The "Pathological Effect" of AMP-activated Kinase α_1-subunit Knock-out on Mouse Heart

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Abstract

Heart failure causes one million hospitalizations and contributes to 250000 deaths a year in the USA alone. Cardiac hypertrophy is a poor prognostic sign for heart failure. Cardiac hypertrophy caused by exercise training is the exception, and exercised hearts are protected against heart failure. Our laboratory has shown that hearts from α1-AMPK KO mice present with increased heart weight and myocyte diameter, with no myofibrillar disarray, fetal gene reactivation or increased mortality compared to the wild type (WT) mice. Further, function of these hearts is enhanced similar to that seen for exercised animals. Our objective is to determine if these hearts are also resistant to development of pathological hypertrophy and heart failure. For this study, heart failure will be induced by treatment with angiotensin II for 2 weeks delivered by subcutaneously implanted Alzet minipumps. Mice will be divided into 4 groups: KO with Ang II, KO with saline WT with Ang II, WT with saline. Echocardiography will be performed before and 14 days after surgery. After sacrifice, hearts will be compared by histopathology for changes in heart and myocyte size, connective content and myofibrillar array. It is reported that energy depletion inhibits protein synthesis to conserve cellular energy. Our data suggest loss of α1 AMPK allows increased beneficial protein synthesis. We predict that hearts lacking α1-AMPK will be less susceptible to development of pathological hypertrophy and heart failure similar to that seen in exercised hearts

Introduction

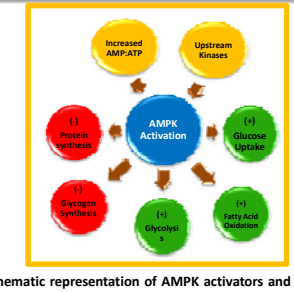

Figure 1. Schematic representation of AMPK activators and downstream targets. AMPK exists as a heterotrimeric, serine/threonine kinase. The alpha subunit contains the catalytic activity and exists in two isoforms (α1, and α2). Regulatory subunits, βγ also exists in multiple isoforms. Tissue distribution of isoforms varies.

Objective

Our objective is to test the effect of conditions that increase cardiac work on the hypertrophic response of α_1-AMPK knock out (KO) mice. α_1-AMPK KO mice have congenital hypertrophy that does not appear to be pathological. Both KO and wild type (WT) mice will be studied. Overwork induced hypertrophy will be caused by exercise and by hypertension. Mice will be provided access to running wheels in their cages for 2 months of exercise. Hypertension will be caused by treatment with angiotensin II for 2 weeks. Hypertrophic response will be measured by comparison of heart weight to body weight and tibial length. Development of heart failure will be assessed from histopathology images.

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Sedentary α_1-AMPK KO Hearts are Hypertrophic

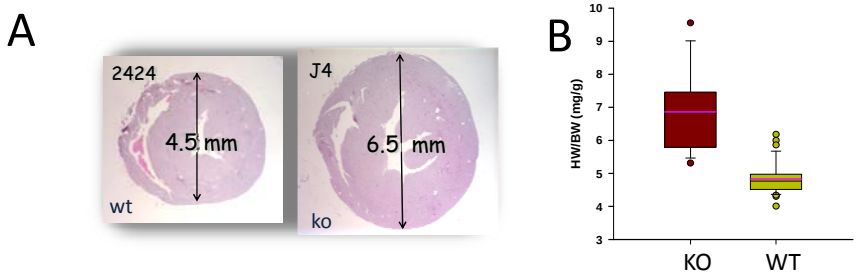

Figure 2. Evidence for Cardiac hypertrophy in α_1-AMPK knock out . (A) Coronal section showing increased cardiac mass of KO with proportional increases in chamber volume. (B) Averaged data of heart weight to body weight ratio for n = 33 WT, 22 KO. *, indicates significantly different at p< 0.05. KO hearts showed no evidence of increased glycogen deposition, fibrosis or fetal Gene RNA.

Sedentary α_1-AMPK KO Hearts Have Increased Function

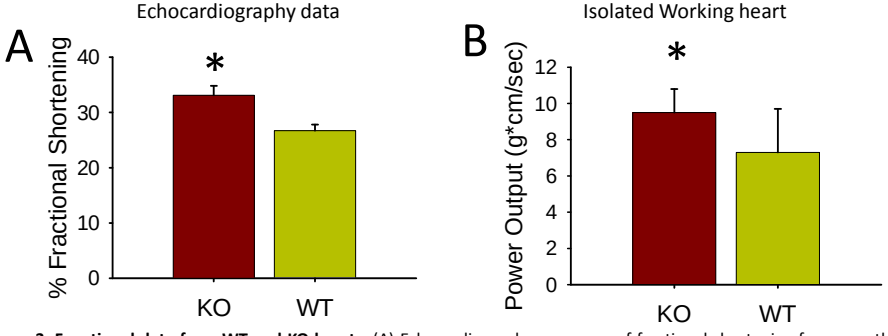

Figure 3. Functional data from WT and KO hearts. (A) Echocardiography measures of fractional shortening from anesthetized mice demonstrates increased ventricular force in KO compared to WT (n=12 each). (B) Isolated working hearts (n=4 each) show increased force of contraction in the KO compared to WT. *, indicated significantly different at p<0.05.

These data suggest α_1-AMPK KO hearts resemble hearts from exercised mice that typically are hypertrophied with increased performance. Exercised hearts also have been shown to be protected from development of heart failure. We tested whether α_1-AMPK KO hearts would develop heart failure if they were forced to hypertrophy further. Hypertrophy was induced by either 1) exercise training for 2 months or 2) treatment with angiotensin II.

Effect of Exercise

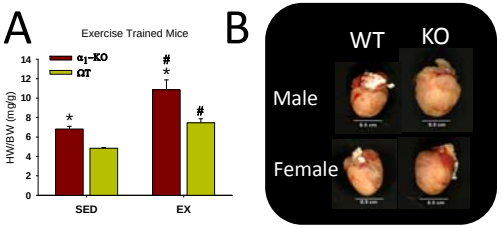

Figure 3. (A) Similar exercise training in the WT and KO induces significantly more hypertrophy in KO. Distance run was not different between WT and KO. KO hearts are also still larger than WT. (B) Representative examples of hearts after exercise from WT and KO. *, significantly different from WT; #, significantly different from sedentary.

Effect of Hypertension

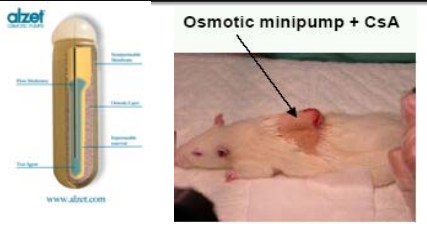

Figure 4. KO and WT mice have been surgically implanted with ALZET mini-pumps, each containing 100ul of fluid containing either saline (sham) or angiotensin II (2mg/kg/day). Angiotensin will be released for 14 days. Cardiac function will be assessed by echocardiography before pumps are implanted and again at 14 days, prior to sacrifice. After sacrifice hearts will be weighed, fixed in formalin and stained with Mason's trichrome for measures of connective tissue.

Summary and Conclusions

Increased cardiac work due to exercise training caused hypertrophy of WT heart and further hypertrophy of KO. KO heart size was still greater than WT. There was no evidence of heart failure due to increased heart size. A subset of mice were implanted with osmotic pumps containing Ang II. 14 days post surgery, mice will receive echocardiograms to assess heart function *in-vivo*. After sacrifice, HW/BW and HW/tibial size ratios will be made. Hearts then will be assessed by histopathology for connective tissue changes indicative of heart failure. I hypothesize that the KO's "physiological hypertrophy" will protect them more than the WT from development of heart failure. Therefore, I expect to see less cardiac damage in the KO.