Letter to the Editor

Akt2 knockout preserves cardiac function in high-fat diet-induced obesity by rescuing cardiac autophagosome maturation

Dear Editor,

Accumulating studies have demonstrated that the autophagy–lysosome pathway, a major pathway governing protein and organelle degradation and recycling, is a house keeper in cardiomyocytes under physiological conditions (Mizushima and Klionsky, 2007). However, the role of autophagy in the heart under pathological conditions is still controversial (Nemchenko et al., 2011). In vivo studies depicted that inhibition of mammalian target of rapamycin (mTOR), a primary inhibitory regulator of autophagy, is capable of attenuating pressure overload-induced cardiac dysfunction (McMullen et al., 2004). To the contrary, recent studies have also indicated that suppressing autophagy is beneficial for cardiac hypertrophy (Cao et al., 2011). Along the same line, activated autophagy has been proved detrimental for pressure overload-induced heart failure (Zhu et al., 2007). However, the role of autophagy in the heart in high-fat diet (HFD)-induced obesity is poorly understood.

To date, there is little evidence suggesting a role of autophagy in heart anomalies associated with diet-induced obesity, although a number of upstream regulators of autophagy have been identified to play a role in HFD-induced obesity. For example, the primary inhibitor of autophagy mTOR may be hyperactivated by an HFD and contribute to the development of cardiac dysfunction (Birse et al., 2010). As the major activator of mTOR, the Akt family of serine–threonine kinases is also activated by an HFD in the heart. However, the precise role of Akt2, one of the three Akt isoforms predominantly found in the heart, in autophagy regulation in HFD-induced obesity still remains elusive. To this end, the present study was designed to evaluate the role of autophagy and autophagy flux in HFD feeding-induced cardiac geometric and functional changes with a special focus on Akt2 signaling.

HFD intake significantly increased body and organ (heart, liver, kidney and adipose tissue) weights compared with low-fat diet (LFD) feeding (Supplementary Table S2). Western blot analysis confirmed the absence of Akt2 in hearts from Akt2—/— mice (Supplementary Figure S1A and B). Interestingly, HFD feeding upregulated cardiac expression of Akt2 (Supplementary Figure S1A and B) but not that of Akt1 (Supplementary Figure S7A and B) and Akt3 (Supplementary Figure S7A and C). Akt2 knockout did not affect body or organ weight in LFD-fed mice (Supplementary Table S2). However, Akt2 knockout effectively nullified HFD-induced gain in body and organ/tissue weights, in particular the heart (Supplementary Table S2). Accumulating studies have demonstrated that Akt regulates cell growth and lipid biosynthesis through mTORC1. Accordingly, we found that an HFD-activated Akt (Supplementary Figure S6A and B) and mTORC1 (Supplementary Figure S7A and I) in the heart, both of which were mitigated by Akt2 knockout. These data depict a beneficial effect of Akt2 knockout against HFD-induced weight gain possibly through the inhibition of Akt-mTORC1 activation. In addition, HFD feeding significantly increased the level of triglyceride, the effect of which was ablated by Akt2 knockout (Supplementary Figure S1C). Further scrutiny of glucose metabolism using intraperitoneal glucose tolerance test revealed overt glucose intolerance following HFD intake in the wild type (WT) which was partially attenuated in the Akt2—/— mice (Supplementary Figure S1D and E).

HFD feeding significantly compromised myocardial geometry and function as evidenced by overtly increased LV ESD, LV EDD and LV mass, as well as decreased fractional shortening associated with unchanged septum and posterior wall thickness. Interestingly, Akt2 knockout ameliorated HFD feeding-induced cardiac geometric and contractile anomalies (Figure 1A and B and Supplementary Figure S2A–E). Further assessment of cardiomyocyte contractile function revealed consistent findings. HFD feeding dampered cardiomyocyte contractile capacity (decreased peak shortening and maximal velocity of shortening/re-lengthening) associated with unchanged duration of shortening and re-lengthening, which was recovered by Akt2 knockout (Figure 1C and D and Supplementary Figures S2F–I and S3A–C). Besides, Akt2 knockout significantly ameliorated intracellular Ca2+-handling dysfunction induced by an HFD in the WT mice (Supplementary Figure S2J–O). Additionally, HFD feeding induced cardiac hypertrophy (Supplementary Figure S4A–H), interstitial fibrosis (Supplementary Figure S5A and B), and activated cardiac protein synthesis pathway (Supplementary Figures S6A–K and S7A, J, and K), which were obliterated by Akt2 knockout. Taken together, these results supported that Akt2 knockout protected murine hearts against HFD-induced cardiac pathological hypertrophy.

Interestingly, our data revealed that the expression of LC3B I (microtubule-associated protein light chain 3 I) and type B) was dramatically increased following HFD feeding in both the WT and Akt2—/— mice, indicating that HFD feeding may trigger the initial autophagy steps (Figure 1E and Supplementary Figure S8A, C–G, J, and L–O). LC3B II integrates onto the autophagosomal membrane, and is widely used as a marker of autophagosomes. Nonetheless, an increase in LC3B II may represent either an increase in autophagosome formation (initiation of autophagy) or a
blockade in autophagolysosome formation (completion of autophagy) (Mizushima et al., 2010). To further examine the expression of p62, a specific autophagy adaptor preferentially degraded by autophagy (Bjorkoy et al., 2005). Remarkably, we found significantly increased level of p62 following high-fat feeding, the effect of which was nullified by Akt2 knockout (Figure 1E and Supplementary Figure S8F and K). In addition, although accumulation of autophagosomes was noted in both WT and Akt2−/− mice fed HFD, only Akt2−/− mice displayed HFD-induced accumulation of the single membrane vacuoles, representing autophagolysosomes (Figure 1F and Supplementary Figure S8L). Since the accumulation of LC3B and p62 can be caused by either a defect in autophagosome formation or inhibition of lysosomal function, we further examined the lysosome function. Interestingly, no significant change in lysosome function was observed in the presence of LFD or HFD in WT and Akt2−/− mouse heart tissues (Supplementary Figure S7A and H). Additionally, we did not find any significant changes in polyubiquitinated protein levels (Supplementary Figure S7A and S), reminiscent of our previous findings. At the same time, Beclin1 was significantly elevated by an HFD in the Akt2−/− mice, although it was unaffected by an HFD in the WT mice (Supplementary Figure S8A and G). To further confirm cardiac autophagy activity, we measured the autophagic flux in WT and Akt2−/− mouse hearts. Further accumulation of LC3B II was observed in the presence of chloroquine in the heart tissue from WT-LFD, Akt2−/−/LFD and HFD but not the WT-HFD group (Supplementary Figure S8B and M–O). Although HFD-induced obesity in most of the WT mice, there were still several WT mice which were lean through the experimental trial (WT-HFD lean mice). We studied cardiac autophagy activity from these lean WT mice on HFD to examine whether weight gain in other tissues can influence cardiac autophagy status. As expected, HFD disrupted cardiac autophagosome maturation in lean WT mice (Supplementary Figure S9A–E), in a manner similar to HFD-induced obese group. Taken together, these data suggested that HFD is capable of initiating cardiac autophagy in the WT and Akt2−/− mice. However, the maturation of autophagosomes was disrupted by the HFD in the WT mice, the effect of which was rescued by Akt2 knockout.

Recent evidence suggested a role of Rab7, a small GTPase protein capable of stimulating lysosomal biogenesis and maturation of autophagic vacuoles by promoting their fusion with endosomes and lysosomes, in the regulation of autophagic flux under various stresses (Harharian et al., 2010). Indeed, HFD significantly suppressed the expression of Rab7 the effect of which was abolished by Akt2 knockout (Supplementary Figure S8A and I), indicating that Rab7 may play a role in the HFD- and Akt2 knockout-induced response of autophagosome maturation process.

Our study revealed that the beneficial role of Akt2 knockout in rescuing HFD-induced cardiac anomalies was associated with facilitating cardiac...
autophagosome maturation process, an effect recapitulated by palmitic acid (PA) *in vitro* (Supplementary Figure S10). Interestingly, PA-induced disruption of autophagosome maturation occurred in a time-course manner (Supplementary Figure S11A–E). More importantly, Akt2 depletion rescued the disrupted autophagosome maturation as early as 3 h and preserved through 6 h following treatment with PA (Supplementary Figure S11A–E). Despite Akt2 knockout prevented the autophagic flux disruption and preserved cardiac function at the same time, these observations do not provide direct evidence with regard to the cause–effect relationship between reactivation of autophagic flux and beneficial role of Akt2 knockout.

To prove that the beneficial role of Akt2 knockout is based on its influence on cardiomyocytes, we challenged isolated cardiomyocytes with PA (100 μM, 2 h at room temperature) to test the protective effect of Akt2 knockout on cardiomyocyte contractile functions. Our data revealed that PA elicited cardiomyocyte contractile dysfunction in the WT mice, consistent with our previous report (Figure 1G and H and Supplementary Figure S12). Of interest, Akt2 knockout abolished PA-induced cardiomyocyte contractile dysfunction, reminiscent of our *in vivo* studies described above (Figure 1G and H and Supplementary Figure S12).

To demonstrate that autophagosome maturation process is playing a role in PA-induced cardiomyocyte contractile dysfunction, and the cardioprotective effect of Akt2 knockout is relying on its facilitating cardiac autophagosome maturation process. The salient findings from this work indicated a protective role of Akt2 knockout against HFD-induced geometric and functional anomalies. Although previous data suggested that Akt1 and Akt3 play a key role in mediating cardiac hypertrophy in various animal models, our data showed that cardiac expression of Akt1 and Akt3 was unaffected by HFD. To the contrary, we found that Akt2 played a predominant role in HFD-induced cardiac hypertrophy and contractile dysfunction. More importantly, our data revealed that the autophagosome maturation process is involved in HFD- and Akt2 knockout-induced myopathic changes. Our *in vivo* observations indicated that HFD promoted the initiation of autophagy and accumulation of autophagy, although it disrupted autophagosome maturation probably at the step of autophagosomes fusion with lysosomes. However, although Akt2 knockout did not affect the initiation of autophagy by HFD, it rescued HFD-induced disruption of autophagosome maturation process and facilitated the transition from autophagosomes to autophagolysosomes. Although the underlying mechanisms of cardioprotective effect of cardiac autophagy in the presence of HFD is still a mystery, our data suggested that the autophagosome dysfunction was associated with increased apoptosis (Supplementary Figure S7A, D, and E), mitochondrial injury (Supplementary Figure S7A, F, and G), intracellular Ca²⁺ dysregulation (Supplementary Figure S7A, P–R) and contractile proteins dysfunction (Supplementary Figure S7A, L–O). Rescuing of the cardiac autophagy by Akt2 knockout inhibited apoptosis and improved mitochondrial performance, intracellular Ca²⁺ homeostasis and contractile protein levels (Supplementary Figure S7A, D–G, and L–R).

Perhaps the most intriguing finding from our present study is that rescuing defective autophagosome maturation process is pivotal for the cardioprotective effect of Akt2 knockout against HFD-induced obesity (Supplementary Figure S13). This is supported by the observation from Baf A1 treatment, which inhibits the fusion of autophagosomes with lysosomes. Although Baf A1 did not further deteriorate cardiomyocyte contractile dysfunction triggered by PA, it mitigated the beneficial effect of Akt2 knockout against PA-induced cardiomyocyte contractile anomalies.

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References


