5-2023

EFFECTS OF ATAXIA TELANGIECTASIA-MUTATED KINASE (ATM) DEFICIENCY ON CARDIAC REMODELING IN RESPONSE TO WESTERN-TYPE DIET (WD) PRIOR TO AND FOLLOWING MYOCARDIAL INFARCTION

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by

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An Undergraduate Thesis Submitted in Partial Fulfillment

of the Requirements for the University Honors Program

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ABSTRACT

Cardiovascular diseases are the leading cause of mortality worldwide. Ataxia telangiectasia mutated kinase (ATM) is a checkpoint protein involved in cell cycle regulation. It is activated in response to genotoxic mediators such as double-stranded DNA damage or oxidative damage. Mutations in the ATM gene result in a multisystemic disease called ataxia-telangiectasia (A-T). Independently, a Western-type Diet (WD) and ATM protein deficiency are linked with heart disease, exacerbated cardiac remodeling, and myocardial infarction (MI). Our laboratory has previously shown that in male mice, the consumption of a WD during ATM deficiency is associated with the exacerbation of cardiac remodeling. This study investigated the effect of ATM deficiency on WD-induced cardiac remodeling parameters before and 1-day post-MI in a sex-specific manner using female and male mice. Age-matched wild-type (WT) and ATM heterozygous knockout (hKO) mice were fed with normal-chow (NC) or WD for 14 weeks. MI was induced by ligation of the left anterior descending coronary artery (LAD) with a 7-0 polypropylene suture. After the study period, 14 weeks post-WD feeding and 1-day post-MI, the heart was removed through an opening in the diaphragm region. Heart sections were stained with Masson's trichrome to quantify fibrosis, TUNEL-stained to quantify apoptosis, infarct size, and infarct thickness, and wheat germ agglutinin-stained to quantify myocyte hypertrophy. In WT female mice, WD increased myocardial fibrosis, myocyte hypertrophy, and apoptosis at baseline compared to NC. However, in hKO-WD female mice, apoptosis was significantly lower, and hypertrophy was significantly higher than in WT-NC female mice at baseline. Intriguingly, no significant difference in apoptosis, infarct size, and infarct thickness was observed in both genotypes and genders 1-day post-MI. Thus, our data suggest that 1) ATM deficiency plays a cardioprotective role in female mice responding to WD, as it reduces apoptosis and increases

hypertrophy at baseline, and 2) sex-specific cardioprotective effects of ATM deficiency in female mice were not observed 1-day post-MI in response to WD.

ACKNOWLEDGEMENTS

First, I would like to express my deepest gratitude to Dr. Krishna Singh for her unwavering support and dedication throughout my research project. Her guidance and mentorship have been invaluable to my personal and academic growth, as she encouraged me to expand my knowledge and skills as a researcher and think critically in the lab. Her open-door policy and the teamwork environment she fostered in the laboratory made me feel welcomed and supported. I have learned so much under her supervision over the past few years and will always be grateful for this remarkable experience.

Next, I would like to thank Dr. Mary Wingard for her invaluable support and guidance throughout my undergraduate research project. I am also grateful to my lab group, including Paige Shook, Dr. Suman Dalal, Bobbie, and Dr. Mahipal Singh, for their commitment and patience in teaching me complex concepts and techniques. Their encouragement and constructive feedback helped me grow as a researcher and contributed significantly to the success of this project.

Lastly, I would like to thank my family. Their encouragement, love, and guidance have been instrumental in shaping me into the person I am today. They have always been there for me, cheering me on during my successes and failures. I am particularly grateful to my parents, who have sacrificed so much to provide me with the opportunity to pursue higher education. Their unwavering support and belief in me have been a constant source of motivation. I am truly blessed to have such a wonderful family, and I could not have accomplished this without their love and support.

*The data presented in the thesis was also a part of Mary Wingard's thesis (a graduate student) as I worked with Mary Wingard to collect the data. Part of the data is published in Physiol Rep. 2022:e1543 (PMID 36117462).

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INTRODUCTION

Heart Disease

Heart disease is the leading cause of death in both men and women in the United States. According to the Centers for Disease Control and Prevention, approximately 697,000 people die yearly due to heart disease (1). Notably, the heart disease mortality rate is substantially higher in the Appalachian region of the United States. This disparity is attributed to numerous factors, such as prominent levels of obesity, tobacco abuse, physical inactivity, and unregulated cholesterol and blood pressure. However, heart disease remains a significant medical issue requiring extensive research to develop more efficacious medical interventions.

Ataxia Telangiectasia Mutated Kinase

Ataxia telangiectasia mutated kinase (ATM) is a high molecular weight serine/threonine kinase with a molecular mass of ~370 kDa and belonging to the phosphoinositide 3-kinase (PI3K) family (2). ATM performs multifaceted functions and is distributed across subcellular compartments such as the nucleus, cytoplasm, and mitochondria (3, 4). The signaling pathways mediated by ATM encompass a plethora of downstream targets that modulate critical cellular processes, including but not limited to DNA damage repair, programmed cell death or apoptosis, cell cycle arrest, metabolic regulation, cellular proliferation, and oxidative stress sensing (2). In the nucleus, ATM is involved in regulating cell cycle checkpoints, DNA damage response, telomere maintenance, and chromatin unfolding (5). Under normal circumstances, ATM exists as an inactive dimer/tetramer. However, its activation is rapidly induced in response to genotoxic agents such as DNA double-stranded breaks, reactive oxygen species, oxidative damage, and ionizing radiation (6). Upon DNA double-stranded breaks, the phosphorylation of a histone

(H2AX) within the chromatin serves as a binding platform for repair. The MRE11-RAD50-NBS1 (MRN) complex recruits ATM to the site of double-stranded breaks, where it undergoes autophosphorylation of ser1981 to activate the monomer form of ATM (7, 8). This activation is essential for homologous recombinant DNA repair. The complex also phosphorylates a series of accessory proteins (BRCA1, MDC1/NFBD1, 53BP1) in an ATM-dependent manner (9). ATM then regulates the cell cycle to prevent cell division concomitantly with DNA repair (9). In addition to DNA damage repair, ATM serves as a redox sensor, regulating metabolic control, mitochondrial function, and oxidative stress signaling (4). Moreover, ATM exerts its cytoplasmic functions by modulating critical cellular processes, such as regulation of autophagic activity, angiogenic response, glucose metabolism, and mitochondrial as well as peroxisomal functions during oxidative stress (4, 6). ATM plays a crucial role in modulating structure and function of the heart (14). Further, ATM plays a role in the normal development and activity of various bodily systems, including the cardiovascular, nervous, and immune systems. Thus, efficient repair of damaged DNA strands mediated by ATM is vital in maintaining the stability of the cell's genetic information.

Ataxia-Telangiectasia

Mutations in ATM cause a multisystemic disease known as Ataxia-telangiectasia (AT). AT is an autosomal recessive disorder that occurs due to mutations in the ATM gene and is characterized by multiorgan system dysfunction, including cardiovascular, immune, endocrine, and neurological abnormalities (11). The severity of the AT phenotype depends on the type and quantity of mutations that occur (12). Several ATM gene mutations have been identified, including nonsense, missense, and splicing site variations. However, the primary ATM gene mutation is the nonsense mutation, which results in a nonfunctional ATM kinase function (13). AT heterozygotes

(hKO), individuals with an ATM mutation in one allele make up ~2% of the population (around 2%) (14). While patients with an ATM mutation typically display less severe systemic dysfunction, they are still at an exponentially higher risk of developing cancer, insulin resistance, diabetes mellitus, ischemic heart disease, heart failure, and die ~7-8 years earlier (4, 6, 14, 40). ATM heterozygous mutations are also associated with increased cardiovascular-related morbidity and mortality (12). Despite extensive research on the role of ATM in DNA damage signaling, its involvement in the heart and myocardial remodeling in response to a Western-type diet (WD) remains to be explored.

Western-Type Diet

Over the past few decades, the prevalence of obesity has been escalating. It now ranks among the leading causes of mortality globally, with more than four million deaths annually associated with being overweight or obese in 2017, according to the global burden of disease (15). The incidence of overweight and obesity continues to rise in adults and children. As of 2016, over 1.9 billion adults aged 18 years or older were overweight, and more than 650 million were obese (15). Notably, low- and middle-income countries, particularly in urban areas, are currently experiencing an upsurge in obesity, a trend once limited to high-income countries (15). In parallel, chronic disorders such as diabetes and cardiovascular disease, which are linked to obesity, are now among the leading causes of mortality in the United States. The medical cost for obesity was $173 billion in 2019, $1,861 higher than that of non-obese patients (16). Numerous factors have been attributed to this epidemic, with the WD significantly contributing to the growing incidence of obesity in the United States. Despite current lifestyle trends emphasizing the significance of healthy eating, foods high in fat, sugar, and carbohydrates remain major constituents of the WD, correlating with the steady increase in obesity (15).

Chronic consumption of WD can lead to metabolic diseases, increased oxidative stress, and chronic inflammation, and can result in cardiac structural and functional anomalies (17). Obesity resulting from poor diets is independently associated with heart failure development (18). Obesity cardiomyopathy, a myocardial disorder, can be attributed to the duration and severity of obesity as well as concomitant comorbidities such as diabetes mellitus (DM), hypertension, and insulin resistance (18). The deleterious changes in cardiac structure and function associated with adiposity can include eccentric/concentric hypertrophy, cardiac output, right ventricular dysfunction, fibrosis, systolic dysfunction, atherosclerosis, and left ventricular dilation (17, 18). Animal models (rats, mice, rabbits, and dogs) have demonstrated various cardiovascular abnormalities similar to humans, including apoptosis, hypertrophy, ventricular stiffness, atherosclerosis, and increased arrhythmic and ischemic events (17, 19). Although diet-induced obesity is usually investigated in male subjects, it is acknowledged that there exist sex-related disparities in energy metabolism, adipose tissue distribution, and sex hormone equilibrium that may have an effect on its outcomes (53).

Cardiac Remodeling

Cardiac remodeling is characterized by a series of cellular and molecular changes that modify the size, mass, geometry, and function of the heart in response to cardiac disease or cardiac damage (20). Various intricate molecular processes such as fibrosis, hypertrophy, and apoptosis are typical features of cardiac remodeling (17, 20). Structurally, a remodeled heart exhibits loss of myocytes, increased fibrosis, and increased size of existing myocytes. Although early cardiac remodeling is a compensatory mechanism that protects the heart, changes in myocardial structure can lead to cardiac dysfunction and heart failure (18). Adverse structural alterations in the heart reduce the efficacy of the left ventricle, the thickest chamber responsible for propelling oxygenated

blood and nutrients to the entire body. Impairment of the left ventricular function can culminate in heart failure, characterized by the heart's inability to meet the body's metabolic demands. Mitigation of cardiac remodeling is widely acknowledged to improve the prognosis subsequent to cardiac damage. Additionally, adiposity or obesity has been shown to affect cardiac performance through alterations in cardiometabolic demands, circulating blood volume, pressure overload, and wall tension (21). Therefore, excessive adipose tissue is associated with hemodynamic-induced alterations in cardiac function and morphology (18, 21).

Apoptosis

The myocardium is composed of myocytes which are the fundamental contractile cells of the heart and are essential for its rhythmic contractions and relaxations. Myocytes play a critical role in cardiac remodeling (6). However, myocardial injury induces irreversible damage through programmed cell death or apoptosis. In response to an apoptotic stimulus, the major apoptosis pathway is triggered by the release of cytochrome c from the mitochondria (22, 23). The released cytochrome c combines with apoptotic protein-activating factor-1 and caspase-9 in the presence of dATP, forming an activation complex that activates downstream caspases and ultimately leads to the execution of morphological and biochemical changes (22, 23). Since myocytes are terminally differentiated and non-regenerative, new myocytes will not regenerate to replace those that have been lost from an injury. Thus, myocyte survival is crucial.

The etiology of heart failure entails various agents and conditions; however, the gradual loss of cardiac myocytes is recognized as one of the most significant pathogenic components (22). Research indicates that myocyte apoptosis is associated with reduced cardiac function and the progression of heart failure (24). High-fat diets have been demonstrated to induce cardiac injury

by promoting apoptosis, disrupting autophagy, and causing endoplasmic reticulum stress (25). Moreover, the lack of metabolic adaptability observed in WD and obesity induces cardiac cell apoptosis by exacerbating toxic byproducts (21). Furthermore, WD and obesity are associated with increased oxidative stress and inflammation, which promote and exacerbate cardiac cell apoptosis (14). Independently, ATM-deficient cells exhibit exacerbated mitochondrial dysfunction and increased apoptosis coupled with an increase in reactive oxygen species (ROS) (6, 26). If prolonged, this phenomenon inactivates mitophagy and activates cell death pathways (6, 27). Therefore, ROS-induced mitochondrial dysfunction and mitophagy impairment may contribute to the elevation of apoptosis in cardiac myocytes due to ATM inhibition (6).

There is mounting evidence from both human and animal models suggesting that apoptosis may represent a crucial mode of cell death in heart failure (22). Interestingly, studies show that suppressing apoptosis mitigates adverse remodeling and the consequent progression to heart failure in models of ischemia/reperfusion (28), myocardial infarction-induced heart failure (29), and non-ischemic cardiomyopathy (22).

Fibrosis

Cardiac injury triggers a reparative program that leads to the deposition of extracellular matrix (ECM) proteins in the heart due to the heart's limited regenerative capacity, known as fibrosis (30). The activation of cardiac fibroblasts into myofibroblasts is a critical step in the development of fibrosis. Under stress, fibroblasts undergo proliferation and differentiation into myofibroblasts, which acquire the ability to contract and produce collagen to maintain the structural integrity of the ECM following cardiac myocyte death (30, 31). Scar tissue formation following cardiac injury is attributed to the process of fibrosis, in which areas of myocyte dropout

are replaced by scar (32). While this process is initially protective and integral for healing, excessive ECM deposition can become detrimental to the heart when it persists, leading to myocardial stiffness and impaired contractability (32). As a result, cardiac fibrosis contributes to morbidity and mortality in many forms of heart disease. Cardiac fibrosis is a common pathophysiologic process in most heart diseases and is caused by numerous factors such as oxidative stress, inflammation, apoptosis, hypertension, diabetes, obesity, and WD (32, 33). Obesity and a WD can cause increased inflammation, oxidative stress, and metabolic abnormalities, which have deleterious effects on the myocardium, initiating cardiac fibroblast activation (17). Previous studies have indicated that the absence of ATM elicits structural and functional alterations in the heart, accompanied by enhanced myocardial fibrosis and myocyte hypertrophy (10, 41, 42).

Fibrosis is a significant cause of organ dysfunction in many different diseases and is characterized by the expansion of the cardiac interstitium due to the net accumulation of ECM proteins (31). The pathophysiological process of fibrosis is widespread in numerous cardiac disorders, including myocardial infarction (MI), hypertensive heart disease, and various forms of cardiomyopathy (34, 35). MI is a typical example of reparative fibrosis, as the sudden death of a large number of cardiomyocytes stimulates inflammation and subsequent activation of reparative myofibroblasts, leading to the formation of a scar (31). Fibrosis can provoke chamber dilation, cardiomyocyte hypertrophy, and apoptosis, resulting in congestive heart failure (31, 36). While fibrosis acts as a protective mechanism, unfortunately, augmented levels of cardiac fibrosis have been associated with reduced electrical conductivity and mechanical function (30).

Hypertrophy

Cardiac remodeling progressively continues following fibrosis formation by provoking cardiac myocyte hypertrophy (37). Cardiac hypertrophy is a physiological response to various stimuli, including blood pressure or volume stress, sarcomeric mutations, or loss of contractile mass following MI (38). Due to the limited ability of fully differentiated cardiac myocytes to reenter the cell cycle, cardiomyocytes adapt to such stimuli by enlarging, elevating protein synthesis, and enhancing the organization of the sarcomere (38). Two distinct hypertrophic phenotypes can be discerned: concentric hypertrophy from pressure overload and eccentric hypertrophy from volume overload or previous infarction (38). Pressure overload-induced hypertrophy is considered compensatory by reducing wall stress and oxygen consumption to preserve function temporarily; hence, it is an adaptive process (38, 39). Simultaneously, maladaptive hypertrophy is associated with a significantly increased risk of heart failure and arrhythmia (37).

Hypertrophic growth is frequently observed in cardiovascular diseases such as ischemic disease, hypertension, heart failure, and valvular disease (38). Further, excessive adipose tissue and WD can cause an increase in circulating blood volume, stroke volume, and cardiac output, which may result in left ventricle (LV) enlargement, hypertension, and elevated wall stress (18). Obesity cardiomyopathy has been associated with both right ventricular hypertrophy and eccentric or concentric LV hypertrophy (18). Moreover, ATM deficiency has been independently linked to elevated levels of cardiac fibrosis, hypertrophy, and cardiac dysfunction (10, 41, 42). Although short-term hypertrophic remodeling has a protective effect, serving to normalize wall stress and oxygen demand, prolonged activation of this response is detrimental, leading to ventricular dysfunction and LV dilation (18,38).

Myocardial Infarction

Cardiovascular disease, a group of disorders affecting the heart and blood vessels, is the primary cause of mortality in the United States. Among these diseases, MI, also known as heart attack, plays a significant role in developing heart failure. In the United States, MI frequently occurs, with 805,000 incidents each year (43). The economic burden of heart disease in the United States is substantial, with an estimated cost of $229 billion annually from 2017 to 2018, including healthcare services, medicines, and lost productivity due to death (44). Multiple factors contribute to an increased risk of MI, including obesity, cardiac remodeling, diabetes, smoking, lack of physical activity, and elevated blood cholesterol levels, pressure, and sugar (45).

MI occurs when the oxygen supply to the myocardium, the heart muscle, is significantly reduced or obstructed, leading to considerable myocardial injury (6). Cardiomyocytes, the specialized cells that form the heart muscle, undergo structural and functional changes shortly after myocardial ischemia, the inadequate blood flow to the heart muscle, occurs typically within 30 minutes. Cardiac ischemia can harm cardiomyocytes by activating the inflammatory cascade and leading to cell death (6, 47). During the inflammatory phase of MI, damage-associated molecular patterns trigger the recruitment of neutrophils and macrophages to the site of infarction (46). Subsequently, dead cardiomyocytes and damaged extracellular matrix are digested and removed from the injury site (32). During the reparative/proliferative phase, fibroblasts, a type of cell responsible for synthesizing the extracellular matrix, activate and differentiate into myofibroblasts, which initiate collagen deposition (fibrosis) (46). Finally, the extracellular matrix becomes cross-linked in the maturation phase, causing the scar to mature and myofibroblasts to deactivate (46). The mature scar can reduce myocardial contraction, promote left ventricular dilation, and

contribute to the development of heart failure (46). MI can also trigger chronic systemic inflammation and increase the circulation of inflammatory chemokines (48).

WD has been linked to the development of obesity and increased cardiovascular risk (49). The risk of MI is raised by 35% in those who consume WD (50). Adiposity, with or without concurrent metabolic syndrome, also elevates the risk of MI. In patients with diabetes, the risk of subsequent MI is increased by approximately 40%, and in non-insulin-dependent diabetic patients, MI is the primary cause of death (49). High-fat diets have been shown to increase nitric oxide, oxidative stress, and exacerbate infarct size and cardiac damage following MI in animal models (51). Further, our lab has previously investigated the functional alterations in ATM-deficient mice 4 hrs., 1-, 3-, 7-, 14-, and 28-days following MI (6, 26, 41, 42). The results suggest that ATM deficiency may attenuate cardiac dysfunction, delay inflammatory response, and increase fibrosis and apoptosis early post-MI (52). However, ATM deficiency late post-MI is associated with exacerbated cardiac structural and functional parameters, abolishing the protective effects (41, 42).

Specific Aims

Current scientific knowledge regarding the effects of ATM deficiency on cardiac outcomes prior to and following MI in response to WD is limited. Previous research from our laboratory has indicated that male ATM-deficient mice experience accelerated weight gain and exacerbation of cardiac remodeling, including increased hypertrophy and apoptosis. This study aimed to explore the role of ATM deficiency in WD-induced cardiac remodeling parameters before and 1-day post-MI using male and female WT and ATM hKO mice. The specific goals of the study were 1) to investigate the role of ATM deficiency in WD-induced cardiac remodeling parameters using female mice, and 2) to examine the role of ATM deficiency on MI-induced cardiac changes in

response to WD using female and male mice. The major findings of this study are that 1) in female mice, ATM deficiency plays a cardioprotective role in response to WD with a decrease in apoptosis and increase in hypertrophy at baseline, and 2) the sex-specific cardioprotective effects of ATM deficiency in female mice were not observed 1-day post-MI in response to WD.

MATERIALS AND METHODS

Animal Care and Treatment

This investigation adheres to the *Guide for the Care and Use of Laboratory Animals*, a publication by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The execution of all experiments followed the protocols approved by the East Tennessee State University Committee on Animal Care. ATM-deficient mice (129S6/ SvEvTac) were obtained from Jackson Laboratory for breeding (stock #002753). Since ATM KO mice have a restricted lifespan (~2 months), ATM heterozygous knockout (hKO) mice were used for breeding. All mice were genotyped using PCR with primers recommended by the Jackson Laboratory, both prior to diet assignment and following exsanguination. Age-matched (~6 weeks old) WT and hKO mice were subjected to a 14-week trial in which they were placed on either a normal chow (NC; Envigo 8604) or a Western-type diet (WD; Envigo TD 88137). The NC's energy composition is—32% kcal protein, 14% kcal fat, 54% kcal carbohydrate, and 4% sugar (by weight), whereas the WD's energy composition is—15.2% kcal protein, 42.0% kcal fat, 42.7% kcal carbohydrate, and 34% sugar (by weight). The mice had access to food and water ad libitum and were maintained on a 12-hour light/dark cycle. Whenever it was practically feasible, the experiments were conducted in a blind manner.

Myocardial Infarction

MI was conducted as previously described (41). In brief, mice were anesthetized with a combination of isoflurane (2%) and oxygen (0.5 l/min) through inhalation, and their ventilation was controlled using a rodent ventilator (Harvard Apparatus). The body temperature of the mice was maintained at ~37°C through the use of a heating pad. The heart was exposed by a left

thoracotomy. The left anterior descending coronary artery (LAD) was ligated with a 7-0 polypropylene suture. The same procedure was performed on sham-operated mice excluding the LAD ligation. At the end of the study period, one-day post-MI, the heart was excised through an opening in the diaphragm region. To ensure blood clearance, the heart was perfused with Krebs-Henseleit buffer, arrested in diastole using 30 mM KCl, and weighed. The isolated heart was then divided into two transverse sections (base/mid and apex) and embedded in paraffin.

Masson's Trichrome Staining

Mid-cardiac transverse sections (5µm thick) were subjected to deparaffinization and rehydration using xylene and ethanol washes, respectively. Masson's trichrome staining was used to quantify fibrosis. Masson's trichrome is a three-dye-based protocol that selectively stains muscle and cytoplasm red, collagen blue, and nuclei dark brown. Fibrosis was assessed by acquiring ten randomly selected septal images using an M7000 EVOS microscope and analyzed using Nikon NIS software. Percent fibrosis was calculated by dividing the total fibrosis area by the total tissue area of each image, multiplied by 100.

Terminal Deoxynucleotidyl Transferase Nick End Labeling (TUNEL) Assay

Mid-cardiac transverse sections (5µm thick) were subjected to staining using TUNEL kit according to the manufacturer's instructions (In Situ Cell Death Detection Kit: Roche) to measure apoptosis. Tissue sections were counterstained with rhodamine-conjugated wheat germ agglutinin (WGA, RI-1022, Vector) to visualize myocytes and Hoechst 33258 (10 µM, Sigma) to visualize nuclei. The number of Hoechst-positive nuclei was determined as an index of the total number of nuclei. The detection of TUNEL-positive staining observed within WGA-stained cells and Hoechst-positive nuclei allowed the identification of apoptotic myocytes. For each animal, ten

randomly selected images from the LV region of the heart were obtained with M7000 EVOS microscope and analyzed with Nikon NIS software. The index of myocyte apoptosis was calculated as the percentage of myocyte apoptotic nuclei divided by the total nuclei and multiplied by 100. Total cardiac cell apoptosis was measured by counting the TUNEL-positive and Hoechst-positive nuclei. The index of cardiac cell apoptosis was calculated as the percentage of total apoptotic nuclei divided by the total nuclei and multiplied by 100.

Myocyte Cross-Sectional Area

Mid-cardiac cross-sections (5μm think), stained with rhodamine-conjugated wheat germ agglutinin (WGA), were utilized for measuring myocyte cross-sectional area. For this, images were obtained using EVOS M7000 imaging system, and quantification of myocyte cross-sectional area was performed with Nikon NIS software as previously described (52). The cross-sectional area was measured using ten different images from the LV region. The suitability of myocytes for cross-sectional area measurement was defined by the presence of a circular cell with a centrally located nucleus.

Myocardial Infarct Size and Thickness

Mid-cardiac transverse sections (5μm thick) were stained using TUNEL staining were used to measure infarct size and infarct thickness. The area of infarct and non-infarct regions was used to measure infarct size utilizing Nikon NIS software as previously described (42). The percentage of infarction was calculated by dividing the total area of infarct by the area of the non-infarcted heart section and multiplying by 100. To measure infarct thickness, six separate transverse measurements were taken from each infarct area of the heart and averaged.

Statistical Analysis

All data presented in this study are expressed as means ± SE. Data were analyzed using a 2-tailed Student's *t*-test or two-way analysis of variance (ANOVA) followed by the Newman-Keuls test (GraphPad Prism 9 software). Statistical significance was set at a probability (p) value of < 0.05. The number (n) indicates the number of biological replicates.

RESULTS

Apoptosis for Female Baseline Hearts

Quantitative analysis of apoptosis using TUNEL assay indicated a significant increase in myocyte and total cardiac cell apoptosis in hKO-NC compared to WT-NC. WD resulted in a significant increase in myocyte apoptosis in both genotypes versus NC. Notably, the increase in myocyte apoptosis was significantly lower in hKO-WD compared to WT-WD (Figure 2B). Similarly, a significant increase in total cardiac cell apoptosis was observed in both genotypes on WD. Total cardiac cell apoptosis was significantly lower in hKO-WD versus WT-WD (Figure 2C).

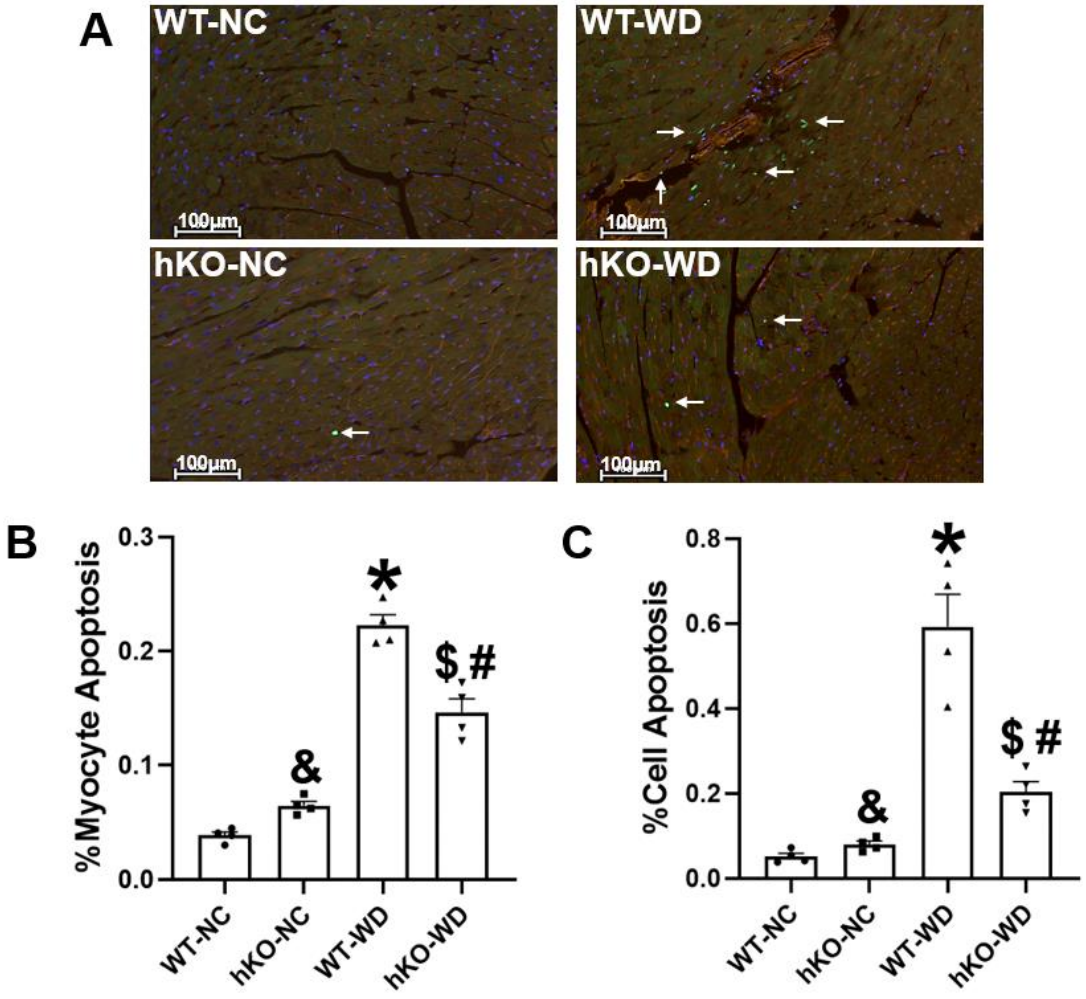


Figure 1. ATM Deficiency Attenuates Apoptosis in the Heart in Response to Western-Type Diet.

A) Representative images of TUNEL-stained cross sections of the heart. Green (TUNEL) staining indicates apoptotic nuclei, red (WGA) staining indicates myocyte, and blue (Hoechst) staining indicates nuclei. B) Quantitative analysis of myocyte apoptosis. C. Quantitative analysis of cardiac cell apoptosis. $^{\&}p<0.05$ vs WT-NC, $^{*}p<0.05$ vs WT-NC, $^{\$}p<0.05$ vs hKO-NC, $^{\#}p<0.05$ vs WT-WD, n=4.

Fibrosis for Female Baseline Hearts

Quantitative analysis of myocardial fibrosis in the heart at baseline using Masson's trichrome staining showed a significant increase in percent fibrosis in hKO-NC versus WD-NC. WD led to an increase in fibrosis in both genotypes. Interestingly, a significant increase in fibrosis was only observed in the WT group, not in the hKO group (Figure 1).

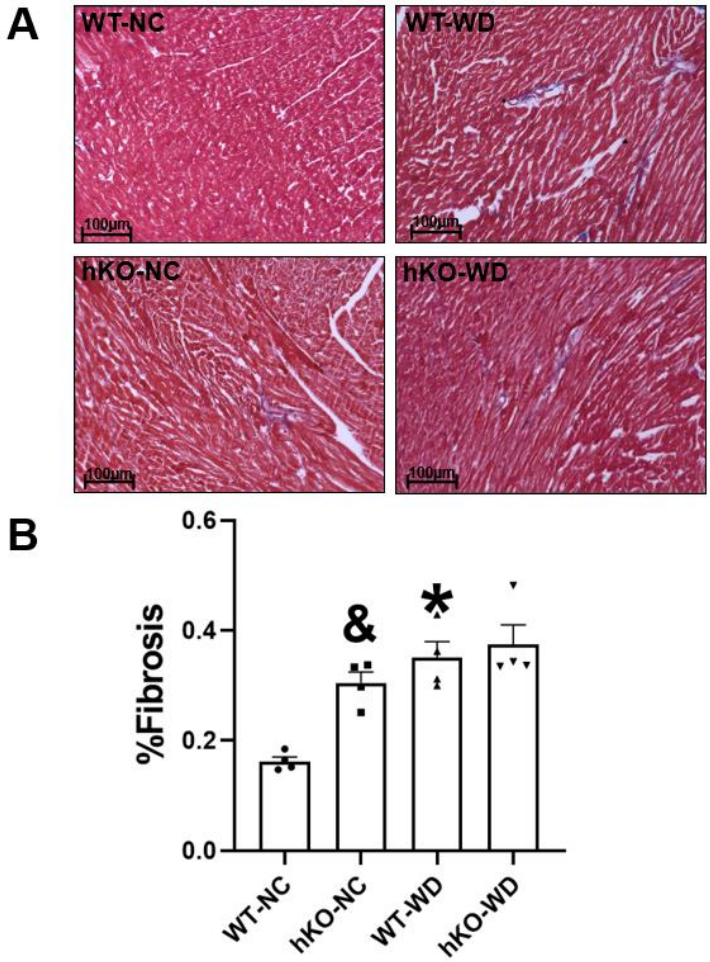
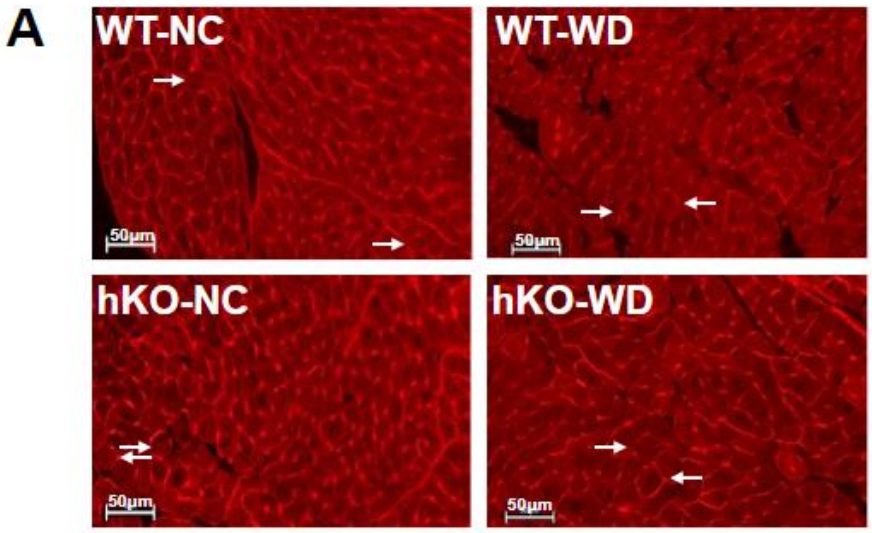


Figure 2. WD Exacerbates Fibrosis in Wild-Type Mice.

A) Cross sections of the heart stained with Masson's trichrome for each group and associated diet. Red staining indicates cardiac tissue, while blue staining indicates fibrosis. B) Quantitative analysis of fibrosis. $^{\&}p<0.05$ vs WT-NC, $^{*}p<0.05$ vs WT-NC, n=4.

Hypertrophy for Female Baseline Hearts

Hypertrophy is assessed by the measurement of the myocyte cross-sectional area. The analysis revealed a significant increase in myocyte cross-sectional area in hKO-NC compared to WT-NC. WD induced a significant increase in myocyte cross-sectional area in both genotypes compared to NC. However, hKO-WD displayed a significantly higher myocyte cross-sectional area compared to WT-WD (Figure 3).



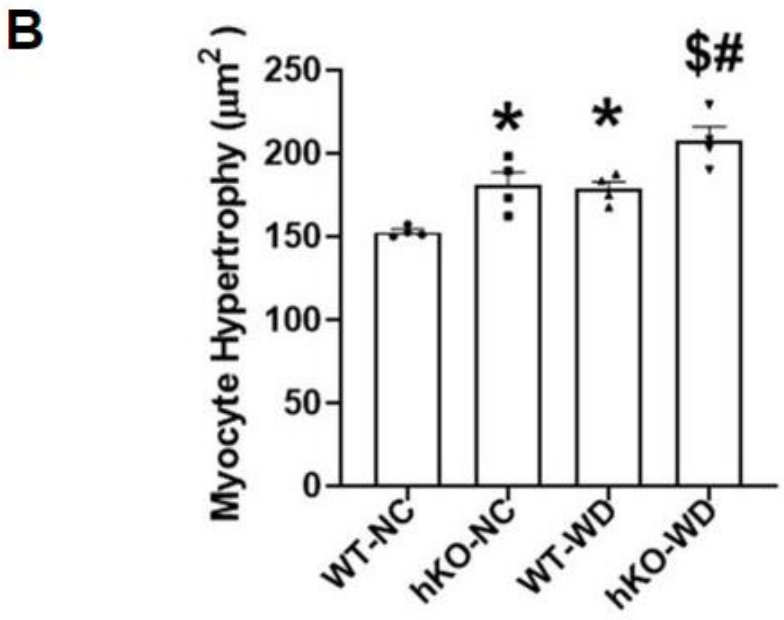


Figure 3. ATM Deficiency Increases Myocyte Hypertrophy in Response to Western-Type Diet.
A) Representative images of wheat germ agglutinin (WGA)-stained cross sections of the heart indicating myocytes. B) Quantitative analysis of myocyte cross-sectional area. $^{\&}p<0.05$ vs WT-NC, $^{*}p<0.05$ vs WT-NC, $^{\#}p<0.05$ vs WT-WD, $^{\$}p<0.05$ vs hKO-NC, n=4.

Apoptosis 1-Day Post-MI

Quantitative analysis of apoptosis using TUNEL assay showed that MI induced apoptosis in all groups similarly in the infarct region 1-day post-MI (Figure 4).

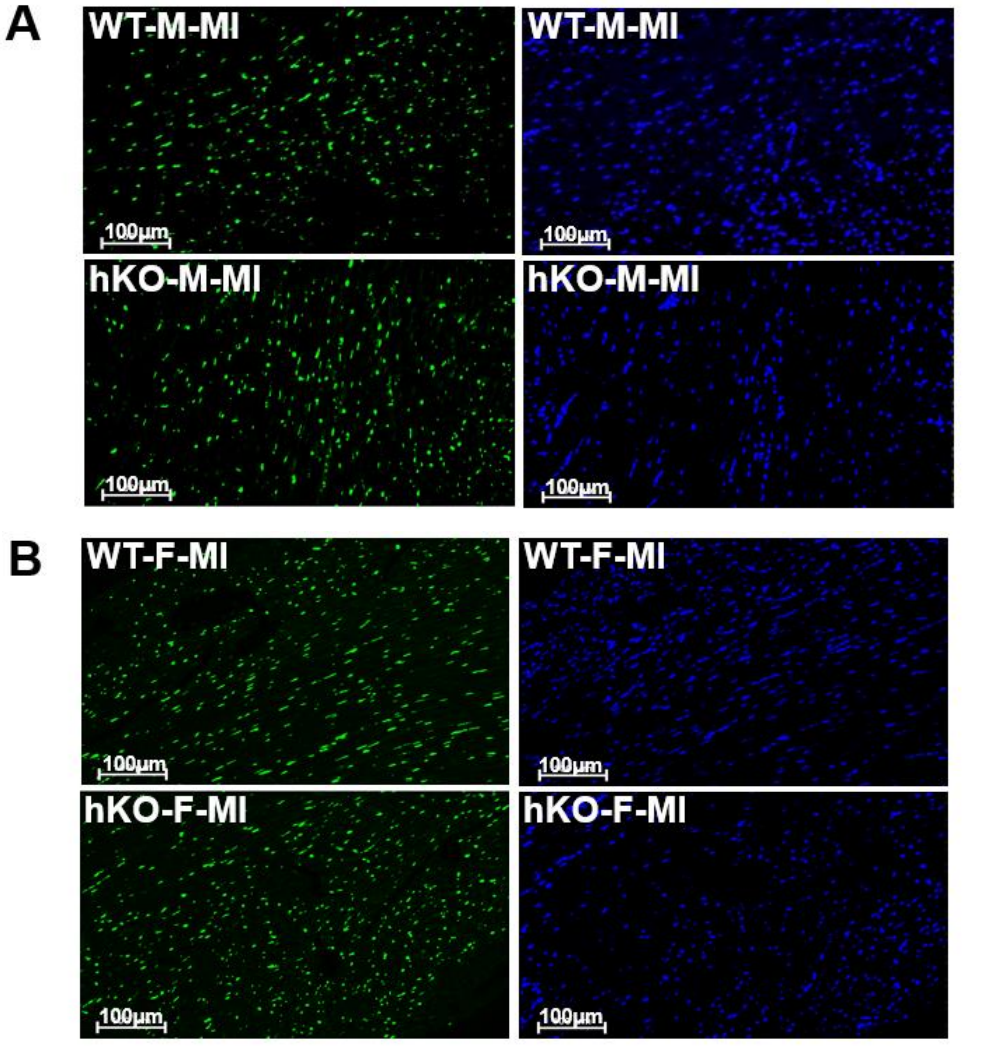
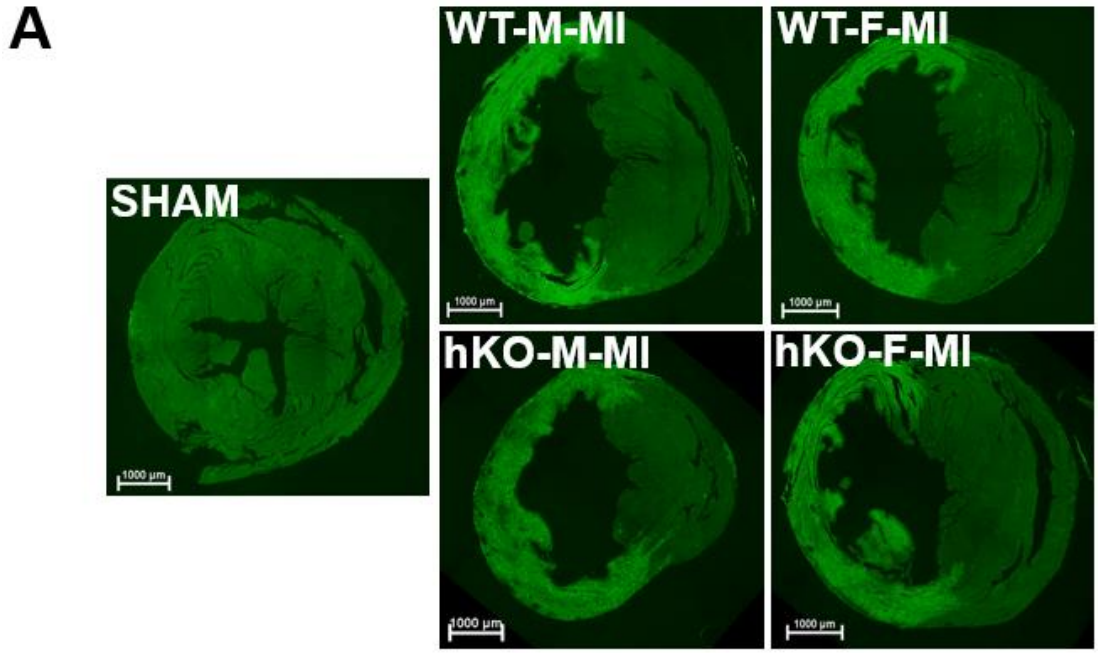

A
WT-M-MI
WT-M-MI
100μm
100μm
hKO-M-MI
hKO-M-MI
100μm
100μm
B
WT-F-MI
WT-F-MI
100μm
100μm
hKO-F-MI
hKO-F-MI
100μm
100μm
C
% Myocyte Apoptosis
50
40
30
20
WT-WD-M
hKO-WD-M
WT-WD-F
hKO-WD-F
D
% Cell Apoptosis
50
40
30
20
WT-WD-M
hKO-WD-M
WT-WD-F
hKO-WD-F

Figure 4. ATM Deficiency has no Effect on Apoptosis in the Heart 1 Day Post-MI in Response to Western-Type Diet.
A) Representative images of TUNEL-stained cross sections of the heart. Green (TUNEL) staining indicates apoptotic nuclei, while blue (Hoechst) staining indicates nuclei. B) Quantitative analysis of myocyte apoptosis in infarct region of LV. C) Quantitative analysis of cardiac cell apoptosis. n=5-6.

Infarct Size and Infarct Thickness 1-Day Post-MI

Quantitative analysis of infarct size and infarct thickness using TUNEL assay revealed no significant differences among all groups 1-day post-MI.



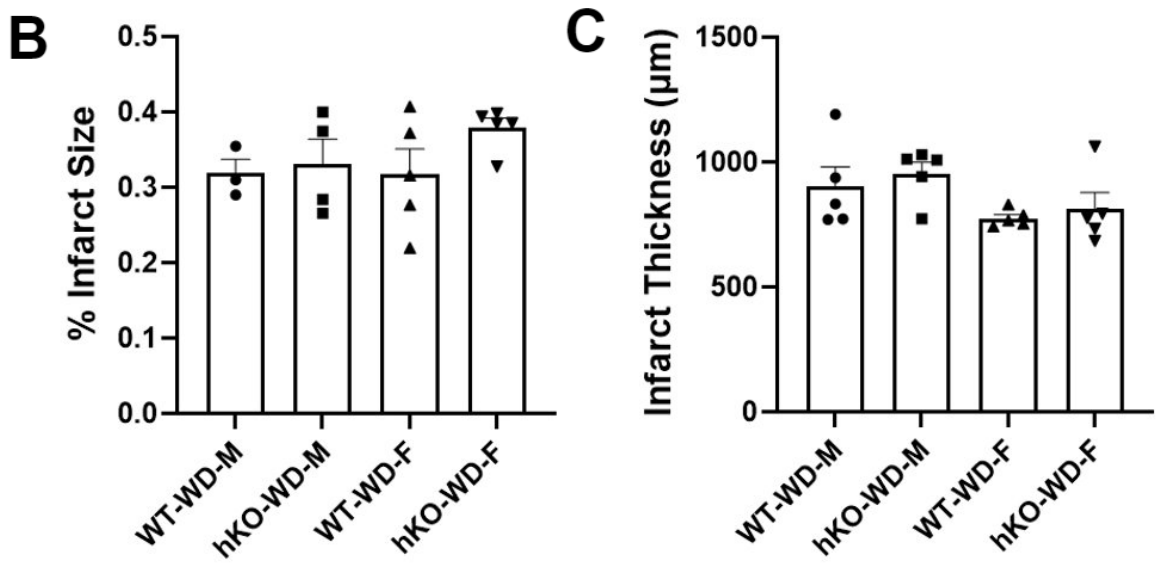


Figure 5. ATM Deficiency has no Effect on Infarct Size or Infarct Thickness 1 Day Post-MI in Response to Western-Type Diet.

A) Representative images of TUNEL-stained cross-sections of the heart illustrating area of infarct. B) Quantitative analysis of percent infarct size. C) Quantitative analysis of infarct thickness. n=5-6.

DISCUSSION

Female Mice Prior to MI

The prolonged consumption of WD can trigger metabolic disorders, augment oxidative stress, promote chronic inflammation, and consequently provoke alterations in cardiac structure and function (17). Obesity resulting from a poor dietary regimen is independently associated with the development of heart failure (18). A-T individuals comprise a substantial portion of the population, around ~1.4%-2.0%, and exhibit increased susceptibility to ischemic heart disease (14). Individuals carrying a single mutated allele of the ATM gene exhibit a reduced lifespan of approximately ~7-8 years compared to non-carriers, which can be attributed to an increased incidence of ischemic heart disease (40). Therefore, the consumption of WD may exacerbate the risk of developing heart disease in A-T carriers. Previously, our lab has demonstrated that in male mice, WD consumption during ATM deficiency is associated with an exacerbation of cardiac remodeling characterized by an increase in myocyte and cardiac cell apoptosis and induction of hypertrophy (52). This study aimed to investigate the effects of ATM deficiency on cardiac remodeling in female mice fed with a WD. Our findings indicate that when exposed to a WD, ATM deficiency in female mice leads to preservation of cardiac function with 1) increase in myocyte hypertrophy, and 2) inhibition of WD-induced cardiac cell apoptosis (54).

The induction of cardiac injury leads to irreparable harm via the activation of programmed cell death pathways, known as apoptosis. Obesity-related chronic increases in blood volume can induce maladaptive cardiac remodeling and stimulate apoptosis of cardiac cells (25). Moreover, WD and obesity are linked to elevated oxidative stress and inflammation, thereby augmenting and exacerbating the apoptosis of cardiac cells (14). Independently, ATM-deficient cells exhibit

heightened mitochondrial dysfunction and increased apoptosis, concomitant with a rise in reactive oxygen species (ROS) levels (6, 26). A previous study from our lab demonstrated that ATM-deficient male mice displayed increased basal levels of myocyte and total cardiac cell apoptosis in comparison to WT. WD resulted in a significant increase in myocyte apoptosis in both genotypes. However, ATM-deficient male mice showed a significant increase in myocyte apoptosis when fed with WD compared to their WT counterparts (52). The results of this study demonstrated that ATM-deficient female mice had a significant increase in myocyte and total cardiac cell apoptosis in hKO-NC compared to WT-NC. WD induced a significant increase in myocyte apoptosis in both genotypes versus NC. Notably, the increase in myocyte and total cardiac cell apoptosis was significantly lower in hKO-WD compared to WT-WD. Given the recognition of the gradual loss of cardiac myocytes as a significant pathogenic component, the reduced extent of apoptosis observed in ATM-deficient female mice may function as an additional cardioprotective mechanism in response to WD-induced cardiac remodeling.

Moreover, cardiac fibrosis is a compensatory response activated by cardiac injury, leading to the scarring of the heart tissue (30). Cardiac fibrosis is characterized by decreased contractile capability, hardening of the ventricular walls, and impaired overall cardiac function (32). While this process is initially protective, it may become detrimental to the heart if it persists, resulting in decreased mechanical and electrical function and ultimately culminating in heart failure (30). Chronic consumption of a WD and ATM deficiency are each independently associated with elevated cardiac fibrosis (10, 18, 41, 42). Our lab has previously shown that ATM-deficient male mice exhibited a significant increase in percent fibrosis fed with NC conditions. WT male mice showed a significant increase in fibrosis levels when subjected to a WD compared to NC. However, the male hKO mice group did not display any significant increase in fibrosis on WD

compared to their NC counterpart (52). Similarly, this study revealed that female mice with ATM deficiency also demonstrate increased fibrosis under basal conditions. Even though WD induced elevated levels of fibrosis in both genotypes, interestingly, only the WT genotype demonstrated a significant increase in fibrosis, while the hKO genotype did not. Obesity- and WD-induced heightened levels of inflammation, oxidative stress, and metabolic disturbances can result in the exacerbation of fibrosis and cardiac remodeling (32, 33). It is plausible that the observed augmented basal cardiac fibrosis levels in female ATM-deficient mice may function as a protective mechanism against WD-induced cardiac dysfunction.

Further, cardiac hypertrophy is a physiological response to various stimuli, such as blood pressure or volume stress, sarcomeric mutations, or loss of contractile mass following MI (38). Cardiomyocytes respond to these stimuli by undergoing cellular enlargement. Obesity cardiomyopathy has been associated with right ventricular hypertrophy as well as eccentric or concentric left ventricular hypertrophy (18). In addition, ATM deficiency has been independently linked to elevated cardiac hypertrophy (10, 41, 42). Our lab previously demonstrated that ATM deficiency in male mice is associated with increased hypertrophy at basal levels, as indicated by an increase in myocyte cross-sectional area. Moreover, it was found that WD-induced increase in hypertrophy was more significant in ATM-deficient male mice compared to their WT counterparts (52). Our present study shows that female ATM-deficient mice also exhibit increased levels of hypertrophy at basal levels, which is consistent with previous findings in male mice. We observed that WD significantly increased hypertrophy in both genotypes compared to NC. However, WD-induced myocyte hypertrophy remained higher in ATM-deficient female mice compared to their WT counterparts. The progression of fibrosis typically precedes the development of hypertrophy in cardiac tissue. While fibrosis diminishes oxygen diffusion, cardiac hypertrophy reduces wall

stress and oxygen consumption in cardiac pathologies (38, 39). Thus, the observed increase in cardiac hypertrophy in female ATM-deficient mice may play a protective mechanism against WD-induced cardiac dysfunction.

In conclusion, our data and previous research in male mice have revealed sex-specific differences in ATM deficiency during WD. While WD may increase myocardial fibrosis, apoptosis, and hypertrophy in WT female mice, our study demonstrates that female ATM-deficient mice show preserved cardiac function following WD (54). This preservation may be linked to reduced myocyte apoptosis and increased myocardial hypertrophy. It should be emphasized that our study used mice with a diet onset age of 6 weeks. However, the effects of WD may vary depending on the age of diet onset; thus, it is important to investigate the influence of other onset ages (young adults and middle-aged) during ATM deficiency. Moreover, examining whether hormones, such as estrogen, play a role in preserving cardiac function in ATM-deficient WD-fed female mice may help explain the observed sex-specific differences. Ultimately, understanding the molecular mechanisms involved in preserving heart function during ATM deficiency in WD-fed female mice could lead to better sex-specific treatment and nutritional counseling for patients with ATM deficiency.

Mice 1-Day Post-MI

The risk of MI is associated with multiple factors, including obesity, diabetes, smoking, lack of physical activity, and elevated blood cholesterol levels, pressure, and sugar (45). The WD has been linked to increased cardiovascular risk and obesity development, resulting in a 35% increase in MI risk (49, 50). Approximately 1.4%-2.0% of the population comprises A-T individuals, who exhibit increased susceptibility to ischemic heart disease (14). Consequently, WD

consumption may exacerbate the risk of MI in A-T patients. Nonetheless, there is limited knowledge regarding the effects of ATM deficiency on cardiac outcomes before and after MI in response to WD. As previously shown, studies have demonstrated that WD induces sex-specific changes in the heart during ATM deficiency at baseline. In male ATM-deficient mice, WD exacerbates cardiac remodeling characterized by increased myocyte and cardiac cell apoptosis and hypertrophy induction (52). Conversely, ATM deficiency in female mice exhibits a cardioprotective role against the deleterious effects of WD, resulting in preserved cardiac function, decreased apoptosis, and increased myocardial hypertrophy (54). In the current study, we aimed to investigate the role of ATM deficiency in early MI-induced cardiac changes in response to WD in female and male mice. Our results indicate a similar increase in apoptosis, infarct size, and infarct thickness in both genotypes and sexes.

After the interruption of arterial perfusion due to occlusion of the coronary vessel, immediate death of cardiomyocytes follows (37). High-fat diets have been demonstrated to promote apoptosis, disrupt autophagy, and cause endoplasmic reticulum stress leading to cardiac injury (25). Furthermore, increased cardiac cell apoptosis greatly increases the risk of MI and the development of heart failure (47). Our previous research demonstrated that a WD significantly increases myocyte and total cardiac cell apoptosis in WT and ATM-deficient male hearts versus their NC groups (52). As previously discussed, WD increased myocyte and total cell apoptosis similarly in female WT and ATM-deficient hearts versus their NC groups. However, WD-induced apoptosis was significantly lower in ATM-deficient female mice compared to WT female mice (54). Our lab also previously shown that ATM deficiency increased myocardial apoptosis in the infarct LV region in pooled male and female mice fed with NC 1- and 3-days post-MI (41). Conversely, total cardiac cell apoptosis was significantly reduced in the infarct LV region of ATM-

deficient mice compared to WT mice 7-days post-MI fed with NC (26). In this study, we show that MI induces a similar increase in apoptosis in the infarct region 1-day post-MI with no significant difference among genotypes and sexes fed with WD. This suggests that WD during ATM deficiency does not impact cardiac cell apoptosis 1-day post-MI. However, further investigation into myocyte hypertrophy and myocardial fibrosis may provide a better understanding of the effects of WD on cardiac remodeling in ATM-deficient mice 1-day post-MI.

Furthermore, infarct scar size and thickness alterations can eventually impact infarct expansion and contribute to diastolic dysfunction (26). Previous research indicated that ATM deficiency on NC did not affect infarct size 1-, 3-, and 7-days post-MI in pooled male and female mice (26,41). Nevertheless, infarct thickness was higher in ATM-deficient mice versus WT mice fed with NC 7-days post-MI (26). In the current study, there was no difference in infarct size or thickness among groups fed with WD. These results suggest that ATM deficiency 1-day post-MI does not affect infarct size or thickness following a WD. However, it would be interesting to investigate whether there are any changes induced by a WD compared to NC.

In summary, MI induced a similar increase in percent myocyte and total cardiac cell apoptosis, infarct size, and thickness in all genotypes and sexes. This suggests that WD does not induce apoptosis and morphological changes in ATM-deficient mice 1-day post-MI. Moreover, the sex-specific differences observed in female mice with WD during ATM deficiency at baseline are not observed 1-day post-MI. It should be emphasized that our data were obtained 1-day post-MI on WD. Thus, the role of ATM deficiency during WD should be assessed at additional time points such as 3- and 7-days post-MI or during late MI. Further, examining myocyte hypertrophy and myocardial fibrosis may better elucidate the effects of ATM deficiency on cardiac remodeling

in response to WD. Lastly, it would be interesting to study the role of ATM in the induction of inflammatory response following WD at the 1-day post-MI time point.

FUNDING SOURCES

This work was supported by Merit Review awards (I01BX004045) from the Biomedical Laboratory Research and Development Service of the Veterans Affairs Office of Research and Development, National Institutes of Health (R15HL156214), and funds from the Institutional Research and Improvement account.

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