Combination Exercise Regimen and Resveratrol Intake Can Be Considered For Cardiac Rejuvenation Therapy in the Aging Process

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ABSTRACT
Aging is an inevitable process of life. Become progressively disorganized and degraded with age occurring as a consequence of physiological aging. This process has many different exercise regime or supplementary resveratrol intake molecular biology mechanisms to regulate different aging grade. Indeed, their combination in the molecular biology interaction is our intriguing in this study. We purchased natural aging mice and gene type senescence-accelerated SAMP8 mice to examine different molecular biology interaction of survival and apoptosis signaling after exercise training, supplementary resveratrol intake, and combination. Histological pathophysiology of age-related cardiac disease was examined using hematoxylin-eosin and Masson's trichrome staining. Apoptosis cells were determined using TUNEL staining. Results showed PGC-1α and SIRT-1 increased were observed in natural aging after exercise training and supplementary resveratrol intake (p<0.05). In the genetic type of genetic senescence-accelerated mice SAMP8, left ventricular weight observed decreased after exercise training, supplementary resveratrol intake, and their combination (p<0.05). However, TUNEL analysis results showed combination exercise training and supplementary resveratrol intake apoptosis cells has significant decreases (p<0.05). We suggest resveratrol intake can help exercise training therapy age-related cardiac disease.

Keywords
Exercise training, Resveratrol intake, Aging, SAMP8 mice, TUNEL analysis.

Introduction
The process of aging refers to divisions into the young old (65-74 years old), the middle old (75-84 years old), and the oldest old (85+ years old). Distinctions may be made between “physiological aging” and “pathological aging”. The most well recognized risk factor for many chronic diseases in physiological aging [1,2]. Interactions between the aging process and aged-related disease has not been seriously addressed or systematically explained. Aging is an inevitable process of life [3]. Because there is a decline in food intake will make the lifespan shorter. This physiologic change in aging will take place older man at risk of developing pathological weight loss [4], when they develop diseases states [5,6]. This phenomena has been described as the physiological anorexia of aging and may be due to altered hedonic qualities of food, early satiation because of changes in adaptive relaxation and an excess satiating effect of cholecystokinin [7]. Although aging is inevitable, with advancing age, even in healthy adults, eventually, the perform certain physical task capacity reduced results in increased incidence of function disability [8]. Physiological aging changes are not caused by disease or environmental influences [9]. Both the incidence and the severity of atherosclerosis and cardiovascular disease increase with age. These changes to the heart throughout life are the result of maturational changes beyond sexual maturity, which cause hypertrophy of myocytes and hyperplasia of capillary endothelial cells and interstitial fibroblasts [10]. Age-related cardiac disease is associated with numerous molecular and biochemical changes in the heart. These changes affect protein function and cardiac morphology resulting in alterations in cell death signaling [11]. Old age is a strong independent predictor of death and morbidity in patients
with structural heart disease. Therefore, old age is a major risk factor that is associated with poor cardiovascular outcome and that reduces endogenous cardioprotection [12]. Both the incidence and the severity of atherosclerosis and cardiovascular disease increase with age. Exercise training, an active lifestyle, is a useful in the prevention of several chronic diseases such as atherosclerosis and cardiovascular disease. Exercise training functionally is responsible for the optimizes of \( O_2 \) transport and utilization of \( O_2 \) for the eventual synthesis of ATP [13]. Various nutritional, resveratrol, is being investigated to mimic the beneficial effects of exercise training on cardiac system to maintain \( O_2 \) transport. Resveratrol is a polyphenol produced by plants and is widely considered to adaption in \( O_2 \) transport unit system [14]. However, the effects of combining resveratrol and exercise is still unclear, especially in different age. This study will compare the effects of exercise training and resveratrol on the coordinative adaptation of cardiac mitochondrial biogenesis.

**Methods**

**Animals**

Rats were purchased from the National Science Council Animal Center, Taipei, Taiwan fed to 18-months old. The animals were housed in individual cages in an environmentally controlled temperature and humidity. Tap water was free provided and standard chow diet. Gene type senescence accelerated mice, SAMP8 mice, also from the National Science Council Animal Center, Taipei, Taiwan fed to 6-months old. All experiments animals were handled in accordance to the guide for the Taiwan Society for Laboratory Animals Sciences for the care.

**Experiment designs**

An 18-month-old as an old group of mice and SAM aging promoted aged, 6 months, mice treated exercise training and resveratrol administration to anti-aging. Exercise training program is place each rat on one lane of the stationary treadmill turn the treadmill on to a slow walking speed, 6 m/min train all rats to walk on the treadmill for 5-10 min/day for 3 consecutive days one week during this to ensure that potential effects observed from the forced exercise regimen for 4 weeks. Mice were administered resveratrol (20 mg/kg) via daily oral gavage and continuing for a period of 4 weeks. Combined exercise with resveratrol carried out this experimental, resveratrol intake was the period of four weeks during habitual treadmill running. All animals were sacrificed after last exercise session.

**Histological analysis by and Masson’s trichrome staining**

Cross sections of cardiac tissue were cut at a thickness of 10 µm and placed on slides. For assessment of left ventricle cross-sectional collagen area and extracellular space, cross sections were stained with masson’s trichrome staining. Slides deparaffin embedded in xylene for 10 minutes, and then hydrated by placing in 80%, 70%, 65% ethanol for 5 minutes each. In order to detect collagen accumulation of cardiac tissue cross sections were stained with masson’s trichrome staining. Stained sections were then rinsed with PBS and air dried before mounting. After gently rinsing with water, the slides were rehydrated through a graded alcohol series for 15 min, cleaned in xylene.

**Western blotting**

Proteins were separated by 10.5~12.5% SDS polyacrylamide gel electrophoresis at 100 V for 1 hr. Electrophoresis proteins were transferred to PVDF membrane using a Bio-Rad Mini Trans-Blot Electrophoretic Transfer Cell Instruments at 150 mA for 2 hr. Membranes were blocked in 1~5% non-fat milk for 1 hr at room temperature. After washes, probed with PGC-1α, SIRT-1 and tubulin antibodies (Santa Cruz Lab) for 2 hr. After washes, membranes were incubated with enzyme conjugated anti-rabbit, or anti-goat, or anti-mouse IgG horseradish peroxidase for 1 hr at room temperature. The membranes were then washed in blotting buffer for 10 min three times. Color development was performed by ECL chemiluminescence.

**Terminal Nucleotidyl transferase-mediated dUTP nick-end-labeling (TUNEL) and DAPI stained**

Cardiac tissue sections of young and old were dewaxed in xylene, and then rehydrated by hydrate by placing in 95%, 70%, 50%, 30% ethanol for 10 minutes each and preincubated with proteinase k (20 ug/ml in 10 Mm Tris-HCl (pH 7.6) at room temperature. cardiac tissue sections were rinsed to TUNEL mixture, at 37°C, one hour in a humidified chamber. After rinses, sections were stained DAPI, and then slides were mounted with a propidium iodide solution and analyzed under fluorescent microscope.

**Statistical analysis**

Data were collected and analyzed using SigmaStat software. All data are expressed as the mean ± standard error of the mean (SEM). Comparison between groups was conducted using a two-way analysis of variance (ANOVA). \( p \) values of less than 0.05 and 0.01 were considered to be statistically significant and highly statistically significant.

**Results**

**Exercise training and resveratrol reduced cardiac fibrosis**

Physicochemical changes in the chemical and thermic contraction have been demonstrated in collagen fibers of different ages. Moreover, biochemical changes in the tissues such as a decrease in the content of extractable collagen show a relationship with increasing age, and the total collagen content in certain tissues has been found to increase with age. From Masson’s trichrome staining, exercise training, resveratrol intake, and combining resveratrol with exercise training aging heart tissue observed collagen accumulation decreases. Figure 1 result showed blue color collagen expression levels gradually decreased in the exercise training, resveratrol intake, and combining resveratrol with exercise training aging heart. Senescent cardiac fibrosis is the consequences of the disruption of the equilibrium between the synthesis and degradation of collagen molecules. Myocardial fibrosis is results in an excessive accumulation of collagen fibres. Exercise training, resveratrol intake, and combining resveratrol with exercise training reverses aging situation.
PGC-1 and SIRT-1 play an important role in mitochondria apoptosis in exercise training, resveratrol intake, and combining resveratrol with exercise training aging heart tissue

Aging metabolic and related cardiovascular disease reduce mitochondrial function in cardiac tissue, thereby reducing exercise capacity. Indeed, increased PGC1α is observed in exercise trained aging heart. Resveratrol has been reported to activate PGC1α through its ability to activate pathways in aging heart (P<0.05) (Figure 2). The dependence of resveratrol on SIRT1 to stimulate PGC1 demonstrated in SIRT1 deficient mice. SIRT1 may be involved in PGC1α stimulation demonstrate that resveratrol and exercise training do act through similar PGC1α-dependent mechanisms to stimulate mitochondrial biogenesis. Resveratrol is a compound SIRT1 activation by activating PGC1α, thereby affecting the number with apoptosis (P<0.05) (Figure 2). In addition to the potential of a direct SIRT1-PGC1α signaling pathway as a mediator of the beneficial effects of combining resveratrol and exercise training in aging heart tissue. Combining resveratrol and exercise training in aging heart may not be involved in direct SIRT1-PGC1α signaling pathway was found in Figure 2.

Figure 1: Histological changes of cardiac tissue sections in exercise training, resveratrol intake, and combining resveratrol with exercise training aging heart tissue by Masson’s trichrome staining. Blue color representation collagen accumulation. Scale bar= 100 um.

Figure 2: Mitochondria proteins expression levels of PGC-1 and SIRT-1 in mitochondria apoptosis in exercise training, resveratrol intake, and combining resveratrol with exercise training aging heart tissue by western blotting analysis. (A). Proteins expression levels of PGC-1 and SIRT-1 by western blotting analysis. (B). Values shown are means ± SEM. *Significant difference, P<0.05.

Figure 3: Various organs weights in exercise training, resveratrol intake, and combining resveratrol with exercise training in gene type senescence accelerated mouse (SAMP8) heart. Heart, spleen, lung, kidney, muscle, left ventricle weights. Values shown are means ± SEM. *Significant difference, P<0.05.

Cells apoptosis of gene type senescence accelerated mouse (SAMP8) in exercise training, resveratrol intake, and combining resveratrol with exercise training in aging heart

Programmed cell death is a recognized mechanism for the elimination of redundant cells in the pathogenesis of human cardiac disorders in the elderly. We compare the effects of exercise training and resveratrol on the coordinative adaptation of the organs that comprise the gene type senescence accelerated mouse (SAMP8). According to Figure 3 result showed left ventricular weight has significantly difference in exercise training, resveratrol intake, and combining resveratrol with exercise training in aging heart (P<0.05). Aging cardiac has no significant different change in exercise training, resveratrol intake, and combining resveratrol with exercise training. Apoptosis cells observed in aging heart. However, gradually decreased in exercise training, resveratrol intake, and combining resveratrol with exercise training using TUNEL staining (Figure 4). The fact that apoptosis is strictly associated with this process including eliminating redundant, damaged and infected cells is generally accepted.
Discussion
Aging is considered as a major risk factor cardiovascular diseases. Various age-associated changes in the cardiovascular system may lead to pathological outcomes including cardiomyocyte death by alterations in structure and function of the heart and vasculature that will ultimately affect cardiovascular performance [14]. Cardiac remodeling during aging includes cardiomyocyte loss, reactive hypertrophy of the remaining cells and increased interstitial tissues [15]. These changes may result in a decline in the biological and physiological functions of the heart (Figure 1).

Fibrosis occurs in most injuries and results from changes in the balance between synthesis and degradation of extracellular matrix components. In addition, resveratrol has additive effects on the heart when administered in combination with exercise training. The biochemical changes also affect the expression levels of mitochondrial membrane anti-apoptosis and apoptosis proteins [16,17]. Human cardiac aging generates a complex phenotype. Experimental evidence in animal models has indicated attenuation of cardioprotective pathways with aging, yet information regarding myocardial dysfunction in old age is limited. Similar data are available regarding age-related changes in the human heart [18-20]. Resveratrol also dose-dependently increases SIRT1/PGC1α levels (Figure 2). However, resveratrol and exercise training may also regulate separate cellular pathways since we have recently demonstrated in two separate of natural and gene type mice models that resveratrol and exercise training has effects on the heart. Interestingly, resveratrol improved exercise capacity in natural and gene type SAMP8 mice with underlying chronic health conditions, resveratrol also enhanced endurance and strength in healthy, especially left ventricular hypertrophy (Figures 3 and 4).

Similarly, resveratrol improved aerobic endurance and muscle strength that were selectively bred for high capacity running. Aging is associated with impaired vascular function [21]. Exercise training counteracts the detrimental effects of aging on vascular function [22]. Evidence for cardiovascular health effects of resveratrol in aged humans is lacking. Resveratrol can hamper training induced adaptations in the cardiovascular system [23]. Physical activity should remain to be promoted for cardiovascular system [23]. Aging is associated with impaired vascular function [21]. Exercise training counteracts the detrimental effects of aging on vascular function [22]. Evidence for cardiovascular health effects of resveratrol in aged humans is lacking. Resveratrol can hamper training induced adaptations in the cardiovascular system [23]. Physical activity should remain to be promoted for cardiovascular system [23].

Conclusions
Therefore, the mechanisms responsible for the effects of resveratrol on strength and endurance parallel the adaptations observed in response to exercise training, suggesting that common pathways are activated by exercise as well as resveratrol.