Chronic moderate-intensity exercise can induce physiological hypertrophy in aged cardiomyocytes through autophagy, with minimal Yap/Taz involvement

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Abstract. Aging is known to cause increased comorbidities associated with cardiovascular decline. Physical exercises were known to be an effective intervention for the age-associated decline in cardiac function. Exercise caused physiological hypertrophy influenced by Yap/Taz, autophagy and myosin heavy chain (MHC) dynamics. However, whether exercise-induced changes are associated with aging has yet to be determined. The present study explored the effects of moderate-intensity exercises on autophagy, MHC dynamics, and Yap/Taz activity to understand their complex interactions at the molecular effects on the cardiac function of aging cardiac tissue. The present study used male Wistar (Rattus norvegicus) rats (80 weeks-old) randomly divided into two groups (n=12): control and intervention. The intervention group was given an intervention using an animal treadmill. After 8 weeks, the animal was sacrificed, and data were collected. Statistical analysis was conducted using an independent t-test or Mann-Whitney U test when appropriate. Exercise in aged rats can induce physiological hypertrophy, as shown by gross measurement and histological features. Yap/Taz did not mediate the effects of exercise on hypertrophy. Autophagy function was shown to increase, which may cause the low expression of Yap/Taz. In conclusion, exercise is a viable intervention in increasing heart mass and potentially delaying the decline in function associated with aging.

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Introduction

The WHO predicts that by 2050, the elderly population in the world will increase in 2020 to 2.1 billion from 1.4 billion individuals, due to increasing healthcare coverage and life expectancy, and up to 2/3 of this population will live in low- and middle-income countries (1). The increase in the elderly changes population structure and disease distribution, with degenerative and metabolic diseases and cancers gaining prominence (2). The physiology of aging must be considered since numerous bodily functions are significantly impaired and present significant morbidity. For example, cardiovascular function is known to decline with increasing age, with fibrosis, reduced contractility and reduced blood flow, to name a few effects (3,4). Several studies have focused on promoting 'healthy aging' to promote healthy function despite old age (5-7). Lifestyle interventions such as physical exercises are well known to counteract the age-associated decline and promote cardiac function with increasing age (5,8). However, up to 1/3 of adults aged ≥45 o lack physical activity. These sedentary lifestyles cause at least 3.2 million deaths per year (9,10). Studies from the early 2000s have already proven strong evidence associating a sedentary lifestyle with cardiovascular mortality. Currently, a sedentary lifestyle is associated with a 30% increased risk for all-cause mortality.

Therefore, physical exercise is essential in promoting survival and function in the aging heart.

Physical exercise has various physiological and psychological benefits. The physiological benefits include the improvement of the function of the cardiovascular system and increasing the heart's resistance to injury (11,12). This improvement is also contingent on the intensity, type and duration of the physical exercise, thereby highlighting the diversity of molecular effects of exercise on the heart. One of the most used recommendations is by the American Heart Association, which states that for older adults, a minimum of 150 min of moderate-intensity physical exercise per week is needed, coupled with medium-intensity strength training at least two times per week (13,14). Previous studies have shown that physical exercises induce physical adaptations of the heart through the remodeling and growth of cardiomyocytes (15). Although taken to the extreme, this remodeling may increase the risk of sudden cardiac death. These so-called physiological cardiac hypertrophies were shown to promote longevity and confer protective benefits to the heart. However, the mechanisms by which moderate-intensity physical exercise affects the aging heart remain unknown.

Autophagy is a cellular homeostasis mechanism responsible for recycling organelles and proteins, especially during aging (16). Studies have found declining levels of autophagy associated with old age, corresponding with increased levels of damaged organelles and mitochondria. These accumulations may contribute to the decline in cardiac function (17,18). Previous studies have also shown that this pathway interacts with the components of the Hippo pathway with Yes-associated protein 1 (Yap1) and Taz proving to be a substrate to autophagy (19,20) and also influences autophagy activation (21). Yap1 is one of the components of the Hippo pathway involved in cell regeneration and hypertrophy. Yap1 is a mechanoreceptor whose activation relies on stretch and mechanical tension (22). Several studies on skeletal muscles prove the ability of Yap1 to induce hypertrophy (23,24). Yet, research on cardiomyocytes revealed that Yap1 induced proliferation instead of cellular hypertrophy reflecting the complex regulation of this protein between tissues (25). Studies on Yap have shown that it influences cardiomyocyte proliferation and regeneration, protecting against ischemic injury (26). Increased Yap1 activity, either through inhibition of the Hippo pathway, a known negative regulator of Yap1 or through gene overexpression, was identified to induce cardiac overgrowth with increased proliferation and protect against doxorubicin-induced cytotoxicity (27,28). Yap1 was also shown to mediate the effects of pathological hypertrophy caused by mechanical stress overload (29). Therefore, although increasing Yap1 may be able to increase cardiomyocyte growth and proliferation, it is unknown whether this protein played a role in the physiological hypertrophy induced by moderate-intensity exercises.

Physiological hypertrophy of the heart is also related to other functions besides the Yap1 protein. The distribution of the myosin-heavy chain is also affected in the aging heart. Several reviews have explored the consequences of aging in myosin heavy chain (MHC) distributions, which affect contractile and metabolic functions (30,31). Autophagy is a well-known physiological process involved in aging, and its influence may regulate cardiac mass. Several studies have shown that autophagy influences Yap1 activity, showing their complex interactions (19,21). Furthermore, autophagy has been demonstrated to regulate myosin-heavy chain dynamics (32). Singh *et al* (33) found that the administration of Rapamycin, or caloric restriction, which induces autophagy function, lessens the effects of hypertrophic cardiomyopathy caused by alterations in genes encoding or affecting the MHCs (32-34). Autophagy is essential in maintaining mitochondrial homeostasis in the heart and further proving its important role in maintaining cardiac function. Therefore, in the present study, the molecular effects of moderate-intensity exercises focusing on the autophagy process, MHC dynamics and Yap1 activity were explored.

Materials and methods

Animal models. A total of 24 male Wistar rats (Rattus norvegicus) obtained from PT. Biofarma (Bandung, Indonesia). Animals were housed from 8th weeks of age and had free access to food and water. The animals were housed in cages measuring 30x40x60 cm^3, lined with husk bedding, and maintained at a room temperature of 25-27˚C with a 20-40% humidity level. A 12/12-h light/dark cycle was implemented, and the bedding was replaced every other day. The animals were included in the study if they weighed at least 200 g, could acclimatize, and were healthy during the experiment. The animals were raised until the 80th week and were allocated to control and exercise groups. The rats were raised until the 80th week since it correlates with human in 45 years of age (35). The present study was approved (approval no. 598/UN6.KEP/EC/202598/UN6.KEP/EC/2022) by the Research Ethics Committee of Universitas Padjadjaran (Bandung, Indonesia). *Exercise intervention*. In the 80th week, the rats were randomly allocated into two groups (n=12): intervention and no treatment. Ronny *et al* (36) and Gunadi *et al* (37) determined exercise intensity according to the lactate accumulation levels, and from the aforementioned studies, the moderate intensity treatment protocol was derived. The exercise intervention was given using an animal treadmill at a speed of 20 meters per min for 30 min per day, repeated five days per week, and lasted 8 weeks. For the no-treatment (control) group, the rats were kept on the immobile treadmill. The rats were euthanized using 5% isoflurane for 1 min, followed by cervical dislocation. After death confirmation, the heart was isolated and harvested. The organ was weighed, and 500 mg of it was frozen using liquid nitrogen and stored at -80˚C for RNA extraction.

The rat's body weight, organ weight and tibia length were measured. Several of these measurements were also normalized with body weight or tibia length (38). For histopathological examination, 500 mg of the heart muscle originating from the left ventricle was fixed using 10% neutral-buffered formalin at room temperature for 72 h. Histopathological sections with a thickness of 4 μm were made and stained with hematoxylin for 5 min, followed by Eosin for 2 min, both conducted at room temperature before being evaluated by a pathologist. H&E staining was chosen since it provides a balanced visualization of tissue structure, cellular morphology, and fibrosis assessment. This provided a thorough evaluation of all the parameters needed. The review was performed on five fields

Table I. Histological assessment criteria used in this research.

Cardiomyocyte hypertrophy	Myofiber disarray	Focal fibrosis
0: No hypertrophy	0: No myofiber disarray	0: No focal fibrosis
1: Cardiomyocyte diameter is 3-4 RBCs large.	1: Disarray in 1-25% of heart muscle	1: 1-5 focal fibrosis
2: Cardiomyocyte diameter is 4-5 RBCs large.	2: Disarray in 26-50% of heart muscle	2: 6-10 focal fibrosis
3: Cardiomyocyte diameter is >5 RBCs large.	3: Disarray in >50% of heart muscle	3: >10 focal fibrosis

Table II. Primer pairs used in the present study.

Gene name	Primer sequence (5'-3')	Base pairs	Annealing (˚C)
Myh6	F: GAGCAGGAGCTGATCGAGAC R: CCTCTGCGTTCCTACACTCC	151	60
Myh7	F: GCGGACATTGCCGAGTCCCAG R: GCTCCAGGTCTCAGGGCTTCACA	133	59.5
Yap	F: GATCCCTGATGATGTACCACTGCC R: GCCATGTTGTTGTCTGATCGTTGTG	101	57
Taz	F: CATGGCGGAAAAAGATCCTCC R: GTCGGTCACGTCATAGGACTG	242	57
PIK3ca	F: ACCTCAGGCTTGAAGAGTGTCG R: CCGTAAGTCGTCGCCATTTTTA	137	59
mTOR	F: CTGATGTCATTTATTGGCACAAA R: CAGGGACTCAGAACACAAATGC	170	57
Lc3	F: GGTCCAGTTGTGCCTTTATTGA R: GTGTGTGGGTTGTGTACGTCG	153	59.5
p62	F: CTAGGCATCGAGGTTGACATT R: CTTGGCTGAGTACCACTCTTATC	116	56
GAPDH	F: GTTACCAGGGCTGCCTTCTC R: GATGGTGATGGGTTTCCCGT	177	61

F, forward; R, reverse.

per sample with an Olympus CX21 light microscope at x100 magnification. The assessment criteria were cardiomyocyte hypertrophy, myofiber disarray and focal fibrosis (Table I).

RNA extraction was performed using TRIsure (cat. no. BIO-38032; Bioline), and RNA purity ratios were examined using spectrophotometry at 260/280 nm. A semiquantitative PCR was carried out using the Bioline one-step RT-PCR kit (cat. no. BIO-72005; Bioline), with GAPDH as a housekeeping gene. The primer sequences are included in Table I. A 10% agarose gel electrophoresis was carried out and stained using SYBRSafe (cat. no. S33102; Invitrogen; Thermo Fisher Scientific, Inc.). The gel was visualized using the BluePad Detection System (BP001CU; Bio-Helix Co., Ltd.) and quantified using ImageJ software version 1.46r (National Institutes of Health). The primer pairs used for PCR in the present study are shown in Table II.

Statistical analysis. The data was presented as the mean ± standard deviation (SD) or median (min-max). The normality test was conducted using the Shapiro-Wilk test; Levene's test was used to determine the homogeneity of variance. Statistical analysis was performed using the independent t-test for normally distributed data or the Mann-Whitney U test for non-parametric data. SPSS V.20 software (IBM Corp.) was used for analysis.

Results

Chronic moderate-intensity physical exercise causes cardiac muscle hypertrophy in old rats. The heart weight between the control and exercise groups was compared to ascertain the relationship between physical exercise and cardiac muscle. The heart weight between the control and exercise groups was significantly different, with a higher weight in the exercise group, as shown in Table III. The difference was still significant even after normalization with the body weight or tibia length of rats. Therefore, chronic moderate-intensity physical exercise was shown to be able to significantly increase heart weight, reflecting the hypertrophy process caused by exercise.

Hypertrophy caused by exercise is not associated with myofiber disarray and focal fibrosis. The histological appearances of

Table III. Gross characteristics of control vs. intervention groups.

	Control [mean ± SD/ median (min-max)]	Intervention [mean ± SD/ median (min-max)]	P-value
Heart weight (g)	1.15 (0.92-1.68)	1.65 (1.22-1.92)	0.01
Body weight (g)	362.5 (347-407)	315.5 (233-372)	0.001
Tibia length (cm)	4.5 (4-5.3)	4.55 (4-4.9)	0.843
Heart to body weight ratio	0.003±0.001	0.01±0.001	<0.001
Heart to tibia length ratio	0.28±0.07	0.36±0.05	0.003

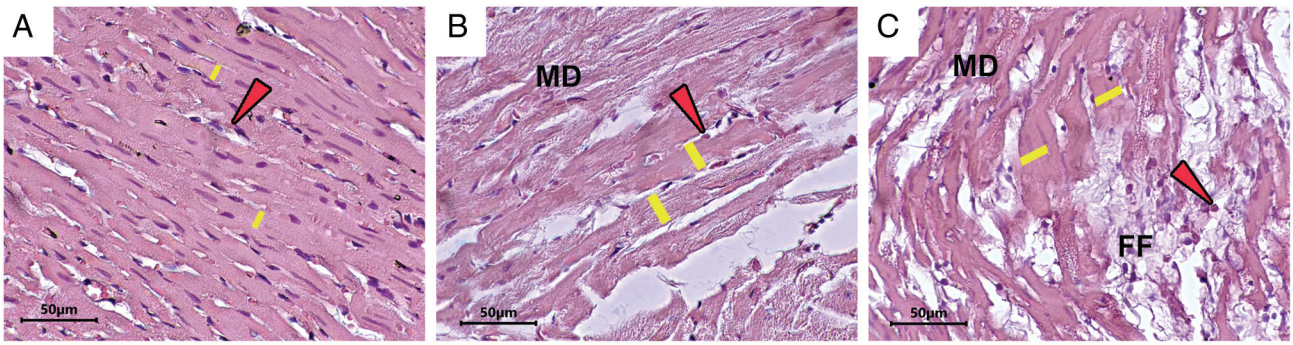

Figure 1. Representative histological features used in assessing microstructure changes in each group. (A) Normal cardiomyocyte diameter with no myofiber disarray. (B) Cardiomyocyte hypertrophy with disarrayed heart muscle. (C) Cardiomyocyte hypertrophy, disarrayed heart muscle and focal fibrosis. Images captured with Olympus CX21 and Optilab advance plus in x400 magnification. MD, muscle disarray; FF, focal fibrosis; yellow line, cardiomyocyte diameter; red arrow, erythrocytes.

cardiomyocytes were quantified by scoring system (37), which compared three main changes i.e., cardiomyocyte hypertrophy, myofiber disarray and focal fibrosis (Fig. 1). However, the authors differed from the earlier planned criteria and the assessment requirements were combined to only yes/no for all histological criteria. The main difference among the three quantified changes is in cardiomyocyte hypertrophy, with the exercise group showing significantly higher hypertrophy than the control group. No significant differences were found for myofiber disarray and focal fibrosis (Table IV).

Chronic moderate-intensity physical exercise causes a significant increase in Myh6 but not Myh7. Two MHC gene isoforms, Myh6 and Myh7, corresponding to α-MHC and β-MHC isoforms, were quantified using conventional PCR (Fig. 2). There was a significant increase in the relative expression of Myh6, with 1.2-fold higher expression in the intervention group (Table V). No significant increase was found in the relative expression of Myh7 corresponding to increased α-MHC expression with no significant changes in β-MHC expression. The ratio between the expression of these two genes also showed significant results with a difference of 1.27-fold increase in the intervention group (P<0.001). The results revealed that moderate-intensity physical activity caused an increase in cardiac muscle mass with MHC isoform distribution consistent with physiological hypertrophy.

Yap and Taz are downregulated due to chronic moderate-intensity physical exercise. The expression of Yap and Taz, effectors of the Hippo pathway, was measured. Semiquantitative measurement demonstrated a significant underexpression of both genes after chronic moderate-intensity physical exercise. Yap and Taz were significantly less expressed in the intervention compared with the control group, with 0.8 and 0.9-fold lower expression levels, respectively (Table VI).

Lc3 and p62 gene expression reveals that exercise potentially increased autophagy activity. In the present study, autophagy pathways were evaluated by measuring Lc3 expression, which is involved in autophagosome formation, and p62, which is constantly degraded by autophagy.

The intervention group exhibited a significantly higher expression of Lc3, coupled with a significantly lower expression of p62 (Fig. 3). This expression pattern suggests potential alterations in autophagy activity in the intervention group compared with the control group. Additionally, genes upstream of the autophagy pathway were examined, specifically the mTOR and PI3KCA genes. Both genes were also significantly underexpressed after chronic moderate-intensity physical exercise. This finding suggested that exercise may cause higher autophagy activity by influencing its upstream regulators, specifically downregulating mTOR and PI3K. However, further analysis using protein-level assays is needed to determine whether these changes reflect an overall increase in autophagy.

Discussion

Research exploring the effects of exercise on the heart were conducted. It was found that even in aged rats, exercise can

Table IV. Comparison of histological characteristics.

	Control (n=12)	Intervention (n =12)	P-value
Any Cardiomyocyte Hypertrophy[a]	1 (8%)	7 (58%)	0.014
Any Myofiber Disarray[a]	1 (8%)	1 (8%)	1
Any Focal Fibrosis[a]	0 (0%)	1 (8%)	1

[a]Fisher's exact test.

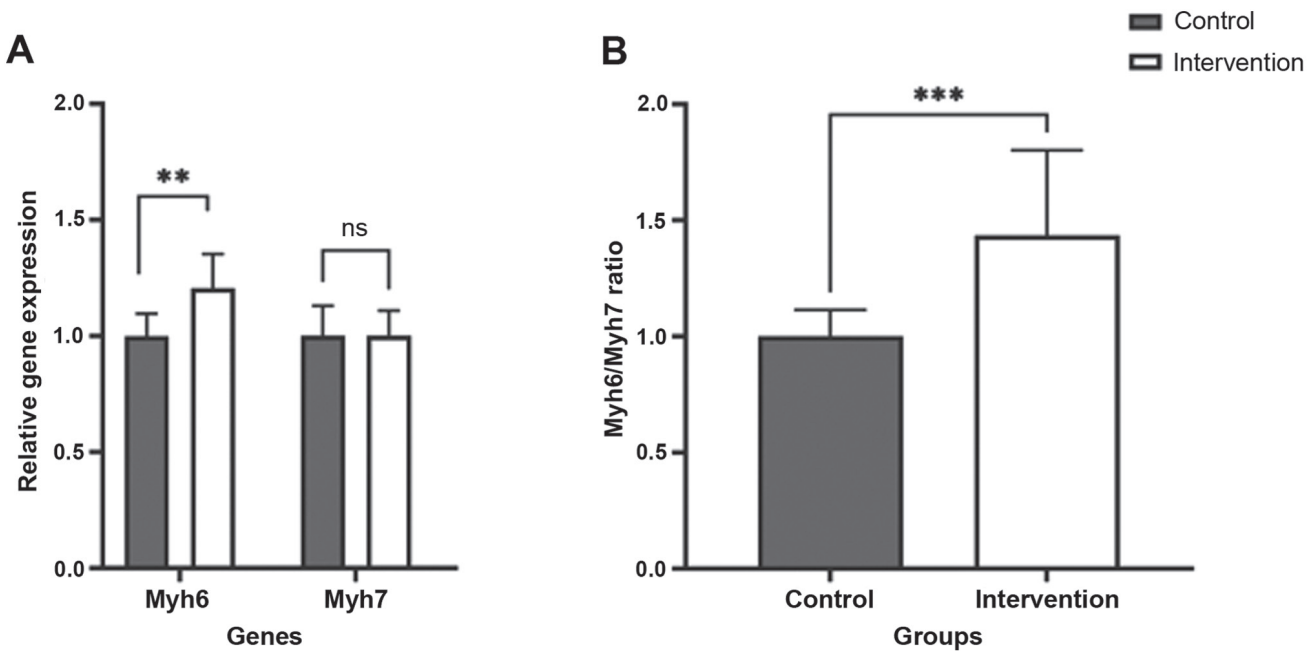

Figure 2. Relative Gene Expression of Myh6, Myh7 and Myh6/Myh7 ratio in control and intervention groups. (A) The bar graph denotes the relative gene expression levels of Myh6 and Myh7, and (B) the Myh6/Myh7 ratio measured using conventional PCR. Grey bars are the control group and the white bars are the intervention group. Data are presented as the mean ± SD. Myh6 expression was significantly increased in the intervention group compared with the control (P<0.01), while Myh7 expression showed no significant difference between the two groups. However, the Myh6/Myh7 ratio was significantly higher in the intervention group (P<0.01). Statistical significance was determined using a t-test for Myh6 and Myh7 and Mann-Whitney U test for the Myh6/Myh7 ratio. **P<0.01 and ***P<0.001. ns, no significance (P>0.05).

still cause an increase in heart weight. A previous study in skeletal muscle showed that aging caused a blunted hypertrophic response to resistance training (39). However, in the heart, several evidence point out that ventricular hypertrophy is one of the physiological changes (40). Ventricular hypertrophy in aging is associated with cardiac fibrosis and lower heart function, signs of pathological hypertrophy (41). These changes cause hypertrophy as a mechanism for compensation to maintain body perfusion. This research shows that exercise can still induce a hypertrophic response in cardiac muscle even during aging, as demonstrated by the increased cardiac weight. Even after normalization, a significant difference in the body weight and tibia length was still found to ensure that the increased weight was not due to body size differences. However, further exploration of microscopic appearances and gene expression levels is needed to ascertain whether it is physiological or pathological.

Several studies have found that α-MHC content is decreased during aging, similar to cardiac changes caused by overload or heart failure (42,43). In the present study, it was validated that even during aging, chronic-moderate-intensity physical exercises can still induce physiological hypertrophy of the heart. The findings of the present study demonstrated that physical exercise caused a preferential increase of alpha myosin over beta myosin, as shown by the higher expression of alpha myosin and the ratio between both myosin isoforms. The increase in alpha myosin is consistent with physiological hypertrophy, which was found with a higher alpha myosin content (41). The α-MHC isoform has the highest myosin ATPase activity and contractile speed, with several research showing that a higher combination of this protein is associated with increased contractile velocity (42). Targeting α-MHC has been the focus of several research studies, and it has been found that overexpression of α-MHC allowed a modest improvement in ventricular function after myocardial infarction (44), and interventions aiming at this protein also alleviated heart failure (45). Therefore, the present findings extended previous findings of increased α-MHC in cardiac muscle after exercise and that the increase of α-MHC still occurs in the context of aging.

Table V. The expression of Myh6 and Myh7 in control vs. intervention groups.

	Control (median (min-max)/mean ± sd)	Intervention (median (min-max)/mean ± sd)	P-value
Myh6	1±0.097	1.204±0.148	0.001
Myh7	1±0.130	1±0.11	0.992
Myh6/Myh7[a]	0.97 (0.85-1.26)	1.27 (1.15-2.623)	<0.001

[a]The Mann-Whitney U Test was used for the Myh6/Myh7 ratio, and independent t-tests were used for the rest of the genes.

Table VI. Comparison of Yap/Taz and autophagy-related genes.

	Control (median (min-max)/mean ± SD)	Intervention (median (min-max)/mean ± SD)	P-value
Yap[a]	0.98 (0.89-1.34)	0.85 (0.67-0.96)	0.001
Taz	1±0.09	0.92±0.05	0.014
mTOR	1±0.09	0.89±0.06	0.003
PI3KCA	1±0.08	0.91±0.06	0.005
Lc3	1±0.11	1.17±0.05	<0.001
P62[a]	0.99 (0.84-1.20)	0.92 (0.83-0.96)	0.005

[a]The Mann-Whitney U Test was used for the p62 and Yap genes, and Independent t-tests were used for the rest of the genes.

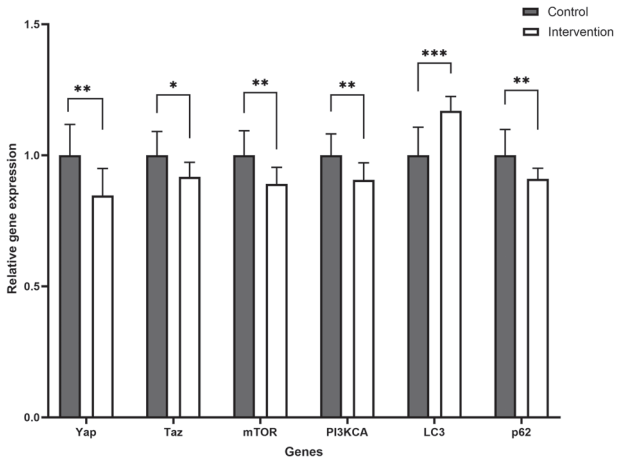

Figure 3. Expression level of genes related with autophagy in the control vs. intervention groups. The bar graph demonstrated the relative expression level of autophagy-related genes (LC3, p62, mTOR and PI3K) and the Hippo pathway genes (Yap and Taz). Grey bars are the control group and the white bars are the intervention group. The data are presented as the mean ± SD. Note the expression pattern of LC3 and p62 implicated increased autophagy in the intervention group. The expression of mTOR and PI3K, both involved in regulating autophagy, is also shown, with statistically significant downregulation in the intervention group compared with the control group. Statistical significance was determined using the Mann-Whitney U Test for the p62 and Yap genes, and independent t-tests were used for the LC3, p62, mTOR and PI3K genes. *P<0.05, **P<0.01 and ***P<0.001.

The increase in alpha myosin contrasts with hypertrophy caused by cardiac overload. It has been previously reported that chronic pressure overload preferentially increases beta myosin (43). Beta myosin has a lower ATPase activity but is more economically efficient in the contractile force it generates. Therefore, the increased force needed for heart function will cause a preferential increase of this isoform. The role of beta myosin is probably better explained by mutations of this gene, which is one of the known causes of hypertrophic cardiomyopathy (46,47). Mutations cause up to a 30% reduction in contractile speed and force generation (46,48), which causes compensatory hypertrophy to generate enough force for heart function. In the present study, it was found that chronic moderate-intensity exercise in old rats did not increase the β-MHC gene expression, supporting the role of exercise in restoring cardiac function even during old age (Fig. 4).

Microscopy further supports the role of exercise in causing physiological hypertrophy. In pathological hypertrophy, a significant amount of fibrosis usually occurs in the cardiac tissue (41). These were considered to be due to several factors: Chronic injury, mismatch between vascular and cardiomyocyte growth, imbalances between pro- and antifibrotic proteins, intense exercises and aging (49-53). Although exercises were known to reduce cardiac fibrosis (49,54,55), it is unknown whether the same effects can be observed on the aging heart, especially considering the lower functional capacity of aged hearts. The current research found that exercise did not cause an increase in cardiac tissue fibrosis compared with control. Additionally, since there is no evidence of fibrosis, a sign of pathological hypertrophy (41), it can be further supported that the hypertrophy caused by exercise, even during old age, is consistent with features of physiological hypertrophy.

Yap/Taz is the effector of the Hippo pathway, which was previously known to cause skeletal muscle hypertrophy. Yap/Taz was activated by several mechanoreceptors and stretch, causing hypertrophy in skeletal muscles (22). Therefore, during exercise, which causes physical stress on the heart muscle, it was initially postulated that Yap/Taz expression would increase. The authors' assumption was supported

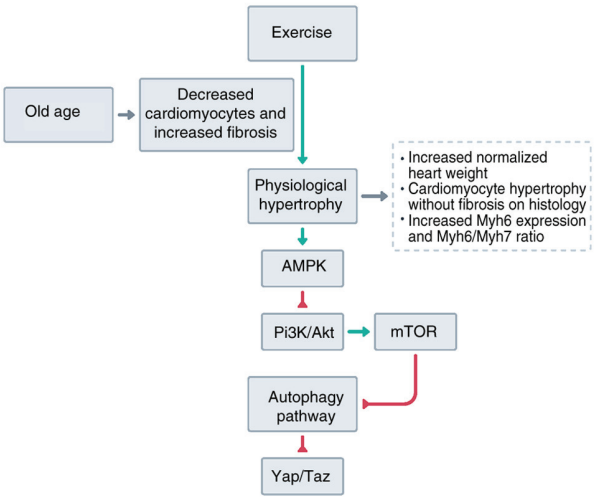

Figure 4. Diagram demonstrating the relationship between hypertrophy and genes measured in the present study. Shown is a diagram depicting the relationship between old age and physical exercise. Old age is known to cause decreased cardiomyocytes and may be reversed with exercise. Exercise was found to induce physiological hypertrophy through activation of the AMPK pathway and inhibition of PI3K/AKT. This inhibition, in turn, inhibits mTOR function, which increases the activity of the autophagy pathway. This increased activity inhibited Yap/Taz function because these proteins are the substrate of autophagy.

by previous studies showing the essential function of Yap in cardiomyocyte function after cardiac injury and embryonic development (56,57). However, the present study identified that their expression is reduced in the exercise group compared with the control. Therefore, it is considered that although the cardiac muscle experienced hypertrophy, it is not due to increased expression of Yap and Taz. Since cardiac muscle hypertrophy involves the enlargement of cardiomyocytes instead of proliferation (41), perhaps the role of Yap and Taz is not that pronounced in cardiac muscle. The current findings are consistent with previous studies that showed Yap is necessary for cardiomyocyte proliferation (25,56,58). Since physical exercise causes hypertrophy through enlargement, the effect of exercise on Yap/Taz is negligible.

Autophagy is a pathway that has been previously researched as a beneficial process in the heart induced by exercise (59-62). However, this function gradually decreases during aging (63), and it was explored whether exercise can still induce autophagy in the aged heart. Therefore, expression of genes involved in autophagy functions such as Lc3 and p62, was determined. The intervention group exhibited an expression pattern consistent with increased autophagy function with high Lc3, markers of autophagosome formation, and low p62, a specific autophagy substrate. However, due to resource constraints, further protein-level analysis which is necessary to confirm the present findings, could not be conducted. The suggested increase in autophagy is supported by previous studies reporting that exercise is one of the main factors inducing autophagy (61,62). Autophagy confers several benefits, such as allowing clearance of damaged mitochondria, which prevents cardiomyocyte injury and apoptosis (61). Interestingly, lower autophagy has been associated with cardiomyocyte hypertrophy. However, this hypertrophy is associated with pathological changes such as lower contractility (64) and increased fibrosis (65). A study by Yan *et al* (66) demonstrated the essential role of autophagy in mediating the beneficial effects of exercise. Without autophagy, exercise caused an increase in fibrosis, impaired mitochondrial biogenesis and fetal gene reprogramming. Therefore, a balanced autophagy level is needed to ensure physiological hypertrophy of the cardiomyocytes. Additionally, the increased autophagy found may explain the lower expression of Yap/Taz in our research. Several studies have found Yap as a substrate for autophagy (21). However, further research is needed to ascertain this conclusion.

The gene expression pattern suggesting increased autophagy activity also occurs with decreased expression of upstream regulators of autophagy, specifically PI3K and mTOR. These findings are counterintuitive since both proteins were usually upregulated during resistance exercises in skeletal muscles, considering their function in activating protein synthesis (67). mTOR is notorious for enhancing protein synthesis and was found to be essential for muscle hypertrophy (68). However, previous research used acute resistance exercise (67,69,70); in the present study, the authors opted for chronic exercise on aged animal models. The current results are supported by studies in rats, which found chronic exercise caused the downregulation of mTOR and PI3K phosphorylation in the brain (71) and the downregulation of mTORC1 activity in the skeletal muscle (70). These findings perhaps reflected the lower anabolic signaling in chronic exercise. Therefore, the main benefit of chronic exercise lies in the ability of autophagy to maintain mitochondrial health and prevent myocardial injury in the heart. This effect is still observed even in aging animal models. Future studies should focus on the expression of protein levels and explore the impact on female rats to account for hormonal differences.

However, several limitations are apparent in the present study. The results are based on rats with significantly different distributions of MHC isoforms, and other mechanisms might exist in humans. It is acknowledged that only the left ventricle was examined in the current study since it plays a critical role in systemic circulation, and its hypertrophic changes are more likely to be reflective of overall heart function in response to exercise. Additionally, specific markers of fibrosis were not investigated using Masson's Trichrome or Picrosirius red staining, which would allow for clearer visualization of collagen fibers and a more accurate assessment of fibrotic changes. Gene expression data were only measured, and thus post-translational modifications may alter the results of the present study. Nevertheless, it can be concluded that even in old age, exercise remains a potent and viable intervention in increasing heart mass and potentially delays the decline in function associated with aging.

In conclusion, the current research has shown that chronic moderate-intensity exercise can induce hypertrophic response in the heart of old rats. This hypertrophic response is consistent with features of physiological hypertrophy with minimal fibrosis and increased α-MHC isoforms and ratio in the intervention group. Additionally, this hypertrophy is not dependent on Yap/Taz expression. Hypertrophy is associated with low anabolic signaling through the PI3KCA and mTOR expression but with gene expression patterns implicating high autophagy function, suggesting that autophagy function may be more critical during regular exercise compared with anabolic signaling.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YL, VV, DKJ, and RL conceptualized the present study. YL, VV, JWG and RL designed the methodology. YL, TL, JWG, KDMW and DKJ performed experiments and statistical analysis. YL, TL, and JWG created the original draft of the manuscript. YL, VV, DKJ, and RL produced the final version of the manuscript. All authors read and approved the final version of the manuscript. YL, JWG and RL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved (approval no. 598/UN6.KEP/EC/2022) by the Research Ethics Committee of Universitas Padjadjaran (Bandung, Indonesia).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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