

# L-Carnitine Treatment Improves Cardiac Performance and Restores High-Energy Phosphate Pools in Cardiomyopathic Syrian Hamster

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Hamsters with either the dilated (BIO 53.58) or hypertrophic (BIO 14.6) form of cardiomyopathy and an inbred control strain of hamster (F1B) were treated for 6 months with high-dose L-carnitine (1 g/kg/day i.p.). After treatment, the animals were killed and their hearts perfused by the isolated working technique. Mechanical performance (as indicated by the double product of heart rate and left ventricular [LV] peak systolic pressure) of both carnitine-treated cardiomyopathic groups was increased significantly above their respective sham-treated groups. Associated with these increases in mechanical performance were significant increases in both peak-positive LV dP/dt (index of contractility) and peak negative dP/dt (index of relaxation) in both carnitine-treated myopathic groups. Serum carnitine levels were increased 10–15 times within 2 hours after injection of L-carnitine in all 3 groups. Myocardial free-carnitine levels were increased twofold in both control and dilated myopathic hearts above their respective sham-treated groups, restoring the level in the dilated hearts comparable to those of controls. Myocardial carnitine levels in the hypertrophic group were not significantly affected by treatment. Total high-energy phosphate stores, i.e., ATP plus creatine phosphate, were restored to control levels by L-carnitine treatment in both cardiomyopathic groups. Levels of the breakdown products of ATP were maintained primarily in the more readily convertible adenosine diphosphate and adenosine monophosphate forms in all three treated groups. These changes resulted in significantly higher ratios of (ATP)/(ADP + AMP + adenosine) and (creatine phosphate)/(creatine) in the treated hearts. This is the first study demonstrating that high-dose L-carnitine treatment results in improved cardiac performance and increased myocardial total high-energy phosphate stores in the Syrian hamster model with one of two distinct forms of cardiomyopathy, i.e., dilated or hypertrophic. The mechanisms for these effects of exogenous L-carnitine treatment cannot be totally explained by changes in oxidative energy metabolism. (*Circulation Research* 1987; 61:396–408)

Most treatment modalities for congestive (dilated) cardiomyopathy in the clinical setting have involved use of digitalis, diuretics, and vasodilators to help relieve any congestive heart failure. Several years ago, excessive catecholamines were reported as possible causes of myocardial injury; this led to the suggestion of a possible “stress cardiomyopathy” by Selye.<sup>1</sup> A subsequent series of studies indicated that  $\beta$ -adrenergic receptor blockade was beneficial in the treatment of congestive cardiomyopathy.<sup>2–4</sup> However, in another study, treatment of these patients with the  $\beta$ -blocker propranolol resulted in decreased cardiac function and exercise intolerance.<sup>5</sup> Staley et al<sup>6,7</sup> using propranolol in the turkey model of congestive cardiomyopathy also found that  $\beta$ -adren-

ergic blockers were not very useful in chronic therapy of this disease. In contrast, others have treated cardiomyopathic patients with  $\beta_1$ -agonists, such as prenalterol, and have demonstrated improvement in their clinical condition.<sup>8</sup> Thus, to date, there is not a consensus on the best treatment regimen for congestive cardiomyopathy.

Calcium overload has been postulated as another factor that contributes to some forms of heart failure. The cardiomyopathic hamster has a defect in calcium handling that results in intracellular calcium overload in the myocardium.<sup>9–11</sup> Sarcoplasmic reticular function is abnormal in both the hypertrophic BIO 14.6<sup>12–14</sup> and dilated BIO 53.58<sup>14</sup> strains of these cardiomyopathic hamsters as well as in failing human hearts.<sup>15</sup> Investigators have demonstrated that when hamsters with the hypertrophic form of cardiomyopathy are treated with verapamil, the degree of myocardial necrosis and scarring is reduced but with preservation of myocardial contractility.<sup>16,17</sup> A more recent study by Wikman-Coffelt et al<sup>18</sup> has shown a preservation of high-energy phosphate stores in these hamsters after verapamil treatment. In the clinical setting, several groups have demonstrated that the use of calcium channel blockers

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in patients with hypertrophic cardiomyopathy has resulted in improved clinical conditions, in lower death rates, and in little need for surgery to relieve left ventricular outflow tract obstruction in both adults<sup>19–22</sup> and children.<sup>23</sup>

Another etiologic factor of cardiomyopathy that has been proposed is a deficiency of myocardial L-carnitine, an essential cofactor for oxidative metabolism of long chain fatty acids. It functions to transport long chain fatty acids from the cytosol into mitochondrial matrix sites of  $\beta$ -oxidation. Earlier studies from this and other laboratories have demonstrated that myocardial carnitine stores are markedly reduced in Syrian hamsters with either the dilated (BIO 53.58) or hypertrophic (BIO 14.6) forms of cardiomyopathy.<sup>24–29</sup> Furthermore, a defect has been suggested in the myocardial membrane transport of carnitine in these cardiomyopathic hamsters.<sup>26</sup> In this regard, serum carnitine is compensatorily increased in both patients<sup>30</sup> and Syrian hamsters<sup>29</sup> with either dilated or hypertrophic cardiomyopathy. York et al<sup>26</sup> were unable to show in these myopathic hamster hearts an increase of myocardial carnitine levels to normal levels after treatment with L-carnitine via their drinking water. However, more recently, Yamashita et al<sup>28</sup> did demonstrate that daily intraperitoneal administration of L-carnitine or verapamil to hamsters with hypertrophic cardiomyopathy (BIO 14.6) for approximately 2 months significantly increased total myocardial carnitine stores and decreased myocardial necrosis, fibrosis, and calcification in the treated hamsters. They concluded that lowered carnitine concentrations in hearts of these cardiomyopathic hamsters may play a pathophysiological role in the development of this disease and that L-carnitine or verapamil may be beneficial in the treatment of cardiomyopathy in this animal model.

The first purpose of the present study was to determine whether the defect in carnitine transport could be bypassed by markedly elevating serum carnitine levels to an extent that would result in increased passive diffusion of carnitine into the myocardial cell. Assuming that the cardiomyopathic cell can still bind L-carnitine, near-normal myocardial carnitine levels could be restored in the myopathic hamsters by this treatment. Marked elevation of serum carnitine would be accomplished by chronic treatment with high-dose L-carnitine via injection. After the myocardial carnitine levels were restored to normal in these myopathic hamsters, the second purpose of this study was then to determine whether cardiac performance would improve during isolated-heart perfusion with maintenance of high-energy phosphate stores comparable to normal control hearts. Decreased high-energy phosphate stores are associated with the Syrian hamster model of cardiomyopathy<sup>18,29,31,32</sup> and other models of heart failure, along with depressed adenosine nucleotide pools<sup>18,34</sup> and a reduction in the release of free energy by ATP hydrolysis.<sup>35</sup> Therefore, high-energy phosphate levels were assayed and used, along with hemodynamic parameters of cardiac function, to indicate functional integrity of the myocardium.

## Materials and Methods

### Animals

Twenty male Syrian hamsters with dilated cardiomyopathy (BIO 53.58) and 20 males with hypertrophic cardiomyopathy (BIO 14.6) were obtained for the study (Bio-Breeders, Inc, Fitchburg, Mass.). Twenty male, age-matched Syrian hamsters (F1B: inbred, normal, and golden) served as controls. The BIO 53.58 strain develops a dilated, i.e., congestive, form of cardiomyopathy characterized by thin ventricular walls and dilated chambers throughout most of its life (personal communication with Dr. Cornelius VanDongen of Bio-Breeders, Inc). This model is morphologically similar to the idiopathic congestive cardiomyopathy, clinically recognized over 100 years ago,<sup>36</sup> and the acquired cardiomyopathies due to such cardiotoxins as alcohol<sup>37</sup> and some anticancer agents.<sup>38</sup> The hypertrophic cardiomyopathic strain (BIO 14.6), which has been used more frequently in previous investigations, develops a myopathy characterized by thickened ventricular walls and septum.<sup>39,40</sup> This model is grossly more similar to idiopathic hypertrophic subaortic stenosis<sup>39</sup> and catecholamine-induced cardiomyopathy.<sup>41</sup> These hypertrophic cardiomyopathic hamsters were treated and subsequently killed prior to the development of any significant ventricular dilation, which is characteristic in late terminal stages of the disease in these animals.<sup>39</sup>

All hamsters were obtained shortly after weaning, i.e., at approximately 1 month of age. Ten hamsters from each of the 3 strains were given a 1 g/kg/day dosage of L-carnitine beginning 1 week after arrival (about 40 days of age). The carnitine was administered twice daily in a normal saline solution by injection (0.25 ml i.p.). The remaining strain, sex-, and age-matched hamsters were given injections of the normal saline carrier. These latter hamsters made up the sham-treatment group. Treatments were carried out for a period of approximately 6 months, during which time the hamsters were observed and weighed daily while being housed in individual hanging cages with food and water ad libitum. During the time the hamsters were housed in the animal facility, the following deaths occurred: during the first week prior to beginning treatments, 3 hamsters died (1 dilated, 2 hypertrophic), possibly because of the stress of adapting to a new environment; after treatments began, 2 hypertrophic hamsters were dropped during injection and died; finally, 5 hearts were lost because of technical problems with cannulation of the vessels prior to perfusion (1 control, 2 dilated, and 2 hypertrophic). None of the deaths during the study were directly attributable to the treatments.

### Perfusion Technique

After the animals were anesthetized with sodium pentobarbital (40 mg/kg i.p.), the hearts were rapidly excised and placed in ice-cold saline solution prior to cannulation for working-heart perfusion according to the method of Neely et al.<sup>42</sup> After cannulation of the aortic stump, a 10-minute retrograde Langendorff

perfusion was begun during which the pulmonary vein was cannulated. During the subsequent 30-minute antegrade working-perfusion period, left atrial filling pressure was maintained at 7.5 mm Hg, and the afterload was kept at 120 mm Hg by a 160-cm column of recirculating perfusate in the aortic outflow tract. The perfusion period of 30 minutes was chosen because the isolated perfused heart requires 20–25 minutes to reach a stable steady state (unpublished observations). All hearts were electrically stimulated at 240 beats/min during working perfusion. This rate resulted in approximately 95% of maximal maintainable peak systolic pressure for the sham-treated myopathic hearts. Thus, none of the myopathic hearts were perfused at a work load severe enough to cause markedly declining left ventricular pressure and overt acute heart failure. Both control and myopathic hearts were, therefore, subjected to similar levels of work throughout the 30-minute perfusion with constant coronary perfusion pressures. It was believed to be very important to maintain coronary perfusion pressure constant so that oxygen and substrate supply would not become limiting during this period.

The perfusion medium consisted of 37° C Krebs-Henseleit bicarbonate buffer containing (in mM): NaCl 118, KCl 4.7,  $\text{KH}_2\text{PO}_4$  1.2,  $\text{MgSO}_4$  1.2,  $\text{CaCl}_2$  3.0,  $\text{NaHCO}_3$  25, and Na EDTA (ethylenediaminetetraacetic acid) 0.5. This perfusate was gassed with 95%  $\text{O}_2$ –5%  $\text{CO}_2$ . Substrates in the perfusate were 11 mM D-glucose during the initial retrograde perfusion and, additionally, 1.0 mM palmitate bound to 3% bovine serum albumin (Pentex Fraction V, Miles Scientific, New Haven, Conn.) during the 30-minute working perfusion. Heart rate, left ventricular (LV) pressure development, and dP/dt were monitored throughout the perfusions. After 30 minutes, hearts were frozen at the temperature of liquid nitrogen using Wollenberger clamps and were stored in liquid nitrogen until analyzed for metabolic intermediate levels.

#### *Estimation of Tissue Metabolites*

The frozen tissue was powdered in a liquid-nitrogen-cooled mortar. This powder was extracted into ice-cold 6% perchloric acid, and the neutralized extract was used to estimate acid soluble metabolite levels. The perchloric acid precipitates were further extracted by alkaline hydrolysis for determination of long chain acylcarnitine and coenzyme A (CoA) derivatives. Sections of liver, kidney, and diaphragm were also frozen at the time of killing for determination of total carnitine levels. Samples of blood were aspirated from the hamsters' chest cavities after excising the heart. Additional 6-month-old animals of each group were used just for these serum carnitine time-course studies. For these acute serum studies, 2 blood samples were obtained from each animal prior to killing. Serum was then obtained by centrifugation for measurement of total carnitine. Elapsed time from carnitine injection to killing was noted.

All enzymes and cofactors used in the assay protocols were purchased from the Sigma Chemical Com-

pany, St. Louis, Mo. Adenine nucleotides, creatine phosphate, creatine, lactate and pyruvate levels were determined by standard spectrophotometric enzymatic procedures as outlined in Bergmeyer.<sup>43</sup> CoA was estimated fluorometrically using the  $\alpha$ -ketoglutarate dehydrogenase reaction.<sup>44</sup> Free L-carnitine levels were estimated by the radioisotopic procedure of McGarry and Foster.<sup>45</sup> Levels of acetyl-CoA were measured using a malate dehydrogenase and citrate synthetase system.<sup>46</sup> Subsequently, acetylcarnitine was estimated by coupling the acetyl-CoA system to the carnitine–acetyl-CoA transferase reaction.<sup>47</sup> Myocardial levels of long chain acyl-CoA were estimated as free CoA after alkaline hydrolysis of the acid precipitates at pH 12.0 for 15 minutes at 55° C.<sup>48</sup> Long chain acylcarnitine was assayed as free carnitine after alkaline hydrolysis at pH 13.0 for 60 minutes at 70° C.<sup>48</sup> Liver, kidney, and diaphragm levels of total L-carnitine were similarly extracted by the pH 13.0 alkaline hydrolysis to release free carnitine for assay.

Tissue metabolite levels were expressed as per gram of noncollagen protein (NCP) to eliminate any contribution from connective tissue present due to scarring that occurs in these myopathic hearts. NCP was extracted into 0.05N sodium hydroxide, and the protein was assayed by the Bradford<sup>49</sup> dye-binding technique (Bio-Rad Protein Assay).

Statistical comparisons between respective carnitine-treated and sham-treated strains were done by the unpaired, two-tailed Student's *t* test with significance level being  $p < 0.05$ . Values given are mean  $\pm$  SEM.

#### **Results**

High-dose L-carnitine treatment did not adversely affect weight gain within the control or either cardiomyopathic strain during the 6 months of treatment (Figure 1). The carnitine-treated hamsters maintained the same growth patterns as their corresponding sham-treated groups, with controls having significantly higher weights ( $p < 0.001$ ) throughout the treatment period. L-carnitine treatment did not affect the heart wt:body wt ratio in any of the strains (Figure 2). Both hypertrophic cardiomyopathic groups had similar ratios that were significantly greater than either the control or dilated groups. Furthermore, carnitine treatment did not affect NCP levels in the hearts of any group of hamsters. NCP levels obtained were comparable to those previously published.<sup>29</sup> Animals did not display any changes in behavior, sleeping patterns, or appetite during the daily handling. Also, no evidence of inflammation developed on the abdomens of these animals as a result of the repeated injections. At the time of killing, no gross evidence of trauma was noted within the abdominal cavity. Neither the carnitine-treated nor the sham-treated cardiomyopathic animals demonstrated any gross signs of heart failure as evidenced by ascites fluid in the abdominal cavity.

#### *Serum Carnitine Levels With Treatment*

The serum concentrations of total L-carnitine markedly increased within the 1–2 hours after the intra-

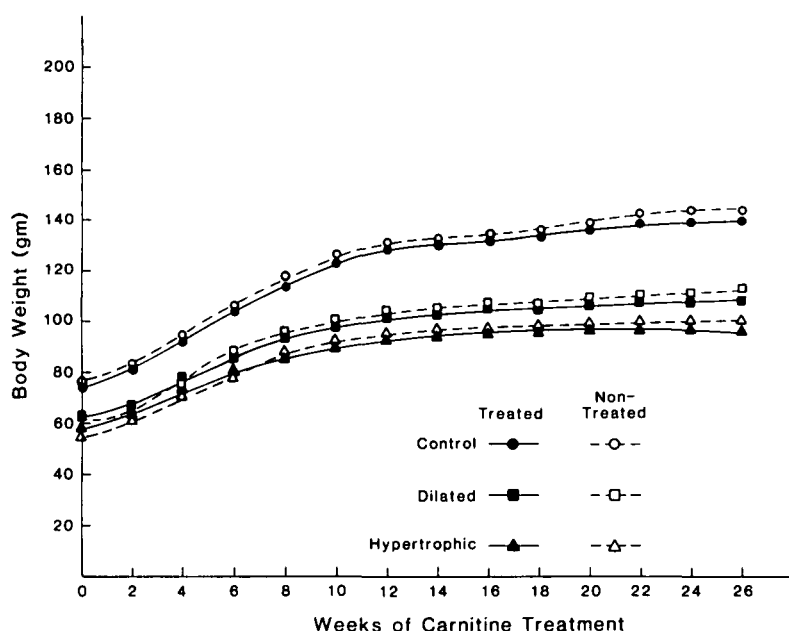


FIGURE 1. Time course of body weight increases in L-carnitine- and sham-treated control and cardiomyopathic hamsters. Weights were taken each day prior to morning injections. Values are mean weights of all animals in each of 6 groups of hamsters over two-week time intervals.  $\circ$ , control hamsters;  $\square$ , dilated cardiomyopathic hamsters;  $\triangle$ , hypertrophic cardiomyopathic hamsters. Solid symbols represent L-carnitine-treated hamsters, and open symbols represent sham-treated hamsters.  $p < 0.001$  at all time points for control vs. both cardiomyopathic groups, carnitine-treated or sham-treated.

peritoneal injection of the concentrated L-carnitine solution (Figure 3). Serum carnitine increased 10–15 times during this period from the baseline concentrations of  $18.9 \pm 1.2$  mM in controls vs.  $26.8 \pm 2.4$  or  $25.7 \pm 1.6$  mM in the dilated and hypertrophic

hamsters, respectively. The maximum levels, which peaked somewhat higher in the controls (not statistically significant), declined over the next 5–6 hours to concentrations that were still approximately 3 times higher than untreated baseline levels.

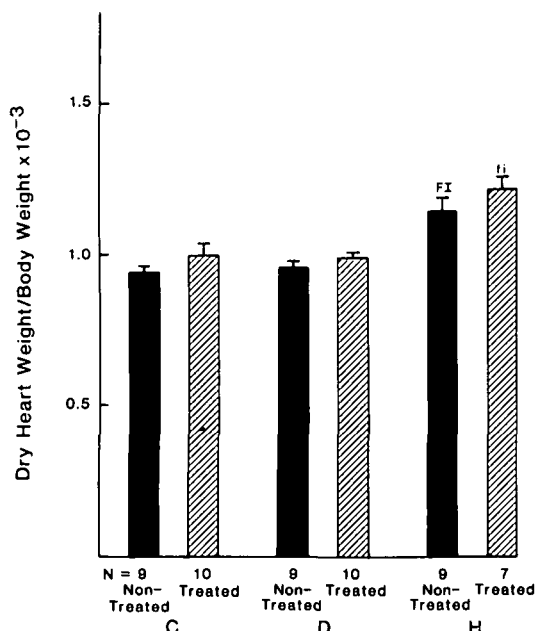


FIGURE 2. Dry heart weight to body weight ratios in L-carnitine- and sham-treated control and cardiomyopathic hamsters. Bars represent mean  $\pm$  SEM. Numbers under bars indicate number of hearts in each group (N). Solid bars represent sham-treated hamsters, and hatched bars represent L-carnitine-treated hamsters. C, control hamsters; D, dilated cardiomyopathic hamsters; H, hypertrophic cardiomyopathic hamsters. Notations of significant differences indicated above bars: capital letters indicate comparisons between sham-treated groups; small case letters indicate comparisons between L-carnitine-treated groups;  $^a p < 0.001$  for C vs. H; and  $^b p < 0.001$  for D vs. H.

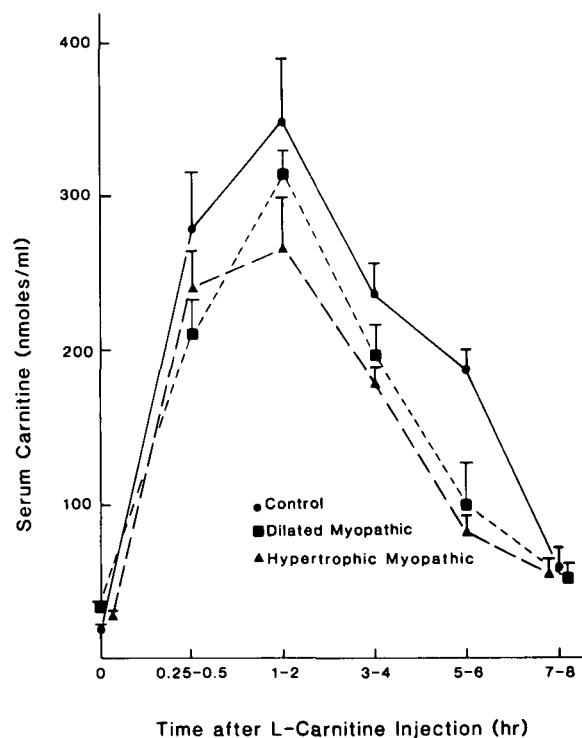


FIGURE 3. Time course of total serum carnitine levels after L-carnitine injection. Values are mean  $\pm$  SEM.  $\bullet$ , control hamsters;  $\blacksquare$ , dilated cardiomyopathic hamsters;  $\blacktriangle$ , hypertrophic cardiomyopathic hamsters. n, 9 serum samples for each group at zero time, and n, 4–5 for each of other time points within each group.

### Effects of L-Carnitine on Cardiac Perfusion Hemodynamics

During the constant work load imposed on the hearts by isolated working-heart perfusion, neither the coronary flows nor the cardiac outputs differed between carnitine-treated and sham-treated strains (data not shown). Peak LV systolic pressures in both sham-treated cardiomyopathic groups were significantly lower ( $p < 0.05$ ) than the control group (Figure 4A). Carnitine treatment resulted in restoration of the systolic pressures to control levels. Since neither of the

sham-treated cardiomyopathic groups manifested gross congestive heart failure at the time of killing and due to the maintenance of coronary perfusion pressure by the manner in which the afterload was constantly maintained, it was not surprising that there was only a small, but still significant, drop in developed LV systolic pressure below controls. Diastolic pressures (data not shown) were essentially identical in all 6 groups. Since all hearts were electrically paced at similar rates, the increased LV systolic pressure in the carnitine-treated myopathic groups significantly in-

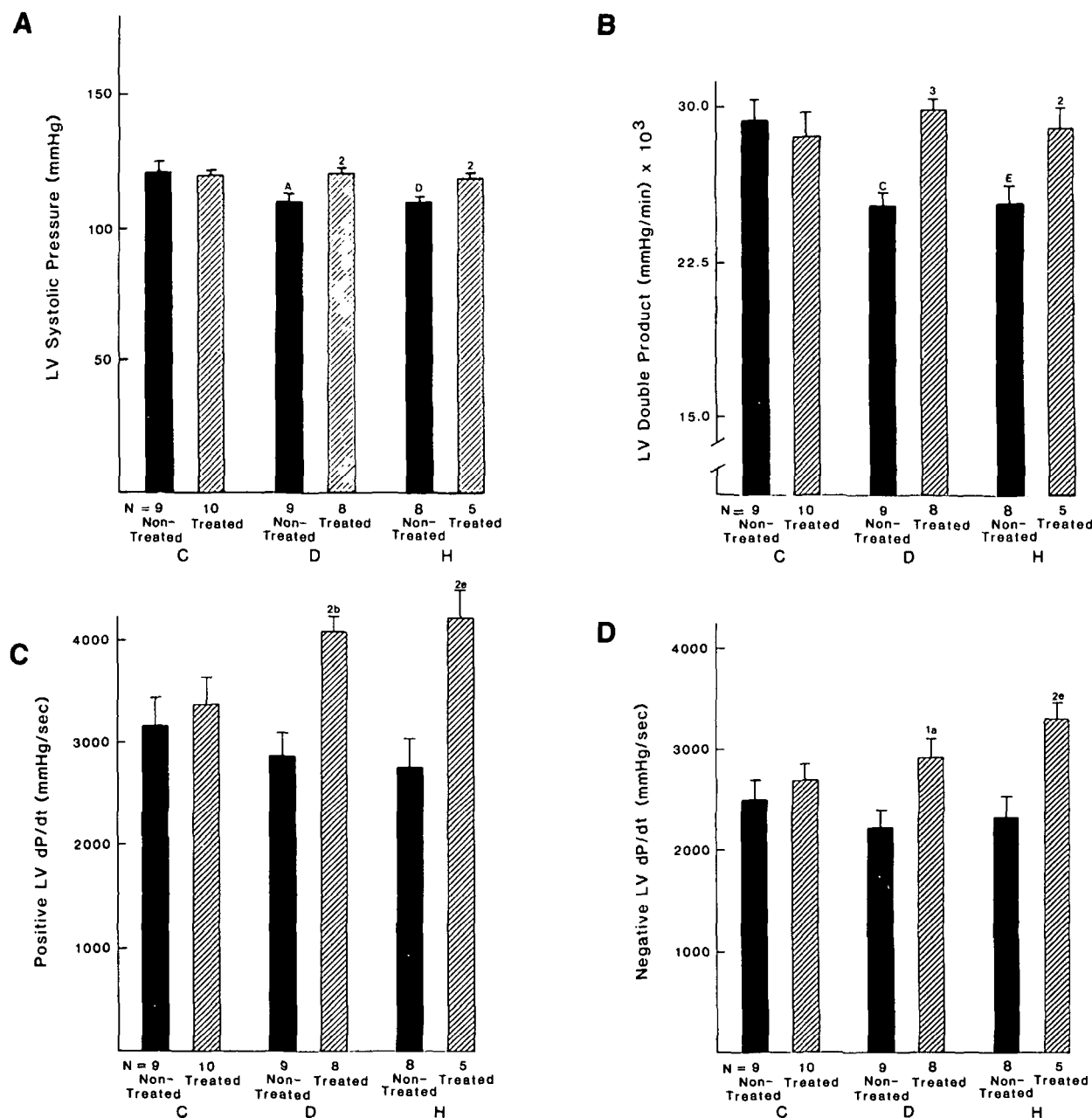


FIGURE 4. Histogram comparisons: A, left ventricular systolic pressures; B, double products of heart rate and LV systolic pressure; C, positive LV dP/dt; D, negative LV dP/dt in isolated working perfused hearts from L-carnitine- and sham-treated control and cardiomyopathic hamsters. Perfusate was Krebs-Henseleit bicarbonate buffer gassed with 95%  $O_2$ -5%  $CO_2$  containing 11 mM glucose and 1 mM palmitate. Histograms and abbreviations as described in Figure 2. Significance notations, <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$  for C vs. D; <sup>d</sup> $p < 0.05$ , <sup>e</sup> $p < 0.01$  for C vs. H; and <sup>1</sup> $p < 0.05$ , <sup>2</sup> $p < 0.01$ , <sup>3</sup> $p < 0.001$  for sham-treated vs. carnitine-treated within group.

creased the double product of heart rate and systolic pressure comparable to levels of the control groups (Figure 4B).

Similar effects of carnitine treatment occurred in the peak-positive LV dP/dt of both cardiomyopathic groups (Figure 4C). L-carnitine treatment resulted in marked increases of 32–53% higher positive dP/dt levels in the dilated and hypertrophic groups than their respective sham-treated groups. These increased levels of cardiac function were also significantly higher than controls. These findings were associated with significantly increased rates of relaxation, as indicated by increased peak-negative dP/dt levels, in the 2 treated cardiomyopathic groups (Figure 4D). In both cardiomyopathic groups, carnitine treatment thus resulted in significant enhancement of cardiac function to levels above those of controls.

#### *Myocardial Levels of Carnitine and Coenzyme A Derivatives*

In both control and dilated myopathic hearts, L-carnitine treatment resulted in an increase in free carnitine 2 times higher than the level in respective sham-treated groups (Figure 5A). Acetylcarnitine increased significantly with carnitine treatment in the control and myopathic hearts compared with respective sham-treated groups, but the degree of increase was less marked in the dilated hearts (Figure 5B). Both groups of myopathic hearts, however, still had much lower acetylcarnitine levels when compared with controls, whether carnitine- or sham-treated. Long chain acylcarnitine levels were not restored to control levels by treatment that caused no effect on absolute levels within either myopathic group (Figure 5C). These changes in tissue-free carnitine and derivative levels resulted in total carnitine levels that were greatly increased in control and dilated myopathic hearts but were not significantly changed in the hypertrophic hearts (Figure 5D). Thus, only carnitine levels in the dilated myopathic hearts were restored to normal levels by prolonged high-dose L-carnitine treatment.

In contrast, myocardial levels of CoA and its derivatives were not changed by carnitine treatment (data not shown). The levels of free CoA and its derivatives were not affected by the disease process, with control and myopathic levels being essentially the same.

#### *Myocardial Levels of Adenine Nucleotides and Creatine Phosphate*

High-energy phosphate stores in the hearts after 30 minutes of working perfusion from carnitine-treated and sham-treated hamsters are shown in Figure 6. Both sham-treated myopathic groups had significantly lower levels of creatine phosphate than respective control groups (Figure 6A). Creatine phosphate stores in the sham-treated dilated myopathic hearts were also significantly lower than hypertrophic heart levels. Adenosine triphosphate (ATP) levels in the sham-treated dilated hearts were significantly lower than both the control and hypertrophic hearts (Figure 6B). In hearts

from the carnitine-treated groups, creatine phosphate stores were increased 30–60% in the myopathic hearts, restoring the levels to those found in the controls. ATP stores in the dilated hearts were increased by 36% to levels comparable to control hearts. ATP levels of the hypertrophic hearts were unaffected by the disease or carnitine treatment. Any transient decrease of ATP stores in the sham-treated hypertrophic group was probably buffered by creatine phosphate, which did not significantly decrease with the disease, via the creatine phosphate shuttle mechanism.

Myocardial tissue levels of adenosine diphosphate (ADP), monophosphate (AMP), and free adenosine were significantly altered by carnitine treatment (Figure 7). After perfusion, all 3 treated groups had moderately to markedly increased levels of both ADP (Figure 7A) and AMP (Figure 7B). Adenosine levels were decreased, and the carnitine-treatment groups had much lower levels than the sham-treated control and both myopathic groups (Figure 7C). With carnitine treatment, these various changes in ATP and other adenine nucleotide derivatives occurred with maintenance of total adenosine stores within the myocardium of all animals. Thus, there were no significant differences between total adenosine stores in any of the groups. The carnitine-treated myopathic groups, however, did maintain these stores primarily in high-energy ATP and in more readily convertible forms, i.e., ADP and to a lesser extent AMP, with reciprocal decreases in adenosine. Creatine levels were not significantly altered by L-carnitine treatment (Figure 7D) within each group, but levels in both myopathic groups were somewhat lower than the controls. Since the creatine levels within each group were unaffected by treatment, total creatine stores approximated the changes in creatine phosphate levels.

#### *Liver, Kidney, and Diaphragm Levels of Total Carnitine*

Total carnitines in the control livers were increased twofold by treatment; however, both myopathic groups had insignificant increases with treatment (Figure 8A). Total liver carnitine in the sham-treated dilated cardiomyopathic hamsters was significantly increased above both control and hypertrophic groups, as has been previously reported.<sup>29</sup> Kidney carnitine levels were increased 2–3 times by carnitine treatment in both control and myopathic hamsters (Figure 8B). These increased kidney levels most likely reflected urinary excretion of high serum carnitine since this is the major route of excess carnitine elimination from the blood. Tissue sections of diaphragm, which were sampled to represent a form of skeletal muscle, had marked increases in total stores of carnitine in both carnitine-treated myopathic strains (Figure 8C). This occurred even though the diaphragm samples from sham-treated myopathic groups had carnitine levels comparable to controls that were unaffected by carnitine treatment.

#### **Discussion**

The present study was designed to evaluate the effects of high dose L-carnitine treatment on cardiac

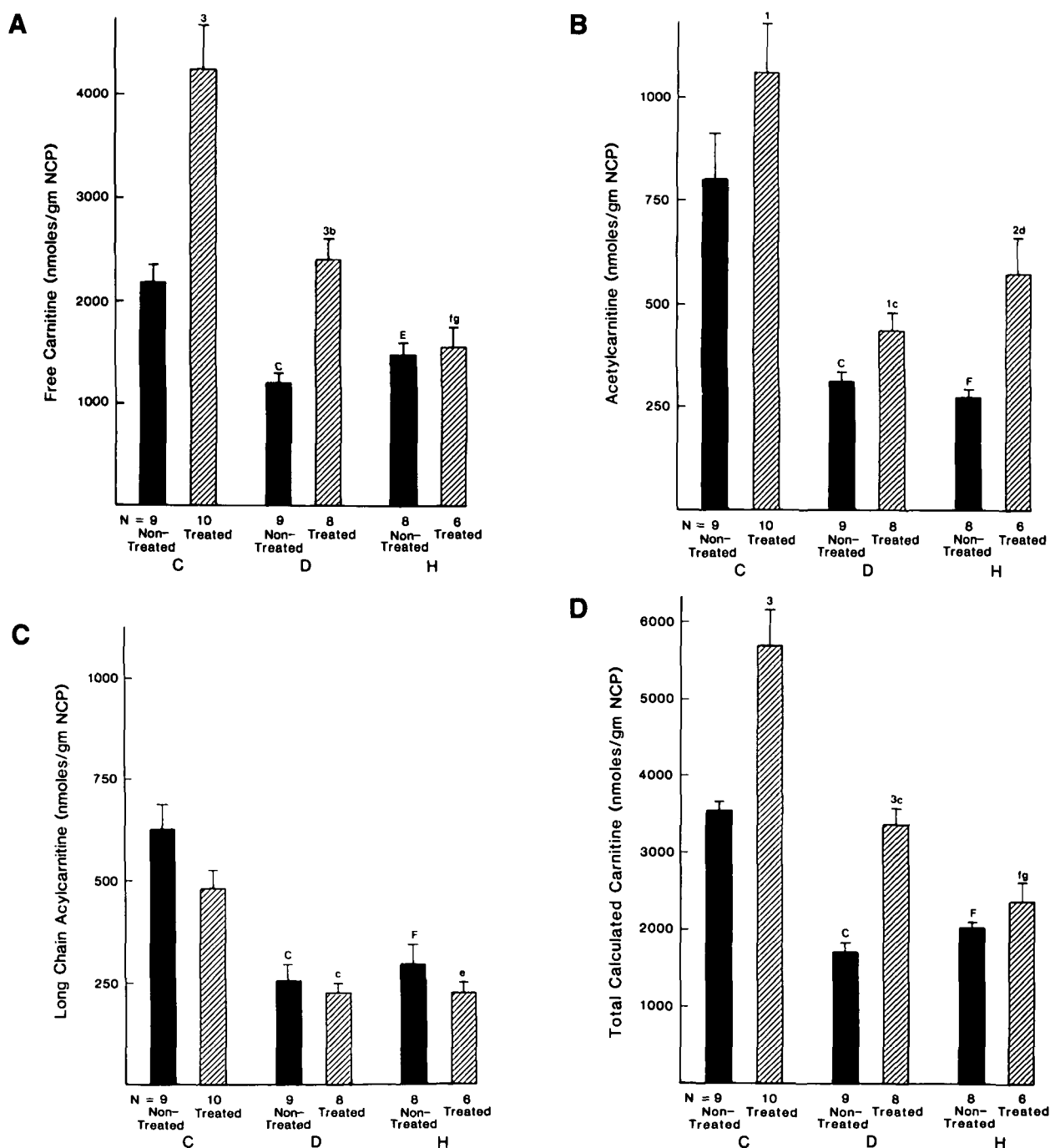


FIGURE 5. Histogram comparisons of tissue levels: A, free carnitine; B, acetylarnitine; C, long chain acylcarnitine; D, total calculated carnitine in hearts from L-carnitine- and sham-treated control and cardiomyopathic hamsters after 30 minutes of working perfusion. Conditions of perfusion were as described in Figure 4. Histograms and abbreviations as described in Figure 2 except NCP, Noncollagen protein. Significance notations, <sup>b</sup>p < 0.01, <sup>c</sup>p < 0.001 for C vs. D; <sup>E</sup>p < 0.01, <sup>F</sup>p < 0.001 for C vs. H; <sup>g</sup>p < 0.05 for D vs. H; and <sup>1</sup>p < 0.05, <sup>2</sup>p < 0.01, <sup>3</sup>p < 0.001 for sham-treated vs. carnitine-treated within group.

function in the hamster model of dilated and hypertrophic cardiomyopathy during isolated heart perfusion and to correlate any beneficial effects of the treatment with changes in myocardial energy stores and other metabolic intermediates. High-energy phosphate stores and hemodynamic parameters of cardiac function were used to assess myocardial functional integrity. The results of carnitine treatment in the myopathic hearts demonstrated 1) restoration of total work ca-

pacities compared with controls, as indicated by increased LV double products; 2) significantly increased cardiac contractility to levels significantly above those of control hearts, as indicated by significantly increased positive LV dP/dt; and 3) increased rates of ventricular relaxation, as indicated by the significantly increased negative LV dP/dt. These improved parameters of cardiac function were associated with restoration of myocardial creatine phosphate

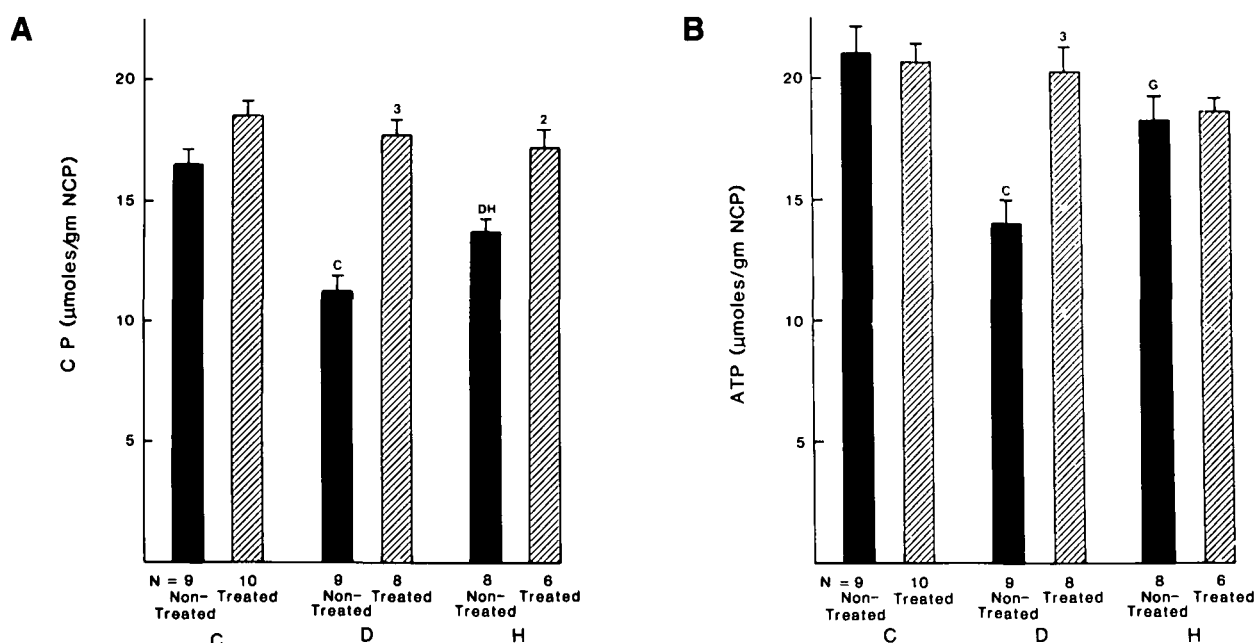


FIGURE 6. Histogram comparisons of tissue levels: A, creatine phosphate; and B, adenosine triphosphate in hearts from L-carnitine- and sham-treated control and cardiomyopathic hamsters after 30 minutes of working perfusion. Conditions of perfusion were as described in Figure 4. Histograms and abbreviations as in Figure 2. CP, creatine phosphate; ATP, adenosine triphosphate. Significance notations, <sup>c</sup>p < 0.001 for C vs. D; <sup>d</sup>p < 0.05 for C vs. H; <sup>e</sup>p < 0.05, <sup>f</sup>p < 0.01 for D vs. H; and <sup>g</sup>p < 0.01, <sup>h</sup>p < 0.001 for sham-treated vs. carnitine-treated within group.

levels in both myopathic groups and with restoration of ATP levels in the dilated group compared with controls. The total energy stores, i.e., ATP plus creatine phosphate, of the sham-treated hypertrophic myopathic hearts were less severely affected at the stage of disease studied. ATP levels in the hypertrophic hearts were probably maintained by an active creatine phosphate shuttle that is believed to exist in cardiac muscle and that allows the high level of free-energy change of ATP hydrolysis to occur.<sup>50</sup> Myocardial energy stores were maintained during the high perfusion work load despite increased oxygen consumption, which is implied by the increased LV double products in these carnitine-treated myopathic hearts.

In the past, carnitine has been used clinically in patients with systemic carnitine deficiency<sup>51–55</sup> and in children with hypertrophic<sup>56</sup> or idiopathic congestive cardiomyopathy.<sup>57</sup> In the experimental animal, myocardial tissue stores of L-carnitine significantly decrease with age.<sup>58</sup> Long-term administration of L-carnitine (105 days) to rats 27 months old resulted in restoration of myocardial carnitine stores to levels found in young animals.<sup>59</sup> Furthermore, the study demonstrated that carnitine treatment resulted in improved cardiac output and work during isolated heart perfusion in both the old (27 months) and young (6 months) rat hearts.

A 50–60% depletion of carnitine stores can adversely affect cardiac function.<sup>60</sup> Some pathologic conditions in which myocardial carnitine decreases to approximately this degree include pressure-overload hypertrophy<sup>61</sup> and diabetes.<sup>62,63</sup> L-carnitine treatment has demonstrated prevention of decreased carnitine

stores of streptozotocin-induced diabetic rat hearts. These hearts were then less vulnerable to ischemia, leading to improved function.<sup>63</sup> Liedtke et al<sup>64,65</sup> have shown protective effects of L-carnitine treatment against experimentally induced myocardial ischemia. These studies, with the knowledge that myocardial stores are reduced in cardiomyopathic hamsters,<sup>24–29</sup> led to the present carnitine-treatment study.

Studies demonstrating that myocardial carnitine stores increased in dilated cardiomyopathic and control hamsters after high-dose treatment but did not increase in hypertrophic hearts indicate that uptake and binding of carnitine is not similarly affected in dilated vs. hypertrophic cardiomyopathy. Yamashita et al<sup>28</sup> showed restoration of total myocardial carnitine stores to normal levels in the hypertrophic BIO 14.6 hamster strain after intraperitoneal injection of L-carnitine for 70 days. The major portion of this increase occurred in the free-carnitine fraction as in the present study. This latter study was a morphologic description demonstrating decreased fibrosis and necrosis after carnitine treatment. Unfortunately, no perfusion studies were performed to assess cardiac function. On the other hand, York et al,<sup>26</sup> who administered L-carnitine via the drinking water, were not able to restore myocardial carnitine stores to control levels in these hypertrophic (BIO 14.6) hamsters. They did, however, demonstrate a significant increase of carnitine in these treated myopathic hamsters compared with the untreated ones.

Results from the present study indicate that exogenous L-carnitine treatment has multifactorial effects that benefit cardiac function in such a way that it cannot simply be explained by improved oxidative metabo-



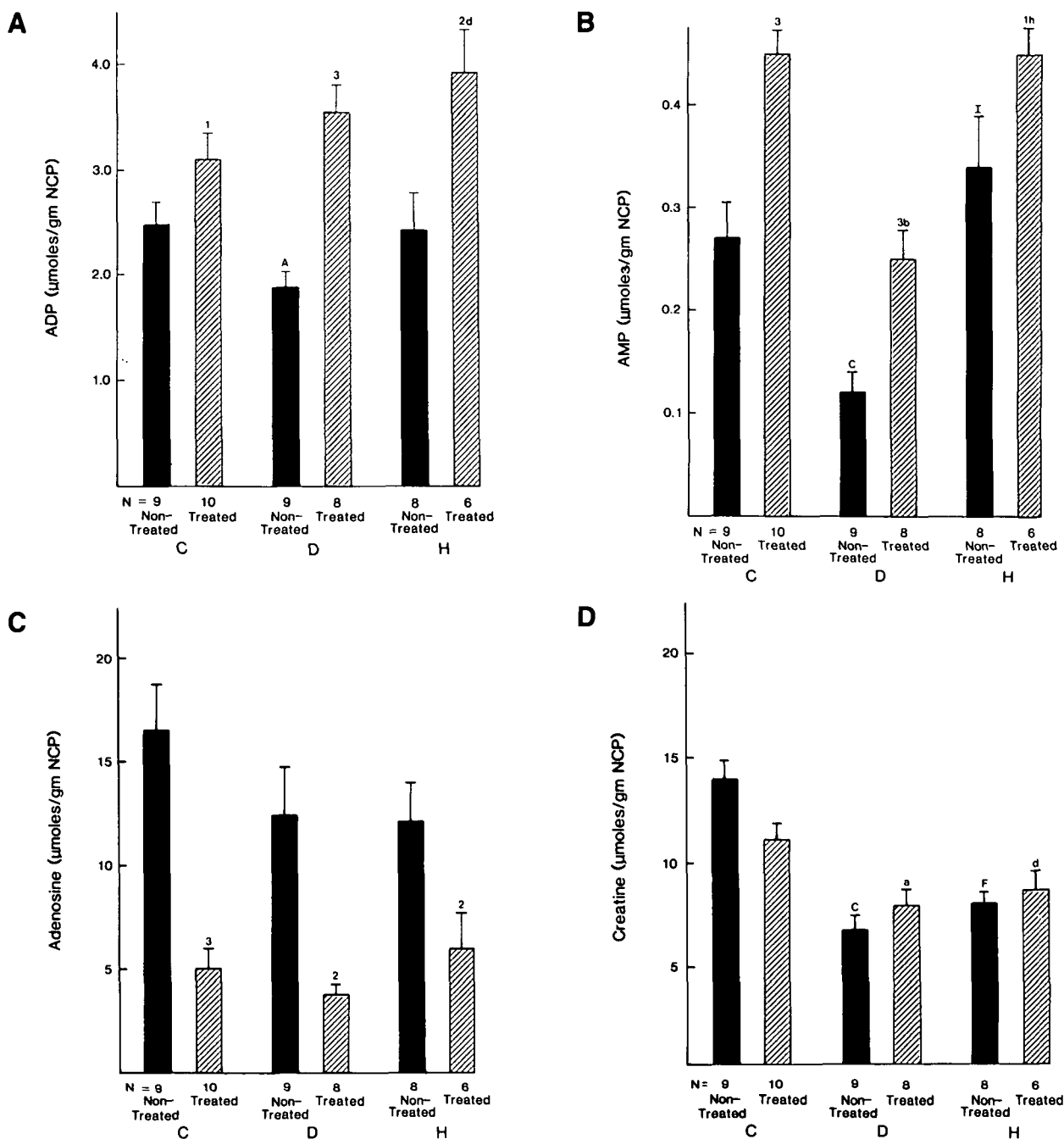


FIGURE 7. Histogram comparisons of tissue levels: A, adenosine diphosphate; B, adenosine monophosphate; C, free adenosine; D, free creatine in hearts from L-carnitine- and sham-treated control and cardiomyopathic hamsters after 30 minutes of working perfusion. Conditions of perfusion were as described in Figure 4. Histograms and abbreviations as in Figure 2. ADP, adenosine diphosphate; AMP, adenosine monophosphate. Significance notations, <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$  for C vs. D; <sup>d</sup> $p < 0.05$ , <sup>e</sup> $p < 0.001$  for C vs. H; <sup>f</sup> $p < 0.01$ , <sup>g</sup> $p < 0.001$  for D vs. H; and <sup>h</sup> $p < 0.05$ , <sup>i</sup> $p < 0.01$ , <sup>j</sup> $p < 0.001$  for sham-treated vs. carnitine-treated within group.

lism. This was most apparent in the marked improvement of cardiac contractility (positive  $dP/dt$ ) and rates of relaxation (negative  $dP/dt$ ) in both dilated and hypertrophic hearts after carnitine treatment. These functional changes occurred despite the fact that carnitine stores were unaffected by treatment in the hypertrophic hearts. In control hearts, these parameters of cardiac function were unchanged after carnitine treatment even though total carnitine stores were

markedly increased. Liedtke et al.<sup>65</sup> have suggested that acute L-carnitine administration beneficially affects cardiac function by decreasing use of fatty acids; this decrease leads to energy sparing processes noted in both aerobic and ischemic myocardium. Rodis et al.,<sup>66</sup> using a perfused rat heart system, demonstrated that carnitine in the perfusate depressed palmitate uptake and slightly decreased fatty acid oxidation in aerobic muscle. Thus, carnitine may improve use of other

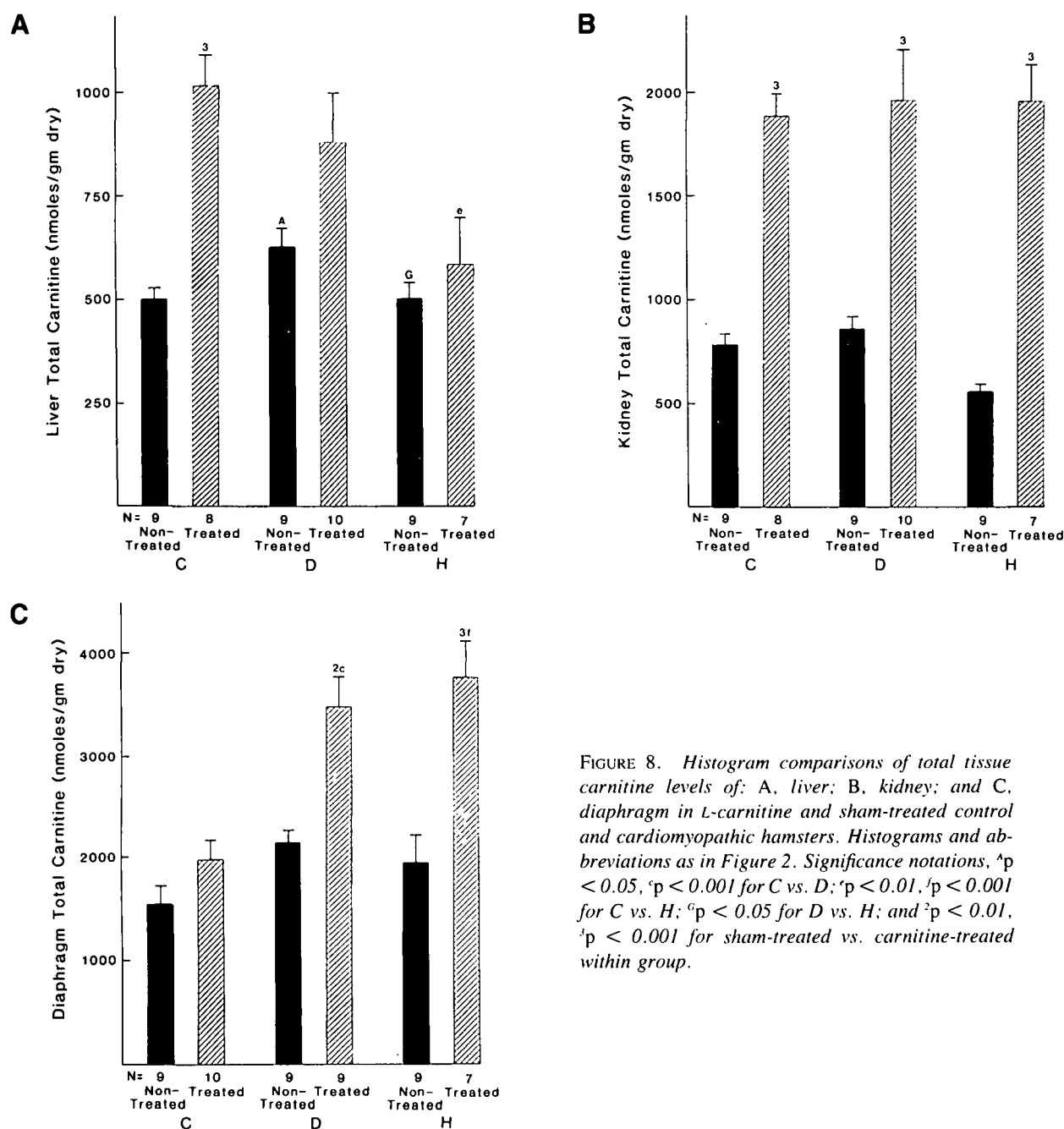


FIGURE 8. Histogram comparisons of total tissue carnitine levels of: A, liver; B, kidney; and C, diaphragm in L-carnitine and sham-treated control and cardiomyopathic hamsters. Histograms and abbreviations as in Figure 2. Significance notations, <sup>a</sup> $p < 0.05$ , <sup>c</sup> $p < 0.001$  for C vs. D; <sup>d</sup> $p < 0.01$ , <sup>e</sup> $p < 0.001$  for C vs. H; <sup>f</sup> $p < 0.05$  for D vs. H; and <sup>g</sup> $p < 0.01$ , <sup>h</sup> $p < 0.001$  for sham-treated vs. carnitine-treated within group.

substrates, which in turn helps maintain high-energy phosphate stores. Opie<sup>67</sup> has proposed that carnitine repletion in rat hearts may accelerate the rate of glycolysis by increasing the activity of pyruvate dehydrogenase, possibly by its effect on the mitochondrial acetyltransferase system. Carnitine, therefore, may be affecting energy metabolism by mechanisms opposite to what one would expect, i.e., by decreasing rather than increasing fatty acid use. In the present study, no substrate-oxidation rate studies were performed that could address this issue.

Another possible mechanism of exogenous carnitine effect may be its ability to increase coronary blood flow.<sup>68</sup> Chronic treatment with high-dose L-carnitine could have improved perfusion of the myopathic hearts

to the extent that oxygen and substrate delivery were not limiting during periods of stress. The myopathic hearts would have been maintained in a more viable condition by improved energy metabolism and in this way would have helped to slow progression of the disease. Increased serum carnitine levels may also have prevented coronary microvascular spasms that sometimes occur in the BIO 53.58 cardiomyopathic hamster.<sup>69</sup> In the present study, no differences were seen in coronary flow rates during the isolated-heart perfusions; however, L-carnitine was not included in the perfusate.

Total adenine nucleotide stores were maintained in 6–7-month-old untreated cardiomyopathic hamsters of both strains. This contrasts with the results of Wikman-

Coffelt et al,<sup>18,70</sup> who found significantly decreased nucleotide stores in 240-day-old hamsters of the UM-X7.1 strain, which is a derivative of the BIO 14.6 strain. They also found decreased ratios of both creatine phosphate or ATP and their respective breakdown products; however, in the present study, these ratios were not significantly different between sham-treated cardiomyopathic and control hearts. Furthermore, other studies by this same group<sup>16,18,70</sup> demonstrated as much as a 50% reduction in cardiac performance in the UM-X7.1 strain by 240 days of age. These discrepancies result most likely from a slowing of the cardiomyopathic disease process in the BIO 14.6 and BIO 53.58 strains of Syrian hamster with subsequently increased longevity in these animals. These changes have occurred after many years of inbreeding.<sup>71,72</sup> Both strains used in the present study now have a lifespan of over a year before fatal congestive heart failure develops. The BIO 14.6 strain lives on average 2–3 months longer than the BIO 53.58 strain (personal communication with Dr. Cornelius VanDongen of Bio-Breeders, Inc.). The UM-X7.1 strain, which was more recently developed, has a faster progression of the disease that results in a significantly shorter lifespan.<sup>72</sup> Thus, even though the animals in this study and the above-mentioned studies were of similar ages, the actual stage of disease studied was probably significantly less advanced in the present study. The energy stores and cardiac functional changes seen in the sham-treated BIO 53.58 and BIO 14.6 hamsters, therefore, would be expected to be of a lesser severity. Also, the work-load conditions of heart perfusion in this and the above studies were different. The perfusions of the present study were done in a manner that maintained constant work load, which was estimated by preliminary studies to be approximately 95% of maximal LV pressure for the sham-treated myopathic hearts. This work load was selected so that the myopathic hearts did not develop acute failure during the 30-minute working perfusion. Comparisons of changes in energy stores and/or cardiac contractility could then be attributed to inherent differences in the myocardium itself rather than to some perfusion-induced variable such as a decline in coronary perfusion pressure.

Interestingly, treatment with L-carnitine resulted in a shift of the adenine nucleotide stores toward more readily reconvertible forms, i.e., ADP and to a lesser extent AMP, with a reciprocal decrease in adenosine levels in both cardiomyopathic and control hearts. This redistribution of the nucleotide stores was also reflected in increased energy-charge ratios of (ATP)/(ADP + AMP + adenosine) in carnitine-treated vs. sham-treated groups (2.40 vs. 1.09 C, 2.64 vs. 1.02 D, and 1.77 vs. 1.03 H). This redistribution occurred with minimal changes in total adenine nucleotide stores in both carnitine-treated and sham-treated animals:  $29.1 \pm 1.0$  vs.  $35.3 \pm 2.7$   $\mu\text{mol/gmol}$  NCP in C;  $27.6 \pm 1.2$  vs.  $29.1 \pm 2.7$  in D; and  $25.7 \pm 1.8$  vs.  $34.7 \pm 2.5$  in H. All of these carnitine-treated hearts would seem to be more capable of maintaining and tolerating

increased work loads and prolonged stresses as suggested by the increased energy charge ratios. One could speculate that with significant increases in the acetyl-carnitine pools in all carnitine-treated groups, this treatment may have resulted in an improvement of mitochondrial nucleotide translocase effect as proposed by Shug et al<sup>73</sup> for ischemic hearts. Even though significant changes were not found in the total tissue acyl-CoA levels, since 95% of total myocardial CoA stores are intramitochondrial,<sup>74</sup> small cytosolic compartmental increases in long chain acyl-CoA derivatives of sham-treated hamsters could have had significant effects on adenine nucleotide translocation across the mitochondrial membrane. Since no such compartmental measurements were made in this study, these hypotheses remain purely speculative. The shifts in adenine nucleotide pools with significant improvements in cardiac contractility and relaxation for both models of cardiomyopathy, however, suggest that high-dose exogenous L-carnitine treatment resulted in effects that cannot solely be explained by changes in fatty acid oxidation and energy production.

Many of the degenerative changes associated with this cardiomyopathy begin in the first few days of life prior to weaning,<sup>39,72</sup> which was before any treatment had been started. Therefore, it was no surprise that L-carnitine did not improve growth patterns, did not affect NCP levels, and did not change the heart-weight to body-weight ratios in either the dilated or hypertrophic cardiomyopathic hamsters. On the other hand, neither control nor myopathic groups were adversely affected by the high dosage of L-carnitine that was administered twice daily to these animals. If anything, the carnitine-treated myopathic hamsters were subjectively more alert and active than their respective sham-treated groups.

As was demonstrated in this and the earlier study,<sup>29</sup> low myocardial carnitine levels in dilated myopathic hamsters were associated with significantly increased carnitine levels in the liver, where most endogenous carnitine is synthesized. It could be speculated that, through a feedback mechanism, synthesis of endogenous carnitine was increased in the myopathic hamsters, and this led to significantly increased serum carnitine levels with resultant increased supply of carnitine to the deficient myocardium. In the kidneys, massive increases in carnitine of all three treated groups reflected urinary excretion of high serum carnitine. Somewhat surprisingly, the diaphragm levels in both groups of myopathic hamsters also greatly increased with treatment, despite no significant differences between control and untreated myopathic groups. The latter groups did, however, have trends of slightly increased carnitine stores, probably reflecting endogenously increased serum carnitine levels. Thus, even though there was not a carnitine deficiency in this representative sample of skeletal muscle from the myopathic hamsters, the diaphragms responded to carnitine treatment by binding and storing larger quantities of carnitine than the controls. No studies were carried out to determine if this treatment affected

oxidative metabolism in the diaphragms from either type of myopathic hamster.

In summary, this is the first study to have demonstrated a beneficial effect from the administration of high-dose L-carnitine on LV myocardial contractility, rate of LV relaxation, and improved energy stores in animal models of two different distinct forms of cardiomyopathy, i.e., dilated and hypertrophic. These effects occurred with no resulting morbidity in the control or either group of cardiomyopathic hamster throughout the treatment period. Total myocardial carnitine levels were restored to normal in the L-carnitine-treated dilated myopathic hearts, but no increase of total stores was found in the hypertrophic hearts. L-carnitine treatment did, however, increase total high-energy phosphate stores, i.e., ATP plus creatine phosphate, in both cardiomyopathic groups. Results from this study suggest that exogenous L-carnitine treatment results in beneficial effects on the myopathic heart that cannot totally be explained by restoration of myocardial carnitine stores. L-carnitine may have improved use of other substrates such as glucose, resulting in increased energy metabolism and improved cardiac function in the different forms of cardiomyopathy. Elucidation of how L-carnitine exerts these beneficial effects is beyond the scope of this investigation and will be the subject of future studies.

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KEY WORDS • carnitine • cardiomyopathy • cardiac function • Syrian hamsters • ATP