Effect of a myocardial volume overload on lactate transport in skeletal muscle sarcolemmal vesicles

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Effect of a myocardial volume overload on lactate transport in skeletal muscle sarcolemmal vesicles. Am J Physiol Reg-ulatory Integrative Comp Physiol 281: R176–R186, 2001.—This study sought to determine the effect of a myocardial volume overload (MVO) on sarcolemmal (SL) lactate (La−) transport and the aerobic profile of skeletal muscle. SL vesicles were obtained from female rats 10 wk after either a MVO was induced by creation of an infrarenal fistula (n = 10), or sham surgeries were performed (n = 11). Influx of 14C-labeled L(+)−La− was measured at various unlabeled La− concentrations under zero-trans conditions. La− transport kinetics were determined using a Michaelis-Menten equation with an added linear component to discriminate between carrier-mediated and diffusional transport. Although heart and lung weights were significantly increased (P < 0.0001) in the MVO group, left ventricular function was only modestly altered (P < 0.05). A significant reduction in type I myosin heavy chain (MHC) in the soleus and a strong trend (P = 0.06) for a reduced type IIx MHC in the plantaris were observed in MVO rats, but no differences in citrate synthase activity or monocarboxylate transporter proteins (MCT)-1 expression were noted in any muscle. Carrier-mediated La− influx into SL vesicles was similar between sham and MVO (Km = 12 ± 1 and 18 ± 3 mM; apparent Vmax = 772 ± 99 and 827 ± 80 nmol·mg−1·min−1), respectively. Total influx at 100 mM was lower in MVO, and this was due to a 30% reduction in membrane diffusion. In conclusion, a 10-wk MVO did not alter MCT-mediated La− transport or protein expression but was associated with modest changes in myofibrillar proteins and impaired SL diffusive properties.

congestive heart failure; monocarboxylate transporter proteins; monocarboxylate; membrane diffusion

CONGESTIVE HEART FAILURE (CHF) may be characterized by a low functional capacity that is due to a rapid onset of exertional fatigue. It is now well known that changes in skeletal muscle structure, function, and metabolism are concomitant with the progression of CHF. Some of these changes include reduced concentrations of ATP and phosphocreatine (4), metabolic abnormalities (10, 22), a reduced activity of mitochondrial enzymes, regardless of muscle fiber composition (12), and a general transition of fiber types toward those that possess a “glycolytic” profile (37). Accordingly, some investigators have reported that experimental and human forms of CHF are associated with a greater reliance on glycolytic metabolism (10) and a more profound development of acidosis (H+ accumulation) at a given work rate (23). These observations clearly demonstrate that the progression of CHF is associated with changes in the oxidative profile of skeletal muscle that may contribute to exertional intolerance.

In agreement with a diminished aerobic capacity, Weber and Janicki (43) reported that the exertional threshold required to reach a “lactate threshold” in a group of CHF patients was inversely related to the severity of the disease. They attributed this observation to a greater rate of lactic acid (HLa) production due to insufficient peripheral oxygen delivery in CHF patients. The large majority of HLa formed during activity dissociates to a lactate (La−) anion and H+ within the physiological pH range. Because accumulation of these metabolites in the myoplasm has been shown to impair function of the sarcoplasmic reticulum (16), key metabolic enzymes (40), Ca2+ sensitivity of myofilaments (15), and the rates of force production and relaxation (1), an elevated La− concentration ([La−]; and associated H+ concentration ([H+]]) in muscle and blood during activity may explain some of the functional impairment associated with CHF.

The role of skeletal muscle in systemic La− dynamics is the cornerstone of the “lactate shuttle hypothesis” (7). Briefly, this hypothesis holds that La− production and distribution throughout the body are a major mechanism by which intermediary metabolism is coordinated in different tissues and cells within those tissues. With respect to skeletal muscle, it is now known that substantial La− exchange occurs between muscle and blood, between active and inactive muscles, between active muscles and fibers within muscles (7),

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and even between myocellular compartments as part of an intracellular La$^-$ shuttle (8). In fact, skeletal muscle represents the greatest source of La$^-$ production and consumption in the body due to its relative mass. Therefore, whole body La$^-$ dynamics are ultimately regulated in, and by, skeletal muscle (32).

Movement of La$^-$ and H$^+$ across the sarcolemma (SL) of striated muscle occurs primarily via two isoforms of SL-bound monocarboxylate transporter proteins (MCT1 and MCT4). La$^-$ transport (19) and uptake (3, 28), as well as MCT expression (3, 31), have been shown to be strongly related to the oxidative capacity of skeletal muscle. Furthermore, La$^-$ transport and expression of MCT proteins in skeletal muscle have also been shown to be increased by contractile activity and training (25, 26, 29), whereas La$^-$ transport is decreased by hypodynamia (14).

Given the close dynamic relationship between La$^-$ and H$^+$ transport and the oxidative properties of skeletal muscle and that oxidative properties are altered during the development of CHF, it is plausible that SL La$^-$ transport may be influenced as well. In the context of the “lactate shuttle” (7), the acidoses observed in skeletal muscle of individuals with CHF during activity may not only be explained by an accelerated HLa production, but a reduced capacity for La$^-$ extrusion from active muscle fibers due to alterations in SL transport properties. Similarly, blood [La$^-$] may also remain elevated for longer periods of time because of impaired uptake into consuming tissues (e.g., active and inactive skeletal muscle) due to a reduced capacity for La$^-$ oxidation and SL transport. Combined, these effects would result in a rapid, exaggerated development of systemic lactacidosis during activity and a prolonged recovery of acid-base status on cessation. This could partly explain the low exertional threshold observed in individuals afflicted with CHF.

The purpose of this investigation was to examine the effect of a myocardial volume overload (MVO), which is a commonly used model to study the progression of CHF, on skeletal muscle La$^-$ transport characteristics. Purified SL vesicles were chosen for this end in an attempt to circumvent confounding variables such as blood flow, multiple membrane barriers, and cellular metabolism and to control the environments on both sides of the membrane. It was our hypothesis that a chronic (10 wk) MVO would have deleterious effects on La$^-$ transport in vesicles isolated from mixed rat skeletal muscle. Furthermore, we hypothesized that this would be associated with changes in metabolic and myofibrillar properties of various hindlimb muscles.

**MATERIALS AND METHODS**

**Animals, Housing, and Care**

All procedures in the current investigation involving animals were reviewed and approved by the Auburn University Institutional Animal Care and Use Committee. Twenty-one female Sprague-Dawley rats were obtained at 3 mo of age and were randomly assigned to either an MVO (n = 10) or sham-operated (n = 11) group. All animals were housed in pairs (1 MVO + 1 sham-operated per cage), maintained on a 12:12-h light-dark cycle (6:00 AM to 6:00 PM light), and received food and water ad libitum.

**Induction of MVO**

A MVO was surgically induced in rats by creation of an aortocaval fistula as described by Garcia and Diebold (17). Briefly, animals were anesthetized with a mixture of ketamine and xylazine (60 and 7 mg/kg, respectively), and an abdominal laparotomy was performed to expose the inferior vena cava and descending aorta. Circulation was impeded via light finger pressure, and a beveled 18-gauge needle was used to create a fistula between both vessels just below the renal arteries. The resulting puncture was sealed with a few drops of cyanoacrylate, and patency of the fistula was confirmed by visualization of pulsatile flow of oxygenated blood into the vena cava. The abdomen was flushed with saline, and the musculature and skin were closed with sutures and autoclips. This technique greatly increases venous return and results in a progressive, biventricular eccentric hypertrophy of the myocardium (9). Sham surgeries, consisting of an abdominal laparotomy without creation of the fistula, were also performed on the remaining rats. All animals were kept in a warm, dry room after surgery until they were ambulatory and showed no signs of discomfort. Rats were killed 10 wk postsurgery for experimental procedures.

**Activity Monitoring**

To ascertain whether skeletal muscle properties may have been influenced by changes in activity levels, gross locomotor activity was monitored during the middle (week 5) and final (week 10) weeks of the experimental period after surgery. This was achieved by placing the rats on an enclosed activity wheel that was interfaced with a revolution counter. Activity was monitored during two consecutive 12-h dark cycles of each week, and data were recorded as a 2-day average number of revolutions per 12-h cycle.

**Myocardial Function Measurements**

All animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). A tracheotomy was performed, and the rats were placed on mechanical ventilation (40 breaths/min) for the remainder of the surgical procedure and functional measurements. The thoracic cavity was exposed, and an ultrasonic flow probe (Transonic T-101) was placed around the ascending aorta for measurement of cardiac output (Q). A 16-gauge beveled needle was passed through the apex of the heart, and a 3-Fr microtip pressure transducer (Millar SPR-249A) was advanced into the left ventricle (LV) for measurement of end-diastolic (LVEDP) and ventricular pressures throughout the cardiac cycle. The analog signals from the flow probe and pressure transducer were digitized and interfaced with a chart recorder and computer for calculation of the derivative of LV pressures (±dp/dt).

Both hindlimbs were quickly skinned after functional measurements, and the musculature was removed. The soleus, plantaris, and extensor digitorum longus (EDL) were bilaterally excised and snap-frozen in liquid N2 for subsequent biochemical and morphological analyses. The remaining hindlimb muscle groups were removed for preparation of SL vesicles.
Citrate synthase. Frozen muscle samples were thawed, trimmed free of connective tissue, weighed, and minced in a 20:1 volume of a 100 mM phosphate buffer with 0.05% bovine serum albumin, pH 7.4 at 25°C. The minced muscle was homogenized in a glass–glass tissue grinder, brought to a final dilution of 101:1, and spun at 400 g for 10 min at 4°C. The supernatant was removed and stored at −80°C until used for protein (6) and enzymatic analysis.

Citrate synthase (CS) activity of supernatants was determined spectrophotometrically according to the method of Srere (38), with modifications. Briefly, background deacylase activity was determined by incubation of 10 μl of muscle homogenate in a medium consisting of 100 μM 5,5′-dithiobis(2-nitrobenzoic acid), 98.3 μM acetyl CoA, and 0.07% Triton X-100, pH 7.4 at 25°C. The change in absorbance at 412 nm was followed for 3 min, at which time the CS reaction was initiated by addition of oxaloacetate (final concentration = 504 μM). The reaction was followed for an additional 3 min. The difference between total and background activities represented the CS activity (expressed as μmol-100 mg protein−1·min−1).

Separation of myosin heavy chains. Remaining soleus, plantaris, and EDL samples were homogenized with a glass–glass tissue grinder in a 10:1 volume of buffer consisting of (in mM) 250 sucrose, 100 KCl, 5 EDTA, 20 Tris, pH 6.8. Protein concentrations of homogenates were determined by the method of Bradford (6) using bovine serum albumin as a standard. Electrophoretic separation of myosin heavy chains (MHC) was performed according to the detailed methodology of Talmadge and Roy (41). Homogenates were diluted in 2× sample buffer (21) to a final protein concentration of 0.125 mg/ml, boiled for 2 min, and 1.25–1.50 μg of protein was loaded into each well of an 8% SDS-PAGE gel. The electrophoresis system (BioRad Mini Protein II) was run at a constant 80 V for 24 h at 4°C. Gels were stained in a solution containing 40% methanol, 10% acetic acid, 0.1% Coomassie brilliant blue R-250, and destained in 40% methanol and 10% acetic acid. The resulting bands were quantified with a scanning densitometer (Alpha Innotech IS-2000), and the proportion of each MHC isoform was taken from the relative area under each peak.

Western immunoblotting of MCT1. A portion of each MHC homogenate was spun at 600 g (4°C) to pellet erythrocytes, and the resulting supernatants were removed for determination of MCT1 expression. Fifty micrograms of supernatant proteins were separated on 12% SDS-PAGE gels and transferred to nitrocellulose membranes (60 min at 100 V). Fifty micrograms of supernatants prepared from ventricular myocardium were also run on each gel as a standard. Membranes were first blocked for 4 h in Tris-buffered saline with 0.05% Tween-20 and 5% nonfat milk, incubated overnight on a rocker (4°C) with a chicken α-MCT1 polyclonal antibody (Chemicon, 1:2,500), then for 1 h at room temperature with a horseradish peroxidase-conjugated α-chicken secondary antibody (1:2,500). MCT1 bands were detected via enhanced chemiluminescence and quantitated using computerized densitometry.

Preparation of Purified SL Vesicles

Skeletal muscle SL vesicles were purified by the method of Grimditch et al. (18), with modifications. Twelve to fifteen grams of skeletal muscle (gastrocnemius, quadriceps, and gluteus maximus groups) were quickly excised from rat hindlimbs and placed into an ice-cold homogenizing medium (HM) consisting of 250 mM sucrose and 20 mM HEPES, pH 7.4 at 4°C. The tissue was trimmed free of visible connective tissue, fat, nerve, and blood vessels, minced with blunt scissors, and subjected to mechanical homogenization at 80% of maximal power (Fisher Powergen 700). The sample was transferred to a Teflon-glass tissue grinder for 10 passes with the pestle under a mechanical stirrer (Cafamo RZR1) at 50–75% of maximal power. The sample was brought to a volume of 40 ml with HM, transferred to ultracentrifuge tubes, and termed crude homogenate (CH). A 200-μl sample of CH was removed, diluted 1:2 with HM, snap-frozen in liquid N2, and stored at −80°C until determination of protein concentration (6) and SL marker enzyme analysis. A volume of KCl medium (3 M KCl, 250 mM Na-pyrophosphate) equal to 10% of the final CH volume was added and vigorously mixed to salt out contractile proteins.

The CH was spun in a Beckman L8–70 ultracentrifuge with a 60-Ti fixed-angle rotor at 222,000 g for 90 min (4°C) in a vacuum. The supernatant from this first spin was discarded, and the inner walls of the ultracentrifuge tubes were washed. A sample of any remaining pellets was subjected to mechanical homogenization and isopycnic centrifugation

Graduates were subjected to isopycnic centrifugation in a Beckman SW-27 swinging-bucket rotor at 68,000 g for 16 h, and SL vesicles were harvested from the 27 and 30% layers. The membranes were diluted with Krebs-Ringer-HEPES buffer (KRH; in mM: 135 NaCl, 5 KCl, 1.2 MgSO4, and 50 HEPES, pH 7.4 at 25°C) and pelleted in a fixed-angle rotor (No. 17.5 Ti, 50,000 rpm, 1.5 h at 4°C). The resulting purified vesicles were suspended in KM and pelleted in a fixed-angle rotor for 90 min at 222,000 g. The resulting purified vesicles were suspended in KRH buffer to a protein concentration of 1–1.5 mg/ml. A 50-μl aliquot was partitioned for subsequent determination of protein concentration (6) and SL marker enzyme analysis. After protein analysis and final suspension, vesicles were snap-frozen in liquid N2 and stored at −80°C until used for transport experiments.

Purity of the vesicle preparation vs. CH was determined by measuring the activity of the SL marker enzyme, K+-stimulated p-nitrophophatase (KpNPPase). KpNPPase is a domain of the Na”+–K”+ adenosine triphosphatase that is located on the extracellular surface of an intact SL and is regarded as a good indicator of vesicle purity (14, 18). Activity was assayed in a medium consisting of (in mM) 40 HEPES, 4 MgCl2, 0.8 EGTA, and 20 KCl, pH 7.4, 37°C. Basic phosphatase activity was also assayed in the same medium but without KCl. The reaction was initiated by addition of p-nitrophophosphate as a substrate (final concentration = 5 mM) and stopped with 1 ml of 1 N NaOH. The difference between the K”+-stimulated and basic phosphatase activity (expressed as μmol p-nitrophophol form·mg protein−1·h−1) represents the KpNPPase. A purification index, defined as the ratio of SL-to CH-specific activities, was used to estimate quality of the final preparation. SL yield was expressed as milligrams of SL protein obtained per gram of trimmed starting tissue.

Preparation of Purified SL Vesicles
La− Transport Assays

La(+)−La− transport in SL vesicles was studied under zero-trans conditions at 25°C, as previously described (33, 34). Sixty microliters of an isosmotic KRH solution containing various concentrations (2, 5, 10, 20, and 100 mM, pH 7.4 at 25°C) of unlabeled L(+)−La− and 0.5 μCi of uniformly labeled 14C-labeled L(+)−La− tracer were placed on the bottom of a 1.5-ml Eppendorf tube. The concentration of NaCl was decreased accordingly with the addition of L(+)−La− to maintain isosmolarity of the intra- and extravesicular milieu. Twenty microliters of vesicle suspension were carefully pipetted onto the side of the tube. Transport was initiated by vortexing the tube and halted at various times (2, 3, 4, 5, 10, 15, and 20 s) by addition of 1 ml of ice-cold KRH containing 3 mM HgCl2. A metronome and stopwatch were used for time measurement during all transport experiments. In an attempt to validate the mathematical determination of the linear diffusive component of influx, assays were also performed at 20 mM extravesicular [La−] on SL vesicles preincubated for 1 h at room temperature with 1 mM p-chloromercuri phenylsulfonic acid (pCMBS), a sulfhydryl group reagent. Background binding in vesicles was assessed by incubating vesicles in the various tracer-containing media that were diluted with ice-cold KRH/HgCl2 stop solution. The radioactivity from these background experiments was subtracted from that obtained in each influx assay. The stopped, diluted vesicles were immediately vacuum-filtered through a protein-binding cellulose-ester membrane (Millipore, 0.45-μm average pore size) and washed twice with 3 ml of KRH/HgCl2 stop solution. The vesicle-containing filters and standards were placed in vials with 5 ml Scintiverse BD (Fisher Scientific) cocktail, and radioactivity was counted in a Wallac 1515 Winspectral liquid-scintillation counter.

Specific influx of La− (defined as nmol/mg SL protein) during the 0- to 20-s time interval was fitted to a monoeponential equation of the type

\[ y_t = C - Ae^{-kt}, \]

where \( y_t \) is La− influx at time \( t \), \( C \) is the asymptote of influx with increasing time, \( A \) is a constant, \( e \) is the base of natural logarithms, and \( k \) is the rate constant for the approach of La− influx toward the asymptote. Initial rates of influx were determined as the solution to the first derivative of the exponential equation at time \( 0 \) (C−k). These initial rates from total influx trials were then plotted as a function of extravesicular [La−] and applied to a Michaelis-Menten plus linear equation

\[ V = (V_{max} - S)S + K_m + (S)M; \]

where \( V \) is the rate of influx, \( V_{max} \) is the maximal rate of carrier-mediated influx, \( S \) is the extravesicular [La−], \( K_m \) is the Michaelis-Menten affinity constant, and \( M \) is the linear slope to determine the relative contributions of carrier-mediated transport and passive diffusion at each [S]. Specific diffusion into SL vesicles was analyzed by comparing both the rates of transport at each individual [La−] and the Ms for each animal. The rates of carrier-mediated transport were used for determination of maximal velocity of transport (apparent \( V_{max} \)) and transporter-substrate affinity (\( K_m \)). All kinetic analyses were performed using the Kaleidagraph 3.09 for Windows software package (Synergy software).

Statistical Analysis

Differences between sham-operated and MVO rats were analyzed with an independent Student’s t-test. However, activity measurements and La− influx rates in saturation experiments were analyzed with a two-way repeated-measures ANOVA and a Student-Newman-Keuls post hoc test. A significance level of 0.05 was selected for all analyses in this investigation.

RESULTS

Morphological Parameters

Mean body and organ weight data from sham-operated and MVO rats are provided in Table 1. Although initial body weights did not differ between groups, MVO rats demonstrated a 67% greater increase in body weight during the 10 wk after surgery (P < 0.01). Mean heart and lung weights were also significantly higher in the MVO group, even when expressed relative to body weight (P < 0.0001 for all comparisons).

Hemodynamic Measurements

Hemodynamic measurements in situ are presented in Table 2. As expected, creation of an arteriovenous fistula resulted in a 2.5-fold increase in total Q after 10 wk (P < 0.0001). This, taken together with the increase in heart weight, indicates that the fistula surgery successfully induced a chronic MVO and cardiac hypertrophy. LVEDPs were significantly greater in MVO rats (P < 0.05), although these values (mean = 6.4 mmHg) were not outside normal physiological ranges. Additionally, although the rates of LV pressure changes (±dP/dt) tended to be lower in the MVO group, the differences in means were not statistically significant (P > 0.05). Thus it appears that the MVO rats in this investigation were still in a state of myocardial compensation at the time of death.

Activity Measurements

Gross locomotor activity levels, reported as the average number of activity wheel revolutions achieved during the 10 wk after surgery, were not outside normal physiological ranges. Additionally, although the rates of LV pressure changes (±dP/dt) tended to be lower in the MVO group, the differences in means were not statistically significant (P > 0.05). Thus it appears that the MVO rats in this investigation were still in a state of myocardial compensation at the time of death.

Table 1. Body and organ data from sham-operated and MVO rats

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (11)</td>
</tr>
<tr>
<td>Initial body wt, g</td>
<td>252 ± 4</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>285 ± 6</td>
</tr>
<tr>
<td>%Gain</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>1,000 ± 29</td>
</tr>
<tr>
<td>Heart-to-body wt ratio, mg/g</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>Lung wt, mg</td>
<td>1,171 ± 44</td>
</tr>
<tr>
<td>Lung-to-body wt ratio, mg/g</td>
<td>4.1 ± 0.1</td>
</tr>
</tbody>
</table>

All values are reported as means ± SE. MVO, myocardial volume overload. *P < 0.01 vs. Sham, †P < 0.0001 vs. Sham.

Table 2. Hemodynamic measurements in situ of sham-operated and MVO rats 10 wk after surgery

<table>
<thead>
<tr>
<th>Measurement n</th>
<th>Sham (7)</th>
<th>MVO (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q, ml/min</td>
<td>94.4 ± 10.9</td>
<td>240.6 ± 21.4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>−1.9 ± 1.1</td>
<td>6.4 ± 1.4*</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>3,458 ± 602</td>
<td>2,905 ± 272</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>2,159 ± 261</td>
<td>1,850 ± 174</td>
</tr>
</tbody>
</table>

Values are reported as means ± SE; n, no. of animals measured. Q, cardiac output; LVEDP, left ventricular end-diastolic pressure; ±dP/dt, rate of change in left ventricular pressure. *P < 0.05 vs. Sham, †P < 0.0001 vs. Sham.
significant increases in both IIa and IIb isoforms. This effect was associated with minute, nonsignificant deviations from the heart value, no significant differences were observed in the other muscles examined, there were additional significant differences in MHC expression between groups in any of the muscles studied (P > 0.05).

Skeletal Muscle Aerobic Profile

CS. Specific activities of the mitochondrial enzyme CS (μmol citrate formed·100 mg protein⁻¹·min⁻¹) are reported for soleus, plantaris, and EDL supernatants in Table 4. No significant differences in CS activities were observed between groups in any of the muscles supernatants assayed (P > 0.05 for all comparisons).

MHC analysis. A scanned image of a representative polyacrylamide gel is provided in Fig. 1, and the proportions of MHC isoforms I, IIa, IIx, and IIb in various hindlimb muscles are presented in Table 5. Soleus samples from MVO rats demonstrated a significantly lower (−5%) proportion of type I MHC with a concomitant increase in type IIa MHC (P < 0.05). Although no additional significant differences in MHC expression were observed in the other muscles examined, there was a strong trend for a lower percentage of type IIx isoforms in plantaris samples from MVO rats (P = 0.06). This effect was associated with minute, nonsignificant increases in both IIa and IIb isoforms.

MCT1 expression. Results of the Western immunoblot analysis of MCT1 content of soleus, plantaris, and EDL samples are shown in Fig. 2. When MCT1 expression levels were normalized as a percentage of the heart value, no significant differences were observed between the trials at the 5th and 10th wk (P > 0.05 for trial and group by trial interaction).

Table 3. Gross locomotor activity of sham-operated and MVO rats during the 10-wk experimental period

<table>
<thead>
<tr>
<th>Measurement period</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (11)</td>
</tr>
<tr>
<td>Middle (wk 5)</td>
<td>997 ± 150</td>
</tr>
<tr>
<td>Final (wk 10)</td>
<td>1,131 ± 93</td>
</tr>
</tbody>
</table>

Values are reported as means ± SE; n, no. of animals observed. All values reported as means ± SE; n, no. of animals observed.

Fig. 1. Myosin heavy chain (MHC) isoforms I, IIa, IIx, and IIb of various hindlimb muscles were separated by SDS-PAGE. Diaphragm standards (DIA) expressing all four MHC isoforms were used as internal standards on each gel. Soleus muscles from myocardial volume overload (MVO) rats demonstrated a significantly lower percentage of type I MHC compared with those from sham-operated rats (P < 0.05).

Table 4. Citrate synthase activities of soleus, plantaris, and EDL muscles from sham-operated and MVO rats

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Group n</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham (10)</td>
</tr>
<tr>
<td>Soleus</td>
<td>31.3 ± 2.2</td>
</tr>
<tr>
<td>Plantaris</td>
<td>22.0 ± 1.8</td>
</tr>
<tr>
<td>EDL</td>
<td>17.7 ± 1.5</td>
</tr>
</tbody>
</table>

Values are reported as means ± SE; n, no. of muscles examined. Citrate synthase activity expressed as micromoles citrate formed per 100 mg of assay protein/min. EDL, extensor digitorum longus.

Table 5. Relative percent of MHC isoforms in soleus, EDL, and plantaris muscles from sham-operated and MVO rats

<table>
<thead>
<tr>
<th>Muscle</th>
<th>MHC Isoform, %</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Soleus</td>
<td>Sham</td>
</tr>
<tr>
<td></td>
<td>MVO</td>
</tr>
<tr>
<td>Plantaris</td>
<td>Sham</td>
</tr>
<tr>
<td></td>
<td>MVO</td>
</tr>
<tr>
<td>EDL</td>
<td>Sham</td>
</tr>
<tr>
<td></td>
<td>MVO</td>
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</table>

Values are reported as means ± SE; n = 10 for both groups. *P < 0.05 vs. Sham, †P = 0.06 vs. Sham. MHC, myosin heavy chain.
ensuing curves increased in a manner accordant with extravesicular \([\text{La}^-]\). These curves were fitted by monoeXponential equations \((r^2 = 0.93–0.99)\) for determination of initial rates of influx at various \([\text{La}^-]\).

Initial rates of influx, expressed as nanomoles of \([\text{La}^-]\) transported per milligram of SL protein per minute, are plotted as a function of extravesicular \([\text{La}^-]\) in Fig. 5. Total influx rates (carrier-mediated + diffusion; Fig. 5A) rose progressively with \([\text{La}^-]\) and were significantly lower in the MVO rats at 100 mM \((P < 0.05)\). A trend for reduced influx in the MVO group was also observed at 20 mM \((P = 0.08)\). The data for total influx were closely fit \((r^2 = 0.96–0.99)\) by an equation containing both Michaelis-Menten and linear components.

The specific rates of lactic acid diffusion (nmol \(\text{mg}^{-1} \cdot \text{min}^{-1}\); Fig. 5B) increased in a linear manner with \([\text{La}^-]\) and were consistently lower in vesicles from MVO rats. In fact, the slope of diffusion was reduced by 30% in the MVO group \((8.9 \pm 0.9 \text{ vs. } 12.9 \pm 1.4, P < 0.05)\). A trend for reduced diffusion in the MVO group was also observed at 20 mM \((P = 0.08)\). The data for total influx were closely fit \((r^2 = 0.96–0.99)\) by an equation containing both Michaelis-Menten and linear components.

The specific rates of lactic acid diffusion (nmol \(\text{mg}^{-1} \cdot \text{min}^{-1}\); Fig. 5B) increased in a linear manner with \([\text{La}^-]\) and were consistently lower in vesicles from MVO rats. In fact, the slope of diffusion was reduced by 30% in the MVO group \((8.9 \pm 0.9 \text{ vs. } 12.9 \pm 1.4, P < 0.05)\). Similar to what was observed for total influx, the differences in diffusion became greater with increasing \([\text{La}^-]\) and reached statistical significance at 100 mM \((P < 0.05)\).

The diffusive component, when expressed relative to total influx (Fig. 6), increased from ~20 to 60% between 2 and 100 mM extravesicular \([\text{La}^-]\), respectively, and did not differ between sham-operated and MVO rats at any concentration \((P > 0.05)\). In an attempt to validate the addition of a linear diffusive component to the Michaelis-Menten equation, the equation-derived percent diffusion was compared with results from influx assays performed at 20 mM \([\text{La}^-]\) on SL vesicles that were preincubated with \(\text{pCMBS}\), a sulfhydryl group reagent. Because no differences in the relative percent diffusion were observed between groups, the data from sham-operated and MVO groups were pooled for this comparison. At 20 mM extravesicular \([\text{La}^-]\), the equation-derived diffusive contribution of 35 ± 3% was very similar to the 33 ± 5% determined from the \(\text{pCMBS}\) trials.

The difference between total \([\text{La}^-]\) influx and diffusion of lactic acid at each concentration was taken to represent the MCT-mediated component of transport and is illustrated in Fig. 7. This estimated carrier-mediated transport displayed saturation kinetics with increasing \([\text{La}^-]\) and affinity constants \((K_m)\) of 12 ± 1 and 18 ± 3 mM and apparent \(V_{\text{max}}\) of 772 ± 99 and 827 ± 80 nmol \(\text{mg}^{-1} \cdot \text{min}^{-1}\) in the sham-operated and MVO groups, respectively. In contrast to

Table 6. Characteristic data of mixed skeletal muscle sarcolemmal vesicles purified from sham-operated and MVO rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sham (7)</td>
<td>MVO (9)</td>
</tr>
<tr>
<td>CH KpNPPase, (\mu\text{mol}-\text{mg}^{-1} \cdot \text{h}^{-1})</td>
<td>0.40 ± 0.02</td>
<td>0.40 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SL KpNPPase, (\mu\text{mol}-\text{mg}^{-1} \cdot \text{h}^{-1})</td>
<td>15.74 ± 0.62</td>
<td>16.92 ± 0.92</td>
<td></td>
</tr>
<tr>
<td>Purity index</td>
<td>40.49 ± 2.87</td>
<td>43.35 ± 2.90</td>
<td></td>
</tr>
<tr>
<td>SL yield, mg SL protein/g</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Values are reported as means ± SE; \(n\), no. of preparations. CH, crude homogenate; SL, sarcolemmal vesicle; KpNPPase, specific activity of \(K^+\)-stimulated \(p\)-nitrophenolphosphatase in \(\mu\)moles substrate converted per milligram of assay protein per hour; purity index, ratio of SL KpNPPase to that of the CH; SL yield, milligrams of SL protein obtained per gram of starting tissue.
total influx and specific diffusion, no significant differences between groups were observed at any [La$^{2-}$] or in either kinetic parameter ($K_m$, $V_{max}$) examined ($P > 0.05$ for all comparisons).
DISCUSSION

The primary finding of this investigation is that a 10-wk MVO did not alter the rates of MCT-mediated SL La\(^{\text{\textsuperscript{-}}}\) transport at any concentration examined, the apparent \(V_{\text{max}}\) of transport, or the MCT1 content of various hindlimb muscles. Although there appeared to be a slight decrease in carrier-substrate affinity (indicated by an increased \(K_{m}\)), the difference between groups was not statistically significant. Therefore, a 10-wk MVO in this investigation did not appear to alter the SL-bound MCT La\(^{-}\)H\(^{+}\) cotransport system. Surprisingly, however, SL diffusional properties were impaired in animals with an MVO.

The \(K_{m}\) values obtained in SL vesicles from sham-operated and MVO rats in this study (12 and 18 mM, respectively) are consistent with those reported by McDermott and Bonen (27) using the SL purification method of Klip et al. (20) and with values obtained in intact soleus strips (3). However, they are considerably lower than values of \(40\) mM reported by others (33–35) using the method of Grimditch et al. (18) that was modified for this study. We attribute this to the fact that the \(K_{m}\) values reported here are specific to MCT-mediated transport, whereas those reported by Roth and Brooks (33–35) appear to be for total La\(^{-}\) influx (MCT mediated + diffusion). In fact, the \(K_{m}\) values for total La\(^{-}\) influx in the current investigation typically ranged between 30 and 40 mM, which is consistent with their (33–35) results.

The \(V_{\text{max}}\) values obtained in this investigation are the highest reported for any “classic” SL preparation. In fact, the \(V_{\text{max}}\) of zero-trans MCT-mediated transport was six- to eightfold greater than those of total influx reported by Roth and Brooks (33–35) and others (13, 14). This discrepancy can be explained primarily by the calculation of initial rates of transport and is presented graphically in Fig. 8. In other investigations (13, 14, 33–35), initial rates were typically determined by linear regression analysis of 5- to 20-s influx measure-

ments. In the present study, La\(^{-}\) influx was also measured before 5 s and the data were fitted by a monoexponential equation (see MATERIALS AND METHODS). As Fig. 8 clearly demonstrates, influx during zero-trans conditions is substantially more rapid during the first 5 s, and calculation of initial velocities with the current model yields rates that are more than 10-fold greater than those derived from a 5- to 20-s linear slope. Therefore, we propose that the values reported in this study are more representative of true initial rates of transport.

To our knowledge, this is the first investigation to appropriately add a linear function to a Michaelis-Menten equation when analyzing total La\(^{-}\) influx kinetics. The linear function was added to discriminate between carrier-mediated transport and diffusion of lactic acid and was in agreement with assays performed at 20 mM [La\(^{-}\)] after incubation of vesicles with pCMBS. With the use of this model, we observed that total influx was reduced at 20 and 100 mM and that this was explained by an alteration in diffusive properties of the SL vesicles. Analysis of the linear slope of diffusion revealed a 30% deficit in MVO rats, suggesting that diffusional properties of the SL were impaired over a wide range of concentrations (2–100 mM) in these animals. At the present time, we have no direct explanation for this observation. It has been shown that the progression of CHF is associated with an accumulation of interstitial collagen in skeletal muscle and that this may be secondary to hypoperfusion, deconditioning, and/or increased concentrations of ANG II (36). Additionally, alterations in the cholesterol-to-phospholipid and/or lecithin-to-sphingomyelin ratios of biological membranes are associated with the pathogenesis of many diseases. Both of these phenomena could certainly have profound effects on membrane fluidity and could possibly explain the change in diffusive properties that were observed in this study (11).

The progression of CHF is associated with deleterious effects in skeletal muscle that seem to be related to
the severity of the disease (36). With the use of a rodent model of myocardial infarction, Delp et al. (12) observed that reductions in skeletal muscle mitochondrial enzyme activity and alterations in fiber-type distributions were only present in rats with severe LV dysfunction (LVEDP > 20 mmHg and an ~50% reduction in \(dP/dt\)). Similar results were obtained by Simoni et al. (37), who observed that skeletal muscle alterations were only present in a group of rats with an LVEDP > 15 mmHg. The MVO rats in the present study demonstrated significant elevations in heart, lung, and body weights, suggesting the development of both pulmonary and peripheral edema and progression to a state of CHF. However, measurements of \(dP/dt\) and LVEDP indicated a very moderate change in LV function in this group. In fact, the highest individual LVEDP measured in the MVO group was 13 mmHg, which is in the “moderate LV dysfunction” range reported by Delp et al. (12). Analysis of \(\text{La}^-\) influx in vesicles obtained from a single rat with evidence of decompensated CHF (e.g., rapid weight gain, severe generalized edema, heavily labored breathing, extreme lethargy) demonstrated a \(V_{\text{max}}\) of 110.1 nmol·mg\(^{-1}\)·min\(^{-1}\), which is only 22% of the lowest value observed in the 10-wk MVO rats. Additionally, a trend for a negative correlation was observed between the \(V_{\text{max}}\) of total \(\text{La}^-\) influx and heart weight in MVO rats \((r = -0.64, P = 0.07)\). Therefore, a likely reason for the lack of effect on skeletal muscle with respect to MCT-mediated \(\text{La}^-\) transport and CS activity in this investigation was that the 10-wk MVO period did not induce a sufficiently severe disease state. Nevertheless, our results do make the important point that MVO animals in a compensated state with only mild myocardial dysfunction are generally similar to control animals with regard to skeletal muscle-facilitated \(\text{La}^-\) transport and oxidative capacity.

Another noteworthy observation was that activity levels were nearly 30% lower in MVO rats and that this was associated with a significant reduction in type I MHC expression in soleus muscles. Additionally, there was a strong trend for a lower percentage of type IIX MHC in the plantaris. These results are congruent with other investigations that demonstrated alterations in skeletal muscle MHC expression and fiber-type distributions in humans (39, 42) and animal models of CHF (12) as well as reduced contractile activity (5, 14, 24). Consequently, these results raise questions as to (1) whether the changes in MHC expression in this study were primarily due to the MVO or reduced activity levels and 2) why CS activity, MCT1 expression, and MCT-mediated \(\text{La}^-\) transport activities were unaffected given the observed changes in MHC isoforms.

Although reduced activity levels may play some role in skeletal muscle alterations in CHF, several lines of evidence argue against this as the primary stimulus for peripheral abnormalities. Simoni et al. (37) reported that although hindlimb muscle samples from rats with large myocardial infarctions had reductions in the activity and mRNA levels of mitochondrial enzymes as well as type I fibers, physical activity levels were not different from those of sham-operated rats. Furthermore, indexes of disease states such as LVEDP, infarction size, and ventricular hypertrophy were poorly correlated with activity levels \((r \approx 0.16\) for each relationship) but were significantly related to the observed changes in skeletal muscle. Delp et al. (12) noted that enzymatic perturbations occurred in muscles composed of primarily glycolytic type IIB fibers that would not be recruited to a great extent during normal cage activity (2). Finally, Vescovo et al. (42) demonstrated that whereas gastrocnemius samples from CHF patients exhibited a typical slow-to-fast transition in MHC profile, those obtained from chronically bed-ridden stroke patients actually showed a profound increase in the proportion of type I MHC isoforms. In the present study, a marked reduction in activity \((30\%)\) was associated with modest changes in types I and IIA isoforms in the soleus and type IIX isoforms in the plantaris. Therefore, it is tempting to speculate that these effects are primarily due to hypodynamia. However, this is unlikely given the evidence cited above, the fact that MHC isoforms throughout the slow-to-fast continuum appeared to be affected, and that all animals were confined in cages that limited physical activity. Additionally, it is uncertain how accurately intermittent measurement periods on a locomotor wheel or in a photoelectric cell (37) reflect chronic activity levels under these conditions.

It is well documented that changes in \(\text{La}^-\) transport characteristics and MCT expression are closely related to changes in oxidative enzyme activities and fiber characteristics of skeletal muscle (3, 14, 30). Therefore, it was interesting to note in the current study that although MHC profiles were altered, CS activity, MCT1 expression, and MCT-mediated transport were unchanged. Because the observed changes in MHC compositions were relatively small, a possible explanation is that the primary stimuli (e.g., MVO and/or hypodynamia) were not great enough to elicit changes in \(\text{La}^-\) transport. This is supported by the fact that only types I and IIA MHC isoforms were detected in soleus samples from MVO rats, which is a typical observation in normal, healthy rats. Dubouchaud et al. (14) observed that a reduction of influx into classic SL vesicles, at 1 mM \(\text{La}^-\), after hindlimb suspension was associated with expression of type IIB MHC in the soleus. Therefore, it is possible that had more profound changes occurred (e.g., expression of IIX and/or IIB isoforms), MCT-mediated transport would have been altered as well.

Another possible explanation for the lack of difference in carrier-mediated transport was the use of SL vesicles from mixed skeletal muscle. Roth and Brooks (35) noted that \(\text{La}^-\) influx kinetics were nearly identical in mixed muscle SL vesicles from control, sprint-, and endurance-trained rats, despite increases in CS activity with both training paradigms. Although subsequent efforts using mixed muscle SL preparations have noted changes in \(\text{La}^-\) transport in response to chronic stimuli (14, 24), the contributions of carrier-mediated transport and diffusion were not identified.
Because expression and distribution of both MCT1 and MCT4 in striated muscle appear to be fiber-type specific (31, 44), it is possible that discrete changes in \(\text{La}^-\) transport may have been confounded by the use of mixed muscles in the present study. However, MCT1 expression levels, as indicated by Western immunoblotting, were not different in the various muscles examined. Therefore, it seems unlikely that discreet changes in MCT-mediated transport were masked in this investigation.

This is not the first study to report a dissociation between changes in biochemical, structural, and functional properties in skeletal muscle. Pilegaard et al. (29) observed in humans that total \(\text{La}^-\) transport and MCT1 and MCT4 expression were increased in biopsies from trained legs after 8 wk of high-intensity one-legged cycling. However, no differences were present between trained and untrained legs with respect to muscle CS activity, fiber-type distribution, or capillarity. Similarly, hindlimb unweighting in rats has been shown to induce a slow-to-fast transition in cytosolic enzymes and myofibrillar proteins in postural muscles (5). Therefore, although there is a strong correlation between changes in biochemical, structural, and functional properties in skeletal muscle, it seems evident that regulation of these properties by contractile activity and/or chronic disease states is complex but not entirely interdependent. In fact, Pilegaard et al. (29) suggest that even individual \(\text{La}^-\) transport proteins MCT1 and MCT4 are subject to differential regulation in skeletal muscle.

In conclusion, the results of this investigation demonstrate that a 10-wk MVO in rats did not alter carrier-mediated \(\text{La}^-\) transport in mixed skeletal muscle SL vesicles. However, diffusive properties of the membranes appeared to be reduced by an MVO, and this observation was associated with a modest slow-to-fast transition in myofibrillar proteins. Additional research should be conducted to fully elucidate possible alterations in systemic \(\text{La}^-\) dynamics that are associated with CHF. Subsequent endeavors should use a longer MVO period or an alternate model of CHF to allow the development of a more severe state of LV dysfunction and disease.

**Perspectives**

In the context of the \(\text{La}^-\) shuttle hypothesis (7), changes in either metabolic regulation (e.g., enzyme activities) or \(\text{La}^-\) transport across the SL are important. More specifically, exaggerated systemic lactacidosis in individuals with CHF is likely to be explained eventually by alterations in one or more components of the \(\text{La}^-\) shuttle. In the current study, changes in oxidative capacity, MHC isoforms, MCT1 expression, and saturable \(\text{La}^-\) transport across the SL were minor. However, significant changes were noted in SL \(\text{La}^-\) diffusion, which suggests that the SL itself may represent an important component of the \(\text{La}^-\) shuttle. This observation may also suggest that an alteration in \(\text{La}^-\) diffusion is an early indicator of membrane changes and other perturbations in skeletal muscle during the progression of CHF. It should be emphasized that as transmembrane \(\text{La}^-\) concentration gradients increase, a greater proportion of total \(\text{La}^-\) flux is due to diffusion (see Fig. 6). To this point, no studies have directly addressed this phenomenon, which needs to be considered in future research with respect to exercise training, disuse, and possibly a variety of chronic disease states.

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