Increased cardiac muscle calcium sensitivity is associated with impaired coronary vascular flow – Implication for hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is primarily a genetic heart disease that is associated with thickening of the myocardium (heart muscle), which is termed hypertrophy [1]. It is generally accepted that hypertrophy of the myocardium impacts upon the function of the heart by compressing and restricting the flow through the microvessels within the muscle (see Fig. 1, illustrating the main coronary arteries and microvessels within the muscle wall) [2]. The coronary microvessels supply vital oxygen and nutrients to the cardiomyocytes to drive ATP production and meet the energetic demands of the beating heart. Ultimately, this microvascular compression and impaired blood flow accelerates the development of heart failure in HCM patients [1]. On the other hand, however, some patients who are carriers of the genetic mutations in myofilament proteins that are yet to develop pathological hypertrophy demonstrate reduced coronary blood flow, suggesting additional factors underly coronary flow impairment beyond hypertrophy *per se* [1].

Under physiological conditions, blood flows freely though the coronary arteries into the microvessels as the heart is filling with blood during the relaxation (diastolic) phase of the cardiac cycle (Fig. 1(a)), whereas during the contraction (systolic) phase, flow is reduced as the coronary vessels are naturally compressed in a “squeezing” motion [3]. In HCM one of the first pathological features that develops is hypercontractility, where the cardiac muscle becomes hypersensitive to the Ca^{2+}-ion (i.e., calcium sensitivity) and the heart contracts more strongly at much lower concentrations of Ca^{2+} than is normally required [1]. Given that both hypercontractility and impaired coronary blood flow are frequently observed prior to the development of pathological hypertrophy in HCM, we hypothesized that the hypercontractility associated with enhanced Ca^{2+} sensitivity will also impair coronary blood flow (Fig. 1(b)) [1].

This brief study was conducted at SPring-8 **BL20B2**. Absorption imaging with monochromatic X-rays at 34 keV, just above the *K*-edge of iodinated contrast agent provides high spatial and temporal resolution to achieve visualization of coronary vessels < 50 μm in diameter in rodents [4]. We used adult wild-type (WT) C57BL/6J mice (n=4) to avoid potential confounding effects associated with transgenic mouse models of HCM (e.g., myocardial wall thickening, fibrosis) that may mask the effects of hypercontractility [1]. Hypercontractility was pharmacologically induced in mice with an acute bolus of EMD-57033 (10 μM, 150 μL intravenous bolus), which elevates myocardial Ca^{2+} sensitivity and induces a hypercontractile state similar to what is observed in HCM. After inducing deep anesthesia mice were first imaged under baseline conditions (vehicle solution, 150 μL i.v.), then subsequently imaging was repeated 10 minutes post EMD-57033 treatment (Fig. 2). Image frames representing diastolic and systolic phases were manually selected for analysis. Visualized vessel area was determined for three to four microvessels that could be visualized across the entire cardiac cycle for each mouse (Fig. 2).

Under baseline conditions, the coronary microvessel area was reduced by 30 ± 13% during the systolic phase relative to the diastolic phase, which is an expected physiological response (Fig. 3(a)) [1]. By contrast, the

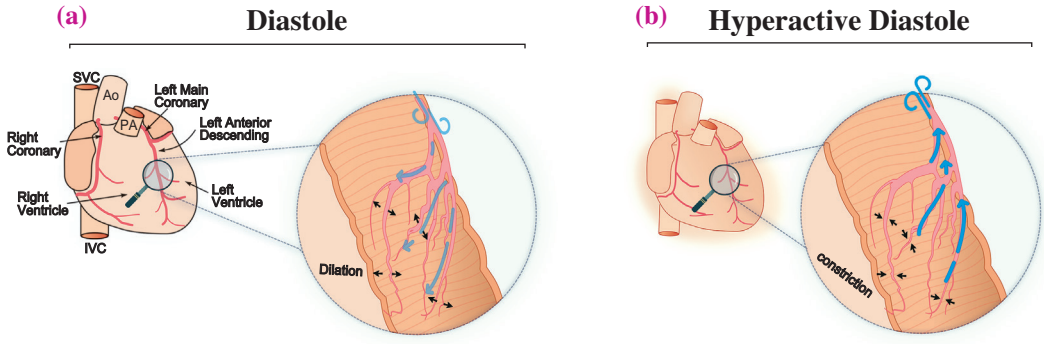

Fig. 1. Hypothesized coronary artery constriction due to hypercontractility. **(a)** In normal diastole, microvessels remain open to support blood flow. **(b)** In hyperactive diastole, excessive contraction compresses microvessels, reducing oxygen supply to deeper muscle within the wall. Ao, aorta; PA, pulmonary artery; IVC, inferior vena cava; SVC, superior vena cava.

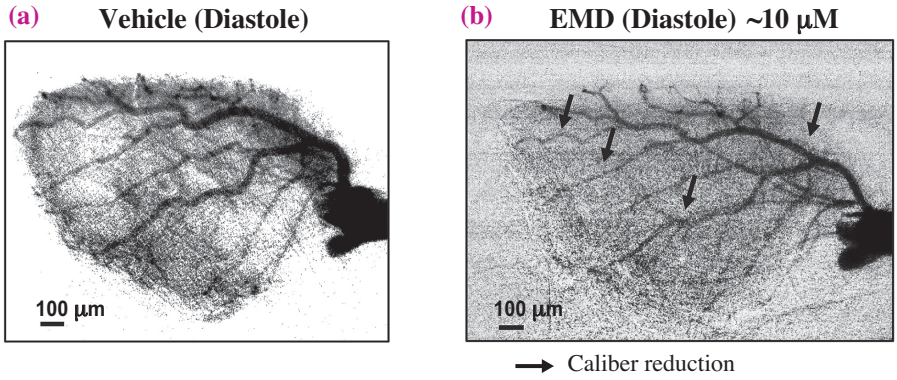

Fig. 2. Synchrotron microangiography showing change in microvascular function. **(a)** Baseline angiogram in diastole after vehicle infusion and **(b)** angiogram 10 minutes post-EMD-57033 infusion shows reduced vessel caliber (arrows). Four wild-type mice were imaged, with 13 microvessels analyzed in total. [1]

coronary vessel area actually increased by 8 ± 7% from the diastolic to systolic phase post-EMD-57033, suggesting that myocardial hypercontractility impaired diastolic coronary flow. Indeed, compared to vehicle baseline, EMD-57033 reduced both the diastolic and systolic vessel areas by 82 ± 16% and 32 ± 11% respectively (Fig. 3(b)). The pronounced constriction of the coronary microvessels observed in this study indicates that myocardial hypercontractility as a result of increased Ca^{2+} sensitivity may be sufficient to impair coronary blood flow.

Our findings in this study have important implications for the management of HCM. Many of the current HCM treatments focus on alleviating the structural manifestations of the myocardium, however, targeting the myocardial hypercontractility from an early stage of HCM may be just as an effective approach. One promising treatment is mavacamten, a novel myosin inhibitor, which is shown to reduce myocardial hypercontractility. Our future studies will further investigate if mavacamten can improve coronary blood flow in animal models of HCM.

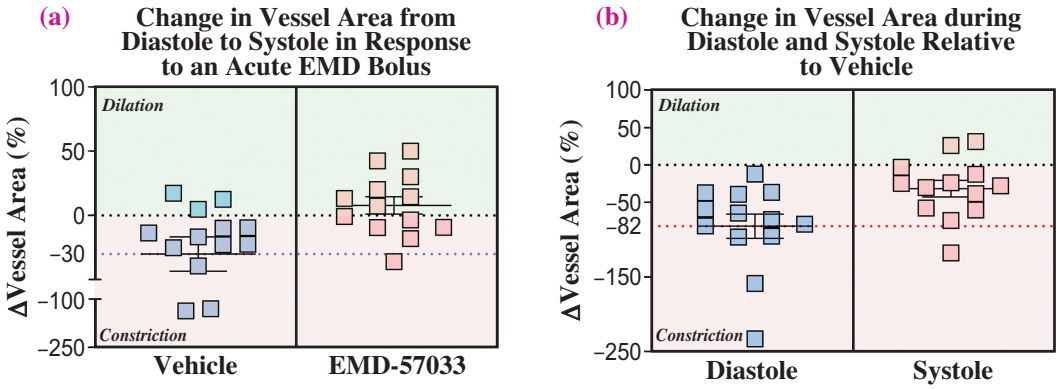

Fig. 3. Percentage changes in vessel area during diastole and systole, with and without EMD-57033. **(a)** Changes from diastole (relaxation) to systole (contraction) following vehicle and EMD-57033 administration. **(b)** Reduction in vessel area in response to the hypercontractile state induced by EMD-57033. Blue and red-dotted lines indicate the mean in the vehicle period and diastole for clarity. [1]

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