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New insights into assessment of mitochondrial dysfunction and oxidative stress in preclinical models of diabetic cardiomyopathy

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Introduction: Mitochondrial dysfunction and oxidative stress are major contributors to the development of diabetic cardiomyopathy. Traditional treatments for diabetic cardiomyopathy are often ineffective as they do not specifically target the underlying pathological mechanisms. This is partially attributed to the lack of experimental models that faithfully mimic the mitochondrial phenotype in human diabetes. In this study, we sought to characterise and understand the cardiac functional, mitochondrial and oxidative stress phenotype in mouse, rat, and *ex vivo* human models of diabetic cardiomyopathy.

Methods and results: To examine their functional, mitochondrial and oxidative stress phenotype, human cardiomyocytes (hCM) were derived from induced pluripotent stem cells (iPSC; foreskin-2 cell line). hCM were exposed to 5.55mM glucose for 5 days, followed by either 5.55mM glucose (control) or type 2 diabetes (T2D) conditions (30mM glucose; palmitate, 0.25mM; linoleic acid, 0.1mM; oleic acid, 0.1mM; endothelin-1, 10nM; cortisol, 1µM) for a further 2 days, with endpoint analyses undertaken on day 7. hCM contraction was recorded using a brightfield microscope (Olympus IX71 with DP72 camera) and revealed a significant increase in total contraction duration in T2D hCM (506±14 vs. 343±31 ms respectively; $P<0.001$), and prolonged relaxation time, compared to control hCM (308±18 vs 178±21 ms respectively; $P<0.001$). T2D hCM also exhibited ~2-fold higher mitochondrial superoxide production compared to control hCM (2.2±0.1 vs. 1.0±0.10 fold respectively; $P<0.0001$). However, T2D milieu did not affect mitochondrial membrane potential (determined by tetramethylrhodamine methyl ester fluorescence intensity). The impact of diabetes on mitochondrial function in snap-frozen rodent left ventricle (LV) using the Agilent Seahorse Bioanalyser was also assessed. Type 1 diabetes was induced in male Sprague Dawley rats (8-week-old) with a single dose of streptozotocin (STZ, 65 mg/kg i.p.) or citrate vehicle and followed for 8 weeks of diabetes. Male C57BL/6NTac mice (8-week-old) received 12 weeks of high-fat diet (HFD; 59% lipids) or a standard chow diet as control. At study end, rodent LV were snap-frozen and stored at -80°C. STZ diabetic rat LV exhibited significantly lower mitochondrial complex 1 oxygen consumption rate (OCR) compared to non-diabetic rats (238±30 vs. 318 ±15 $pmol \cdot s^{-1} \cdot mg^{-1}$ respectively; $P<0.05$). However, no differences were observed in mitochondrial complex 2 or 4 OCR between groups. After 12 weeks of HFD, mouse LV exhibited a significant increase in mitochondrial complex 4 OCR (159 ± 28 vs. 218 ±16 $pmol \cdot s^{-1} \cdot mg^{-1}$ respectively; $P<0.05$) compared to normal chow. No differences were observed in mitochondrial complex 1 and 2 OCR between groups.

Conclusion: Our findings suggest that myocardial mitochondrial changes were consistently present in human and rodent models of diabetes. Interestingly, STZ diabetic rats exhibited mitochondrial dysfunction at the level of complex 1, whereas HFD mice exhibited complex 4 dysregulation, and T2D hCM exhibited mitochondria-induced oxidative stress. Although these models of diabetes mimic some of the myocardial mitochondrial changes observed in human diabetes, these changes appear to differ in mechanisms, highlighting the need to further interrogate complementary models of diabetes for novel drug discovery and clinical transition.