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## CHANGES IN MELATONIN CONCENTRATION AFTER PHYSICAL EXERCISE OF VARIABLE INTENSITY

**Key words:** melatonin, maximal exercise, PWC<sub>170</sub>.

### ABSTRACT

The aim of the study was to investigate changes in blood melatonin concentration in volleyball players after performing an exercise of moderate intensity (test PWC<sub>170</sub>) and of intensity increasing to the maximum. The sample consisted of eleven volleyball players from the UKS "Budowlanka" Poznań sport club, aged between 15 and 17 years old (mean age 16.0±0.45). Tests and measurements were carried out between 8 a.m. and 11 a.m. The exercise test of an intensity increasing to the maximum induced significant disturbances (at 1% confidence level) in the acid-base balance. The pH value declined from 7.38±0.01 to 7.23±0.03, pCO<sub>2</sub> by 8 mmHg, and the bicarbonates by 10 mmHg. The base deficit was increased from 0.48±1.03 to 12.40±1.78 mmol/l. The lactic acid concentration was increased by 7.7 mmol/l. The aerobic exercise (PWC<sub>170</sub>) induced a decrease in blood pH from 7.37±0.01 to 7.34±0.02, a decrease in bicarbonates by 1.84 mmol/l, and the base deficit by 2.1 mmol/l (p<0.01). The concentration of the lactic acid increased from 1.22±0.23 to 2.07±0.72 mmol/l (p<0.01). The concentration of melatonin in the blood serum after exercise of an intensity increasing to the maximum was higher by 3.4 pg/ml (p<0.05), and after the PWC<sub>170</sub> test was lower by 7.3 pg/ml (p<0.05). In conclusion, a single physical exercise, depending on its intensity, causes diverse changes in blood melatonin concentration.

### INTRODUCTION

The main hormone of the pineal body – melatonin, was discovered in 1958, and chemically identified in 1959 by Lerner et al. [9]. Initially, the pineal body was considered a typical endocrine gland in mammals, and numerous studies indicated its regulative role, mostly with respect to the reproductive system [5]. This influence, most likely mediated by receptors in the pituitary gland, was for many years acknowledged as the major effect of

melatonin [5, 8, 21]. More universal functions of melatonin appeared in later studies which showed its effect on the immunological system, carcinogenesis, circadian rhythms and aging processes [18, 21]. The first report on antioxidative properties of melatonin was published in 1993 by Tan et al. [18]. Since then melatonin has become the subject of numerous studies carried out on animals and humans [6, 12, 13, 14, 17, 18, 22].

The existence of an indole cycle in the molecule of melatonin is the reason for its antioxidative properties [6, 15]. Tan et al. [18] and Pieri et al. [13] have revealed that melatonin remo-

ves hydroxyl (OH<sup>•</sup>) and peroxy (CCl<sub>3</sub>O<sub>2</sub><sup>•</sup>) radicals, and Hara et al [7] have reported that melatonin protects from peroxidative injuries induced by physical exercise. These authors have made experiments on rats performing 30-minute swimming exercises. Administration of 1 mg of melatonin per 1 kg of the body mass before the physical exercise caused no changes of the GSH/GSSG ratio in the liver, as compared with the control group. The antioxidative function of melatonin is also manifested by detoxication of hydrogen peroxide and lipid peroxide radicals [10, 12, 16]. According to Pieri et al. [13], melatonin evokes a twice stronger effect in neutralizing ROO<sup>•</sup> as compared with vitamin E – the main antioxidant of lipids. In vitro studies carried out by these authors showed that melatonin was five times more effective than glutathione and ten times more effective than mannitol in removing the most reactive and toxic hydroxyl radical. They have also reported that melatonin is more effective in removing the peroxy radical than vitamins E and C as well as the reduced glutathione.

Most studies on the antioxidative functions of melatonin have been performed in vitro [6, 15] or on animals [5]. Results of studies carried out on human subjects are less representative and have been rather performed under incomparable conditions [4, 5, 11]. This inspired us to undertake a study aiming at determination of changes in melatonin concentration in volleyball players following a single physical exercise of moderate intensity or an intensity increasing up to the maximum.

## METHODS

Eleven volleyball players from the UKS “Budowlanka” Poznań sport club participated in the study. They were between 15 and 17 years old (mean age 16.0±0.45), their mean body mass was 73.0±8.46 kg, the mean BMI 21.9±2.34 kg/m<sup>2</sup> and the mean training experience 4.3±2.45 years. The research was conducted during the starting period (March 2003). Subjects performed an exercise of an intensity increasing up to the maximum on the cycloergometer (Kettler CX-1, Germany) and an aerobic exercise (PWC<sub>170</sub>, Physical Work Capacity test). During tests the subjects’ heart rate was measured with the use of the Polar Accurex sport tester (Finland).

Tests and measurements were conducted between 8 a.m. and 11 a.m. The exercise test of an

intensity increasing to the maximum started from the load of 60W which was subsequently increased by 5W every 15 minutes until refusal. During the PWC<sub>170</sub> test, subjects performed two 5-minute submaximal exercises at a 20-minute interval, with a load adjusted individually on the basis of body mass, according to the following formula:

- 1<sup>st</sup> load = 0.75 W x kg of the body mass;
- 2<sup>nd</sup> load = 1.25 W x kg of the body mass.

On the basis of a load adequate to heart rates of 130 bpm and 150 bpm, the PWC<sub>170</sub> value was determined from a chart showing interdependence between parameters.

Before the exercise, at a fasting state, the venous blood was taken from the antecubital vein and the capillary blood from the fingertip. After a light meal (bread and butter and a cup of tea) the subjects performed the exercise test, and 3 minutes after its completion the venous and capillary blood was taken again. In the blood serum the melatonin concentration (MLT) was assessed using the ELISA immunoenzymatic method (ICN Biomedicals, Inc., USA). In the capillary blood the acid-base balance parameters and haemoglobin concentration were determined by the Automatic Blood Gaz System Al 995 Hb (Austria), and the lactic acid concentration by the enzymatic method (Boehringer – Mannheim).

The results were statistically analysed with Wilcoxon’s test.

The study was conducted with the subjects’ consent and was granted the approval of the Local Ethics Committee of Scientific Research at the University School of Medical Sciences in Poznań.

## RESULTS

The profile of test exercises performed by subjects under the study is presented in Table 1. The maximum load during the physical exercise was assessed by measurement of the heart rate, which amounted to the mean of 193.7±9.48 bpm. During the first load of the test PWC<sub>170</sub> the mean pulse rate amounted to 109.0±10.59, while during the second load to 134.70±10.59 bpm.

The mean values of the acid-base balance parameters and of blood lactate concentration in the players before and after the physical exercise test of an intensity increasing to the maximum are presented in Table 2. The exercise test induced a significant, at 1% confidence level, disturbance of the acid-base balance. The pH value of the

blood decreased from  $7.38 \pm 0.01$  to  $7.23 \pm 0.03$ , the  $p\text{CO}_2$  value decreased by 8 mmHg, the concentration of bicarbonates by 10 mmHg, and the base deficit increased from  $0.48 \pm 1.03$  to  $12.40 \pm 1.78$  mmol/l. The lactic acid concentration increased by 7.7 mmol/l.

The acid-base balance parameters and parameters of lactate blood concentration in the subjects during the  $\text{PWC}_{170}$  test are presented in Table 3. The comparative analysis of mean values measured at rest with values calculated after the exercise tests revealed a significant decrease, at the 1% confidence level, of blood pH from  $7.37 \pm 0.01$  to  $7.34 \pm 0.02$ , of  $\text{HCO}_3^-$  concentration by 1.84 mmol/l; and the increase of the base deficit by 2.1 mmol/l. The lactic acid concentration increased from  $1.22 \pm 0.23$  to  $2.07 \pm 0.72$  mmol/l ( $p < 0.01$ ).

Changes in the blood melatonin concentration at rest and after performing the exercise tests are shown in Figures 1 and 2. The exercise of an intensity increasing to the maximum resulted in a significant, at 5% confidence level, increase of melatonin concentration in the blood serum by 3.4 pg/ml. The  $\text{PWC}_{170}$  test caused a significant (5% of confidence level), decrease in the melatonin concentration by 7.3 pg/ml.

## DISCUSSION

Physical exercise, depending on its intensity, duration and time of its performance, evokes different changes in melatonin blood concentration [4, 5, 11]. Monteleone et al. [11] investigated concentra-

**Table 1.** Characteristics of the exercise tests of an intensity increasing to the maximum (n=11)

|                         | Load [W]<br>$\bar{x} \pm \text{SD}$ | Time [s]<br>$\bar{x} \pm \text{SD}$ | Heart rate<br>[bpm]<br>$\bar{x} \pm \text{SD}$ |
|-------------------------|-------------------------------------|-------------------------------------|--|
| Maximum exercise        | $286.1 \pm 37.07$                   | $708 \pm 128$                       | $193.7 \pm 9.48$                               |
| $\text{PWC}_{170}$ test | $174.7 \pm 27.1$                    | (I) 300<br>(II) 300                 | $109.0 \pm 10.59$<br>$134.7 \pm 14.01$         |

**Table 2.** Mean values of the acid-base balance parameters, lactic acid and hemoglobin concentrations in the blood of competitors during the exercise test of an intensity increasing to the maximum

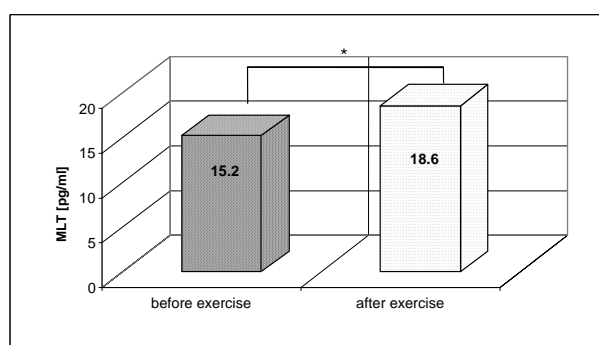
| Parameter                 | At rest<br>$\bar{x} \pm \text{SD}$ | After the exercise<br>$\bar{x} \pm \text{SD}$ | Difference<br>(rest – exercise) | Wilcoxon's<br>test |
|---------------------------|------------------------------------|---|---------------------------------|--------------------|
| pH                        | $7.38 \pm 0.01$                    | $7.23 \pm 0.03$                               | 0.15                            | $p \leq 0.01$      |
| $p\text{CO}_2$ (mmHg)     | $42.60 \pm 2.49$                   | $34.79 \pm 1.99$                              | 7.81                            | $p \leq 0.01$      |
| $p\text{O}_2$ (mmHg)      | $72.32 \pm 6.21$                   | $92.33 \pm 5.19$                              | -20.01                          | $p \leq 0.01$      |
| $\text{HCO}_3^-$ (mmol/l) | $24.29 \pm 1.21$                   | $14.12 \pm 1.43$                              | 10.17                           | $p \leq 0.01$      |
| BE (mmol/l)               | $-0.48 \pm 1.03$                   | $-12.40 \pm 1.78$                             | 11.92                           | $p \leq 0.01$      |
| LA (mmol/l)               | $1.46 \pm 0.23$                    | $9.14 \pm 1.06$                               | -7.68                           | $p \leq 0.01$      |
| Hg (g/dl)                 | $15.35 \pm 0.95$                   | $15.37 \pm 1.30$                              | -0.02                           | NS                 |

$p\text{CO}_2$  – pressure of  $\text{CO}_2$  in the blood;  $p\text{O}_2$  – pressure of  $\text{O}_2$  in the blood; BE – base excess; LA – lactic acid; Hg – hemoglobin; NS – non-significant

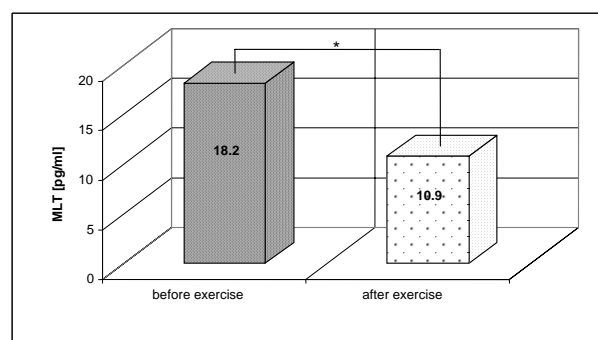
**Table 3.** Mean values of the acid-base balance parameters, lactic acid and hemoglobin concentrations in the blood of competitors during the PWC<sub>170</sub> test

| Parameter                              | At rest<br>$\bar{X} \pm SD$ | Following exercise<br>$\bar{X} \pm SD$ | Difference<br>(rest – exercise) | Wilcoxon's test |
|--|-----------------------------|--|---------------------------------|-----------------|
| pH                                     | 7.37 ± 0.01                 | 7.3 ± 0.02                             | 0.07                            | p ≤ 0.01        |
| pCO <sub>2</sub> (mmHg)                | 69.28 ± 11.51               | 75.24 ± 6.16                           | -5.96                           | NS              |
| pO <sub>2</sub> (mmHg)                 | 42.93 ± 2.59                | 42.0 ± 1.77                            | 0.95                            | NS              |
| HCO <sub>3</sub> <sup>-</sup> (mmol/l) | 24.04 ± 1.45                | 22.20 ± 0.96                           | 1.84                            | p ≤ 0.01        |
| BE (mmol/l)                            | -0.78 ± 1.10                | -2.95 ± 1.06                           | 2.17                            | p ≤ 0.01        |
| LA (mmol/l)                            | 1.22 ± 0.23                 | 2.07 ± 0.72                            | -0.85                           | p ≤ 0.01        |
| Hg (g/dl)                              | 14.90 ± 0.83                | 15.01 ± 0.74                           | -0.11                           | NS              |

pCO<sub>2</sub> – pressure of CO<sub>2</sub> in the blood; pO<sub>2</sub> – pressure of O<sub>2</sub> in the blood; BE – base excess; LA – lactic acid; Hg – hemoglobin; NS – non-significant



**Figure 1.** Influence of the physical exercise of an intensity increasing to the maximum on the melatonin concentration (MLT) in the blood serum. The asterisk indicates statistically significant difference at p < 0.05



**Figure 2.** Influence of the aerobic exercise (PWC<sub>170</sub> test) on the melatonin concentration (MLT) in the blood serum. The asterisk indicates statistically significant difference at p < 0.05

tions of melatonin and cortisol regularly every hour or two hours between 8 p.m. and 8 a.m. in six untrained persons at the mean age 29.8±1.9 years. Between 10 p.m. and 11 p.m. the subjects performed a 30-minute physical exercise with the mean load of 211.6±33.7 W. This caused a decrease in their melatonin concentration for one hour. Between 12 p.m. and 4 a.m. the concentration of melatonin regularly increased up to values comparable with those measured at rest. The authors explained such a considerable drop in the melatonin concentration by the impact of physical exercise on the secretory activity of the pineal body. They also suggested that glyocorticosteroids might inhibit functions of this endocrine organ. On the other hand, Carr et al. [4] investigated seven women after

a 60-minute physical exercise between 1 p.m. and 6 p.m. and noted a 100-200% increase of melatonin secretion into the blood. Elias et al. [5] monitored the melatonin concentration during 3 hours in seven men aged 22-42 years after a running exercise with a velocity of 0.765 m s<sup>-1</sup> performed between 9.30 a.m. and 10 a.m. and did not observe any significant changes in the level of melatonin in the blood.

In the present study we analyzed effects of single physical exercises on the melatonin blood concentration using the PWC<sub>170</sub> test and the exercise test of an intensity increasing to the maximum. These tests were performed between 8 a.m. and 11 a.m. and enabled us to determine the influence of either aerobic or anaerobic exercises

on the melatonin concentration as well as to observe differences in the melatonin level depending on exercise intensity. The exercise of an intensity increasing to the maximum performed during the starting period resulted in severe disturbances of acid-base balance parameters being an effect of the considerable contribution of anaerobic processes to covering energetic costs of the effort. This was determined by an increase in the lactic acid concentration by the mean of 7.68 mmol/l ( $p \leq 0.01$ ), and a decrease of blood pH from  $7.38 \pm 0.01$  to  $7.23 \pm 0.03$  ( $p \leq 0.01$ ). This anaerobic exercise caused a statistically significant, (5% confidence level), increase of melatonin concentration from 15.2 to 18.6 pg/ml that was correlated significantly with the lactic acid concentration ( $r = 0.585$ ;  $p < 0.05$ ). Our results remain in agreement with those published by Theron [19], Carr [4] and Buxton [3].

According to Vaughan and Reiter [20] the post-exercise increase of the melatonin concentration is due to the increase of concentration of catecholamines which stimulate the pineal body through beta-1 adrenergic receptors to produce melatonin de novo. This hypothesis was supported by studies of Arendt [2] who revealed that atenolol block of beta-1 adrenergic receptors caused the total inhibition of melatonin secretion in the pineal body.

In order to study effects of a single aerobic exercise on the level of melatonin in the blood serum the subjects performed the PWC<sub>170</sub> test. The aerobic character of this effort was confirmed by very little changes in the lactic acid concentration (mean increase by 1 mmol/l as compared with the resting state) assessed in the capillary blood three minutes after the exercise. This test resulted in a statistically significant decrease of the melatonin concentration by 7.3 pg/ml ( $p < 0.05$ ). Such opposite direction of changes in the hormone blood concentration enabled us to hypothesize that the aerobic exercise unaccompanied by the increase of adrenergic stimulation was an insufficient stimulus to induce melatonin synthesis de novo and to its blood secretion (as melatonin is not stored in the pineal body and secreted in response to an adequate stimulus).

The results of the study are partly in agreement with reports published previously by Buxton [3]. He concluded that the exercise of intensity between 40 and 60% of  $VO_{2max}$  did not cause changes in melatonin concentration, but a considerable increase in the concentration of this hormone

was induced by exercises of an intensity amounting to 75% of  $VO_{2max}$ .

Our study results supported by the reports of the aforementioned authors indicate that the melatonin secretion may be increased, decreased or unchanged following a single physical exercise. However, in all cases, changes are maintained for no longer than three hours after the exercise completion. It can be thus concluded that changes in the melatonin concentration in the blood serum depend not only on exercise intensity, but on conditions of the study, i.e., time and duration of an exercise, as well as subjects' age and sex. Appenzeller and Wood [1] revealed that the increase of the melatonin concentration after the exercise might be determined by age; and Buxton et al. [3] claim that it may depend on subjects' change from the seating to standing position.

In conclusion, we can state that a single physical exercise, depending on its intensity, induces diverse changes of melatonin blood concentration. Exercises of an intensity increasing to the maximum cause an increase in melatonin concentration; while aerobic exercises lead to its decrease.

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