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High-Frequency Echocardiographic Assessment of Cardiac Function in the Naked Mole-Rat

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SUMMARY:

This protocol describes standardized high-frequency echocardiography for assessing cardiac structure, function, and physiological reserve in naked mole-rats, with an emphasis on species-specific considerations, including anesthesia, thermoregulation, heart rate, imaging-plane optimization, and the interpretation of cardiovascular function.

ABSTRACT:

The naked mole-rat (NMR) is an exceptionally long-lived rodent that maintains cardiovascular function into advanced age and exhibits physiological adaptations associated with hypoxia tolerance, metabolic efficiency, and resistance to age-related decline. These characteristics have increased interest in the NMR as a model for investigating cardiovascular resilience and healthy aging. However, its distinct cardiovascular physiology and sensitivity to anesthesia and changes in body temperature create technical challenges when conventional rodent echocardiographic methods are applied without modification. This protocol describes a standardized high-frequency echocardiographic workflow for assessing cardiac structure and function in the NMR. The procedure includes animal preparation, physiological monitoring, acquisition of parasternal long-axis, parasternal short-axis, apical, M-mode, and Doppler images, and optimization of imaging conditions to obtain reproducible cardiac measurements. Particular attention is given to

maintaining appropriate body temperature, minimizing anesthesia-related cardiovascular depression, controlling image acquisition at suitable heart rates, and recognizing species-specific limitations in the interpretation of resting cardiac parameters. The approach supports assessment of ventricular morphology, systolic and diastolic function, and, when experimentally indicated, cardiac reserve using stress echocardiography. This standardized workflow provides a reproducible framework for cardiovascular phenotyping and for investigating cardiac resilience, aging, and responses to physiological or experimental stress in naked mole-rats.

INTRODUCTION:

Human aging is associated with progressive declines in cardiovascular reserve and adaptive capacity, resulting in structural and functional changes that compromise cardiac performance and vascular integrity. Humans and commonly used laboratory rodents exhibit age-associated cardiovascular alterations characterized by arterial stiffening, ventricular remodeling, oxidative stress accumulation, fibrosis, endothelial dysfunction, and reductions in cardiac reserve¹⁻⁴. These changes contribute to increased susceptibility to hypertension, ischemic injury, heart failure, and other cardiovascular diseases later in life. Although traditional rodent models have provided substantial insight into cardiovascular aging, their relatively short lifespans and compressed aging trajectories limit their ability to capture mechanisms responsible for long-term cardiovascular maintenance and healthy aging⁵. These limitations have increased interest in alternative animal models capable of preserving cardiovascular function throughout an extended lifespan⁶⁻⁸.

The naked mole-rat (NMR; *Heterocephalus glaber*), a subterranean rodent native to the hypoxic underground burrows of East Africa, represents a unique model of exceptional longevity and physiological resilience⁹. Despite a body size comparable to that of a mouse, the NMR exhibits a lifespan exceeding 40 years and demonstrates negligible senescence, a phenotype characterized by minimal age-related increases in mortality, disease incidence, and physiological decline¹⁰. The extreme environmental conditions experienced by this species in the arid subterranean burrows of East Africa have driven adaptations that support metabolic efficiency, oxygen conservation, and cellular stress resistance¹¹. Unlike conventional laboratory rodents, NMRs preserve cardiovascular function despite advanced age, avoiding many age-associated changes, including ventricular hypertrophy, arterial stiffening, and diastolic dysfunction^{12,13}. In addition, NMRs maintain substantial cardiac reserve despite relatively low basal cardiac activity, indicating an energy-efficient cardiovascular system capable of adapting rapidly to increased physiological demand^{13,14}. These characteristics make the NMR a valuable model for investigating cardiovascular resilience and for developing approaches to assess cardiac function and adaptability during aging.

Echocardiographic assessment in small laboratory animals presents technical challenges because the small size of the heart, rapid cardiac motion, limited access to standard imaging planes, and the sensitivity of cardiovascular measurements to anesthesia can increase variability and complicate image acquisition and interpretation^{15,16}. These considerations are particularly important in NMRs because their cardiovascular physiology differs from that of conventional laboratory rodents. Their relatively low basal cardiac activity, sensitivity to anesthetic depth, and dependence on environmental temperature can substantially affect heart rate and contractility

89 during imaging. Standardized imaging conditions are therefore necessary to distinguish species-
90 specific physiological characteristics from measurement variability and anesthesia-associated
91 cardiovascular depression.

92
93 Echocardiography provides a noninvasive and repeatable approach for evaluating cardiac
94 morphology, function, and physiological reserve in vivo. Baseline echocardiographic
95 measurements enable assessment of cardiac structure and systolic and diastolic function,
96 whereas stress echocardiography, including dobutamine challenge, can provide insight into
97 cardiac reserve and the ability of the cardiovascular system to respond to increased demand. This
98 approach is particularly valuable for aging research because reductions in cardiac reserve may
99 occur before overt structural abnormalities become apparent. Although echocardiography is
100 routinely used in conventional rodent models^{15,16}, detailed methodological considerations for
101 performing cardiac imaging and stress testing in NMRs remain limited.

102
103 The NMR provides a unique opportunity to study cardiovascular resilience through optimized
104 functional assessment methods because of its exceptional longevity, resistance to age-associated
105 cardiovascular decline, and sustained physiological adaptability. Previous studies have identified
106 multiple adaptations contributing to this resilience, including enhanced genome stability,
107 proteostasis, stress resistance, metabolic efficiency, and maintenance of cellular homeostasis^{17,18}.
108 Additional physiological adaptations associated with the NMR include heterothermy and marked
109 thermal sensitivity, which can influence cardiovascular measurements during anesthesia and
110 echocardiographic imaging^{19,20}. However, the physiological mechanisms that preserve
111 cardiovascular adaptability throughout the lifespan remain incompletely understood,
112 underscoring the need for reliable, standardized approaches to evaluate cardiac function in this
113 species.

114
115 This protocol describes the optimization and application of echocardiographic approaches for
116 assessing cardiovascular function and stress responsiveness in the NMR. The protocol provides a
117 standardized framework for evaluating cardiac structure, baseline function, and physiological
118 reserve, with particular attention to species-specific considerations related to thermoregulation,
119 anesthetic depth, heart rate, and image acquisition. This framework supports future
120 investigations of cardiovascular aging, resilience, and disease resistance in this unique
121 mammalian model.

122 123 **PROTOCOL:**

124 The procedures were approved by the University of Illinois Chicago Institutional Animal Care and
125 Use Committee (IACUC) under protocol number 2022115. The protocol is based on methods
126 described by Lindsey *et al.*¹⁵ and Salerno *et al.*¹⁶, with modifications for use in NMRs. The research
127 tools used in this protocol are listed in the **Table of Materials**.

128
129 A schematic overview of the experimental workflow for naked mole-rat (NMR) echocardiography
130 is shown in **Figure 1**.

131 132 **1. Prepare the ultrasound imaging system**

133
134 1.1. Power on the imaging system

135
136 1.1.1. Switch on the main power located at the rear of the Vevo 2100 imaging system.

137
138 1.1.2. After the display initializes, press the computer standby button to start the workstation.

139
140 1.2. Initialize the imaging software

141
142 1.2.1. Log in to the workstation using the laboratory-assigned user account.

143
144 1.2.2. From the Study Management window, select the appropriate operator profile.

145
146 1.2.3. Select New Study and verify that the Animal Information window opens before proceeding.

147
148 1.3. Prepare the imaging workstation

149
150 1.3.1. Install the imaging platform configured for the NMR. Use a mouse-sized imaging platform.

151
152 1.3.2. Connect the nose cone and temperature probe to the imaging platform.

153
154 1.3.3. Connect the high-frequency ultrasound transducer (MS400-0140) to Port 1 and secure it in
155 the transducer holder.

156
157 1.3.4. Turn on the heart rate and temperature controller and set the heating pad to 37.0 °C.

158
159 1.3.5. Fill the isoflurane vaporizer to the recommended fill level.

160
161 **CAUTION:** Isoflurane is a volatile anesthetic. Fill the vaporizer in a well-ventilated area or under
162 an approved anesthetic scavenging system while wearing appropriate personal protective
163 equipment.

164
165 **2. Prepare the animal for imaging**

166
167 **2.1. Induce anesthesia**

168
169 2.1.1. Place the animal in the induction chamber after the imaging system has warmed.

170
171 2.1.2. Direct the anesthetic gas to the induction chamber and induce anesthesia using 3.0%
172 isoflurane.

173
174 **NOTE:** Verify the induction isoflurane concentration against the laboratory-approved animal
175 protocol. Isoflurane can depress cardiac function; therefore, use the lowest concentration
176 required to maintain adequate anesthesia¹⁵.

177
178 **2.2. Create the imaging record**

179
180 2.2.1. While anesthesia is being induced, enter the study information into the imaging software.

181
182 2.2.2. Record the study name, experimental time point, animal identification, sex, body weight,
183 and age.

184
185 **2.3. Position the animal**

186
187 2.3.1. Transfer the animal to the imaging platform and position the nose within the nose cone.

188
189 2.3.2. Adjust the nose cone so that all four paws comfortably contact the electrocardiogram (ECG)
190 electrodes on the imaging platform.

191
192 2.3.3. Redirect anesthesia delivery to the nose cone and reduce the isoflurane concentration to
193 1.5%.

194
195 2.3.4. Secure the head to the nose cone using tape.

196
197 **2.4. Connect the physiological monitoring equipment**

198
199 2.4.1. Apply electrode gel to each ECG electrode.

200
201 2.4.2. Position each paw on the corresponding ECG electrode and secure the limbs with tape
202 without placing them under tension.

203
204 2.4.3. Lubricate the rectal temperature probe before insertion using petroleum jelly.

205
206 2.4.4. Insert the rectal temperature probe approximately 2 cm into the rectum and secure it in
207 place.

208
209 2.4.5. Maintain the animal's body temperature between 32 °C and 35 °C throughout imaging.

210
211 **2.5. Position the animal for echocardiographic imaging**

212
213 2.5.1. Raise the imaging platform slightly and rotate it approximately 30° toward the animal's left
214 side.

215
216 2.5.2. Apply ultrasound transmission gel to the thoracic imaging area, ensuring that no visible air
217 bubbles are present.

218
219 2.5.3. Position the transducer in the parasternal long-axis (PLAX) orientation, with the orientation
220 notch at approximately the 5 o'clock position (**Figure 2**).

2.5.4. Lower the transducer until gentle contact is made with the gel-covered skin. Avoid excessive pressure on the thorax.

2.6. Optimize the parasternal long-axis image

2.6.1. Begin live image acquisition in the PLAX view.

2.6.2. Configure the imaging settings to use a single focal zone positioned at the region of interest.

2.6.3. Adjust the focal depth, imaging depth, and image offset to center the heart within the field of view.

2.6.4. Adjust the image gain to maximize visualization of the cardiac structures while minimizing background noise.

2.6.5. Verify that the frame rate remains greater than 200 frames/s before acquiring images.

3. Acquire cardiac ultrasound images

3.1. Optimize image acquisition

3.1.1. Monitor the animal's physiological status throughout imaging. Adjust the isoflurane concentration as needed to maintain a stable heart rate between 105 and 175 bpm.

3.1.2. Optimize the transducer position and imaging settings before acquiring each view. Adjust the imaging depth, focal position, gain, and platform orientation to maximize image quality.

3.2. Acquire B-mode images

3.2.1. Adjust the platform orientation, transducer angle, and imaging settings until the relevant cardiac structures are clearly visualized.

3.2.2. **Save** a cine loop of the PLAX view by selecting Cine Store.

3.2.3. Rotate the transducer approximately 90° to obtain the parasternal short-axis (PSAX) view at the level of the papillary muscles (**Figure 2**).

3.2.4. Adjust the transducer position until the left ventricle appears circular and the papillary muscles are clearly visualized.

3.2.5. **Save** a cine loop of the PSAX view by selecting Cine Store.

3.3. Acquire M-mode images

265
266 3.3.1. Select M-mode to activate the cursor.

267
268 3.3.2. Position the M-mode cursor through the center of the left ventricle. Adjust the cursor
269 position as needed to obtain a representative image.

270
271 3.3.3. Acquire the M-mode trace and confirm that the endocardial and epicardial borders are
272 clearly defined.

273
274 3.3.4. **Save** the M-mode image for subsequent cardiac measurements.

275
276 3.3.5. Select B-mode to return to live two-dimensional imaging before acquiring the next view.

277
278 3.4. Acquire the apical four-chamber view

279
280 3.4.1. Reposition the transducer to obtain the apical four-chamber view (**Figure 2**).

281
282 3.4.2. Optimize the image and **save** a cine loop for subsequent analysis.

283
284 **4. Conclude the imaging session**

285
286 4.1. **Save** the imaging study

287
288 4.1.1. Select Study Management and click Close Series to end the imaging session.

289
290 4.1.2. If prompted, select Commit Session Data to save the study.

291
292 4.2. Shut down the imaging system

293
294 4.2.1. Close the imaging software and shut down the workstation.

295
296 4.2.2. Turn off the main power to the imaging system.

297
298 4.3. Clean and disinfect the equipment

299
300 4.3.1. Wipe the transducer with a water-moistened cloth to remove residual ultrasound gel.
301 Return the transducer to its holder and disinfect it using the laboratory-approved disinfectant.

302
303 4.3.2. Turn off the oxygen supply, isoflurane vaporizer, and any additional imaging accessories.

304
305 4.3.3. Clean and disinfect the imaging platform and rectal temperature probe according to the
306 manufacturer's recommendations.

307

4.3.4. Clean and disinfect the induction chamber and nose cone using the laboratory-approved disinfectant.

4.3.5. Disinfect the work surface and surrounding equipment before leaving the imaging area.

NOTE: Confirm that all imaging data have been successfully saved and backed up before powering down the system.

[Place **Figure 1** here]

[Place **Figure 2** here]

RESULTS:

Successful execution of this protocol produces echocardiographic images that permit visualization of cardiac anatomy and acquisition of standard imaging planes in the naked mole-rat (NMR). The high-frequency ultrasound transducer enables visualization of ventricular walls, the left ventricular cavity, the aortic root, and intracardiac blood flow, supporting assessment of cardiac morphology and function. Representative parasternal long-axis (PLAX), parasternal short-axis (PSAX), apical, M-mode, and Doppler images obtained using this protocol are shown in **Figures 3–5**.

Representative PLAX images demonstrate visualization of the left ventricle, left ventricular walls, and aortic root (**Figure 3A**). Simultaneous visualization of the aortic root and well-defined ventricular walls indicates appropriate alignment of the imaging plane and helps confirm that the left ventricle is not foreshortened. Once this orientation is achieved, position the M-mode cursor through the center of the left ventricular cavity, perpendicular to the interventricular septum and posterior wall, to obtain tracings suitable for assessment of ventricular dimensions and systolic function (**Figure 3B**).

Representative PSAX images acquired at the level of the papillary muscles demonstrate a circular left ventricular cavity, visible papillary muscles, and clearly defined myocardial borders (**Figure 4A**). These features indicate appropriate transducer orientation and provide a cross-sectional image suitable for evaluation of ventricular geometry. Positioning the M-mode cursor through the center of the left ventricle produces tracings that permit assessment of ventricular dimensions and wall motion (**Figure 4B**).

Representative apical images are obtained by positioning the transducer at the ventricular apex and progressively adjusting the imaging plane toward the center of the ventricle (**Figure 5A**). Owing to the small size of the NMR heart, cardiac morphology alone may not consistently identify the optimal imaging plane. Color Doppler imaging can therefore be used to guide transducer positioning by identifying intracardiac blood flow. Appropriate alignment is indicated by adjacent red and blue Doppler signals centered within the ventricular cavity. Once the imaging plane is optimized, pulsed-wave (PW) Doppler is used to record transmitral inflow and identify the early filling (E) and atrial filling (A) waves to assess diastolic function (**Figure 5B**).

Representative M-mode tracings from the PLAX and PSAX views (**Figures 3B** and **4B**) show ventricular wall motion throughout systole and diastole with identifiable endocardial and epicardial borders. These tracings permit measurement of left ventricular internal diameter and ventricular wall thickness and support calculation of functional parameters such as fractional shortening and ejection fraction. Successful image acquisition is characterized by consistent visualization of relevant cardiac structures throughout the cardiac cycle without excessive motion artifact, ventricular foreshortening, or loss of endocardial definition.

[Place **Figure 3** here]

[Place **Figure 4** here]

[Place **Figure 5** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Overview of the echocardiographic workflow for cardiovascular assessment in naked mole-rats. Schematic illustrating the experimental workflow for echocardiographic evaluation of cardiac structure and function in naked mole-rats, including animal preparation, baseline echocardiography, experimental interventions, and subsequent data analysis.

Figure 2: Transducer positioning for acquisition of standard echocardiographic imaging planes in the naked mole-rat. Representative transducer positions for obtaining the parasternal long-axis (PLAX), parasternal short-axis (PSAX), and apical four-chamber (A4C) views.

Figure 3: Representative B-mode and M-mode echocardiographic images acquired from the parasternal long-axis view. (A) Representative B-mode image obtained from the parasternal long-axis view showing the left ventricular cavity, left ventricular anterior wall (LVAW), left ventricular posterior wall (LVPW), and aortic root. (B) Corresponding M-mode tracing positioned through the left ventricle for measurement of left ventricular internal diameter (LVID) and ventricular wall thickness during systole and diastole, supporting assessment of ventricular morphology and systolic function.

Figure 4: Representative B-mode and M-mode echocardiographic images acquired from the parasternal short-axis view. (A) Representative B-mode image obtained from the parasternal short-axis view at the mid-papillary level showing the left ventricular cavity, left ventricular anterior wall (LVAW), and left ventricular posterior wall (LVPW). (B) Corresponding M-mode tracing positioned through the left ventricle for measurement of left ventricular internal diameter (LVID) and ventricular wall thickness during systole and diastole, supporting assessment of ventricular morphology and contractile function.

Figure 5: Representative B-mode and pulsed-wave Doppler echocardiographic images acquired from the apical four-chamber view. (A) Representative B-mode image obtained from the apical four-chamber view showing the ventricular chambers and alignment of the Doppler sampling

region. (B) Corresponding pulsed-wave (PW) Doppler tracing of transmitral inflow showing the early filling (E) and atrial filling (A) velocities used for assessment of left ventricular diastolic function.

DISCUSSION:

High-quality small-animal echocardiography requires standardized imaging planes and careful physiological monitoring to minimize measurement variability and imaging artifacts^{15,16}. In naked mole-rats (NMRs), suboptimal image acquisition may be characterized by poorly defined myocardial borders, acoustic shadowing, motion artifact, sternal interference, ventricular foreshortening, or excessive transducer pressure that distorts ventricular morphology. View-specific anatomical landmarks are therefore critical for confirming appropriate alignment. In the parasternal short-axis (PSAX) view, asymmetric visualization of the papillary muscles or a non-circular left ventricular cavity indicates improper transducer positioning and may distort measurements of wall thickness and ventricular dimensions. In the parasternal long-axis (PLAX) view, incomplete visualization of the aortic root or poorly defined ventricular walls suggests suboptimal alignment. During apical imaging, incomplete visualization of the central intracardiac flow pattern or poor alignment of the Doppler signal indicates that further transducer adjustment may be required before pulsed-wave Doppler measurements are acquired. Minor adjustments in transducer position, imaging depth, gain, or cursor placement can improve alignment without requiring complete repositioning of the animal.

Species-specific physiological characteristics create additional challenges when echocardiographic methods developed for conventional laboratory rodents are applied to NMRs. Standard mouse echocardiography must accommodate very rapid cardiac rates and small cardiac dimensions, requiring careful optimization of spatial and temporal resolution^{15,16}. In contrast, NMRs exhibit lower basal cardiac activity and a slower resting heart rate than conventional laboratory mice, together with substantial cardiac reserve^{13,14}. These differences alter both image acquisition and interpretation. Rapid murine heart rates can contribute to fusion of diastolic E- and A-waves, whereas the slower cardiac rate of the NMR may facilitate separation of these filling phases. However, marked reductions in NMR heart rate during imaging can impair image acquisition and may reflect anesthesia- or temperature-associated cardiovascular depression rather than the animal's physiological resting state. Measurements obtained under anesthesia should therefore be interpreted within the standardized experimental conditions used for image acquisition rather than assumed to represent conscious physiological baselines.

Thermoregulation and anesthetic depth are particularly important critical steps in NMR echocardiography. NMRs lack an insulating pelage and exhibit pronounced heterothermy and thermal lability^{19,20}. Their physiology is therefore highly responsive to environmental temperature, and anesthesia can further reduce metabolic rate, body temperature, heart rate, and cardiac contractility. These effects can complicate differentiation between physiological cardiac characteristics and anesthesia-induced cardio-depression. Body temperature should consequently be continuously monitored and maintained within the defined imaging range using a heated platform, while anesthetic exposure should be limited to the minimum concentration required to ensure adequate immobilization. Maintaining stable physiological conditions

throughout image acquisition is essential for obtaining comparable measurements between animals and across longitudinal imaging sessions.

Interpretation of functional measurements also requires consideration of NMR-specific cardiovascular physiology. The highly compliant ventricles and prolonged diastolic filling period of NMRs can produce low-velocity filling profiles that approach the detection limits of high-frequency ultrasound systems, potentially reducing the consistency of early-to-late diastolic filling measurements such as the E/A ratio. In addition, NMRs exhibit relatively low basal cardiac activity despite retaining substantial capacity to increase cardiovascular performance in response to physiological demand^{13,14}. Consequently, resting measures of contractile function should be interpreted cautiously and in the context of the species-specific physiological baseline. Stress echocardiography using dobutamine can provide additional information on cardiac reserve when assessment of stress responsiveness is an experimental objective. The precise role of dobutamine within the standardized workflow, including whether it is required for routine functional phenotyping or represents an optional stress application, should be defined according to the intended experimental endpoint.

Successful application of this protocol is demonstrated by consistent acquisition of standard echocardiographic views with clearly identifiable cardiac structures suitable for quantitative assessment. Important limitations include sensitivity to anesthetic depth and temperature, limited access to optimal imaging planes due to the small cardiac dimensions, and potential difficulty obtaining consistent Doppler-derived diastolic measurements. Nevertheless, the noninvasive and repeatable nature of echocardiography enables longitudinal assessment of ventricular morphology and cardiac function in NMRs, providing a framework for investigating cardiovascular aging, physiological resilience, and responses to experimental stress. Given the preservation of cardiovascular function reported in NMRs throughout much of their extended lifespan^{7,12,13}, this approach may facilitate studies examining mechanisms underlying sustained cardiac performance and resistance to age-related cardiovascular decline.

ACKNOWLEDGMENTS:

DISCLOSURES:

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