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Gene expression profiling of the left ventricles in a rat model of intrinsic aerobic running capacity

S. J. Lee, J. A. Ways, J. C. Barbato, D. Essig, K. Pettee, S. J. DeRaedt, S. Yang, D. A. Weaver, L. G. Koch and G. T. Cicila

Physiol Genomics, September 21, 2005; 23 (1): 62-71.

[Abstract] [Full Text] [PDF]

Power output is linearly related to MyHC content in rat skinned myocytes and isolated working hearts

F. S. Korte, T. J. Herron, M. J. Rovetto and K. S. McDonald

Am J Physiol Heart Circ Physiol, August 1, 2005; 289 (2): H801-H812.

[Abstract] [Full Text] [PDF]

Angiogenic pretreatment improves the efficacy of cellular cardiomyoplasty performed with fetal cardiomyocyte implantation

M. A. Retuerto, P. Schalch, G. Patejunas, J. Carbray, N. Liu, K. Esser, R. G. Crystal and T. K. Rosengart

J. Thorac. Cardiovasc. Surg., April 1, 2004; 127 (4): 1041-1050.

[Abstract] [Full Text] [PDF]

Rapid Effects of Aldosterone and Spironolactone in the Isolated Working Rat Heart

J. C. Barbato, P. J. Mulrow, J. I. Shapiro and R. Franco-Saenz

Hypertension, August 1, 2002; 40 (2): 130-135.

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Myocardial function in rat genetic models of low and high aerobic running capacity

JOHN C. BARBATO, SOON JIN LEE, LAUREN GERARD KOCH, AND GEORGE T. CICILA
Functional Genomics Laboratory, Medical College of Ohio, Toledo, Ohio 43614-5804

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Barbato, John C., Soon Jin Lee, Lauren Gerard Koch, and George T. Cicila. Myocardial function in rat genetic models of low and high aerobic running capacity. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R721–R726, 2002; 10.1152/ajpregu.00367.2001.—We recently evaluated treadmill aerobic running capacity in 11 inbred strains of rats and found that isolated working left ventricular function correlated ($r = 0.86$) with aerobic running capacity. Among these 11 strains the Buffalo (BUF) hearts produced the lowest and the DA hearts the highest isolated cardiac output. The goal of this study was to investigate the components of cardiac function (i.e., coronary flow, heart rates, stroke volume, contractile dynamics, and cross-bridge cycling) to characterize further the BUF and DA inbred strains as potential models of contrasting myocardial performance. Cardiac performance was assessed using the Langendorff-Neely working heart preparation. Isolated DA hearts were superior ($P < 0.05$) to the BUF hearts for cardiac output (63%), stroke volume (60%), aortic $+dP/dt$ (47%), and aortic $-dP/dt$ (46%). The mean α/β -myosin heavy chain (MHC) isoform ratio for DA hearts was 21-fold higher relative to BUF hearts. At the steady-state mRNA level, DA hearts had a fivefold higher α/β -ratio than the BUF hearts. The mean rate of ATP hydrolysis by MHCs was 64% greater in DA compared with BUF ventricles. These data demonstrate that the BUF and DA strains can serve as genetic models of contrasting low and high cardiac function.

aerobic capacity; myosin heavy chains; cardiac output; contractility

ENERGY TRANSFER VIA AEROBIC pathways defines a large part of the biology for essentially all multicellular organisms (1, 23, 31). At the systemic level of organization, aerobic capacity is a complex trait in the sense of being a function of the interaction of both genetic and environmental factors (32). Simple additive models of heredity plus environment have been used to estimate the genetic contribution to variance in human aerobic capacity in twins. Klissouras (15) estimated that ~93% of the interindividual variance in maximal aerobic power was of genetic origin in pairs of monozygotic and dizygotic twins. Bouchard et al. (6) concluded that a genetic component accounts for 70% of the variation in endurance capacity in young adults when measured as

the total work performed during a 90-min maximal ergocycle test.

A long-term goal of our laboratory is to define the genetic basis for variation in aerobic exercise capacity in mammals (16). Given the complexity of this trait (30), the use of inbred models can be of special value for two related reasons: 1) genetic and environmental variation can approach minima and 2) widely divergent strains can be crossed to produce second filial (F_2) populations for use in cosegregation studies (7). In previous work we reported that significant variation exists for endurance running capacity between 11 inbred strains of rats. In addition, we also found that intrinsic cardiac contractility (Langendorff-Neely preparation) correlated ($r = 0.86$) positively with aerobic running capacity among these 11 inbred strains (2). Specifically, we found that the Buffalo (BUF) rats had the lowest intrinsic isolated heart performance and the DA rats the highest (1.9-fold difference).

The purpose of the current study was to evaluate physiological and biochemical pathways that could account for the difference in intrinsic myocardial capacity between hearts from the BUF and DA inbred strains of rats. Specifically, we investigated the components of cardiac function, including coronary flow, heart rate, stroke volume, contractile dynamics, and cross-bridge cycling to characterize these strains as potential genetic models of contrasting myocardial capacity. Because increased cross-bridge (i.e., actomyosin) cycling is associated with a higher α/β -myosin heavy chain (MHC) ratio, differences in the α/β -MHC were investigated at both the protein and mRNA levels (13, 17, 27). In addition, the rates of gamma phosphate hydrolysis were investigated in MHCs isolated from the left ventricles of BUF and DA rats.

METHODS

Animals

The BUF (BUF/NHsd) and DA (DA/OlaHsd) strains were purchased from Harlan Sprague Dawley (Indianapolis, IN) at 7–8 wk of age (18 rats per strain). When not being studied, the rats were housed two per cage and only age- and sex-matched rats of the same strain shared a cage. The rats were provided food and water ad libitum and placed on a 12:12-h

Address for reprint requests and other correspondence: G. T. Cicila, Dept. of Physiology and Molecular Medicine, Medical College of Ohio, 3035 Arlington Ave., Toledo, OH 43614-5804 (E-mail: gcicila@mco.edu).

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light-dark cycle, with the light cycle occurring during the daytime. All procedures were carried out with approval from our Institutional Animal Care and Use Committee and were conducted in accordance with the *Guiding Principles in the Care and Use of Animals*, as approved by the Council of the American Physiological Society.

Estimation of Isolated Cardiac Performance

Preparation of the heart. Isolated heart performance was measured in 13-wk-old rats using the Langendorff-Neely isolated working heart preparation (2, 22). Heparin sodium (300 IU) was injected intraperitoneally 1 h before rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The heart was extirpated via a sternotomy and rapidly placed in chilled Krebs-Henseleit buffer. Subsequently, the aorta was cannulated, and retrograde perfusion was initiated with oxygenated buffer solution.

Perfusion of the working heart. Isolated working heart function was assessed as described in detail previously (2). Briefly, retrograde perfusion through the aorta was initiated at a pressure of 80 mmHg within <1 min after the abdominal cavity was opened. During the subsequent 15 min of equilibration, a cannula, connected to an oxygenated reservoir set at a fixed filling pressure of 15 mmHg, was inserted into the left atrium. After equilibration, anterograde perfusion was initiated at a preload of 15 mmHg and an afterload of 70 mmHg. Aortic flow was collected from the side arm of the afterload column, and coronary effluent was collected from the pulmonary artery every 10 min for 1 min for a total of six volume measurements per heart. In addition, heart rate and the rate of increase and decrease (\pm dp/dt) of aortic pressure with each heart beat were measured with a Statham pressure transducer (model P23dB) attached to the aortic cannula. The analog signal from the pressure transducer was amplified with a SensorMedics R-611 polygraph (Anaheim, CA). The amplified signal was sampled at 250 Hz by a PO-NE-MAH digital data-acquisition and archiving system (Storrs, CT) and stored on disk for subsequent analysis. This included heart rate and aortic pressure measurements corresponding to the duration of each aortic and coronary volume measurement.

Perfusion medium. Hearts were perfused with Krebs-Henseleit bicarbonate buffer that was aerated with 95% O₂-5% CO₂ at 37°C (pH 7.4). Concentrations were as follows (in mM): 118 NaCl, 4.7 KCl, 2.25 CaCl₂, MgSO₄, 1.2 KH₂PO₄, 0.32 EGTA, 25 NaHCO₃, and 11 D-glucose.

Determination of the Ratio of Left Ventricular α/β -MHC mRNA

MHC mRNA analysis. Total RNA was isolated from left ventricular tissue obtained from male DA and BUF rats ($n = 6$ for each strain) at 13 wk of age. Tissue was homogenized in a guanidine thiocyanate-phenol solution (Ultraspec II, Biotecx, Austin, TX) at 1 ml/0.2 g tissue using a polytron (Brinkmann, PT 3000). The homogenate was extracted with chloroform, and the upper aqueous layer was removed, mixed with isopropanol (0.5 volumes), and 0.05 vol RNA Tack resin (Biotecx) was added. The resin was mixed, pelleted, and washed twice with 75% ethanol. RNA was eluted in diethyl pyrocarbonate-treated water. RNA concentration and quality was determined by absorbance at 260 and 280 nm. Only RNA samples having an A₂₆₀/A₂₈₀ ratio between 1.8 and 2.0 were used in this study. RNA integrity was monitored by electrophoresis in 1% agarose gels under denaturing conditions.

cDNA synthesis. cDNA was synthesized by the following procedure. Five micrograms total RNA was heat denatured

for 10 min at 70°C, chilled on ice, and reverse transcribed for 1 h at 42°C in a 20- μ l reaction containing 50 mM Tris·HCl, 75 mM KCl, 3 mM MgCl₂, 0.5 mM dNTP, 10 mM dithiothreitol, 0.5 μ g oligo (dT)₂₀, and 200 U Moloney Murine Leukemia Virus reverse transcriptase (Super Script II, GIBCO-BRL, Gaithersburg, MD). cDNAs were normalized within a linear PCR range using the housekeeping gene glyceraldehyde-3'-phosphate (GAPDH).

PCR amplification of MHCs. α - and β -MHC cDNAs were concomitantly amplified using a single primer set that was designed to bind to identical sequences in both MHC cDNAs. For α -MHC, this region spanned from nucleotide (nt) 4718 to nt 5060, and, for β -MHC, this region spanned from nt 4688 to nt 5030 (19). The resulting 342-bp PCR product was amplified using the following primer set: 5'-CGA GCC CAG CTG GAG TTC AA and 5'-CGA TGG CGA TGT TCT CCT TC. Equal amounts of normalized cDNA were PCR amplified in a total volume of 25 μ l containing 1.5 mM MgCl₂, 2.5 mM stock dNTP, 1 unit of *Taq* polymerase, and 10.0 pmol of each oligonucleotide primer. PCR reactions were overlaid with mineral oil, and 25 cycles of PCR were performed in a PTC-100-96VAg thermocycler (MJ Research, Watertown, MA) using the following program: 5 min at 94°C plus 25 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1.5 min. A final extension at 72°C for 5 min was included to ensure that all reactions were completed. The α - and β -MHC cDNA ratio was determined by restriction digestion of the amplified product with *Bst*X1. This digestion cleaved only the β -MHC cDNA (the restriction site located at nt 4912 in the β -MHC as numbered in the β -cDNA according to Ref. 19). PCR products were diluted to a volume of 50 μ l and digested for 1-2 h at 37°C with 40 units of *Bst*X1 (Gibco-BRL) according to the manufacturer's instructions. Overnight (16 h) *Bst*X1 restriction digestion products were not different from the 1- to 2-h digestion products. Restriction-digested DNA was fractionated electrophoretically on a 4% agarose gel and photographed using positive/negative film (Polaroid). Negatives were scanned using a Hewlett Packard Scan-Jet 3C, and the intensity of the signals was determined using National Institutes of Health Image Analysis software (National Center for Biotechnological Information, Bethesda, MD). Discernment of the α/β -MHC cDNA ratio was independent of the amount of cDNA amplified or the number of cycles used, because the primers were complementary to both cDNAs; the amplified PCR products were the same size and the PCR product sequences nearly identical. In addition, although not shown, α/β -mRNA ratios were measured using an RNase Protection Assay (RPA) kit (Ambion, Austin, TX). A 210-bp portion of the 3' untranslated region of β -MHC was amplified using the following primers (5'-CCAACACCAACCTGTCCAAGTTC, 5'-GGTGCTGTTTCAAAGGCTCCAG), cloned into the pCRII plasmid (Invitrogen, Carlsbad, CA), and used as a protection probe for α/β -MHC mRNAs. With this experimental design, only 126 bp of α -MHC mRNA was complementary to the 210-bp β -MHC probe.

MHC ATPase activity and MHC protein analysis. MHCs were isolated from left ventricular tissue obtained from male DA ($n = 6$) and BUF rats ($n = 6$) at 13 wk of age. Ventricular tissues were homogenized in 10 vol of extraction buffer of the following composition (in mM): 100 Na₄P₂O₇ (pH 8.8), 5 EGTA, and 2 β -mercaptoethanol at 2°C (11). Homogenates were centrifuged at 48,000 *g*, and the supernatant was mixed with an equal volume of glycerol (11). MHC ATPase was measured using the metal fluoride complex inhibition technique (24) on samples homogenized and centrifuged in sodium pyrophosphate extraction buffer (11). MHC ATPase

activity was measured in triplicate in the presence and absence of a metal fluoride by diluting the MHC solution 1:50 with a reaction buffer containing either 50 mM MOPS (pH 7.4), 5 mM ATP, 4 mM $MgCl_2$, or a trapping buffer containing 50 mM MOPS (pH 7.4), 5 mM ATP, 4 mM $MgCl_2$, 0.4 mM ADP, and 10 mM NaF (24) for 6 min at 37°C. Control reactions were treated identically to those incubated with reaction buffer, except they were maintained at 0°C to determine basal inorganic phosphate generated from the $Na_4P_2O_7$ extraction buffer. Reactions were terminated by the addition of 1 ml of 20% trichloroacetic acid, and inorganic phosphate was measured as the amount of heteropolymolybdenum at a wavelength of 660 nm as described by Fiske and Subbarow (7a). The final MHC ATPase activity was determined by subtracting trapped and controls values from reaction values.

MHC SDS-PAGE analysis. Left ventricular tissue was prepared as described by Blough et al. (4) and separated electrophoretically as described by Talmadge and Roy (27). In brief, six hearts from each strain were placed in a cold relaxing solution of the following composition (in mM): 2.0 EGTA, 4.4 Mg ATP, 10 imidazole, 1.0 Mg^{2+} , and 180 KCl, pH 7.00. Left ventricular tissue was dissected, weighed, and placed in 10 vol glycerated relaxing solution that was prepared by adding 50% (vol/vol) glycerol and leupeptin (5 μ l/ml) to the relaxing solution. Samples were homogenized on ice for 30 s and mixed 1:40 with sample preparation buffer containing the following: 8 M urea, 2 M thiourea, 0.05 M Tris base, 0.075 M β -mercaptoethanol, 3% (wt/vol) SDS, and 0.004% (wt/vol) bromophenol blue, at pH 6.8 (4). Samples were heated for 5 min at 100°C before loading onto gels. SDS-PAGE was performed on 0.75-mm-thick vertical slab gels (Mini-Protean II Dual Slab cell electrophoretic system, Bio-Rad, Hercules, CA) as described by Talmadge and Roy (27). The stacking gels consisted of 30% glycerol, 4% acrylamide-*N,N'*-methylene-bis-acrylamide (50:1), 70 mM Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. The resolving gels were composed of 30% glycerol, 8% acrylamide-*N,N'*-methylene-bis-acrylamide (50:1), 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% SDS. The upper tank buffer consisted of 0.1 M Tris (base), 150 mM glycine, and 0.1% SDS. The lower buffer consisted of 50 mM Tris (base), 75 mM glycine, and 0.05% SDS. The pH of the buffers was not adjusted. One to five microliters of sample was loaded into each well and run overnight (16 h) at 4°C under constant voltage (70 V). After electrophoresis, the gels were stained for 3 h at 50°C using Coomassie brilliant blue (R250, Bio-Rad). The α/β -MHC ratio for each strain was determined by scanning the gels and analysis using the NIH-Image program using a Hewlett-Packard Scan-Jet 3C/T scanner.

Data Analyses

Hemodynamic parameters, isoform percentages, and MHC ATPase activities were initially tested for homogeneity of variance using a Levene test. Statistical significance for hemodynamic parameters and isoform percentages were determined using either a Student's *t*- or Wilcoxon Mann-Whitney test. Significant differences in MHC ATPase activity were determined using a one-way analysis of variance. All statistical tests were performed using SPSS statistical software. The 1% level of confidence was arbitrarily used for assigning a difference as significant, and data are presented as means \pm SE.

RESULTS

DA hearts produced an average output of 62 ± 3 $ml \cdot min^{-1} \cdot g$ heart wt^{-1} and BUF hearts produced an average output of 38 ± 2 $ml \cdot min^{-1} \cdot g$ heart wt^{-1} . This represents a 63% difference in intrinsic cardiac output between DA and BUF rats ($P < 0.01$).

The DA rats had coronary flows that averaged 25.5 ± 1 $ml \cdot min^{-1} \cdot g$ heart wt^{-1} tissue and were not statistically different from those of BUF rats, which had coronary flows that averaged 22.8 ± 1 $ml \cdot min^{-1} \cdot g$ heart wt^{-1} . The mean intrinsic heart rates of DA rats (304 ± 3 beats/min) and BUF rats (303 ± 5 beats/min) were also not statistically different. There was, however, a significant difference in average stroke volume between these two strains. DA hearts ejected 202 ± 10 $\mu l \cdot beat^{-1} \cdot g$ heart wt^{-1} , whereas BUF hearts ejected 126 ± 6 $\mu l \cdot beat^{-1} \cdot g$ heart wt^{-1} ($P < 0.01$).

The rate of rise in aortic pressure (+dP/dt) produced by left ventricular ejection was significantly ($P < 0.01$) faster for DA hearts compared with BUF hearts ($+1,042 \pm 32$ vs. $+708 \pm 40$ mmHg/s). The rate of aortic pressure diminution during diastole ($-dP/dt$) was also significantly ($P < 0.01$) faster for DA hearts compared with BUF hearts (-925 ± 60 vs. -632 ± 70 mmHg/s).

Figure 1A shows a representative acrylamide gel demonstrating the separation of left ventricular α - and β -MHC from BUF and DA. Figure 1B illustrates the

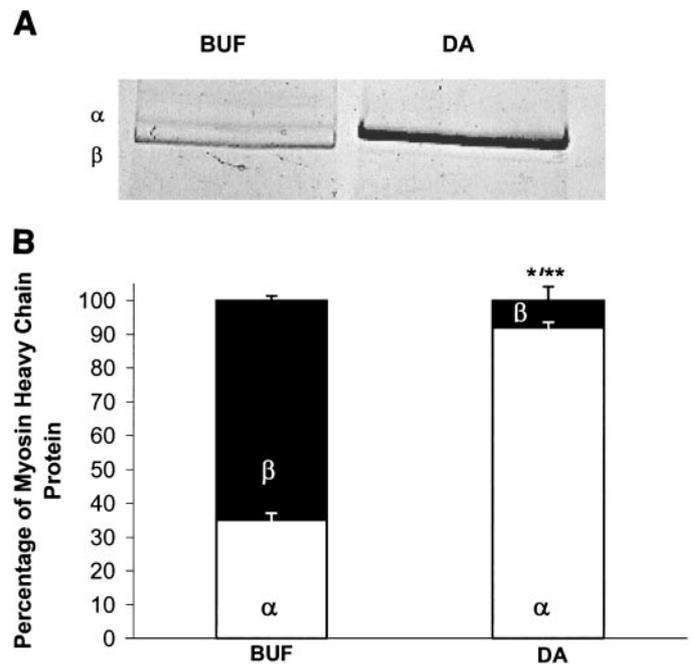


Fig. 1. The relative percentage of α - and β -myosin heavy chain (MHC) protein in the left ventricle of Buffalo (BUF) and DA rats. A: representative polyacrylamide gel demonstrating the separation of α - and β -MHC protein. B: relative percentage of α - and β -MHC as determined by densitometry from Coomassie-stained gels. Bars represent the average percent from 6 male ventricular heart samples from each strain. Error bars represent SE. *Significance relative to the α -MHC BUF value; **significance relative to the β -MHC BUF value as determined by a *t*-test; $P < 0.01$.

average relative percentage of α - and β -MHC protein expressed in six BUF and six DA left ventricular tissue samples. For DA left ventricular tissue, α -MHC represents $92 \pm 1.1\%$ of all MHC protein present in the left ventricle. Conversely, for BUF left ventricular tissue, α -MHC represents $36 \pm 2.1\%$ of the total amount of expressed MHC. Therefore, the ratio of cardiac α/β -MHC for BUF and DA was 0.56 and 11.5, respectively ($P < 0.01$).

Figure 2A illustrates a representative 4% agarose gel demonstrating the fractionation of steady-state α - and β -mRNAs after PCR product digestion with *Bst*X1. For DA rats, the percentage of steady state α -MHC mRNA found in the left ventricle ($94.5 \pm 0.63\%$) was similar to the percentage of α -MHC protein ($92 \pm 1.1\%$). In addition, steady-state β -MHC mRNA ($5.5 \pm 0.66\%$) was

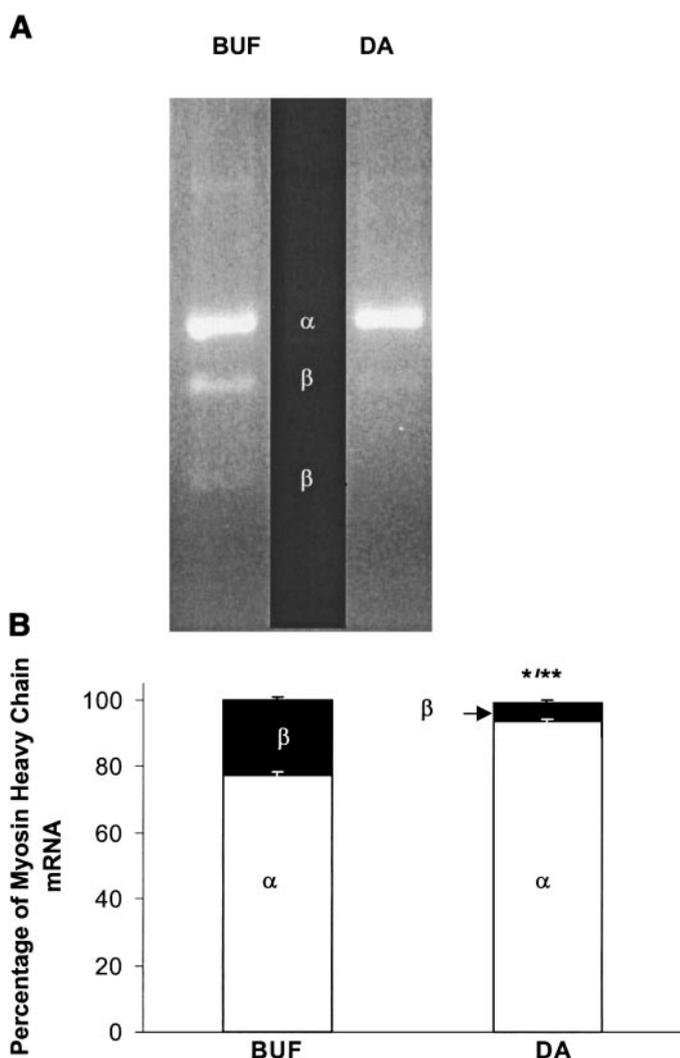


Fig. 2. The relative percentage of α - and β -MHC mRNA in the left ventricle of BUF and DA rats. A: representative 4% agarose gel demonstrating the separation of amplified α - and β -MHC after complete digestion using *Bst*X1. B: relative percentage of α - and β -MHC as determined by densitometry from ethidium bromide-stained gels. Bars represent the average percent from ventricular heart samples from 6 males of each strain. Error bars represent SE. *Significance relative to the α -MHC BUF value; ***significance relative to the β -MHC BUF value as determined by a *t*-test; $P < 0.01$.

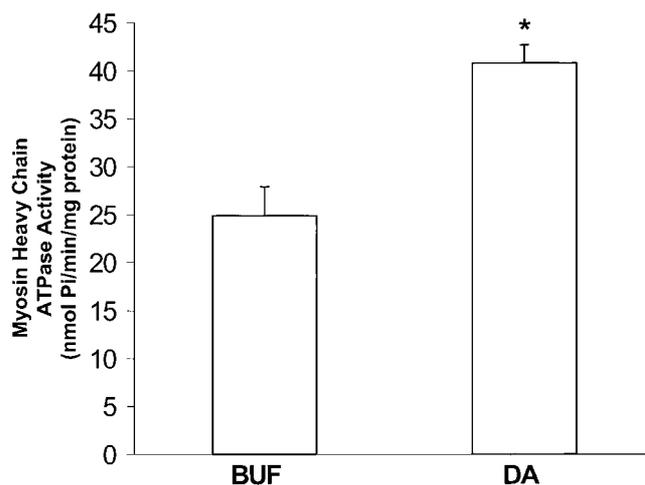


Fig. 3. Mean MHC ATPase activity from 6 BUF and 6 DA rats. Error bars represents the SE. *Significance as determined by Student's *t*-test; $P < 0.01$.

also similar to the percentage of β -MHC protein ($8.0 \pm 1.0\%$). Conversely, for BUF ventricular samples, the percentage of steady state α -MHC mRNA ($77.5 \pm 0.78\%$) was disproportionate to the percentage of α -MHC protein ($36 \pm 2.1\%$). In addition, the percentage of β -MHC steady-state mRNA ($22.5 \pm 0.8\%$) did not correspond to the percentage of β -MHC protein ($64 \pm 0.5\%$). Nevertheless, BUF ventricles had significantly higher steady-state β -MHC mRNA levels compared with DA (22.5 ± 0.8 vs. $5.48 \pm 0.66\%$). Therefore, similar to the α/β -MHC protein ratio, a significant difference ($P < 0.01$) in the steady-state α/β -MHC mRNA ratio between the BUF and DA ventricles was observed (3.4 and 17.1, respectively). The relative amounts of α and β , as determined by RPA, were comparable with those obtained using RT-PCR (data not shown).

Figure 3 illustrates the mean rate of ATP hydrolysis by MHCs taken from left ventricular tissue from male DA and BUF rats ($n = 6$ for each strain). On average, MHC ATPase from DA hearts hydrolyzed ATP at a rate significantly faster ($P < 0.01$) than MHC ATPase derived from BUF rats. Therefore, the difference in MHC ATPase activity between BUF and DA is consistent with difference in α/β -MHC protein present in the left ventricles of these strains (Fig. 1).

DISCUSSION

This study evaluated physiological and biochemical pathways that may account for the difference in isolated cardiac function between the BUF and DA strains of rats. More importantly, this study further characterized these strains as potential contrasting genetic models of myocardial performance. First, at the organ physiological level, we confirmed our previous observation (2) that hearts from DA rats produce a greater output per gram of tissue at a constant preload (15 mmHg) and afterload (70 mmHg) compared with hearts from BUF rats (1.6-fold difference; $P < 0.01$). Separating cardiac output into its components of coro-

nary flow, heart rate, and stroke volume revealed that differences in isolated cardiac output were due to differences in stroke volume between hearts from the two strains. Moreover, because cardiac output was measured under constant preload and afterload conditions (i.e., no change in initial fiber length) (3, 14), the differences in cardiac output were interpreted as differences in intrinsic contractility.

Although myocardial contractility is an aggregate of multiple factors, it is determined primarily by intrinsic mechanical properties related to generation of force and velocity (10, 14). Specifically, myocardial performance is influenced by 1) the number of cross bridges formed between actin and myosin and 2) the velocity at which high-energy phosphates are converted into mechanical energy by MHC ATPase (14, 21). During isolated working heart conditions, DA hearts had faster inotropic and lusitropic velocities compared with BUF hearts. Nonetheless, it is important to note that due to the limiting characteristics of our system (i.e., elastic and inertial factors) and the resultant dynamic error caused by these factors, the inotropic and lusitropic velocities obtained underestimate the absolute velocities. However, irrespective of this, detectable differences in the relative inotropic and lusitropic velocities were observed. Therefore, because the MHC-ATPase affects cross-bridge cycling (14), it is a logical candidate to explain the relative differences in inotropy and lusitropy between the strains. Indeed, the 1.64-fold higher MHC ATPase activity in DA hearts, compared with BUF, should result in more actomyosin cycles per unit time and produce a greater contractility.

Although a measurable amount of α -MHC mRNA was transcribed in the hearts of BUF rats, it was not translated into a proportionate amount of α -MHC protein in this strain (Fig. 1). This suggests that for the BUF strain, there is a difference at either the posttranscriptional and/or translational level that is responsible for the incongruity between steady-state mRNA and protein. The functional extension of this divergence is evidenced by the significant difference found at the protein level (Fig. 1) and the corresponding difference in MHC ATPase activity observed between these inbred strains (Fig. 3). This partly explains the divergence in intrinsic cardiac function between BUF and DA because the MHC ATPase reaction will affect the cycling of actomyosin.

Numerous studies have demonstrated that α -MHC is associated with enhanced myocardial contractility (11, 28, 29) and β -MHC is associated with decreased contractility (18, 20, 28). Specifically, shifts from α -MHC to β -MHC have been reported in rat left ventricular tissue secondary to a variety of conditions, including diabetes mellitus (18, 28), hypothyroidism (20), hypertension (25), aging (5), experimental aortic banding (13), and thyroidectomy (13). In contrast, reversion from β -MHC to α -MHC has been demonstrated in rats subsequent to aerobic exercise conditioning (29), palmitoylcarnitine transferase I blockade (28), and orchietomy (28). In humans, recent studies have demonstrated that decreased myocardial performance

in the failing heart was associated with α - to β -MHC conversion (13, 17). Although these above described conditions are known to produce shifts in the prevalence of the α - and β -MHC isoforms, natural variation of these cardiac MHC isoforms has not been previously described in age-matched animals. For normal adult rats (9–15 wk old), α -MHC makes up >90% of all left ventricular MHCs (13, 17), a value comparable to the percentage found in the DA strain. However, the percentage of α -MHC found in the BUF left ventricle is considerably less than expected for a 13-wk-old rat. Therefore, these differences are important because they represent a natural, presumably heritable, variation in MHC expression that differs from previously described variances in α/β -MHC expression known to occur secondary to environmental alterations such as exercise conditioning (12), diabetes (8), high-carbohydrate diet (26), thyroidectomy (13), and gonadectomy (9).

Perspectives

The significance of this work relates to recent reports of α/β -MHC conversion in the human failing heart (17). Lowes and colleagues (17) observed that human non-failing hearts expressed significant amounts of α -MHC. In addition, Lowes demonstrated that the majority of α -MHC was converted to β -MHC as a consequence of heart failure (17). Therefore, α/β -MHC conversion is a likely candidate responsible for decreased systolic myocardial function in the failing heart (17). However, the mechanism responsible for this conversion is unknown. Therefore, identifying inbred animal models with a natural, heritable disparity in α/β -MHC isoform expression, may help identify the mechanism(s) influencing MHC isoform prevalence. For example, the BUF rat strain may carry genes that specifically reduce the prevalence of α -MHC and could help define risk factors in humans. Therefore, because the BUF and DA rats strains have contrasting phenotypes for myocardial function, they provide a suitable substrate for studying the molecular mechanism(s) underlying the pathophysiology of the failing heart. Furthermore, understanding the natural, heritable disparity in MHC isoform expression between these strains may lead to the development of novel therapeutic strategies for the failing heart.

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REFERENCES

1. **Baldwin JE and Krebs H.** The evolution of metabolic cycles. *Nature* 291: 381–382, 1981.
2. **Barbato JC, Koch LG, Darvish A, Cicila GT, Metting PJ, and Britton SL.** Spectrum of aerobic endurance running performance in eleven inbred strains of rats. *J Appl Physiol* 85: 530–536, 1998.
3. **Berne RM and Levy MN.** *The Cardiovascular System*. St. Louis: Mosby, 1998.

4. **Blough ER, Rennie ER, Zhang F, and Reiser PJ.** Enhanced electrophoretic separation and resolution of myosin heavy chains in mammalian and avian skeletal muscles. *Anal Biochem* 233: 31–35, 1996.
5. **Boluyt MO, Bing OH, and Lakatta EG.** The ageing spontaneously hypertensive rat as a model of the transition from stable compensated hypertrophy to heart failure. *Eur Heart J* 16: 19–30, 1995.
6. **Bouchard C, Lesage R, Lortie G, Simoneau JA, Hamel P, Boulay MR, Perusse L, Theriault G, and LeBlanc C.** Aerobic performance in brothers, dizygotic and monozygotic twins. *Med Sci Sports Exerc* 18: 639–646, 1986.
7. **Britton SL and Koch LG.** Animal genetic models for complex traits of physical capacity. *Exerc Sport Sci Rev* 29: 7–14, 2001.
- 7a. **Fiske CH and Subbarow Y.** The colorimetric determination of phosphorous. *J Biol Chem* 66: 375–400, 1925.
8. **Goldspink PH, Thomason DB, and Russell B.** Beating affects the posttranscriptional regulation of α -myosin RNA in cardiac cultures. *Am J Physiol Heart Circ Physiol* 271: H2584–H2590, 1996.
9. **Hajjar RJ and Gwathmey JK.** Cross-bridge dynamics in human ventricular myocardium. *Circulation* 86: 1819–1826, 1992.
10. **Harrison TR and Fauci AS.** *Harrison's Principles of Internal Medicine*. New York: McGraw-Hill, Health Professions Division, 1998.
11. **Hoh JF, McGrath PA, and Hale PT.** Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. *J Mol Cell Cardiol* 40: 1053–1076, 1977.
12. **Ito H, Miller SC, Billingham ME, Akimoto H, Torti SV, Wade R, Gahlmann R, Lyons G, Keddes L, and Torti FM.** Doxorubicin selectively inhibits muscle gene expression in cardiac muscle cells in vivo and in vitro. *Proc Natl Acad Sci USA* 87: 4275–4279, 1990.
13. **Izumo S, Lompre AM, Matsuoka R, GK, and Schwartz K.** Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy. Interaction between hemodynamic and thyroid hormone-induced signals. *J Clin Invest* 79: 970–977, 1988.
14. **Katz AM.** *Physiology of the Heart*. New York: Raven, 1994.
15. **Klissouras V.** Heritability of adaptive variation. *J Appl Physiol* 31: 338–344, 1971.
16. **Koch LG and Britton SL.** Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol Genomics* 5: 45–52, 2001.
17. **Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeyer TJ, Quaife RA, Roden RL, Dutcher DL, Robertson AD, Voelkel NF, Badesch DB, Groves BM, Gilbert EM, and Bristow MR.** Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *J Clin Invest* 100: 2315–2324, 1997.
18. **Malhotra A and Lopez MC.** Troponin subunits contribute to altered myosin ATPase activity in diabetic cardiomyopathy. *Mol Cell Biochem* 151: 165–172, 1995.
19. **McNally EM, Kraft R, Bravo-Zehnder M, Taylor DA, and Leinwand LA.** Full-length rat alpha and beta cardiac myosin heavy chain sequences. Comparisons suggest a molecular basis for functional differences. *J Mol Biol* 210: 665–671, 1989.
20. **Mercadier JJ, Lompre AM, Wisniewsky C, Samuel JL, Bercevic J, Swynghedauw B, and Schwartz K.** Myosin isoenzyme changes in several models of rat cardiac hypertrophy. *Circ Res* 49: 525–532, 1981.
21. **Metzger JM, Wahr PA, Michele DE, Albayya F, and Westfall MV.** Effects of myosin heavy chain isoform switching on Ca^{2+} -activated tension development in single adult cardiac myocytes. *Circ Res* 84: 1310–1317, 1999.
22. **Neely JR, Liebermeister H, Battersby EJ, and Morgan HE.** Effect of pressure development on oxygen consumption by the isolated rat heart. *Am J Physiol* 212: 804–814, 1967.
23. **Nisbet EG and Sleep NH.** The habitat and nature of early life. *Nature* 409: 1083–1091, 2000.
24. **Park S, Ajtai K, and Burghardt TP.** Inhibition of myosin ATPase by metal fluoride complexes. *Biochim Biophys Acta* 1430: 127–140, 1999.
25. **Raizada V, Pathak D, Skipper B, Nakouzi A, and Malhotra A.** Age-related difference in cardiac adaptation to chronic hypertension in rats, with and without nifedipine treatment. *Mol Cell Biochem* 198: 109–112, 1999.
26. **Sindwhani R, Ismail-Beigi F, and Leinwand LA.** Post-transcriptional regulation of rat a-cardiac myosin heavy chain gene expression. *J Biol Chem* 269: 3272–3276, 1994.
27. **Talmadge RJ and Roy RR.** Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J Appl Physiol* 75: 2337–2340, 1993.
28. **Turcani M.** Etomoxir improves left ventricular performance of pressure-overloaded rat heart. *Circulation* 96: 3681–3686, 1997.
29. **Wade ME, Herb RA, Powers SK, and Criswell D.** Exercise and beta-adrenergic regulation of rat cardiac myosin isoforms. *J Sports Med Phys Fitness* 39: 42–46, 1999.
30. **Wagner PD.** New ideas of limitation to VO_2max . *Exerc Sport Sci Rev* 28: 10–14, 2000.
31. **Wagner PD, Hoppeler H, and Saltin B.** Determinants of maximal oxygen uptake. In: *The Lung, Scientific Foundations*, edited by Crystal RG, West JB, Weibel ER, and Barnes PJ. Philadelphia: Lippincott-Raven, 1997, p. 2033–2041.
32. **Williams S and Neuffer PD.** Regulation of gene expression in skeletal muscle by contractile activity. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD: Am Physiol Soc, 1996, sect. 12, chapt. 25, p. 1124–1150.
33. **Zini R, Morin C, Bertelli A, Bertelli AA, and Tillement JP.** Effects of resveratrol on the rat brain respiratory chain. *Drugs Exp Clin Res* 25: 87–97, 1999.