Deficiency of Ataxia Telangiectasia-mutated Kinase (ATM) Preserves Heart Function by Affecting Cardiac Remodeling in Response to Western-type Diet (WD) in Female Mice

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ABSTRACT

Background: Western-type Diet (WD) and deficiency of ATM protein independently associate with heart disease. Previous work demonstrated that WD in male ATM deficient mice induces accelerated body weight gain and heart dysfunction (Am J Physiol Heart Circ Physiol. 2021;320:H2324-H2338). Conversely, WD in female ATM deficient mice attenuates weight gain and preserves heart function (unpublished data). Here, we investigated the mechanism by which ATM deficiency preserves heart function in female mice. It is hypothesized that ATM deficiency attenuates adverse myocardial remodeling in female mice, thereby preserving heart function. **Methods**: Female wild-type (WT) and ATM heterozygous knockout (hKO) mice, aged 6 weeks, were fed with normal chow (NC) or WD for 14 weeks. Heart sections were stained with Masson's trichrome to quantify fibrosis, TUNEL-stained to quantify apoptosis, and WGA (wheat germ agglutinin)-stained to quantify myocyte hypertrophy. Data were analyzed using ANOVA followed by Student-Newman-Keuls test. **Results**: ATM deficiency associated with increased fibrosis, apoptosis (myocytes and non-myocytes) and hypertrophy in hKO-NC vs WT-NC ($p<0.05$; n=4). WD significantly increased fibrosis in WT-WD mice, while no increase in fibrosis was observed in hKO-WD. WD significantly increased apoptosis in both genotypes. However, the increase in apoptosis was significantly lower in hKO-WD vs WT-WD ($p<0.05$; n=4). WD increased hypertrophy in WT group. However, the hypertrophy remained significantly higher in hKO-WD vs WT-WD ($p<0.05$; n=4). **Conclusion:** WD-mediated increase in myocardial fibrosis, apoptosis, and hypertrophy may decrease heart function in WT female mice. The preservation of heart function in ATM-deficient female mice in response to WD may involve decrease in myocardial cell apoptosis.

INTRODUCTION

- Cardiovascular diseases are the leading cause of mortality worldwide.
- Consumption of Western-type diet (WD) often leads to obesity, which induces cardiac dysfunction such as worsening of fibrosis, hypertrophy, biventricular stiffness, and ischemic events (1,2).
- Ataxia telangiectasia mutated kinase (ATM) is a cell cycle check-point protein which becomes activated in response to double-stranded DNA damage, oxidative damage, or by other genotoxic mediators (4).
- Mutations in *ATM* gene cause a multisystemic disease known as ataxia-telangiectasia (A-T). A-T patients prefer to consume a diet high in fat and sugar. Patients with mutation in one allele, constitute ~1.4 to 2% of population, exhibit enhanced susceptibility to ischemic heart disease (3,4).
- Using male deficient mice, our lab demonstrated that WD in male ATM heterozygous knockout (deficient; hKO) mice accelerates body weight gain and induces heart dysfunction (5). Unpublished work from the lab provided evidence that WD in female ATM deficient mice attenuates weight gain and preserves heart function.

HYPOTHESIS

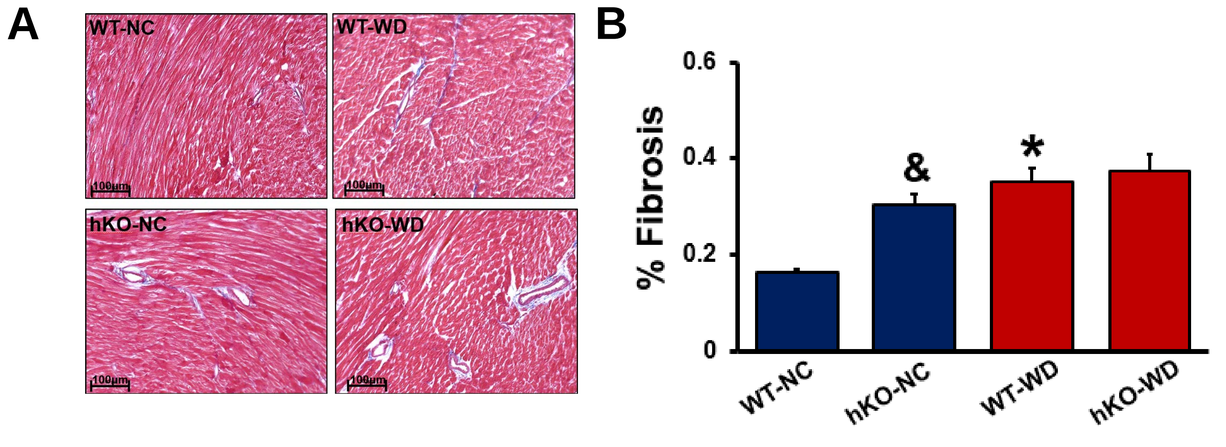
ATM deficiency attenuates adverse myocardial remodeling in female mice via the modulation of myocardial fibrosis, myocyte hypertrophy, and apoptosis.

MATERIALS AND METHODS

- **WD feeding:** Wild-type (WT) and hKO mice, 6 weeks old, were placed on normal chow (NC) or WD for 14 weeks.
- **Masson's Trichrome staining**: Mid-cardiac cross sections (5µm thick) were stained with Masson's Trichrome to quantify fibrosis. Percent fibrosis was calculated by dividing the total fibrosis area (blue staining) by the total tissue area multiplied by 100.
- **TUNEL-staining assay:** Mid-cardiac sections (5µm thick) were stained using TUNEL kit (in Situ Cell Death Detection Kit; Roche) to identify apoptotic cells, followed by counterstaining with rhodamine-conjugated wheat germ agglutinin (WGA) to identify myocytes and Hoechst 33258 to identify nuclei. Index of myocyte or total cell apoptosis was calculated as the percentage of myocyte apoptotic nuclei or total cell apoptotic nuclei / total number of nuclei multiplied by 100.
- **Myocyte Cross-Sectional Area:** WGA-stained cross-sections (5µm thick) were used to measure myocyte cross-sectional area. Suitability of myocytes for cross-sectional area was defined with centrally located nucleus.
- **Statistical analysis:** All the data shown is expressed as mean ± SE. Data were analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test or 2-tailed Student's t-test. Probability (p) values of <0.05 are considered to be significant.

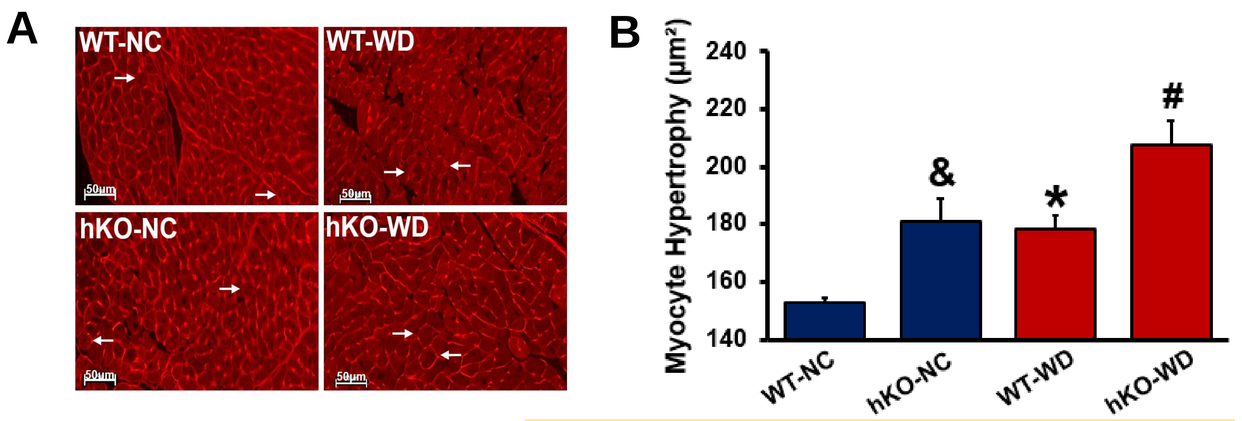
RESULTS

Figure 1. WD exacerbates fibrosis in wild-type mice.



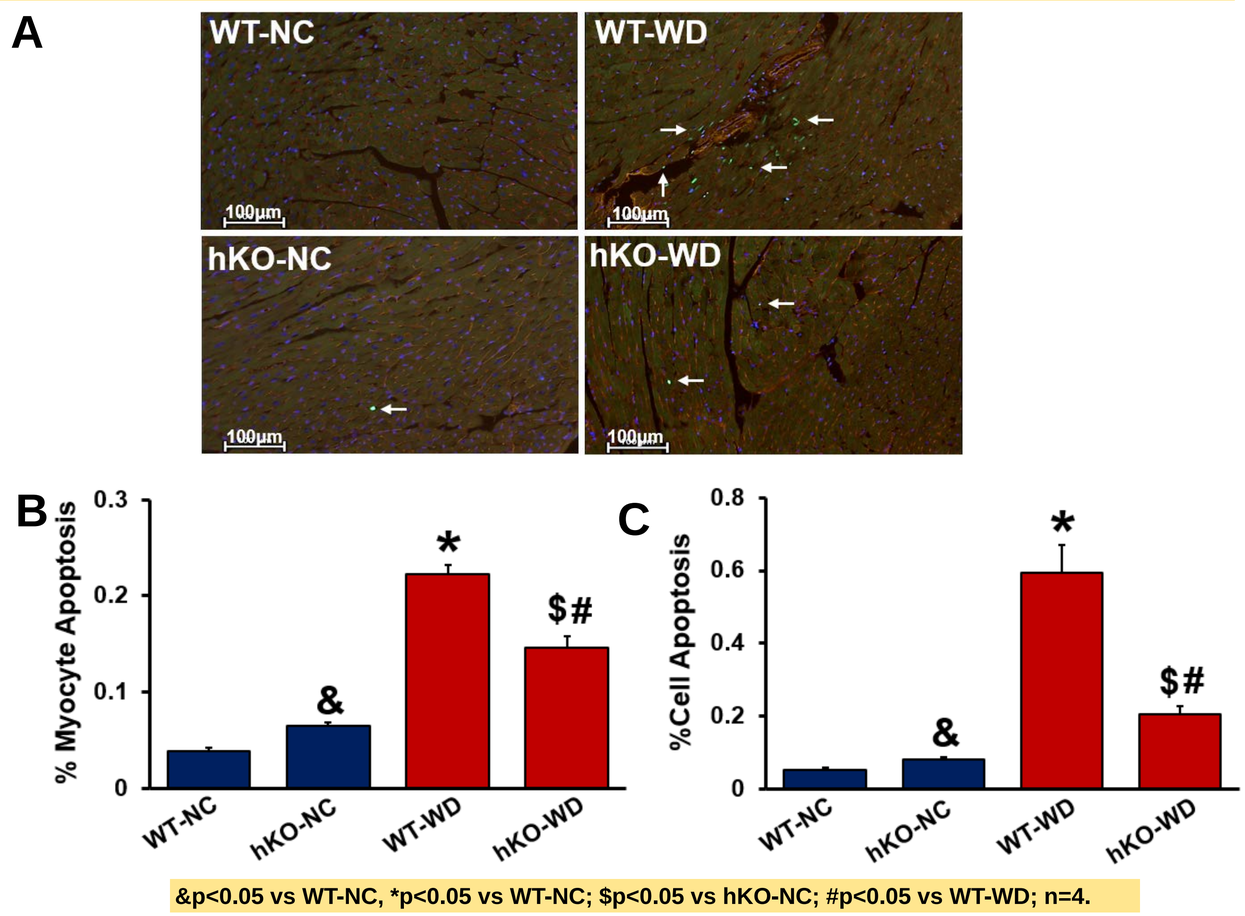
&p<0.05 vs WT-NC, *p<0.05 vs WT-NC; n=4.

Figure 2. WD increases myocyte hypertrophy in WT group, and it remains higher during ATM deficiency.



&p<0.05 vs WT-NC, *p<0.05 vs WT-NC; #p<0.05 vs WT-WD; n=4.

Figure 3. ATM deficiency attenuates apoptosis in the heart in response to WD



&p<0.05 vs WT-NC, *p<0.05 vs WT-NC; $p<0.05 vs hKO-NC; #p<0.05 vs WT-WD; n=4.

SUMMARY

- **Fibrosis, Hypertrophy, and apoptosis are significantly higher in hKO-NC vs WT-NC.**
- **WD significantly increased fibrosis in WT, not in hKO-WD, group.**
- **WD increased myocyte hypertrophy in WT, not in hKO-WD, group. However, myocyte hypertrophy remained significantly higher in hKO-WD vs WT-WD.**
- **WD significantly increased apoptosis (myocyte and non-myocyte) in both genotypes. However, myocyte and non-myocyte apoptosis was significantly lower in hKO-WD vs WT-WD**

CONCLUSION

WD-mediated increase in myocardial fibrosis, apoptosis, and hypertrophy may decrease heart function in WT female mice. The preservation of heart function in ATM-deficient female mice in response to WD may involve decrease in myocardial cell apoptosis.

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