EFFECT OF MELATONIN SUPPLEMENTATION ON PLASMA GLUCOSE AND LIVER GLYCOGEN LEVELS IN RATS SUBJECTED TO ACUTE SWIMMING EXERCISE

OKTAY KAYA[1]*, MEHMET KİLİC[2], ILHAMİ CELİK[3], ABDULKERİM KASİM BALTACİ[4] AND RASİM MOGULKOC[4]

[1]*School of Physical Education and Sports Firat University, Elazig-Turkey*
[2]*School of Physical Education and Sports Selçuk University, Konya, Turkey*
[3]*School of Veterinary, Department of Histology, Selcuk University, Konya, Turkey*
[4]*Meram School of Medicine, Department of Physiology, Selçuk University, Konya, Turkey*

ABSTRACT

The aim of the present study is to examine how melatonin supplementation affects plasma glucose and liver glycogen levels in rats subjected to acute swimming exercise.

Spraque-Dawley species thirty adult male rats were allocated to 3 groups with equal number of animals: general control group which was not subjected to any procedure (Group 1), the group subjected to a 30-minute acute swimming exercise (Group 2), and the group subjected to a 30-minute acute swimming exercise after intraperitoneal (i.p.) melatonin supplementation (3 mg/kg/day) for 4 weeks (Group 3). Blood samples collected from the experimental animals by decapitation method were analyzed in terms of plasma glucose, and glycogen levels were determined in liver tissue samples by histological method.

The highest plasma glucose levels were obtained in group 2 ($p<0,05$). Plasma glucose levels in groups 1 and 3 were not different. Mean liver glycogen level in group 3 was significantly higher than those in the other groups ($p<0,01$), while there was no significant difference between group 1 and group 2 in terms of this parameter.

Results of the study demonstrate that melatonin supplementation can have a protective effect on liver glycogen reserves in rats subjected to acute swimming exercise.

Keywords: Melatonin, swimming, glucose, liver glycogen.

INTRODUCTION

A possible relation between melatonin hormone secreted from the pineal gland and exercise has been put forward (Meeking *et al.,* 1999). It has been argued both that physical activity can change plasma melatonin levels (Hara *et al.,* 1996) and that melatonin supplementation has a performance enhancing effect in exercise (Buxton *et al.,* 1997; Buxton *et al.,* 1997). The fact that melatonin supplementation to exercised rats has been demonstrated to lead to a decline in plasma lactate levels and an increase in muscle and liver glycogen levels (Mazepa *et al.,* 2000) can be presented as an interesting example of the relation between melatonin and exercise. It has been shown that melatonin supplementation successfully prevents lipid peroxidation in rats subjected to swimming exercise (Hara *et al.,* 1996; Hara *et al.,* 1997). High plasma melatonin levels found in women subjected to physical activity relative to controls who were not exercised indicates that the relation between melatonin and exercise is not one-directional (Diaz and Blaxque, 1986; Diaz *et al.,* 1993). However, the report to the effect that nocturnal exercise inhibits melatonin levels (Monteleone *et al.,* 1990) points to the need for many-sided research on this topic. It has been observed that blood glucose levels which increase after intravenous glucose supplementation decline during sleep. The fact that melatonin has been reported to play an important role in this decrease in blood glucose level (Rohr and Herold, 2002) can be cited as proof of a relation between carbohydrate mechanism and pineal gland. Melatonin's increasing the use of liver carbohydrates in rats (Anisimov, 2003) is a significant result supporting this relation. The aim of the present study is to explore how melatonin supplementation affects glucose and liver glycogen levels in rats subjected to acute swimming exercise.

MATERIALS AND METHODS

This study was conducted in Selcuk University Experimental Medicine Research and Application Center (SUDAM) on 30 adult male rats of Spraque-Dawley species, supplied thereof. The experimental animals were divided into 3 groups as follows:

Group 1 (n: 10) Normal Control Group: the group which was not subjected to any procedure and fed on a normal diet.

Group 2 (n: 10) Swimming Control Group: the group which was fed on a normal diet and subjected to 30 minutes acute swimming exercise.

Corresponding author: e-mail: kmoktaykaya@yahoo.com.tr

Group 3 (n: 10) Melatonin-Supplemented Swimming Group: the group which was supplemented with i.p. melatonin (3 mg/kg/day) for 4 weeks and subjected to 30 minutes acute swimming exercise.

Swimming exercise

Exercise was performed in a 50-cm deep pool made of heat-resistant glass and having a thermostat that kept the heat of the water fixed at 37°C. The exercise was a one-time (lasting 30 minutes) acute swimming exercise. Experimental animals were made to swim at the end of the study and before decapitation in groups of two. Rats in group 1 (normal control group) were not subjected to swimming exercise.

Plasma glucose analyses

Glucose analyses were conducted in the blood samples collected from the animals (1 ml) and centrifuged at 3000 rotations for 5 minutes to separate plasma. The analyses were carried out in Olympus AU 560 brand autoanalyzer using glucose kits of the device according to colorimetric method. The results (mg/dl) were read at 540-600 wavelength.

Glycogen analysis in liver tissue by histological method

Tissue samples collected for histological examinations from the livers of the experimental animals constituting the study groups were fixated in Rosmann's fixative fluid (Hopwood, 1990) at +4°C for 24 hours. Tissue samples were kept in 96% renewed alcohol till the color of picric acid in the fixative fluid was removed from the tissues. The tissues passed through degreed alcohols and xylol were put into paraffin blocks. Two 5µm cross-sections were taken from each sample. One of the cross-sections was treated with α amylase enzyme (Sigma A 3410) at 37°C for an hour to prepare the negative control preparations. Slides treated with enzyme and slides known to be positive controls were stained at the same time with Best's Carmine Technique (Cook, 1990). The preparations were examined in Leitz Laborlux-12 laboratory microscope and photographs of the sites which were considered necessary were taken by Leitz Ortholux II light microscope. Findings in the histological examination were determined by scoring method. The cells which were not stained were evaluated as (0), 25% staining as (1), half of the cell's staining as (2), more than half of the cell's staining as (3) and all of the cell's staining as (4).

STATISTICS

Arithmetic means and standard errors of the parameters were calculated in the statistical evaluations about glucose. Variance analysis was employed to determine the differences between groups. Least Significant Difference Test "LSD" was used to compare group means in variance analysis results which were found statistically significant. Mann-Whitney U test was used in the statistical analysis of histological findings and median values were compared (comparison of groups in two).

RESULTS

The highest plasma glucose levels were found in Group 2 ($p<0,05$, LSD: 16,95). Plasma glucose levels of groups 1 and 3 were not different (table 1). Mean liver glycogen level in group 3 was higher than those in the other two groups ($p<0,01$), while there was no significant difference between groups 1 and 2 in terms of the concerned parameter (Table 2) (Pictures 1, 2, 3).

Table 1: Plasma Glucose Levels of the Study Groups

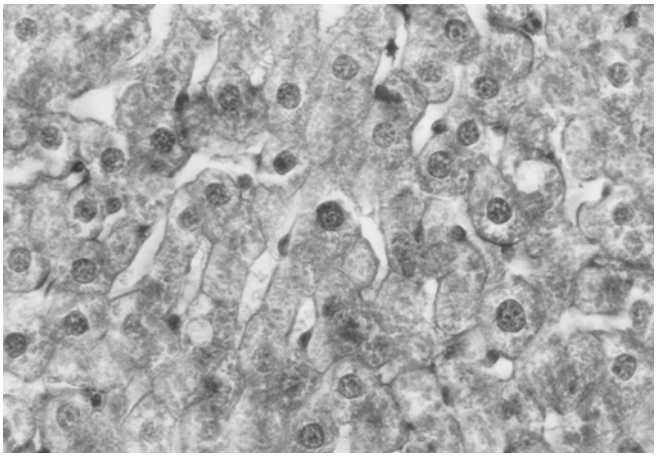
Groups (n=10)	Glucose (mg/dl)
1 Controls	93,20 ± 22,32[b]
2 Swimming Controls	119,60 ± 9,54[a]
3 Melatonin supplemented, swimming	80,50 ± 18,54[b]

*Differences between means with different superscripted letters in the same column are statistically significant ($p<0,05$).

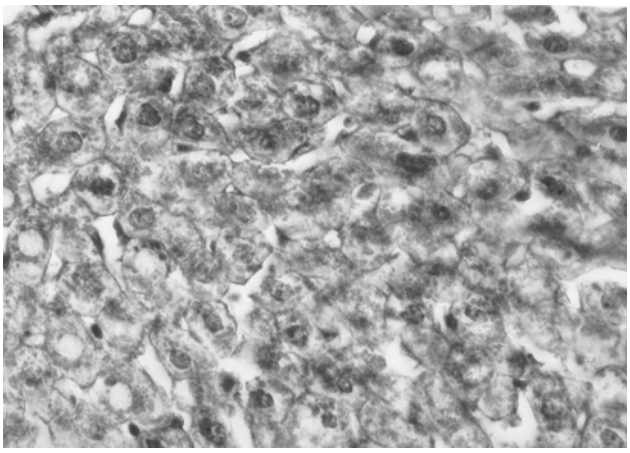
Table 2: Comparison of liver glycogen content

Groups (n=10)	Median Value of Liver Glycogen[1]
1 Controls	3,800 ± 2,394[b]
2 Swimming Controls	3,600 ± 1,838[b]
3 Melatonin supplemented, swimming	8,800 ± 3,155[a]

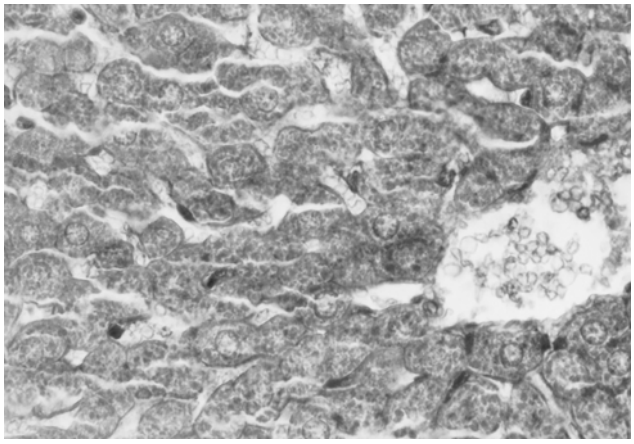
Different letters indicate significant differences, a>b, $p<0.01$
[1]As Best's Carmine paint H.E. 450 X scores.



Picture 1: Glycogen distribution in liver epithelial cells of the nonswimming controls (group 1, score 3.80, Best's Carmine paint, H.E. 459X)



Picture 2: Glycogen distribution in liver epithelial cells of the swimming controls (group 2, score 3.60, Best's Carmine paint, H.E. 459X)



Picture 3: Glycogen distribution in liver epithelial cells of the melatonin supplemented, swimming (group 3, score 8.80, Best's Carmine paint, H.E. 459X)

DISCUSSION

There is a growing body of evidence suggesting that exercise can have both acute and long-term effects on melatonin secretion (Buxton *et al.,* 1997). Exercise brings about acute changes in melatonin levels and leads to changes in melatonin levels secreted at night after 12-24 hours (Van Cauter *et al.,* 1993). Both acute and delayed effects appear dependent on the timing of exercise. Appearance of identifiable acute effects also depends upon the intensity, duration and type of exercise. Exercise performed at night during the escalating phase of melatonin secretion inhibits melatonin levels. It has been noted that, irrespective of severity and intensity, exercise performed during the day does not have a consistent acute impact on melatonin secretion, while nocturnal exercise, intense or moderate, leads to a delay in the beginning phase of melatonin secretion in the following evening (Buxton *et al.,* 1997). As a result, many researchers have pointed to a certain relation between melatonin and exercise.

In the present study, the highest plasma glucose level was found in group 2 (swimming control). Plasma glucose levels in group 3 (melatonin-supplemented swimming group) were lower than those in group 2, but were not different from the levels in group 1. We were not able to find a study with which we could compare the reduced plasma glucose levels we obtained in the melatonin-supplemented swimming group (group 3). However, Rohr and Herold (2002) concluded in a study that blood glucose levels that increased after intravenous glucose supplementation declined during sleep and melatonin played a significant role in the decline of blood glucose level. Results of Rohr and Herold (2002) are consistent with the reduced glucose levels we found in group 3.

Mean liver glycogen level was found significantly higher in group 3, relative to the other two groups, which were not significantly different in terms of the concerned parameter. Exercise requiring endurance is associated with metabolic changes stemming from increased need for energy. Mazepa *et al.* (2000) examined the effects of melatonin on parameters associated with various carbohydrate and lipid mechanisms in exercised and not-exercised rats. Exercise led to a significant decrease in muscle and liver levels of animals and a decrease in plasma lactate levels. When melatonin-supplemented exercised rats were compared with exercised rats not supplemented with melatonin, there was a significant increase in muscle and liver glycogen content and a significant decrease in plasma lactate levels. Mazepa *et al.* (2002) concluded in their study that melatonin protected glycogen reserves via changes in carbohydrate and lipid use in exercised rats.

High liver glycogen levels we found in the melatonin-supplemented swimming group (group 3) are consistent with literature information. It has been reported in studies conducted on Spraque-Dawley species rats subjected to swimming exercise that exercise increases free radical formation in liver, muscle and brain tissues, while melatonin supplementation prevents formation of free radicals by stimulating antioxidant capacity in the same tissues (Hara *et al.,* 1996; Hara *et al.,* 1997). Intense exercises like swimming are known to cause significant metabolic changes (Yaga *et al.,* 1993). The results of our study demonstrate that melatonin supplementation to rats subjected to acute swimming exercise can have a protective impact on liver glycogen reserves.

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