Previous literature and our preliminary results have shown a close association between caloric restriction, or fasting, and preserved cardiac function. Indeed, we found that one-day fasting accelerated while two-day fasting reduced mitophagy flux in the mouse heart, demonstrating time-dependent differential effects of fasting on cardiac mitophagy. Mitophagy is a tightly-regulated process that allows cells to eliminate dysfunctional mitochondria in order to maintain cardiac homeostasis. In this study, we characterized the functional role of mitophagy in the heart during fasting by using FUNDC1 knockout mice (KO).

Our cardiac function results demonstrate that two-day fasting impaired cardiac function in wild-type (WT) mice and that this dysfunction was exacerbated in FUNDC1 KO mice, suggesting that FUNDC1 or FUNDC1-mediated mitophagy is essential to the maintenance of cardiac function during fasting. Moreover, western blotting analysis showed that phosphorylated, but not total, pyruvate dehydrogenase (p-PDH) expression was dramatically decreased in fed FUNDC1 KO compared with the fed WT mice, indicating an activation of PDH by FUNDC1 deficiency and, thus, increased utilization of the glycolytically derived substrate pyruvate in FUNDC1 KO heart.
FUNDC1 is Required for the Maintenance of Normal Cardiac Function During Fasting
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**Fasting increases mitophagy at 24-hours but inhibits it at 48-hours**

**Figure 1:** Western blotting results showed increased expression levels of autolysosome marker LC3-II in the mitochondrial fraction at 24-hr fasting compared to fed control, which was further elevated by PepA and E64d, suggesting increased mitochondrial degradation or mitophagy.

**Figure 2:** 48-hr fasting continued to increase LC3-II-mitochondria association. However, LC3-II expression was not further increased by PepA and E64d compared to 24-hr fasting. These results suggest that mitochondria tend to be preserved under prolonged nutrient deprived conditions.
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We subjected FUNDC1 KO mice to 48-hours of complete caloric restriction.
Mitochondria were isolated from fresh mouse cardiac tissues and used for western blot analysis of mitochondrial-associated proteins and stress markers.
Cardiac functional status was assessed by echocardiography and hemodynamic measurement (via left ventricular catheterization). Quantitative data, acquired from Image J and Western blot analysis, are expressed as mean ± SD and analyzed by GEE or GLE as appropriate with significance level set at p < 0.05.
Comparing cardiac dysfunction in WT and FUNDC1 KO mice after 48-hour fast

Figures A-E: Echocardiography and Invasive left ventricular catheterization were used to assess cardiac functional status after 48-hours of fasting. Pmax, maximal left ventricular pressure (A); ESP, end systolic pressure (B); Tau logistic (C); (D); dP/dt min and max (D,E); WT n=6, FKO n=5, p <0.05; *: difference between fed and fasted mice, #: difference between fasted mice.

Figure 3: Western blotting analysis showed that phosphorylated, but not total, pyruvate dehydrogenase (p-PDH) expression was dramatically decreased in fed FUNDC1 KO compared with the fed WT mice. Fasting markedly increased the p-PDH levels in the WT hearts but to a lesser extent than in the KO heart.
1. Knocking out FUNDC1 exacerbates fasting-induced cardiac dysfunction as determined by echocardiography and hemodynamic measurements.

2. Western blotting analysis showed that phosphorylated, but not total, p-PDH expression was dramatically decreased in fed FUNDC1 KO compared with the fed WT mice, indicating an activation of PDH by FUNDC1 deficiency and, thus, increased utilization of the glycolytically derived substrate pyruvate in FUNDC1 KO heart. Interestingly, fasting markedly increased the p-PDH levels in the WT hearts but to a lesser extent than in the KO heart, suggesting that PDH activity remained relatively higher in the KO heart during fasting.

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