at least 2 weeks prior to the start of each experiment. When the younger cardiomyopathic hamsters were 2.5 months old and the older were 5 months old, they were divided into two groups matched by weight and were randomly assigned to stress (S-2.5-month-body weight 97.5 ± 5.6 g, S-5-month- 105 ± 8.9 g) or nonstress (NS-2.5-month- 109 ± 7.2 g, NS-5-month - 120 ± 4.1 g) conditions along with appropriate numbers of HHs (NS-HHs-150 ± 30.1 g, S-HHs- 146.7 ± 4.6 g). In these experiments, 5-month-old HHs were used as controls. We chose this age group as the control based on previous work done by Hunter *et al.* (7), who showed that cardiac anatomy and function were similar for all age groups of healthy hamsters (i.e., controls). In this regard, we assumed that the 2.5-month-old and 5-month-old controls would respond to stress in a similar way.

The exogenous stressor was a 2-hr period of supine immobilization at a temperature of 4°C, administered daily for 5 consecutive days (and was applied to S-2.5-month, S-5-month, S-HH). This stress regimen was chosen because it was one that produced equal mortality outcomes for younger and older cardiomyopathic hamsters (6). In addition, in our earlier work (8) we studied the coronary vascular resistance of cardiomyopathic hamsters in the same age groups using the same stress regimen. We showed that 2.5-month S-CMHs had hyperreactive coronary vasculature compared to 6.5-month-old S-CMHs and 2.5-month-old NS-CMHs. In the present experiments, nonstressed hamsters from each group (NS-2.5-month, NS-5-month and NS-HHs) were moved out of their home cages to other shoe-box cages with no food or water during the 2-hr period when the stressor was applied to their matched partners. All surviving hamsters were sacrificed 5 days after the last stress treatment, and their hearts were studied during exposure to ischemia/reperfusion using a Langendorff perfusion apparatus in conjunction with ^{31}P NMR (9).

Isolated Perfused Heart Preparation. Hearts were perfused with the Langendorff method (perfusion pressure 100 mm Hg at 37°C) with modified Krebs-Henseleit buffer (NaCl = 118 m*M*, KCl = 4.7 m*M*, $MgSO_4$ = 1.2 m*M*, $CaCl_2$ = 2.0 m*M*, Na_2EDTA = 0.5 m*M*, $NaHCO_3$ = 25 m*M*) to which 11 m*M* glucose was added as previously reported (10). The buffer was equili-

brated with 95%O_2/5%CO_2. The PO_2 of the perfusate was between 450–500 mmHg. The buffer was not recirculated.

^{31}P NMR. The NMR measurements were performed on a Bruker AM-500 spectrometer with a home-built probe, tuned to 202.458 MHz, using a 45° observe pulse and a 2-sec pulse interval (11). Baseline, ischemia, and reperfusion peak areas (ATP, PCr, P_i,) were determined *via* triangulation. pH was calculated using the chemical shift difference between P_i and PCr (12). Representative ^{31}P NMR spectra for baseline NS-HHs, NS-2.5-month and NS-5-month cardiomyopathic hamsters are shown on Figure 1. Baseline ^{31}P NMR spectra for S-CMH were similar to those in NS animals (data not shown).

Physiological Intervention: Acute Ischemia. After baseline ^{31}P NMR measurements, all hearts were exposed to global ischemia (55 min) which was accomplished by stop-flow (i.e., clamping the perfusion tubing to the aorta). Reperfusion was initiated by unclamping the perfusion tubing; ^{31}P spectra were collected during 30 min of reperfusion. During stop flow, the perfusion media surrounding the heart cooled slightly, and despite the heat generated by NMR pulsing, the temperature in the NMR tube (and presumably the heart) decreased to 29°–30°C.

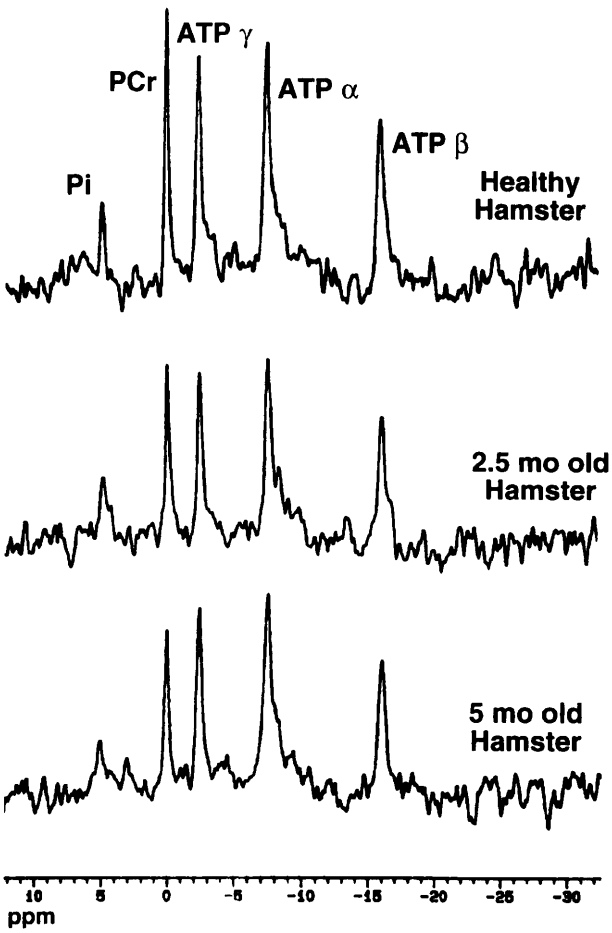

Figure 1. Representative ^{31}P-NMR spectra are shown for baseline nonstressed healthy, and 2.5-mo and 5-mo cardiomyopathic hamster hearts perfused with modified Krebs-Henseleit buffer containing glucose (11 m*M*). Similar baseline spectra were obtained from stressed hamsters (data not shown). P_i, inorganic phosphate. PCr, phosphocreatine. ATP, (γ, α, β) adenosine triphosphate.

Biochemical Analysis *via* HPLC. High energy phosphate content (concentration, μmoles/g wet tissue) from hamster hearts (after 30 min of perfusion and not exposed to ischemia, defined as baseline; and after 30 min of post-ischemic reperfusion) was analyzed by HPLC. Hearts were freeze clamped at liquid nitrogen temperatures while still being perfused on the Langendorff perfusion apparatus. Frozen samples were crushed under liquid nitrogen and resulting powder was extracted with perchloric acid at a 1:4 dilution. The extracts were centrifuged at 5000*g* for 5 min at O°C. The supernatant was neutralized with K_2CO_3. A C-18 reverse-phase column was used for separation of nucleotides. The mobile phase consisted of acetonitrile in a buffer containing 85 m*M* KH_2PO_4 and 5 m*M* TBAHS (tetrabutylammonium hydrogen sulfate) at pH 5.8. Elution was 1 ml/min, and detection was with a UV Spectraphysics detector at 259 nm. Detection of PCr was done with the same column but at 210 nm.

Nucleotides and PCr were quantitated by using integrated areas of each defined peak in conjunction with defined peaks of standards with known concentration.

Statistics. Statistical comparisons among HHs, necrotic stage (2.5-month) and hypertrophic stage (5-month) CMHs were done using repeated measure Analysis of Variance (ANOVA) (Disease × Stress (between groups) × Time (within subjects) to analyze the changes during ischemia and/or reperfusion (NMR data); and 2-way (2 × 3) ANOVA for analysis of HPLC data in conjunction (when appropriate) with *post hoc* comparisons among the groups using the least significant difference (LSD) test. Because the changes in bioenergetic metabolites were different in different time points (fast at the beginning and slow at the end of intervention), we also performed a 2 × 3-way ANOVA for NMR data. All values are reported as mean ± SEM. The significance level was $P < 0.05$.

Results

^{31}P NMR Experiments. Baseline, ischemic and reperfusion values for percentage change of P_i, PCr, ATP, and pH are presented in Figures 2–5 (significance: $P < 0.05$). Findings of note are summarized and presented below. We presented data as percentage change to normalize responses of different subjects with different heart sizes and at different stages of the disease.

Percentage Change of ATP, PCr, P_i and pH During Ischemia (55 min). During ischemia, the ATP content progressively decreased in both healthy and cardiomyopathic hamster hearts (Fig. 2). After 55 min of ischemia, ATP decreased—75.5% ± 6.2% in NS-HHs and −85.5% ± 7.8% in S-HHs. In cardiomyopathic hamster hearts, the percentage of ATP at 55 min of ischemia was as follows: NS-2.5-month: −74.6% ± 8.9%; S-2.5-month: −83.2% ±

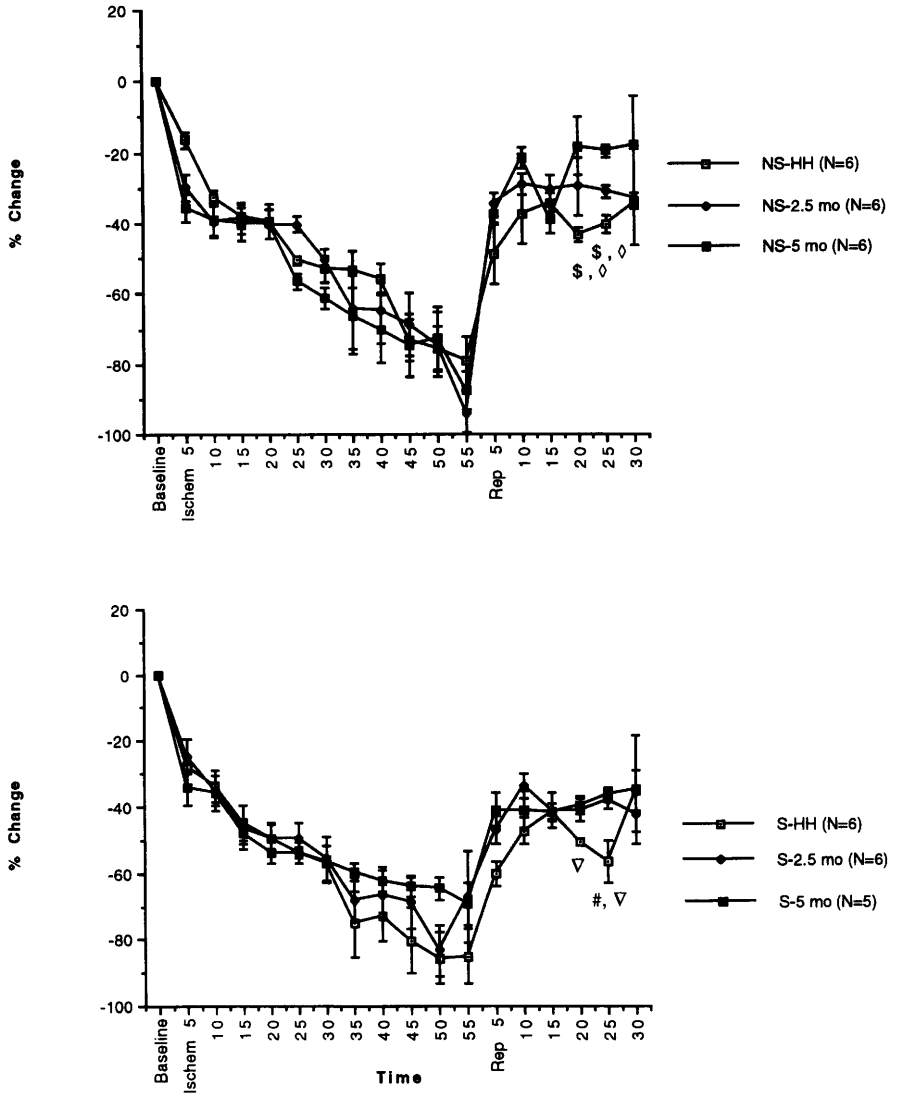

Figure 2. Changes (%) in ATP content during 55 min of global myocardial ischemia followed by 30 min of reperfusion. Hearts were perfused with modified Krebs-Henseleit buffer containing 11 m*M* glucose. Abbreviations: NS, nonstressed hamsters; S, stressed hamsters; N, number of hearts; Rep., reperfusion post 55 min of global ischemia. Values are mean ± SEM. Note: temperature in the NMR tube (and presumably also the heart) decreased to 29°–30°C during stop flow.
Rate of change (slopes) during 5–15 min of reperfusion were statistically significantly different in NS-CMH compared to NS-HHs. Slopes were: NS-HH 0.12 ± 0.06; S-HH 0.08 ± 0.01; NS-2.5-month 0.03 ± 0.01*, S-2.5-month −0.0008 ± 0.02; NS-5 month 0.03 ± 0.02 $; S-5-month 0.006 ± 0.02. Note: These differences could be related to differences in mechanical function (not measured) and/or reperfusion injury.
Notations for statistically significant differences for slopes and for *post hoc* analyses noted in the figure with $P < 0.05$ are:
NS-HH vs NS-2.5-month *, S-HH vs S-2.5-month,NS-HH vs NS-5-month $, S-HH vs S-5-month ∇, NS-2.5-month vs NS-5-month ◊, S-2.5-month vs S-5-month ○.

7.8%; NS-5-month: −72.8% ± 9.2%; S-5-month: −64.6% ± 3.3%. The smallest change in ATP was found in the S-5-month group.

PCr also decreased progressively during ischemia to the point of being NMR undetectable in both HHs and CMHs (Fig. 3).

P_i increased in CMH hearts (NS-2.5-month: 179.6% ± 35.6%; S-2.5-month: 216.7% ± 9.7%; NS-5-month: 118.0% ± 26.6%; S-5-month: 148.6% ± 21.6%) after 50 min of ischemia, but the increase was less than that in HH hearts (NS-HHs: 306.8% ± 52.1%, S-HHs: 222.8% ± 19.0%) (Fig. 4).

The change of myocardial pH was inversely related to the P_i changes. The decrease in intracellular pH after ischemia was −17.7% ± 1.01% for NS-HHs and −20.3% ± 6.5% for S-HHs hearts. Changes in intracellular pH for nonstressed cardiomyopathic hearts were smaller than for nonstressed HHs hearts (−9.7% ± 2.8% for NS-2.5-month: −13.5% ± 0.9% for NS-5-month); however, the changes in pH for stressed hamsters were similar to that of HHs (−18.3% ± 0.7% for S-2.5-month, and −22.1% ± 4.1% for S-5-month) (Fig. 5).

Three-way, repeated-measure ANOVA showed statistically significant time effect for percentage changes in ATP ($F(11,319) = 125.7$, $P < 0.000000$), PCr ($F(11,319) = 808.9$, $P < 0.000000$), P_i ($F(11,132) = 49.98$, $P < 0.000000$), and pH ($F(17,53) = 52.97$, $P < 0.000000$); and disease plus time interaction for P_i ($F(22,132) = 3.15$, $P < 0.000025$).

The overall 2 × 3 ANOVA showed statistically significant disease effects for P_i at 50 min of ischemia ($F(2,18) = 6.24$, $P < 0.009$); and for pH at 5 min ($F(1,29) = 4.04$, $P < 0.03$) and 15 min ($F(1.28) = 4.4$, $P < 0.02$), but not for ATP and PCr. There were no statistically significant chronic stress effects for ATP, PCr, P_i and pH. There was a statistically significant stress plus disease effect for pH at 40 min ($F(1,24) = 4.4$, $P < 0.02$) and 45 min ($F(2,20) = 4.5$, $P < 0.024$).

Significant findings (P value indicated) for post hoc analyses can be found in Figures 2–5.

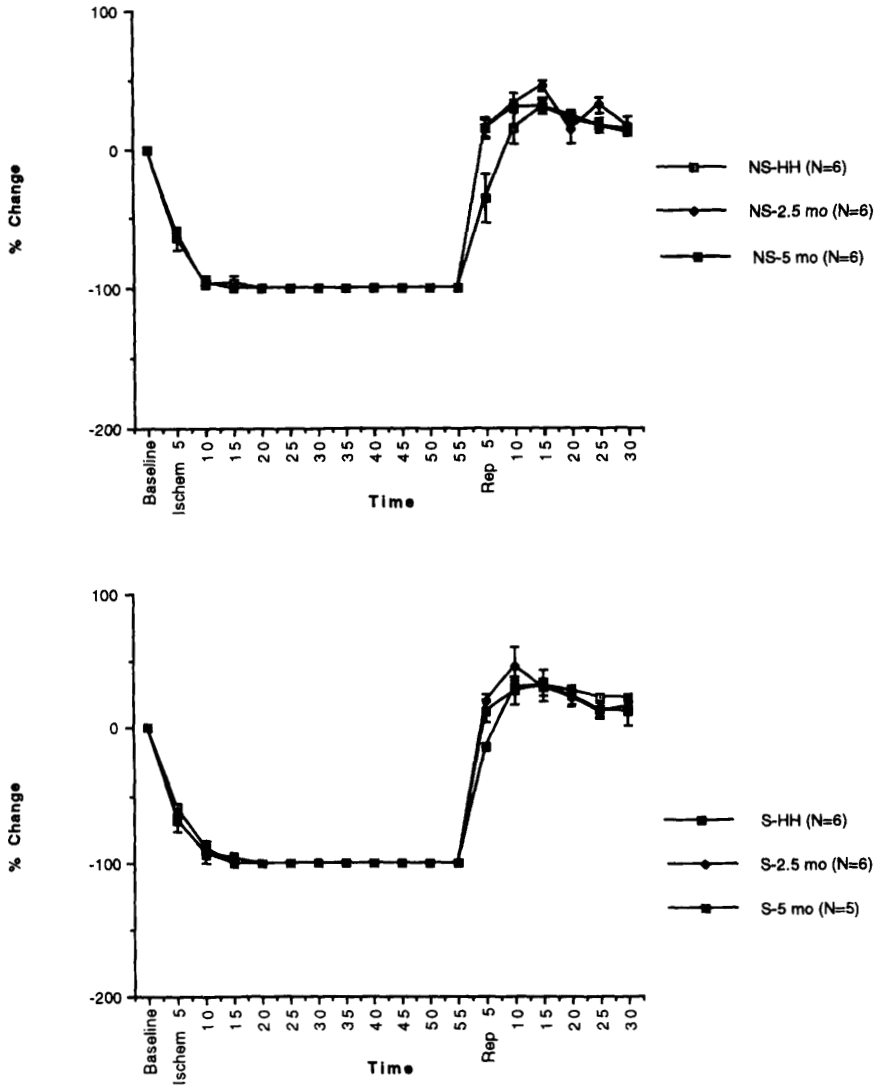

Figure 3. Changes in PCr content during 55 min of global myocardial ischemia followed by 30 min of reperfusion. Perfusion conditions, abbreviations, and notations for statistically significant differences are the same as for Figure 2. Values are mean ± SEM, $P < 0.05$. No significant differences were noted between groups by two-way analysis of variance (ANOVA) and *post hoc* analysis.
Rate of change (slopes) during 5–15 min of reperfusion were statistically significantly different in NS-2.5-month, and NS-5-month compared to NS-HHs, and in S-2.5-month CMH compared to S-HHs. Slopes were: NS-HH 0.202 ± 0.03; S-HH 0.18 ± 0.03; NS-2.5-month 0.09 ± 0.03*; S-2.5-month 0.073 ± 0.02 #; NS-5-month 0.09 ± 0.04 $; S-5-month 0.1 ± 0.03, $P < 0.05$.

Reperfusion (30 min). During reperfusion, ATP recovered to higher levels in the NS-5-month (−18.6% ± 8.2%) and S-5-month CMH (−39.45% ± 2.6%) than in NS-HHs (−43.6% ± 2.05%), and S-HHs (−50.7% ± 0.8%). In NS-2.5-month CMH, recovery was less (−29.6% ± 4.6%) than in NS-5-month CMH, but returned more toward baseline than in NS-HHs. In the stress group, ATP recovery was similar in CMH (2.5-month −40.9% ± 3.4% and 5-month −39.45% ± 2.6%), and both returned more toward baseline than in S-HHs (Fig. 2). In addition, at 25 min, NS-5-month CMHs returned more toward baseline (−19.9% ± 1.7%) than S-5-month (−36.4% ± 2.2%). P_i also returned more toward baseline (Fig. 4) for NS-5-month (−19.4% ± 10.4%) and NS-2.5-month (−9.8% ± 13.2%) CMHs compared to S-HHs (32.4% ± 14.6%) (at 20 min); and for NS-2.5-month (−10.6% ± 12.2%) and NS-5-month (5.2% ± 13.6%) compared to NS-HHs (29% ± 20.9%). There was no significant difference in recovery of PCr (Fig. 3) and pH (Fig. 5) for all six groups.

Three-way repeated measure ANOVA Disease × Stress (between groups) × Time (within subjects) showed statistically significant time effect for ATP ($F(5,80) = 2.69$, $P < 0.027$), PCr ($F(5,75) = 6.7$, $P < 0.000031$) and P_i ($F(5,105) = 4.2$, $P < 0.0016$); statistically significant disease effect with respect to P_i ($F(5,105) = 4.2$, $P < 0.0016$); and disease plus time interaction with respect to ATP ($F(10,80) = 2.2$, $P < 0.025$) and PCr ($F(10,75) = 1.19$, $P < 0.027$).

The overall 2 × 3 ANOVA showed a statistically significant effect of prior chronic stress on postischemic ATP recovery at 20 and 25 min: ($F(1,23) = 12.91$, $P < 0.0015$); and ($F(1,24) = 17.99$, $P < 0.0003$), respectively. Also, ANOVA showed a statistically significant disease effect for ATP and P_i at 20 min of reperfusion: ($F(2,23) = 11.3$, $P < 0.0004$) and ($F(2,27) = 5.5$, $P < 0.0099$), respectively; and at 25 min: ($F(2,24) = 4.65$, $P < 0.025$) and ($F(2,25) = 4.27$, $P < 0.025$), respectively.

Significant findings (P value indicated) for *post hoc* analyses can be found in Figures 2–5.

Slopes. Because it is possible that rates of change of metabolic parameters might be more sensitive to physiologic insult than change of absolute values of individual

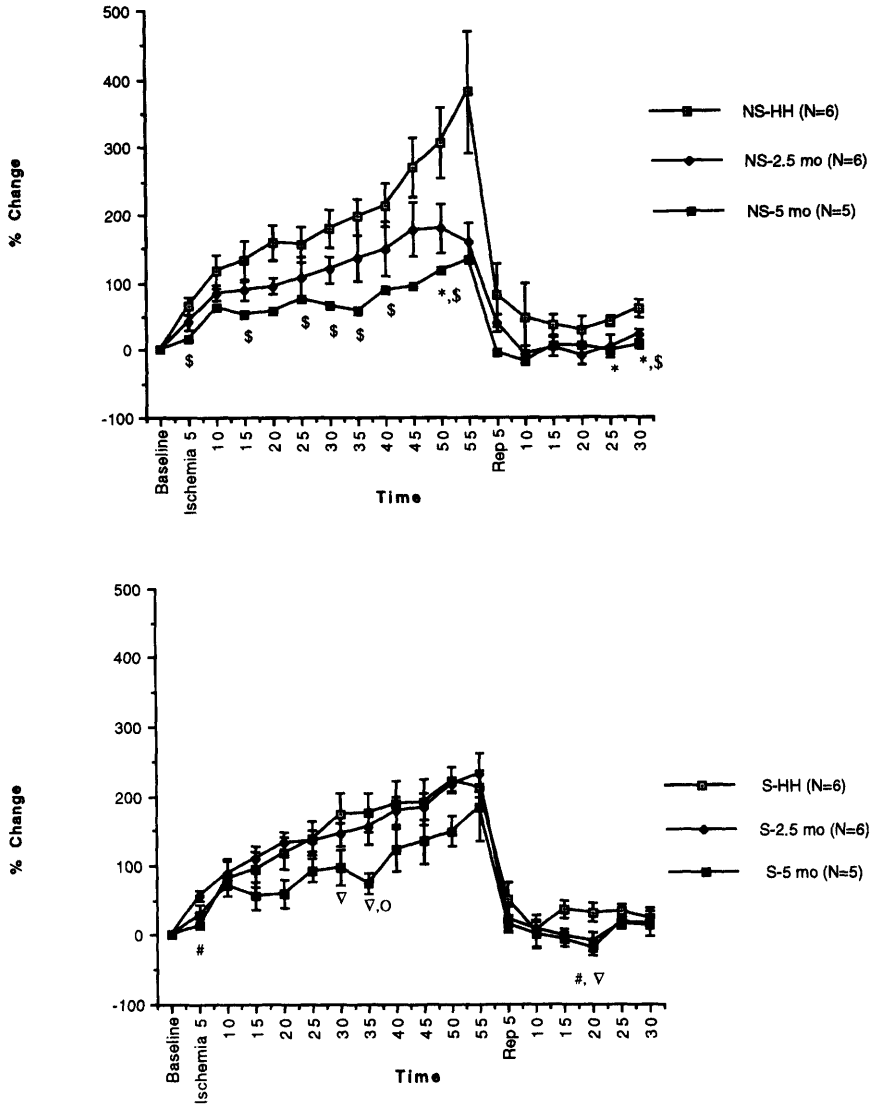

Figure 4. Changes in P_i content during 55 min of global myocardial ischemia followed by 30 min of reperfusion. Perfusion conditions, abbreviations, and notations for statistically significant differences are the same as for Figure 2. Values are mean ± SEM, $P < 0.05$.
Rate of change (slopes) during 5–15 min of reperfusion were statistically significantly different in NS-5-month CMHs compared to NS-HHs. Slopes were: NS-HH-0.08 ± 0.04; S-HH −0.03 ± 0.01; NS-2.5-month −0.06 ± 0.03; S-2.5-month −0.05 ± 0.02; NS-5-month 0.023 ± 0.03 $; S-5-month −0.04 ± 0.02, $P < 0.05$.

metabolites, we calculated the slopes for each metabolic parameter during ischemia and reperfusion, and compared the resulting slope data using 2 × 3 ANOVA. During ischemia and 0–5 min of reperfusion, there were no differences in the slopes for any metabolic parameter between the different groups. We have shown that initial recovery of PCr and ATP toward baseline is rapid; however, shortly later, mechanical function can return, and reperfusion damage can begin. Some causes of this are: Ca^{2+} overload, activation of lipid oxidation, and generation of free radicals, such as OH^-, and O_2^-. Because of this, we decided to evaluate the slopes of changes during 5–15 min of reperfusion. The differences in the recovery process evidenced during this time period might help to distinguish the different groups.

2 × 3 ANOVA showed a statistically significant disease effect with respect to ATP ($F(2,29) = 4.98$, $P < 0.01$) and PCr ($F(2,28) = 72$, $P < 0.003$) during 5–15 min of reperfusion. See Figures 2–5 for statistically significant differences in slope for each of the six groups.

HPLC Data

Baseline (before-acute ischemia) Metabolic Parameters. High-energy phosphate levels from hamster hearts (HHs and CMHs, NS and S) which were perfused for 30 min, but not exposed to acute ischemia, were determined by HPLC (and defined as baseline values), Table I. The overall 2 × 3 ANOVA showed a statistically significant stress effect with respect to ATP levels ($F(1,20) = 38.45$, $P < 0.00001$), but not with respect to PCr, adenosine diphosphate (ADP), adenosine monophosphate (AMP), or nicotinamide adenine dinucleotide (NAD). ANOVA also showed a statistically significant disease effect with respect to PCr ($F(2,18) = 22.63$, $P < 0.00001$), ATP ($F(1,20) = 29.98$, $P < 0.00001$), ADP ($F(1,16) = 3.80$, $P < 0.045$), and NAD ($F(1,19) = 3.90$, $P < 0.038$), but not with respect to AMP. Specific statistically significant differences from *post hoc* analysis can be found in Table I.

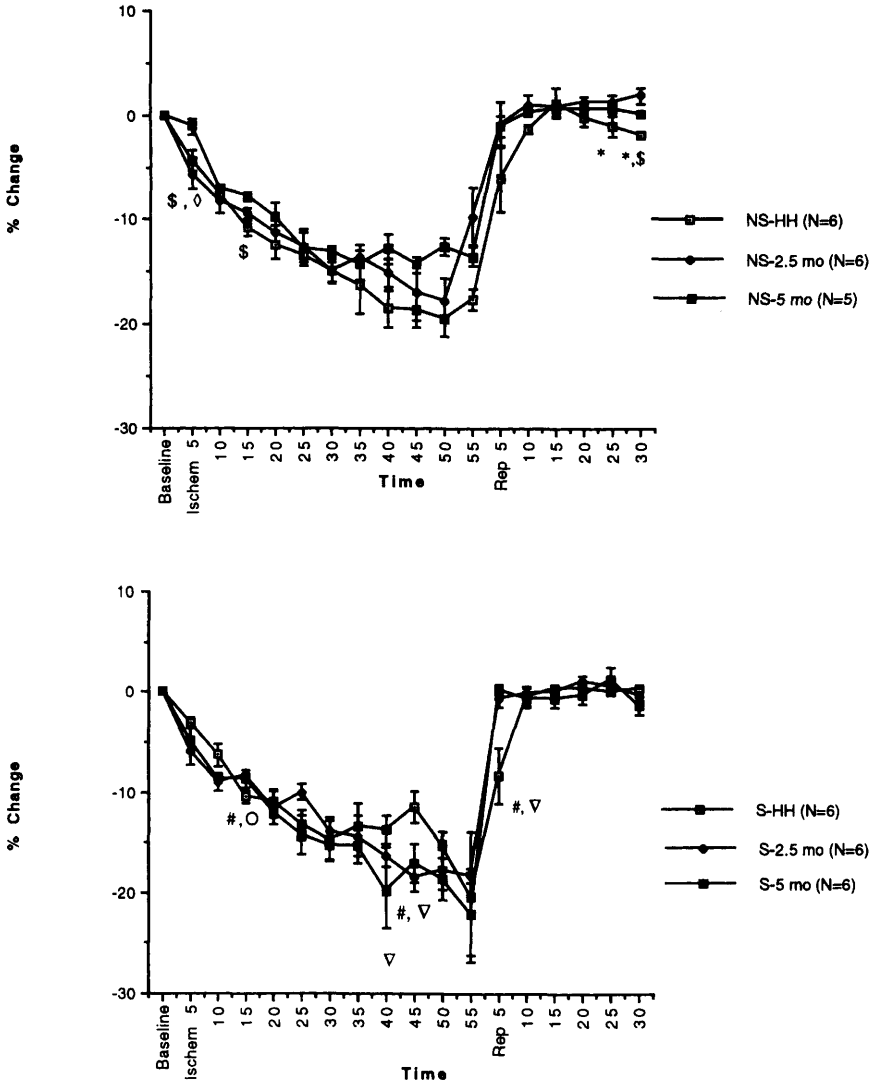

Figure 5. Changes in pH content during 55 min of global myocardial ischemia followed by 30 min of reperfusion. Perfusion condition, abbreviations, and notations for statistically significant differences are the same as for Figure 2. Values are mean ± SEM, $P < 0.05$.
No significant differences were noted in the rate of change (slopes) during the first 15 min of reperfusion.

Table I. Bioenergetic Metabolites Obtained from Hearts from Healthy and Cardiomyopathic Hamsters: Using HPLC; Baseline (Before Ischemia)

Parameter	NS-HH	S-HH	NS-2.5-month	S-2.5-month	NS-5-month	S-5-month
PCr	4.56 ± 0.12	3.81 ± 0.15 a	3.38 ± 0.08 d	3.34 ± 0.23	2.99 ± 0.2 e	3.48 ± 0.12
ATP	3.16 ± 0.08	3.0 ± 0.05	2.79 ± 0.05 d	2.22 ± 0.1 b,g	2.78 ± 0.13 e	2.22 ± 0.08 c,h
ADP	1.00 ± 0.03	1.00 ± 0.02	0.85 ± 0.02 d	0.82 ± 0.08	1.03 ± 0.02 f	0.89 ± 0.1
AMP	0.50 ± 0.02	0.49 ± 0.02	0.47 ± 0.003	0.43 ± 0.08	0.53 ± 0.04	0.47 ± 0.09
NAD	0.87 ± 0.15	0.82 ± 0.04	0.78 ± 0.002 d	0.70 ± 0.04 g	0.72 ± 0.05 e	0.83 ± 0.05 i

Note. Values are mean ± SE in μmoles/g wet tissue, $P < 0.05$. Number of animals in each group $n = 4–5$; NS, nonstressed hamsters; S, stressed hamsters; HH, healthy hamsters. Notations for statistically significant differences are:

a = NS-HH vs S-HH
b = NS-2.5-month vs S-2.5-month
c = NS-5-month vs S-5-month
d = NS-HH vs NS-2.5-month
e = NS-HH vs NS-5-month
f = NS-2.5-month vs NS-5-month
g = S-HH vs S-2.5-month
h = S-HH vs S-5-month
i = S-2.5-month vs S-5-month

Post-Ischemic (Reperfusion) Metabolic Parameters. High-energy phosphate levels from heart samples obtained after exposure to 55 min of ischemia and 30 min of reperfusion were also determined by HPLC, Table II. The overall 2 × 3 ANOVA showed a statistically significant stress effect with respect to ATP levels ($F(1,18) = 4.89$, $P < 0.04$), but not with respect to PCr, ADP, AMP, and NAD. ANOVA also showed a statistically significant disease effect with respect to ATP ($F(2,18) = 11.68\%$ $P < 0.0006$), ADP ($F(2,19) = 4.3$, $P < 0.029$), and PCr ($F(2,18) = 6.39$, $P < 0.007$), but not with respect to AMP and NAD. Specific statistically significant differences from *post hoc* analysis can be found in Table II.

Table II. Bioenergetic Metabolites Obtained from Hearts from Healthy and Cardiomyopathic Hamsters: Using HPLC; Reperfusion (post 55 min ischemia/30 min reperfusion)

Reperfusion*	NS-HH	S-HH	NS-2.5-month	S-2.5-month	NS-5-month	S-5-month
PCr	2.79 ± 0.15	2.79 ± 0.3	1.94 ± 0.2	1.77 ± 0.4 g	1.66 ± 0.3 e	2.06 ± 0.3
ATP	1.80 ± 0.15	1.68 ± 0.2	1.10 ± 0.14 d	0.93 ± 0.14 g	1.66 ± 0.14 f	1.13 ± 0.13 c,h
ADP	0.84 ± 0.12	0.64 ± 0.1	0.47 ± 0.04 d	0.43 ± 0.05	0.69 ± 0.16	0.56 ± 0.06
AMP	0.42 ± 0.066	0.24 ± 0.08 a	0.41 ± 0.006	0.37 ± 0.03	0.37 ± 0.04	0.35 ± 0.04
NAD	0.76 ± 0.2	0.67 ± 0.1	0.92 ± 0.2	0.73 ± 0.08	0.79 ± 0.18	0.85 ± 0.15

Note. Values are mean ± SE in μmoles/g wet tissue, $P < 0.05$. Number of animals in each group $n = 4$; NS, nonstressed hamsters; S, stressed hamsters; HH, healthy hamsters. Reperfusion*, after 55 min of ischemia/30 min of reperfusion. Notations for statistically significant differences are the same as for Table I.

Discussion

In the present set of experiments, we used both ^{31}P NMR and HPLC techniques to evaluate bioenergic processes in a Syrian hamster model of cardiomyopathy. Both 2.5-month (necrotic stage) and 5-month (hypertrophic stage) animals were studied and compared to healthy hamsters. In earlier work, Wrogemann *et al.* (13) did not detect defects in oxidative phosphorylation parameters between cardiomyopathic and control hamsters when pyruvate plus malate, palmityl-L-carnitine plus malate, DL-hydroxybutyrate, or glutamate plus malate were used as substrates. However, previous data obtained in our laboratory indicate that metabolic changes occur in 2.5-month CMHs before clinical manifestations of the disease are evident; and that metabolic abnormalities change throughout the disease process.

In this previous work, we found differential abnormalities of mitochondrial function in cardiomyopathic hamsters of different ages and stress states. Changes in oxidative phosphorylation were small in nonstressed cardiomyopathic hamsters and markedly increased after application of cold immobilization stress. The 2.5-month CMHs reacted to stress by increasing State 4 respiration, possibly related to ischemia resulting from chronic vasospasm (14). The increase in State 4 leads to a decrease in RCI, which indicates that oxidative phosphorylation in mitochondria from cardiomyopathic hearts is loosely coupled. These data are in agreement with the data of Proschek and Jasmin (15), obtained in heart mitochondria from cardiomyopathic hamsters at the peak necrotic period, using pyruvate plus malate as substrate. Improvement of all respiratory function parameters occurred during the healing stage (5–6-month) of the disease.

Baseline Data. HPLC analysis of bioenergetic metabolites showed that baseline ATP and PCr were less in both NS-2.5-month and NS-5-month CMHs compared to NS-HHs. These lowered levels of high-energy phosphates may be related to a decreased activity of creatine kinase (16, 17), myokinase (18), or disturbed function of the ATP/ADP translocase (19), which in turn may contribute to contractile dysfunction. In addition, the lowered NAD levels in both 2.5- and 5-month CMHs suggest a decrease in electron transport capability, further confirming the finding of decreased coupling of oxidation to phosphorylation reported for previous polarographic measurements (20).

Chronic Stress Data. Application of chronic stress to 2.5-month and 5-month CMHs was used in an attempt to further differentiate metabolic characteristics of various stages of disease progression. HPLC data showed that the application of chronic stress resulted in further lowering of ATP levels in both 2.5-month and 5-month CMHs, whereas there was no additional lowering of PCr levels from the pre-stress condition. In both cases, however, ATP and PCr levels were significantly lower than in HHs (both NS and S). Of interest, NAD was lower in S-2.5-month CMHs compared to NS-2.5-month whereas it was higher in S-5-month than in NS-5-month; and in fact NAD levels were significantly higher in S-5-month than those of S-2.5-month. This suggests that chronic stress may induce improved electron transport in the S-5-month group, and therefore, may confer some protection from disease progression due to somewhat better coupling of oxidative phosphorylation.

Acute Stress Data. Application of acute stress, in the form of stop-flow ischemia, to stressed and nonstressed cardiomyopathic and healthy hamsters, produced similar rates of ATP and PCr depletion. It is of interest that both the increase in P_i and the decrease in pH were less in 5-month CMH compared to HHs or 2.5-month CMH. This suggests that progression of the cardiomyopathic process *per sé* may increase the tolerance of the myocardium to acute stress (ischemia). This tolerance (or metabolic stability) may result from improved membrane stability or greater efficiency in the use of energetic intermediates associated with slower and smaller changes in P_i, which in turn may be responsible for smaller pH changes. Further support for this hypothesis was obtained by the observation that recovery of ATP, P_i, and pH in the post-ischemic period was more toward baseline levels in 5-month CMHs compared to HHs. Along similar lines, P_i and pH also recovered to near baseline levels in 2.5-month CMHs.

HPLC Discrepancy. ATP and PCr from heart extracts obtained at the end of the 30-min reperfusion period determined *via* HPLC were somewhat different from ^{31}P NMR data. HPLC data suggested that PCr did not recover in contrast to the recovery found with ^{31}P NMR. This is most likely due to metabolic changes (further ischemia) that oc-

curred during tissue harvesting, freezing, and extracting processes. The post-ischemic hearts are more sensitive to this acute insult than hearts harvested at baseline. PCr is susceptible to rapid enzymatic breakdown during ischemia. ATP, which is less sensitive to the harvesting processes, was similar using both HPLC and NMR techniques.

Summary. These data show that bioenergetic differences (elicited by both acute and chronic stress) can be used to characterize various stages of the cardiomyopathic process, compared to healthy hamsters. It is of interest that application of chronic stress helped to distinguish the two cardiomyopathic groups, whereas application of acute ischemic stress to chronically stressed hamsters did not further separate the differences between these two groups. In addition, the data suggest that chronic stress may uncover pathology at an earlier stage. Further, both exogenously applied chronic stress (in this case, cold immobilization) and the stress of the chronic endogenous disease process (both 2.5- and 5-month exposure to the cardiomyopathic process) may increase tolerance of energy metabolism to acute stress, such as stop-flow ischemia.

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