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The Effects of Resveratrol Supplementation on Bone Tissue SIRT1 levels in old Female Rats with Diabetic

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Abstract – The aim of this study was to investigate how resveratrol administration diabetic elderly female rats affects the levels of bone tissue SIRT1.
The study was performed on female aged rats (16 months old) who were provided by The Experimental Medicine Research and Application Center of Selçuk University. A total of 24 elderly female rats were divided into 4 groups: Group 1. Control, Group 2. Control + Resveratrol, Group 3. Diabetes, Group 4. Diabetes + Resveratrol. In order to induce diabetes, the animals were intraperitoneally (IP) injected with 40 mg/kg streptozotocin (STZ). The animals were supplemented with 5 mg/kg/day resveratrol IP for 4 weeks. At the end of the study, SIRT1 protein gene expression were determined by PCR, on bone tissue samples obtained from the animals sacrificed under general anesthesia.
In our study, the highest bone tissue SIRT1 expression values were obtained in the diabetes + resveratrol group and the lowest bone SIRT 1 levels were found in the diabetes group (G3).
Our results suggest that resveratrol supplementation increases bone SIRT1 expression in diabetic elderly female rats.

Keywords – *old female rats, diabetes mellitus, resveratrol, bone tissue, SIRT1*

I. Introduction

Diabetes and osteoporosis are common diseases with growing prevalence in the aging population [1]. Many recent studies have reported an association between diabetes mellitus and an increased osteoporosis rate [1]. Compared to control subjects, decreased bone mineral density has been observed in patients with type 1 diabetes mellitus, while those with type 2 diabetes display a unique skeletal phenotype of increased bone mineral density, but impaired architectural structure and mineral properties [2]. Accumulation of advanced glycation end products changes collagen structure and suppression of bone turnover causes impairment of repair and adaptation mechanisms [1],[2]. Sirtuins have been identified as a family of antiaging genes and have been shown to increase lifespan in lower organisms [3]. SIRT1 activity declines with age, suggesting the association of SIRT1 with age-related diseases [3]. One of the age-related diseases is bone loss. Therefore, it is likely that SIRT1 functions in bone [3],[4]. Indeed, SIRT1 is expressed in osteoblasts, and Osteoblast-specific or mesenchymal-stem cell-specific Sirt1 deletion results in loss of bone mass [3,4]. On the other hand, SIRT1 overexpression protects against age-induced bone loss in mice, and SIRT1 activation prevents bone loss [3],[4]. Resveratrol has been shown to stimulate differentiation of osteoblast cells in vitro [5]. Resveratrol promotes osteoblast differentiation from mesenchymal stem cells in vitro, reduces the formation of bone-resorbing osteoclasts and may be protective in an experimental model of accelerated bone disease [6]. Therefore, it can be suggested that resveratrol supplementation may be beneficial to bone health as an experimental drug in preventing age-related decline in functional integrity or in disorders of excessive bone destruction [5],[6]. Resveratrol is a natural antioxidant substance of polyphenol which is abundant in grape seeds and is a natural stimulant of Sirtuin-1 (SIRT-1) protein gene activation, which can also affect bone metabolism [6]. Therefore, the use of resveratrol as a potential therapeutic tool to reduce the risk of osteoporosis is promising.

The aim of this study was to investigate how RSV supplementation diabetic elderly female rats affects the levels of bone SIRT1.

II. Materials and Method

The study was performed on elderly female rats (16 months) obtained from Selçuk University Experimental Medicine Research and Application Center. The ethics committee of the same center approved the study. A total of 24 elderly female rats were divided into 4 groups.

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Group 1. Control: The group which was not subjected to any procedure and fed on a normal diet.

Group 2. Control + Resveratrol: The group which was fed on a normal diet and was additionally administered 5 mg/kg/day intraperitoneal (ip) resveratrol for 4 weeks.

Group 3. Diabetes: The group which was induced diabetes with intraperitoneal "50 mg/kg" streptozotocin (STZ)

Group 4. Diabetes + Resveratrol: The group which was induced diabetes with intraperitoneal "50 mg/kg" streptozotocin (STZ) injection and which was then administered 5mg/kg/day intraperitoneal (ip) resveratrol for 4 weeks.

A. Experimental animals

Experimental animals were kept in special steel cages which were washed and cleaned every on a daily basis. They were fed from special steel bowls and water (normal tap water) was given by glass feeding bottles. They were fed on 10 g feed per 100 g body weight daily. They were kept in an environment with 12 hour dark/12 hour light cycles and standard room temperature (21±1ºC). All injections were given at 09:00-10:00 a.m.

After 4-weeks resveratrol treatment period, SIRT1 protein gene expression were determined by PCR, on bone tissue samples obtained from the animals sacrificed under general anesthesia.

B. Experimental procedures

Induction of diabetes in experimental animals

In order to induce diabetes in experimental animals, 40 rats were used as diabetes groups. The rats were injected with 50 mg/kg intraperitoneal streptozotocin (STZ) "Sigma S-0130". Blood glucose levels of the animals were determined in the blood taken from the tail vein of the animals 6 days after the injection by using a diagnostic glucose kit. Animals with blood glucose at or above 300 mg/dlt were accepted diabetic [7].

Resveratrol supplementation

The resveratrol supplemented groups (group 2 and group 4) was given daily intraperitoneal resveratrol (R5010-Sigma; 5 mg/kg) for 4 weeks.

C. Statistics

A computer software package was used in the statistical evaluation of results. Arithmetic means and standard errors of all parameters were calculated. Variance analysis was used to determine differences between groups. The Least Significant Difference "LSD" Test was employed to compare group means in the statistically significant variance analysis results. Differences for which $p<0.05$ were accepted significant.

III. RESULTS

In our study, the highest bone tissue SIRT1 expression values were obtained in the diabetes + resveratrol group and the lowest bone SIRT 1 levels were found in the diabetes group (G3) (Table 1).

Table 1. Bone SIRT1 Gene Activation of Study Groups (2-Δ CT)

Groups (n=6)	**SIRT1 Gene Activation ($2^{-\Delta CT}$)**
G1 Control	0,109±0,099[b]
G2 Control+Resveratrol	0,108±0,103[b]
G3 Diabetes	0,003±0,037[c]
G4 Diabetes+Resveratrol	0,253±0,186[a]

a,b,c: *Means with different superscripted letters in the same column are statistically significant ($p<0.05$).

IV. CONCLUSION

Our results suggest that resveratrol supplementation increases bone SIRT1 expression in diabetic elderly female rats.

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