Evaluating spiny mice (Acomys) as a model for cardiac research

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Abstract

Complex tissue regeneration is extremely rare among adult mammals. An exception, however, is the superior tissue healing of multiple organs in spiny mice (*Acomys*). While *Acomys* species exhibit the remarkable ability to heal complex tissue with minimal scarring, little is known about their cardiac structure and function. In this study, we characterized cardiac structure, anatomy, and *in vivo* function, as well as cardiomyocyte characteristics in *Acomys* compared to the most commonly used cardiac mouse model, the C57BL6 mouse strain (*Mus*). Our results demonstrate comparable cardiac anatomy, structure and function between the two rodent species, but reveal significant differences in their cardiomyocyte characteristics. These findings establish *Acomys* as a new mammalian model for cardiac research.
Introduction

Significant clinical advances in revascularization and medical therapies have improved the mortality rate after myocardial infarction (MI). Unfortunately, there are no therapies to limit myocardial damage during MI and millions of patients progress to develop heart failure (HF). This is because the normal healing response to tissue injury in adult mammals is fibrotic repair; an effective short-term strategy, but one that leads to loss of tissue function. Following injury to the heart, a limited ability to replace functional cardiomyocytes results in irreversible damage, scar formation, and compromised heart function. These events will ultimately lead to HF. Although robust cardiac regeneration occurs in some adult vertebrates (e.g., zebrafish, salamanders, newts, etc.) and neonate mammals, adult mammalian hearts exhibit poor regenerative capacity. The lack of adult mammalian models demonstrating cardiac regeneration has contributed to the current knowledge gap in the field.

The ability to regenerate injured organs is widespread in the animal kingdom. Fishes, newts, and salamanders have extensive regenerative ability, and can functionally replace most tissues and organs after amputation. Specifically, zebrafish have become a well-characterized model for adult cardiac regeneration with the documented ability to recover from a plethora of heart injuries including apical resection, cryo-injury, and coronary artery ligation. In contrast, most adult mammals generally exhibit poor regenerative capacity, especially as it pertains to recovering from heart damage. Spiny mice (*Acomys spp.*) are murid rodents found throughout Africa, the Middle East and Western Asia. These rodents exhibit a number of special traits, tantamount among them is the ability to regenerate skin and complex tissue. Moreover, a recent study examining acute and chronic kidney injury documented enhanced cytoprotection in *Acomys cahirinus* compared to two laboratory mouse strains. At present, the extent to which spiny mice respond to heart injury remains unknown. In order to characterize the heart injury response in a comparative framework, it is imperative to accurately determine *Acomys*
heart structure and function. Here, we report detailed cardiac characterization of *A. cahirinus* in comparison to the commonly used C57BL6 mouse strain. The results of this study demonstrate comparable cardiac anatomy, structure and function between *Acomys* and *Mus* hearts but differences in cardiomyocyte phenotype between species.

**Methods**

**Animal care**

Male *Acomys cahirinus* (6-8 months old, in-house breeding colony) and *Mus musculus* (C57Bl6, 8-12 weeks old, Jackson Laboratory, Bar Harbor, ME) were housed at the University of Kentucky, Lexington, KY. *Acomys* were housed individually in metal wire cages (Quality Cage Company, Portland, OR) and fed a 3:1 mixture by volume of 14% protein mouse chow (Teklad Global 2014, Harlan Laboratories, Indianapolis, IN) and black-oil sunflower seeds (Pennington Seed Inc., Madison, GA).

All animal procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC #: 2019-3254 and 2011-0889).

**Echocardiography**

Echocardiography was acquired with a Vevo 3100 (VisualSonics, Toronto, Canada) equipped with a MX550D 25-55 MHz linear array transducer. Baseline cardiac function was assessed as previously described. A heating pad and rectal temperature probe were utilized to keep body temperature at 37°C during the experiment. Modified parasternal long-axis and short-axis was utilized to determine left ventricular function and volume in M-mode, two-dimensional and Doppler echocardiography modes. We also used M-mode tracings at the mid-papillary level to estimate the systolic and diastolic parameters, and Teichholz formula at end-systole and end-diastole to measure the left ventricle (LV) volumes. All mice were
anaesthetized using 1%–3% isoflurane during Echocardiography to maintain a heart rate of 450–500 BPM for all echocardiographic acquisitions. Echocardiographic imaging and analysis were performed by a blinded investigator.

**Coronary anatomy**

Mice received an i.p. injection of heparin (100 units in 300ul sterile PBS) to prevent coagulation. Ten minutes later, mice were injected with TRITC-Dextran according to the method of Hoffmann et al (2011). Briefly, mice were anesthetized and 0.5ml of TRITC-Dextran (Sigma # 42874, 40 kDa, 100mg/ml) was injected via retro-orbital route and the animal returned to anesthesia. After two minutes, mice were euthanized via cervical dislocation and the heart excised for imaging using the Maestro EX *in vivo* Imaging System (Cambridge Research & Instrumentation Inc., USA) using a blue filter (λex 500 nm; λem 750 nm long in 10-nm steps). Images were imported into Nikon AR (Version 4.51.01) for cropping and scale bar representation.

In a separate cohort, mice received an i.p. injection of heparin (100 units in 300ul sterile PBS) to prevent coagulation. Ten minutes later, under isoflurane anesthesia, hearts were excised and cannulated with a blunt tip 23g needle and clamp. 10 ml of 1x PBS was perfused intra-aortically, followed by 1.5ml Batson’s 17 polymer mixture (2.5ml base monomer + 600ul Catalyst + 3 drops Promoter; Polysciences). Polymer was mixed and ten minutes later was injected over five minutes and then left to cure on ice for 3 hours. Once cured, hearts were briefly washed with ddH2O and then placed in potassium hydroxide (maceration solution) for one hour at room temp. After maceration incubation, hearts were laid with the anterior side up and the left anterior descending artery was imaged using a Nikon Camera (DS-12) after application of several drops of ddH2O to clear overlying tissue.
Tissue collection and Histology

Tissue from all species were collected and weighed immediately at sacrifice. The dry lung weight was collected after 3 days of 65°C incubation. Hearts were perfused with PBS (VWR International) followed by 4% buffered formalin (VWR International) fixation via cannulation of the ascending aorta. Hearts were post-fixed overnight at 4°C. Hearts were then sectioned into 3-mm cross-sectional slices followed by paraffin embedding. Tissue was transferred to 70% ethanol until sectioning into 4-μm sections starting at the level of papillary muscle.

Immunofluorescence

Immunohistochemical assessments were carried out on deparaffinized and rehydrated sections as previously described. After deparaffinization, washing, TRIS heat induced epitope retrieval, and protein and streptavidin/biotin blocking, slides were incubated with primary antibody: Isolectin B4 (L-2140, Sigma, St. Louis, MO) overnight at 4°C. After washing, sections were incubated with secondary antibody conjugated to streptavidin-Alexa Fluor 568 (S11226, Thermo Fisher Scientific, Waltham, MA) for 30 minutes, and cover slipped with mounting medium containing DAPI counterstain (Vector #H-1200). 10–20 adjacent areas per section were examined at 40x magnification using Nikon A1 Confocal Microscope in the University of Kentucky Confocal Microscopy facility. Data were presented as total capillary density per mm².

Cardiomyocyte isolation

Ventricular cardiomyocytes were isolated as previously described. Briefly, mice received an i.p. injection of heparin (0.3ml of 1000 units/ml) prior to sacrifice. Mice were then anesthetized with 1–3 % Isoflurane. Hearts were excised and immediately perfused on a Langendorff apparatus with a high-potassium Tyrode buffer and then digested with 5 to 7 mg
of liberase (Roche Applied Science). After digestion, atria were removed, and ventricular myocytes were mechanically dispersed. Some isolated ventricular cardiomyocytes were used for measuring cell surface area and nuclei, and the others were used for electrophysiological recordings and calcium transients. Calcium concentrations were gradually restored to physiological levels in a stepwise fashion for electrophysiological studies, and only healthy quiescent ventricular myocytes were used for electrophysiological analysis within 12 h.

**Cell surface area, t-tubule quantification, and nuclear number**

Isolated ventricular cardiomyocytes were incubated with primary antibodies: Troponin T (MA512960, Thermo Fisher Scientific, Waltham, MA). After washing, cardiomyocytes were incubated with secondary antibody conjugated to Alexa Fluor 594 (ab175700, Abcam, Cambridge, MA) and Hoechst (H3570, Thermo Fisher Scientific, Waltham, MA) nuclear counterstain. 100 cardiomyocytes per animal were examined using Nikon ECLIPSE E600 Microscope. For t-tubule quantification, live ventricular cardiomyocytes were stained with Di-8-ANNEPES for 30 min to stain the cell membrane. AutoTT was used to measure t-tubule density[^20].

**Ploidy measurement**

Cardiomyocyte ploidy analyses were done with modification.[^21] Isolated ventricular cardiomyocytes were enriched by centrifugation at 3000 RPM for 1 minute and resuspended in PBS supplemented with 1% goat serum. DAPI was added to the cell suspension (1:10,000 dilution) 3 mins prior to FACS. Cardiomyocyte sorting was performed on a cell sorter system (iCyt-Sony) at room temperature. Nucleation was gated on height and width of signal from a 380 nm laser. Spleen cells from each species were also collected and sorted as diploid control.
Electrophysiological recordings and calcium transients

$\text{I}_{\text{Ca,L}}$ was recorded in the whole-cell configuration of the patch clamp technique as previously described. All recordings were performed at room temperature (20 to 22 °C). The pipette solution consisted of (in mmol/liter) 125 Cs-methanosulfonate, 15 TEA-Cl, 1 MgCl2, 10 EGTA, and 5 Hepes, 5 MgATP, 5 phosphocreatine, pH 7.2. Bath solution contained (in mmol/liter) 140 NaCl, 5.4 KCl, 1.2 KH2PO4, 5 Hepes, 5.55 glucose, 1 MgCl2, 1.8 CaCl2, pH 7.4. Once a cell was successfully patched, zero sodium bath solution was introduced into the chamber (mmol/liter) 150 N-methyl-D-glucamine, 2.5 CaCl2, 1 MgCl2, 10 glucose, 10 Hepes, 4-amino-pyridine, pH 7.2. Recordings of isoproterenol response were recorded in zero sodium bath solution containing 300 nM isoproterenol. $\text{I}_{\text{Ca,L}}$ was recorded from a holding potential ($V_{\text{hold}}$) of -50mV. $\text{I}_{\text{Ca,T}}$ and $\text{I}_{\text{Ca,L}}$ was recorded from $V_{\text{hold}}$ -80mV with 300ms depolarization steps to levels as shown in Figure 4.

Calcium transients were recorded from ventricular cardiomyocytes loaded with cell permeable Fura-2-AM (Invitrogen). Cardiomyocytes were field stimulated at 1.0 Hz to determine transient amplitude, upstroke velocity, and rate of decay. All measurements were made following >2 minutes of conditioning of 1 Hz-field stimuli to induce steady state. Transients were recorded at 1 Hz. All Ca$^{2+}$ transient / sarcomere dynamic data were analyzed using IonOptix IonWizard 6.3 (IonOptics Inc., Westwood, MA). Background fluorescence (Fbackground) for F380 and F340 were determined from cell-free regions. Data are expressed as F340/380 and were corrected for Fbackground.

Statistics

Values are expressed as mean ± standard error of mean (SEM). We used unpaired Student t test to estimate differences, as appropriate. Throughout the analyses, a $P$ value < 0.05 was considered statistically significant. All statistical analyses were performed using the Prism
software package (GraphPad, La Jolla, CA).
Results

Heart structure and coronary tree anatomy are similar between *Acomys* and *Mus*

To establish a basis for accurate comparison across species, we first set out to determine whether baseline differences in cardiac gravimetrics exist between *Acomys* and *Mus*. First, we assessed heart weight (HW) and normalized it to body size (body weight [BW] and tibia length [TL]) to account for overall size differences between species. Although *Acomys* demonstrated a higher mean HW than *Mus* (Figure 1B), normalized to BW, *Acomys* exhibited a slightly lower HW than *Mus* (Figure 1C). There were no differences between species when HW was normalized to TL (Figure 1D). *Acomys* demonstrated heavier wet and dry lung weight than *Mus* (Figure 1E and F), but lower wet and dry lung weight after normalization by BW (Figure 1G and H). However, when normalized by TL, dry and wet lung weight were similar across species (Fig 1I and J). The descriptive analyses are summarized in supplemental table 1.

Next, we compared the gross cardiac structure, coronary tree and vasculature between *Acomys* and *Mus*. Overall, cardiac structure was not visibly different between the species both on gross inspection of the whole heart, short or long axis (Figure 2A) and both species demonstrated similar coronary course with the left anterior descending artery (LAD) supplying the anterior wall and the left circumflex artery supplying the lateral aspect of the left ventricle (Figure 2B-D). Vascular density plays an important role in tissue healing and integrity. Therefore, we assessed vascular density using isolectin staining and observed no significant difference in capillary density between *Mus* and *Acomys* (Figure 2E, F). The descriptive analyses are summarized in supplemental table 1.

*Mus* and *Acomys* have comparable baseline heart function

Building on our anatomical analyses of *Acomys* and *Mus* hearts, we next performed
cardiac echocardiography to compare in vivo cardiac function between species. The ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular posterior wall thickness (LVPW), and stroke volume were comparable between Acomys and Mus (Figure 3A-D, F). Left ventricular anterior wall thickness (LVAW) was higher in Acomys when compared to Mus (Figure 3E). However, when normalized to body weight, LV mass and stroke volume were smaller in Acomys (Figure 3G, H). These data support our anatomical data and demonstrate that in vivo cardiac function and structure are comparable between species. Furthermore, our functional data provide a baseline for future studies utilizing Acomys as a model for cardiac research in comparison to Mus. The descriptive analyses are summarized in supplemental table 1.

Acomys cardiomyocytes resemble less mature adult mouse cardiomyocytes

Ventricular cardiomyocyte (CM) phenotype varies across ages and species and plays an important role in cardiac response to injury. During the first week of post-natal life, cardiomyocytes undergo a final burst of DNA replication with and without karyokinesis and therefore most CMs after the first post-natal week are either mononuclear polyploid or multinucleated. In mammals, the presence of mononuclear diploid CMs are indicative of a ‘young’ heart phenotype that is rarely seen in adult mice. To examine CM phenotype between species, we isolated ventricular CMs and stained them with cardiac troponin T (cTnT) and Hoechst. Acomys CMs were smaller than Mus and were more likely to be mononucleated (Figure 4A-C). Acomys CMs also showed reduced t-tubule density and organization compared to Mus (Figure 4D-G). We then assessed the ploidy of ventricular CMs across species using flow cytometry as previously described. Our studies demonstrate a significantly higher percentage of diploid CMs in Acomys with corresponding lower percentage of tetraploid and...
>4N CMs (Figure 4H).

The relatively small cell size and higher mononucleated percentage in *Acomys* suggest a ‘young’ cardiomyocyte phenotype. The expression of low-voltage activated (T-type calcium channels) is a hallmark index of relatively immature ventricle; therefore, we recorded voltage-dependent I$_{\text{Ca}}$ from *Acomys* ventricular myocytes. From a $V_{\text{hold}}$ -80mV, a $V_{\text{test}}$ step to -25mV elicits little if any discernable current in *Mus* (Figure 5A, left); by contrast, for $V_{\text{test}}$ -25mV *Acomys* cardiomyocytes exhibit a transient ‘T-type’ calcium current (I$_{\text{Ca,T}}$). Longer lasting calcium current (I$_{\text{Ca,L}}$) is prominent with larger depolarizations in both *Mus* and *Acomys* (Figure 5A, lower panels). The current-voltage relationship shows a prominent I$_{\text{Ca,T}}$ component with a peak current between $V_{\text{test}}$ -20 and -25mV in *Spiny*, not in *Mus* (Figure 5B).

To dissect I$_{\text{Ca,T}}$ from I$_{\text{Ca,L}}$, we performed current-voltage curve protocols from $V_{\text{hold}}$ -50mV to voltage-inactivate the I$_{\text{Ca,T}}$ component. Current-voltage relationships for I$_{\text{Ca,T}}$-expressing Spiny cells show the prominent I$_{\text{Ca,T}}$ appearing as a low-voltage activated current for $V_{\text{hold}}$ -80mV but not from $V_{\text{hold}}$ – 50 mV (Figure 6A). 33% of *Acomys* ventricular cardiomyocytes show no I$_{\text{Ca,T}}$ (Figure 6B) but there was no observed correlation of cell size to presence of I$_{\text{Ca,T}}$ (Figure 6C). I$_{\text{Ca,T}}$ is present in 67% of *Acomys*, compared to <5% of *Mus* ventricular cardiomyocytes (Figure 6D). Cell capacitance was significantly lower in *Acomys* compared to *Mus* (Figure 6E), consistent with morphometric analysis (above, Figure 4B).

Additionally, maximal conductance density trended greater in *Mus* than *Acomys* (p=0.06, Figure 6F). Voltage-dependent activation and inactivation of I$_{\text{Ca,L}}$ was significantly shifted positive for Acomys compared to *Mus* (Figure 6G and H). Taken together these data are consistent with a ‘young’ adult heart physiological phenotype in the mature *Acomys* heart.

We next explored cytosolic calcium handling. Cytosolic Ca$^{++}$ transients (CaT) have a larger amplitude (Figure 7A, 7B), faster upstroke (Figure 7C), and more rapid decay (Figure 7D) in *Mus* compared to that in *Acomys*. As with CM morphometrics (Figure 4) and the
prevailing expression of T-type calcium current (Figures 5, 6) the smaller, slower CaT segregate
with a less mature CM phenotype. Immature ventricular CM also tend to show reduced β-
adrenergic receptor (β-AR) acute responsiveness. Therefore, we tested the effect of
isoproterenol challenge (ISO). *Acomys* CaT amplitude remained smaller (Figure 7E, F) and
slower (Figure 7G, H). To assess acute responsiveness, we compared within cell before – after
ISO. *Mus* shows increased amplitude and more rapid kinetics (Figures 7I-K). By contrast,*Acomys* CaT amplitude and upstroke velocity were not significantly different. Ca++ re-uptake
was accelerated consistent with only partial β-AR coupling to downstream effectors. Taken
together, the *Acomys* ventricular myocardium exhibits a less mature phenotype than *Mus*. 
Discussion

The lack of adult mammalian models of cardiac recovery following MI has hampered the field’s ability to identify new therapies for heart failure. Based on their enhanced regenerative ability for number of tissue and organs, spiny mice represent a potential model to explore endogenous cardiac repair. In this study, we sought to establish the anatomical and cellular characteristics of *A. cahirinus*, with a specific comparison to the commonly used cardiac murine model, the C57BL6 mouse (*Mus*). Our results demonstrated comparable cardiac structure, anatomy and functional parameters between the two species. Interestingly, we found a significant difference in CM phenotype between species - including smaller cardiomyocyte surface area with less t-tubule density, and more mononucleated, predominantly diploid ventricular cardiomyocytes in mature *Acomys*. Physiological properties reflective of younger cardiomyocytes include expression of I_{Ca,T}, lower I_{Ca,L} density and smaller, slower cytosolic CaT. Overall, the outcome of our investigation suggest that *Acomys* may prove to be a useful model for future cardiac regeneration studies.

In vertebrate regeneration models, (e.g., axolotl limb, zebrafish heart, *Acomys* musculoskeletal tissue, etc.), tissue is removed by surgical excision and the lost tissue is structurally and functionally replaced.\(^6\), \(^16\), \(^27\) *Acomys* has emerged as a bonafide adult mammalian regeneration model for their ability to regrow complex tissues following full-thickness skin excision\(^12\), \(^14\), \(^16\) and removal of musculoskeletal tissue from the ear pinna.\(^13\), \(^15\), \(^28\) In each type of injury, adult *Acomys* were capable of restoring functional, and in some cases, complete scar-free tissues compared to other adult rodents. To establish *Acomys* as an experimental cardiac model, we characterized key anatomical, structural and functional parameters between *Acomys* and the most commonly used experimental *Mus* species (C57Bl/6 mice). Our anatomical studies established gross similarity in coronary anatomy between species. *Acomys* and *Mus* have a LAD artery that courses anteriorly below the left atrial
appendage, and traverses the anterior interventricular groove supplying the majority of the anterior wall.\textsuperscript{29} Similarly, the left coronary artery supplies the lateral and distal anterior wall (Figure 2). Interestingly, \textit{Acomys} hearts were similar structurally and anatomically to \textit{Mus}. Additionally, there were no significant differences for \textit{in vivo} cardiac functional parameters between the two groups (Figure 3). These findings are crucial in planning future myocardial ischemia studies as they indicate that similar techniques can be used to induce comparable injury across species.

The adult mammalian heart loses its regenerative capability after the first week of life and heart damage results in permanent loss of myocardium combined with fibrotic scarring and adverse remodeling.\textsuperscript{3} The loss of regenerative capability corresponds with loss of CM proliferation as CMs enter cell cycle arrest and become mononuclear polyploid or multinucleated.\textsuperscript{3, 30} Therefore, in animal models and humans, cardiac ischemic injury typically leads to the progression of heart failure when the damage is widespread.\textsuperscript{9} However, in other vertebrate species where CMs maintain the lifelong ability to proliferate (e.g., zebrafish and newts), cardiac regeneration is still possible.\textsuperscript{30, 31} One of the hallmarks of regenerative hearts is the relatively high frequency of mononucleated diploid CMs. Indeed, both neonatal mice and adult zebrafish with high percentage of mononuclear diploid CMs can regenerate cardiac tissue after injury via the proliferation of pre-existing CMs.\textsuperscript{32} Taken together, this data suggests that higher proportions of mononuclear diploid CMs are associated with higher regenerative capacity. A recent study further reported that the proportion of mononuclear cardiomyocytes varied more than 7-fold in 120 inbred adult mouse strains, and a higher proportion of mononuclear cardiomyocytes was associated with improved EF after myocardial infarction.\textsuperscript{22} Our study examined the nucleation and ploidy of \textit{Acomys} and \textit{Mus} and established that \textit{Acomys} have a higher proportion of mononucleated and diploid cardiomyocytes that are smaller in size resembling those of neonatal mice (Figure 4A-C, H). Future studies are planned to examine the
proliferative potential of CMs in *Acomys* and *Mus* and their potential to adequately respond to myocardial injury.

There are two types of Ca++ channels in cardiomyocytes, L-type and T-type. L-type Ca++ channels are highly expressed in the adult heart and are important therapeutic targets for the management of various cardiovascular diseases. In contrast to L-type channels, T-type Ca++ channels are rarely found in adults and are present in the hearts of fetal and early postnatal mice that have the ability to regenerate cardiomyocytes. In our cell electrophysiology studies, *Acomys* exhibit T-type Ca++ channels (Figure 6A-D). This feature is consistent with *Acomys* cardiomyocytes exhibiting a ‘younger’ phenotype compared to *Mus*. Furthermore, immature ventricular cardiomyocytes show reduced Ca++ transient amplitude, slower kinetics, and attenuated β-adrenergic receptor responsiveness. In concert with the reduced t-tubule density the overall picture emerges that the adult *Acomys* ventricular cardiomyocytes retain a more ‘youthful’ phenotype.

In conclusion, spiny mice represent a novel model for cardiac research. *Acomys* demonstrate comparable cardiac structure, function and anatomy when compared to *Mus*, aiding in ease of interpretation of future experimental results. However, ventricular CM phenotype in *Acomys* represent a significant departure from those seen in adult *Mus* with distinct features such as smaller surface area, abundance of T-type calcium currents and a higher percentage of mononucleated and diploid cells; phenotypic differences that are typical of species capable of cardiac regeneration. Future studies will focus on the potential of these phenotypic differences to foster regeneration and repair instead of scarring and heart failure in models of cardiac disease.
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**Figure legend.**

**Figure 1. Acomys heart is heavier than Mus, but heart size is no significant different.**

(A) The quantitation of body weight (BW) of Mus (n = 10) and Acomys (n = 22). (B, C, D) The quantitation of heart weight (HW) of Mus (n = 10) and Acomys (n = 23), HW normalized by BW of Mus (n = 10) and Acomys (n = 23), and HW normalized by tibial length (TL) of Mus (n = 6) and Acomys (n = 17). Values are means ± S.E.M. *P < 0.05 compared to Mus. (E-J) The quantitation of wet and dry lung weight (LW) of Mus (n = 10) and Acomys (n = 20), wet and dry LW normalized by BW of Mus (n = 10) and Acomys (n = 20), and wet and dry LW normalized by TL of Mus (n = 6) and Acomys (n = 17) (Values are means ± S.E.M, *P < 0.05 compared to Mus).

**Figure 2. Coronary anatomy in Mus and Acomys.**

(A) Representative whole hearts of Mus (left) and Acomys (right) at baseline. (B) A schematic of the coronary anatomy in mice. Panel C shows representative images of the blood supply of the anterior and posterior wall across species using TRITC-Dextran IV infusion prior to sacrifice. Hearts were imaged using the Maestro EX system (red arrows are left anterior descending artery). The lower panel shows the coronary anatomy of the posterior wall. Panel D demonstrates the left anterior descending artery across species using intra-aortic infusion of Batson’s 17 polymer mixture (Scale bar represents 1 mm). (E) Representative isolectin staining (Red) for capillary density at baseline. Scale bar represents 50 μm. (F) Quantitative analysis of capillary density for Mus and Acomys. n = 4 mice for each group (Values are means ± S.E.M).

**Figure 3. Cardiac function by echocardiograph at baseline.**

M-mode echocardiography of Mus (left) and Acomys (right) at baseline (top images). Ejection fraction (A), fractional shortening (B), left ventricular end-diastolic diameter (LVEDD) (C), left ventricular end-systolic diameter (LVESD) (D), left ventricular anterior wall (LVAW) (E),
left ventricular posterior wall (LVPW) (F), LV mass normalized by body weight (BW) (G), and stroke volume normalized by BW (H) were comparable when measured by echocardiography in Mus and Acomys at baseline (n = 30 Mus and 10 Acomys) (Values are means ± S.E.M, *P < 0.05 compared to Mus).

**Figure 4. Characteristics of cardiomyocyte in Acomys resemble neonatal mouse cardiomyocytes.**

(A) Single-cell ventricular suspension stained with cardiac troponin T (cTnT) (red) and with Hoechst (blue), identifying a small mononuclear cardiomyocyte on Acomys, and to a larger binucleated cardiomyocyte on Mus. Scale bar represents 50 μm. (B) The quantitation of cell surface area of cardiomyocytes. This graph shows the cell surface area of Mus and Acomys. All cells (n = 100, each animal) randomly selected (n = 6 mice for Mus and 4 mice for Acomys, *P < 0.05 compared to Mus. Values are means ± S.E.M). (C) Calculation of percentage of mononuclear cardiomyocytes for Mus and Acomys (n = 4 mice for each group, values are means ± S.E.M, *P < 0.05 compared to Mus). (D, E, F, G) Single cell pooled t-tubule analysis shows significantly greater t-tubule density in transverse (E) and axial directions (F) along with wider t-tubule spacing (G) in Acomys compared to Mus (****p<0.001, scale bar = 50 μm). (H) Calculation of percentage of ploidy in cardiomyocytes for Mus and Acomys by Flow cytometry (n = 2 mice/group, values are means ± S.E.M).

**Figure 5. Calcium current in Acomys and Mus.**

(A) Current traces elicited by $V_{\text{test}}$ -25 mV (upper) & $V_{\text{test}}$ +15mV (lower) from $V_{\text{hold}}$ -80 mV. (B) Current-voltage relationships shows evidence for $I_{\text{Ca,T}}$ in Acomys, but not in Mus. For Acomys, n=9 cells; N=5 animals; for Mus, n=44, N=13 animals.
**Figure 6.** $I_{Ca,T}$ measurements indicating less mature ventricular cardiomyocytes in *Acomys*.

(A, B) Current-voltage curves from $V_{hold}$ -80 mV and -50 mV superimposed for *Acomys* CM exhibiting $I_{Ca,T}$ (A) or no $I_{Ca,T}$ (B). (C) Cell capacitance was not different for *Acomys* CM with or without $I_{Ca,T}$. (D) $I_{Ca,T}$ expression was heterogeneous but more *Acomys* ventricular CMs showed $I_{Ca,T}$ compared to rare occurrences in *Mus*. (E) Cell capacitance was greater in *Mus* than *Acomys*. **$P$<0.01.** (F) Maximal conductance density trended greater in *Mus* than *Acomys*. *$P$=0.06.** (G) Voltage-dependent activation and inactivation (H) of $I_{Ca,L}$ was significantly shifted positive for *Acomys* compared to *Mus* (**$P$<0.001**).

**Figure 7.** Calcium transients in *Mus* and *Acomys*.

(A) Representative calcium transients from isolated ventricular cardiomyocytes loaded with fura2-AM, *Mus* (top, red) and *Acomys* (bottom, blue) paced at 1 Hz. Scale bar: 2 seconds. (B) Amplitude of the transients (**$P=0.0004$**). (C) Velocity at which calcium enters the cytosol (upstroke of the transient: *$P=0.04$**). (D) Calcium transient decay (**$P=0.0005$**). (E) Representative calcium transients treated with 100 nM isoproterenol (ISO), *Mus* (top, red) and *Acomys* (bottom, blue), paced at 1 Hz. Scale bar: 2 seconds. (F) Amplitude of the transients with ISO (*$P=0.04$**). (G) Velocity at which calcium enters the cytosol with ISO (*$P=0.05$**). (H) Calcium transient decay with ISO (**$P<0.0001$**). (I) Before and after ISO Amplitude of the transients (**$P=0.007$**). (J) Before and after ISO Velocity at which calcium enters the cytosol (*$P=0.02$**). (K) Calcium transient decay (**$P=0.002$**). *N = 7 animals and 37 cells for Mus*; and *4 animals and 31 cells for Acomys*. 
## Supplemental table 1.

|                     | Mus |          |          | Acomys |          |          | P-value |
|---------------------|-----|----------|----------|--------|----------|----------|---------|
|                     | n   | Mean     | SEM      | n      | Mean     | SEM      |         |
| **Structural parameters** |     |          |          |        |          |          |         |
| BW (mg)             | 10  | 26.92    | 1.12     | 22     | 50.26    | 2.18     | <0.0001*|
| HW (mg)             | 10  | 167.58   | 10.41    | 23     | 231.07   | 12.92    | 0.0034* |
| HW / BW             | 10  | 6.22     | 0.29     | 23     | 4.59     | 0.34     | 0.0058* |
| HW / TL             | 6   | 96.60    | 8.31     | 17     | 95.81    | 5.59     | 0.8648  |
| Wet LW (mg)         | 10  | 161.40   | 12.55    | 20     | 204.03   | 9.92     | 0.0222* |
| Dry LW (mg)         | 10  | 34.91    | 2.42     | 20     | 41.83    | 2.71     | 0.0479* |
| Wet LW / BW         | 10  | 5.98     | 0.36     | 20     | 4.00     | 0.20     | <0.0001*|
| Dry LW / BW         | 10  | 1.30     | 0.07     | 20     | 0.81     | 0.05     | <0.0001*|
| Wet LW / TL         | 6   | 80.62    | 5.99     | 17     | 92.72    | 5.45     | 0.2273  |
| Dry LW / TL         | 6   | 18.19    | 1.15     | 17     | 18.38    | 1.39     | 0.6088  |
| **Cardiac function** |     |          |          |        |          |          |         |
| EF (%)              | 30  | 62.94    | 0.94     | 10     | 65.93    | 1.89     | 0.1314  |
| % FS                | 30  | 33.63    | 0.67     | 10     | 35.01    | 1.35     | 0.3626  |
| LVEDD (mm)          | 30  | 3.72     | 0.04     | 10     | 3.79     | 0.11     | 0.3964  |
| LVESD (mm)          | 30  | 2.46     | 0.04     | 10     | 2.47     | 0.10     | 0.7179  |
| LVAW (mm)           | 30  | 0.78     | 0.02     | 10     | 0.88     | 0.02     | 0.0014* |
| LVPW (mm)           | 30  | 0.8210   | 0.0164   | 10     | 0.8951   | 0.0351   | 0.0707  |
| LV mass / BW        | 30  | 3.31     | 0.07     | 10     | 2.42     | 0.10     | <0.0001*|
| Stroke volume / BW | 27 | 1.59 | 0.03 | 7 | 0.95 | 0.06 | <0.0001* |
|-------------------|----|------|------|---|------|------|-----------|

**Cardiomyocyte characteristic**

| Cell surface area (μm²) | 6  | 2719.18 | 193.29 | 4 | 699.69 | 48.01 | 0.0095* |
|-------------------------|----|----------|--------|---|--------|-------|---------|
| % mononuclear           | 4  | 6.32     | 1.89   | 4 | 16.90  | 0.82  | 0.0061* |
| Vessel density (1/mm²)  | 4  | 2736.29  | 209.44 | 4 | 2431.86| 249.80 | 0.6857 |

BW; body weight, HW; heart weight, TL; tibial length, LW; lung weight, EF; ejection fraction, LVEDD; left ventricular end-diastolic diameter, LVESD; left ventricular end-systolic diameter, LVAW; left ventricular anterior wall, LV mass; left ventricular mass (*P < 0.05).
Figure 1
Figure 2

A. **Mus** vs. **Acomys**

- **Anterior**
- **Short axis**
- **Long axis**

B. **Heart Diagram**
- Left Circumflex artery
- Right coronary artery
- Left Anterior Descending artery

C. **Anterior View**

| Mus | Acomys |
|-----|--------|

C. **Posterior View**

| Mus | Acomys |
|-----|--------|

D. **Heart Views**

| Mus | Acomys |
|-----|--------|

E. **Iselectin & DAPI**

F. **Vessel Density**

- **Mus**
- **Acomys**

Vessel density/mm²
Figure 3

**Mus**

A. Ejection Fraction (%)

B. Fractional shortening (%)

C. LVEDD (mm)

D. LVESD (mm)

E. LVFW (mm)

F. LVPW (mm)

G. LV mass / BW

H. Stroke volume / BW

**Acomys**

Figure 5

A.  

Mus

B.  

Acomys

Vtest (mV)  

Current Density (pA/pF)
**Figure 6**

**A.** Cells With T-Current

- $V_{\text{test}}$ (mV)
- $V_{\text{hold}}$ -80 mV
- $V_{\text{hold}}$ -50 mV
- Current Density (pA/pF)
- $n = 6$

**B.** Cells Without T-Current

- $V_{\text{test}}$ (mV)
- Current Density (pA/pF)
- $n = 3$

**C.**

- Cell Capacitance (pF)
- T-Current
- No T-Current

**D.** Prevalence of $I_{\text{Ca},T}$

|                  | Mus | Acomys |
|------------------|-----|--------|
| Total cells      | 44  | 9      |
| $I_{\text{Ca},T}$ Positive | 2   | 6      |
| Fraction of cells with $I_{\text{Ca},T}$ | <5% | 67%*** |

***$p < 10^{-3}$: Fisher’s exact test

**E.**

- Cell Capacitance (pF)
- Mus
- Acomys

**F.**

- Maximal Conductance (pS/pF)
- Mus
- Acomys

**G.**

- Activation Midpoint (mV)
- Mus
- Acomys

**H.**

- Inactivation Midpoint (mV)
- Mus
- Acomys

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