

# Cardiac-targeted NADPH oxidase 4 in the adaptive cardiac remodelling of the murine heart

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## Abstract

**Background** The mechanisms that determine whether the heart adapts to overload stress, or fails, are poorly understood. NADPH oxidase (NOX) proteins produce reactive oxygen species (ROS) involved in redox signalling, and our recent studies have found that an increase in Nox4 during pressure overload protects the heart against failure. We aimed to identify novel Nox4-driven cardioprotective mechanisms that promote adaptive cardiac remodelling.

**Methods** We first undertook a proteomic comparison of heart tissue from cardiac-targeted Nox4-overexpressing mice and controls. The Nox4 cardiac metabolome was then investigated by <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy. Effects on cardiac metabolism were assessed by ex-vivo working heart perfusions and isolated mitochondrial respiration studies. Ex-vivo cardiac energetics were assessed by <sup>31</sup>P NMR. Alterations to cardiac fatty acid oxidation were explored in primary cardiomyocytes (extracellular flux analysis).

**Findings** Cardiac-targeted Nox4 overexpression profoundly remodelled the cardiac proteome in an isoform-specific manner, both in the unstressed and stressed heart. Glycolysis and fatty acid oxidation were identified as the most enriched pathways that were altered by Nox4. Metabolomic analysis showed a 2.2 times increase in acetylcarnitine concentrations ( $p=0.002$ ). Ex-vivo heart perfusions demonstrated a profound increase in palmitate oxidation relative to wild-type hearts (3.6 times increase,  $p=0.01$ ), with opposite findings observed in primary cardiomyocytes with a knockdown of *Nox4*. A preference for fatty acid oxidation in Nox4 hearts correlated with a better energetic state (phosphocreatine:ATP ratio) when subjected to increasing doses of isoprenaline stress under baseline and pressure-overload.

**Interpretation** In this study we identified a novel role for Nox4 in the regulation of cardiac fatty acid oxidation. Cardiomyocyte-targeted Nox4 hearts preferentially oxidised fatty acids for energy provision, improving myocardial energetics under stress. Enhancing fatty acid oxidation might have an adaptive role in the setting of pressure-overload hypertrophy. These data provide novel insights into ROS-dependent metabolic programming.

**Funding** UK Medical Research Council, British Heart Foundation.

## Contributors

AN co-conceived the project; designed experiments; performed the proteomic work immunoblots, extracellular flux analyses, metabolite extractions, and statistical analyses; and wrote the abstract. AH performed the ex-vivo perfusion studies. TE supervised the <sup>1</sup>H NMR and <sup>31</sup>P NMR studies. XY performed the proteomic work with AN. AB initially generated the *Nox4* transgenic mice. MZ provided the murine tissue for proteomic analyses. AS and MM are supervisors for AN's PhD.

## Declaration of interests

We declare no competing interests.

Published Online  
February 26, 2015

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