Reduced mechanical efficiency of rat papillary muscle related to degree of hypertrophy of cardiomyocytes

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Wong YY, Handoko ML, Mouchaers KT, de Man FS, Vonk-Noordegraaf A, van der Laarse WJ. Reduced mechanical efficiency of rat papillary muscle related to degree of hypertrophy of cardiomyocytes. Am J Physiol Heart Circ Physiol 298: H1190–H1197, 2010. First published January 29, 2010; doi:10.1152/ajpheart.00773.2009.—Isolated rat papillary muscles were subjected to sinusoidal length changes at 37°C and 5 Hz with a peak-to-peak amplitude of 15% of Lmax. Isometric tension at Lmax was similar in control and hypertrophied muscles. Work was assessed from the area encompassed by force-length loops. Work per loop was 0.93 ± 0.11 and 0.84 ± 0.11 μJ/mm3 (means ± SE) for control and hypertrophied muscles, respectively (P = 0.591). Suprabasal O2 uptake per work loop was 5.7 ± 0.7 μmol/mm3 in control muscles and 8.7 ± 1.7 μmol/mm3 in hypertrophied muscles (P = 0.133). Net mechanical efficiency was calculated from the ratio of work output and suprabasal O2 uptake. The efficiency of hypertrophied muscles was 29.1 ± 3.7% and was smaller than in control muscles (43.7 ± 2.2%, P = 0.016). The right ventricular cardiomyocyte cross-sectional area increased from 272 ± 17 μm2 in control muscles to 396 ± 31 μm2 in hypertrophied muscles (P < 0.003). Mechanical efficiency correlated negatively with right ventricular wall thickness and cardiomyocyte cross-sectional area [Spearman rank correlation coefficients of −0.50 (P = 0.039) and −0.53 (P = 0.024), respectively]. We conclude that efficiency decreases with increasing cardiomyocyte hypertrophy. Thus, the reduced efficiency of diseased whole hearts can be at least partly explained by reduced efficiency at the cardiomyocyte level.

Oxygen consumption; work output; monocrotaline; pulmonary hypertension; right ventricular hypertrophy

Patients with chronic heart failure have reduced myocardial efficiency (3, 44, 51). Reduced myocardial efficiency in vivo can be due to an increase in afterload, changes in the geometry and activation of the heart, or intrinsic changes in remodeling cardiomyocytes. Molecular studies (21, 27–29) have found contractile changes due to a myosin isoform shift from α to β and increase in Ca2+ sensitivity in the failing myocardium. Such changes are expected to increase the efficiency of cardiomyocytes during cardiac hypertrophy and heart failure, which is opposite to the findings in vivo in diseased hearts. It follows that other factors are more important determinants of myocardial efficiency. To elucidate the underlying cause, the mechanical efficiency of the diseased myocardium has to be measured in a setting isolated from the hemodynamic load of the heart.

Papillary muscle is, due to the size and arrangement of cardiomyocytes, the most widely used simplified model of the heart to study work output and energetics (2, 26, 34–36, 49). Only Coleman and coworkers (8, 9, 18) have combined measurements of normal and hypertrophied papillary muscle mechanics and O2 consumption. They found increased O2 consumption and reduced shortening velocity and isometric tension in pressure-induced hypertrophied muscles (18). The authors attributed the increased O2 consumption to mitochondrial Ca2+ cycling on the basis of experiments on isolated mitochondria (9). They did not report mechanical efficiency values, but their results suggested that mechanical efficiency might be decreased in hypertrophied cardiomyocytes due to a decrease of ATP/O2.

We have determined, for the first time, the net mechanical efficiency of hypertrophied and control papillary muscles from work loops and O2 consumed. These muscles were isolated from the right ventricle (RV) of rats. By subjecting papillary muscle to sinusoidal length changes, the muscle performs movements that simulate the contraction and relaxation of the pumping myocardium (23).

We hypothesized that mechanical efficiency decreases with increasing hypertrophy.

Materials and Methods

Pulmonary hypertension was induced in 11 male Wistar rats (Harlan Laboratories, Horst, The Netherlands) by an injection of monocrotaline (MCT; Sigma Aldrich, Zwijndrecht, The Netherlands, 60 mg/kg sc at a body mass of 190–200 g). Seven untreated rats served as controls in the experiments at 37°C. For a comparison with experiments at 27°C (2, 36), untreated rats (n = 6) were used. This study was performed in accordance with national guidelines and with the permission of the local Institutional Animal Care and Use Committee.

After 3 wk and 1 day before the experiment, rats underwent echocardiography under isoflurane anesthesia to assess RV function and the degree of pulmonary hypertension. To avoid papillary damage by invasive right heart catheterization, RV pressures were not measured. Instead, pulmonary artery acceleration time (PAAT) per cycle length (CL) was obtained by echocardiography. A decrease of PAAT/CL in pulmonary hypertension has been shown to correlate inversely with an increase of RV systolic pressure (20, 22). Other parameters of RV function were tricuspid annular plane systolic excursion, stroke volume, cardiac output, and RV end-diastolic volume (20). RV wall thickness was determined as a measure of hypertrophy. In parallel experiments, we found RV systolic pressures of 26 ± 2.3 (n = 20) and 48 ± 3.5 (n = 18) Torr in control and MCT-injected rats, respectively (19).

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Papillary muscle preparation. Rats were anaesthetized with isoflurane in air, and the heart was rapidly excised and perfused with Tyrode solution at 10°C to remove blood and stop contraction. The Tyrode solution contained (in mM) 120 NaCl, 5 KCl, 1.0 CaCl2, 27 NaHCO3, 2 Na2HPO4, 1.2 MgSO4, 10 glucose, 20 2,3-butanedione monoimine (BDM), and 10 N-acetylcyesteine and was equilibrated with 95% O2 and 5% CO2 (carbon: pH 7.4).

The RV free wall was opened via the pulmonary artery, and papillary muscles were exposed. Cylindrically shaped papillary muscles were excised with care including the tricuspid valve on one end and the septum on the other end. Platinum hooks made of 50-μm-diameter wire were tied with 20-μm-diameter nylon thread to the tendon as close to the muscle as possible and at the base of the muscle. Excess muscle tissue from the septum was removed or squeezed with a pair of forceps to minimize O2 consumption due to damaged cardiomyocytes. The slack length (excluding the tendon and damaged tissue) was measured under the microscope at ×50 magnification using an ocular scale before the experiment. The larger and smaller diameters of the muscle were measured at three levels. The volume of the muscle was calculated as the product of average cross-sectional area assuming an elliptical shape and slack length. Isometric active and passive tension were normalized by the average cross-section area at optimum length, which was calculated from the volume of the muscle divided by the length at which active tension was maximal.

Experimental setup. The preparation was mounted in a 381-μl glass oxygen chamber, similar to the one described previously (15), containing Tyrode solution. The muscle was attached to the force transducer via a tungsten wire, which left the chamber via a capillary, and to the pivot of the spinner. The force transducer was connected to a model for elliptical cross sections (54).

A polarographic electrode was used to measure PO2 (25). The electrode current in Tyrode solution equilibrated with carbogen varied with and without a papillary muscle in the chamber. The PO2 decrease with and without a papillary muscle in the chamber. The PO2 decrease in the chamber without a muscle varied between experiments from 78 and 160 pmol/s in Tyrode solution equilibrated with carbogen. Suprabasal O2 consumption of the papillary muscle was calculated after the decrease of PO2 in the chamber, the decrease of PO2 in the chamber at which the steady rate of O2 consumption during stimulation started to decrease, the rate of O2 consumption at that time (including the resting rate), and the dimensions of the preparation using a Hill-type diffusion model for elliptical cross sections (54).

Histochemistry and morphometry. To determine the degree of myocardial hypertrophy and morphology of the experimental muscles, sections of experimental papillary muscles and RV free walls were cut. Cryostat sections (5 μm thick) of all preparations were incubated as previously described in detail for succinate dehydrogenase (SDH) activity (14) and myoglobin concentration (52). In papillary muscle cross sections, the interstitial space (the volume not contributing to work output) was determined in sections incubated for SDH activity as previously described (54). Cross-sectional area was measured in cardiomyocytes stained with hematoxylin and eosin and was normalized to a sarcomere length of 2 μm in the papillary muscle. The sarcomere length was determined in longitudinal sections of the papillary muscle. For all histochemical analyses, mean values were obtained by measuring 20–30 cardiomyocytes/preparation.

Statistical analyses. Values are mean ± SE. Normality was checked using the Kolmogorov-Smirnov test. When the data were not distributed normally (muscle volume, O2 uptake per workloop, and mechanical efficiency), the Mann-Whitney U-test was used. Student’s t-test with unequal variances was used to evaluate differences between means of normally distributed data. P values of <0.05 were considered significant.

RESULTS

Development of pulmonary hypertension. MCT-injected rats developed clear signs of pulmonary hypertension with shortening of PAAT/CL and reduced cardiac output. In addition, lung wet mass and RV wall thickness increased (Table 1) (20).

Efficiency measurements. The dimensions of the experimental papillary muscles did not differ between control and hypertrophied hearts. Isometric active and passive tensions were similar at Lmax and 0.5 Hz (Fig. 1 and Table 2). Isometric contraction time was increased in hypertrophied papillary muscles, but half-relaxation time was similar in control and hypertrophied muscles (Fig. 1 and Table 2).

The muscle O2 uptake at rest was 0.024 ± 0.003 and 0.036 ± 0.007 nmol-mm−2-s−1 in control and hypertrophied papillary muscles, respectively. It was not possible to determine the rate of O2 consumption at rest using cyanide, as cyanide reduced the decrease of PO2 in the chamber without muscle preparation, resulting in an apparent (Krogh’s) diffusion coefficient for O2 in the preparation at 37°C was calculated from the PO2 in the chamber at which the steady rate of O2 consumption during stimulation started to decrease, the rate of O2 consumption at that time (including the resting rate), and the dimensions of the preparation using a Hill-type diffusion model for elliptical cross sections (54).
Sinusoidal length changes of the relaxed preparations resulted in clockwise work loops (inducing energy transfer from the motor to the preparation, or negative work), corresponding to 0.19 ± 0.16 and 0.11 ± 0.10 μJ/mm³ per loop in control and hypertrophied muscles, respectively. These values were not significantly different, indicating that the viscoelastic properties of the preparations were similar.

The stimulus phase at which maximum work output was reached was at 24 ± 1 ms before reaching maximum length in control muscles, which was similar to that in hypertrophied muscles (20 ± 7 ms).

Figure 2 shows the work output and O₂ uptake of electrically stimulated control and hypertrophied papillary muscles. Work output usually transiently peaked after the start of stimulation (Fig. 2, A and C) but stabilized within 1.5–2 min. After 20 s of stimulation, the rate of O₂ uptake by the papillary muscle became constant. This was maintained until the uptake rate decreased slightly at the end of the 4-min stimulation period (arrowheads in Fig. 2, C and D). The efficiency of the papillary muscles was calculated from the work output and O₂ uptake when the rate of O₂ uptake was steady. These values are shown in Table 2. Work output and O₂ uptake per loop of the control and hypertrophied papillary muscles were not significantly different, whereas the efficiency of the control muscles was higher than the efficiency of hypertrophied papillary muscles.

After the 4-min contraction period and 3-min recovery period, the Tyrode solution in the chamber was replaced, and the preparation was stimulated for a second efficiency measurement. Work output during the second run was 77 ± 4% of the first run in control muscles and 67 ± 9% in hypertrophied muscles. Efficiencies during the first and second runs were the same.

The mechanical efficiency did not correlate with the average cross-sectional area of the papillary muscle (r = 0.15, P = 0.55; Supplemental Fig. S1).

In five control and eight hypertrophied muscles, it was possible to determine the PO₂ in the chamber at which the steady rate of O₂ consumption started to decrease. This critical PO₂ was used to calculate the apparent diffusion coefficient for O₂ in the preparation, assuming that the rate of O₂ consumption decreased because the center of the preparation started to develop an anoxic core. We found 1.9 ± 0.9 and 3.5 ± 0.9 nM-mm⁻²-Torr⁻¹-s⁻¹ in control and hypertrophied muscles, respectively (P = 0.008).

Work output in the Tyrode solution containing 2 mM cyanide was similar in control and hypertrophied muscles at 15 ± 15 nM/mm³ per work loop (n = 2 and 4, respectively), which was <3% of work output during the first measurement in carbogen. Cyanide inhibition of work output was partly reversible.

Histochemistry and morphometry. RV hypertrophy in the MCT-injected rats was histologically confirmed by the larger cardiomyocyte cross-sectional area in both the RV free wall (14, 19, 37) and experimental papillary muscles (Table 3). Figure 3 shows cross sections of a control papillary muscle and a hypertrophied papillary muscle incubated for SDH activity. Mean cardiomyocyte cross-sectional area doubled, and heterogeneity in the cardiomyocyte cross-sectional area and in SDH activity in cardiomyocytes of the hypertrophied muscle increased.

Myoglobin concentration and mean SDH activity were similar in control and hypertrophied papillary muscles (Table 3).
Mechanical efficiency and hypertrophy. Figure 4 shows the relationships between the efficiency and RV free wall thickness and myocyte cross-sectional area in papillary muscles. Net mechanical efficiency of papillary muscles correlated negatively with increasing RV free wall thickness and cardiomyocyte cross-sectional area. Note the larger variation of myocyte cross-sectional area of papillary muscles in the hypertrophied group, indicating heterogeneity in the progression and the severity of pulmonary hypertension development. There were no correlations between mechanical efficiency and other echocardiographic parameters.

Efficiency at 27°C. To compare the efficiency measured with the present setup with those of Baxi et al. (2) and Mellors et al. (36) in control muscles, we performed similar experiments at 27°C and 2-Hz sinusoidal length changes. Isometric contraction and half-relaxation times at Lmax and 0.5 Hz were slower than at 37°C and were 148 ± 5 and 147 ± 10 ms, respectively (P < 0.001). The efficiency under these conditions was 26.5 ± 1.7%, which was significantly less than at 37°C and 5 Hz (P < 0.001; Table 2). Work and suprabasal O2 uptake per loop at 27°C were 1.7 ± 0.3 μJ/mm3 and 14.2 ± 2.6 pmol/mm3, respectively.

DISCUSSION

Net mechanical efficiency, calculated from direct measurements of O2 consumption during sinusoidal contractions by papillary muscles at 37°C, was reduced in hypertrophied muscles compared with control muscles. As far as comparison allows, this result confirms and extends the results regarding efficiency by Coleman et al. (9, 18) in cat papillary muscle. We quantified mechanical efficiency in mammalian heart muscle for the first time by measuring power output and O2 consumption and found that it can be reduced considerably with only minor changes in contractile characteristics in hypertrophied papillary muscles.

The variation in myocyte cross-sectional area, and in the efficiency between hypertrophied papillary muscles (Fig. 4), may be explained by the severity of pulmonary hypertension induced by MCT. Moreover, the results shown in Fig. 4 suggest that efficiency drops by more than half once cardiomyocytes have reached a certain maximum size (400–500 μm2 at 2-μm sarcomere length), indicating a limitation of progressive cardiomyocyte hypertrophy, which may be the cause of heart failure.

Table 3. Histology of experimental papillary muscle myocytes

<table>
<thead>
<tr>
<th></th>
<th>Control (μm²)</th>
<th>Hypertrophy (μm²)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-sectional area, μm²</td>
<td>272 ± 17</td>
<td>396 ± 31</td>
<td>0.003</td>
</tr>
<tr>
<td>Myoglobin concentration, mM</td>
<td>0.73 ± 0.07</td>
<td>0.78 ± 0.07</td>
<td>0.637</td>
</tr>
<tr>
<td>SDH activity, A660 nm</td>
<td>0.19 ± 0.01</td>
<td>0.21 ± 0.02</td>
<td>0.420</td>
</tr>
<tr>
<td>Interstitial space of papillary muscle, %</td>
<td>8.8 ± 3.9</td>
<td>12.9 ± 6.7</td>
<td>0.162</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7 control papillary muscles and 11 hypertrophied papillary muscles. SDH, succinate dehydrogenase; A660 nm, absorbance at a wavelength of 660 nm. Cross-sectional area was corrected at 2 μm of sarcomere length of the papillary muscles.
Reduced efficiency of hypertrophied papillary muscle

Fig. 4. Net mechanical efficiency was plotted against right ventricular (RV) wall thickness, determined by echocardiography (A), and CSA of cardiomyocytes (B) of the experimental papillary muscles. Mechanical efficiency decreased with increasing wall thickness and cardiomyocyte CSA [Spearman correlation coefficient: $-0.50 (P = 0.039)$ in A and $-0.53 (P = 0.024)$ in B]. Solid circles indicate control papillary muscles, and open circles indicate hypertrophied papillary muscles.

Force development and work output in hypertrophic and control muscles. Despite the echocardiographic signs of heart failure, increase in lung mass, and increase in cardiomyocyte cross-sectional area, we observed only minor changes in isotropic contractile characteristics at $L_{\text{max}}$: active tension and half-relaxation time were similar, whereas contraction time increased by 14%. This differs from the results of Coleman and coworkers (9, 18), who found a significant decrease in isometric tension in pressure-induced hypertrophied cat papillary muscles, indicating that the cat model and the present results differ in this respect. The reduced isometric force reported by Cooper et al. (9, 18) could be due to an increase in interstitial space, since Marino et al. (33) reported a significant increase in connective tissue in the pressure-overloaded cat heart. The normal contractile characteristics of hypertrophied cardiomyocytes and the clear signs of heart failure demonstrated by echocardiography indicate that either the load of the myocardium or the O$_2$ supply in situ are responsible for the reduced cardiac output rather than the contractile characteristics of hypertrophied cardiomyocytes.

Layland et al. (30–32) obtained work output from normal rat papillary muscles during sinusoidal length changes of 1.43–1.51 µm/J/mg. Compared with their data, work output from our control papillary muscles was lower, 0.93 µm/J/mg. This difference may be due to the higher Ca$^{2+}$ concentration used in the experiments of Layland et al. (2 mM; we used 1.5 mM).

Work output of control muscles per loop obtained at 27°C of 1.74 µm/J/mg was higher than the work output reported by Baxi et al. (2) (0.75 µm/J/mg) and Mellors et al. (36) (1.29 µm/J/mg). This difference may be related to the smaller sizes of the present preparations or to the use of different rat strains.

Work per loop of hypertrophied papillary muscles was similar to control muscles, also after a correction for the interstitial space (Tables 2 and 3). These results indicate that the contractile properties of cardiomyocytes do not drastically change in this model of RV hypertrophy.

Our experimental protocol was designed to determine efficiency at 37°C and 5 Hz to simulate the in vivo situation of the rat heart. However, we did not subject isolated papillary muscles to adrenergic stimulation. The addition of catecholamines can double maximum power output in control preparations (32), but adrenergic stimulation may be chronically increased in MCT-injected rats (55). This may explain why the power output of control muscles (5 µW/mm$^2$; Table 2) was lower than the power output of the rat RV in situ [12 µW/mm$^2$, calculated as follows: cardiac output of 110 ml/min × mean developed pressure of 22 Torr/(2 × free RV wall volume of 440 mm$^3$)].

$O_2$ uptake of hypertrophied and control papillary muscles. $O_2$ uptake per loop varied considerably between hypertrophied papillary muscles. Care was taken to prevent anoxic cores in the muscle, as we determined efficiency when work output and $O_2$ uptake were approximately steady, whereas PO$_2$ in the experimental chamber decreased. We did not observe any increase in passive tension during the experiments, long-lasting recovery $O_2$ uptake, or myoglobin release, which would be indicative of reoxygenation injury.

The steady rate of $O_2$ consumption during the first minutes usually declined during the stimulation period while the PO$_2$ in the chamber decreased. Taking this PO$_2$ as the PO$_2$ at which the center of the preparation developed an anoxic core, we calculated the apparent diffusion coefficient for $O_2$ in the preparation. As previously reported (54), the value of the diffusion coefficient in hypertrophied muscles was larger than in control muscles. The value in control muscles was 20% smaller than reported before in thin trabeculae contracting isometrically at 10 Hz, and the value was 42% smaller in hypertrophied muscles. These differences may be due to the smaller amount of interstitial space in the present preparations (for discussion, see Ref. 54). This result indicates that hypoxic cores can develop at the end of the stimulation period. However, even when anoxic cores are present, the effect on efficiency is likely to be small because anaerobic work output (determined using cyanide) was negligible.

Efficiency comparison with previous studies in whole hearts. In control muscles, we found an efficiency of 43.7%, which is relatively high compared with efficiency values found in classical studies. The values of myocardial efficiency found in those studies using normal perfused hearts ex vivo at 37°C ranged from 6% to 23% (13, 16, 39, 43). One explanation for the lower values is that $O_2$ uptake in these studies included basal $O_2$ consumption. Assuming that basal metabolism is about one-fifth of the total $O_2$ consumption (4), maximum net mechanical efficiency would increase by a factor of 1.25 in whole hearts after correction. Bing and Michal (4) reported mechanical efficiencies of normal in situ hearts of 37% in dogs and 39% in humans after the correction of basal $O_2$ consumption. A second factor to explain the lower efficiency found in whole hearts is that $O_2$ consumption was derived from the difference of arterial and coronary sinus $O_2$ content, which includes $O_2$ consumption of the right heart and the whole septum, whereas only left ventricular power output was measured. Schipke (44) already noted that efficiencies measured ex vivo are lower than in vivo. The reason for the discrepancy is not clear. The results of Bing and Michal (4) were confirmed
by Thompson et al. (51), who found a net efficiency of 39.4% in control human hearts. In patients with mild to moderate heart failure or idiopathic cardiomyopathy, efficiencies, without a correction for basal O_2 consumption, have been reported of 25–34% (6, 45–47). We therefore argue that mechanical efficiency values for normal whole hearts can be considerably higher than the commonly accepted values of 20–25%.

Comparison with previous studies in papillary muscles. Syme (49) reported the only study that measured work and O_2 consumption simultaneously to calculate net mechanical efficiency, but frog papillary muscles were used. He found a net efficiency of 13% at 20°C.

We found a higher net mechanical efficiency of control papillary muscles compared with the values found by others in normal papillary muscles. Net mechanical efficiency, calculated from work derived from isotonic contractions and suprabasal heat production, was found to be 5–20% at 27°C (2, 26, 34–36). Both Baxi et al. (2) and Mellors et al. (35) found a maximum net mechanical efficiency of rat papillary muscle of ~15% in experiments at 27°C. Mellors et al. (36) demonstrated that the efficiency of rat papillary muscle obtained from force-length-loop analysis was similar to the protocol using isotonic contractions. The efficiency of our present study, conducted at 27°C and 2 Hz, was 26.5% and was considerably closer to the efficiencies found by Baxi et al. (2) and Mellors et al. than the efficiency measured at 37°C and 5 Hz. This suggests that the difference between the present efficiency value at 37°C and the results of Baxi et al. (2) and Mellors et al. (36) is, at least in part, due to a difference in experimental temperature and/or stimulus frequency.

Potential causes for the lower efficiency in hypertrophy. The reduction in myocardial efficiency has been ascribed to increased mechanical afterload or to chronic pressure overload in patients and in experimental models. Mechanical loading conditions, however, cannot explain why isolated hypertrophied papillary muscles have reduced mechanical efficiency.

As far as comparison allows, our lower efficiency in pressure-overload hypertrophied papillary muscles is in agreement with the findings of Coleman and coworkers (9, 18). They attributed the increased O_2 consumption in hypertrophied cat papillary muscles to nonphosphorylation mitochondrial respiration linked to Ca^{2+} transport (9). This may reduce mitochondrial efficiency (ATP/O_2). Judging from the increased variation of SDH activity among cardiomyocytes in hypertrophied rat papillary muscles (Fig. 3) and the clustering of mitochondria that has been previously described in the RV of MCT-injected rats (24), it is obvious that mitochondrial changes occur. It has already been shown that cytochrome c can be released in hypertrophic cardiomyocytes (53), which may lead to the oxidation of cytosolic NADH by cytochrome c, and oxygen radicals may be formed at complex II of the respiratory chain (41). Furthermore, NADPH oxidase can use O_2 to produce ATP and increase oxidative stress in MCT-injected rats (41).

All these mechanisms reduce ATP/O_2. The magnitudes and effects of these reactions remain to be determined in the present preparation. Judging from the magnitude of the decrease in mechanical efficiency, we expect the functional effects to be considerable compared with the changes in myosin heavy chain (MHC) isoform shifting from α-MHC to the more slower contracting β-MHC and alterations in Ca^{2+} handling, e.g., downregulation of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (27, 42, 55), and/or an increase in Ca^{2+} sensitivity (27, 29). Even though the contractile properties may have changed in the hypertrophied myocardium, the net work remained similar to control muscles at 37°C.

It has been suggested that increased cytoskeletal stiffness and viscosity contribute to decreased mechanical efficiency (10, 50). Although our setup was not designed to test this point, we did not observe that the work done by the motor on the papillary muscles during sinusoidal length without stimulation was larger in hypertrophied muscles compared with control muscles. There was no significant increase in interstitial space in the hypertrophic papillary muscles, indicative of fibrosis.

There is growing evidence that non-ATP-producing reactions like ROS formation in cardiac disease may be an important cause for reduced cardiac efficiency. In patients with idiopathic dilated cardiomyopathy, a selective xanthine oxidase inhibitor, allopurinol, was given intracoronary, after which myocardial efficiency improved from 34 to 45%, whereas stroke work remained unchanged (6). The authors conclude that the decrease in O_2 cost for left ventricular contraction was a result of xanthine oxidase inhibition by allopurinol and that xanthine oxidase contributes to mecha-noenergetic uncoupling in human heart failure. Increased xanthine oxidoreductase was also found in hypertrophied and failing RVs in the MCT model (12). Other mitochondrial sites, i.e., monoamine oxidase and nitric oxide synthase, can also be potential sites for oxidative stress production, causing a decrease in ATP/O_2 in cardiomyocytes of the hypertrophied or failing myocardium (17). To explain the reduction of efficiency in terms of radical formation would require a substantial fraction of the O_2 consumed to be converted to radicals. Murray et al. (38) reported that the reduced efficiency of isolated infarcted rat hearts was due to an upregulation of uncoupling protein 3, which could reduce oxidative stress. However, uncoupling protein 3 mRNA is not upregulated in decompensating hearts of MCT-injected rats (5). Further analyses are required to quantify all possible mechanisms causing reduced efficiency in this model of RV hypertrophy.

Consequences of lowered efficiency. The rate of O_2 consumption of 28.6 pmol·mm^{-3}·s^{-1} in control papillary muscles (Table 2) corresponds to only 5.3% of the maximum rate of O_2 consumption (14). When efficiency is reduced to 10.5% (the lowest value we observed; Fig. 4) and developed pressure is increased from 22 to 70 Torr (20), the suprabasal rate of O_2 consumption, assuming no change in cardiac output and no hypertrophy, would increase 13.5-fold and approaches the maximum rate of O_2 consumption already at resting heart rate of 5 Hz, suggesting that O_2 supply can become rate limiting under these conditions. Interestingly, MCT-injected rats (60 mg/kg) do not tolerate moderate exercise (19). Using immunohistochemistry (14) and Western blot analysis (41, 48), our laboratory previously found an upregulation of hypoxia-inducible factor-1α in the hypertrophied RV myocardium in this model, suggesting that hypertrophied cardiomyocytes become hypoxic. It has been shown that the shift in MHC isoforms from α to β in the rat heart can be induced by hypoxia (40). It is plausible that cardiomyocyte hypoxia develops partly due to the decrease in efficiency during myocardial hypertrophy, suggesting that the efficiency decrease is an early event in the development of chronic heart failure. In addition, the fact that the reduced cardiac output coincides with the normal power
output of isolated muscle suggests that reduced efficiency is an early event in the development of heart failure in this model (Table 2). Reduced contractility of isolated papillary muscles has been described in other models of pressure overload (e.g., Refs. 9 and 18). Our results indicate that the reduced cardiac output (Table 1) is due to increased pulmonary resistance (19) rather than reduced systolic pressure (deduced from the decreased PAAT/CL; Table 1) (22).

We conclude that mechanical efficiency is reduced with increasing cardiomyocyte hypertrophy in pulmonary hypertension. Further experiments are needed to discover the mechanism(s) behind the reduction of efficiency and to verify whether the reduction of efficiency with increasing free wall thickness and cardiomyocyte cross-sectional area is a general phenomenon.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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