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Original Article

Effect of Moderate Exercise on the level of DHEAS hormone and lymphocyte apoptosis in healthy subjects

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Summary:

Background: Physical exercise is essential for performance of the immune system. The integrity of immune response is dependent on the intensity and duration of the exercise performed. During exercise the metabolic and hormonal changes that occurred can induce lymphocyte apoptosis. Dehydroepiandrosteron sulphate (DHEAS) is one of the hormones that is affected by exercise, it is an androgen released from the adrenal cortex in response to adrenocortiocotropin releasing hormone (ACTH). DHEAS is regarded as an anti-aging hormone; it is primarily immune enhancing, providing some degree of counterbalance to the potentially detrimental effects of catecholamine and cortisol which are immunosuppressive if chronically elevated, it also had a role in the elimination of harmful lymphocytes which may give rise to chronic inflammation and possibly to autoimmunity.

Methods: Sixty healthy subjects were involved in this study with a mean age of (37.05 ± 13.02) year. 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 samples were collected before and after exercise to examine its effect on the level of DHEAS hormone and apoptosis of peripheral blood lymphocyte.

Results: This study showed significant increment ($P < 0.05$) in the level of DHEAS hormone and the percentage of lymphocyte apoptosis after exercise in comparison to pre exercise values for each week along the period of the test.Three months exercise showed a significant correlation with the levels of DHEAS hormone ($P = 0.000$, $r = 0.75$), and with percentage of lymphocyte apoptosis ($P = 0.001$, $r = 0.701$).

Conclusion: in this prospective study, following moderate exercise for three months; there was increase in the percentage of peripheral blood lymphocyte apoptosis and increased level of DHEAS hormone in the first two months while in the third month this anti aging hormones had reached to a steady state after the subjects get trained to exercise.

Keywords: moderate exercise, DHEAS, lymphocyte apoptosis.

Introduction:

Exercise is an important activity to improve health, maintain fitness and it is important as a means of physical rehabilitation. Physical exercise is important for strengthening the immune system. The immune responses to exercise are dependent on the intensity and duration of the exercise performed.1Exercise accompanied with the hormonal changes which may affect lymphocyte mobilization. It reverses immunosenescence by increasing the production of endocrine hormones, which may contribute to less accumulation of autoreactive immune cells by enhancing apoptosis.2 Several factors, such as reactive oxygen species (ROS), DNA damage, cytokine and hormone levels, are involved in the regulation of apoptosis in various Cell types.3 Apoptosis is a physiological type of cell death; its active, well-controlled genetic program of cell death that does not require the participation of the inflammatory process. It is responsible for focal elimination of unwanted cells during normal embryonic development, organ homeostasis, immune regulation and defense without causing stress to the neighboring cells.4

One of these hormones that are modulated by exercise is the DHEAS hormone, it is a sulphated form of DHEA which is a C19 steroid synthesized from cholesterol in the zona reticularis of the adrenal cortex, in response to adrenocortiocotropin releasing hormone (ACTH). The process requires two enzymes-P450scc and P450c17. A significant proportion of DHEA is converted to DHEAS (DHEA- sulphate) by the hydroxysteroid sulfotransferase SULT2A1.5 In the blood, most DHEA is found as DHEAS with levels that are about 300 times higher than those of free

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DHEA since the interconversion rate between them is high. The levels of both DHEAS falls with age, a process termed as the adrenopause. However, although DHEAS levels fall with age the production of glucocorticoids such as cortisol is remarkably unaltered resulting in a relative excess of cortisol over DHEAS and an imbalance of immune suppression over immune enhancement.6 DHEAS providing some degree of counterbalance to the potentially detrimental effects of catecholamine and cortisol which are immunosuppressive if chronically elevated, it also had a role in the elimination of harmful lymphocytes which may give rise to chronic inflammation and possibly to autoimmunity.7 Previous studies had shown that DHEAS levels increase in response to exercise; regular moderate exercise can buffer the negative impact of stress on immune responses. For example, while cortisol suppresses neutrophil function, this can be overcome by co incubation with DHEAS. 8

Subjects and Methods:

This study was carried out during the period from September (2007) to July (2008), it included (60) healthy subjects and had not been involved in any regular exercise for at least the previous 6 months ;(30 male), (30 female), their age range from (15-62) year (37.05 ± 13.02). Each subject had full history and medical exams, alcohol drinkers, smokers were excluded from the study as well as hypertensive, diabetics, or if they had history of drugs that affect immune function (i.e. aspirin, anti-inflammatory drugs) or if they had any recent (before 3 months) surgery or if they have allergic conditions. Pregnant females were excluded from the study and if they were used contraceptive pills.

The moderate exercise done in the morning in the sport club, each exercise session lasts for 60 minute, 3 times/ week for three consecutive months. Subjects were fasting and they volunteered for this research and complete the session of exercise till the end of three months. The minimum threshold of moderate exercise intensity of improvement in fitness had been proposed to be 50-60% of heart rate reserve.9 which was applied for each subject participated in this study and monitored by using Karvonen formula. 10

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; the program continued for 30 min. 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:

First: 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, Second: 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 machine (Scientific type, CYBEX, XELG90, USA) for 30 min; 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% (moderate exercise) + 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 & 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 the method of Boyum 11 to detects the following: Lymphocyte count and viability by trypan blue exclusion test according to Dolye and Griffiths 12.and Morphology of apoptotic lymphocytes by DNA-binding fluorescent dyes (acridine orange) according to Vacca 13. Second part (2 ml) was used for serum separation to estimate the DHEAS by electrochemiluminescence immuno assay (ECLIA) (DHEAS reagent kit, Type cobas®, Roche Diagnostics GmbH, D-68298 Mannheim, USA).

Statistical analysis:

A paired sample students T-test was used to compare between the data before and after exercise and the difference was considered statistically significant when P value was <0.05. The different types of relationships accomplished in this work between the duration of exercise and:

- DHEAS hormone level - Percentage of lymphocyte apoptosis, were examined using bivariate Pearson's correlation coefficient (two-tailed) test (Woolson, 1987).

Results:

A significant difference ($P < 0.05$) was observed in the level of DHEAS and percentage of lymphocyte apoptosis before and after exercise for each week along the period of the experiment as shown in table (1). Figure (1) Clarify the weekly changes in the level of DHEAS before and after exercise; DHEAS increased along the period of exercise until it reached a steady state. The highest level of DHEAS reached after exercise was in the tenth week (245.84+0.49), while the lowest value was in the first week (234.18+1.13). After

exercise there was a significant correlation (P= 0.000, r= 0.75) between the level of DHEAS hormone and the exercise duration as shown in figure (2); Illustrate changes in DHEAS level after exercise (3 times per week for 12 weeks). Figure (3) shows that the level of DHEAS hormone after exercise was highest in the third month in comparison to the first and second months. The highest percentage of lymphocyte apoptosis reached after exercise was 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).Figure (5) shows a positive correlation (r=0.701) between exercise duration and percentage of lymphocyte apoptosis after exercise.

Table (1): Weekly differences in the level of DHEAS and percentage of apoptotic lymphocyte before and after exercise along the experiment period

WEEKS	DHEAS µg/dl		% ofLymphocyte Apoptosis	
	Before	After	Before	After
Week 1	222.95± 4.23	234.18± 1.13	2.07± 0.25	3.36± 0.16
P	0.033*		0.002*	
Week 2	222.54± 2.84	235.54± 1.32	2.35± 0.16	4.09± 0.49
P	0.004*		0.015*	
Week 3	233.79± 6.26	242.17± 4.5	1.88± 0.23	4.11± 0.19
P	0.02*		0.001*	
Week 4	223.92± 2.83	238.26± 2.24	1.83± 0.04	4.14± 0.13
P	0.003*		0.0042*	
Week 5	228.03± 4.34	238.9± 2.59	2.05± 0.04	4.43± 0.07
P	0.01*		0.0021*	
Week 6	227.73± 1.51	240.35± 0.65	2.17± 0.57	4.49± 0.06
P	0.007*		0.0017*	
Week 7	234.64± 1.82	244.57± 0.64	1.87± 0.05	4.37± 0.14
P	0.009*		0.001*	
Week 8	236.72± 1.84	244.75± 1.64	2.1± 0.34	4.66± 0.01
P	0.006*		0.006*	
Week 9	232.07± 2.09	241.77± 2.94	2.61± 1.11	4.38± 0.16
P	0.004*		0.003*	
Week 10	238.46± 1.83	245.84± 0.49	2.66± 0.07	4.6± 0.09
P	0.0012*		0.0023*	
Week 11	238.62± 1.11	244.42± 0.33	2.33± 0.14	4.7± 0.15
P	0.008*		0.001*	
Week 12	236.08± 2.34	243.88± 1.59	2.94± 0.23	4.4± 0.21
P	0.019*		0.008*	

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

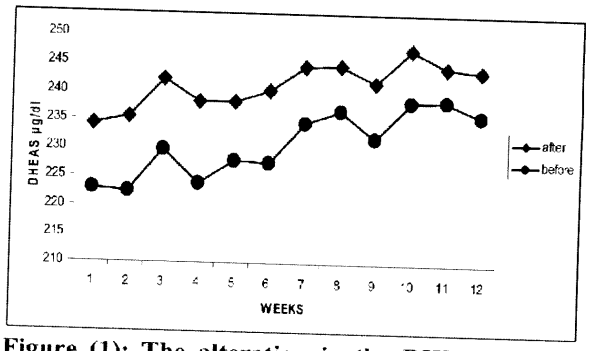

Figure (1): The alteration in the DHEAS levels for each week before and after exercise

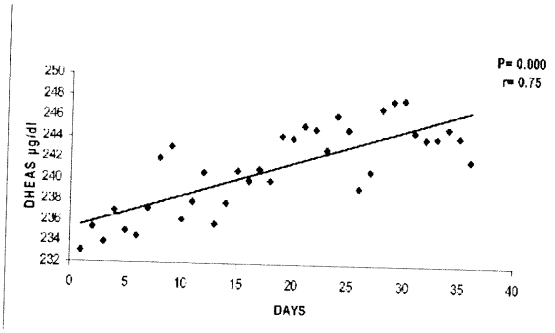

Figure (2): Correlation between exercise duration and DHEAS level after exercise.

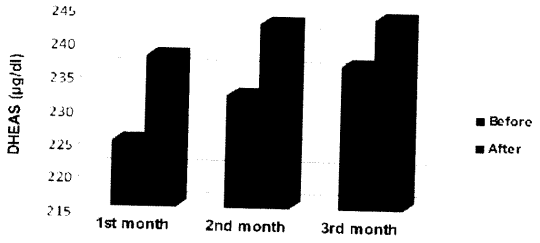

Figure (3): Monthly differences in the level of DHEAS before and after exercise

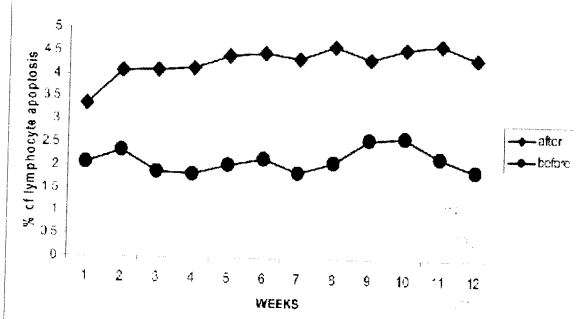

Figure (4): The alteration in the percentage of lymphocyte apoptosis for each week before and after exercise

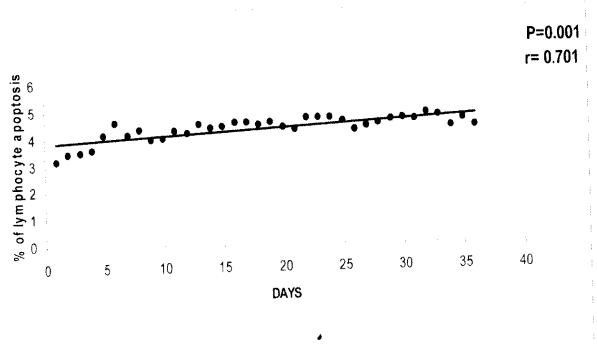

Figure (5): Correlation between exercise duration and percentage of lymphocyte apoptosis after exercise.

Discussion:

The current study demonstrated that the level of DHEAS hormone was elevated significantly after exercise as compared to the pre exercise level ($P < 0.05$) from the first week to the last week of the study table (1), figure (1). This result is in harmony with previous studies which had shown that DHEAS levels increase in response to exercise. 14 Exercise increased the production of DHEAS from adrenal cortex through the Hypothalamus-Pituitary-Adrenal axis. 15 It is obvious from this study that after exercise there was a highly significant correlation ($P= 0.000$, $r= 0.75$) between the level of DHEAS hormone and the duration of exercise, i.e as the days of the experiment was progressed the level of DHEAS hormone was significantly increased figure (5), this result was in a accordance with other study done by Tremblay et al.16 The percentage of apoptosis in the peripheral blood lymphocyte after exercise was significantly increased to ($4.32\% \pm 0.62\%$) figure (4).17 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.18 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.19 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 DHEAS hormone as the daily exercise was progressed, DHEAS hormone had multiple functions on T cells, which are mediated through binding to a group of intracellular receptors, or a specific receptor mediating many different pathways. The increment in the percentage of lymphocyte apoptosis was associated with increase DHEAS hormone this could be due to increased expression of the Fas and Fas-L that induces thymocyte apoptosis 20 .So DHEA may induce apoptosis through Fas/Fas-L pathway. In the third month the level of DHEAS hormone reached to a steady state due to the effect of endurance training as showed in figure (3).

Conclusion:

This prospective study had shown an increment of anti-aging hormone (DHEAS) along three months of moderate exercise, and this increment in hormone level is associated with increment in the percentage of peripheral blood lymphocyte apoptosis.

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