

## The Effects of Melatonin Administration 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 melatonin 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 + Melatonin, Group 3. Diabetes, Group 4. Diabetes + Melatonin. 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 melatonin 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 + melatonin group and the lowest bone SIRT 1 levels were found in the diabetes group (G3). Our results suggest that melatonin supplementation increases bone SIRT1 expression in diabetic elderly female rats.

**Keywords** – melatonin, bone tissue, sirt1, diabetic, old rats

### I. INTRODUCTION

In patients with diabetes mellitus, higher risks of impaired bone metabolism are widely reported [1]. Long-term exposure to a diabetic environment leads to changes in bone metabolism and impaired bone micro-architecture through a variety of mechanisms on molecular and structural levels [2]. These changes predispose the bone to an increased fracture risk and impaired bone healing [2]. As a result Impaired bone quality and increased fracture risk have become recognized complications of diabetes mellitus [1,2]. It was demonstrated in a study which included rats with streptozotocin-induced diabetes that diabetes caused bone destruction by significantly increasing urinary excretion of calcium-phosphorus [3]. Consequently numerous studies also attest to the fact that diabetes has a negative effect on bone metabolism [1,3]. The radical scavenger antioxidant melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted mainly by the pineal gland, which has the ability to stimulate antioxidant enzymes that neutralize free radicals and ROS [4]. Melatonin also contributes to the maintenance of bone health by promoting osteoblast differentiation and limiting osteoclastic activity [4]. Bone healing process consists of inflammatory, proliferative, and remodeling phases [4]. The production of free radicals causes cell damage and disruption of bone healing process due to the chain reactions of protein and lipid peroxidation [5]. Melatonin participates in the physiological functions of bone cells, promotes angiogenesis and, through its free radical scavenging properties, it may also serve as a preventive agent against radical-induced hard tissue damages

[4,5]. Melatonin has been reported to be a potent activator of SIRT1 in many diseases [6]. Similarly It has been reported that the neuroprotective effects of melatonin against bone destruction is via the SIRT1 signaling pathway [7]. The aim of this study was to investigate how melatonin administration diabetic elderly female rats affects the levels of bone tissue 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.

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

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

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

Group 4. Diabetes + Melatonin: The group which was induced diabetes with intraperitoneal “50 mg/kg” streptozotocin (STZ) injection and which was then administered 5mg/kg/day intraperitoneal (ip) melatonin 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 melatonin 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 40 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 (8).

**Melatonin supplementation**

After 40 mg of melatonin (Sigma M-5250) was dissolved in pure ethanol, this suspension was kept capped and in the dark in a deepfreeze, until it was used. Of the stock solution, 0.1 ml was taken, added 0.9 ml NaCl (5 mg/kg/day) and injected to rats at 09:00 a.m. through intraperitoneal route. Melatonin supplementation was carried out at the same hour for 4 weeks.

**C. Statistic.**

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 + melatonin group and the lowest bone SIRT 1 levels were found in the diabetes group (G3).

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

Groups (n=6)	SIRT1 Gene Activation (2 <sup>-Δ CT</sup> )
G1 Control	0,109±0,099 <sup>b</sup>
G2 Control+Melatonin	0,114±0,103 <sup>b</sup>
G3 Diabetes	0,003±0,001 <sup>c</sup>
G4 Diabetes+Melatonin	0,184±0,083 <sup>a</sup>

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

**IV. CONCLUSION**

The findings of our study show that bone tissue damage can be prevented by melatonin supplementation in diabetic elderly female rats. This protective effect of melatonin supplementation shows the bone SIRT1 activity in diabetic elderly rats.

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