Exercise and cardiomyocyte regeneration

Indira Vidiari Juhanna1*, I Nyoman Adiputra1, I Putu Gede Adiatmika1, I Made Muliarta1, Ni Made Linawati2, I Putu Adiartha Griadhi1

ABSTRACT
Cardiovascular disease is the most common cause of death. Many researchers have evaluated the effect of exercise on heart function improvement, but studies about how exercise can affect heart regeneration are rare. Most of the previous studies only assess biomarkers that indicate heart damage. GATA-4 Transcription Factor is one of the transcription factors that form heart cells, showing the heart's ability to function properly under pressure. Cardiac-restricted zinc-finger TF GATA-4 is a survival factor that can break the vicious cycle of post-MI heart failure through increased myocardial angiogenesis, decreased apoptosis, and increased c-kit cell generation. Further research is needed because of its critical role in heart regeneration, whether GATA-4 can be used as an excellent cardiac biomarker in the future, and how the role of exercise or physical exercise on GATA-4 protein and its expression in cardiac regeneration.

INTRODUCTION
Cardiovascular disease is the number one cause of death globally. An estimated 17 million people died from cardiovascular disease in 2005, representing 30% of all global deaths.1 The number of myocardial infarction and stroke cases is 32.4 million worldwide each year. Patients with myocardial infarction (MI) and stroke are at the highest risk for future coronary and cerebral infarction. Patients with MI have an increased risk of recurrent infarction with an annual death rate of 5%, six times higher than the common population.2

Most recent studies about cardiac fitness evaluated how exercise improved heart function, but the studies that assess exercise's effect on myocardial regeneration are rare. Most of the existing studies only assessed the extent of myocardial damage (e.g., by evaluating Cardiac Troponin (cTn)), which consists of cardiac Troponin T (cTnT) and cardiac Troponin I (cTnI), which is the gold standard for the diagnosis of Acute Myocardial Infarction (AMI).3–5

However, cardiac regeneration was deemed possible after discovering a population of stem cells that have a biologically significant role in heart function and regeneration. Recent studies have identified gene products that can induce embryonic characteristics in adult cardiomyocytes.6 These features raise the possibility of cardiac regeneration by activating several genes that can induce embryonic characteristics, enabling the cells to divide and differentiate into new cardiomyocytes. Additionally, the stem cells also support the damaged cardiomyocytes, which enhance their survival under hypoxic and inflamed conditions. Cardiomyocyte survival is critical to ongoing hemodynamic performance and remodeling due to fibrosis and scarring's simultaneous formation. Multiple signaling pathways are involved in cardiomyocyte survival, mainly through inhibition of aging, anti-inflammatory, differentiation, and angiogenesis.7

GATA-4 transcription factor is one of the transcription factors that have a vital role in cardiomyocyte regeneration. However, recent findings also supported its importance for the heart's adaptability under stress.8,9 GATA4 is a transcription factor containing a zinc finger that
belongs to the GATA superfamily. GATA4 is widely expressed in cardiomyocytes where GATA 4 regulates transcription of α- and β-myosin heavy chain (MHC), atrial natriuretic (ANP), and β-type natriuretic peptide (BNP), which are essential in cardiac function and blood pressure regulation. GATA4 is also critical for adaptive responses such as cardiomyocyte survival, hypertrophy, exercise response, and ischemic response. Therefore, this review describes in detail the evidence-based developments in understanding the effect of exercise on increasing the GATA-4 Transcription Factor Protein as a cardioprotective agent.

**CARDIAC REGENERATION**

Cardiac remodeling and heart failure have been critical problems after myocardial infarction, although treatment and reperfusion strategies have been improved. An experimental study on a mammalian model found that complete regeneration of the heart could occur after a severe ischemic heart injury. This evidence has sparked intense efforts to uncover molecular and cellular pathways in regeneration after ischemic injury, a finding that could someday be applied to post-cardiac patients. Various studies have shown that cardiomyocyte regeneration in adult mammals occurred at a very slow pace, mainly from pre-existing cardiomyocytes. However, it has been known that cardiomyocyte is a terminally differentiated cell and external stimulations are needed to induce the regeneration from this cell-type. The reason for this phenomenon is because of the endogenous progenitor cells within the myocardium. A study from Bergmann showed that 0.5-1% of postnatal human cardiomyocytes could change each year so that about 50% of cardiomyocytes are reconstituted during human life. Cardiomyocyte proliferation in adult rat hearts was also confirmed using multi-isotope imaging mass spectroscopy combined with genetic labeling of pre-existing cardiomyocytes.

A new perspective suggests that stem cells and fibroblasts only contributed to a relatively small population in the myocardium. Endothelial cells, which are noncardiomyocyte components, tend to play a more significant role in physiological function and response to injury. Immunohistochemistry revealed that endothelial cells consisted of more than 60% of the responded cells, while hematopoietic-derived cells were only comprised of 5% to 10%, and fibroblasts were less than 20% of non-myocytes in the heart.

The heart is one of the least regenerating organs in the body, with lower regeneration capacity than many other organs, such as the liver, skeletal muscle, lungs, intestines, bladder, bones or skin. The human left ventricle has 2-4 billion cardiomyocytes, and myocardial infarction can damage 25% of them within a few hours. The ultimate goal of heart regeneration is to regenerate the myocardium after the injury to prevent or treat heart failure. This interdisciplinary field has recently gained support from the advances in stem cells or progenitor cell development, chemical genetics, engineering biomaterials, and other areas to generate a new, electrically and mechanically integrated myocardium. Cardiac stem cells (CSC) can be used as a therapy to repair heart failure. CSCs are defined as heart cells that exhibit clonogenic, self-renewing, and multipotent capacity. CSCs can differentiate into at least three types of primary cells in the myocardium: cardiomyocytes, smooth muscle cells, and endothelial cells. Therefore, CSCs are expected to be an effective and powerful source of cells to replace the damaged cardiomyocytes.

Inflammation naturally occurs in response to injury, regeneration, scar, MI recovery, and response remodeling. The inflammatory response to myocardial injury includes mononuclear cell infiltration, interleukins production and secretion, accumulation of mast cells in the healing of scars, fibroblasts, and extracellular matrix (ECM) remodeling, and regulation of angiogenesis in the development and healing of subsequent infarcts. In healthy heart tissue, collagen homeostasis is tightly regulated through the controlled synthesis of new collagen and old collagen fibers’ degradation. Collagen is degraded by a group of endopeptidases called matrix metalloproteinases (MMPs), and their functions are tightly controlled to maintain homeostatic degradation and synthesis of extracellular matrix (ECM).

Adult mammalian cardiomyocytes can replicate DNA but rarely progress to the cytokinesis stage. Mature mammalian cardiomyocytes have failed to undergo continued division, which correlates with the G2M cell cycle gene’s permanent silencing, similar to the old cells. The genes responsible for the cardiomyocytes’ division become condensed and encased into heterochromatin, which is transcriptionally inactive in the adult heart.

**GATA-4 TRANSCRIPTION FACTOR AS CARDIOPROTECTIVE AGENT**

Until now, there is no effective treatment for repairing damaged tissue after a heart injury because existing therapies only focus on rescuing the damaged tissue. Cell-based therapy using cardiomyocytes produced from stem cells is a promising therapeutic approach that can directly replace the damaged myocardium with healthy...
tissue. However, the molecular mechanisms underlying this role of stem cells are not fully understood. GATA4 is an essential regulator of cardiac differentiation, endoderm development, and mesoderm cardiogenesis in the embryo.\textsuperscript{19} Yilbas et al. in stated that the p300 wave was directly involved in GATA4 gene regulation in the early stages of cardiac specification.\textsuperscript{19} P300 event-related potential (ERP) was used to assess human cognitive function. P300 wave is thought to reflect the information processing cascade associated with attention and memory mechanisms. P300 wave is a positive wave deflection in human event-related potential. Event-Related Potential (ERP) assesses electrical activity duration on the cerebral surface, showing the different phases of cortical processes. ERP reflects the neurophysiological processes related to various cognitive tasks.\textsuperscript{21} Its inducer, valproic acid, is known to effectively induced histone acetylation and cardiac differentiation. Additionally, Dimethyl Sulfoxide (DMSO) induces differentiation of P19 stem cells into cardiomyocytes. DMSO-enhanced-cardiac differentiation was associated with increased histone and p300 acetylation in the GATA4 promoter.\textsuperscript{19} Heterozygous mutations in GATA4 can cause defects in the atrial or ventricular septum and pulmonary valve stenosis.\textsuperscript{22}

GATA4 is a zinc-finger transcription factor that primarily regulates several cardiac-related genes, such as atrial natriuretic peptides, type-B natriuretic peptide, and myosin heavy chain. GATA-4 is also essential for cardiac development because embryos lacking homozygous GATA-4 will die 7 to 10 days post-conception due to defects in ventral morphogenesis and cardiac tube formation.\textsuperscript{20,22,23} GATA-4 appears to have a unique dual role as a mediator of the hypertrophic response and survival factor. GATA-4 is required for cardiomyocytes’ response to hypertrophic stimuli and has been suggested as the stretch-activated response’s nuclear effector.\textsuperscript{25} He et al. reported that exosomes isolated from GATA-4-expressing mice bone marrow mesenchymal stem cells (BMSCs) induced differentiation of BMSC into cardiomyocytes, decreased apoptosis anoxia-induced cardiomyocytes, and improve myocardial function after infarction.\textsuperscript{24} Additionally, induced-expression of GATA4 in MSCs also enhanced its regenerative capacity through myocardial differentiation, enhanced cell viability, and modulated stem cells’ paracrine activity.\textsuperscript{25}

The high mortality rate of stem cells transplanted in myocardial infarction (MI) and low differentiation efficiency of cardiomyocytes suggested that mesenchymal stem cell (MSC) transplantation after MI is ineffective. Csx/Nkx2.5 and GATA-4 are considered as the primary regulators of cardiogenesis. Overexpression of Csx/Nkx2.5 and GATA-4 in MSC transplantation is a new treatment strategy with the potential to improve cardiac function after MI.\textsuperscript{26} Functional studies showed that the differentiation potential of MSC GATA-4 was reduced by insulin-like growth factor-binding protein (IGFBP)-4 knockdown. GATA-4 overexpression significantly increases MSC differentiation into myocardial phenotypes related to IGFBP-4 regulation.\textsuperscript{27} The use of biomaterials is a viable strategy for improving cell survival and cardiac repair. However, there are limitations to combinational cell biomaterial therapy since the microenvironment and physical characteristics 28 influence cellular behavior. GATA 4–6 plays an essential role in the development of the endoderm and mesoderm. Functional redundancy between GATA4 and GATA5 is acquired during cardiac development and involves GATA5 as a candidate gene modifier for congenital heart disease.\textsuperscript{28}

The transcription code programming cardiac hypertrophy involves a DNA binding factor containing zinc finger-containing DNA binding factors GATA-4 and GATA-6, both of which are required for hypertrophic adult cardiac response. GATA-4 and GATA-6 play a role in programming the hypertrophic growth response of the heart after stimulation of excessive stress, but each has a more complex role in maintaining cardiac homeostasis and resistance to heart failure after non-compensatory injury.\textsuperscript{29} Heart failure is often a consequence of less optimal heart regeneration. Reconstitution of cardiac GATA4 levels by adenoviral gene transfer significantly increased cardiac regeneration after cryoinjury at day seven postnatal. In contrast, myocardial scarring was more extensive in cardiomyocyte-specific GATA4 knockout (CM-G4-KO) after cryoinjury on postnatal day 0, indicating impaired regeneration and accompanied by decreased cardiomyocyte proliferation and myocardial angiogenesis. Cardiomyocyte proliferation is also reduced in CM-G4-KO and reduced expression of GATA4 in isolated cardiomyocytes. Mechanically, the decrease in GATA4 levels leads to the downregulation of several pro-regenerative genes (interleukin-13) in the myocardium. Interestingly, systemic administration of IL-13 can save regeneration of damaged hearts in CM-G4-KO mice and could be evaluated as a future therapeutic strategy.\textsuperscript{30}

The transcriptome is a pool of all the transcribed elements in a cell regulated by interactions between different molecular levels, involving epigenetic, transcription, and post-transcription. Integrating mRNA profiles with DNA binding of major cardiac
transcription factors (Gata4, Mef2a, Nkx2.5, and Srf), activated histone modification (H3ac, H4ac, H3K4me2, and H3K4me3), and microRNA profiles obtained by wild-type and RNAi-mediated knockdown may facilitate cardiogenesis. However, some epicardial progenitor-genes may also contribute to cardiomyogenesis, such as T-box transcription factor 5 (Tbx5), homeodomain protein Islet1 (Isl1), and epicardial progenitors by Wilms tumour-1 (WT1) or T-box transcription factor 18 (Tbx18). Other cardiogenic factors identified by embryonic lineage tracing or analysis of gene silencing include homeobox protein nkx2.5, the myocyte enhancer factor 2C (Mef2c), and GATA4.

GATA-4 TRANSCRIPTION FACTOR EXPRESSION AFTER EXERCISE

Evidence supports that all exercise types with different intensities can improve heart function, stem cell activity, and cardiomyocyte marker. In some cases, reduced infarct size was also observed. Biomolecular assessment of MI patients who underwent moderate exercise showed that the level of GATA4, Nkx2.5 and c-Kit were significantly increased. The high-intensity exercise group experienced more remarkable improvement than the low and moderate-intensity exercise group. In the high-intensity exercise group, the level of Sca-1 and CITED4 were also increased, which was higher than the low-intensity exercise group. On the other hand, the mRNA and C/EBPβ protein levels decreased after training, with greater magnitude was observed in the low or moderate-intensity high-intensity exercise group. This study suggests that high-intensity exercise has a cardioprotective effect on cardiac dysfunction in the MI experimental model.

Bahramian et al. presented other findings using the left coronary artery (LAD) ligation in adult male Wistar rats. The animals were randomly divided into the MI-sedentary group (MI-Sed) or the MI-Low Intensity Interval Training (LIIT) group, the MI-Moderate Intensity Interval Training (MIIT) group, and the MI-High Intensity Interval Training (HIIT) group and all of them were evaluated using echocardiography before experimentation. The study was conducted for six weeks (five days/week), and the reported results showed down-regulation of C/EBP β mRNA expression on HIIT. Other effects reported included decreased C/EBP β expression, increased CITED4 expression, C/EBP β down-regulation, and Serum Response Factor (SRF) to bind the target gene promoter. These components are involved in activating the exercise gene set (GATA-4, Tbx5, Nkx2.5, and Mef2c) and, ultimately, cardiac regeneration.

Experimental research by Xiao et al. in 2013 at Shanghai University also reported another supporting evidence. They discovered that swimming induced cardiac physiological hypertrophy. In-depth molecular analysis showed increased mRNA level of Binding Protein GATA-4, Atrial Natriuretic Peptide (ANP), brain natriuretic peptide (BNP), endogenous hepatocyte growth factor (HGF), and Insulin-like Growth Factor 1 (IGF) from the liver which were determined by Real-Time Polymerase Chain Reaction (RT-PCR). This study also reported an increase in the Heart Weight (HW) to bodyweight ratio, HW to tibia length ratio, and increased level of GATA4 mRNA. Meanwhile, no change was reported in the ANP and BNP mRNA levels did not change. The C-kit and Sca-1 positive cardiac progenitor cells were also activated by swimming exercise as well as increased endogenous production of HGF and IGF at the mRNA level.

CONCLUSION

GATA-4 effectively improves stem cell cardiogenic differentiation capacity, both through direct differentiation or paracrine effect. However, research on GATA-4 is still scarce, especially studies that study the effect of exercise on increasing GATA4 expression. Conversely, understanding the role of GATA-4 could potentially improved stem cell therapy and overall cardiovascular disease treatment. Therefore, further research is needed to evaluate the link between exercise, GATA-4, and cardiovascular function because GATA-4 reflects both the heart's regenerative and functional status. It is also interesting to assess the potency of GATA-4 as cardiac regeneration and functional biomarker in the future.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

AUTHOR CONTRIBUTION

All authors contributed equally in writing and revising this article.

FUNDING

This article was self-funded with no third party involvement.

ETHICS APPROVAL

Not Applicable
REFERENCES

11. Senyo SE, Lee RT, Kuhn B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. Stem Cell Res. Published online 2014. doi:10.1016/j.scr.2014.09.003
12. Lin Z, Pu WT. Strategies for cardiac regeneration and repair. Sci Transl Med. Published online 2014. doi:10.1126/scitranslmed.3006681
14. Laflamme MA, Murry CE. Heart regeneration. Nature. Published online 2011. doi:10.1038/nature10414
22. Rysa J, Tenhunen O, Serpi R, et al. GATA-4 is an angiogenic survival factor of the injured heart. Circ Hear Fail. Published online 2010. doi:10.1161/CIRCHEARTFAILURE.109.889642
24. He JG, Li HR, Han JX, et al. GATA-4-expressing mouse bone marrow mesenchymal stem cells improve cardiac function after myocardial infarction via secreted exosomes. Sci Rep. Published online 2018. doi:10.1038/s41598-018-34615-5
32. Steinhauser ML, Lee RT. Regeneration of the heart. EMBO Mol Med. Published online 2011. doi:10.1002/emmm.201100175