The Need for Speed
Mice, Men, and Myocardial Kinetic Reserve
Paul M.L. Janssen, Brandon J. Biesiadecki, Mark T. Ziolo, Jonathan P. Davis

The in-bred laboratory mouse (hereafter referred to as simply mouse) has in the past decades emerged as the go-to animal model in the arena of cardiovascular research. The reasons are straightforward: the relative ease of genetic manipulation coupled with short gestation times, the much cheaper cost of maintaining large numbers, and a large number of similarities to human genomics, proteomics, and many aspects of function. These advantages make the mouse a powerful tool for a wide array of cardiac research.

The resting heart rate in the conscious unrestrained mouse is ≈500 to 700 bpm,1,3 roughly 10× faster than in a resting human. In addition, the mouse modulates heart rate, and, thus, contraction/relaxation kinetics, rather minimally; even during strenuous exercise, it only increases from the resting rate by 50% at most,1 typically by only ≈10% to 20%,3,4 whereas in humans, the heart rate can increase by ≤300%, typically 100% to 200%. As a result, exercise-induced increases in cardiac output (heart rate combined with an increase in stroke volume) can be 5- to 10-fold in the human, but are only 1.3- to maximally 2-fold in the mouse. In this viewpoint, we will convey how the high murine heart rate limits myocardial kinetic reserve (ie, the ability for the cardiac muscle to increase contraction and relaxation speed) and focus on the ramifications for extrapolating the regulation of contractile kinetics to human health and disease. In addition, we will discuss the use of contracting human tissue as a potential powerful ally in our quest to further understand contractile and kinetic regulation in health and disease.

Heart Rate and Heart Rate Changes in the Mouse
Critical differences are evident in the contractile/relaxation kinetics of cardiac muscle. Despite the fact that all the proteins of the contraction process are the same or similar between human and mouse cardiac muscle, these components are geared and tuned to operate at an entirely different level; the speed of the processes involved is vastly faster in the mouse.3,4 The timing of the contractile process is a complex interaction, including ion-channel function, sarcoplasmic reticulum calcium handling, thin filament properties, and crossbridge cycling.5 Thus, these processes and their interactions are quantitatively and qualitatively different in mouse versus human. These prominent, well-known differences are found in the action potential, excitation-contraction (EC) coupling, and myofilament control (Table).

As can be derived from the table, dimension-wise, the myocyte and sarcomere are nearly identical in both species. Also, the blood pressure achieved, as well as the force generated per cross-sectional area of the myocardium, is virtually the same because the size of the heart is scaled similarly to body weight so that the relationship between pressure, volume, and resistance results in adequate tissue perfusion pressure. In addition, the stoichiometry of the myofilament proteins is virtually identical, with differences in the myofilament protein isoforms. However, these differences in the isoform are critical because they are at the basis of a major difference between mouse and human: the speed/kinetics at which the contractile machinery operates. These components interact with each other dynamically and are tuned to operate at the animal’s specific heart rate. For the mouse, the cycle length is roughly 100 ms at 600 bpm (rest) and ≈80 ms at 750 bpm (peak exercise), whereas in the human, all these processes are tuned to each other to perform in 1000 ms at 60 bpm (rest) to 350 ms at 170 bpm (peak exercise).

There is no need for a large kinetic reserve in the mouse because of the high heart rate necessitated by the metabolic need coupled with biophysical constraints. Because even during strenuous exercise, mice do not drastically elevate heart rate, they can function with a relatively minor increase in cardiac output. Also, there are biophysical constraints with the higher heart rates, with calcium diffusion into the sarcomere as a limiting factor. When calcium enters the myocyte’s cytoplasm from the t-tubular region, it diffuses to the center of the sarcomere, severely limiting activation of contraction. In sharp contrast, this diffusion limitation in the human sarcomere has a negligible impact, given the much lower heart rate, even when heart rate increases by 200%.

Heart Rate Regulation and Impact on Kinetics
The larger the mammal, the slower the resting heart rate,8 and this is coupled with an increased need for modulation of cardiac output by heart rate. This vast range of heart rates

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Viewpoints
necessitates a large kinetic reserve. Humans heavily rely on the ability of the heart to increase cardiac output when demand for oxygen increases, such as during exercise. On increased cardiac output, the healthy human heart beats stronger and contracts and relaxes faster, effectively boosting cardiac output. The failing heart has trouble increasing its power and maintaining appropriate speeds of contraction/relaxation, decreasing kinetic reserve. The inability to sufficiently accelerate kinetics on an increase in heart rate leads to improper ventricular filling, resulting in insufficient cardiac output, and ultimately death. Thus, acceleration of contractile and relaxation kinetics with increased heart rate is a prominent feature of the healthy human heart and is critically deranged in heart failure. Understanding the defective regulation of contractile/relaxation kinetics to restore kinetic reserve is essential for finding a future cure to heart failure.

The mouse is a powerful model and should continue to be used to study a wide array of cardiovascular processes. Research on small rodents has translated into treatments in areas of vascular biology/blood pressure regulation. However, few applicable treatment strategies have been derived from murine EC coupling and contractile work, specifically as it relates to contractile/relaxation kinetics. For instance, a critical difference is in the quantitative and qualitative use of the sarcoplasmic reticulum to cycle calcium intracellularly. When the sarcoplasmic reticulum (SR) is pharmacologically inhibited, the mildly positive force–frequency relationship, critical for enhancing cardiac output, becomes extremely negative in the rat (and mouse), whereas even in absence of a functional SR, this important force–frequency relationship remains positive in larger mammals, such as the rabbit. When the SR is rendered not functional, the L-type calcium current, which accounts for ≈30% of the calcium cycling each beat, can provide sufficient calcium for activation of the thin filament in larger mammals and humans. In contrast, in the mouse and rat, this L-type calcium contribution to the calcium transient is ≈10-fold less, and this much smaller L-type calcium current is insufficient to adequately activate the myofilaments when the SR is not functioning. Although the force–frequency aspect mainly impacts inotropy, the vast majority of inotropic drugs also are lusitropic. As a result, the lack of inotropic efficiency may in part stem from the inability to use this kinetic reserve in diseased human myocardium. The drastically limited kinetic reserve in the mouse is, unfortunately, remote from human. Thus, investigation of impaired regulation of contraction/relaxation kinetics, prevalent in human heart failure, is limited in the murine model and prevents unambiguous translation of findings into significant advancements in human cardiac muscle care.

### Use of Contracting Human Myocardial Tissue

Several laboratories have, over the past decades, used human cardiac muscle to determine kinetic properties and their

### Table. Values Are Illustrative/Approximated Based on Literature Averages and Our Laboratory Experiences

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human</th>
<th>Mouse</th>
<th>Difference (Mouse vs Human)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anatomic/systemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocyte size, μm</td>
<td>140×20×15</td>
<td>140×20×15</td>
<td>Not different</td>
</tr>
<tr>
<td>Sarcomere length, μm</td>
<td>2.0–2.2</td>
<td>2.0–2.2</td>
<td>Not different</td>
</tr>
<tr>
<td>Force per myocyte, μN</td>
<td>10–15</td>
<td>10–15</td>
<td>Not different</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>110</td>
<td>110</td>
<td>Not different</td>
</tr>
<tr>
<td><strong>EC coupling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-SR Ca²⁺ cycling per beat, % total</td>
<td>30</td>
<td>3</td>
<td>≈10-fold lower</td>
</tr>
<tr>
<td>APD 90%, ms</td>
<td>300</td>
<td>30</td>
<td>≈10-fold faster</td>
</tr>
<tr>
<td>Myosin isoform*</td>
<td>Human β (MYH7)</td>
<td>Mouse α (MYH6)</td>
<td>&gt;5-fold faster</td>
</tr>
<tr>
<td><strong>Baseline kinetics (at rest)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate, bpm</td>
<td>50–70</td>
<td>500–700</td>
<td>≈10-fold higher</td>
</tr>
<tr>
<td>dP/dt&lt;sub&gt;max&lt;/sub&gt;, mmHg/s</td>
<td>1500</td>
<td>15000</td>
<td>≈10-fold higher</td>
</tr>
<tr>
<td>TTP, ms</td>
<td>185</td>
<td>35</td>
<td>&gt;5-fold faster</td>
</tr>
<tr>
<td>RT50%, ms</td>
<td>130</td>
<td>20</td>
<td>&gt;5-fold faster</td>
</tr>
<tr>
<td><strong>Kinetic reserve</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in HR, % rest</td>
<td>100–200</td>
<td>10–50</td>
<td>&gt;5-fold less</td>
</tr>
<tr>
<td>Acceleration of TTP, % rest</td>
<td>35</td>
<td>5</td>
<td>&gt;5-fold less</td>
</tr>
<tr>
<td>Acceleration of RT50%, % rest</td>
<td>40</td>
<td>5</td>
<td>&gt;5-fold less</td>
</tr>
</tbody>
</table>

Kinetic reserve values reflect differences at maximal heart rate compared with the species’ resting rate. ADP indicates action potential duration to 90% repolarization; EC, excitation-contraction; HR, heart rate; MYH, myosin heavy chain; RT50%, time from peak tension to 50% relaxation in an isometrically contracting myocardial muscle preparation; SR, sarcoplasmic reticulum; and TTP, time to peak tension in an isometrically contracting myocardial muscle preparation.

*Quantitative myosin isoform difference reflects the combined difference in species and isoform.
regulation. Although the scientific relevance of such experiments is obvious, logistics and economic reasons hamper its widespread use in cardiovascular research.

Outcomes of human heart experiments are inherently more variable because of the genomic and phenotypic variability of the patients and donors. Rather than demonize the large variability between these human samples by citing human-to-human variability as a limitation of a research study or proposal, we must celebrate and embrace this biological variability. The human diversity is an added opportunity to interpret, fine-tune, and classify the outcome findings, as well as consider it as a validation of the applicability of the findings for the highly variable human population. In addition, in a recent viewpoint article in this journal, it was postulated that the lack of variability between subjects in inbred mice does not reflect human variability. Experiments on a single inbred mouse strain pose a significant disadvantage to the interpretation of the outcomes to human relevance. The logistical down-side to the inherent biological variability of the human specimens is that it requires study of a large number of subjects. This higher n-number typically exceeds the numbers for similar inbred animal experiments, and several past studies on human myocardium have suffered from a lack of adequate statistical power. Coupled with the often sparse and irregular availability of human tissue, an adequately powered study on human muscle tissue takes years to complete, rather than weeks or months. In the current climate of publish or perish, costly, labor-intensive, multi-year studies with large n-numbers are not practical to undertake, but they are critically needed to further our understanding of human cardiac kinetics in health and disease.

Another hurdle for using human tissue is the data quality, which critically depends on the quality of the initial procurement and the chosen experimental conditions. Unless cold cardioplegia is expertly applied immediately (ie, seconds) before or after the heart is removed from the patient or donor, the quality of the live myocardium will likely suffer. A related difficulty, inherent in kinetics research, is that if experimental conditions are not chosen to be near physiological, kinetic rates are vastly affected. The quantitative, and often qualitative, outcome of studies on kinetic rates of contracting tissue critically depends on the pacing rates, temperature, and mechanical loading conditions. Kinetic studies conducted at body temperature, and appropriate pacing frequencies, and in presence of pre/after load, will be significantly more relevant to human (patho-)physiology than those in cold, slow, and unloaded conditions.7

The vast majority of live human tissue research is done on end-stage failing tissue because the vast majority of past studies were performed on tissue obtained after a cardiac transplant surgery. Obtaining nonfailing controls, to contrast/compare the data obtained from failing tissue, using the same rigorous procurement procedures (immediate cold cardioplegic), is of tremendous value to these studies. It is critical going forward that nonfailing controls be included in human tissue studies, and coupled with the sparse availability of this nonfailing tissue, sharing such resources to the fullest is critically needed. The above logistical and economical difficulties can and are being overcome to exploit the vast scientific progress that can be made in research on contractile kinetics. Successful collaborations between cardiologists, surgeons, basic scientists, organ procurement organizations, granting agencies, and legislature result in a critically needed resource: the availability of live human cardiac nonfailing and failing tissue to a wide group of investigators. Outcomes of this live tissue research can and should also be correlated to investigations on the same heart for protein expression/localization, RNA and genomic data, as well as with biometric data from that particular donor or patient, and the clinical/pharmacological treatment history of that heart. If we are to halt progression, or in the future even find a cure for impaired regulation of cardiac contractile kinetics, we need to develop and widely incorporate live human cardiac tissue into our quest to cure or curb cardiac contractile dysfunction, even though this is a long, arduous, and challenging task.

Summary

Kinetic reserve, that is, the ability of the myocardial muscle to accelerate contraction and relaxation on an increase in heart rate is a critically important aspect of human cardiac health and is negatively impacted in cardiac pathologies. The in-bred laboratory mouse exhibits minimal kinetic reserve, limiting extrapolation of this particular aspect of this model to human relevance. Use of human tissue research, in parallel with animal experiments, is critically needed to advance our understanding of kinetic reserve and needed to evaluate potential treatment strategies.

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None.

References


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