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Effects of Combined Submaximal Aerobic Exercise and Anaerobic Exercise on Serum Human Growth Hormone in Undergraduate Students

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ABSTRACT

The serum human growth hormone (GH) levels at pre- and post-experiment based on designed submaximal aerobic exercise and anaerobic exercise program for cardiovascular endurance in physical education students were investigated in comparison to the aged-match sedentary control medical student group. According to a paired-samples t-test of physical education students, there were statistically significant differences between the pre- and post-exercise program at week 0 $(t=-2.724, p=0.013)$ and week 4 $(t=-1.340, p=0.018)$, whereas no significant differences between pre- and post-exercise programs were observed at week 8. It may be possible that the intensity of the designed exercise program was ineffectually strenuous to prolong the activation of GH secretion. Hence, the improvement by adjusting the exercise program in each week should be designed to prevent the body from becoming acclimatized to the exercise program.

Keywords: Exercise; Growth hormone; Physical education

1. Introduction

In humans, growth hormone (GH) is generated and secreted from anterior pituitary gland that can be altered by various stimulating factors such as exercise, food, sleep and inhibiting factors including free fatty acid [1]. The highest peaks of GH secretion occur approximately an hour after

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starting sleeping (13-72 ng/mL plasma levels) [2]. There is a variation of growth hormone secretion all through the day. Generally, its individual level ranges from 5 to 45 ng/mL [3] of which its basal level is usually less than 5 ng/mL [2]. In the circulation, serum GH levels are commonly reported as total GH. The serum GH levels fluctuate due to pulsatile secretion pattern from the anterior pituitary gland. The serum GH in the basal level ranges between 0.01 and 1 ng/mL while that in the secretory pulse range from 1 and 100 ng/mL. Usually, the secretion of GH has reached the highest level during slow-wave sleep at night, ranging between 10-20 mg/mL. The other peaks observed during the day usually range between 2-10 ng/mL. However, other factors such as age, gender, food, exercise, stress, and hormones can affect the GH secretion [4]. According to age, GH secretion rate in young adolescents is approximately 700 ug/day while that in healthy adults is around 400 ug/day [5]. It is known that insufficient sleep can suppress the GH secretion, particularly in adults [6]. Concerning hormones that affect GH secretion, the growth hormone-releasing hormone (GHRH) operates via binding to the growth hormone-releasing hormone receptor (GHRHR) [7] while ghrelin operates via binding to growth hormone secretagogue receptors (GHSR) [8]. In addition, sex hormones increase the GH secretion via androgen secretion during teenage years [9].

Weltman et al. [10] reported that both continuous and intermittent acute aerobic exercise for 30 minutes caused an increase in 24-h GH secretion. Also, Kanaley et al. reported that 90 min of aerobic submaximal exercise caused an increase in GH level secretion that remained for more than 6 hours after exercise [11].

Herein, the average serum human growth hormone levels were compared between two groups of volunteers. Group 1 consisted of 20 healthy sedentary medical students who did not regularly perform exercise: approximately ≤ 2 times a week. Group 2 comprised 20 healthy physical education students with routine training under the designed submaximal aerobic exercise for cardiovascular endurance/fitness. Resting blood pressure, resting heart rate, resting body temperature, body weight and height were recorded as the indications of healthy subjects from both groups. This study will be beneficial for improvement of the physical capacity and efficiency in physical education students representing active undergraduate students based on a combined aerobic and anaerobic exercise program.

2. Materials and Methods

2.1 Ethics

This research was approved by Ethic Committees, Srinakharinwirot University under Ethic Approval No SWUEC/F-125/2558.

2.2 Selection of volunteers

The double-blind sampling volunteers consist of 20 males and females aged 18-20 studying in Faculty of Medical and Faculty of Physical Education, Srinakharinwirot University.

2.2.1 Inclusion criteria

- No-smoking for 1 month before participation in the program
- No-alcohol drinking for 1 month before participation in the program
- Have enough sleep before the venous blood collection appointment

2.2.2 Exclusion criteria

- Have underlying diseases such as heart diseases, hypertension, diabetes mellitus, and asthma.

2.3 Volunteers and exercise program

Volunteers were recruited from physical education and medical students studying at Faculty of Physical Education and Faculty of Medicine, Srinakharinwirot Uni-

versity. The activities were conducted in the Faculty of Medicine, Srinakharinwirot University (Prasarnmit Campus) and Faculty of Physical Education, Srinakharinwirot University (Ongkharak Campus). After the approval of the Ethics Committee in Human Research, the experiment was performed according to the ethical guidelines for research on human subjects in Thailand. The data of medical history including blood pressure, heart rate, body temperature measurements and venipuncture blood collection were worked out. Volunteers were separated into two groups. Group 1 was the control group containing 2nd year medical students $(n = 20)$. Second year medical students (preclinical phase) usually studied hard and they had no free time to perform exercise regularly. Thus, they were assumed to be sedentary/control group representing sedentary human growth hormone levels. Group 2 was an experimental group consisting of the 2nd year physical education students $(n = 20)$ because they had to do more physical activities due to the compulsory studying program for physical education. In this study, they had to perform following the assigned exercise program at weeks 0, 4 and 8. For exercise group 2, venipuncture bloods were collected before and after the exercise program. Venous blood collection in control group 1 was performed without exercise intervention at the same time as that of the experimental group. The serum hGH level of each sample was determined in triplicate with 4 weeks interval using vacutainer serum tubes.

2.4 Exercise program for the experiment group (Physical Education students)

The volunteers exercise three times a week (Monday, Wednesday, and Friday) which includes aerobic and anaerobic exercises. The aerobic exercises include interval, fat burn, and cardiovascular training for 30 minutes at 65 % submaximal exercise of maximum heart rate (Table 1a-1c). The anaerobic exercises include weight training for every part of the body including chest, back, biceps, triceps, shoulder, legs, and abdomen for 25 minutes as listed in (Table 2).

Table 1. (a) Anaerobic exercise program for Monday.

Body parts	Weight training	Number of set	Repetition per set	Rest between sets (minute)
Chest Upper back Biceps Triceps Shoulder	Standard Push Ups	2	10-12	1
	Side to Side Push Ups	2	10-12	1
	Burpee	2	10-12	1
	Bear Crawl Push Ups	2	10-12	1
	Prone Cobra	2	10-12	1
	Towel Pull Ups	2	10-12	1
	Side to Side Pull Ups	2	5-10	1
	Chair Dips	2	10-12	1

Table 1. (b) Anaerobic exercise program for Wednesday.

Body parts	Weight training	Number of sets	Repetition per set	Rest between sets (minute)
Abdomen Lower Back	Sit Ups	2	10-12	1
	Knee Elbow Crunch	2	10-12	1
	Reverse Cruncher	2	10-12	1
	Toe Toucher	2	10-12	1
	V-up	2	10-12	1
	Planks	2	1 (15 sec)	0.5
	Side Planks	2	1 (15 sec)	0.5
	Superman	2	10-12	1

Table 1. (c) Anaerobic exercise program for Friday.

Body parts	Weight training	Number of sets	Repetition per set	Rest between sets (minute)
Legs	Squats	2	10-12	1
	Back Lunges	2	10-12	1
	Front Lunges	2	10-12	1
	Wide Jump Squats	2	10-12	1
	Natural Glute Ham Raise	2	10-12	1
	Front Box Jump	2	10-12	1
	Single Leg Box Jump	2	10-12	1
	Calf Raises	2	10-12	1

Table 2. Submaximal aerobic exercises program.

Day	Program	Target Heart Rate (beat/min)	Time (minute)
Monday	Stretching then warm up (jogging) for 5 minutes, 30 seconds sprint with 30 seconds rest for 20 minutes, and then cool down (jogging) for 5 minutes and stretching	65 % Max HR	30
Wednesday	Stretching then warm up (jogging) for 5 minutes, 30 seconds sprint with 30 seconds rest for 20 minutes, and then cool down (jogging) for 5 minutes and stretching	65 % Max HR	30
Friday	Stretching then warm up (jogging) for 5 minutes and running for 20 minutes then cool down (jogging) for 5 minutes and stretching	65 % Max HR	30

2.5 Preparation of serum human and determination of growth hormone levels

Serum samples were manipulated according to Thavasu et al. [12] with some modification. Briefly, test tubes containing whole blood specimens were left for clotting in the upright position at room temperature for 30 minutes. Subsequently, the clotted blood samples were centrifuged at 1,000 x g for 10 minutes prior to transferring each serum into 1.5 mL autoclaved microcentrifuge tubes. Then, the serum samples were stored at -80 °C until use.

Results were reported as mean ± standard errors of the means (SEM) determined from the absorbances of each triplicate standard or samples and subtracted by the baseline value. The standard curve was constructed using Microsoft Excel 2013 where the standard concentration and absorbance were on the x-axis and y-axis, respectively. The best-fit straight line was created through standard point including the equation of the line (data not shown). A paired sample t-test using SPSS 19 version was achieved for analysis of the difference among the mean of control and treatment groups in each experiment using statistically significant difference at $p < 0.05$.

2.6 Enzyme-linked immunosorbent assay (ELISA)-standard curve

The standard curve of serum hGH concentration was determined using Ray Bio Human GH ELISA Kit (Ray Biotech, Inc., Georgia, United States). ELISA assay was performed according to manufacturer's protocols. Briefly, the GH standard protein (Item C) was added to 400 uL of Assay Diluent A (Item D) and mixed thoroughly. Next, total volume of 8 uL of dissolved GH standard protein (Item C) was mixed with 658.7 uL of Assay Diluent A (Item D, PBS supplemented by 0.09% sodium azide) in 1.5 mL autoclaved microcentrifuge tubes to prepare a 600 pg/mL stock standard solution. After that, serial dilution of standard solution was prepared by pipetting 300 uL Assay Diluent A (Item D) into each 1.5 mL micro centrifuge tube. Finally, a total volume of 200 uL of stock standard solution was added into 300 uL Assay Diluent A

(Item D), mixed thoroughly and then transferred to the next dilution. The concentrations of hGH standard solution for the standard curve were 600 pg/mL, 240 pg/mL, 94 pg/mL, 38.4 pg/mL, 15.4 pg/mL, 6.1 pg/mL, 2.5 pg/mL and 0 pg/ mL (as the zero standard).

2.7 Determination of serum growth hormone levels using ELISA

The ELISA assay was carried out according to manufacturer's protocols. All of the standards and samples were worked out in triplicate. Firstly, total volume of 100 uL of each standard and the sample were added into the 98-wells plate and incubated for 2.5 hours at room temperature. Then, the solution in each well was removed, and washed four times with 300 uL of 1X Wash Buffer (Item B). After the last washing, the remaining wash buffer was removed by inverting the plate and blotting against clean paper towels. Afterwards, total volume of 100 uL of 1XDetection Antibody GH (Item F) was added to each well and incubated for another 1 hour at room temperature with gentle agitating. Next, the solution was removed and the plate was washed three times with washing buffer. Then, 100 uL of 1XHorseradish Peroxidase-Streptavidin (Item G) was added to each well and incubated for 45 minutes at room temperature with gentle shaking. After that, the solution was removed, and the plate was washed three times. Subsequently, 100 uL of Tetraethyl Benzidine One-Step Substrate Reagent (Item H) was added to each well and incubated in the dark with gentle shaking for 30 minutes at room temperature. Finally, 50 uL of Stop solution (Item I, sulfuric acid) was added to each well. Absorbances were measured immediately or within 15 minutes after stop solution at 450 nm using BioTek Synergy Readers 9600.

3. Results and Discussion

3.1 Determination of human serum growth hormone levels

The average growth hormone levels in human serums were determined from two groups of volunteers. Group 1 was the 20 healthy sedentary medical students who did not perform exercise regularly (≤ 2 times a week). Group 2 was the 20 healthy physical education students with routine training including submaximal aerobics for cardiovascular training and anaerobic exercise by weight training programs. Blood pressure, heart rate, body temperature, body weight and height were recorded and revealed that both groups were healthy.

A paired-samples t-test was established to compare pre-experiment and post-experiment conditions in medical and physical education students. For the medical student group, mean serum hGH concentrations of pre-experiment at weeks 0, 4, and 8 were 0.4377, 0.9640, and 2.1021 ng/mL while those of post-experiment were 0.1084, 0.8509, and 0.5661 ng/mL, respectively (Table 3). Referring to the physical education student group, mean serum hGH concentrations of pre-experiment at weeks 0, 4, and 8 were 0.4588, 0.4015, and 1.0324 ng/mL, whereas post-experiment means were 0.8351, 0.8427, and 1.4373 ng/mL, respectively (Table 3). As per a paired-samples t-test of medical student group, significant differences between pre- and post-experiments at week 0 $(t = 2.434, p = 0.025)$ and week 8 $(t = 6.021, p = 0.000)$ were observed (Table 4). Similarly, significant differences were also elucidated in the physical education student group in pre-experiment and post-experiment at week 0 $(t = -2.724, p = 0.013)$ and week 4 $(t = 1.340, p = 0.018)$, respectively (Table 4).

Table 3. The mean serum hGH concentration (Triplicated reactions) in medical and physical education students at pre- (week 0) and post-experiments (week 4 and 8) according to ELISA data.

Weeks	Mean hGH concentration (ng/mL) ± standard errors of means (SEM)			
	Medical students (n= 20)		Physical Education students (n= 20)	
	Pre-experiment	Post-experiment	Pre-experiment	Post-experiment
0	0.438 ± 0.127	0.108 ± 0.014	0.449 ± 0.067	0.835 ± 0.154
4	0.964 ± 0.290	0.851 ± 0.128	0.402 ± 0.086	0.843 ± 0.125
8	2.102 ± 0.225	0.566 ± 0.168	1.032 ± 0.135	1.437 ± 0.261

Table 4. Paired-samples t-tests in medical (MED) and physical education (PEd) students.

Comparison Period	Mean hGH (SEM) (ng/mL)	Mean hGH (SEM) (ng/mL)	t
PreExWk0-PostExWk0(MED)	0.4377 (0.13)	0.1084 (0.01)	2.434[a]
PreExWk4-PostExWk4(MED)	0.9640 (0.30)	0.8509 (0.13)	0.326
PreExWk8-PostExWk8(MED)	2.1021 (0.23)	0.5661 (0.17)	6.061[b]
PreExWk0-PostExWk0(PEd)	0.4588 (0.07)	0.8351 (0.16)	-2.724[c]
PreExWk4-PostExWk4(PEd)	0.4015 (0.09)	0.8427 (0.13)	-2.597[d]
PreExWk8-PostExWk8(PEd)	1.0324 (0.14)	1.4373 (0.27)	-1.340

PreExWk0: Pre experiment at week 0, PreExWk4: Pre experiment at week 4, PreExWk8: Pre experiment at week 8
PostExWk0: Post experiment at week 0, PostExWk4: Post experiment at week 4, PostExWk8: Post experiment at week 8
[a,b] Statistically difference ($p<0.05$, 0.001) observed between PreEx and PostEx at Wk0 and Wk8 in MED students respectively.
[c,d] Statistically difference ($p<0.01$, 0.05) observed between PreEx and PostEx at Wk0 and Wk4 in PEd students respectively.

4. Conclusion

Concerning the average serum hGH level in the control group, there was a statistically significant difference between pre- and post-treatment at weeks 0 and 8 where serum hGH concentration in each week was slightly dispersed. The data was correlated to a previous report which stated that growth hormone levels in each individual fluctuate depending on various factors including gender, age, stress, hormones, food, and exercise [13]. According to the experimental group data, there was a statistically significant difference between pre- and post-exercise treatment in weeks 0 and 4, but not in week 8. The significant difference data in the experimental group at week 4 should be the acute effect of combined submaximal aerobic exercise and anaerobic exercise on human serum GH in physical education students. Additionally, the prohibited/ avoided physical activities for experimental participants may be the risk factors which cause fluctuation in serum hGH secretion in experimental subjects at week 8. It was notable that serum hGH levels of the post-exercise program at all three periods were greater than those of the pre-exercise program. This confirmed that submaximal aerobic exercise actually enhanced growth hormone secretion [14-15]. Considering the hGH level of physical education students at week 8, there were no statistically significant differences between pre- and post-exercise programs. It is possible that the intensity of submaximal aerobic exercise program in this study was not quite strenuous enough to prolong an increase in GH [16]. Hence, the improvement and/or adjustment of the exercise intensity program in each week should focus on strenuous effectiveness and be specifically designed to prevent subjects' adaptation/acclimatization to the exercise program [17-18].

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