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COMMENTARY

Sarcomere length in the beating heart: Synchronicity is optional

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Anyone who has studied cardiac function at the myofilament level knows how critical it is to control the sarcomere length at which the myofibril, myocyte, muscle strip, or heart operates. Measuring sarcomere length in the single myofibril or myocyte is straightforward and can be done by using simple video-based brightfield microscopy. With muscle strips thicker than ∼250 µm, visualizing sarcomeres is more challenging. Sarcomere lengths can still be detected using laser diffraction technologies (see Pitoulis et al., 2020 for references), but robust measurements throughout the cardiac cycle are difficult to achieve. Measuring sarcomere length in the whole heart has been elusive to say the least. Several attempts have been made in the past. The most successful measures, however, have relied upon examination of fixed preparations (Sonnenblick et al., 1967; Grimm et al., 1980; LeWinter et al., 2010) or after arresting the heart (Bub et al., 2010). The Fukuda group, featured in an earlier issue of the *Journal of General Physiology*, is the first to measure sarcomere lengths in a beating heart (Kobirumaki-Shimozawa et al., 2016, 2021). They achieved this by locally expressing a fluorescent protein in the Z band, and by using a spinning disc confocal microscope they were able to visualize the same population of sarcomeres at a rate of 100 frames/s, without losing focus throughout the cardiac cycle. Several previous studies have been published on this topic by this group in recent years (e.g., Kobirumaki-Shimozawa et al., 2016), but their *tour de force* has not yet received the attention it deserves.

This study by Kobirumaki-Shimozawa et al. (2021) is unique in its tracking up to 30 sarcomeres in the same myofibril for up to six consecutive contractile cycles in vivo. This first-of-its-kind technology allows examination of interactions among sarcomeres in consecutive cycles. The study demonstrates that average sarcomere shortening nicely follows the pressure development in the ventricle, but there is always a significant fraction of sarcomeres that do not shorten at all, or even stretch during systole. The authors express the relative synchrony of sarcomere shortening as a "contribution index," ranging from −1 for asynchronous (for lengthening when a comparator sarcomere is shortening) to 1 for synchronous. As much as ∼25% of the sarcomeres in a myofibril register a contribution index < 0, and therefore do not appear to contribute to contraction.

The Fukuda group is not the first to report an inhomogeneity in sarcomere length distribution. Earlier reports (Stehle et al., 2002; de Souza Leite and Rassier, 2020) mostly examined isolated myofibrils in the relaxation phase whereas the present study is focused on systole of the intact heart. Thus, multiple levels of observation point to the same phenomenon and it is worthwhile to pay attention, as the implications are quite profound.

The generally accepted assumption is that sarcomere shortening is a self-balancing system due to length-dependent activation: if one sarcomere shortens more than its neighbor, its force production would decline faster, and the neighbor should catch up. The new data show that this assumption is clearly not valid, at least not within a heartbeat. Once a sarcomere is behind in shortening during systole, it does not catch up. Neighboring sarcomeres showed the least amount of correlation in sarcomere length shortening, if not a negative correlation. It is not that these non-contributing sarcomeres are broken, as they tend to be contracting strongly during the next contractile cycle. It appears that serially linked sarcomeres are not self-balancing, but rather that they are a fundamentally unbalanced system that realigns itself during diastole.

It is intriguing to speculate why there is such beat-by-beat inhomogeneity in sarcomere length. It is safe to assume that over six contractions (Kobirumaki-Shimozawa et al., 2021), not much changes in the inotropic and lusitropic state of the heart, so we are solely examining the interplay between the sarcomeres in the myofibril. Among the most striking figures in Kobirumaki-Shimozawa et al. (2021) is Fig. 2, B and C, which shows that the amount of shortening of a given sarcomere during the systolic phase correlates well with the diastolic length of the sarcomere, but not with end systolic sarcomere length. These data imply that the presumed consequences of length-dependent activation are alive and well at the sarcomere level but is set by diastolic sarcomere length alone. In some of our own studies, where we measured force and sarcomere

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length in attached intact myocytes (Helmes et al., 2016; Najafi et al., 2019), we also found that peak force production was strongly correlated with the average end-diastolic sarcomere length and not with the end-systolic sarcomere length. These collective results imply that the length-dependent activation is fully set before the contraction starts. In other words, length-dependent activation is a pre-activation phenomenon, if you will, and is not augmented as contraction progresses.

This finding can be explained by realizing that, in a twitch contraction, force development is time limited by the duration of the calcium transient. For a given ionotropic/lusitropic state, the rate at which force develops is therefore the main determinant of how much total force will be developed. This is in contrast to the classic experiments on permeabilized preparations, where activation usually is maintained until a steady equilibrium force is reached. Such equilibrium is absent during a twitch contraction, and once a sarcomere is behind in force development, it will never catch up and it shortens less or even lengthens during systole (the force–velocity relationship is of course still valid, so it can develop a balancing force in spite of not shortening). This sarcomere, however, is now predisposed for a strong subsequent contraction, as it is now longer than its neighbor at the end of diastole and primed to win the next round. This scenario means that, in the heart, sarcomere length inhomogeneity doesn't really matter that much from a functional standpoint. Those sarcomeres that shorten significantly in one cycle make up for the non-shortening sarcomeres, and force developed by the entire myofibril will not be affected in spite of a significant percentage of non-shortening sarcomeres.

Our viewpoint is speculative, but it is based on the wealth of fascinating raw data that the paper presents. We encourage you to explore the paper yourself and come up with your own conclusions!

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