Effect of Moderate Exercise on the Level of Melatonin Hormone and Lymphocyte Apoptosis in Healthy Subjects

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ABSTRACT:

BACKGROUND:

Physical exercise is important for promotion of the immune system. The immune responses to exercise are dependent on the intensity and duration of the exercise performed. During exercise, metabolic and hormonal changes occurred that can induce lymphocyte apoptosis.

Melatonin is one of the hormones that are affected by exercise, it is a pineal gland hormone, its production and secretion followed a circadian pattern, onset of darkness stimulate its secretion, while day light suppress its production.

Melatonin is regarded as an anti-aging hormone; it has a role in the elimination of harmful lymphocytes which may give rise to chronic inflammation and possibly to autoimmunity.

OBJECTIVE:

This prospective study aimed to elucidate the effect of moderate endurance exercise on melatonin hormone and the percentage of peripheral blood lymphocyte apoptosis in healthy subjects.

METHODS:

Sixty healthy subjects with a mean age of (37.05 ± 13.02) year, participated in the study. They were subjected to moderate exercise session which lasted for 60 min and repeated 3 times per week for 3 months. The exercise intensity was 50-60% of heart rate reserve (according to Karvonen formula). Two exercise stages were performed; the first stage include 30 minute of physical activity program and the second stage include 30 minute of treadmill exercise.

Blood sample were collected before and after exercise to examine its effect on the level of melatonin hormone and apoptosis of peripheral blood lymphocyte.

RESULTS:

This study showed significant increment in the level of melatonin hormone and the percentage of lymphocyte apoptosis after exercise in comparison to pre exercise values for each week along the period of the test ($P < 0.05$).

Three months exercise showed a significant correlation with the levels of melatonin hormone ($P= 0.008$, $r= 0.44$), and with percentage of lymphocyte apoptosis ($P = 0.001$, $r= 0.701$).

CONCLUSION:

It can be concluded that moderate exercise for three months was associated with increased percentage of periphral blood lymphocyte apoptosis and increased level of melatonin hormone in the first two months while in the third month this anti aging hormone had reached a steady state after the subjects get trained to exercise.

KEY WORDS: moderate exercise, melatonin, lymphocyte apoptosis.

INTRODUCTION:

Physical exercise is the performance of some sort of activity for maintaining physical fitness including healthy weight; building and maintaining healthy bones, muscles, and joints; promoting physiological well-being and reducing surgical risks [(1)].

Exercise is important for strengthening the immune system. The characteristics of the immune responses to exercise are dependent on the intensity and duration of exercise performed [(2)].

Exercise accompanied with the hormonal changes which may affect lymphocyte mobilization. Moderate exercise has been suggested to reverse immunosenescence by increasing the production of endocrine hormones, which may contribute to less accumulation of autoreactive immune cells by enhancing the programmed cell death (apoptosis) [(3)].

Apoptosis is a physiological type of cell death that occurs in all multicellular organisms as a part of normal development, deletion of un-needed cells and tissues, regulation of growth and cell number, and elimination of abnormal dangerous cells [(4)].

Moderate exercise modulates several factors, such as reactive oxygen species (ROS), DNA damage, cytokine and hormone levels, which are involved in

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the regulation of apoptosis in various cell types [5].
One of these hormones is the melatonin hormone, which is secreted from pineal gland; the pineal gland is an end organ of the visual system [6].
Melatonin (N-acetyl-5 methoxytryptamine) is the principal hormone of the vertebrate pineal gland. It is a compound that is synthesized from tryptophan; tryptophan undergoes oxidation to serotonin, and it is eventually converted to its active form [7].
Melatonin production and secretion follows a circadian pattern. Research on melatonin secretion patterns in humans had shown that serotonin levels decrease and melatonin levels rise with onset of darkness [8].
Melatonin has a diverse range of physiological effects one of them is immune enhancing effects, and can counteract the immune depression that may follow acute stress, drug treatment, and viral diseases or aging [9].
Melatonin was noted to up-regulate tumor necrosis factor, stem cell factors, interleukins, and interferon[10]. Conversely, pinealectomy reduces antibody activity and therefore impairs the immune response [11].
Exercise may have both rapid and delayed effects on human melatonin secretion and it had been shown to alter the pattern of melatonin secretion in different ways depending on the time of the day and also depends on the duration, intensity, and type of exercise [12].

SUBJECTS AND METHODS:

The study included (60) healthy subjects ;(30 male), (30 female), their age range from (15-62) year (37.05 ± 13.02).
Each subject had full history and medical exams, they were excluded if they were alcoholics, smokers, hypertensive, diabetics, or if they were taking any medications (i.e. aspirin, anti-inflammatory drugs) known to affect immune function, or if they had any recent (before 3 months) surgery or if they have a previous history of cancer, arthritis, or immune disorders, female excluded if they were pregnant.
The exercise was done in the morning; each exercise session lasts for 60 minute, 3 times/ week for three consecutive months. The minimum threshold of moderate exercise intensity of improvement in fitness had been proposed to be 50-60% of heart rate reserve [13,14,15]. Which was applied for each subject participated in this study and monitored by using Karvonen formula.[16, 17, 18]
Karvonen formula was calculated after determining resting heart rate and the intensity of exercise, then applying the formula:
(220 = predicted maximum heart rate)
220 - Age = Maximum Heart Rate
Max Heart Rate – Resting Heart Rate = Heart rate reserve
(Heart rate reserve x training intensity %) + resting heart rate = target heart rate
- The first stage of exercise was with physical activity program continued for 30 minutes.
- The initial part (5 minutes) consisted of exercises preparing the body for physical exertion simply called "warm up" preferred to be done in stand positions by walking whenever possible.
- The main part (20 minutes) of different intensity consisted of:

a- Marching and standing, reaching, throwing, catching, kicking, chair stands, bending down, toe and heel raises – aimed at improving the condition of the circulatory and respiratory system,
b- Workout in sitting positions – in squat, on hands and knees, lying on one side, in prone position, which intended to strengthen the kinetic system.

- The final part (5 minutes) "cool down" and relaxation exercises done in low positions such as Lying and squat positions.
- The second stage of exercise was with the treadmill type (CYBEX, XELG90, USA) for 30 minutes.

- Minutes 0-2: walk at 2.2 mph (Mile/hour), 0% grade
- Minutes 2-5: acquisition of heart rate range by increasing speed
- Minutes 5-25: exercised at 50-60% + 5 bpm of calculated training zone of targeting heart rate.
- The exercise finished with 5 minutes "cool down" by gradual decreasing of treadmill speed.

During exercise the heart rate was monitored every minute by the treadmill sensor and the velocity was corrected if the heart rate was below or above the calculated zones of training heart rate for each subject.
Four ml of venous blood were aspirated from each subject before and after exercise at the recovery period after 5 minutes from the cool down; the sample was divided into two parts:
- First part (2 ml) was processed for peripheral blood lymphocytes separation (PBL) according to. Boyum and Mendelson[19,20]to detect the following:
- Lymphocytes count and viability by trypan blue exclusion test according to Dolye and Griffiths [21].
- Morphology of apoptotic lymphocytes by DNA-binding fluorescent dyes (acridine orange) according to Vacca [22].

- Second part (2ml) was used for serum separation to estimate the melatonin by Enzyme linked immuno sorbant assay (ELISA) (The type of ELISA kit was Ref: RE54021. IBL gesellschaft fur Immunochemie und Immunbiologie MBH, Flughafenstrasse 52a. D-22335 Hamburg, Germany).

RESULTS:

A significant difference ($P < 0.05$) was observed in the level of melatonin and percentage of lymphocyte apoptosis before and after exercise for each week along the period of the experiment as shown in table (1).

There is an obvious increase in the level of melatonin hormone after exercise in comparison to pre exercise along the period of experiment (figure1).

Figure (2) showed that the level of melatonin in the third month was near its level in the second month.

After exercise there was a significant correlation ($P= 0.008$, $r= 0.44$) between the level of melatonin hormone and the exercise duration as shown in figure (3).

The highest percentage of lymphocyte apoptosis reached after exercise in the eleventh week (4.7 ± 0.15). In the last three weeks the percentage of lymphocyte apoptosis after exercise are nearly equal to each other as demonstrated in figure (4). A significant positive correlation ($P = 0.001$, $r= 0.701$) was found between the percentage of lymphocyte apoptosis and exercise duration as shown in figure (5).

Table 1: Weekly differences in the level of melatonin and percentage of apoptotic lymphocyte before and after exercise along the experiment Period

Weeks	Melatonin Pg/ml		% of Lymphocyte Apoptosis	
	Before	After†	Before	After†
Week 1	4.04± 0.77	7.4±0.37	2.07±0.25	3.36± 0.16
Week 2	3.63±0.42	6.9±0.95	2.35±0.16	4.09± 0.49
Week 3	3.99±0.22	7.53±0.65	1.88±0.23	4.11± 0.19
Week 4	4.12±0.08	6.86±0.96	1.83±0.04	4.14± 0.13
Week 5	4.52±0.43	8.24±0.98	2.05±0.04	4.43± 0.07
Week 6	4.73±0.43	7.61±1.77	2.17±0.57	4.49±0.06
Week 7	4.47±0.71	8.74±1.88	1.87±0.05	4.37± 0.14
Week 8	4.41±1.18	8.66±1.18	2.1±0.34	4.66±0.01
Week 9	4.16±0.23	8.43±1.4	2.61±1.11	4.38± 0.16
Week 10	4.51±0.4	8.6±0.53	2.66±0.07	4.6± 0.09
Week 11	4.63±0.36	7.91±0.68	2.33±0.14	4.7± 0.15
Week 12	4.93±0.68	9.2 ±1.06	2.94±0.23	4.4±0.21

A probability value < 0.05, values represent mean ± **SD**

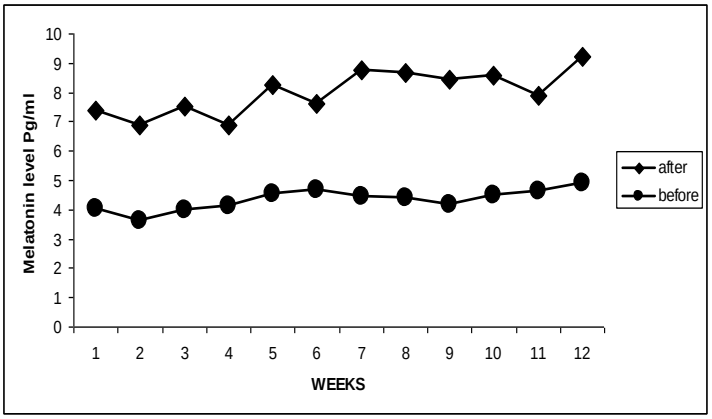

Figure 1: The alteration in the melatonin levels for each week before and after exercise

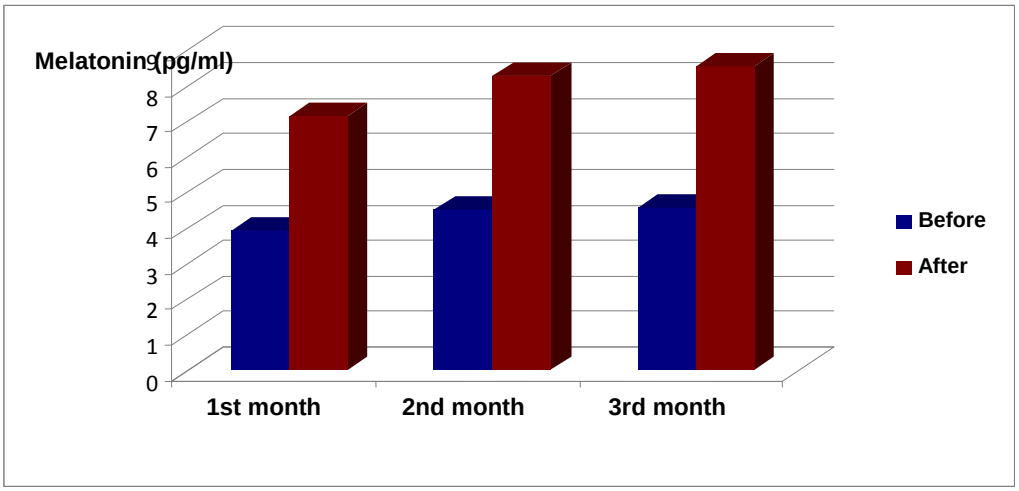

Figure 2: Monthly differences in the level of melatonin before and after exercise

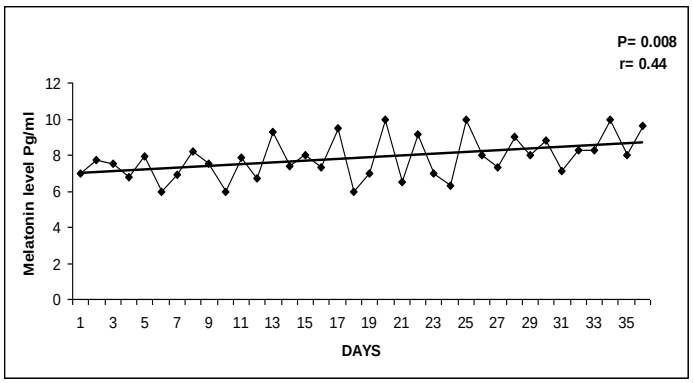

Figure 3: Correlation between exercise duration and melatonin level after exercise.

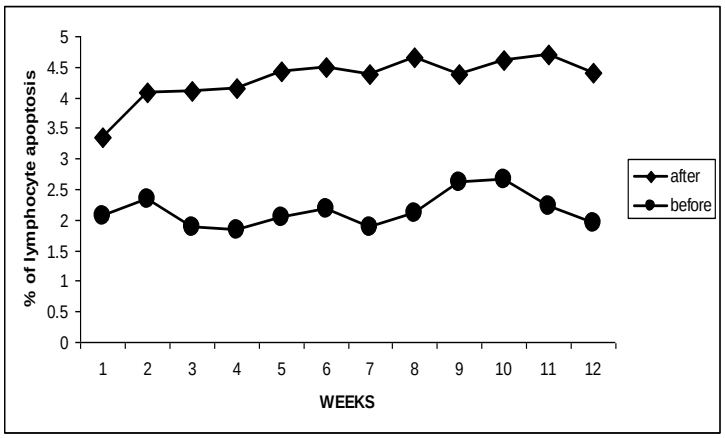

Figure 4: The alteration in the percentage of lymphocyte apoptosis for each week before and after exercise

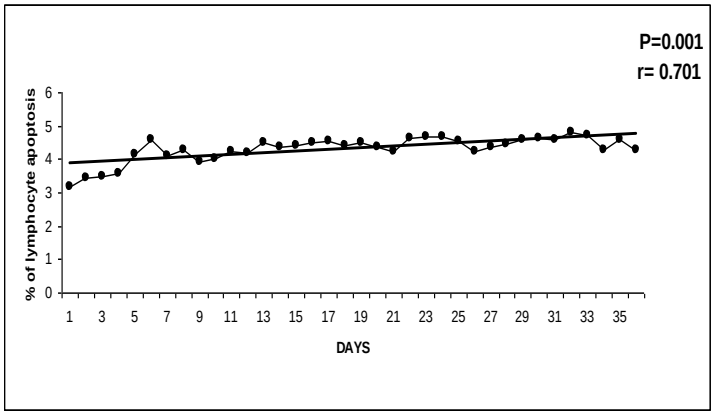

Figure 5: Correlation between exercise duration and percentage of lymphocyte apoptosis after exercise.

DISCUSSION:

This study showed that the melatonin hormone increased significantly (P< 0.05) after exercise in comparison to pre exercise level from the first week to the last week of experiment table (1), figure (1).

Different studies have reported that melatonin concentration increase, decrease or remain unaffected by bouts of exercise. Such conflicting findings may be explained by inter-study differences in lighting conditions, the time of day, the study participants have exercised and age, fitness status have also been identified as intervening factors in exercise-mediated changes in melatonin concentration.

The current study was in agreement with previous studies which indicated that daytime melatonin levels increased when the subjects were subjected to the exercise.[23, 24]

Regulation of melatonin secretion is under neural control, as exercise has a role in increment of the sympathetic discharge which will release noradrenalin that has played a major role in elevation of tryptophan level in pineolocytes; tryptophan is regarded as the precursor of melatonin synthesis and thus leads to increment in melatonin release.[7, 25, 26]

In the third month the level of melatonin hormone reached to a steady state due to the effect of endurance training as showed in figure (3).

The research illustrated that after exercise there was a significant correlation (P= 0.008, r= 0.44) between the level of melatonin hormone and the duration of exercise i.e. as the days of the experiment were progressed the level of melatonin was significantly increased figure (2), this result was in agreement with the study of Knight and coworkers who stated that the increment in melatonin production continues as long as the exercise activity was being carried out [27].

This study had shown that the percentage of lymphocyte apoptosis before exercise were within normal range (2.15% ± 0.38%) along the period of the experiment, this result is near the estimated percentage of apoptotic lymphocyte isolated from peripheral blood of normal subjects (3.36% ± 0.54%)[28].

The percentage of apoptosis in the peripheral blood lymphocyte after exercise was significantly increased to (4.32% ± 0.62%) figure (4) and this in agreement with previous study which found that exercise intensity threshold for inducing an increase in lymphocyte apoptosis occurs between 40 and 60 % VO2max [29].

Mars and coworkers were the first to document lymphocyte apoptosis after exercise; they found that T lymphocytes with a senescent phenotype are mobilized and subsequently removed from the blood stream in response to exercise [30]. Apoptosis might be induced via the interaction of death ligands and receptors, Fas receptor (CD95) and Fas ligand (CD95L)[31]; an up-regulation of Fas receptor, which serves as another indicator of cell activation, had been shown after treadmill exercise tests.

Exercise had the ability to mobilize T lymphocytes from the peripheral lymphoid compartments, and these lymphocytes are likely to be at a more advanced stage of biological aging and have a reduced capacity for clonal expansion than blood-resident T cells and at the same time these lymphocytes are more sensitive to apoptosis than other lymphocytes [32].

Based on the results of this study, the significant correlation (P = 0.001, r= 0.701) between the percentage of lymphocyte apoptosis and the

duration of the experiment figure(5), was associated with a significant elevation of melatonin hormone as the daily exercise was progressed, this can be explained as; the melatonin hormone had a role in induction of peripheral blood lymphocyte apoptosis through its effect on up-regulation of TNF which was regarded as the major extrinsic mediator of apoptosis [33].

Or through the antiproliferative or apoptotic effect of melatonin in thymus and lymph nods by measuring the level of mRNA, melatonin inhibits lymphocyte division in the thymus when administered chronically [34].

CONCLUSION:

There is a gradual increase in melatonin level after exercise; a steady state of melatonin is reached after endurance exercise (3 months). Increment in melatonin leads to increase in the percentage of lymphocyte apoptosis after three months of exercise.

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