Irisin and exercise activate renal anti-aging pathways and enhance survival in young mice: a translational insight into muscle-kidney crosstalk

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Abstract

Irisin, a myokine released by skeletal muscle during physical activity, has emerged as a key regulator of energy metabolism, cellular stress responses, and longevity pathways. While previous studies have focused on aged animal-models or pathological states, the long-term impact of early-life interventions on molecular aging pathways remains poorly understood. This study investigated whether early-life irisin administration and physical exercise could modulate the renal-expression of Klotho and HSP70—two hallmark genes of cellular protection and anti-aging in young adult NMRI mice. Animals underwent 8 weeks of resistance training, endurance training, or irisin injection. Plasma irisin was quantified via ELISA, and renal Klotho and HSP70 expression levels were assessed using qPCR and Western-blotting. All interventions significantly increased circulating irisin and upregulated Klotho and HSP70 at both transcriptional and protein levels, with resistance training inducing the most pronounced effects. A 20-month survival analysis showed a trend toward improved longevity in all intervention groups. These findings suggest that early-life exercise and irisin exposure may activate renoprotective and longevity-associated pathways before the onset of molecular aging, supporting their potential as preventive strategies in translational geroscience.

Key Words: aging, exercise, irisin, Klotho, HSP70, preventive intervention, renal gene expression.

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Aging is a progressive and multifactorial process characterized by the gradual deterioration of physiological systems, with the kidney being particularly vulnerable due to its high metabolic demand and pivotal role in systemic homeostasis.[1-3] Central to renal aging is the decline of Klotho, a transmembrane and soluble protein predominantly expressed in the kidney, known for its anti-inflammatory, antioxidative, and metabolic regulatory functions.[4,5] Diminished Klotho levels have been linked to accelerated aging phenotypes and increased risk of chronic kidney disease and other age-related disorders.[6]

Physical activity has emerged as a non-pharmacological intervention capable of enhancing Klotho expression, possibly via muscle-kidney crosstalk mediated by exercise-induced myokines.[7,8] Among these, irisin—secreted by skeletal muscle during physical exertion—has shown promise in modulating metabolic and stress-response pathways, suggesting it may act as an exercise mimetic.[9,10] Another relevant molecular target is Heat Shock Protein 70 (HSP70), a chaperone protein involved in proteostasis and cellular resilience, which, like Klotho, declines with age and contributes to cellular vulnerability.[3,11,12] its potential interplay with Klotho in renal tissue remains an area of active exploration.

Although many studies have investigated these molecules in aged or disease-prone models, less is known about how early-life interventions such as physical training or irisin administration might preemptively activate protective molecular pathways before the onset of age-related dysfunction. Understanding such early modulation is crucial for the development of preventive strategies aimed at extending health span rather than merely treating disease.

In this study, we examined the effects of two exercise modalities—resistance and endurance training—alongside exogenous irisin administration on the renal expression of Klotho and HSP70 in young adult NMRI mice. By evaluating molecular and survival outcomes, we aimed to explore the potential of these interventions to enhance renoprotective and anti-aging mechanisms during early adulthood, thereby offering insight into novel strategies for delaying systemic aging.

Materials and Methods

Materials

Recombinant irisin (Cat. No. 067-29) was purchased from Phoenix Pharmaceuticals (CA, USA). TRIzol reagent, cDNA synthesis kits, and BCA assay reagents were from Thermo Fisher Scientific. SYBR Green PCR Master Mix (BioFACT, Korea) was used for qPCR. Antibodies against Klotho, HSP70, GAPDH, and HRP-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology. Other reagents (e.g., EDTA, Triton X-100) were analytical grade (Sigma-Aldrich).

Animal husbandry and experimental design

Seventy-six adult male NMRI mice (7 weeks old, 18±2 g) were obtained from the Pasteur Institute of Iran and housed under standard conditions (12-h light/dark cycle, 23±1°C, 50±3% humidity) with ad libitum food and water. After a 2-week acclimation, animals were assigned to two cohorts:
Cohort 1 (n=28; 4 groups, n=7): molecular analyses (gene/protein expression, plasma irisin).
Cohort 2 (n=48; 4 groups, n=12): survival analysis over 20 months.
All protocols were approved by the Animal Ethics Committee of Isfahan University of Medical Sciences.[13]

Exercise training and injection protocol

Endurance training involved 8 weeks of treadmill running with progressive intensity (from 5 m/min, 0° incline, 10 min/day to 10 m/min, 5°, 15 min/day). Resistance training consisted of ladder climbing (80° incline) with tail weights progressively increased from 30% to 200% of body weight. Irisin-treated mice received intraperitoneal injections of 100 µg/kg recombinant irisin (in 1% DMSO), three times per week for 8 weeks.[12]

Measurement of plasma irisin concentration

Seventy-two hours' post-intervention, mice were anesthetized with isoflurane (2% in oxygen), and blood was collected via cardiac puncture into EDTA tubes. Samples were centrifuged at 15 min at 4°C, and the resulting plasma was stored at -80°C until analysis. Irisin concentrations were measured using a commercial ELISA kit (Phoenix Pharmaceuticals, CA, USA) according to the manufacturer's instructions.

Sample collection and protein extraction

Seventy-two hours' post-intervention, mice were anesthetized with isoflurane (2% in oxygen), and kidney tissues were excised, snap-frozen in liquid nitrogen, and stored at -80°C. For protein extraction, 50-100 mg of tissue was homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate) supplemented with protease/phosphatase inhibitors (Roche, Germany). Homogenates were centrifuged at 12,000 rpm for 20 min at 4°C, and supernatants were quantified using the BCA assay (Thermo Fisher Scientific, USA).

Real-time PCR for Klotho and HSP70 gene expression quantification

Total RNA was extracted from kidney tissue using TRIzol reagent (Thermo Fisher Scientific, USA), and 1 µg RNA was reverse-transcribed into cDNA (Thermo Fisher Scientific, USA). qPCR was performed on an ABI StepOnePlus system using SYBR Green Master Mix (BioFACT, Korea) with primers for Klotho (F: 5'-CTGCAGTGAAGCAGTGTGGA-3', R: 5'-TGTCGAGCGGAGTTCAGC-3'), HSP70 (F: 5'-GGTGGAATGAGCGGAGTACC-3', R: 5'-CCTGTCCAGTTCGTCACAGT-3'), and GAPDH (F: 5'-CAGAACATCATCCCAGCCTCC-3', R: 5'-TTGGCAGGTTTCTCAAGACGG-3'). The amplification protocol consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 40 s. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the housekeeping gene for normalization.

Western blot analysis of protein expression

Western blot analysis was used to measure the expression levels of the Klotho, HSP70, and GAPDH proteins. Proteins (30 µg) were separated by 12% SDS-PAGE, transferred to nitrocellulose membranes (Bio-Rad, CA, USA), and blocked with 5% non-fat milk in TBST. Membranes were probed with anti-Klotho (1:1000), anti-HSP70 (1:1000), and anti-GAPDH (1:2000) antibodies (Santa Cruz Biotechnology, CA, USA) overnight at 4°C, followed by HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Bands were visualized using an ECL kit (GE Amersham, UK) and quantified with ImageJ software, normalized to GAPDH.

Survival analysis

Following the 8-week interventions, mice from Cohort 2 were observed under standard housing for 20 months. No further treatments were administered. Survival was defined as natural death or humane euthanasia due to severe morbidity. Data were analyzed using the Kaplan-Meier method; differences between groups were assessed using the Log-rank (Mantel-Cox), Gehan–Breslow–Wilcoxon, and trend tests (GraphPad Prism 8.0). Statistical significance was set at $p<0.05$.

Statistical analyses

Group comparisons for molecular data were performed using one-way ANOVA with Tukey's post-hoc test. Pearson correlation was used to examine relationships between

plasma irisin and gene expression. All results are reported as mean±SD or survival curves as appropriate.

Results

Plasma irisin levels

Plasma irisin levels were significantly elevated in all intervention groups compared to the Control group (4.35±0.45 ng/ml, $p<0.0001$). The Endurance Exercise group showed a 46.7% increase, with a mean concentration of 6.38±0.65 ng/ml, while the Resistance Exercise group exhibited the greatest rise (65.5%), reaching 7.42±0.35 ng/ml. The Irisin Injection group displayed a 62.1% increase, with levels at 7.27±0.66 ng/ml. No significant differences were observed among the intervention groups ($p>0.05$). These data are presented in Figure 1, which illustrates the mean plasma irisin concentrations across all groups.

Renal mRNA expression of Klotho and HSP70

Real-time PCR analysis revealed significant upregulation of Klotho and HSP70 mRNA in kidney tissues across all intervention groups compared to Control ($p<0.001$). Klotho mRNA expression increased by 2.12±0.3-fold in the Endurance Exercise group, 3.85±0.5-fold in the Resistance Exercise group, and 3.31±0.4-fold in the Irisin Injection group, relative to Control (1.0±0.1). Compared to Irisin Injection, Resistance Exercise significantly increased Klotho expression ($p<0.05$), while Irisin Injection showed higher expression than Endurance Exercise ($p<0.05$). HSP70 mRNA expression was elevated by 2.53±0.3-fold in Endurance Exercise, 3.49±0.4-fold in Resistance Exercise, and 3.26±0.3-fold in Irisin Injection groups, compared to Control (1.0±0.1). Compared to Irisin Injection, Resistance Exercise showed no significant difference in HSP70 expression ($p>0.05$), while Irisin Injection had significantly higher expression than Endurance Exercise ($p<0.05$). These data are presented in Figure 2 (panels E and F).

Renal protein expression of Klotho and HSP70

To confirm the qPCR results at the protein level, Western blot analysis was performed on kidney tissues, with GAPDH as the loading control. Densitometric quantification (normalized to GAPDH) is shown in Figure 2 (panels B and D). All intervention groups showed higher Klotho and HSP70 protein levels compared to Control ($p<0.001$). Fold changes relative to Control were calculated by dividing the normalized intensity values of each group by the average normalized intensity of the Control group. Klotho protein expression increased by 1.50-fold in the Endurance Exercise group, 3.48-fold in the Resistance Exercise group, and 3.12-fold in the Irisin Injection group, relative to Control. Compared to Irisin Injection, Resistance Exercise showed no significant difference in Klotho expression ($p>0.05$), while Irisin Injection significantly increased expression compared to Endurance Exercise ($p<0.001$). HSP70 protein expression was elevated by 1.30-fold in Endurance Exercise, 2.80-fold in Resistance Exercise, and 2.48-fold in Irisin Injection groups, relative to Control. Compared to Irisin Injection, Resistance Exercise showed no significant difference in HSP70 expression ($p>0.05$), while Irisin Injection significantly increased expression compared to Endurance Exercise ($p<0.001$).

Statistical analysis of correlations

Correlation analyses between plasma irisin levels (ng/ml) and relative mRNA expression of Klotho and HSP70 (measured by Real-Time PCR and normalized to GAPDH) are presented in Figure 3. Plasma irisin levels showed a strong positive correlation with Klotho expression ($R=0.9498$, $p<0.0001$) and a moderate positive correlation with HSP70 expression ($R=0.7511$, $p<0.003$), as determined by a two-tailed Pearson correlation test.

Survival analysis

A 20-month Kaplan–Meier survival analysis was conducted to evaluate the long-term effects of resistance training, endurance training, and irisin administration on mortality in NMRI mice (Figure 4). Over the follow-up period, the Control group exhibited the highest number of deaths (n=8), whereas the Resistance Exercise group showed the lowest mortality (n=3). The Endurance Exercise and Irisin Injection groups experienced 5 and 4 deaths, respectively. Median survival time was 480 days in the Control group, while it was not reached in any of the intervention groups.

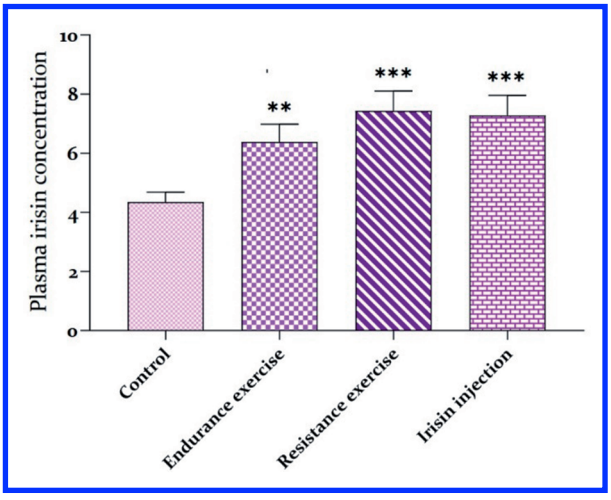

Figure 1. *Plasma Irisin Levels in NMRI Mice in Response to Irisin Injection and Physical Exercise. The control, resistance, endurance, and irisin injection groups' plasma irisin concentrations were measured 72 hours after the last intervention. When compared to the control group, both types of exercise and irisin injection significantly increased plasma irisin levels, with resistance exercise exhibiting the highest rise. The data is displayed as mean±SD. Significant values were defined as* ***p≤*0.001*, **p≤*0.01*.

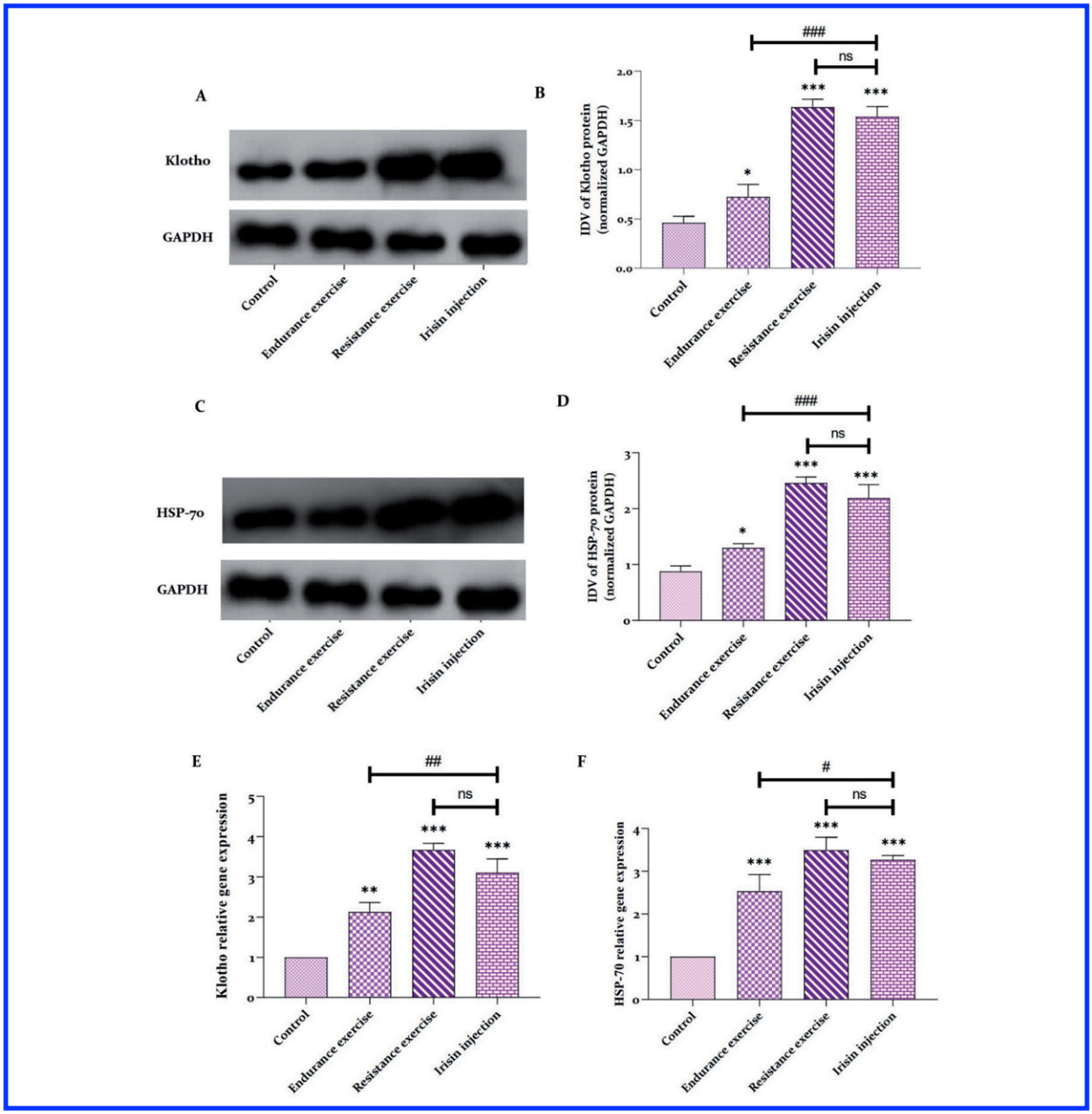

Figure 2. *Effect of Exercise and Irisin Injection on Klotho and HSP70 Expression in Kidney Tissue of NMRI Mice. (A, B) Western blot (A) and densitometric quantification (B) of Klotho protein levels (~62 kDa), normalized to GAPDH (~37 kDa) (shown as normalized intensity values). (C, D) Western blot (C) and densitometric quantification (D) of HSP70 protein levels (~70 kDa), normalized to GAPDH (~37 kDa) (shown as normalized intensity values). All intervention groups showed significantly higher expression compared to Control (*$p<0.001$). Compared to Irisin Injection, Resistance Exercise showed no significant difference in Klotho or HSP70 expression (ns, $p>0.05$), while Irisin Injection significantly increased both Klotho and HSP70 expression compared to Endurance Exercise ($p<0.001$). (E, F) Relative mRNA expression of Klotho (E) and HSP70 (F) in kidney tissue, measured by real-time PCR and normalized to GAPDH. All intervention groups showed significantly higher expression compared to Control ($p<0.001$). Compared to Irisin Injection, Resistance Exercise significantly increased Klotho expression (*$p<0.05$), while Irisin Injection showed higher expression than Endurance Exercise $p<0.01$). For HSP70, Irisin Injection had significantly higher expression than Endurance Exercise (*$p<0.05$), but no significant difference was observed compared to Resistance Exercise (ns, $p>0.05$). qPCR data (E, F) are presented as mean±SD. Statistical significance is denoted as follows: ***$p<0.001$, **$p<0.01$, $p<0.05$ compared to Control; #$p<0.05$ for comparisons between Irisin Injection and each exercise group; ns indicates no significant difference ($p>0.05$).*

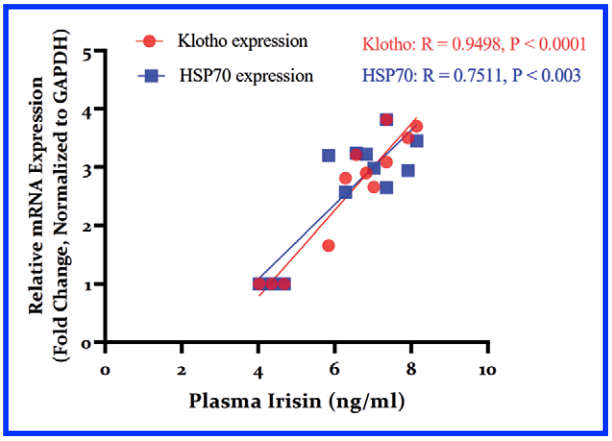

Figure 3. *Correlation Analysis between Plasma Irisin Levels and Gene Expression of Klotho and HSP70 in Kidney Tissue of NMRI Mice. Scatter plot showing the relationship between plasma irisin levels (ng/ml, x-axis) and relative mRNA expression (fold change, normalized to GAPDH, measured by Real-Time PCR, y-axis) of Klotho (red circles) and HSP70 (blue squares). A strong positive correlation was observed between plasma irisin levels and Klotho expression (R=0.9498,* p*<0.0001), while a moderate positive correlation was found for HSP70 expression (R=0.7511,* p*<0.003), as determined by a two-tailed Pearson correlation test.*

Although the Log-rank (Mantel–Cox) test did not reveal a statistically significant difference among groups (χ^2=6.425, df=3, p=0.0927), the Log-rank test for trend indicated a significant linear trend toward improved survival across the intervention groups (χ^2=4.125, p=0.0423). This suggests that early-life interventions, particularly resistance training and irisin administration, may contribute to enhanced long-term survival.

These findings, although preliminary and derived from healthy young mice, support the hypothesis that short-term early interventions can exert lasting effects on physiological resilience. However, further studies using aged or disease-prone models and extended follow-up are needed to validate these effects and determine their relevance to aging-related mortality.

Discussion

This study investigated the effects of resistance training, endurance training, and exogenous irisin administration on plasma irisin levels, the expression of Klotho and HSP70 (at both mRNA and protein levels) in renal tissue, and long-term survival in NMRI mice. While the study employed young mice as a preventive model to simulate early-life interventions, our findings demonstrate that all interventions significantly increased plasma irisin concentrations, upregulated Klotho and HSP70 expression, and improved survival rates compared to the control group,

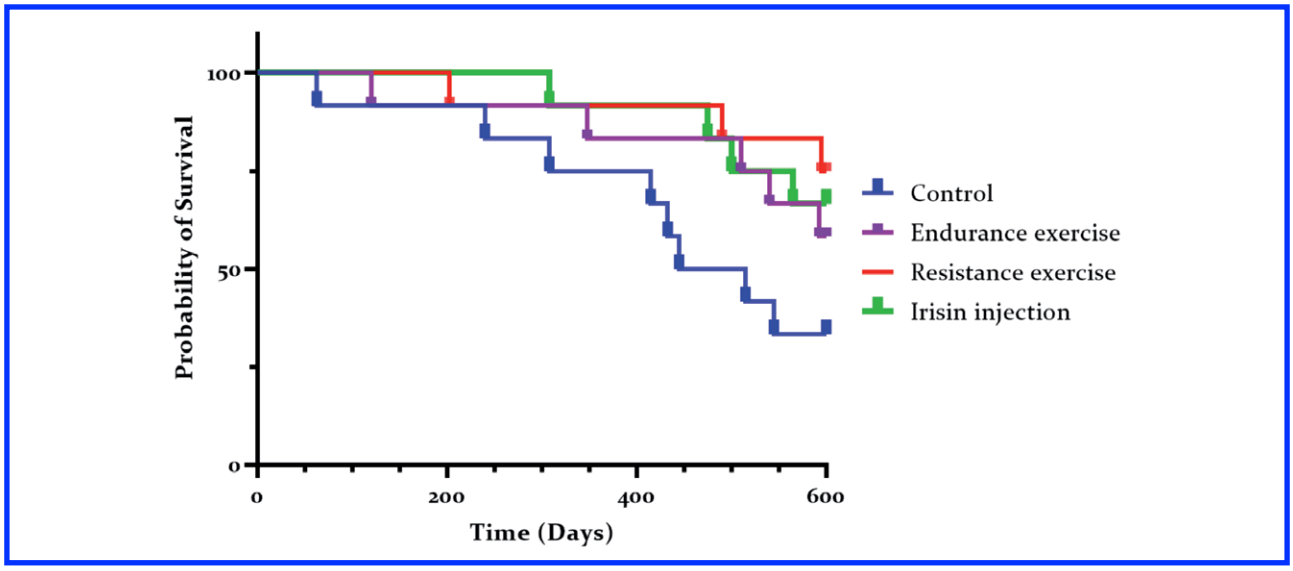

Figure 4. *Kaplan–Meier survival curves for Control, Endurance Exercise, Resistance Exercise, and Irisin Injection groups over a 20-month period. Male NMRI mice (n=12 per group) were monitored daily following an 8-week intervention. The Control group (blue) showed the highest mortality (8 deaths), while the Resistance Exercise group (red) had the lowest (3 deaths). The Irisin Injection (green) and Endurance Exercise (purple) groups exhibited 4 and 5 deaths, respectively. The median survival time was reached only in the Control group (480 days), whereas it remained undefined in all intervention groups. Statistical analysis using the Log-rank (Mantel–Cox) test did not reveal significant differences among the groups (*p*=0.0927), but the Log-rank test for trend indicated a significant survival trend favoring the intervention groups (*p*=0.0423). These results suggest a potential long-term benefit of early-life physical interventions and irisin administration on survival.*

highlighting their potential to enhance cellular resilience and systemic health. These results position irisin as a promising exercise-mimetic agent, particularly for individuals with limited mobility such as the elderly, post-surgical patients, or those with severe neuromuscular or orthopedic impairments.

Physical exercise is widely recognized as a potent intervention for promoting health and mitigating cellular stress, largely through the upregulation of protective factors like Klotho and HSP70, which are implicated in cellular resilience and longevity.[14] Previous studies have consistently shown that exercise enhances the expression of these genes across various models. For instance, Mostafidi *et al.* reported that regular aerobic exercise increased plasma Klotho levels via PGC-1α activation in humans,[15] while Ji *et al*. found that both intermittent and continuous aerobic exercise in Sprague Dawley rats significantly elevated Klotho mRNA and protein levels, reduced Reactive Oxygen Species (ROS), and improved survival, suggesting a role for Klotho in exercise-mediated longevity.[16] More recently, a 2024 study demonstrated that 8 weeks of endurance and resistance exercise increased serum FGF23 and soluble Klotho levels, improving glucose metabolism in type 2 diabetic women.[17] Additionally, Shamsi *et al.* reported that resistance training in rats enhanced HSP70 expression in skeletal muscle, supporting its role in cellular protection and stress response.[18]

Our results align with these findings, as both resistance and endurance training significantly upregulated Klotho and HSP70 expression in the renal tissue of NMRI mice (Figure 2). Resistance training exerted the most pronounced effect, increasing Klotho mRNA by 3.85-fold and HSP70 mRNA by 3.49-fold ($p<0.05$), compared to 2.12-fold and 2.53-fold increases, respectively, with endurance training ($p<0.05$). At the protein level, resistance training led to a 3.48-fold increase in Klotho and a 2.80-fold increase in HSP70, while endurance training induced more modest elevations (1.50-fold for Klotho and 1.30-fold for HSP70). This disparity likely stems from the broader mechanical and metabolic stimuli induced by resistance training, which activate a more diverse range of protective signaling pathways compared to endurance training, which is more associated with metabolic health and inflammation reduction.[19] This distinction suggests that the type and intensity of physical activity may differentially influence anti-aging gene expression, with implications for designing targeted exercise interventions to support renal function and systemic health in populations at risk for kidney dysfunction.

A key finding of this study is the ability of exogenous irisin to replicate many of the beneficial effects of exercise, supporting its potential as a therapeutic alternative for individuals unable to engage in physical activity. Irisin injection increased plasma irisin levels by 62.1% (7.27±0.66 ng/mL), closely mirroring the 65.5% increase observed with resistance training (7.42±0.35 ng/mL) and surpassing the 46.7% increase with endurance training (6.38±0.65 ng/mL) (Figure 1). Irisin injection also significantly upregulated Klotho and HSP70 expression at both mRNA and protein levels, with effects comparable to those of resistance and endurance training (Figure 2). These findings are consistent with prior studies reporting that irisin, a myokine released during exercise, can mimic exercise-induced benefits by enhancing metabolism and upregulating protective gene expression.[12,20,21] The mechanism underlying irisin's effects likely involves the activation of PGC-1α and FNDC5 in skeletal muscle, leading to irisin production, which subsequently engages downstream pathways such as MAPK and PI3K/Akt in target tissues like the kidney.[22,25] These pathways may enhance mitochondrial function, reduce oxidative damage, and upregulate longevity-related genes, thereby protecting against renal stress and injury—a critical consideration given the kidney's vulnerability to age-related decline. For instance, a recent study demonstrated that irisin administration in a mouse model of renal ischemia-reperfusion injury reduced levels of renal injury markers (e.g., serum creatinine, BUN, Kim-1, and NGAL) and preserved mitochondrial integrity by upregulating autophagy-related proteins (PINK1, PARK2, LC3) while downregulating mitochondrial membrane proteins (TOM20, TIM23), highlighting its renoprotective potential.[26]

Correlation analyses further elucidate irisin's regulatory role, revealing a strong positive correlation between plasma irisin levels and Klotho mRNA expression ($R=0.9498$, $p<0.0001$), suggesting that irisin may directly enhance Klotho expression, a protein with well-documented anti-aging and renoprotective properties (Figure 2). A more moderate but significant correlation was observed with HSP70 expression ($R=0.7511$, $p<0.003$), indicating that irisin also influences stress-protective pathways, albeit to a lesser extent (Figure 2). The coordinated upregulation of Klotho and HSP70 suggests a potential synergistic effect in mitigating oxidative stress and inflammation, key drivers of renal injury and aging. This is supported by Sugiura *et al.*, who demonstrated that Klotho gene therapy in a mouse model of renal ischemia-reperfusion injury reduced apoptosis by upregulating HSP70, highlighting Klotho's role in oxidative stress mitigation.[27] Additionally, a recent study found a positive correlation between hippocampal irisin levels and HSP70 expression in rats with high-fat, high-fructose diet-induced cognitive dysfunction, suggesting that irisin contributes to stress response pathways across different tissues.[28]

Although our primary focus was on the molecular modulation of anti-aging pathways, we also conducted an exploratory long-term survival analysis to evaluate whether early-life interventions could influence longevity trajectories. Despite the lack of statistically significant differences among groups based on the Log-rank (Mantel-Cox) test ($p=0.093$), a significant linear trend was detected using the Log-rank test for trend ($p=0.042$), indicating a potential dose–response relationship between intervention intensity and survival benefit.

By the end of the 20-month follow-up, the control group exhibited the highest mortality (8/12), while the resistance training group showed the lowest (3/12), followed by irisin injection (4/12) and endurance training (5/12). Notably, the median survival time was reached only in the control group (480 days), whereas it remained undefined

in all intervention groups—suggesting prolonged survival. These findings align with growing evidence that early-life molecular enhancement—particularly via Klotho and HSP70—may contribute to long-term physiological resilience. For instance, Feng *et al.* reported that three months of swimming exercise in young C57BL/6J mice significantly extended health span, though not lifespan, supporting the concept that early interventions confer persistent systemic benefits.[29]

Our data complement this concept by showing a modest, yet consistent trend toward improved survival following early resistance training or irisin administration. Moreover, our results resonate with studies demonstrating that increased Klotho expression extends lifespan and attenuates age-related tissue degeneration, and that preconditioning exercise protocols can reduce mortality in disease models by modulating inflammation and organ integrity.[6,30-33]

It is worth emphasizing that our survival data were obtained in young, healthy animals without disease burden, and without repeated intervention post-treatment. Therefore, the observed differences in mortality likely reflect the long-term programming effects of transient early-life interventions, rather than continuous stimulation.

Taken together, this study supports the hypothesis that brief, early-life exercise or irisin treatment may enhance longevity potential by initiating protective molecular programs, particularly in tissues like the kidney, where Klotho expression is pivotal. These preliminary findings warrant further investigation in aged or disease-prone models and raise the prospect of developing early-life or one-time exercise-mimetic interventions to promote healthy aging trajectories.

Conclusions

This study provides compelling evidence that both physical exercise, particularly resistance training and exogenous irisin administration can significantly upregulate the renal expression of Klotho and HSP70, two critical regulators of cellular stress resistance and longevity. Our findings demonstrate that early-life interventions not only induce favorable molecular adaptations but may also confer long-term survival benefits, even in the absence of continuous stimulation. Notably, irisin mimicked many of the beneficial effects of exercise, supporting its potential as a promising exercise-mimetic candidate for populations unable to engage in physical activity.

Although the survival analysis did not yield statistically significant differences across all tests, a meaningful trend toward improved survival in the intervention groups was observed, reinforcing the hypothesis that early-life molecular enhancement may promote physiological resilience during aging. These findings highlight the translational potential of muscle-derived signaling molecules like irisin in targeting systemic aging pathways.

Future studies using aged or disease-prone models, as well as investigations into tissue-specific effects beyond the kidney, will be essential to fully elucidate the therapeutic potential of irisin and refine strategies for delaying age-related decline. Overall, our work adds to the growing body of evidence that skeletal muscle exerts far-reaching endocrine effects with implications for aging, renal health, and systemic homeostasis.

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Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

Contributions

ZSA, conceptualization, methodology, investigation, data curation, writing – original draft; MH, methodology, investigation, formal analysis; HR, validation, resources, writing – review & editing; SZ, conceptualization, methodology, supervision, project administration, funding acquisition. All authors read and approved the final manuscript.

Ethics approval

All experimental procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, USA) and were approved by the Institutional Animal Care and Use Committee (IACUC) of by Isfahan University of Medical Science (Approval Code: IR.MUI.MED.REC.1401.024). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Informed consent

Not applicable.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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