

The Interactive Effects of Swimming High Intensity Interval Training and Resveratrol Supplementation on E1 α -Subunit Pyruvate Dehydrogenase Enzyme in the Hippocampus of Aged Rats

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Abstract

Introduction: Pyruvate Dehydrogenase E1 enzyme is a heterotetrameric α -ketoacid decarboxylase that oxidizes Pyruvate to Acetyl-CoA. Increasing in the age results in phosphorylation of E1 α -subunit Pyruvate Dehydrogenase Enzyme (PDH-E1 α) in mitochondria. Furthermore, PDC activity in brain is related to PDH-E1 α expression. Evidence shows that specific structure of hippocampus makes great responses to exercise. Prior studies have reported biological activities of resveratrol (RSV) such as antioxidant, anti-inflammatory and neural protection activities. Therefore, the purpose of this study was to investigate the interactive effects of swimming high intensity interval training (HIIT) and resveratrol supplementation on PDH-E1 α in hippocampus of aged rats.

Methods: 45 male Wistar rats with an age of 20 months were randomly divided into five groups (control (C), Swimming HIIT (S-HIIT), Swimming HIIT with resveratrol supplementation (S-HIIT-R), resveratrol supplementation (R) and solvent of resveratrol supplementation (SR). Swimming HIIT and resveratrol supplementation groups performed the exercise and received resveratrol(10 mg/kg/day, gavage) for six weeks. All rats were sacrificed and the brain was extracted immediately and the hippocampus was separated. Shapiro-Wilk test was used to normalize the research data. Statistical analysis was performed using one-way analysis of variance (ANOVA) and repeated measures with Tukey's post hoc test at a significant level of 0.05. The hypothesis test was performed using SPSS software version 20.

Results: Western Blot method showed that Swimming HIIT with resveratrol supplementation and resveratrol alone increased PDH-E1 α level significantly (P=0.007 and P=0.047, respectively). Furthermore, Swimming HIIT increased PDH-E1 α level but not significantly (P=0.383).

Conclusion: It appears that resveratrol alone and with exercise can influence levels of hippocampus mitochondrial metabolism key proteins such as PDH-E1 α in aged rats and regulated PDC activity, TCA cycle and hippocampal cell metabolism.

Keywords: Aging, Training, Resveratrol, PDH-E1 α , Hippocampus

Introduction

Age increasing is the main risk factor in progression of chronic diseases, and is accompanied with changes in metabolic processes and mitochondrial dysfunction (1). Different studies about aging process reveal some of the cellular indicators of this process

such as proteostasis decline, mitochondrial dysfunction, cellular senescence and stem cells exhaustion. It seems that mitochondrial disorder is a common factor which links these various indicators to each other, directs the aging process, and progressively affects the

tissues all over the body (2). Brain cells (neuron and glial) are quite sensitive to mitochondrial dysfunction which is caused by high demand of Adenosine triphosphate (ATP) (3). Mitochondrial dysfunction has been attributed to various aspects of aging process such as decreased activity of metabolic enzymes (4). Pyruvate Dehydrogenase Complex (PDC) is a mitochondrial enzyme which has an important role in aerobic metabolisms (5) through reversible oxidation catalyst of pyruvate derived from glucose to Acetyl-coA. It possesses several copies of enzymes E1, E2 and E3 which are structurally different but functionally dependent on each other. Pyruvate dehydrogenase E1 enzyme is a heterotetrameric ($\alpha_2\beta_2$) of α -keto acid decarboxylase which oxidizes pyruvate into Acetyl-coA in the presence of thiamine pyrophosphate (as the co-factor) (6). Pyruvate dehydrogenase (PDH) is the key mitochondrial enzyme that bonds glycolysis to the tricarboxylic acid cycle (TCA cycle). PDH activity is regulated through reversible phosphorylation of serine residues (S232, S293, S300) in E1 α subunit of PDH enzyme by pyruvate dehydrogenase kinase (PDK1-4), which decreases the use of pyruvate in TCA cycle. Dephosphorylation of these sites by pyruvate dehydrogenase phosphatase (PDP1-2) stimulates PDH activity (7). It has been revealed that age increasing leads to phosphorylation of E1 α (catalytic subunit of E1) PDH enzyme and PDH inhibition (8) which consequently restrains Acetyl-coA production of pyruvate in mitochondria (9). In case of dephosphorylation of this subunit and the activation of PDH, pyruvate in mitochondria will change in to Acetyl-coA by this enzyme (10). After entering the Krebs cycle, it is used as the ATP substrate synthesis. In fact the brain neurons use glucose and astroglial cells lactate to maintain their functions. Both of the energy substrates produce pyruvate; therefore, neurons need functional PDC to assist oxidative phosphorylation. Consequently, PDC activity

is related to expression of E1 α subunit of this enzyme in brain (6). Any strategy which avoids the problems caused by lack of brain access to ATP production substrates can be of benefit to brain function, especially Hippocampus in aging process. There is evidence showing that age-related decline of energy metabolism (use of glucose) in rat's brain cell is related to decline of learning and memory function in Hippocampus and Prefrontal cortex (11). Moreover, the results of studies have indicated that in aging process the amount of ATP in the white matter of rat brain has decreased, and it is correlated with mitochondrial structural changes (12). Evidence shows that brain is a limb with high level of compatibility in morphological, metabolic and functional responses to exercise (13). Specifically, it has been proved that Hippocampus has a structure that responds the most to exercise. This has been verified with numerous animal and human studies (14). On the other hand, among vegetative and natural influences on mitochondrial functions, polyphenols and flavonoids are remarkably influential (15). Resveratrol is a polyphenolic composition which is found in grape and different types of berries (16). Previous studies have shown a wide variety of biological activities of Resveratrol including antioxidant, anti-apoptotic, anti-inflammatory and neural protection (16). Resveratrol can also regulate mitochondria's function (17). As a result, Resveratrol supports the survival of cell's mitochondria by activating various mechanisms. In addition, intensive training affects mitochondria's activity. This activities as well as mitochondrial enzymes increase the continuity of energy production. On the other hand, the studies conducted till today, have mainly investigated the amount of this protein in muscle cells (18, 19). There are also studies that have worked on the impact of high intensity training and Resveratrol (20, 21). Another study has found out that after high intensity training for 8 weeks the content of PDH- E1 α protein of skeletal muscle in trained

healthy old men was significantly more than that of untrained ones (18). Many research studies have investigated the anti-aging and antioxidant effects of resveratrol in morphology (22) and molecular and cognitive (23, 24) level of Hippocampus. In another study, swimming has been proven that functions better than groups of exercises related to running in terms of Hippocampus-related behaviors such as spatial memory and retention in aged rats (18 months old) (25). Other groups of studies have shown that the efficiency of trainings caused by (intensive resistance training) in the older adults decreases compared to the younger adults (26). A gap here in this filed is that whether any of the exercise intervention or Resveratrol – alone– or both can compensate for the decline of PDH-E1 α protein amount caused by age increasing in brain or not. Due to the significance of PDH-E1 α protein related to the mitochondrial metabolism of brain cells through age increasing, finding a strategy for compensating the decline of PDH- E1 α accompanied by aging process and age-related diseases as well as regarding the inadequate research studies in this field, this research program attempts to answer this question: Can swimming high intensity interval training and Resveratrol supplementation affect the amount of this protein in Hippocampus of aged rates?

Methods

This study is a developmental research and in terms of methodology it belongs to the group of experimental research of pre-test and post-test type studies that was conducted by five groups (control (C), Swimming High Intensity Interval Training (HIIT) (S-HIIT), Swimming HIIT with resveratrol supplementation (S-HIIT-R), resveratrol supplementation (R) and solvent of resveratrol supplementation (carboxymethyl cellulose) (SR). In this research, 60 male 20-months old Wistar rates (22, 27) (weighing 350-450 g) were purchased from Neuroscience Research Center of Kerman University of Medical Sciences.

Regarding the fact that cognitive decline is the outstanding indicator of aging process (28), in order for cognitive assessment of rats (as the aging indication) and also for non-interference of that assessment with exercise protocol, the rats underwent the Novel object recognition test one month before the initiation of the research. Moreover, to investigate the lack of motion impairment in aged rats, the open filed test was also administered (29). Then, 45 rats whose Novel object recognition test results were negative and had no motion impairment participated in the study. All the rats experienced the pool habituation stage for one week. The animals' swimming pool was 180 cm in diameter, and 80 cm in depth (30). On the first day, the rats were put in the depth of 50 cm water and average temperature of 30 ± 0.5 °C (31). They started to swim at their own favorite velocity for 5 minutes. In the later sessions, the rats were quite familiar with the animal pool. Still again, in later sessions, the rats were well familiarized with animals' pool and in order to learn the type of interval training, they swam for one minute and then were removed from water by the rest plate and released into the water again. Forty-eight hours after the last familiarization session, the rats were randomly divided into five groups: C group (n =9, weighting 415 ± 34 g), S-HIIT group (n =9, weighting 404 ± 30 g), R group (n =9, weighting 400 ± 30 g), S-HIIT-R group (n =9, weighting 401 ± 31 g) and SR group (n =9, weighting 400 ± 31 g). The rats of EX group underwent swimming HIIT in 14 bouts, 20-sec with a 10-sec rest between all the 14 bouts. This protocol continued for 6 weeks (3days/week). In interval training, the amount of primarily applied load (in the first week) was %9 of a rat's weight and every week, %1 of the weight was added to it. In the last week, the rats exercised while %14 percent was added to each rat's weight (31). The rats in group C did not undergo training protocol. The rats of group R only received Supplementation of resveratrol (Serva-10700-Usa) solution in carboxymethyl cellulose %1 through gavage

(32) (10 mg/kg/day) (33). Rats in group S-HIIT-R did swimming HIIT and received resveratrol. SR Group received carboxymethyl cellulose %1 (G201505-13-China) through gavage. The gavage of rats in three groups of S-HIIT-R, M and R was administered in the morning; therefore, in order to remove the stress caused by gavage, the rats of S-HIIT-R group were starting their training eight hours after gavage. Swimming HIIT (similar to S-HIIT group) was done in the evening (which is the best possible training time for natural activity rhythm of rats) under red light (which cuts down the stress level) (34). To assess the training intensity, in every session the blood lactate of rats in both training groups (S-HIIT, S-HIIT-R) was immediately after 14th bout measured through the blood sample from the tail vein (Lactate Scout-EKF-German) (35). The swimming speed and distance of each rat was recorded by smart video tracking system (Noldus Ethovision ® system, Netherland, version 7) which is connected to a computer monitor. In the controlled environmental conditions, the rats in all five groups were kept in cage of rodents (3 rats in each cage) made of poly carbonate. Temperature was $23 \pm 1^\circ\text{C}$ and the light/dark cycle was 12/12h (light on: 7:00 a.m till 7:00 p.m) (31). The rats had free access to food and water. In order to remove the acute effects of training, uncontrollable variables, and the rats' stress while conducting the training, 48 hours after the last training session (31) the rats underwent light anesthesia (36) in desiccator attached to carbon dioxide capsule and sacrificed. This was done based on principles in the Ethics Committee of Neuroscience Research Center of Kerman University of Medical Sciences (KNRC/24-96/EC). After the rats underwent a light anesthesia, the brains were immediately extracted and the Hippocampus was separated on ice and fixated in liquid nitrogen tank for 15 to 30 seconds. They were collected and prepared for Western Blotting. Until assessing the amount of PDH-E1 α , they were frozen at (-80°C). To prepare the tissues, immediately

after taking the Hippocampus out of freezer, and weighing it, homogenous Buffer including RIPA Lysis Buffer and inhibitors Aprotinin (A1153sigma), leupeptin (L2023 sigma), Phenylmethylsulfonyl fluoride (P7626sigma) and Sodium orthovanadate (S450243 sigma) (37) was added to the tissue to homogenize on the ice. The amount of added buffer was 5 times the sample weight. Then they were centrifuged (Eppendorf- USA) with 14000 rpm for 20 minutes in 4°C . The supernatant was transferred to clean micro tubes. Using the standard curve and by Bradford method, the appropriate density of the samples protein was calculated. In equal portion, sample buffer of %4 was added to supernatant and heated for 5 minutes in 95°C in order for denaturation of the proteins. For administering Western Blot method, equal amounts of protein were separated by %12.5 Polyacrylamide gel SDS-PAGE. Vertical electrophoresis stage was conducted by 80-100V. Next, separated proteins in gel were transferred to PVDF membrane (0.45 mm sc-3723) by special tank (Bio-Rad) including transfer buffer, 220 mA current for 80 minutes. The membranes were put in %5 blocking solution for two hours. After that, they were incubated in the primary antibody (PDH-E1 α (D-6) sc-377092) and β actin (Monoclonal-A2228) which were diluted with %2.5 skim milk/TBS-T-Tween20 in a 1/1000 ratio. The incubation took 18 hours in 4°C . In the next step, the membranes were washed in washing solution (TBS-T-Tween20) 3 times and each time for 5 minutes. Then for one hour, were incubated in room atmosphere in the secondary antibody (Rabbit anti-mouse IgG-HRP: sc-358914) with %2.5 skim milk/TBS-T-Tween20 in a 1/2000 ratio. After being washed, the membranes were incubated to ECL (GERPN2232) and the emitted light of luminescence reaction was recorded on the film (Roche-000000011666657001) in dark room. Then, using photo processing solutions, the images were appeared. The ImageJ software was used to investigate the density of the films (37). Shapiro-Wilk test showed

normal distribution of dependent variables in each group. The significant difference among different groups was evaluated through one-way analysis of variance, and if a significant difference was observed, the inter group difference was determined through Tukey's post hoc test in ANOVA. Using repeated measures to determine the differences of weeks in the groups (S-HIIT, S-HIIT-R). The significance level for all calculations was considered $p < 0.05$. All statistical operations were performed with SPSS 20.

Results

The variation of rat's weight in all groups are reported in Table-1. one-way analysis of variance of the preset study indicated that average amounts of E1(α) catalytic subunit of Pyruvate Dehydrogenase enzyme (Table-2) and Tukey's post hoc test (fig.1) in resveratrol supplementation group and Swimming HIIT with resveratrol supplementation group (R and S-HIIT-R), compared to SR group

significantly increased ($p=0.007$ and $p=0.047$ and respectively). It was also found out that the amount of PDH-E1 α in SR group, compared to C group did not have significant difference ($p=0.99$). This result shows that using carboxymethyl cellulose solvent in resveratrol supplementation had no impact on average amounts of PDH-E1 α in intervention groups. Moreover, it was also realized that the amount of this subunit in Swimming HIIT group compared to the control group increased but not significantly ($p=0.383$) (fig-1). Moreover, repeated measures of the present study indicated that the average changes speed and distance in swimming HIIT in each bout and section of aged rat ($M \pm SE$) in 1st, 4th and 6th weeks reported in Table-3. Moreover, the average changes of lactate amount in C, S-HIIT and S-HIIT-R group in 3rd and 6th weeks are reported in Table 4.

Table 1. The variation of weight in different groups in 1st, 4th and 6th weeks ($M \pm SD$)

Group	weight		
	First week	Fourth week	Sixth week
C	415.66 \pm 34.88	430.77 \pm 33.09	425.55 \pm 35.21
M	400.22 \pm 31.95	416.56 \pm 24.95	427.89 \pm 22.33
R	400.22 \pm 30.19	392.11 \pm 33.57	390.67 \pm 34.67
S-HIIT-R	401.44 \pm 31.59	388.23 \pm 22.11	380.33 \pm 17.03
S-HIIT	404.44 \pm 30.68	404.67 \pm 27.73	405.22 \pm 29.95

Table 2. One-way analysis of variance for level of PDH-E1 α protein ($M \pm SD$)

Group	$M \pm SD$	F	P
Control (C)	1.0 \pm 0.00	5.476	0.001
Solvent (SR)	1.0 \pm 0.01		
Resveratrol Supplementation (R)	1.05 \pm 0.02		
Swimming HIIT with Resveratrol Supplementation (S-HIIT-R)	1.04 \pm 0.02		
Swimming HIIT (S-HIIT)	1.03 \pm 0.05		

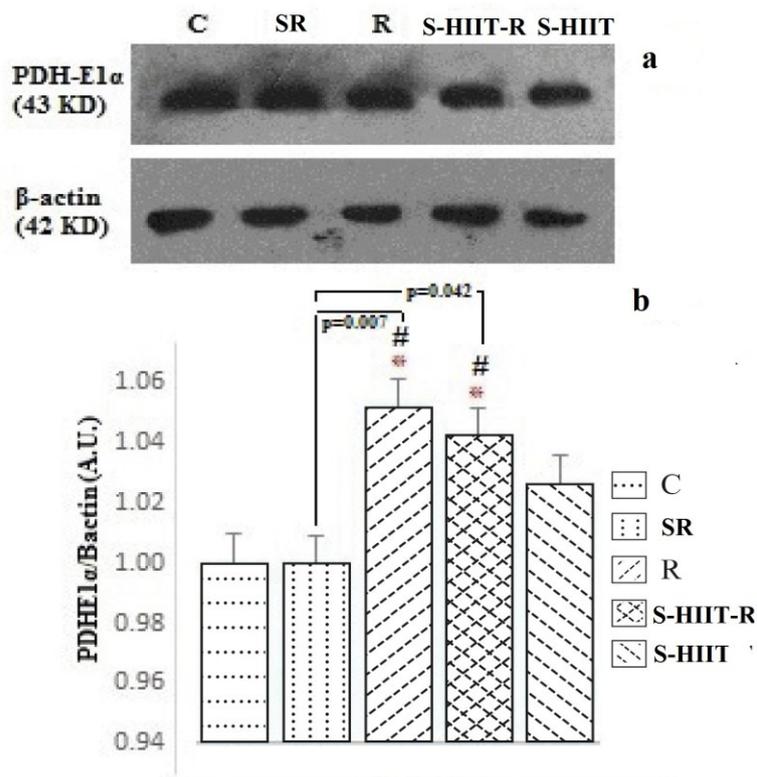


Figure.1 Comparison in the studied groups. a) Immunoblotting images of PDH-E1α protein and β-actin protein are shown as control loading in the hippocampus tissue. b) The PDH-E1α protein quantified bands are against the control loadings. PDH-E1α protein level in resveratrol supplementation group (R) ($p=0.007$) and swimming HIIT with resveratrol supplementation group (S-HIIT-R) ($p=0.047$), compared to solvent of resveratrol supplementation (SR) group has increased. swimming HIIT group (S-HIIT), has increased PDH-E1α protein level compared to control (C) group but not significantly ($p=0.383$). (#: Significant difference compared to the SR group).

Table 3. Average speed and distance in swimming HIIT of aged rat in 1st, 4th and 6th weeks (M±SD)

Time	Group	Swimming speed	Swimming distance in each bout	Total swimming distance in each section
		cm/sec	cm	cm
First week	S-HIIT-R	9.59±1.28	173.27±32.57	2425.86±456.05
	S-HIIT	8.47±1.31	154.79±26.09	2167.11±365.30
Fourth week	S-HIIT-R	18.59±1.26*	314.18±27.93*	4398.59±391.11*
	S-HIIT	17.97±0.71*	310.31±26.51*	4344.35±371.18*
Sixth week	S-HIIT-R	10.14±1.08 [†]	177.52±25.88 [†]	2485.32±362.35 [†]
	S-HIIT	9.49±1.47 [†]	174.37±29.71 [†]	2441.31±416.03 [†]

The swimming speed, swimming distance in each bout and total swimming distance in each section in 4th weeks in the aged rats of S-HIIT and S-HIIT-R group Significant increased compared to the 1st week ($P=0.0001$). In contrast, the swimming speed, swimming distance in each bout and total swimming distance in each section in 6th weeks in aged rats of S-HIIT and S-HIIT-R Significant declined compared to the 4th week ($P=0.0001$). (*: Significant difference compared to the first week and [†]: Significant difference compared to the Fourth week).

Table 4. Average Changes in blood lactate of aged rats in swimming HIIT in 1st, 3rd and 6th weeks (M±SD)

Variable	Group	First week	Third week	Sixth week
Blood lactate (mMol/L)	C	2.03±0.15	2.00±0.17	2.01±0.12
	S-HIIT-R	8.50±1.79	9.80±0.27*	10.00±0.28*
	S-HIIT	8.68±1.48	8.80±0.51	6.11±0.29*

The amount of blood lactate in 6th weeks in the aged rats of S-HIIT group declined compared to the first week (P=0.0001). In contrast, the amount of blood lactate in 3rd and 6th weeks in aged rats of S-HIIT-R group increased (P=0.0001). (*: Significant difference compared to the first week).

Discussion

The main finding of this study was that the amount of PDH-E1 α in Swimming HIIT with resveratrol supplementation group and resveratrol supplementation group increased significantly. It was also indicated that in the Swimming HIIT group, the amount of PDH-E1 α has increased but it was not significant. Following this increase in the amount of E1 α catalytic subunit of Pyruvate Dehydrogenase enzyme, the activity rate of this enzyme may also rise. Since, biochemically accepted that lack of PDC activity is rooted in defect or decline of X gene which belongs to the subunit of E1 α of PDH enzyme (6). Also, there is evidence to show that if phosphorylation of PDH-E1 α increases due to exercise training, a similar increase in the content of PDH-E1 α protein have taken place. This suggests that a similar section of PDH-E1 α molecules has been phosphorylated in the trained and untrained conditions (18). Saunier *et al.* (2017) whose research findings are in line with our study, it was indicated that resveratrol, has significantly decreased the phosphorylation of serine residue 232 (S232) of E1 α subunit enzyme of PDH. The activity of PDH enzyme of cancer cells in large intestine was almost 2.5 times more. Regarding the results of this study in which the resveratrol supplementation increased the amount of PDH-E1 α protein in aged rats' Hippocampus, and also due to this fact that age increasing leads to

phosphorylation of E1 α subunit of PDH enzyme and consequently to inhibition of PDH complex (8); therefore, it is possible that resveratrol supplementation for six weeks leads to phosphorylation decline of E1 α subunit of PDH enzyme through decreasing the phosphorylation S232, and increasing the amount of protein PDP1 expression and no modulation of protein PDP2 expression (7). This, as a result, leads to dephosphorylating of PDH-E1 α and finally, the activity of PDH enzyme in mitochondria of rat's Hippocampus cells increases. Although few studies have looked at the mitochondrial metabolic factors in the hippocampus and the exact mechanism of resveratrol protective effects due to increased PDH-E1 α proteins in the Hippocampal neurons has not been ascertained, but it has been shown that any morphological changes such as increasing dendritic length and dendritic column density in neurons of the hippocampus and prefrontal regions of the 20-month-old male rat of Sprague Dawley has occurred after resveratrol supplementation for eight weeks and 20 mg/kg/day (22). Moreover, it was indicated that resveratrol maintains its neuroprotective

role by decreasing the mitochondrial dysfunction, oxidative damage and prolonged inflammation (38). This study agrees with our research study in that any change in morphology (22) and neuron protection (23) caused by resveratrol supplementation and increasing the E1 α subunit of PDH enzyme in old rat's Hippocampus can activate the mitochondrial metabolic pathways in the cells, and compensate the decreased age-related mitochondrial metabolism due to decreased mitochondrial sirtuins (1). It could also prevent cell death caused by glucose and oxygen deficiency in the Hippocampus (39). Therefore, it can be said that the amount of PDH-E1 α is immensely affected by exercise and resveratrol – as an antioxidant. On the other hand, though the results show that the impact level of the resveratrol supplementation is 67% and the resveratrol supplementation with exercise was 33% compared to mere exercise in old rats; the researchers of the present study raise the idea that the amount of PDH-E1 α protein in Hippocampus of old rat, is more affected by the resveratrol than by swimming HIIT. This is because in aged rats which took resveratrol supplementation with swimming HIIT, the exercise decreased positive effects of the supplementation in Hippocampus by half. The results of this study contradict Gudiksen *et al.* (2017) who stated that PDH-E1 α protein is not affected by one bout submaximal intensity to exhaustion exercise. Primarily these differences can be traced back in to the functional and structural differences of brain cells (neuron and glial) of Hippocampus and muscle cells (which was examined and investigated in Gudiksen *et al.* research), but regarding this post translational regulation mechanism, it is found out that the difference of brain and muscle cell can cause difference in the rate of Mitochondrial metabolism of cells depending on what each cell needs. This can induce the difference between expression and activity of PDP1-2

and PDK1-4 proteins in response to exercise which are responsible to covalent regulation of the subunit sites of the E1 α of PDH enzyme of the hippocampal neural cell. This can undergo additional studies in future. Second, due to differences in the nature of exercise programs (high intensity interval vs. progressive to exhaustion exercises), two separate research studies are suggested to be conducted because PDH-E1 α protein in various intensities of exercise, responds differently; but in HIIT training it can induce reconstruction opportunity, though little, of expression or more exercise. On the other hand, referring to the results of this study, since HIIT provides an opportunity to reconstruct the energy supplies, substrate flux continues to PDC. This indicates a lot of adaptation with exercise (40). As a result, the amount of PDH- E1 α as well as the activity of this key subunit of PDH increases. Thirdly, the type of exercise in Gudiksen *et al.* was to pedal on the ergometer cycle; while in our study, the type of exercise was swimming. Consequently, regarding the result of the research conducted by Irandost *et al.* (2014) and also the increasing PDH-E1 α in present study, it can be claimed that not only swimming leads to better functioning of Hippocampus related (cognitive) behaviors in aged rats, but also leaves more effect on PDH-E1 α protein expression and mitochondrial function in aged rats' Hippocampus. Other than the type of exercise, the differences in results may have induce training period time, which was six weeks in this study while it was a bout of the Gudiksen research (25). It was also found out high and low-intensity combined exercise for eight weeks on ergometer cycle among 60 to 72 years old men, has increased the amount of PDH-E1 α protein in muscular cells (18). What is noticeable in both this study and Bienso *et al.* is that elderly people can also undergo high intensity training programs. Since in untrained people – when VO₂ reaches to maximum 60% - 70% and in trained people the VO₂ reaches

to 75%-80%, the lactate threshold is too much. Therefore, in elderly people, the supra-lactate threshold special for high intensity training have to be noticed. To this end, many studies have shown that aerobic exercise of 60% to 80% Vo_{2max} is possible and practical (27). The training intensity, however must follow scientific principles. At present study, swimming HIIT was carried out with the load fastened to the rat's tail (6 weeks, between 9% to 14% of each rat's weight; and every week 1% of the rat weight is added to previous weight). This caused the exercise intensity to increase. Also, in Biensø *et al.* study, strength training as well as crossfit, and high intensity pedaling on ergometer cycle were carried out. PDH-E1 α protein increase, caused by these high intensity training types in elderly people shows that the PDH-E1 α protein amount can increase due to the intensity and type of exercise. Undertaking the covalent changes is necessary for PDH-E1 α activity and consequently for PDH enzyme complex. It can also induce exercise and adaptations caused by exercise. In contrast, some researchers rely on the findings of their own studies and oppose our reasons and arguments. For instance, Ringholm *et al.* (2013) investigated the impact of running on treadmill and resveratrol supplementation on the amount of PDH-E1 α protein in 3-month-old and 15-month-old rats. They concluded that exercise itself causes mitochondrial enzymes such as PDH-E1 α protein to increase; and the protein amount in training group is even more than in training with resveratrol group. It is worth mentioning that the protein amount in training and training with resveratrol groups in 15-month-old rats is even more than young group (3-month-old rats) and the 15-month-old group of rats without training and resveratrol. The results of our study, on the other hand, indicate that the impact of resveratrol alone on PDH-E1 α protein is higher than the impact of exercise and exercise with resveratrol supplementation. In this case, age difference of the subjects, kind of training program and even the way of

resveratrol intake must be taken into account. In this study, the rats received resveratrol through gavage, but in Ringholm *et al.* study, the rats received resveratrol accompanied with food. Moreover, the amount of received resveratrol can also change and affect the results, because in the recent study 4g of resveratrol per 1kg food was given to rats, but in our study, the rats received 10mg resveratrol per 1kg of their body weight. To summarize, the results of this study supports this view that resveratrol and swimming HIIT as bioenergetic interventions can affect the key proteins of mitochondrial metabolism such as E1 α catalytic subunit of Pyruvate Dehydrogenase enzyme in the Hippocampus of old rats and regulate PDH complex activity, TCA cycle and mitochondrial metabolism in hippocampal cell. Also, lactate which is a by-product of muscle exercise enters the brain and there, it is used as the source of energy (27). Moreover, this study recommends the swimming HIIT to old people, because it elevates the lactate level in the body and can compensate the lack or decline of metabolic flexibility caused by aging process (41). Finally, this study found out that if exercise and resveratrol supplementation can affect the amount of PDH-E1 α protein in brain cells, the opposite may also happen and age and the gender of the subjects can impact the creation of useful effects of exercise and resveratrol.

Conclusion

It appears that resveratrol alone and with exercise can influence levels of hippocampus mitochondrial metabolism key proteins such as PDH-E1 α in aged rats and regulated PDC activity, TCA cycle and hippocampal cell metabolism.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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References

1. Van de Ven RA, Santos D, Haigis MC. Mitochondrial sirtuins and molecular mechanisms of aging. *Trends Mol Med*. 2017; (4): 320- 331.
2. Lane RK, Hilsabeck T, Rea SL. The role of mitochondrial dysfunction in age-related diseases. *Biochim Biophys Acta Mol Basis Dis*. 2015; 1847 (11): 1387-1400.
3. Jardim FR, de Rossi FT, Nascimento MX, da Silva Barros RG, Borges PA, Prescilio IC, et al. Resveratrol and brain mitochondria: A review. *Mol Neurobiol*. 2018; 55 (3): 2085- 2101.
4. Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. *Mol Cell*. 2016; 61 (5): 654- 666.
5. Nuzzo E, Berg KM, Andersen LW, Balkema J, Montissol S, Cocchi MN, et al. Pyruvate dehydrogenase activity is decreased in the peripheral blood mononuclear cells of patients with sepsis. A prospective observational trial. *Ann Am Thorac Soc*. 2015; 12 (11): 1662- 1666.
6. Ojano-Dirain C, Glushakova LG, Zhong L, Zolotukhin S, Muzyczka N, Srivastava A, et al. An animal model of PDH deficiency using AAV8-siRNA vector-mediated knockdown of pyruvate dehydrogenase E1 α . *Mol Genet Metab Rep*. 2010; 101 (2): 183- 191.
7. Saunier E, Antonio S, Regazzetti A, Auzeil N, Lapr evote O, Shay JW, et al. Resveratrol reverses the Warburg effect by targeting the pyruvate dehydrogenase complex in colon cancer cells. *Sci. Rep*. 2017; 7 (1): 6945.
8. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, et al. Regulation of cellular metabolism by protein lysine acetylation. *Science*. 2010; 327 (5968): 1000- 1004.
9. Jing E, O'Neill BT, Rardin MJ, Kleinridders A, Ilkeyeva OR, Ussar S, et al. Sirt3 regulates metabolic flexibility of skeletal muscle through reversible enzymatic deacetylation. *Diabetes*. 2013: DB- 121650.
10. Nagaraj R, Sharpley MS, Chi F, Braas D, Zhou Y, Kim R, et al. Nuclear localization of mitochondrial TCA cycle enzymes as a critical step in mammalian zygotic genome activation. *Cell*. 2017; 168 (1-2): 210- 223.
11. Camandola S, Mattson MP. Brain metabolism in health, aging, and neurodegeneration. *EMBO Rep*. 2017; 36 (11): 1474- 1492.
12. Stahon KE, Bastian C, Griffith S, Kidd GJ, Brunet S, Baltan S. Age-related changes in axonal and mitochondrial ultrastructure and function in white matter. *J Neurosci*. 2016; 36 (39): 9990- 10001.
13. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *J Stem Cells*. 2006; 24 (4): 857- 864.
14. Erickson KI, Leckie RL, Weinstein AM. Physical activity, fitness, and gray matter volume. *Neurobiol Aging J*. 2014; 35: S20- 28.
15. Eghbaliferiz S, Iranshahi M. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanisms and catalyzing metals. *Phytother Res*. 2016; 30 (9): 1379- 1391.

16. Shi Z, Qiu W, Xiao G, Cheng J, Zhang N. Resveratrol attenuates cognitive deficits of traumatic brain injury by activating p38 signaling in the brain. *Med Sci Monit.* 2018; 24: 1097.
17. Carrasco-Pozo C, Mizgier ML, Speisky H, Gotteland M. Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. *Chem Biol Interact.* 2012; 195 (3): 199- 205.
18. Biensø RS, Olesen J, Gliemann L, Schmidt JF, Matzen MS, Wojtaszewski JF, et al. Effects of exercise training on regulation of skeletal muscle glucose metabolism in elderly men. *J Gerontol A Biol Sci Med Sci.* 2015; 70 (7): 866- 872.
19. Pilegaard H, Birk JB, Sacchetti M, Mourtzakis M, Hardie DG, Stewart G, et al. PDH-E1 α dephosphorylation and activation in human skeletal muscle during exercise: effect of intralipid infusion. *Diabetes.* 2006; 55 (11): 3020- 3027.
20. Gliemann L, Schmidt JF, Olesen J, Biensø RS, Peronard SL, Grandjean SU, et al. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J Physiol.* 2013; 591 (20): 5047- 5059.
21. Ringholm S, Olesen J, Pedersen JT, Brandt CT, Halling JF, Hellsten Y, et al. Effect of lifelong resveratrol supplementation and exercise training on skeletal muscle oxidative capacity in aging mice; impact of PGC-1 α . *Eep Gerontol.* 2013; 48 (11): 1311- 1318.
22. Monserrat Hernández-Hernández E, Serrano-García C, Antonio Vázquez-Roque R, Díaz A, Monroy E, Rodríguez-Moreno A, et al. Chronic administration of resveratrol prevents morphological changes in prefrontal cortex and hippocampus of aged rats. *Synapse.* 2016; 70 (5): 206- 2017.
23. Kodali M, Parihar VK, Hattiangady B, Mishra V, Shuai B, Shetty AK. Resveratrol prevents age-related memory and mood dysfunction with increased hippocampal neurogenesis and microvasculature, and reduced glial activation. *Sci. Rep.* 2015; 5: 8075.
24. Kumar V, Pandey A, Jahan S, Shukla RK, Kumar D, Srivastava A, et al. Differential responses of trans- resveratrol on proliferation of neural progenitor cells and aged rat hippocampal neurogenesis. *Sci Rep.* 2016; 6: 28142.
25. Irandost Kh, Taheri M, Sadeghi A. The effects swimming and running training protocol on motor function, learning, spatial memory of old rats. *J Mot Learn Dev.* 2014; 6 (2): 259- 270.
26. Stec MJ, Mayhew DL, Bamman MM. The effects of age and resistance loading on skeletal muscle ribosome biogenesis. *J Appl Physiol.* 2015; 119 (8): 851- 857.
27. Lezi E, Burns JM, Swerdlow RH. Effect of high-intensity exercise on aged mouse brain mitochondria, neurogenesis, and inflammation. *Neurobiol Aging.* 2014; 35 (11): 2574- 2583.
28. Dutt Way W, Soga T, Parhar IS. Aging and chronic administration of serotonin-selective reuptake inhibitor citalopram upregulate Sirt4 gene expression in the preoptic area of male mice. *Front Genet.* 2015; 6: 281.
29. Walsh RN, Cummins RA. The open-field test: a critical review. *Psychol Bull.* 1976; 83 (3): 482.
30. Farzanegi P, Habibian M, Alinejad H. The combined effect of regular aerobic exercise with garlic extract on renal apoptosis regulatory factors in aged rats with chronic kidney disease. *J Arak Univ Med Sci.* 2016; 19 (3): 62- 70.
31. Ramos-Filho D, Chicaybam G, de-Souza-Ferreira E, Martinez CG, Kurtenbach E, Casimiro-Lopes G, et al. High intensity interval training (HIIT) induces specific changes in respiration and electron leakage in the mitochondria of different rat

- skeletal muscles. *PLOS ONE*. 2015; 10 (6): e0131766.
32. Shadfar S, Couch ME, McKinney KA, Weinstein LJ, Yin X, Rodríguez JE, et al. Oral resveratrol therapy inhibits cancer-induced skeletal muscle and cardiac atrophy in vivo. *Nutr Cancer*. 2011; 63 (5): 749- 762.
33. Ungvari Z, Orosz Z, Labinskyy N, Rivera A, Xiangmin Z, Smith K, et al. Increased mitochondrial H₂O₂ production promotes endothelial NF- κ B activation in aged rat arteries. *Am J Physiol Heart Circ Physiol*. 2007; 293 (1): H37- 47.
34. Shafiee A, Kordi MR, Gaeini AA, Soleimani M, Nekouei A, Hadidi V. The effect of eight week of high intensity interval training on expression of mir-210 and ephrinA3 mRNA in soleus muscle healthy male rats. *J Arak Univ Med Sci*. 2014; 17 (3): 2- 8.
35. Casimiro-Lopes G, Ramos D, Sorenson MM, Salerno VP. Redox balance and mitochondrial glycerol phosphate dehydrogenase activity in trained rats. *Eur J Appl Physiol*. 2012; 112 (11): 3839- 8346.
36. Marosi K, Bori Z, Hart N, Sárga L, Koltai E, Radák Z, et al. Long-term exercise treatment reduces oxidative stress in the hippocampus of aging rats. *J Neurosci*. 2012; 226: 21- 28.
37. Braidy N, Poljak A, Grant R, Jayasena T, Mansour H, Chan-Ling T, et al. Differential expression of sirtuins in the aging rat brain. *Front Cell Neurosci*. 2015; 9: 167.
38. Kulkarni SS, Cantó C. The molecular targets of resveratrol. *Biochim Biophys Acta Mol Basis Dis*. 2015; 1852 (6): 1114- 1123.
39. Zhang H, Schools GP, Lei T, Wang W, Kimelberg HK, Zhou M. Resveratrol attenuates early pyramidal neuron excitability impairment and death in acute rat hippocampal slices caused by oxygen-glucose deprivation. *Eep Neurol*. 2008; 212 (1): 44- 52.
40. Gudiksen A, Bertholdt L, Stankiewicz T, Tybirk J, Plomgaard P, Bangsbo J, et al. Effects of training status on PDH regulation in human skeletal muscle during exercise. *Pflugers Arch*. 2017; 469 (12): 1615- 1630.
41. Consitt LA, Saxena G, Saneda A, Houmard JA. Age-related impairments in skeletal muscle PDH phosphorylation and plasma lactate are indicative of metabolic inflexibility and the effects of exercise training. *AM J Physiol Endoc M*. 2016; 311 (1): E145- 145.