TJEM TURKISH JOURNAL OF ENDOCRINOLOGY AND METABOLISM

ORIGINAL ARTICLE
Turk J Endocrinol Metab. 2022;26(4):203-207

The Evaluation of the Relationship of Klotho and Telomerase in Obese Patients

ABSTRACT

Objective: Obesity maintains its importance as an important public health problem with its increasing morbidity and mortality in all ages and social groups all over the world. A new protein Klotho, discovered in 1997, has an important role in the aging process. It has been already known that another factor related to aging is telomere length and telomerase activity. Telomere lengths shorten and aging occurs with decreasing telomerase activity. The purpose of the present study was to evaluate the relationship between Klotho protein, telomerase enzyme activity, and obesity, which is a risk factor for aging.

Methods: A total of 39 obese patients and 42 normal-weight patients were included in the study. Three tubes of blood were collected from the participants to determine the expression levels of the human telomerase reverse transcriptase gene and the Klotho and glycated hemoglobin levels. The Statistical Package for the Social Sciences version 21 was used in the statistical analysis of the study data.

Results: Although no significant differences were detected between the obesity group and the control group in terms of Klotho levels, telomerase activity was decreased in the obese group. When the relationship between Klotho and telomerase in the obese and control group was compared, no statistically significant relationships were detected in either group.

Conclusion: Although the comorbidities brought by obesity pose a risk for aging, this could not be demonstrated in the present study in terms of Klotho level and telomerase activity. Further studies are needed on this subject.

Keywords: Klotho, obesity, telomerase activity

Introduction

Obesity is a chronic and progressive disease with high mortality and morbidity because of the additional diseases and social problems it brings to the individual and draws attention as a common public healthcare issue with a gradually increasing importance. Obesity is the second most important cause of preventable deaths after smoking.[1] For this reason, many experimental and clinical studies on obesity have been started and health conditions caused or may be caused by obesity have begun to be investigated.

The Klotho gene, which was first discovered in 1997 in studies that were conducted on mice with multiorgan damage and shortened life span, is a gene encoding the Klotho protein, which is an anti-aging protein. This gene prolongs life span when overexpressed in mice, accelerates senescence when it is disrupted, and in this way this gene was found to be involved in suppressing aging phenotypes. The amount of Klotho protein decreases with aging, which was associated with chronic renal failure, atherosclerosis, emphysema, osteoporosis, hyperphosphatemia, skin and muscle atrophy, growth retardation, premature death, infertility, and arterial calcification.[2] Generally, the Klotho family includes 3 isoforms of the Klotho protein, including α, β, and γ Klotho, which have pleiotropic functions in vivo. Studies showed that α-Klotho can affect insulin and Wnt signaling, reduce oxidative stress, and regulate mineral homeostasis, and c-Klotho was associated with metabolic regulation, glucose and fatty acid metabolism, and bile acid synthesis. However, the function of γ-Klotho remains largely unclear.

Telomeres are sequences that are located at the end regions of eukaryotic chromosomes preserving the integrity of the chromosomes and preventing them from joining. This sequence contains 5′-TTAGGG-3′ repetitions in humans. The preservation of telomere sequences

Esma Pehlivan Köroğlu[1]
Bakiye Göker Bağca[2]
Ilgın Yıldırım Şimşir[1]
Cumhur Gündüz[3]
Lütfiye Füsun Saygılı[1]

[1]*Department of Endocrinology and Metabolism, Ege University, İzmir, Turkey*
[2]*Department of Medical Biology, Adnan Menderes University, Aydın, Turkey*
[3]*Department of Medical Biology, Ege University, İzmir, Turkey*

Corresponding author:
Esma Pehlivan Köroğlu
✉ esmapehlivan@gmail.com

Received: May 17, 2022
Accepted: September 19, 2022
Publication Date: December 1, 2022

Cite this article as: Pehlivan Köroğlu E, Göker Bağca B, Yıldırım Şimşir I, Gündüz C, Saygılı LF. The evaluation of the relationship of klotho and telomerase in obese patients. *Turk J Endocrinol Metab.* 2022;26(4):203-207.

DOI: 10.5152/tjem.2022.22057

during DNA replication occurs via the "telomerase enzyme." However, telomere sequences shorten in the aging process, and cell division is stopped after a certain number of divisions with the shortening of telomeres.[3] The expression level of the human telomerase reverse transcriptase (hTERT) gene, which encodes the catalytic subunit of the human telomerase enzyme, can be employed to determine the telomerase enzyme activity.

Determining the possible relationship between α-Klotho protein and hTERT gene expression levels, which are known to have anti-aging effects, the body mass index (BMI), and the probability of a direct organic association between obesity and aging constituted the hypothesis of this study.

Materials and Methods

Determining the Study Group

The present study was conducted in Ege University Faculty of Medicine Internal Diseases Department, Endocrinology and Metabolism Diseases Clinic between January and February 2017. Obese patients with a BMI of 35-39.9 kg/m^2 were included in the study as the patient group among the individuals aged 30-50 years, and individuals with a BMI of 18.5-24.9 kg/m^2 were taken as the control group. Individuals who were younger than 30 years of age or older than 50 years of age, those who had a history of surgery in the last 1 week, individuals with acute kidney failure or chronic kidney failure, pregnant women, those in the lactation period, and those with malignancies were not included in the study. Ethics committee approval was received for this study from the Ethics Committee of Ege University (approval no: 16-2.1/14). An informed consent form was obtained from all patients participating in the study. Ege University Scientific Research Project support was received for the study (16-TIP-003).

Determining the Klotho Level

The Klotho level was examined with commercially available ELISA kits (MYBIOSOURCE, HUMAN SOLUBLE ALPHA-KLOTHO [SAKL] ELISA KIT) based on the "Sandwich Enzyme Immunoassay." The blood sample was collected in flat tubes and centrifuged at 1500 × *g* (or 5000 rpm) for 20 minutes within 30 minutes of sample collection. Supernatants were collected carefully and stored at −80°C until assayed. After all the samples were collected, the samples that were taken from −80°C were allowed to thaw at room temperature and were then studied in line with the manufacturer's instructions.

Determining Telomerase Gene Expression Level

The expression level of the hTERT gene, which encodes the catalytic subunit of the human telomerase enzyme, was determined with the quantitative reverse transcripatse polymerase chain reaction method for the evaluation of telomerase enzyme activity. For this purpose, total RNA isolation from patient and control group samples was made by using the QIAamp RNA Blood Mini Kit (Qiagen). The measurement of the concentrations and the determination of purity values of the RNA samples were made by using the NanoDrop 1000 (Thermo Scientific) device and its software. The quantification of hTERT mRNA from the RNA samples that had appropriate purity and concentration was made with the TeloTAGGG hTERT Quantification Kit (Roche) and LightCycler 480 (Roche) device. Porphobilinogen deaminase (PBGD) housekeeping gene was used for the normalization. The copy numbers of the hTERT and PBGD gene expressions were determined with the LightCycler Quantification Software according to the Ct values with a certain standard, and the normalized copy number (hTERT mRNA copy number/PBGD mRNA copy number × 100) was calculated. The fold change was determined by dividing the hTERT normalized copy number of the patient group by the hTERT normalized copy number of the control group.

Statistical Analysis

The data obtained from the patients were recorded and evaluated in the Microsoft Office Excel program. The Statistical Package for the Social Sciences version 21.0 (IBM Corp.; Armonk, NY, USA) was used in the statistical analysis of the study data. The Mann–Whitney *U* test was used for the pairwise comparison of numerical variables of independent groups, and the chi-square test was used for pairwise comparison of categorical variables. The Spearman's correlation test was used to evaluate the relationships between intra-group numerical variables and $P < .05$ was taken as statistically significant.

Results

A total of 81 people, 42 in the control group and 39 in the patient group, were included in the present study (43 female and 38 male). When the participants were evaluated according to BMI scores, the mean BMI of the obesity group was 36.66 ± 1.68, and the mean BMI of the control group was 22.5 ± 1.86. The sociodemographic characteristics of the participants (e.g., gender, marital status, educational status, smoking history, and BMI) are given in Table 1.

Relationship Between Obesity and Klotho Levels

Among the patients who were included in the study, the mean age of the obesity group was found to be 40.97 ± 5.17 and that of the control group was 38.69 ± 5.80. In the obesity group, when the relationship between α-Klotho levels and age was evaluated, it was found that there was no relationship ($P = .461$). Similarly, when the α-Klotho level was compared with age in the control group, no relationship was detected in this group ($P = .453$). No decrease was detected in α-Klotho levels with age for both groups in the present study.

When the relationship between α-Klotho level and BMI, which is one of the evaluations that made up the main target of the study, was evaluated, no relationships were detected in the obesity group or in the control group ($P = .509$ and $P = .920$, respectively).

MAIN POINTS

- Obesity is a chronic and progressive health problem increasing in frequency and has become a pandemic.
- Obesity is one of the most investigated healthcare problems all over the world, and there are many studies on its effect on life expectancy. Klotho level and telomerase activity, which are 2 anti-aging factors, were evaluated in the present study.
- No relationships were detected between body mass index and Klotho levels in the present study.
- An inverse relationship was detected between body mass index and telomerase activity in the study. Telomerase activity decreased as the body mass index increased.
- No correlations were detected between Klotho levels and telomerase activity for both the obese group and the control group.

Table 1. Sociodemographic Characteristics

	Obesity Group (n = 39)	Control Group (n = 42)
Gender		
Female (n = 43) (53.1%)	21 (53.8%)	22 (52.4%)
Male (n = 38) (46.9%)	18 (46.2%)	20 (47.6%)
Marital status		
Single (n = 13) (16%)	5 (12.8%)	8 (19%)
Married (n = 68) (84%)	34 (87.2%)	34 (81%)
Education status		
Primary school (n = 10) (12.3%)	8 (20.5%)	2 (4.8%)
Middle school (n = 6) (7.4%)	6 (15.4%)	0 (0%)
High school (n = 10) (12.3%)	5 (12.8%)	5 (11.9%)
University (n = 55) (67.9%)	20 (51.3%)	35 (83.3%)
Cigarette		
Smoker (n = 54) (66.7%)	29 (74.4%)	25 (59.5%)
Non-smoker (n = 27) (33.3%)	10 (25.6%)	17 (40.5%)
BMI (kg/m^2)	3666 ± 1.68	22.50 ± 1.86

BMI, body mass index.

When the relationship between α-Klotho and glycated hemoglobin (Hba1c) levels of obese patients was examined, a statistically significant difference was detected ($P = .042$). As the Hba1c level increased, the α-Klotho level decreased. The mean Hba1c of the patients was found to be 5.91 ± 1.23. Four patients were diagnosed with newly diagnosed type 2 diabetes mellitus (DM), and these 4 patients were in the obesity group in the study. When the obesity group was evaluated in terms of α-Klotho and Hba1c levels after excluding these 4 patients with DM (n = 35), the statistical significance continued and the relationship between them became stronger ($P = .010$). After the correction, it was found that the Hba1c average of the patients was 5.57 ± 0.30 ($P = .640$). The relationship between Hba1c levels and α-Klotho levels of the patients is given in Table 2.

Considering the relationship between smoking and α-Klotho in the obesity and control groups, no relationship was detected in the groups.

The Relationship Between Telomerase and Obesity

The relationship between hTERT gene expression level and age was evaluated in the obesity group; however, no relationship was found in this regard ($P = .251$). Similarly, the relationship between hTERT gene expression level and age was evaluated in the control group, and statistical significance was not detected in this group ($P = .394$).

Among the participants who were divided into groups according to BMI scores, a correlation was detected between the hTERT gene expression levels and BMI scores in the obese group ($P = .002$). As BMI increased, hTERT gene expression level decreased. When this relationship was evaluated in the control group, it was found to be statistically significant ($P = .029$) (as BMI increased, telomerase level decreased).

Table 2. Relationship between Klotho Level and Hba1c

	Hba1c	Hba1c After Excluding 4 DM Patients in the Obesity Group (n = 35)
Obesity group (n = 39)	5.91 ± 1.23 ($P = .042$)	5.57 ± 0.30 ($P = .010$)
Control group (n = 42)	5.17 ± 0.29 ($P = .640$)	

Hba1c, glycated hemoglobin; DM, diabetes mellitus.

When the relationship between hTERT gene expression levels and Hba1c levels was evaluated in the obese and control groups, no statistical significance was detected in the groups ($P = .171$-$.808$).

When the relationship between smoking and hTERT gene expression level was evaluated in the obese group, a statistically significant relationship was detected ($P = .021$). As smoking increased, hTERT gene expression level also increased. There was no such relationship in the control group ($P = .808$).

When the relationship between α-Klotho and hTERT gene expression levels was evaluated, which was the main target of the present study, no relationship was detected in the obese and control group ($P = .795$-$.224$).

Discussion

Obesity is among the most investigated healthcare problems all over the world, and there are many studies on its effect on life expectancy. However, this study was conducted to determine the relationship between α-Klotho protein and hTERT gene expression levels and obesity, and as far as we know, it is one of the first studies in this field. In the present study that compared obese patients with a BMI of 35-39.9 kg/m^2 and individuals with a BMI of 18.5-24.9 kg/m^2, no significant difference was detected in the level of Klotho and hTERT gene expression. The hTERT gene expression level was lower in obese patients when compared to the control group. The decrease in hTERT gene expression level in the obese group was the most significant finding in the study and is also important in terms of data proving that obesity shortens life expectancy.

Studies reporting a possible relationship between Klotho protein level and aging in humans are still limited. There are studies supporting that the Klotho gene plays a role in the aging process and aging-related diseases in mammals. Age-like phenotypes occur with the decreased Klotho levels. Another study reported that overexpression of the Klotho gene increases life expectancy by 20%-30%[2,4,5]. Interestingly, although the Klotho gene is expressed in a limited number of tissues, it was reported that damage to the Klotho gene causes many senescence-like phenotypes in all tissues and organs.[2] The excessive synthesis of Klotho slows down the aging process at significant levels prolonging the life span by providing resistance to oxidative stress. When the results of the present study were evaluated, no significant correlation was detected between Klotho and age in the obese and control group. This may be because of the small number of participants in the study and the narrow age range.

The relationship between Klotho and obesity was evaluated in 11 obese, 12 anorexia nervosa, and 11 control patients who were grouped by Amitani et al[6] and it was shown that the plasma Klotho levels were lower in the obese and anorexia nervosa patients compared to the control group. Also, in another study by Jackson et al,[7] it was shown that the Fibroblast growth factor (FGF)-Klotho axis plays a role in eating behavior and energy homeostasis as well as other signaling systems in obese patients. In the present study, however, no difference was detected between the obese group and the control group in terms of Klotho levels. This may be because the obese group

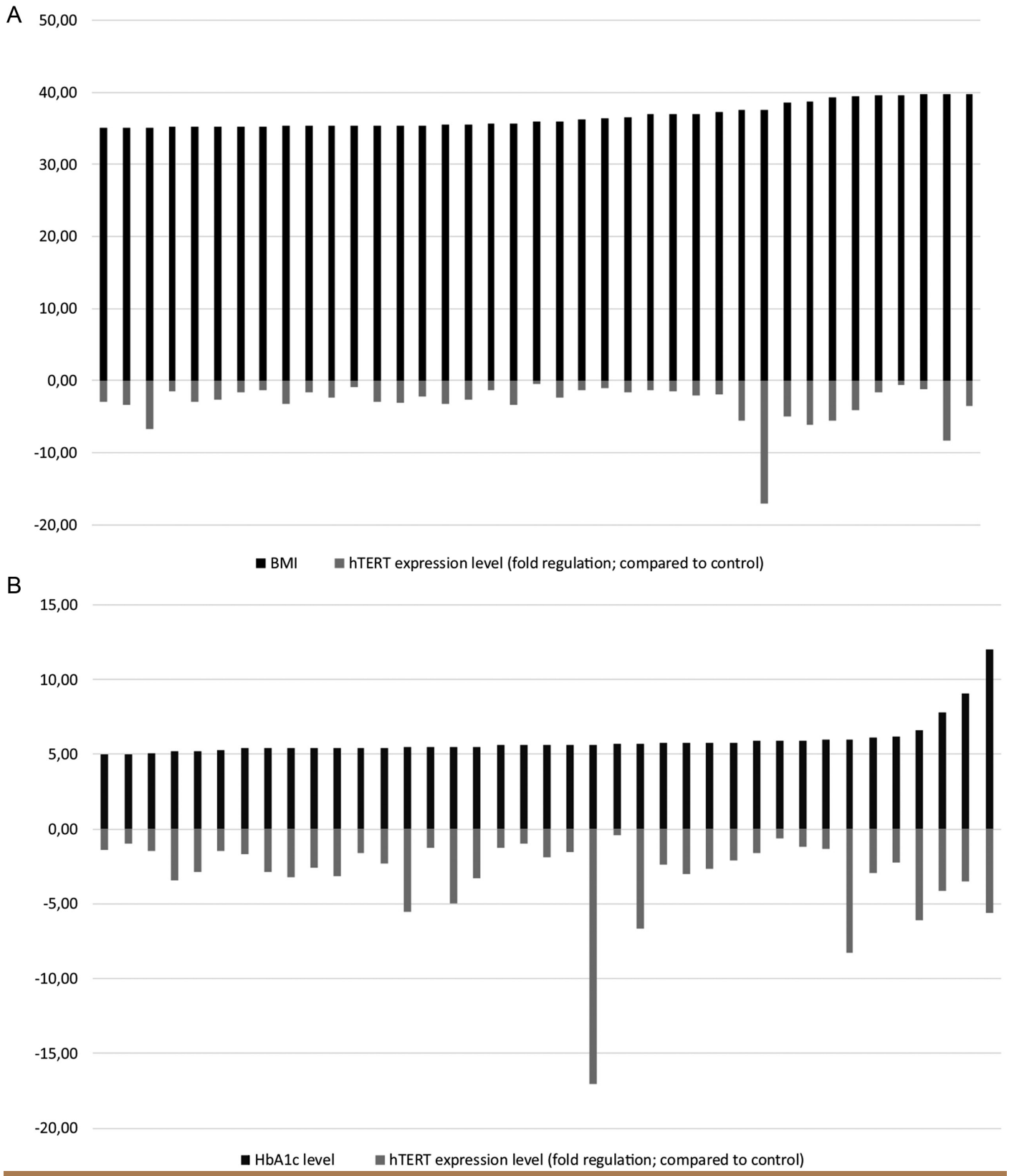

Figure 1. (A) The fold change between BMI and hTERT gene expression levels for the obese group. (B) The fold change between Hba1c and hTERT gene expression levels for the obese group.

that was included in our study consisted of Stage 2 obese patients with a BMI of 35-39.9 kg/m^2.

An inverse relationship was detected between α-Klotho and Hba1c levels in the obesity group in the present study. As the Hba1c level increased, the α-Klotho level decreased. Four patients were diagnosed with type 2 DM. The persistence of a significant correlation between Klotho and Hba1c, even when cases with type 2 DM were excluded, indicates that there is a relationship independent of the presence of type 2 DM. In the literature review, it was found that there are studies evaluating this issue for patients with type 2 DM. In one study, a negative relationship was reported between α-Klotho and fructosamine and Hba1c in a type 2 DM model. In the same study, a negative relationship was also found between β-Klotho and fructosamine and Hba1c. However, no significant correlations were detected between α-Klotho or β-Klotho and serum glucose levels.[8] In another study, type 2 DM patients were reported to have lower α-Klotho and β-Klotho levels than healthy controls, but serum α-Klotho and β-Klotho levels were not significantly correlated with Hba1c levels in type 2 DM patients. Contrary to the previously mentioned study this one shows a negative correlation between serum levels of Klotho protein and glucose, independent of Hba1c levels, which suggested that serum Klotho levels were directly affected by high blood glucose.[9]

The relationship between telomere length and telomerase activity is already known. Telomeres become shorter with the decreased telomerase activity, and this is defended as one of the theories that cause aging. The expression level of the hTERT gene, which encodes the catalytic subunit of the human telomerase enzyme, is important in terms of indicating telomerase activity. It is also known that telomere length shortens with advancing age.[10] In the study by Takubo et al[11] it was found that human tissues other than the brain and myocardium always show telomere shortening with aging. In the present study, no relationship was detected between age and hTERT gene expression levels in the obese and control group.

The literature display that there are many studies on the relationship between obesity and short telomere length, but limited number of studies evaluated telomerase activity in relation to obesity. In different studies conducted on telomere and telomerase effects, it was reported that various environmental factors, lifestyles (i.e., regularly consumed alcohol and cigarettes, diet, and exercise), existing free radicals, and some antioxidants affect telomere length and accelerate the aging process.[12-14]

The small number of patients and the inclusion of the Stage 2 obese group were the limitations of this study. However, our study is of interest in terms of evaluating obesity and anti-aging conditions. It is already well known that 3%-5% weight loss in adults greatly improves adverse health outcomes. In this study, it was determined that telomerase activity decreased, which once again supports that obesity is a condition that shortens life expectancy. Now, longitudinal studies on obese patients are needed to show if telemorase level increase by weight loss.

Ethics Committee Approval: The study was approved by the ethical committee of Ege University (approval no: 16-2.1/14).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.P.K., I.Y.Ş., L.F.S; Design – E.P.K., I.Y.Ş., C.G., L.F.S.; Supervision – E.P.K., I.Y.Ş., C.G., L.F.S.; Funding – E.P.K., B.G.B., C.G., L.F.S.; Materials – E.P.K., B.G.B., C.G., L.F.S.; Data Collection and/or Processing – E.P.K., B.G.B., C.G., L.F.S.; Analysis and/or Interpretation – E.P.K., B.G.B., C.G.; Literature Review – E.P.K., L.F.S.; Writing – E.P.K.; Critical Review – E.P.K., L.F.S.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: This study was funded by the Ege University, BAP Grant/Award (number: 16 TIP 003).

References

1. Obesity, Lipid Metabolism and Hypertension Working Group. *Obesity Diagnosis and Treatment Guide*. *Turkish Society of Endocrinology and Metabolism*; Ankara, Turkey. 2017:11-19.
2. Kuro-o M, Matsumura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997;390(6655):45-51. [CrossRef]
3. Kusoglu A, Goker Bagca B, Ozates Ay NP, Gunduz C, Biray Avci C. Telomerase inhibition regulates EMT mechanism in breast cancer stem cells. *Gene*. 2020;759:145001. [CrossRef]
4. Koyama D, Sato Y, Aizawa M, et al. Soluble αKlotho as a candidate for the biomarker of aging. *Biochem Biophys Res Commun*. 2015;467(4):1019-1025. [CrossRef]
5. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone klotho. *Science*. 2005;309(5742):1829-1833. [CrossRef]
6. Amitani M, Asakawa A, Amitani H, et al. Plasma klotho levels decrease in both anorexia nervosa and obesity. *Nutrition*. 2013;29(9):1106-1109. [CrossRef]
7. Jackson VM, Breen DM, Fortin JP, et al. Latest approaches for the treatment of obesity. *Expert Opin Drug Discov*. 2015;10(8):825-839. [CrossRef]
8. Zhang L, Liu T. Clinical implication of alterations in serum klotho levels in patients with type 2 diabetes mellitus and its associated complications. *J Diabetes Complications*. 2018;32(10):922-930. [CrossRef]
9. Nie F, Wu D, Du H, et al. Serum klotho protein levels and their correlations with the progression of type 2 diabetes mellitus. *J Diabetes Complications*. 2017;31(3):594-598. [CrossRef]
10. Hewakapuge S, van Oorschot RA, Lewandowski P, Baindur-Hudson S. Investigation of telomere lengths measurement by quantitative real-time PCR to predict age. *Leg Med (Tokyo)*. 2008;10(5):236-242. [CrossRef]
11. Takubo K, Aida J, Izumiyama-Shimomura N, et al. Changes of telomere length with aging. *Geriatr Gerontol Int*. 2010;10(suppl 1):S197-S206. [CrossRef]
12. Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology*. 2005;6(1):71-75. [CrossRef]
13. Arsenis NC, You T, Ogawa EF, Tinsley GM, Zuo L. Physical activity and telomere length: impact of aging and potential mechanisms of action. *Oncotarget*. 2017;8(27):45008-45019. [CrossRef]
14. Denham J, O'Brien BJ, Charchar FJ. Telomere Length Maintenance and Cardio-Metabolic Disease Prevention Through Exercise Training. *Sports Med*. 2016;46(9):1213-1237.