# Metabolic flux changes with hyperpolarized $^{13}$C MRS correlate with mitochondrial loss and impairment in a rat model of doxorubicin-induced cardiotoxicity

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**Introduction:** Doxorubicin (DOX) is a chemotherapeutic for the treatment of a wide range of cancers. However, DOX can cause serious cardiac side effects leading to heart failure[1]. The mechanism for this toxicity is not yet fully understood, although mitochondrial oxidative stress and altered cardiac energetics are thought to play a key role in the pathology[2]. Hyperpolarized $^{13}$C MRS can assess real-time metabolic fluxes *in vivo* and the technique has recently transitioned into clinical trials in oncology[3], offering a unique tool to study the metabolic effects of DOX on the heart[4].

**Methods:** Male Wistar rats were treated for 6 weeks with weekly iv injections of either 3mg/kg DOX (n=8) or saline (n=8). Cardiac function and cardiac metabolic fluxes were assessed at weeks 1, 3 and 6 with CINE MRI and hyperpolarized [1-$^{13}$C]- and [2-$^{13}$C]pyruvate MRS, respectively in a 7T spectrometer (Varian). CINE images were acquired as described previously[5]. [1-$^{13}$C]- and [2-$^{13}$C]pyruvate were hyperpolarized as described previously[6] and 1 mL of 80 mM pyruvate was injected into the tail vain over 10s. $^{13}$C MR spectra were acquired every second for 60s using a 72-mm dual-tuned birdcage volume transmit $^{1}$H/$^{13}$C coil and a $^{13}$C two-channel surface receive coil (Rapid Biomedical; 10º hard pulse; 10kHz bandwidth). Multicoil spectra were added in phase, and the first 30s of spectra from appearance of the pyruvate peak were summed and quantified with AMARES/jMRUI[7]. From a second cohort of rats treated for 6 weeks weekly with either 3mg/kg DOX (n=6) or saline (n=6) hearts were collected and mitochondria isolated for oxygen consumption and electron transport chain complex activity measurements. Mitochondrial number was assessed in tissue extracts with qPCR of a mitochondrial marker (cytB) compared to a nuclear marker (GAPDH).

**Results:** We established a rat model of DOX-induced cardiotoxicity characterized by decreased left ventricular ejection fraction and reduced cardiac index. Real-time metabolic flux measurements in the myocardium of these rats using hyperpolarized [1-$^{13}$C]- and [2-$^{13}$C]pyruvate MRS revealed decreased tricarboxylic acid cycle flux and glucose oxidation that correlate with cardiac function (Fig. 1). We also observed reduced $^{13}$C label incorporation into the acetyl-carnitine pool, suggesting reduced acetyl-CoA buffering capacity in the myocardium. We furthermore found a decrease in mitochondrial number and mitochondrial mass in hearts taken from DOX-treated rats. Mitochondrial oxygen consumption and mitochondrial complex activity were also reduced.

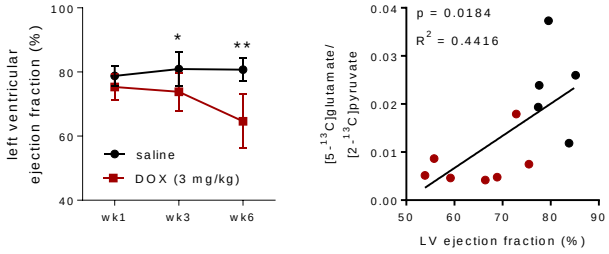


**Figure 1.** CINE MRI (left) showing decreased left ventricular ejection fraction in hearts from DOX-treated rats, which correlates with $^{13}$C-labelling of TCA cycle-derived glutamate from injected hyperpolarized [2-$^{13}$C]pyruvate (right).

**Discussion and conclusion:** We found that metabolic fluxes into oxidative catabolic pathways are reduced in the hearts of rats treated with DOX and this correlates with metabolic fluxes. Furthermore, metabolic fluxes appear to be reduced due to loss and impairment of mitochondria. This study shows the benefit of hyperpolarized MRS to detect impairments in metabolic fluxes directly reflecting mitochondrial impairment non-invasively. These findings suggest that adjuvant treatments that protect the heart from the metabolic effects of DOX may offer new potential for the prevention of DOX-induced cardiotoxicity.

**References:** [1] Moslehi (2016) *N Engl J Med* 375:1457-1467 [2] Tokarska-Schlattner (2006) *J Mol Cell Cardiol* 41:389-405 [3] Nelson *et al.* (2013) *Science translational medicine* 198ra108 [4] Tyler *et al.* (2017) *Proc. Intl. Soc. Mag. Reson. Med.* 25:0726 [5] Dodd *et al.* (2012) *Cardiovascular Research* 95:69-76 [6] Dodd *et al.* (2014) *Circ. Cardiovasc. Imaging* 7:895-904 [7] Vanhamme *et al.* (1997) *J Magn Reson* 129:35-43.