Aerobic activity combined with Q10 supplementation has a positive effect on serum Klotho levels in the elderly

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النشاط الهوائي مع مكملات Q10 له تأثير إيجابي على مستويات كلوثو في المصل لدى كبار السن

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الملخص:-

كان الهدف الرئيسي من الدراسة الحالية هو التحقيق في تأثير التمارين الهوائية المعتدلة مع مكملات أنزيم Q10 على مستويات كلوثو في المصل ونوعية الحياة لدى كبار السن في طهران. وفقًا لذلك، تم اختيار ٤٠ شخصًا مسنًا في طهران بمتوسط عمر ٦٧.٧٢ ± ٣.٧٩ عامًا، ومتوسط طول الأشخاص ١٦١.٩٥ ± ٥.٩١ سم، ومتوسط الوزن ٦٨.٣٥ ± ٥.٠٠٥ كجم، ومتوسط مؤشر كتلة الجسم ٢٦.٠٤ ± ١.٠٧ كجم / م ٢. تم تقسيم الأشخاص إلى ٤ مجموعات: التدريب الهوائي، ومكملات أنزيمQ10 ، ومكملات التمارين الهوائية، وأنزيمQ10 ، ومجموعة التحكم. قامت مجموعة التمارين الهوائية بتدخل تمريني بكثافة ٦٠-٦٥٪ من الحد الأقصى لمعدل ضربات القلب لمدة ٣٠ دقيقة لكل جلسة و٣ أيام في الأسبوع لمدة ٨ أسابيع، وتلقت مجموعة CoQ10 جرعة ٢٠٠ ملغ/يوم من هذا المكمل. في بداية ونهاية هذه الدراسة، تم أخذ عينات دم لتقييم مستويات مصل كلوثو واستبيان SF36 لتقييم جودة الحياة. أظهرت نتائج هذه الدراسة أن التدريب الهوائي ومكملات التمارين الهوائية من أنزيم كيو ١٠ أدت إلى زيادة كبيرة في مستويات مصل كلوثو لدى كبار السن (P = 0.0001) ونوعية الحياة (P = 0.0001).أظهرت نتائج تحليل التباين أحادي الاتجاه وجود فرق كبير بين قيم كلوثو ونوعية حياة كبار السن في المجموعات المدروسة بعد دورة تدريبية هوائية مع مكملات أنزيم كيو ١٠.(P = 0.0001)

بشكل عام، أظهرت نتائج هذه الدراسة أن مكملات CoQ10وممارسة التمارين الرياضية الهوائية متوسطة الشدة لمدة ٨ أسابيع أدت إلى تحسين جودة الحياة وزيادة بروتين Klotho في المصل كعلامة مضادة لارتفاع ضغط الدم لدى كبار السن. ويبدو أن القيام بذلك معًا من شأنه أن يمنع الوفيات الناجمة عن الأمراض المرتبطة بالشيخوخة.

الكلمات المفتاحية: الشيخوخة، Klotho، Q1O، التمارين الرياضية.

Abstract:-

The main objective of the present study was to investigate the effect of moderate aerobic exercise with coenzyme Q10 supplementation on serum Klotho levels and quality of life in elderly people in Tehran. Accordingly, 40 elderly people in Tehran with an average age of 67.72 ± 3.79 years, mean height of subjects were 161.95 ± 5.91 cm, mean weight 68.35 ± 5. 005 kg, and mean BMI, 26.04 ± 1.07 kg / m 2 were selected. Subjects were divided into 4 groups: aerobic training, supplementation of coenzyme Q10, aerobic exercise supplementation, coenzyme Q10, and control group. The aerobic exercise group performed an exercise intervention with intensity 60-65% of maximum heart rate for 30 minutes per session and 3 days per week for 8 weeks, and the CoQ10 group received 200 mg / day of They consumed this supplement. At the beginning and the end of this study, blood samples were taken to evaluate the serum levels of Klotho and SF36 questionnaire for assessing quality of life. The results of this study showed that aerobic training and aerobic exercise supplementation of coenzyme Q10 significantly increased the serum levels of elderly Klotho (P = 0.0001) and quality of life (P = 0.0001). The results of one-way ANOVA showed that there is a significant difference between the Klotho values and the quality of life of the elderly in the studied groups after an aerobic training course with supplementation of coenzyme Q10 (P = 0.0001).

Overall, the results of this study showed that CoQ10 supplementation and moderate-intensity aerobic exercise for 8 weeks improved quality of life and increased serum Klotho protein as an antihypertensive marker in the elderly. And it seems that doing these together would prevent deaths from diseases associated with aging.

Keywords: Aging, Klotho, Q1O, Exercise.

Introduction

The increase in the elderly population in Iran, like in other countries, due to the decrease in birth rates, improvement in health status, and increase in life expectancy, has made it necessary to pay attention to the issue of health, wellness, ensuring the comfort and well-being of the elderly in society, and also to address the needs and problems of this segment of society. On the other hand, with increasing age and the biological changes that have occurred, the elderly are susceptible to chronic diseases such as diabetes, cancer, cardiovascular diseases, bone-related diseases, etc., as well as decreased balance in walking and hiking, including falls [28]. Very few studies have compared active and inactive elderly people in different dimensions. Most studies have shown the effect of one or more types of intervention, such as performing various sports activities or using supplements or various medicinal plants, on their various physiological parameters. In this context, we can mention the studies of Ilkhani et al. (2017) [29], Khademi et al. (2012) [30], Moein et al. (2017) [31], Nilsson et al. (2017) [32] and Akri et al. (2006) [33]. The health benefits of physical activity in the elderly (i.e., reduction of cardiovascular diseases, diabetes, blood pressure, cancer and mortality) are well known [34-36]. Studies have also shown that with increasing age and the onset of the aging process, serum levels of Klotho protein decrease significantly [37]. Maintaining independence in performing activities of daily living is important for increasing health and improving the quality of life of the elderly. Recent studies have shown that poor physical performance and low physical fitness are important risk factors for the inability to perform activities of daily living in the elderly. Klotho, as an anti-aging protein, plays an important role in the progression of disability in the elderly and other individuals. Epidemiological studies have shown that elderly individuals with low circulating Klotho concentrations have poorer muscle strength and are at higher risk of death [38]. In this regard, Crasto et al. (2011) studied the relationship between low circulating Klotho levels and disability in daily activities and showed that elderly individuals with high Klotho levels had greater independence in performing daily activities [37]. However, very few studies have examined the levels of circulating Klotho in individuals. The physical fitness status of active and inactive elderly individuals is also of great

importance. Most studies have shown that individuals with high physical ability have a higher quality of life [18, 21, 32]. On the other hand, the use of antioxidant supplements seems to be important in reducing aging and related diseases; One study reported that elderly people who took a combination of selenium and CoQ10 over a 4-year period reported improvements in dizziness, physical function, and quality of life. However, no study has examined the effect of coenzyme Q10 supplementation or its combination with exercise on Klotho levels in humans or animals.

Research method

Participations

The present study is of a semi-experimental and laboratory type, and is cross-sectional in terms of the length of time and in terms of the use of applied results, which was conducted as a pre-test, post-test, and with a control group. The subjects of this study were human samples and were randomly divided into 4 groups of 10 people.

The statistical sample of the present study consisted of 40 elderly men and women (60-75 years old), who were selected purposively and conveniently and randomly assigned to 4 groups (exercise (10 people), coenzyme Q10 supplementation (10 people), exercise-supplement group (10 people), and placebo or control group (10 people)). The present study included all sedentary elderly (60-75 years old) in Zabol city who had referred to clinics and health centers and parks and recreational areas. The inclusion criteria for this study were being over 60 years old, having Iranian nationality, being able to speak Persian, not suffering from a known mental illness (psychosis), being fully conscious at the time of the study, being able to communicate, being able to answer the study questions, and residing in Zabol city. The exclusion criteria were being active according to the questionnaire, refusing to answer the questionnaire during work and interviews, and refusing to implement the desired exercise or supplement program, as well as any unwanted events.

The sampling method of this study will be as follows: the researcher and colleagues will be present at the desired places (places such as parks, swimming pools, morning exercise stations, mosques, elderly care centers, and retirement centers) where the elderly will gather, and after conducting a short interview and

explaining the purpose and subject of the research, they will select people who were willing to cooperate. Then, explanations were given about the subject and purpose of the research and the participants were asked to complete the questionnaires. Each person completed a questionnaire while respecting privacy. The elderly who could not read and write were asked to read the questions to them through an interview method and the questionnaire items were completed based on their opinions and choices. The elderly who could read and write were given the questionnaire to complete its items. The questionnaire forms were collected while the interviewer was present. If a questionnaire had incomplete information, the participant was asked to complete the information. In order to investigate the physical activity level of the elderly in this study, the subjects were asked to indicate their physical and sports activities during the week in a questionnaire. Accordingly, the elderly were divided into two active and inactive groups. The active elderly group included people who had at least two 45-minute sessions of physical and sports activities per week; such as walking, jogging, morning exercises, swimming, etc. The inactive group consisted of people who, according to their statements, did not do any physical or sports activities during the week. Active subjects were excluded from the study and inactive people were selected.

Then, the subjects were asked to attend between 8 and 11 in the morning and fasting blood was taken from the subjects. The weight of the subject was measured in light clothes and without shoes using a Seca scale with an accuracy of half a kilogram, and height was measured in a standing position and without shoes using a tape measure mounted on the wall. Blood sampling was performed to measure the level of circulating Klotho and was measured by ELISA using the soluble alpha Klotho ELISA assay kit (Eastbiofarm, China).

The study consisted of two stages: pre-test and post-test. Between these two stages, the subjects performed the relevant activities for 8 weeks. The purpose and program of the study were explained to the subjects. The variables of age, weight, height, blood pressure, and health were examined by a doctor, then blood was drawn. Blood sampling was performed by a specialized laboratory after at least 12 hours of overnight fasting at 9 am. The initial blood sample of 4 cc was taken from the subjects' anterior brachial vein by

the laboratory's blood collection specialists and frozen at -35 °C. After the necessary coordination was made with the subjects, the physical activity intervention was implemented as follows.

The exercise program began with warming up and ended with cooling down. The subjects were randomly assigned to 4 groups of 10. The aerobic exercise group participated in an 8-week exercise program with 3 aerobic exercise sessions per week, including warm-up, stretching, cooling, walking, and slow running in green spaces of parks, under the supervision of the researcher and a fellow trainer. This exercise program was previously conducted by Mir Moezi et al. (2015) on elderly people similar to the present study.

A total daily dose of 200 mg of Q10 in two 100 mg capsules in the morning and evening with food was given to subjects in the supplement group and the combination of supplement and exercise group. The placebo consisted of wheat flour, which was prepared in the same packaging, color, and taste as Q10 and was given to the control and exercise groups. All stages of supplementation were performed by the researcher's colleagues in a double-blind manner [59].

Blood sampling

Blood sampling was performed in two stages, 12 hours before the start of the first training session (pre-test) and 48 hours after the last training session (end of the eighth week). The collected blood was placed in sterile tubes and then the plasma serum was separated by centrifugation (4555 rpm for 4 minutes) and frozen at 35°C until measurement. After 8 weeks of training, similar blood sampling was performed. All blood samples were removed from the freezer on the same day and the desired tests were performed to measure serum Klotho levels.

After data collection, SPSS version 22 statistical software was used to analyze them; in a way that the values of central tendency and mean dispersion and standard deviation were used to estimate descriptive statistics of the research. The Shapiro-Wilks statistical test was used to examine the distribution of data and the t-test was used to compare means within groups and the analysis of covariance one-way (ANOVA) test was used to compare between groups. The significance level was considered to be P<%5. All data analysis was performed using SPSS22 software.

Result

Fifty-three participants were enrolled, 10 participants withdrew due to health complications unrelated to the study (AT+Q10s: N =3; AT: N =2; Q10s: N =2; Control: N =3) and 2 dropped out due to unforeseen family and work interruptions (AT+Q10s: N =1; Q10s: N =1). 40 participants completed the full intervention (AT+Q10s: N =10; AT: N =10; Q10s: N =10; Control: N =10). with an adherence rate between 85-100%. Participant characteristics from the eligibility screening (Table 1) and all baseline outcome variables were similar between groups ($p > 0.050$). A Wilcoxon test revealed no differences in pre-to-post SLIQ for each group ($p > 0.050$) indicating participants maintained their current lifestyle behaviors throughout the intervention period.

The results of one-way analysis of variance (ANOVA) showed that there was a significant difference between the Klotho values of the elderly in the study groups after a period of aerobic exercise with the use of coenzyme Q10 supplementation (P=0.0001) (Fig-1).

In order to find the location of the differences, Tukey's test was used to compare the groups pairwise. There is a significant difference between the aerobic exercise group with coenzyme Q10 supplementation and the aerobic exercise group in the amount of Klotho in the elderly (P=0.0001). There is a significant difference between the aerobic exercise group with coenzyme Q10 supplementation and the coenzyme Q10 supplementation group in the amount of Klotho in the elderly (P=0.0001). There is a significant difference between the aerobic exercise group with coenzyme Q10 supplementation and the control group in the amount of Klotho in the elderly (P=0.0001). There is no significant difference between the aerobic exercise group and the coenzyme Q10 supplementation group in the amount of Klotho in the elderly (P=0.46). There is no significant difference between the aerobic exercise group and the control group in the amount of Klotho in the elderly (P=0.0001). There is a significant difference between the coenzyme Q10 supplementation group and the control group in the amount of Klotho in the elderly (P=0.007).

Discussion

The results of the present study showed that an 8-week period of CoQ10-aerobic exercise supplementation (Q10-AT) significantly

increased serum Klotho levels and quality of life (QoL) in elderly people aged 65 to 75 years in Tehran.

One of the results of the present study was an increase in serum Klotho levels in elderly people in the post-test compared to the pre-test, after performing 8 weeks of moderate-intensity aerobic exercise (60-65% of maximum heart rate). On the other hand, the results of this study showed that there was no significant difference in the AT group compared to the control group. While there was no significant difference between the AT group and the Q10 group. In fact, the findings of this study showed that performing aerobic exercise for 8 weeks increased serum Klotho levels compared to the control and pre-test groups. However, there was no significant difference between the two intervention groups separately. On the other hand, other results of this study showed that the combination of Q10 supplementation and aerobic exercise (Q10-AT) for 8 weeks had a significant effect on serum Klotho levels in the elderly compared to the AT and Q10 groups. Although there was a significant difference between the two Q10 and control groups in serum Klotho levels in the elderly, taking a Q10 supplement at a dose of 200 g/day was not more beneficial than performing moderate-intensity aerobic exercise 3 days/week.

In general, many studies have not compared the effects of Q10 supplementation and aerobic exercise in the elderly and other groups or research samples. However, several studies have been conducted on the effect of aerobic exercise on serum Klotho levels. For example, Moin and colleagues showed in a study on rats that eight weeks of aerobic exercise on a treadmill (3 days a week and at an intensity of 60 to 80% VO2MAX) increased Klotho protein and significantly reduced the level of the inflammatory protein IL-6; However, in this study, healthy and young 8-week-old animal samples were used in comparison with the present study (Moin et al., 2017). Another study also showed that serum Klotho levels were significantly higher in patients with coronary artery disease compared with untrained patients and their healthy counterparts (39). Another study also examined 1270 elderly subjects (over 65 years of age) and showed that individuals with lower plasma Klotho had lower wrist strength. Although plasma Klotho is not an independent predictor of wrist strength, it is a strong marker of mortality risk and an anti-aging factor. Their study also showed that participants with lower Klotho

concentrations were at higher risk of mortality (Semba et al., 2012). In another study, Crasto et al. showed that plasma Klotho levels in elderly individuals are inversely and significantly associated with the prevalence of disability in daily activities. The results of Matsubara et al.'s study on the effect of aerobic exercise on plasma Klotho levels and arterial stiffness - as a cardiovascular risk factor and aging - are similar to the present study. In this study, plasma Klotho levels increased and arterial stiffness decreased following a period of aerobic exercise in postmenopausal women aged 50 to 76 years (Matsubara et al., 2014). Recently, Sagyu et al. studied the effect of aerobic and anaerobic activities on circulating soluble Klotho and IGF-1 in young and elderly individuals and coronary artery patients. They stated that Klotho has an important effect on improving quality of life, reducing the negative effects of age on physical strength capacity, and possibly increasing lifespan. In this review, different models of dynamic exercise and their effects on circulating levels of soluble Klotho and IGF-1 in young adult athletes, untrained young individuals, trained healthy elderly individuals, untrained healthy elderly individuals, and individuals with coronary artery disease were reviewed and discussed. They showed that long-term (chronic) aerobic exercise is likely an anti-aging agent that counteracts aging and the process of coronary artery disease by increasing circulating soluble klotho and reducing IGF-1 levels. However, the opposite effects occur following anaerobic exercise (Sagiou et al., 2017).

As a suppressor of aging, Klotho is an important molecule in the aging process, and its overexpression leads to increased lifespan. For many reasons, the insulin/IGF-1 pathway has been considered a key pathway in aging research. Since Klotho leads to IGF-I and insulin resistance, these findings are inconsistent with previous evidence of increased lifespan in dwarf mice by reducing IGF-I and insulin levels and increasing insulin sensitivity (Bartke, 2006).

Soluble Klotho in the blood inhibits IGF-1 and insulin receptor signaling by inhibiting the tyrosine phosphorylation of both its receptors and their downstream signaling proteins (Dunnit-Correa et al., 2014), thus extending lifespan in mice (Corso et al., 2005). Furthermore, blocking IGF-I signaling by circulating soluble klotho has been shown to increase resistance to oxidative stress, thereby increasing survival (Yamamoto et al., 2005).

Due to genetic factors, work capacity declines with the onset of aging regardless of lifestyle. As a result, maximal oxygen uptake decreases. Such complications contribute to the aging-related frailty syndrome, thus severely limiting performance, quality of life, and lifespan (Ofilalo et al., 2009; Sagiou et al., 2017). Apart from genetic issues, an individual must also interact with environmental factors associated with longevity. One of these factors is maintaining a high level of physical activity (Baldwin and Haddad, 2010). Chronic endurance training will reduce the decline in maximal oxygen uptake associated with aging (Buford et al., 2014). In addition, anti-aging effects have been attributed to aerobic exercise (Bartke et al., 2006). The association between muscle function and soluble Klotho expression was recently demonstrated in longitudinal cohort studies (Barton et al., 2012).

Recently, a significant association between circulating soluble Klotho and IGF-I was observed following aerobic exercise (Sagiou et al., 2015). This study showed that circulating soluble Klotho levels were similar in healthy young elite runners and professional athletes. This response appears to be dependent on the level of aerobic fitness (Reimers et al., 2012). Furthermore, a study showed that Klotho levels were significantly higher in both young and old age groups when trained versus untrained individuals were compared (Lee et al., 2014).

Since aging is an independent risk factor for age-related diseases and mortality, there is a growing effort in gerontological research to slow aging and increase healthy lifespan. A population studied from birth to 91 years of age by ELISA showed that Klotho levels decrease with aging. In other words, at any age, elderly people with aerobic capacity experience a longer life compared to inactive people (Apor and Rady., 2010; Crasto et al., 2012).

Inhibition of IGF-1 signaling by IGF-1R haploinsufficiency, reduction of IGF-1 ligand levels, or deletion of the insulin receptor in adipose tissue increases lifespan. Increased Klotho expression inhibits insulin and IGF-1 signaling. Therefore, Klotho slows down aging directly by signaling target tissues and indirectly by inhibiting insulin signaling and inducing the FOXO1 mediator in adipose tissue. In addition, the antioxidant, antifibrotic, anticancer, calcium transporter enzyme, and antiphosphatemia properties contribute to

the antiaging role of Klotho. Klotho can protect organs and delay the development of chronic diseases, which it does by creating beneficial and beneficial signals in vital cells of the body. High extracellular phosphate concentrations are toxic to cells, and impaired urinary excretion of phosphate; It increases serum phosphate levels and stimulates premature aging. Urinary phosphate levels increase with excessive phosphate intake, which induces tubular damage and interstitial fibrosis. Klotho prevents hyperphosphatemia, protects the vessels and renal system, and thus prolongs life. In addition, 1,25D, in cooperation with Klotho, delays vascular calcification by inducing osteoponin. In patients with renal failure due to loss of renal mass, 1,25D and Klotho play a key role in improving kidney and vascular health. Klotho protects against endothelial dysfunction and regulates nitric oxide production: Klotho has an anti-aging and anti-apoptotic role in vascular endothelial cells, thereby protecting against endothelial dysfunction. Klotho is known as a humoral factor and is constantly present in vascular endothelial cells and plays an important role in controlling vascular tone, releasing nitric oxide in response to specific agonists such as acetylcholine. As a result, Klotho upregulates nitric oxide. Urinary excretion of Klotho metabolites such as NO2 and NO3 significantly reduced Klotho in mice. Klotho deficiency reduces NO production and consequently impairs endothelial function. Klotho protein acts as a factor for converting inactive precursors into biologically active ones (Saito et al., 2000; Bien et al., 2015).

In general, the effect of coenzyme Q10 supplementation on serum Klotho levels has not been studied, but the anti-aging effects of CoQ10 have been discussed to some extent. A significant decrease in the rate of CoQ biosynthesis occurs during the aging process and age-related diseases (Hernandez-Camacho et al., 2017). However, there is controversy regarding the relationship between CoQ levels and the progression of aging. Mice lacking one allele of the COQ7 gene (mCOQ7/mCLK1 gene) show prolonged lifespan, even though their CoQ levels are similar to wild-type mice, suggesting that a factor other than endogenous CoQ may be responsible for the extended lifespan in these animals (Lapointe and Hakimi, 2008). However, other in vivo studies have reported a direct relationship between lifespan and mitochondrial CoQ levels in the Samp1 mouse model of accelerated aging (Tian et al., 2014).

Ubiquinone supplementation has been shown to activate mitochondrial biogenesis control mechanisms (Schmelzer et al., 2010) and delay aging (Tian et al., 2014).

Plasma CoQ10 concentrations in older adults are directly related to physical activity levels and cholesterol concentrations (Del Pozo-Cruz et al., 2014a,b), as well as to lower lipid oxidative damage. Antioxidant protection by CoQ10 is associated with skeletal muscle function during aging, suggesting that the CoQ10H2/CoQ10 ratio is associated with increased muscle strength (Fischer et al., 2016). Conversely, the CoQ10H2/CoQ10 ratio can be considered as a predictor of sarcopenia in humans. Elderly individuals who received a combination of selenium and coenzyme Q10 over a 4-year period showed improvements in quality of life, exercise performance, and vitality (Johansson et al., 2015). Furthermore, CoQ10 supplementation has been shown to confer health benefits in older adults by inhibiting chronic oxidative stress associated with cardiovascular disease and neurodegeneration (Gonzalez-Guardia et al., 2015). Despite this evidence, most validated geriatric clinical trials have primarily focused on CoQ10 as an effective anti-aging treatment (Varela-Lopez et al., 2016).

Conclusion:-

In general, the results of the present study show that performing aerobic exercise and taking Q10 supplements simultaneously for 8 weeks improves serum Klotho levels in the elderly, thereby reducing mortality due to aging and improving the living conditions and independence of the elderly.

References:-

1. Autry, A. E., & Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacological reviews, 64(2), 238-258.
2. Bell, L., Lamport, D. J., Butler, L. T., & Williams, C. M. (2015). A review of the cognitive effects observed in humans following acute supplementation with flavonoids, and their associated mechanisms of action. Nutrients, 7(12), 10290-10306.
3. Bing, Y., & Zhaobao, W. (2010). Effects of Ginkgo biloba extract on free radical metabolism of liver in mice during endurance metabolism. African Journal of Traditional, Complementary and Alternative Medicines, 7(4).

4. Bonaccorso, S., Sodhi, M., Li, J., Bobo, W. V., Chen, Y., Tumuklu, M.,... Meltzer, H. Y. (2015). The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism is associated with increased body mass index and insulin resistance measures in bipolar disorder and schizophrenia. Bipolar disorders, 17(5), 528-535.
5. Cotman, C. W., Berchtold, N. C., & Christie, L.-A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends in neurosciences, 30(9), 464-472.
6. Damirchi, A., Tehrani, B. S., Alamdari, K. A., & Babaei, P. (2014). Influence of aerobic training and detraining on serum BDNF, insulin resistance, and metabolic risk factors in middle-aged men diagnosed with metabolic syndrome. Clinical Journal of Sport Medicine, 24(6), 513-518.
7. DeFeudis, F., & Drieu, K. (2000). Ginkgo biloba extract (EGb 761) and CNS functions basic studies and clinical applications. Current drug targets, 1(1), 25-58.
8. Defeudis, F. V. (2002). Bilobalide and neuroprotection. Pharmacological research, 46(6), 565-568.
9. Do, H. T., Bruelle, C., Tselykh, T., Jalonen, P., Korhonen, L., & Lindholm, D. (2013). Reciprocal Regulation of Very Low Density Lipoprotein Receptors (VLDLRs) in Neurons by Brain-derived Neurotrophic Factor (BDNF) and Reelin INVOLVEMENT OF THE E3 LIGASE Mylip/Idol. Journal of Biological Chemistry, 288(41), 29613-29620.
10. Drew, P. D., & Chavis, J. A. (2000). Inhibition of microglial cell activation by cortisol. Brain research bulletin, 52(5), 391-396.
11. Fujinami, A., Ohta, K., Obayashi, H., Fukui, M., Hasegawa, G., Nakamura, N.,... Ohta, M. (2008). Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: relationship to glucose metabolism and biomarkers of insulin resistance. Clinical biochemistry, 41(10), 812-817.
12. Gelfo, F., Tirassa, P., De Bartolo, P., Croce, N., Bernardini, S., Caltagirone, C.,... Angelucci, F. (2012). NPY Intraperitoneal Injections Produce Antidepressant-Like Effects and Downregulate BDNF in the Rat Hypothalamus. CNS neuroscience & therapeutics, 18(6), 487-492.
13. Halima, B. H., Sonia, G., Sarra, K., Houda, B. J., Fethi, B. S., & Abdallah, A. (2017). Apple Cider Vinegar Attenuates Oxidative Stress and Reduces the Risk of Obesity in High-Fat-Fed Male Wistar Rats. Journal of medicinal food.
14. Hashimoto, M. (2014). Omega-3 fatty acids and cognition. Nihon rinsho. Japanese journal of clinical medicine, 72(4), 648-656.
15. Hashimoto, M., Inoue, T., Katakura, M., Tanabe, Y., Hossain, S., Tsuchikura, S., & Shido, O. (2013). Prescription n-3 Fatty Acids, But Not Eicosapentaenoic Acid Alone, Improve Reference Memory-Related

Learning Ability by Increasing Brain-Derived Neurotrophic Factor Levels in SHR. Cg-Leprcp/NDmcr rats, A Metabolic Syndrome Model. Neurochemical research, 38(10), 2124-2135.

16. He, S.-X., Luo, J.-Y., Wang, Y.-P., Wang, Y.-L., Fu, H., Xu, J.-L.,... Liu, E.-Q. (2006). Effects of extract from Ginkgo biloba on carbon tetrachloride-induced liver injury in rats. World journal of gastroenterology: WJG, 12(24), 3924.

17. Jodayree, S., Patterson, Z. R., MacKay, H., Abizaid, A. B., & Tsopmo, A. (2014). Blood and liver antioxidant capacity of mice fed high fat diet supplemented with digested oat bran proteins. International Journal of Food Science and Nutrition Engineering, 4(1), 9-14.

18. Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 444(7121), 840-846.

19. Karczewska-Kupczewska, M., Strączkowski, M., Adamska, A., Nikołajuk, A., Otziomek, E., Górska, M., & Kowalska, I. (2011). Decreased serum brain-derived neurotrophic factor concentration in young nonobese subjects with low insulin sensitivity. Clinical biochemistry, 44(10), 817-820.

20. Kawada, T., Hagihara, K., & Iwai, K. (1986). Effects of capsaicin on lipid metabolism in rats fed a high fat diet. J Nutr, 116(7), 1272-1278.

21. Klop, B., Elte, J. W. F., & Cabezas, M. C. (2013). Dyslipidemia in obesity: mechanisms and potential targets. Nutrients, 5(4), 1218-1240.

22. Kuo, Y.-C., Lin, J.-C., Bernard, J. R., & Liao, Y.-H. (2015). Green tea extract supplementation does not hamper endurance-training adaptation but improves antioxidant capacity in sedentary men. Applied Physiology, Nutrition, and Metabolism, 40(10), 990-996.

23. Li, B., Lang, N., & Cheng, Z.-F. (2016). Serum levels of brain-derived neurotrophic factor are associated with diabetes risk, complications, and obesity: a cohort study from Chinese patients with type 2 diabetes. Molecular neurobiology, 53(8), 5492-5499.

24. Liu, X., Zhu, Z., Kalyani, M., Janik, J. M., & Shi, H. (2014). Effects of energy status and diet on Bdnf expression in the ventromedial hypothalamus of male and female rats. Physiology & behavior, 130, 99-107.

25. London, E., Lala, G., Berger, R., Panzenbeck, A., Kohli, A. A., Renner, M.,... Castonguay, T. W. (2007). Sucrose access differentially modifies 11β-hydroxysteroid dehydrogenase-1 and hexose-6-phosphate dehydrogenase message in liver and adipose tissue in rats. The Journal of nutrition, 137(12), 2616-2621.

26. MacLullich, A. M., Deary, I. J., Starr, J. M., Ferguson, K. J., Wardlaw, J. M., & Seckl, J. R. (2005). Plasma cortisol levels, brain volumes and cognition in healthy elderly men. Psychoneuroendocrinology, 30(5), 505-515.

27. Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American journal of clinical nutrition, 81(1), 230S-242S.

28. Manni, L., Nikolova, V., Vyagova, D., Chaldakov, G. N., & Aloe, L. (2005). Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. International journal of cardiology, 102(1), 169-171.

29. Masters, R. K., Reither, E. N., Powers, D. A., Yang, Y. C., Burger, A. E., & Link, B. G. (2013). The impact of obesity on US mortality levels: the importance of age and cohort factors in population estimates. American journal of public health, 103(10), 1895-1901.

30. Meng, R., Zhu, D.-L., Bi, Y., Yang, D.-H., & Wang, Y.-P. (2011). Anti-oxidative effect of apocynin on insulin resistance in high-fat diet mice. Annals of Clinical & Laboratory Science, 41(3), 236-243.

31. Negrón, A., Beymer, M., Yu, G., Warren, K., & Acosta-Martínez, M. (2015). Prolonged hyperglycemia & hyperinsulinemia increases BDNF mRNA expression in the posterior ventromedial hypothalamus and the dorsomedial hypothalamus of fed female rats. Neuroscience, 303, 422-432.

32. Noble, E. E., Billington, C. J., Kotz, C. M., & Wang, C. (2011). The lighter side of BDNF. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 300(5), R1053-R1069.

33. Pedersen, B. K., Pedersen, M., Krabbe, K. S., Bruunsgaard, H., Matthews, V. B., & Febbraio, M. A. (2009). Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals. Experimental physiology, 94(12), 1153-1160.

34. Rathod, R. S., Khaire, A. A., Kale, A. A., & Joshi, S. R. (2015). Beneficial effects of omega-3 fatty acids and vitamin B12 supplementation on brain docosahexaenoic acid, brain derived neurotrophic factor, and cognitive performance in the second-generation Wistar rats. Biofactors, 41(4), 261-272.

35. Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R.,... Jaenisch, R. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Molecular endocrinology, 15(10), 1748-1757.

36. Russo-Neustadt, A., Beard, R., Huang, Y., & Cotman, C. (2000). Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience, 101(2), 305-312.

37. Seifert, T., Brassard, P., Wissenberg, M., Rasmussen, P., Nordby, P., Stallknecht, B.,... Nielsen, H. B. (2009). Endurance training enhances BDNF release from the human brain. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 298(2), R372-R377.

38. Slusher, A., Whitehurst, M., Zoeller, R., Mock, J., Maharaj, A., & Huang, C. J. (2015). Brain-Derived Neurotrophic Factor and Substrate Utilization Following Acute Aerobic Exercise in Obese Individuals. Journal of neuroendocrinology, 27(5), 370-376.

39. Suwa, M., Kishimoto, H., Nofuji, Y., Nakano, H., Sasaki, H., Radak, Z., & Kumagai, S. (2006). Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female patients with type 2 diabetes mellitus. Metabolism, 55(7), 852-857.

40. Szatmari, E., Kalita, K. B., Kharebava, G., & Hetman, M. (2007). Role of kinase suppressor of Ras-1 in neuronal survival signaling by extracellular signal-regulated kinase 1/2. Journal of Neuroscience, 27(42), 11389-11400.

41. Tsuchida, A., Nonomura, T., Nakagawa, T., Itakura, Y., Ono-Kishino, M., Yamanaka, M.,... Noguchi, H. (2002). Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice. Diabetes, Obesity and Metabolism, 4(4), 262-269.

42. Ude, C., Schubert-Zsilavecz, M., & Wurglics, M. (2013). Ginkgo biloba extracts: a review of the pharmacokinetics of the active ingredients. Clinical pharmacokinetics, 52(9), 727-749.

43. Ueda, M., & Ashida, H. (2012). Green tea prevents obesity by increasing expression of insulin-like growth factor binding protein-1 in adipose tissue of high-fat diet-fed mice. Journal of agricultural and food chemistry, 60(36), 8917-8923.

44. Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. European Journal of Neuroscience, 20(10), 2580-2590.

45. Vendrell, J., Broch, M., Vilarrasa, N., Molina, A., Gómez, J. M., Gutiérrez, C.,... Richart, C. (2004). Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. Obesity, 12(6), 962-971.

46. Vilar, J., Leite, K., & Chen, L. C. (2009). Antimutagenicity protection of Ginkgo biloba extract (Egb 761) against mitomycin C and cyclophosphamide in mouse bone marrow. Genet. Mol. Res, 8(1), 328-333.

47. Wang, H., Ward, N., Boswell, M., & Katz, D. M. (2006). Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. European Journal of Neuroscience, 23(6), 1665-1670.

48. Wang, J., Zhou, S., Bronks, R., Graham, J., & Myers, S. (2007). Supervised exercise training combined with ginkgo biloba treatment for patients with peripheral arterial disease. Clinical rehabilitation, 21(7), 579-586.

49. Williamson, G., & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. The American journal of clinical nutrition, 81(1), 243S-255S.

50. Wong, D., Sullivan, K., & Heap, G. (2012). The pharmaceutical market for obesity therapies. Nature Reviews Drug Discovery, 11(9), 669-670.

51. Zhou, Y.-H., Yu, J.-P., Liu, Y.-F., Teng, X.-J., Ming, M., Lv, P.,... Yu, H.-G. (2006). Effects of Ginkgo biloba extract on inflammatory mediators (SOD, MDA, TNF-α, NF-κBp65, IL-6) in TNBS-induced colitis in rats. Mediators of inflammation, 2006.

52. Zoladz, J., Pilc, A., Majerczak, J., Grandys, M., Zapart-Bukowska, J., & Duda, K. (2008). Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. J Physiol Pharmacol, 59(Suppl 7), 119-132.

Table 1. Participant baseline characteristics from eligibility screening. Data are means (±SD).

GROUP	AGE (yrs)	BMI (kg/m2)
AT+ Q1Os	66.90 (3.69)	25.98 (1.20)
AT	67.90 (3.98)	26.02 (1.006)
Q1Os	67.80 (3.39)	25.89 (1.16)
Control	98.30 (4.11)	26.27 (0.92)

Fig 2. change in blood klotho marker.