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The Role Of Map Kinase Phosphatase-5 In Cardiac Adaptations To Endurance Exercise

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THE ROLE OF MAP KINASE PHOSPHATASE-5 IN CARDIAC ADAPTATIONS TO ENDURANCE EXERCISE

by

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Chapter 1: Introduction

1.1 Exercise and Health Benefits

Physical activity improves exercise capacity and physical fitness, leading to many health benefits [1]. Physical activity is defined as any form of muscular activity that results in energy expenditure above resting levels [2]. Exercise is a subcategory of physical activity that is planned, structured and repetitive with a goal of improving or maintaining physical fitness [2]. Physical fitness is defined as the ability to perform physical activity [2]. It has been well known that active individuals appear to have lower rates of all-cause mortality due to a decrease in chronic diseases, including cardiovascular disease [3]. A meta-analysis demonstrated that active individuals had a reduction in the risk of cardiovascular death by 35% and a decrease in the risk of all-cause death by 33% over 20 years [4]. Specifically, endurance exercise, also known as aerobic exercise, is effective in the prevention and treatment of cardiovascular diseases [5]. It has also been shown that regular engagement of endurance exercise not only reduces cardiovascular risk factors, but also obesity, abdominal fat and high blood pressure [3]. The types and characteristics of endurance exercise are often debated by medical professionals on physiological influence in specific populations. Endurance exercise is defined as exercise that is maintained by using oxygen delivered by the blood to the exercising muscles [6]. Endurance exercise utilizes the large muscle groups with alternate contraction and relaxation, forces to deep breath, heart to pump more blood with adequate tissue oxygenation, resulting in the improvement of cardiovascular functions [7]. A longitudinal study revealed that just 5~10 minutes of running per day at less than 6 mph was sufficient to reduce all-cause mortality by 38% [8], suggesting that regular endurance exercise contributes to a reduction in cardiovascular diseases and all-cause morbidity and mortality.

1.2 Endurance Exercise and Cardiac Function

Substantial evidence has focused on the effects of endurance exercise on myocardial changes. The mammalian heart is primarily made of a thick muscle, which is responsible for pumping blood through the circulatory system and nutrient to organs and transporting carbon dioxide back to the lungs [9]. The heart displays remarkable plasticity in response to exercise, particularly endurance exercise [10]. Cardiac structure and morphology are profoundly altered following endurance exercise, as supported by initial evidence from cross-sectional studies reporting that hearts in endurance athletes are significantly larger than those of the general population [11]. Their hearts show increased ventricular chamber dimensions, wall thickness and mass due to the high endurance exercise volume [12-14]. The increased heart size and mass are called cardiac hypertrophy, which decreases ventricular wall stress and maintains or even augments cardiac pump function [9]. The cardiac hypertrophy is a mild form of growth characterized by a 10~20% increase in heart weight normalized to body weight [15]. This cardiac morphological adaptation to endurance exercise results in enhanced cardiac function, such as myocardial contractility, leading to increased cardiac output [16, 17]. Cardiac output is the amount of blood pumped by the left ventricle per one minute. Cardiac output is determined by stroke volume and heart rate. Both stroke volume and heart rate increase during endurance exercise. Although an increase in heart rate is responsible for most cardiac output augmentation during endurance exercise, maximal heart rate is unaltered, or even slightly decreased [18].

The increase in cardiac output after endurance exercise is mainly a result of a larger stroke volume due to an increase in left ventricle end-diastolic volume and a decrease in end-systolic volume [10]. The increase in cardiac output after endurance exercise is a nearly universal finding in studies in healthy individuals and is arguably the most studied effect [10,

19]. The increased heart size and mass induced by endurance exercise led to increased cardiac output during exercise, facilitating a significantly higher maximal oxygen consumption [20]. The enhanced cardiac function following endurance exercise improves a transport of blood and oxygen to skeletal muscles, leading to increased exercise capacity [21].

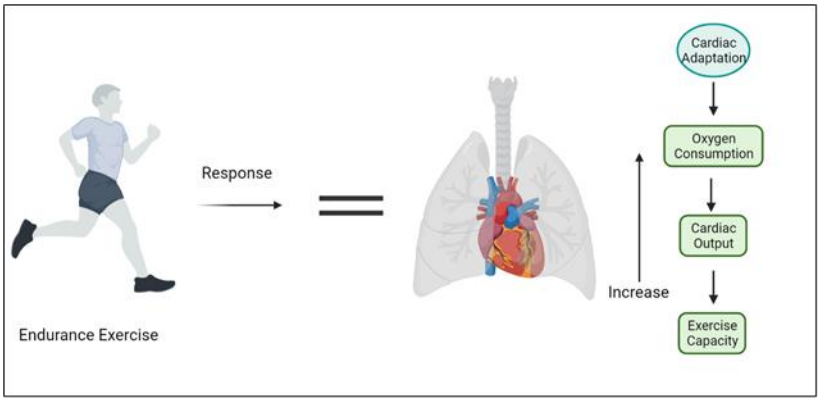

Figure 1. According to literature, endurance exercise provides substantial beneficial components to physiological change within the body [12-14]. Increases in cardiac function primarily, is one of the key factors to increase in exercise capacity.

1.3 Molecular Mechanisms and Endurance Exercise-Induced Cardiac Adaptations

Endurance exercise has been established to activate specific pathways, which facilitate cardiac adaptation and cardiac benefits. Specifically, phosphoinositide 3-kinase (PI3K)-protein kinase B(Akt)-mammalian target of rapamycin (mTOR) pathways is activated in cardiac muscle during and/or following endurance exercise, leading to cardiac growth and enhanced cardiac function [22]. PI3K has been known as a critical mediator of cardiac adaptation in response to endurance exercise [23]. The PI3K is composed of separate regulatory (p85) and catalytic (p110 α, β, or δ) subunits [24]. The p110α is robustly expressed in the heart [25]. Importantly, PI3K is activated in the heart during endurance exercise and the activation of PI3K is also cardioprotective against cardiomyopathy [26, 27]. Akt, also known as protein kinase B, is an effector kinase downstream of PI3K and plays a key role in cardiac adaptation to exercise [28].

There are three isoforms (Akt1, Akt2, and Akt3) of Akt. The Akt1 and Akt2 are abundantly expressed in the heart [29]. Activated Akt phosphorylates a variety of intracellular substrates that regulate growth, metabolism, and survival. Specifically, activation of Akt leads to myocardial hypertrophy with enhanced cardiac function [30], whereas disruption of Akt signaling inhibits exercise-induced hypertrophy [28]. Indeed, Akt knockout mice showed a blunted hypertrophic response to swim training [28]. mTOR is serine/threonine protein kinase in the PI3K-related protein kinases family, which senses various environmental and intracellular changes, including nutrient availability, and energy status [31]. Thus, mTOR regulates cell growth, differentiation, autophagy, cell survival, and metabolism [32]. mTOR constitutes the catalytic subunit of two distinct complexes known as mTOR complex: mTORC1 and mTORC2 [32]. Activated mTOR regulates two targets involved in the regulation of protein translation, including 4E-binding protein-1 (4E-BP1) and p70 ribosomal S6 protein kinase (p70S6K) [33]. mTOR has been proven to have an essential role in exercise-induced cardiac hypertrophy [34, 35]. mTOR is highly phosphorylated in the myocardium after long-term moderate endurance exercise, whereas mTOR inhibitor, rapamycin, diminishes exercise-induced cardiac hypertrophy [36].

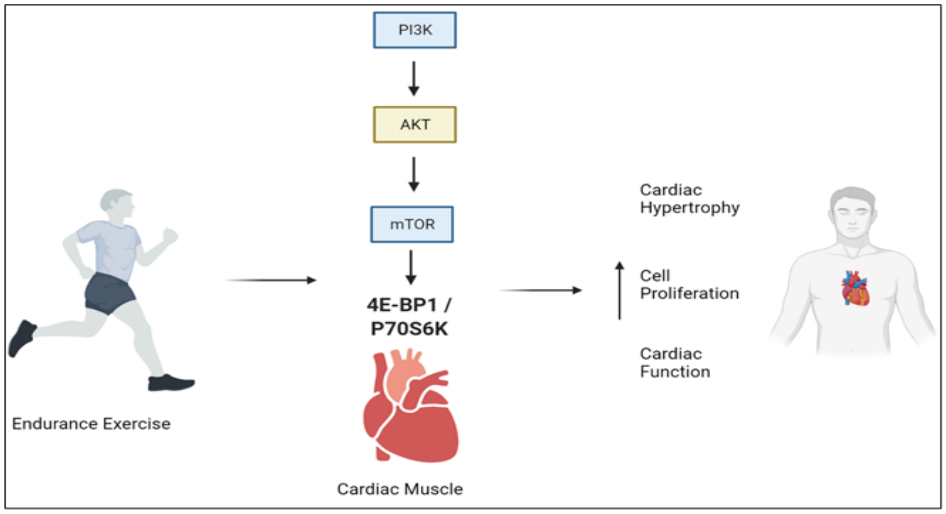

Figure 2. Activation of PI3K/Akt/mTOR provides well-supported Cardiac Benefits. Cardiac Function, Protection and Cell Capacity are enhanced with activation due to Endurance Exercise [26, 27, 29].

Interestingly, additional molecular mechanisms involved within endurance exercise continue to be researched as to responses of not only cardiac function but also hyper/hypo generative activity. In recent studies related to endurance exercise mechanisms, it has been proposed that endurance exercise during cardiomyopathy, can show both positive and negative results towards cardiac myopathy populations [57]. Studies suggest intensity, duration and application of endurance exercise provides mechanisms in restrictive cardiomyopathy populations to show a variety of results alluding to exercise [57].

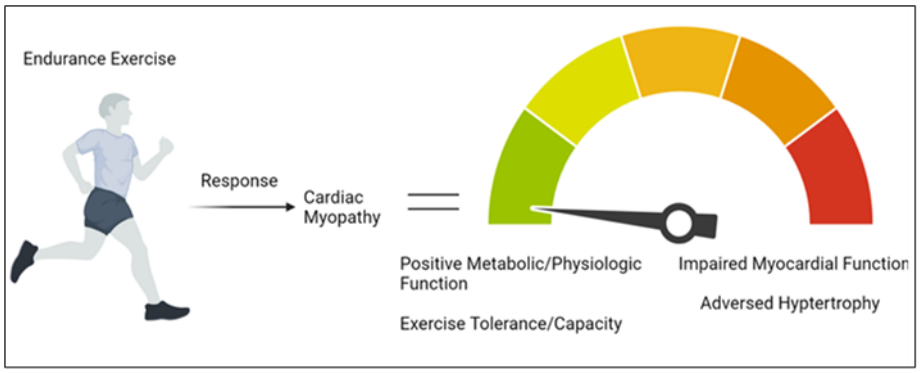

Figure 3. The responses and mechanisms of endurance exercise still remains an unclear application to cardiac myopathy. Although variation of exercise has been shown to provide positive/negative results. Exercise has been seen to be a positive regulator of cardiac health regardless of mechanism.

1.4 Mitogen-Activated Protein Kinase (MAPK) and MAP Kinase Phosphatase (MKP) Relationship in Cardiac Muscle

Growing evidence shows that the MAPK plays an important role in the regulation of PI3K/Akt/mTOR pathway [37-39]. The MAPKs are highly conserved serine/threonine protein kinases that participate in a multitude of signal transduction pathways [40]. MAPKs are activated by a wide variety of extracellular stimuli, including mitogens, growth factors, cytokines, and cellular stresses associated with physiological mechanisms [41]. The MAPK family includes p38 MAPK, c-Jun NH2-terminal kinases (JNKs), and extracellular signal-regulated kinases 1 and 2

(ERK1/2) [42]. It has been reported that the MAPKs are activated in cardiac muscle in response to exercise, facilitating cardiac benefits and adaptation [43-45]. According to literature, it has been found that activation of the MAPK PI3K/AKT/mTOR pathways along with the p38/JNK/ERK1/2 kinases due to endurance exercise have both benefits in cardiac and skeletal muscle [53, 54]. In where cardiac/skeletal benefits include: improvements in fuel homeostasis, cell proliferation, muscular hypotrophy and prevention of atrophy [21]. The level of p38 MAPK phosphorylation is increased in response to endurance exercise, stimulating mitochondrial biogenesis [43]. Chronic low-intensity exercise upregulates JNK phosphorylation in the heart and improves ejection fraction [46, 47]. Increased ERK1/2 expression improves oxygen consumption during treadmill exercise [48]. The activity of MAPKs is regulated by both MAPK kinases (MKKs) and MAP kinase phosphatases (MKPs) through phosphorylation events [49]. Specifically, The MKPs constitute one group of the dual-specificity phosphatases (DUSPs) that exhibit the capacity to dephosphorylate MAPKs on regulatory threonine and tyrosine residues (Figure 4) [49, 50].

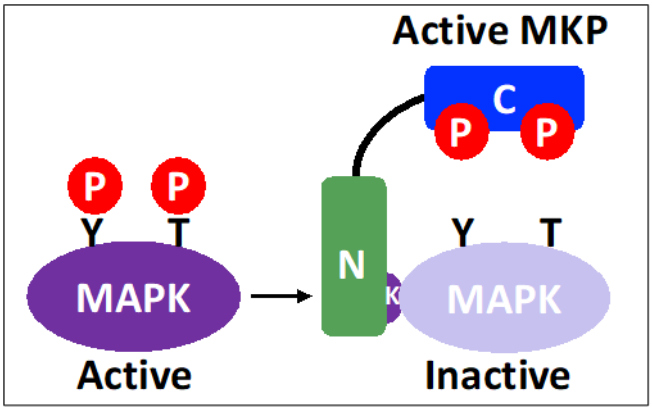

Figure 4. MKP dephosphorylates the regulatory tyrosine and threonine residue on MAPK

There are 10 MKPs that preferentially regulate the activity of MAPKs depending on cellular stress [49]. Among the MKPs, MKP-5 is highly expressed in cardiac and skeletal muscle, suggesting that it plays an important functional role in the muscle metabolisms [40, 51]. MKP-5 has been shown to regulate regenerative myogenesis in response to muscle injury through activation of muscle stem cells, satellite cells [50]. MKP-5 inactivation also exhibited enhanced myofiber survival through mitochondrial-mediated apoptosis [40]. Recently our group demonstrated that MKP-5 gene expression is significantly increased in the heart of mice subjected to transverse aortic constriction (TAC), which causes pressure overload-induced cardiomyopathy (Figure 5) [51]. Remarkably, we discovered that the heart of MKP-5-deficient mice (Mkp-$5^{-/-}$) completely preserves cardiac function against the cardiomyopathy (Figure 6). Our findings suggest that MKP-5 may represent a novel therapeutic target against heart disease.

Guided by our data, we asked whether MKP-5 can contribute to improvement of cardiac function. Since enhancement of cardiac function is required to perform endurance exercise [52], endurance exercise capacity was measure with progressive exercise stress test following endurance exercise. Interestingly, Mkp-$5^{-/-}$ mice exhibited significantly improved endurance exercise capacity (Figure 7). Our preliminary data indicates that MKP-5 may play a decisive role in cardiac adaptation to endurance exercise, leading to enhanced endurance exercise capacity. Although MAPKs are involved in the PI3K/AKT/mTOR pathways and/or cardiac adaption to exercise, the mechanisms of whether MKP-5-mediated MAPKs activate PI3K/AKT/mTOR pathway has not been explored in the cardiac muscle.

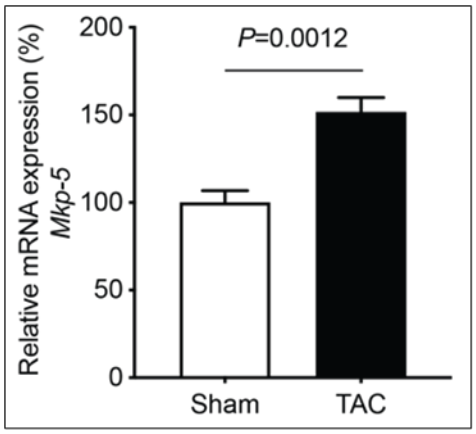

Figure 5. MKP-5 is activated in cardiomyopathy [53]. Relative mRNA expression of MKP-5 in cardiac muscle of wild type (C57BL-6J) mice was measured at 4 weeks after sham and transverse aortic constriction (TAC). Results are the mean ± SEM and were analyzed by unpaired t-test (n=5 per group).

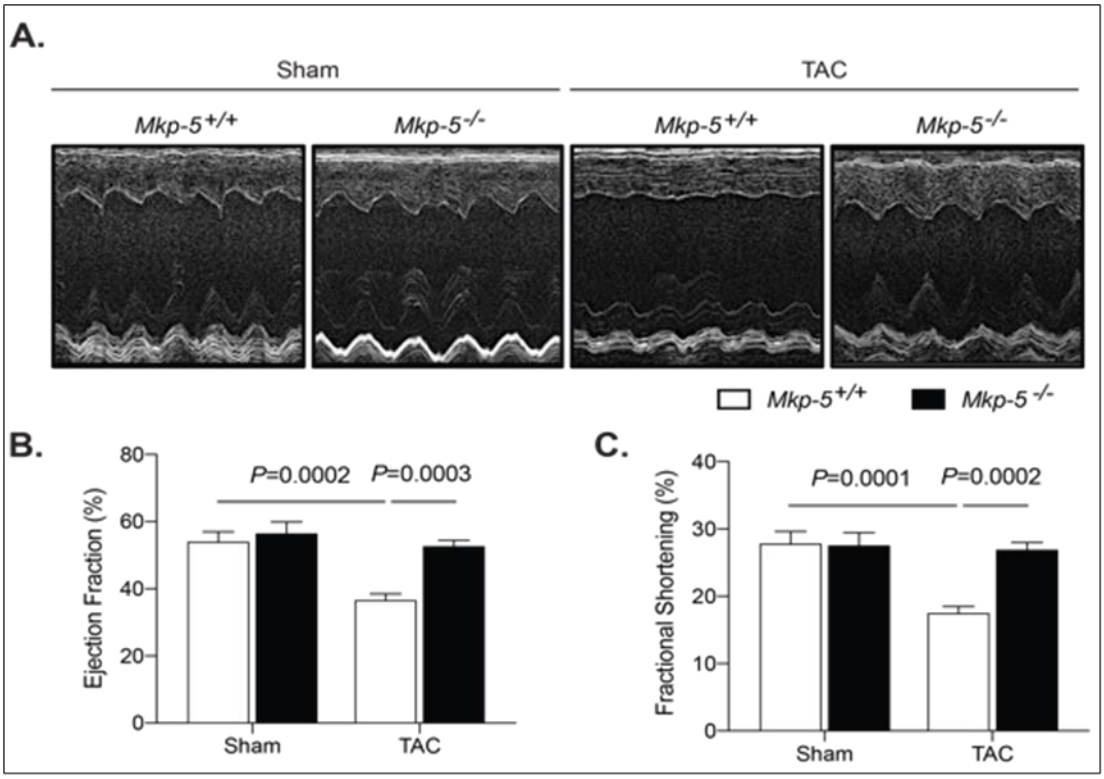

Figure 6. MKP-5 deficiency exhibits cardio protection against cardiomyopathy [53]. (A) Echocardiographic images, (B) ejection fraction and (C) fractional shortening of Mkp-$5^{+/+}$ and Mkp-$5^{-/-}$ mice at 4 weeks after sham and TAC. Results are the mean ± SEM and were analyzed by 2-way ANOVA and Tukey's multiple comparison test (n=9 for Sham; n=10 for TAC per group).

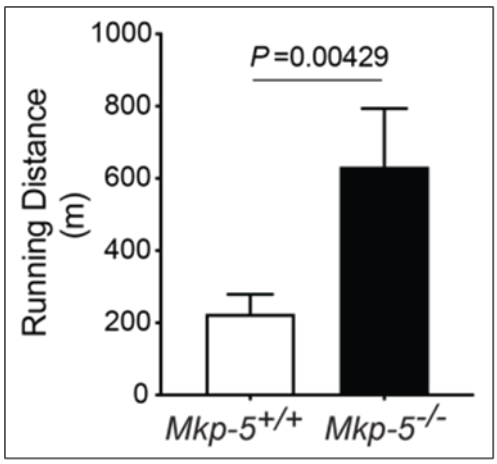

Figure 7. MKP-5 inactivation in the heart improves endurance exercise capacity. Endurance exercise capacity was measured by a graded increase in treadmill speed until exhaustion. Results are the mean ± SEM and were analyzed by unpaired t-test (n=5 per group).

Chapter 2: Scientific Applications

2.1 Hypothesis

Based on our findings, preliminary data, and literature reviews, the objective of this project is to investigate whether MKP-5 plays a role in facilitating cardiac adaptation to endurance exercise. Our central hypothesis is that MKP-5-mediated MAPK activity is involved in cardiac adaptation to endurance exercise via PI3K/AKT/mTOR pathway (Figure 8).

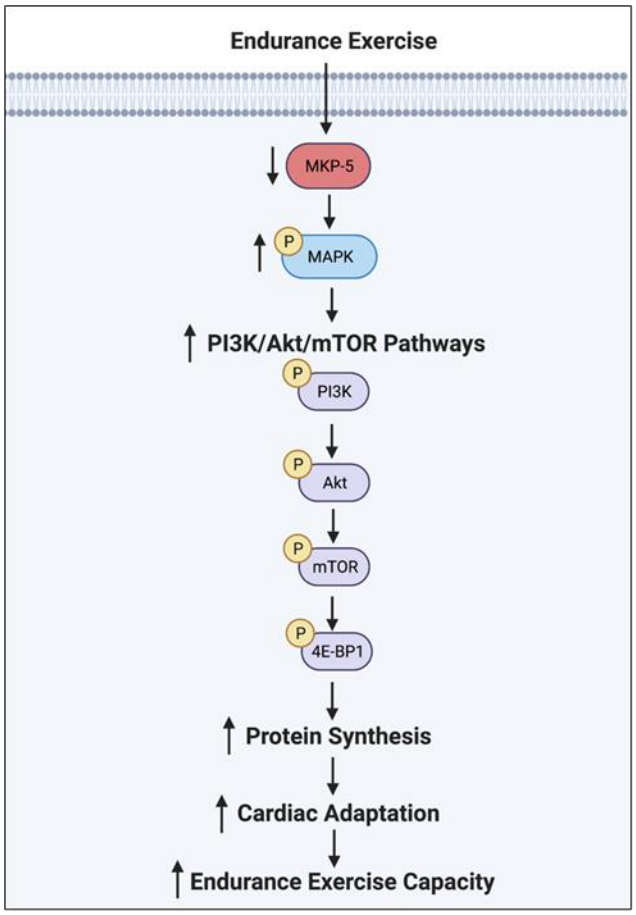

Figure 8. Hypothesis. Endurance exercise promotes cardiac adaptation, improving endurance exercise capacity

Chapter 3: Materials and Methods

3.1 Endurance Exercise Training

To investigate the role of MKP-5 in cardiac adaptation to endurance exercise, Mkp-$5^{+/+}$and Mkp-$5^{-/-}$ mice were subjected to endurance exercise. 8-week-old male mice were habituated to running in a rodent treadmill by increasing the duration and speed of running for 5 consecutive days. After the habituation, the mice were rested for 2 days and then performed 5 consecutive days of treadmill running for 60 min/day at 15 m/min and 0% grade. This exercise intensity is known to maintain 70~75% of VO2max [54]. After performing the exercise training protocol, we performed a progressive exercise stress test in day 6. In this test, treadmill speed was gradually increased from 2 m/min to 6 m/min every 5 minutes until the point of exhaustion. Mice were considered exhausted when they could no longer move forward from behind of the lane for 5 consecutive seconds three times or 10 consecutive seconds once without attempting to run on the treadmill again. Then, the running distance was calculated.

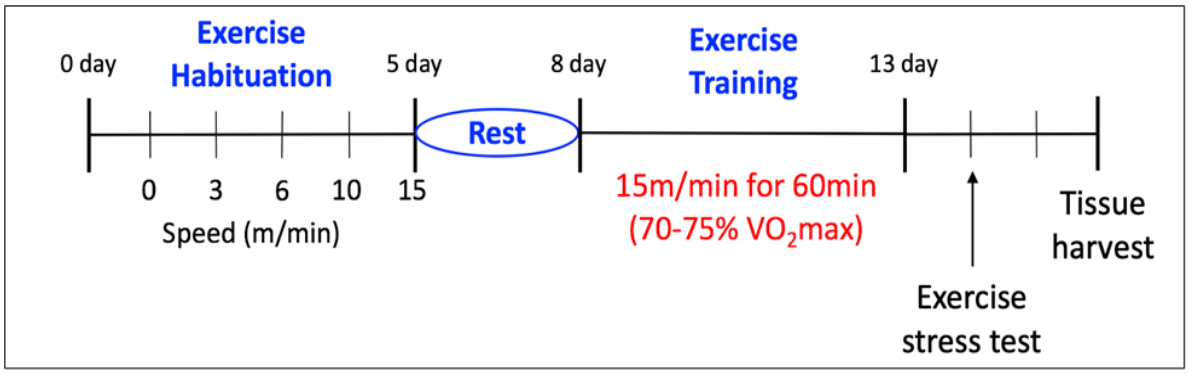

Figure 9. Endurance exercise training and progressive exercise test protocol

3.2 Immunoblotting

The left ventricle of cardiac muscle was collected from Mkp-$5^{+/+}$and Mkp-$5^{-/-}$ mice at 48 hours after endurance exercise training and test. The cardiac muscle tissues will be used for

immunoblotting. The cardiac muscle will be homogenized and lysed on ice in lysis buffer containing 100 mM Tris HCL (pH 7.4) and 2.5 mM EDTA. The lysis buffer will be supplemented with protease and phosphatase inhibitors (1 mM Na3VO4, 10 mM NaF, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstain A, 5µg/ml aprotinin, 5 µg/ml leupeptin). The lysates will be incubated at 4□C for 30 min and clarified by centrifugation at 14,000 rpm at 4ºC for 10 min. The protein concentration will be determined using the bicinchoninic acid reagent. Lysates will be resolved by SDS-PAGE and transferred onto Nitrocellulose membranes (Bio-Rad). Membranes will be blocked with 5% BSA in Tris-buffered saline/Tween-20 (TBST) for 1 h at room temperature. Primary antibodies, including anti-phospho-p38 MAPK (T180/Y182), JNK (T183/Y185), ERK (T202/Y204), PI3K (Y607), Akt (S473), mTOR(S2448) and 4E-BP1 (T37/46) will be diluted in 5% BSA in TBST. After primary antibody incubations overnight at 4ºC, the membranes will be washed in TBST three times for 10 min. The membranes will be then incubated in secondary antibodies (LI-COR). The sites of antibody binding will be visualized and be quantified using the Odyssey CLx Imaging System.

3.3 Data Analysis

All data will present the means ± standard errors of the means (SEM). Differences between groups will be assessed by a student's t test using Prism software (GraphPad Software). The levels of phosphorylation of each protein will be normalized with the levels of total expression of each protein.

Chapter 4: Results

4.1 The Effect of MKP-5 on Activity of MAPKs in Endurance Exercised Hearts

Much evidence has demonstrated that the MAPKs are activated in cardiac muscle for adaptive response to endurance exercise, leading to cardiac benefits [43, 44, 47]. Although the signaling pathways underlying cardiac adaptation to endurance exercise have been extensively studies, little is known about the precise mechanisms of how the MAPKs and subsequent MAPK signaling cascades are activated in exercised cardiac muscle. Since MKP-5 has been responsible for the inactivation of MAPKs through dephosphorylation, phosphorylation of MAPKs, including p38 MAPK, JNK and ERK was measured in endurance exercised cardiac muscle derived from Mkp-$5^{+/+}$ and Mkp-$5^{-/-}$ mice. Our data showed that Mkp-$5^{-/-}$ mice exhibit enhanced phosphorylation of p38 MAPK and JNK in response to endurance exercise, as compared to Mkp-5+/+ mice (Figure 10 A and B). However, the phosphorylation of ERK was not different in endurance exercised cardiac muscle derived from Mkp-$5^{+/+}$ and Mkp-$5^{-/-}$ mice (Figure 10 C). Our results are consistent with previous studies showing that MKP-5 dephosphorylates p38 MAPK and JUN, but not ERK in skeletal muscle and cardiac muscle [50, 51]. These data demonstrate that MKP-5 deficiency activates MAPKs during the progression of cardiac adaption in response to endurance exercise, leading to the improvement of endurance exercise capacity.

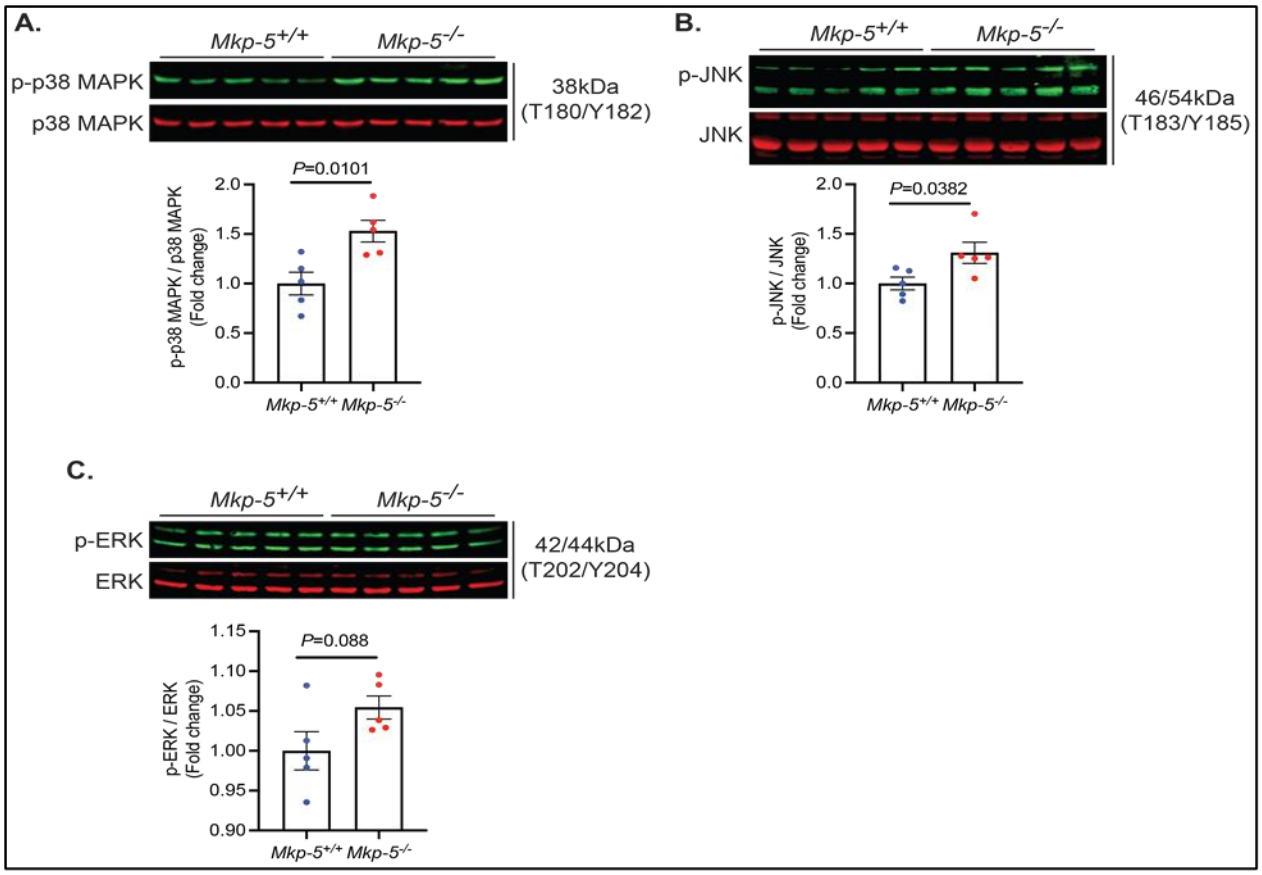

Figure 10. MKP-5 dephosphorylates MAPKs in endurance exercised hearts. Cardiac muscle derived from endurance exercise Mkp-$5^{+/+}$ and Mkp-$5^{-/-}$ mice were immunoblotted for phosphorylation of (A) p38 MAPK, (B) JNK and (C) ERK. The graphs represent the ratio of p-p38 MAPK / P38 MAPK, p-JNK / JNK, and p-ERK / ERK. Results are the mean ± SEM and were analyzed by unpaired t-test (n=5 per group).

4.2 The Effect of MKP-5 on Activity of Phosphoinositide 3-Kinase in Endurance Exercised Hearts

Phosphoinositide 3-kinase (PI3K) has been shown to be involved in cardiac adaptation to endurance exercise [23]. Further, PI3K is required to induce physiological cardiac growth and cardio protection against heart disease [55, 56]. To investigate whether MKP-5 is involved in the activation of PI3K in cardiac muscle following endurance exercise, phosphorylation of PI3K was measured in cardiac muscle isolated from endurance exercised Mkp-$5^{+/+}$ and Mkp-$5^{-/-}$ mice. Our finding revealed that Mkp-$5^{-/-}$ mice show increased phosphorylation of PI3K in cardiac muscle in

response to endurance exercise, as compared with Mkp-5$^{+/+}$ mice (Figure 11). Our data demonstrated that MKP-5 deficiency improves endurance exercise capacity through the activation of PI3K in cardiac muscle.

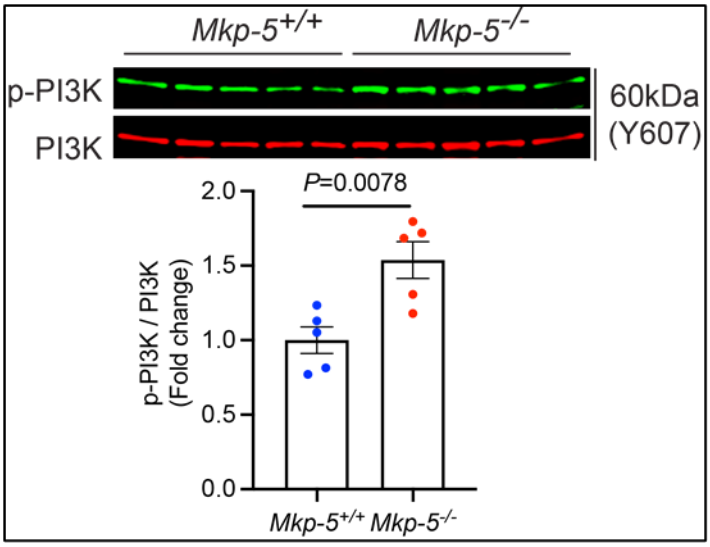

Figure 11. MKP-5 deficiency increases phosphorylation of PI3K in endurance exercised hearts**.** Cardiac muscle derived from endurance exercised *Mkp-5*$^{+/+}$ and *Mkp-5*$^{-/-}$ mice were immunoblotted for phosphorylation of PI3K. The graphs represent the ratio of p-PI3K / PI3K. Results are the mean ± SEM and were analyzed by unpaired t-test (*n*=5 per group).

4.3. The Effect of MKP-5 on Activity of Protein Kinase-B in Endurance Exercised Hearts

One of the downstream targets of PI3K is protein kinase-B, also known as Akt [57]. It has been reported that activation of Akt also leads to the enhancement of cardiac function in response to exercise [58]. To test the effect of MKP-5 in activation of Akt in endurance exercised-hearts, phosphorylation of Akt was measured in cardiac muscle isolated from endurance exercised Mkp-5$^{+/+}$ and Mkp-5$^{-/-}$ mice. The level of phosphorylation of Akt was significantly increased in cardiac muscle of Mkp-5$^{-/-}$ mice in response to endurance exercise, as compared with Mkp-5$^{+/+}$ mice (Figure 12). This finding suggests that MKP-5 deficiency promotes activation of Akt, which is required for physiological cardiac adaptation.

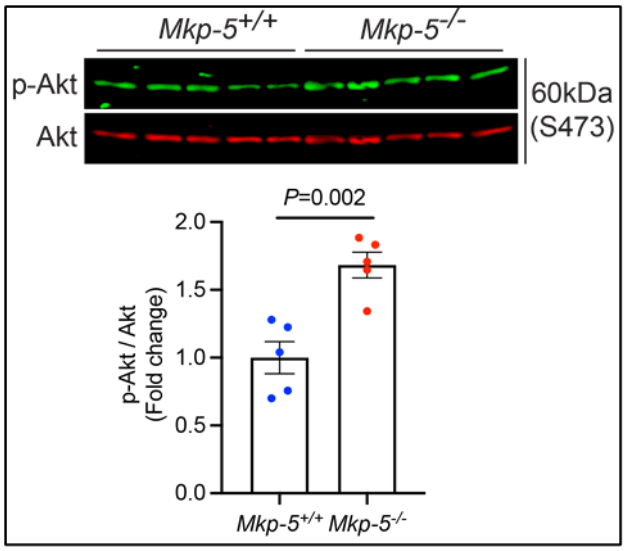

Figure 12. MKP-5 deficiency promotes phosphorylation of Akt in endurance exercised hearts**.** Cardiac muscle derived from endurance exercised *Mkp-5*$^{+/+}$ and *Mkp-5*$^{-/-}$ mice were immunoblotted for phosphorylation of Akt. The graphs represent the ratio of p-Akt / Akt. Results are the mean ± SEM and were analyzed by unpaired t-test (*n*=5 per group).

4.4 The Effect of MKP-5 on Activity of Mammalian Target of Rapamycin in Endurance Exercised Hearts

Cumulative evidence has indicated that mammalian target of rapamycin (mTOR) is a key regulator for cardiac hypertrophy [59, 60]. To evaluate whether MKP-5 regulates mTOR activity in cardiac muscle in response to endurance exercise, mTOR activation was determined by immunoblotting with anti-phospho-mTOR antibody in cardiac muscle isolated from endurance exercised Mkp-5$^{+/+}$ and Mkp-5$^{-/-}$ mice. Our dada indicate that MKP-5 deficiency significantly increases phosphorated mTOR proteins in cardiac muscle in response to endurance exercise (Figure 13). It suggests that MKP-5 may play an important role in mTOR pathway to promote cardiac adaptation and improve endurance exercise capacity.

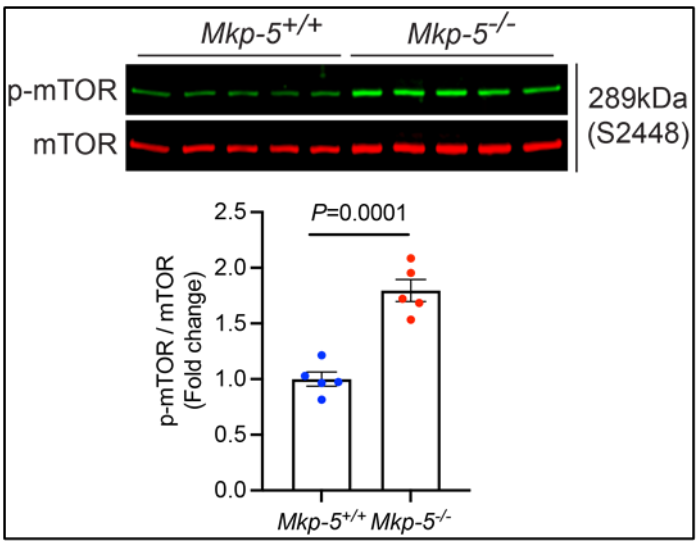

Figure 13. MKP-5 deficiency increases phosphorylation of mTOR in endurance exercised hearts**.** Cardiac muscle derived from endurance exercised *Mkp-5*$^{+/+}$ and *Mkp-5*$^{-/-}$ mice were immunoblotted for phosphorylation of Akt. The graphs represent the ratio of p-mTOR / mTOR. Results are the mean ± SEM and were analyzed by unpaired t-test (*n*=5 per group).

4.5 The Effect of MKP-5 on Activity of 4E-Binding Protein 1 in Endurance Exercised Hearts

mTOR protein promotes protein synthesis through activation of its downstream target 4E-binding protein 1 (4E-BP1) in exercised cardiac muscle [61]. To test the effect of MKP-5 on activation of 4E-BP1 in cardiac muscle following endurance exercise, cardiac muscle lysates isolated from endurance exercised Mkp-5$^{+/+}$ and Mkp-5$^{-/-}$ mice were immunoblotted with anti-phospho-4E-BP1 antibody. Western blot assays exhibited that the phosphorylation of 4E-BP1 is significantly increased in cardiac muscle of Mkp-5$^{-/-}$ mice following endurance exercise, as compared with Mkp-5$^{+/+}$ and Mkp-5$^{-/-}$ mice (Figure 14). This result suggests that MKP-5 deficiency activates mTOR pathway in endurance exercised cardiac muscle.

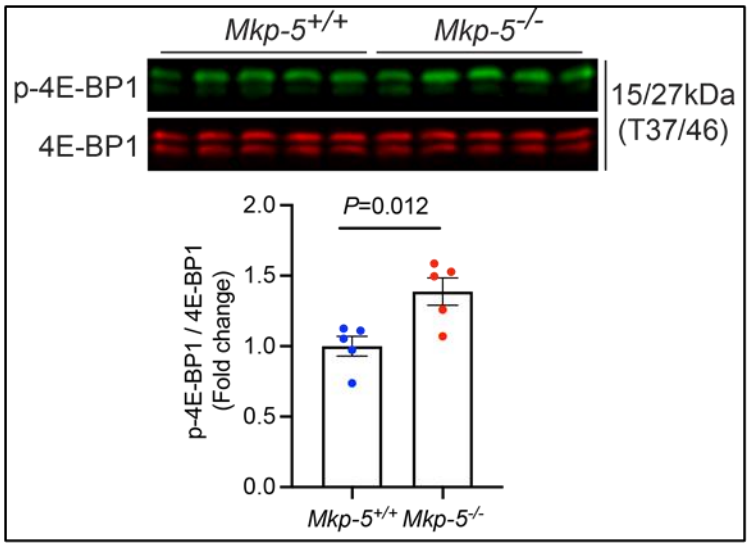

Figure 14. MKP-5 deficiency activates 4E-BP1 through phosphorylation in endurance exercised hearts**.** Cardiac muscle derived from endurance exercised *Mkp-5*$^{+/+}$ and *Mkp-5*$^{-/-}$ mice were immunoblotted for phosphorylation of 4E-BP1. The graphs represent the ratio of 4E-BP1 / 4E-BP1. Results are the mean ± SEM and were analyzed by unpaired t-test (*n*=5 per group).

Chapter 5: Discussion

5.1 Endurance Exercise and Physiologic Cardiac Adaptations

The human body during exercise is able to adapt and configure many physiological components that make a healthy lifestyle. Specifically, endurance exercise has been a strong indicator of not only a healthy lifestyle in terms of fitness, but also as research shows, regular aerobic physical exercise protects against atherosclerotic cardiovascular diseases and certain malignances [67]. Studies have also shown that, the heart develops several myocardial adaptations to endurance exercise training, causing physiological remodeling [22]. Additionally, while cardiac and physiologic adaptations have been well documented in response to endurance exercise training [68], the mechanisms activated via response have yet to be fully understood.

Importantly, additional studies of physiological adaptations found that due to the high cardiovascular demands of endurance exercise, the most profound cardiac adaptations, have shown significant increases in left ventricle and right ventricle cavity enlargement and mass. [67, 22, 4]. According to experiments on mice/rat models during endurance exercise training, increases in left ventricle size, mass and diameter, showed significant increases in exercise capacity during training [68]. Data on this form of cardiac adaptation in response to endurance exercise shows evidence that physiologic adaptation take place within the heart in response to exercise.

5.2 MAPK and MKP-5 in Cardiac and Physiological Adaptations

While physiological adaptations and cardiac adaptations respectively are well documented, the specific mechanisms behind these adaptations have yet to be fully understood. One of the prominent signaling pathways involved in the progression of exercise-induced cardiac

benefits is the mitogen-activated protein kinase (MAPK) pathway [45, 41, 43]. There are three major MAPK family, including p38 MAPK, c-Jun NH2-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERKs) [43]. Ludlow et al. has showed that phosphorylation of p38 MAPK is highly increased in cardiac muscle of mice after a single bout exercise [40]. [69] investigated whether treadmill exercise activates JNK in the cardiac muscle. In this study, rats were subjected to supervised treadmill exercise with low, moderate, and high intensity for 6 weeks. The high exercise intensity resulted in a 12% increase in heart mass, as compared with untrained rats. JNK activity was increased 2.5-fold in the cardiac muscle of rats subjected to high exercise intensity compared to untrained rats [69]. The phosphorylation of ERK has been shown to promote protein synthesis through enhanced cell proliferation [70].

Further, exercise training has protective effects against cardiomyocyte death through activation of ERK. However, inhibition of ERKs leads to cardiomyocyte death and impaired heart function [71]. Although the activity of MAPKs is regulated by either their upstream MAPK kinases (MKKs) and MAP kinase phosphatases (MKPs) through phosphorylation events [40], MKP-mediated MAPK signaling has been overlooked in the cardiac function in response to exercise. Consistent with other studies, our data also show that phosphorylation of p38 MAPK and JNK in cardiac muscle isolated from Mkp-$5^{-/-}$ mice that exhibited improved endurance exercise capacity was significantly increased as compared to Mkp-$5^{+/+}$ mice (Figure 10). It suggests that MKP-5 may be a key regulator for the activation of MAPKs in exercised cardiac muscle, and MKP-5-mediated MAPK signaling may play a decisive role in cardiac adaptation to endurance exercise, leading to improved physical fitness.

5.3 PI3K/Akt/mTOR Pathway and Endurance Exercise-Induced Cardiac Adaptation

It has been well known that MAPKs activate molecular signaling pathways that alter cardiomyocytes and enhance cardiac muscle function. PI3K/Akt/mTOR pathway is one of the MAPK-induced pathways [37, 38, 39]. Growing evidence suggests that PI3K/Akt/mTOR pathway have been shown to be associated with cell growth, metabolism survival and angiogenesis [74]. Therefore, PI3K/Akt/mTOR pathway is one of potential molecular signaling pathways in MKP-5-mediated MAPK signaling that promotes cardiac adaptation to endurance exercise.

5.4 MKP-5 Deficiency Enhances PI3K Phosphorylation in Endurance Exercised Cardiac Muscle

Phosphoinositide 3-kinase or (PI3K) provides numerous cellular responses and mechanistic activity through cellular stress [26]. It has been proposed that high phosphorylation levels of PI3K can be used to enhance physiological adaptations for left ventricle hypertrophy during exercise [74]. Ma et al., measured PI3K phosphorylation on mice and determined that PI3K phosphorylation was significantly increased in exercised mice as compared to non-exercised mice [74]. Moreover, Weeks K.T. et al. demonstrated that PI3K is essential for exercise-induced cardio protection against heart failure. In this study, animals were subjected to 4 weeks of exercise training followed by 1 week of pressure overload to induce pathological remodeling. Untrained animals exhibited pathological cardiac hypertrophy, depressed systolic function, and lung congestion. However, this phenotype was attenuated in animals subjected to 4-week exercise training. Importantly, animal with constitutively active PI3K were protected from pathological remodeling independent of exercise status [56]. In this regard, our data also

show that MKP-5 deficiency increased phosphorylation of P13K in cardiac muscle in response to endurance exercise (Figure 11). It suggests that MKP-5 is involved in PI3K activity in cardiac muscle, leading to improved endurance exercise.

5.5 MKP-5 Deficiency Increases Akt Activity in Endurance Exercised Cardiac Muscle

PI3Ks downstream substrate, known as Akt is also an essential protein in cellular growth mechanisms and physiological adaptations within cardiac muscle. In the study conducted by Ma et al, [74], isoforms of Akt know to be (Akt1, Akt2 and Akt3) respectively, are expressed in all areas of skeletal muscle tissue and heart tissue [74]. Essentially, this study evaluated the expression levels of Akt1 due to the proposed intervention of physiological adaptation response to left ventricle hypertrophy [74]. Results showed that Akt in mice showed a significant level of phosphorylation in exercised mice as compared to non-exercised. Although Akt has general purposes towards cardiac hypertrophy during exercise, alteration of Akt isoforms remain to be fully understood [74, 75], Our current data suggest similar attributions of phosphorylated Akt in MKP-5 deficient-mice as compared to wild-type mice during exercise training. The phosphorylation of Akt was significantly increased in cardiac muscle of exercised Mkp-$5^{-/-}$ mice, as compared with exercised Mkp-$5^{+/+}$ mice (Figure 12). This founding suggests that MKP-5 deficiency promotes activation of Akt in cardiac muscle in response to endurance exercise.

5.6 MKP-5 Deficiency Activates mTOR in Endurance Exercised Cardiac Muscle

It has been established that mammalian target of rapamycin (mTOR) also contributes to protein synthesis, leading to cardiac hypertrophy following exercise [36]. mTOR phosphorylation becomes activated through initial activation of Akt through the MAPK pathway [36]. mTOR has tow distinct serine/threonine kinase complexes, mTORC1 and mTORC2

[67,74]. mTORC1/mTORC2 both regulate cell growth and survival and have been associated with controlling adaptive growth of the heart [44, 67, 74]. In a study observed by Ma et al, phosphorylation of mTOR was significantly increased in the exercise group as compared to non-exercise mice, suggesting involvement of cardiac adaptations during exercise [74]. It has been found that high phosphorylated levels of mTOR could transmit a signal to its downstream signal molecules, such as 4E-BPI and, p70S6K [74, 77]. Association of mTOR phosphorylation showed that post-exercise mice displayed a significant role in physiological hypertrophy and cardiac adaptation in response to exercise [76, 77, 78]. In correlation to this study, Mkp-$5^{-/-}$ mice with enhanced endurance exercise capacity show that phosphorylation of mTOR was significantly increased in cardiac muscle as compared with Mkp-$5^{+/+}$ mice (Figure 13). Our observation indicates that MKP-5 deficiency improves endurance exercise capacity through activation of mTOR that is required for cardiac adaptation to exercise.

5.7 MKP-5 Deficiency Increases 4E-BPI Phosphorylation in Endurance Exercised Cardiac Muscle

One of the important proteins for protein synthesis is 4E-binding protein 1 (4E-BP1). 4E-BP1 has unique roles in regulating translation of mRNA and regulation of protein synthesis [79]. Specifically, 4E-BPI becomes active during phosphorylation of mTOR. Further 4E-BPI becomes phosphorylated during cardiac muscle contractions and mediates cardiac hypertrophy [80, 81]. mTOR-mediated phosphorylation of 4E-BP1 prevents its inhibitory action of the eukaryotic initiation factor 4E (elf-4E), stimulating the initial step of protein synthesis [82]. Our data show that phosphorylation of 4E-BP1 was significantly increased in cardiac muscle lacking MKP-5

following endurance exercise (Figure 14). It suggests that MKP-5 deficiency may promote protein synthesis in cardiac muscle in response to endurance exercise.

Chapter 6: Conclusion

Endurance Exercise is a critical component of not only a healthy lifestyle, but also towards adaptations of cardiac muscle in response to exercise [68]. Although mechanisms behind physiological adaptations are not yet fully understood, the MAPK pathways involved in cardiac muscle tissue during exercise show to be key factors in determining heart health and function [84] and also in promoting cardiac adaptations that can alleviate injury and pathological adaptations [54, 83]. Given mice lacking MKP-5 showed enhanced endurance exercise capacity and activation of PI3K/Akt/mTOR pathway, it is apparent that the role of MKP-5 deficiency plays a crucial factor in regulating cardiac adaptations in responses to endurance exercise.

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Vita

Jaime Alfredo Perales was born in El Paso, Texas. While attending the University of Texas at El Paso (UTEP), he earned a Bachelor's of Science Degree in Kinesiology, while also completing a Minor in Military Science. While pursuing an education in the health sciences, he also competed in Athletics (Football) for The University of Texas at El Paso for 5 consecutive seasons. Earning Conference USA Commissioners Honor Roll and earning Letterman's for each season of play.

While completing a Master's Degree in Kinesiology under the guidance of Dr. Kisuk Min. Jaime held positions of Graduate Research Assistant and Teaching Assistant, where he developed skills in laboratory techniques and animal handling. As well as instructed Undergraduate Level courses (Coronary Intervention), and guided students into performing Stress Test Exercise Protocols. Jaime's current research is focusing on uncovering specific mechanisms related to cardiac adaptations in response to endurance exercise. In where he hopes to apply and understand many of the physiologic adaptations that are obtained during exercise and towards heart function.

Upon completion of his Master's degree in May 2022, Jaime plans to pursue a Doctorates Degree within his field of Kinesiology and hopes to continue his research during his tenure.