



# Interactive effects of melatonin, exercise and diabetes on liver glycogen levels

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## Abstract

**Background:** This study aimed to examine the effects of melatonin supplementation on liver glycogen levels in rats with streptozotocin-induced diabetes and subjected to acute swimming exercise.

**Material and methods:** Eighty Sprague-Dawley type adult male rats were divided into eight groups: Group 1, general control; Group 2, melatonin-supplemented control; Group 3, melatonin-supplemented diabetes; Group 4, swimming control; Group 5, melatonin-supplemented swimming; Group 6, melatonin-supplemented diabetic swimming; Group 7, diabetic swimming; Group 8, diabetic control. Melatonin was supplemented at a dose of 3 mg/kg/day intraperitoneally for four weeks. Liver tissue samples were collected and evaluated using a Nikon Eclipse E400 light microscope. All images obtained from the light microscope were transferred to PC medium and evaluated using Clemex PE 3.5 image analysis software.

**Results:** The lowest liver glycogen levels in the study were found in group 4. Liver glycogen levels in groups 3, 6, 7 and 8 (the diabetic groups) were higher than group 4, but lower than those in groups 1 and 2. The lowest liver glycogen levels were obtained in groups 1 and 2.

**Conclusions:** The study indicates that melatonin supplementation maintains the liver glycogen levels that decrease in acute swimming exercise, while induced diabetes prevents this maintenance effect in rats. (*Pol J Endocrinol* 2011; 62 (3): 252–255)

**Key words:** melatonin, diabetes, acute swimming exercise, glycogen

## Introduction

A possible relation between melatonin hormone secreted from the pineal gland and exercise has been suggested [1]. It has been argued that physical activity might bring about a change in plasma melatonin levels [2] and that melatonin supplementation has a performance-enhancing effect in exercise [3, 4]. It has been observed that blood glucose which increased after intravenous glucose administration dropped back during sleep [5]. A report that melatonin played a significant role in this decrease in the blood glucose level [5] can be considered to be sound evidence of the relationship between carbohydrate metabolism and pineal gland. Furthermore, the account that melatonin increased the use of carbohydrates in rats [6] is a crucial factor supporting this relationship.

Diabetes mellitus is a chronic metabolic disease, characterised by hyperglycaemia, as well as insufficient or ineffective secretion of endogenous insulin [7]. The beneficial effects of exercise on cardiovascular risk factors, insulin sensitivity, glucose disposal and

body fat distribution in diabetic patients have been well-established [8]. Consequently, exercise is strongly recommended for diabetic patients [9]. However, the search for effective factors regarding glucose metabolism is ongoing [10]. In exercise, muscle cells may use muscle glycogen independent from insulin, and for this, factors of liver glycogen are much more significant.

The present study aimed to examine the effect of melatonin supplementation on liver glycogen levels in rats with streptozotocin-induced diabetes and subjected to acute swimming exercise.

## Material and methods

### *Animal material and groups*

This study was conducted at the Experimental Animals Unit of Selcuk University, Faculty of Veterinary Medicine (Konya, Turkey), using 80 Sprague-Dawley type adult (4–6 month-old and weighing 200–250 g) male rats obtained from the Experimental Medicine Application and Research Centre of Mediterranean University



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(Antalya, Turkey). The study protocol was approved by the ethics committee of the same university. Each experimental animal was fed with 10 g feed (standard rat pellet) per 100 gram body weight daily. Animals were fed standard rat food (metabolic energy: 3,100 kcal/kg), and kept in an environment with a 12 hour light/12 hour dark cycle at a standard room temperature ( $21 \pm 1^\circ\text{C}$ ).

The experimental animals used in the study were allotted to one of eight groups in equal numbers:

Group 1 (n = 10) General control group: fed on a normal diet and not subjected to any procedure.

Group 2 (n = 10) Melatonin-supplemented control group: fed on a normal diet and melatonin supplemented.

Group 3 (n = 10) Melatonin-supplemented diabetic control group: melatonin supplemented, following induction of diabetes by subcutaneous administration of streptozotocin (STZ) 40 mg/kg.

Group 4 (n = 10) Swimming control group: fed on a normal diet and subjected to acute swimming exercise for 30 minutes.

Group 5 (n = 10) Melatonin-supplemented swimming group: fed on a normal diet, melatonin supplemented, and subjected to acute swimming exercise for 30 minutes.

Group 6 (n = 10) Melatonin-supplemented diabetic swimming group: melatonin supplemented and subjected to acute swimming exercise for 30 minutes, following induction of diabetes by subcutaneous administration of streptozotocin (STZ) 40 mg/kg.

Group 7 (n = 10) Diabetic swimming group: diabetes was induced by subcutaneous administration of streptozotocin (STZ) 40 mg/kg and subjected to acute swimming exercise for 30 minutes.

Group 8 (n = 10) Diabetes group: diabetes was induced by subcutaneous administration of streptozotocin (STZ) 40 mg/kg.

Melatonin supplement was for four weeks at 3mg/kg/day intraperitoneally in groups 2, 3, 5 and 6.

### **Experimental animals**

Experimental animals were kept in special steel cages which were washed and cleaned every day. They were fed from steel bowls and normal tap water was given by glass feeding bottles. All injections were given at 09:00–10:00 a.m. At the end of the four-week study period, the animals were decapitated at 09:00–10:00 a.m. and liver tissue samples were collected. Animals were decapitated after 24 hours following the final injection.

## **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). The injections were repeated at the same dose 24 hours later. Blood glucose levels of the animals were determined from blood taken from the tail vein of the animals six days after the last injection by using a diagnostic glucose kit. Animals with blood glucose at or above 300 mg/dlt were deemed to be diabetic [11].

### **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 deep-freeze, until it was used. Of the stock solution, 0.1 ml was added to 0.9 ml NaCl (3 mg/kg/day) and injected into rats at 09:00 a.m. intraperitoneally. Melatonin supplementation was carried out at the same time of day for four weeks.

### **Swimming exercise**

Swimming exercise was conducted in a heat-resistant, glass swimming pool, which was 50 cm in depth and width and had a thermostat to keep the temperature fixed at  $37^\circ\text{C}$ . The exercise was performed once for 30 minutes, 24 hours after the end of procedures. The experimental animals were made to swim in pairs. The animals were decapitated immediately after the swimming exercise and liver tissue samples were collected.

### **Determination of glycogen in liver tissue**

After being fixed in 95% ethyl alcohol, liver tissue samples were processed with autotechnicon, then buried into paraffin and 5  $\mu\text{m}$  cross-sections were obtained using a microtome. The sections were placed on a microscope slide and stained with PAS. The stained areas were evaluated using a Nikon Eclipse E400 light microscope. Digital images of the appropriate sites were taken with a Nikon Coolpix 5000 digital camera connected to the light microscope. For calibration purposes during photography, Nikon Stage Micrometer images were obtained at the same magnification rates. All images were transferred to a computer and assessed using Clemex PE 3.5 image analysis software. Using this, 0.1 mm<sup>2</sup> zones were selected. Hepatocytes which contained glycogen (stained positively with PAS) in these zones were marked and automatically counted. Liver glycogen levels were determined by mentioned methods that this glycogen including cells may account by automatic and a reliable methods [12].

### **Statistical evaluation**

Statistical evaluation of data was conducted using a computer software package. Arithmetic means and standard errors of all parameters were calculated. Variance analysis was employed to determine the differ-

ences between groups. The Least Significant Difference (LSD) test was used to compare group means in the variance analysis results which were found statistically significant. Differences for which  $p < 0.05$  were accepted as significant.

### Results

The lowest liver glycogen levels were obtained in groups 3, 6, 7 and 8 ( $p < 0.05$ ). Liver glycogen levels in group 4 were higher than the levels in groups 3, 6, 7 and 8 ( $p < 0.05$ ), but lower than those in groups 1 and 2 ( $p < 0.05$ ). The highest liver glycogen levels were found in groups 1 and 2 ( $p < 0.05$ , Table I, Figures 1–3).

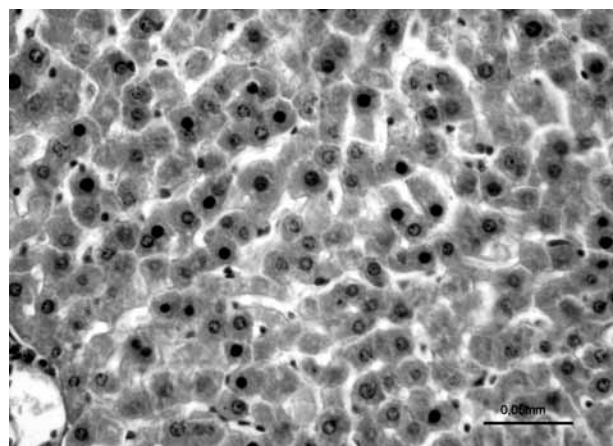
### Discussion and conclusions

The lowest glycogen levels in the study, except the diabetes groups, were found in group 4. The decreased liver glycogen obtained in group 4 (the swimming control) can be considered a result of the acute swimming exercise. Strackovski et al. [13] found that liver glycogen decreased in exercise and that exhaustive exercise brought about defects in the use of carbohydrates. A report that medium- and high-intensity exercise causes an increase in glucose production of liver origin in healthy individuals [14] is consistent with the decreased levels of liver glycogen we found in group 4. Liver glycogen levels in groups 3, 6, 7 and 8 (the diabetic groups) were lower than those in all other groups. This decrease in liver glycogen in the diabetic groups is probably a result of both acute exercise and induced diabetes. Liver plays a key role in glucose balance, as well as lipid and energy metabolisms. Impairment in this balance culminates in the

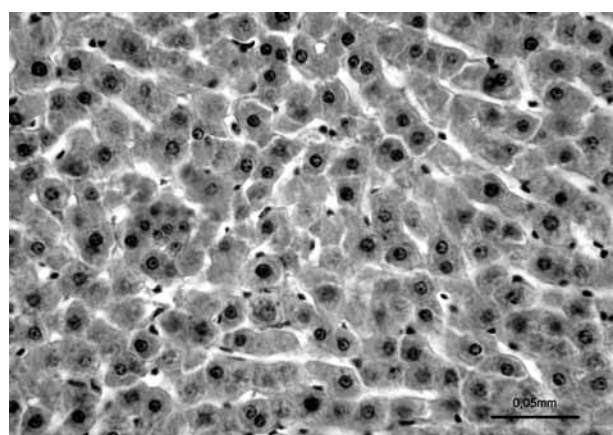
**Table I.** Liver glycogen levels of study groups

Groups (n = 10)	Glycogen (cell number/0.1 mm <sup>2</sup> area)
1 General control	67.43 ± 8.12A
2 Melatonin supplemented control	71.00 ± 7.07A
3 Melatonin supplemented diabetic control	07.80 ± 1.92D
4 Swimming control	37.14 ± 4.22C
5 Melatonin supplemented swimming	42.20 ± 7.56B
6 Melatonin supplemented diabetic swimming	6.20 ± 2.28D
7 Diabetic swimming	02.60 ± 1.52D
8 Diabetes	05.20 ± 1.30D

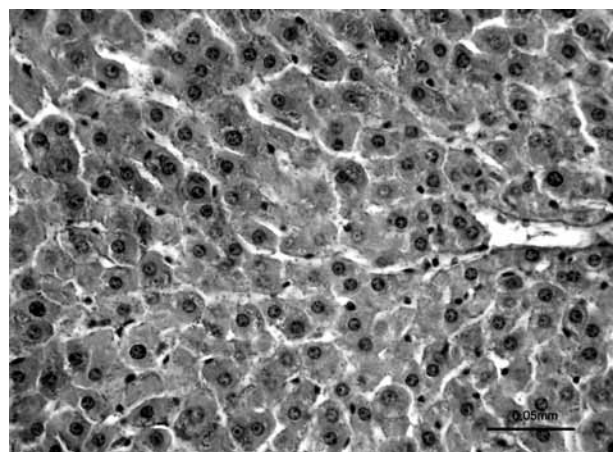
\*Means with different superscripted letters in the same column are statistically significant ( $p < 0.05$ )



**Figure 1.** Reduced glycogen levels (groups 3, 6, 7 and 8)



**Figure 2.** Group 5 liver glycogen levels compared to diabetic groups



**Figure 3.** Liver glycogen (groups 1 and 2)

development of metabolic syndrome and diabetes [15]. Glucose production starts immediately after exercise in the liver, and continues intensely as long as the physical activity goes on, until the end of the exercise [15, 16].

Exercise has been reported to markedly reduce liver glycogen in diabetic animals [17]. Exhaustive exercise has been reported to significantly inhibit liver glycogen by causing defects in the use of carbohydrates and lipids in diabetics [13]. This agrees with the decreased glycogen levels we obtained in groups 3, 6, 7 and 8 (the diabetic groups).

In our study, group 5 (the melatonin-supplemented swimming group) had lower liver glycogen levels than groups 1 (general control) and 2 (melatonin-supplemented control), but higher levels than all other groups.

Mazepa et al. [18] explored the effects of melatonin on various parameters related to carbohydrate and lipid metabolisms in exercised and non-exercised rats. They found that exercise caused a significant decline in muscle and liver levels, but a significant increase in plasma lactate levels. However, as concerns melatonin-supplemented exercised rats, they established a significant increase in glycogen content of the muscles and liver, and a significant decrease in plasma lactate levels, compared to exercised rats not supplemented with melatonin. Consequently, the authors concluded that melatonin protected glycogen stores through changes in carbohydrate and lipid use in exercised rats. The high liver glycogen levels we obtained in the melatonin-supplemented swimming group (group 5) in our study agree with the literature data.

We found the highest liver glycogen levels in group 1 (general control) and group 2 (melatonin-supplemented control). Reduced liver glycogen in all diabetic and/or exercised rats can be regarded as an expected result due to diabetes and exercise.

Results obtained from the study indicate that liver glycogen levels that decrease in acute swimming exercise are maintained by melatonin supplementation, and that diabetes induced in rats prevents this maintenance effect of melatonin.

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