

# Cine DENSE and manganese-enhanced cardiac MRI demonstrate that eNOS does not play a determining role in modulating the effects of $\beta$ -adrenergic stimulation

M. H. Vandsburger<sup>1</sup>, B. A. French<sup>1</sup>, X. Zhong<sup>1,2</sup>, C. M. Kramer<sup>1</sup>, and F. H. Epstein<sup>1</sup>

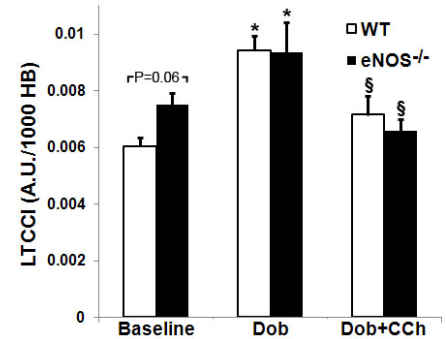
<sup>1</sup>University of Virginia, Charlottesville, VA, United States, <sup>2</sup>MR R&D Collaborations, Siemens Healthcare, Atlanta, GA, United States

**Introduction** Within healthy cardiomyocytes, L-type calcium channel (LTCC) flux and cardiac contractility are linked through the process of calcium induced calcium release (CICR). Stimulation of cardiomyocytes with a  $\beta$ -adrenergic agonist acts to increase LTCC flux and consequently increases contractility. The effects of  $\beta$ -adrenergic stimulation are reversed by muscarinic stimulation. Although abnormal CICR occurs in heart failure, intracellular regulation of CICR in both healthy and disease states is not fully understood. Two isoforms of nitric oxide synthase (NOS), endothelial NOS (eNOS) and neuronal NOS (nNOS), are constitutively expressed in cardiomyocytes and are believed to play roles in modulating CICR and contractility<sup>1</sup>. The prevailing model of NO signaling hypothesizes that eNOS modulates basal CICR by inhibiting LTCC flux<sup>2</sup>. However, modulation of basal LTCC function by eNOS has never been demonstrated *in vivo*. Further, only 2 *in vitro* studies have demonstrated modulation of LTCC function by eNOS either under  $\beta$ -adrenergic stimulation<sup>3</sup>, or following subsequent muscarinic inhibition<sup>4</sup>. Additionally, divergent results in isolated cardiomyocytes from eNOS<sup>-/-</sup> mice<sup>5</sup> suggest that differing *in vitro* preparations may obscure the *in vivo* role of eNOS in contractile function.

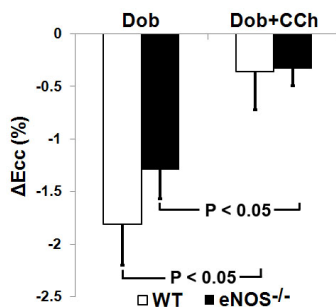
Dynamic Mn-enhanced MRI can be used to assess an index of LTCC function *in vivo* in mice<sup>6</sup>. Also, cine DENSE MRI enables multiphasic strain imaging of cardiac contractile function *in vivo* in mice. The purpose of the present study was to use dynamic Mn-enhanced MRI and cine DENSE to elucidate whether eNOS modulates LTCC function and contractile function *in vivo* at baseline (Bsl), with  $\beta$ -adrenergic stimulation, and with concomitant  $\beta$ -adrenergic and muscarinic stimulation.

**Methods** Measurement of LTCC function using dynamic Mn-enhanced MRI has been previously described in detail<sup>6</sup>. In brief, the uptake of Mn<sup>2+</sup> was probed in two mid-ventricular short axis slices using an ECG-gated saturation recovery pulse sequence with a constant saturation time of 200ms. Images were acquired every 2 to 3 minutes for 90 minutes prior to, during, and after a 30 minute intraperitoneal (IP) infusion of MnCl<sub>2</sub> at a rate of 0.42 mg/kg-min. Signal to noise ratio (SNR) was measured from the entire myocardium and normalized to the SNR of a proton density weighted scan for each slice. Normalized SNR was plotted against the cumulative number of heart beats, and the slope (in units of A.U. per 1000 heart beats) during the infusion period was used as an index of LTCC flux (LTCCI). LTCCI was measured on a 4.7T MR system (Varian, Palo Alto CA) in 8 wild-type (WT) and 8 eNOS<sup>-/-</sup> mice during 3 separate imaging sessions at Bsl, during  $\beta$ -adrenergic stimulation with continuous IP infusion of dobutamine (Dob, 5 $\mu$ g/kg-min), and with concomitant  $\beta$ -adrenergic and muscarinic stimulation with infusion of Dob and carbamylcholine chloride (CCh, 300 $\mu$ g/kg-min) (Dob+CCh).

Contractile function was assessed using a spiral cine DENSE method<sup>7</sup> in 2 mid-ventricular short axis slices. Specifically, peak systolic circumferential shortening ( $E_{cc}$ ) was measured. Cine DENSE was performed sequentially at Bsl, during infusion of Dob, and during infusion of Dob+CCh, during the same imaging session in 9 WT and 9 eNOS<sup>-/-</sup> mice. Imaging was performed on a 7T ClinScan MR system (Bruker, Ettlingen, Germany). Specific imaging parameters included field of view = 32mm, spatial resolution = 250  $\mu$ m<sup>2</sup>, slice thickness = 1mm, TR = 7.1ms, TE = 1ms, averages = 4, number of spiral interleaves = 36, number of cardiac phases = 14, and displacement encoding frequency = 1 cycle/mm. Artifact generating echoes were suppressed using 3-point phase cycling and thru-plane dephasing<sup>8</sup>. For data analysis, segmentation of the heart was performed using a semi-automated technique<sup>9</sup> and displacement and strain were calculated using algorithms described previously<sup>10</sup>. Finally, systolic blood pressure (BP) was measured using a tail cuff system.



**Figure 1.** Measurements of LTCCI indicate that LTCC function is normal in eNOS<sup>-/-</sup> mice (\*P<0.05 vs. BSL, §P<0.05 vs. Dob).



**Figure 2.** Peak  $E_{cc}$  increased in response to Dob in both WT and eNOS<sup>-/-</sup> mice. Inhibition of the Dob response by Dob+CCh was similar in WT and eNOS<sup>-/-</sup> mice.

**Results** Although Bsl LTCCI trended higher in eNOS<sup>-/-</sup> mice (p=0.06 vs. WT), LTCCI was similar in eNOS<sup>-/-</sup> and WT mice in response to Dob, and in response to Dob+CCh (Figure 1). In addition, peak  $E_{cc}$  was similar at baseline in eNOS<sup>-/-</sup> and WT mice (-12.6  $\pm$  0.4% WT vs. -11.6  $\pm$  0.4% eNOS<sup>-/-</sup>, P=NS). Mirroring LTCCI,  $E_{cc}$  increased in both WT and eNOS<sup>-/-</sup> mice in response to Dob, and returned to BSL levels in response to Dob+CCh (Figure 2). Heart rate was significantly higher in WT mice at Bsl (539 $\pm$ 16 BPM WT vs. 495 $\pm$ 12 BPM eNOS<sup>-/-</sup>, p<0.05), but similar in both groups in response to Dob (550 $\pm$ 15 BPM WT vs. 521 $\pm$ 19 BPM eNOS<sup>-/-</sup>, p=NS), and Dob+CCh (547 $\pm$ 16 BPM WT vs. 521 $\pm$ 10 BPM eNOS<sup>-/-</sup>, P=NS). Finally, systolic blood pressure was significantly higher in eNOS<sup>-/-</sup> mice (110  $\pm$  3 mmHg WT vs. 131  $\pm$  2 mmHg eNOS<sup>-/-</sup>, p<0.05).

**Conclusions** Consistent with known modulation of CICR, in WT mice dynamic Mn-enhanced MRI noninvasively detected increases in LTCCI over Bsl with Dob, and an inhibition of the  $\beta$ -adrenergic response with Dob+CCh. Similar findings were observed in eNOS<sup>-/-</sup> mice. Also, in WT mice changes in  $E_{cc}$  with Dob and Dob+CCh were consistent with changes in LTCCI. Again, similar results were observed in eNOS<sup>-/-</sup> mice. Although we detected a trend toward increased basal LTCCI in eNOS<sup>-/-</sup> mice, this is likely explained by the accompanying hypertension in these mice, in whom eNOS is globally deleted. Together, these findings suggest that, counter to the prevailing hypothesis<sup>2</sup>, eNOS does not play a significant role in modulating CICR *in vivo* in the healthy mouse heart. Recent studies have demonstrated increased left

ventricular remodeling in eNOS<sup>-/-</sup> mice after myocardial infarction<sup>5</sup>, although cardioprotective mechanisms remain unclear. A clearer understanding of the role of eNOS in LTCC and contractile function in the healthy heart may help clarify the role of eNOS during LV remodeling.

**References** (1) Belge et al. *Ann NY Acad Sci.* 2005; 1047:173-182. (2) Hare et al. *J. Mol Cell Cardiol.* 2003; 35(7):719-729. (3) Wang et al. *Am J Phys Hrt Circ Phys.* 2008; 294:1473-1480. (4) Han et al. *PNAS.* 1998. 95(11):6510-6515. (5) Rastaldo et al. *Life Sci.* 2007.81:779-793. (6) Vandsburger et al. *Proc ISMRM.* 2009. 17; 807. (7) Zhong et al. *JCMR.* 9:398-399. (8) Zhong et al. *MRM.* 2006. 56(5):1126-1131. (9) Spottiswoode et al. *Med Im Analysis* 200. 13(1):105. (10) Spottiswoode et al. *IEEE Trans Med Imag.* 26:15-30.