Effects of Endurance Training and Breath-holding Training on Breath-holding Time, Maximal Oxygen Uptake, Lung Capacity, Red Blood Cell and White Blood Cell Counts

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Abstract

The purposes of this study were to investigate effects of endurance training, breath-holding training, and combination of endurance training and breath-holding training on breath-holding time (BHT), resting heart rate (RHR), vital lung capacity (VLC), maximal oxygen uptake (VO_2max), hematocrit (Hct), red and white blood cell counts (RBC and WBC) and relationship between each parameter after training. Forty male college students (mean age=20.05 ± 0.88 years and weight=63.946 ± 6.90 kg.) volunteered to be the subjects and were divided into 4 groups of 10 subjects. Group C served as control group. The experimental groups (Group E, BH, E+BH) were trained by either running, breath holding, or a combination of both, respectively. BHT, RHR, VLC, and VO_2 max were collected before training, after the 4th week of training, and after training, while Hct, RBC and WBC were measured before and after training. Means ± S.D., T-test, One-way ANOVA, Tukey's HSD, and Pearson correlation coefficient were used for data analyses. Significant level was set at .05. Results showed that after training, RHR were lower ($p<.05$) in Group E (71.20 ± 1.93, 68.00 ± 2.31, and 66.10 ± 2.23 beats/min) and Group E+BH (70.00 ± 2.98, 67.70 ± 2.31, and 65.30 ± 2.67 beats/min). VLC were higher ($p<.05$) in Group E (3,730 ± 305.69, 4,040 ± 206.56, and 4,390 ± 119.72 ml) and Group E+BH (3,845 ± 457.32, 4,080 ± 413.79 and 4,380 ± 301.11 ml). VO_2 max were higher ($p<.05$) in Group E (41.56 ± 5.41 47.11 ± 5.53 and 53.30 ± 4.48 ml/kg min) and Group E+BH (41.85 ± 6.27, 47.28 ± 5.62, and 51.71 ± 4.56 ml/kg/ min). RBC were higher ($p<.05$) in Group E (5.48 ± 0.51 and 5.66 ± 0.49 $x10^6$cells/mm^3) and Group E+BH (4.90 ± 0.37 and 5.23 ± 0.42 $x10^6$cells/ml^3). HCT were lowered ($p<.05$) in Group E (47.60 ± 2.12 and 43.20 ± 2.25 %) and Group E+BH (47.10 ± 1.79 and 42.80 ± 2.53 %). However, BHT in group BH (43.10 ± 13.36 , 87.21 ± 23.14, and 98.31 ± 19.64 sec) and group E+BH (43.17 ± 11.05 103.63 ± 33.75 and 136.39 ± 25.90 sec) were higher ($p<.05$) after training. However, the combined breath-holding training with running training increased more BHT than breath-holding training alone ($p<.05$). There were no significant differences in RHR, VLC and VO_2 max between Group E and E+BH ($p>.05$). There was no different in HCT, RBC, and WBC between groups ($p>.05$). Paired relationships showed a negative relation between RHR and BHT, VLC, and VO_2 max ($\alpha<.05$). VLC related positively to VO_2max ($\alpha<.05$). HCT related negatively to BHT and positively to WBC ($\alpha<.05$). BHT alone is not enough to estimate the cardiorespiratory condition. The results led to the conclusion that endurance training cannot increase BHT, but improved after breath-holding training. Endurance training combined with breath-holding training increased more BHT than breath-holding training. However, endurance exercise training protocol used in this study had no effect on WBC.

Keywords: Endurance Training/ Breath-holding Training/ Cardiorespiratory Function/Blood Cell Counts

บทคัดย่อ

วัตถุประสงค์การวิจัยครั้งนี้เพื่อการศึกษาผลการฝึกออกกำลังกายประเภททอดทน การฝึกกลั้นหายใจในน้ำ และการฝึกออกกำลังกายประเภททอดทนร่วมกับการฝึกกลั้นหายใจในน้ำที่มีต่อเวลาในการกลั้นหายใจในน้ำ (BHT) อัตราการเต้นของหัวใจขณะพัก (RHR) ความจุปอด (VLC) ปริมาณการใช้ออกซิเจนสูงสุด (VO_2max) ฮีมาโตคริต (Hct) ปริมาณเม็ดเลือดแดง (RBC) และเม็ดเลือดขาว (WBC) และความสัมพันธ์ระหว่างตัวแปรแต่ละตัวหลังจากสิ้นสุดการฝึก ในการศึกษานี้ใช้นักศึกษาอาสาสมัครชายที่กำลังศึกษาในระดับปริญญาตรี (อายุเฉลี่ย 20.05±0.88 ปี, น้ำหนักเฉลี่ย 63.946±6.90 ก.ก.) เป็นกลุ่มตัวอย่าง โดยแบ่งออกเป็น 4 กลุ่ม กลุ่มละ 10 คน ดังนี้ กลุ่ม C เป็นกลุ่มควบคุม กลุ่ม E ฝึกออกกำลังกายด้วยการวิ่ง กลุ่ม BH ฝึกกลั้นหายใจในน้ำ และ กลุ่ม E+BH ฝึกวิ่งและกลั้นหายใจในน้ำประกอบกันเป็นเวลา 8 สัปดาห์ โดยทำการเก็บข้อมูล BHT, RHR, VLC และ VO_2 max ก่อนการฝึก หลังการฝึกในสัปดาห์ที่ 4 และ หลังจากสิ้นสุดการฝึก โดยทำการเก็บตัวอย่างเลือดเพื่อเก็บข้อมูล Hct, RBC และ WBC ก่อนและหลังการฝึก ผลการ ศึกษาครั้งนี้พบว่า RHR ในกลุ่ม E (71.20±1.93, 68.00±2.31 และ 66.10±2.23 ครั้ง/นาที) และกลุ่ม E+BH (70.00±2.98, 67.70±2.31 และ 65.30±2.67 ครั้ง/นาที) ลดลง ($p<.05$) ค่า VLC ในกลุ่มที่ E (3,730±305.69, 4,040±206.56 และ 4,390±119.72 ม.ล.) และกลุ่ม E+BH (3,845±457.32, 4,080±413.79 และ 4,380±301.11 ม.ล.) มีค่าเพิ่มขึ้น ($p<.05$) หลังจากการฝึก VO_2 max ของกลุ่ม E (41.56±5.41 47.11±5.53 และ 53.30±4.48 ม.ล/ก.ก./นาที) และกลุ่ม E+BH (41.85±6.27, 47.28±5.62 และ 51.71±4.56 ม.ล./ก.ก./ นาที) มีค่าเพิ่มขึ้น ($p<.05$) ค่าของ RBCในกลุ่ม E (5.48±0.51 และ 5.66±0.49x10^6 เซล/ม.ม.^{3}) และกลุ่ม E+BH (4.90±0.37และ 5.23±0.42 x10^6 เซล/ม.ล.^{3}) เพิ่มขึ้น ($p<.05$) หลังการฝึก ค่า Hct ของกลุ่ม E (47.60±2.12 และ 43.20±2.25 %) และกลุ่ม E+BH (47.10±1.79 และ 42.80±2.53 %) มีค่าลดลง ($p<.05$) หลังการฝึก ค่าของ BHT ในกลุ่ม BH (43.10 ±13.36, 87.2±23.14 และ 98.31±19.64 วินาที) และ กลุ่ม E+BH (43.17±11.05 103.63±33.75 และ 136.39±25.90 วินาที) มีค่าเพิ่มขึ้น ($p<.05$) หลังการฝึก ผลการวิจัยสามารถอธิบายได้ว่า การฝึกกลั้นหายใจร่วมกับการฝึกวิ่งส่งผลให้ความสามารถในการกลั้นหายใจเพิ่มขึ้นมากกว่า ($p<.05$) การฝึกกลั้น

หายใจเพียงอย่างเดียว ค่า RHR ที่ลดลง VLCและVO_2 max ที่เพิ่มขึ้นซึ่งเป็นผลจากการฝึกวิ่งไม่แตกต่างจากผลของการฝึกวิ่งร่วมกับการฝึกกลั้นหายใจ ($p>.05$) จากการเปรียบเทียบค่า Hct, RBC และ WBC ระหว่างกลุ่มตัวอย่างแต่ละกลุ่มพบว่าไม่แตกต่างกัน ($p>.05$) ในด้านความสัมพันธ์ระหว่างตัวแปรแต่ละตัวพบว่า RHR มีความสัมพันธ์เชิงผกผันกับ BHT, VLC และ VO_2 max ($p<.05$) ค่าVLC สัมพันธ์ตามค่า VO_2max ($p<.05$) ค่า Hct สัมพันธ์เชิงผกผันกับค่าของ BHT แต่สัมพันธ์ตามค่าของ WBC ($p<.05$) ทั้งนี้ค่า BHT แต่เพียงค่าเดียวไม่สามารถชี้วัดสมรรถภาพระบบหัวใจและปอดได้ จากการศึกษานี้สามารถสรุปได้ว่าการฝึกออกกำลังกายประเภทอดทนไม่มีผลต่อการพัฒนาความสามารถในการกลั้นหายใจได้ แต่การฝึกออกกำลังกายประเภทอดทนร่วมกับการฝึกกลั้นหายใจในน้ำสามารถพัฒนาความสามารถในการกลั้นหายใจในน้ำได้และให้ผลที่ดีกว่าการฝึกกลั้นหายใจในน้ำแต่เพียงอย่างเดียว อย่างไรก็ตาม ความหนักของการฝึกออกกำลังกายที่ใช้ในการศึกษานี้ยังไม่เพียงพอที่จะส่งผลต่อปริมาณเม็ดเลือดขาวในเลือด

คำสำคัญ: การฝึกความอดทน การฝึกการกลั้นหายใจ ระบบหัวใจและปอด การนับเม็ดเลือด

Introduction

Recent studies have shown that human diving responses were similar to marine mammals in that bradycardia and vasoconstriction observed in some organs could redistribute blood flow to the hypoxia-sensitive organs such as the heart and brain to sustain apnea. In addition, it had been reported that repetitions of breath holding could delay the depletion of lung oxygen store, with larger reduced peripheral venous oxygen and increase anaerobiosis, and therefore prevent alveolar and arterial oxygen content (PO_2). This improved hypoxia in vital organs could enlarge the diving responses with an oxygen-conserving effect during exercise (Andersson and Schagatay, 1998 and Andersson, Liner, Runow, and Schagatay, 2002). Previous studies had also shown that aerobic capacity depends on the respiratory system for ventilation and gas exchange, the circulatory system to deliver O_2 to the working muscles, and the oxidative enzymes to use the O_2 once it has been delivered (Sherwood, 2004). Endurance training could positively develop the cardiorespiratory function, plasma volume, red blood cell counts, and hemoglobin. These adaptations could improve performance with a concomitant increase in maximal oxygen uptake (VO_2 max), external respiration, muscular and cardiovascular functions and ventilatory threshold. (Robergs & Roberts, 1997) In addition, changes that occurred in white blood cell counts depended on exercise intensity and duration (Plowman and Smith, 2003). Exhaustive exercise suppressed immune system whereas moderate exercise had favorable effect on immune system (Sherwood, 2004; Mackinnon, 2000). Although, it was clear that breath-holding ability, aerobic capacity of an individual depend on the responses and adaptations of the cardiorespiratory fitness as mentioned above, it remains unclear whether or not endurance training could prolong the breath-holding time and in what extent how breath-holding training could improve the fitness condition and blood cell counts. In addition, It is not known whether how breath-holding training in the combination with endurance training could improve the cardiorespiratory condition, breath-holding time, blood cell counts, and confer more benefits than endurance training alone. Therefore, the present study was undertaken in order to answer all those questions.

The purposes of this study were (1) to investigate effects of endurance training, breath-holding training, and endurance exercise training combined with breath-holding training on breath-holding time (BHT), resting heart rate (RHR), maximal oxygen uptake (VO_2max), vital lung capacity (VLC), hematocrit (Hct), red blood cell counts (RBC) and white blood cell counts (WBC), and (2) to see if there are any positive relationships between BHT, RHR, VO_2 max, VLC, Hct, RBC, and WBC among 3 training regimens.

Methods

Subjects

Subjects were±forty healthy male undergrad±uate student volunteers of Burapha University (aged = 20.05 0.876 years; weight = 63.946 6.897 kg.) who never participated in exercise training program. Subjects were divided into 4 groups of 10 subjects; control group C, endurance training group E, breath holding-training program group BH and endurance training combined with breath-holding training group E+BH. The subjects were matched by individual VO_2max and BHT.

Measures and Data Collection

RHR was measured at rest. Åstrand-Rhyming Test (r =0.94; p<.01) was used to assess VO_2max on the bicycling ergometer (Monark Ergomedic 884 ESs, Sweden) for 6 minutes at the speed of 50 rpm. The average heart rate between the 5th and 6th minute was used to determine VO_2max. VLC assessment was done by expiring maximal breathing into a dry spirometer (Spiropet-Windmill Type, Nubion Medical Instrument Co., Ltd.). BHT was measured by a set of three apneic face immersion with 2 minutes interval in 9.5-10.5°C water in a prone position without hyperventilation before apnea (Andersson and Schagatay, 1998) (r = 0.89; p<.01). Blood samples (3-5 cc.) were collected in resting state for RBC, WBC, and Hct determination (Coulter JT). After a pre-test for RHR, VO_2max, VLC, BHT, Hct, RBC, and WBC), the subjects in group E were participated in endurance training (running 3-days a week for 8-weeks, with the starting distance of 2.5 km and 20 % progressive distance after every two weeks) for 8 weeks (Kanchanathaweekul, 1998). The subjects in BH group were participated in 5-repetition of breath-holding training in a prone position in cold water (9.5°C-10.5° C) with a 2-minutes interval without hyperventilation before breath holding and swallowing or expiring during breath holding (Shagatay and andersson, 1999). This was done 3 days a week for 8-weeks. The subjects in E+BH group underwent the breath-holding training as in BH group and followed by the same endurance training as E group after 45-minutes rest. After the 4th week of training, mid-test was conducted to determine RHR, BHT, VO_2max, and VLC of all subjects. All variables were measured again at the end of the 8^{th} week training session.

Data analyses

Means and standard deviations of each parameter (BHT, RHR, VO_2max, VLC, Hct, RBC, and WBC) were determined. One-way ANOVA followed by Tukey's HSD Test (where appropriated) were used for data analysis. Significant level was set at .05. The differences of Hct, RBC and WBC within group were analysed with paired dependent t-test. In addition, Pearson product-moment correlation coefficiency was used for the analysis of paired relationships between each parameter after training.

Physical characteristics of subjects were summarized in Table 1. RHR of all subject groups appear in Table 2. As shown in Table 2, RHR of the control and BH groups had no significant change. However, RHR in the E and E+BH groups were significantly lower after training. A comparison between groups showed no significant difference in RHR between control and BH group. After the experiment, RHR of control and BH groups were significantly higher than E and E+BH group. However, no significant difference in RHR between E and E+BH groups were observed.

BHT at pre-test, mid-test, and post-test were showed in Table 2. It was shown that BHT of control and E groups were not significantly changed. BHT in BH group was significantly higher after the 4th week of training. BHT in E+BH group was also higher after the 4th week of training. When comparisons between groups were done, it was found that BHT of control group was not significantly different from E group. After training,

Results

Table 1 Physical Characteristic of Subjects (Means±S.D.) in each Group (n=10)

	C	E	BH	E+BH	Average
Age (yrs.)	20.00±0.67	20.00±0.94	20.03±0.95	19.90±0.99	20.05±0.89
Weight (kg.)	65.28±6.45	63.12±8.37	65.00±8.10	62.38±4.65	63.95±6.90

Remark: C = Control group, E = Endurance training group, BH = BHT group, and E+BH = Endurance training combined with BHT group

Table 2 Comparisons of RHR, BHT, VLC, VO_2max, HCT, RBC, and WBC among four subject groups

Parameter	Group	Mean ± S.D.		C			E			BH			E+BH		
				Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
		71.40 ± 2.50	**Pre**		- .20	- .60	.20	3.40	**6.60***	.80	1.20	1.00	1.40	3.70	**6.10***
	C	71.60 ± 3.37	**Mid**			- .40	.40	3.60	**6.80***	1.00	1.40	1.20	1.60	3.90	**6.30***
		72.00 ± 2.83	**Post**				.80	**4.00***	**7.20***	1.40	1.80	1.60	2.00	**4.30***	**6.70***
		71.20 ± 1.93	**Pre**					3.20	**6.40***	.60	1.00	.80	1.20	3.50	**5.90***
	E	68.00 ± 2.31	**Mid**						1.90	-2.60	-2.20	-2.40	-2.00	.30	2.70
RHR		66.10 ± 2.23	**Post**							**-5.80***	**-5.40***	**-5.60***	**-5.20***	2.90	.50
(beats/min)		70.60 ± 2.12	**Pre**								- .40	- .20	- .60	2.50	**5.30***
	BH	70.20 ± 2.39	**Mid**									.20	.20	2.70	**4.90***
		70.40 ± 3.19	**Post**										.40	2.30	**5.10***
		70.00 ± 2.89	**Pre**												**4.70***
	E+BH	67.70 ± 2.31	**Mid**												2.40
		65.30 ± 2.67	**Post**												-
		43.35 ± 13.10	**Pre**		- 7.9	-11.0	- 0.6	- 5.8	-21.7	0.3	**-43.9***	**-55.2***	-.18	**-60.3***	**-93.0***
	C	51.27 ± 3.16	**Mid**			- 3.0	- 7.3	2.09	-13.8	8.2	**-35.9***	**-47.2***	-8.21	**-52.4***	**-85.1***
		54.31 ± 13.57	**Post**				-10.4	- 5.1	-10.8	11.2	**-32.9***	**-44.2***	-11.1	**-49.3***	**-82.1***
		43.93 ± 18.10	**Pre**					- 5.3	-21.1	0.8	**-43.3***	**-54.6***	-0.8	**-59.7***	**-92.5***
	E	49.18 ± 21.56	**Mid**						-15.9	6.1	**-38.0***	**-49.3***	-0.6	**-54.5***	**-87.2***
BHT		65.07 ± 32.92	**Post**							22.0	-22.0	**-33.4***	-21.9	**-38.6***	**-71.3***
(seconds)		43.10 ± 13.36	**Pre**								**-44.1***	**-55.4***	-.07	**-60.5***	**-93.3***
	BH	87.21 ± 23.14	**Mid**									-11.3	**44.0***	-16.4	**-49.2***
		98.31 ± 19.64	**Post**										**55.3***	5.1	**-37.9***
		43.17 ± 11.05	**Pre**											**-60.5***	**-93.3***
	E+BH	103.63 ± 33.75	**Mid**												**-32.8***
		136.39 ± 25.90	**Post**												-

Parameter	Group	Mean ± S.D.		C			E			BH			E+BH		
				Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
		3,595 ± 363.97	**Pre**		40	65	-135	**-445***	**-795***	-215	-190	-200	-250	-485	**-785***
	C	3,555 ± 369.27	**Mid**			25	-175	**-485***	**-835***	-255	-230	-240	-290	**-525***	**-825***
		3,530 ± 337.64	**Post**				-200	**-510***	**-860***	-280	-280	-255	-265	**-550***	**-850***
		3,730 ± 305.69	**Pre**					-310	**-660***	- 80	- 55	- 65	-115	-350	**-650***
	E	4,040 ± 206.56	**Mid**						-350	230	255	245	195	- 40	-340
VLC		4,390 ± 119.72	**Post**							**580***	**605***	**595***	**545***	310	10
(milliliters)		3,810 ± 384.27	**Pre**								25	15	- 35	-270	**-570***
	BH	3,785 ± 347.25	**Mid**									- 10	- 60	-295	**-595***
		3,795 ± 381.12	**Post**										- 50	-285	**-585***
		3,845 ± 457.32	**Pre**											-235	**-535***
	E+BH	4,080 ± 413.79	**Mid**												-300
		4,380 ± 301.11	**Post**												-

Remark: C = Control group, E = Endurance training group, BH = Breath holding training group, and E+BH = Endurance training combined with Breath holding training group, and * = significance at the .05 level.

Table 2 (Continued) Comparisons of RHR, BHT, VLC, VO_2max, HCT, RBC, and WBC among four subject groups

Parameter	Group	Mean±S.D.		C			E			BH			E+BH		
				Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
		41.87±7.12	**Pre**		0.39	0.42	0.30	-5.25	**-11.43***	-0.11	-0.24	-0.47	0.02	-5.14	**- 9.85***
	C	41.47±7.81	**Mid**			0.03	-0.09	-5.64	**-11.82***	-0.50	-0.63	-0.86	-0.37	-5.80	**-10.24***
		41.44±7.66	**Post**				-0.12	-5.67	**-11.85***	-0.54	-0.66	-0.90	-0.41	-5.84	**-10.27***
		41.56±5.41	**Pre**					-5.55	**-11.73***	-0.42	-0.54	-0.78	-0.29	-5.72	**-10.15***
	E	47.11±5.53	**Mid**						- 6.19	5.13	5.00	4.77	5.26	-0.17	- 4.60
VO_2max		53.30±4.48	**Post**							**11.32***	**11.19***	**10.96***	**11.45***	6.02	- 1.58
(ml/kg/min)		41.98±5.68	**Pre**								-0.13	-0.36	0.13	-5.30	**- 9.73***
	BH	42.11±5.68	**Mid**									-0.23	0.26	-5.17	**- 9.60***
		42.34±5.53	**Post**										0.49	-4.94	**- 9.37***
		41.85±6.27	**Pre**											-5.43	**- 9.86***
	E+BH	47.28±5.62	**Mid**												- 4.43
		51.71±4.56	**Post**												-
	C	44.50±2.59	**Pre**		-	-.80	-3.10	-	1.30	.70	-	- .60	-2.6	-	1.70
		45.30±3.09	**Mid**				-2.30	-	2.10	1.50	-	0.20	-1.80	-	2.50
HCT	**E**	47.60±2.12	**Post**						**4.40***	**3.80***	-	2.50	0.50	-	**4.80***
(%)		43.20±2.25	**Pre**							-0.60	-	-1.90	**-3.90***	-	0.40
	BH	43.80±2.70	**Mid**									-1.30	-3.30	-	1.00
		45.10±3.73	**Post**										-2.00	-	2.30
	E+BH	47.10±1.79	**Pre**												**4.30***
		42.80±2.53	**Mid**												

Parameter	Group	Mean±S.D.		C			E			BH			E+BH		
				Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
	C	5.37±0.48	**Pre**		-	.172	-.108	-	-.283	-.198	-	-.031	.473	-	.144
		5.23±0.33	**Post**				-.280	-	-.455	-.370	-	-.203	.301	-	-.028
	E	5.48±0.51	**Pre**						-.175	-.090	-	.077	.581	-	.252
RBC		5.66±0.49	**Post**							-.085	-	.252	**.756***	-	.427
(10^6 cells/mm^3)	**BH**	5.57±0.63	**Pre**									.167	.671	-	.342
		5.40±0.54	**Post**										.504	-	.175
	E+BH	4.90±0.37	**Pre**												-.329
		5.23±0.42	**Post**												
	C	6,680± 939.03	**Pre**												
		7,600±1,883.85	**Post**												
	E	7,760±1,474.37	**Pre**												
WBC		7,760±1,224.02	**Post**	ANOVA show that there is no significant difference within and between group (p>.05)											
(cells/mm^3)	**BH**	7,890±2,100.36	**Pre**												
		8,200±3,180.85	**Post**												
	E+BH	7,720±1,599.17	**Pre**												
		7,370±1,564.93	**Post**												

Remark: C = Control group, E = Endurance training group, BH = Breath holding training group, and E+BH = Endurance training combined with Breath holding training group, and * = significance at the .05 level.

BHT of control and E groups were significantly lower than BH and E+BH groups. BHT of the BH group was significantly lower than E+BH group (Table 2).

VLC at pre-, mid-, and post-tests were shown in Table 2. It can be noticed that VLC in control group and BH group were not significantly different between tests. VLC in E group was significantly higher after the 4th week of training. VLC in E+BH group was significantly higher after training. When comparisons between groups were made, it was found that there was no significant difference in VLC between C and BH groups. After the experiment, VLC of C and BH groups was significantly lower than E and E+BH groups. The difference in VLC between E and E+BH groups was not observed (Table 2).

VO_2max at pre-, mid-, and post-tests was shown in Table 3, it can be seen that VO_2 max in C and BH groups was not significantly changed. VO_2max in E and E+BH groups was significantly higher after training. In a comparison between groups, there was no significant difference in VO_2max between C and BH groups. After the experiment, VO_2max of C and BH groups were significantly lower than E and E+BH groups. VO_2max between E and E+BH groups showed no difference.

Hct at pre- and post-tests was shown in Table 3. Hct of C and BH groups had no significant change. Hct of E and E+BH group decreased significantly after training without significant difference between groups (Table 3).

In addition, RBC count at pre- and post-tests was shown in Table 3 without significant difference between groups. However, RBC within E and E+BH groups were significantly changed after training (Table 3).

Table 3 Paired samples test for RBC (x10^6cells/mm3) within each subject group

		Paird Differences							
		Mean	S.D.	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2 tailed)
					Lower	Upper			
C	**Pre-Post**	.15	.28	.09	-.60	.35	1.63	9	.137
E	**Pre-Post**	-.18	.13	.04	-.27	-.08	-4.32	9	.002*
BH	**Pre-Post**	.17	.26	.08	-.02	.35	2.02	9	.074
E+BH	**Pre-Post**	-.33	.28	.09	-.53	-.13	-3.75	9	.005*

Remark: C = Control group, E = Endurance training group, BH = BHT group, and E+BH = Endurance training combined with BHT group, and * = significance at the .05 level.

Table 4 Analysis of Variance of WBC between Groups

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	14,049,500	7	2,007,071.43	.59	.762
Within Groups	245,090,000	72	3,404,027.78		
Total	259,139,500	79			

* = significance at the 0.05 level

As shown in Table 4, WBC count was not found to change within or between groups. Results also showed that RHR related negatively to BHT (BHT= 314.32-3.30 RHR), VLC (VLC = 8775.34-69.43RHR) and VO_2max (VO_2max = 146.09-1.45 RHR). VO_2max related positively to VLC (VO_2max = 11.76+0.01 VLC). HCT related negatively to BHT (Hct = 322.72-5.19 BHT), but related positively to WBC (Hct = 41.19+.001 WBC) as shown in Table 5.

Table 5 Pearson Product Moment Correlation for Dependent Variables

	RHR	BT	VlC	VO_2 max	RBC	Hct	WBC
RHR, Correlation	1	-.320*	-.566**	-.726**	.048	-.251	.128
Sig. (2-tailed)		.044	.000	.000	.770	.411	.131
N	40	40	40	40	40	40	40
BHT, Correlation		1	.235	.187	-.237	-.415*	-.022
Sig. (2-tailed)			.144	.248	.141	.008	.891
N		40	40	40	40	40	40
VLC, Correlation			1	.542**	.033	.102	.014
Sig. (2-tailed)				.001	.840	.018	.929
N			40	40	40	40	40
Vo_2max.Correlat^n				1	.099	-.139	-.037
Sig. (2-tailed)					.542	.393	.820
N				40	40	40	40
RBC, Correlation					1	.182	-.008
Sig.(2-tailed)						.260	.963
N					40	40	40
Hct, Correlation						1	.327*
Sig.(2-tailed)							.039
N						40	40

Remark: RHR=resting heart rate, BHT=breath-holding time, VLC=vital lung capacity, VO_2max =maximal oxygen uptake, RBC=red blood cell counts, Hct=hematocrit, WBC=white blood cell counts,

* Correlation is significant at 0.05 level and ** is significant at 0.01 level (2-tailed).

Discussion

For E group, there was no significant BHT change after training (p>.05) since endurance training led to the cardiorespiratory and muscle metabolic improvement that was not concerned for the improvement of diving reflexes and the brain functions that prolonged the intolerable need to breathe (Robergs and Roberts, 1997; Lindholm, 2002). In contrast, RHR was significantly lower, but VLC was significantly higher after the 4th week of training, while VO_2max was significantly higher after training (p<.05). As the reason, the larger heart dimensions after endurance training increased the resting stroke volume and made the RHR lower, while strength and endurance of the respiratory muscles appeared to improve by training and VLC could be elevated. Furthermore, cardiovascular adaptations that improved cardiac output and a-vO_2diff by training could enhance VO_2max of people in all races and ages. (Robergs and Kateyian, 2003; Willmore, Stanforth, Gagnon, Rice, Mandel, Leon, Rao, Skinner, and Bouchard, 2001 ; Robinson and Kjeldgaard, 1982). Significantly, Hct decreased, but RBC increased after training (p<.05), since endurance training led to the elevation of plasma volume accompanied with the elevation of RBC and hemoglobin (Hb). Nevertheless, the elevated ratio of plasma volume was higher than the elevated RBC that lowered Hct. However, there was no significant WBC difference after training, since intensity of the running protocol for this study were not enough to promote WBC change as the impact of exercise on the immune defense depended on the intensity of the exercise (Wilmore and Costill, 1988; Sherwood, 2004; Convertino, 1991).

As BH group, BHT was higher after the 4th week of breath-holding training (p<.05), since the most ventilatory stimulants (P_ACO_2 and P_AO_2) were delayed that prolonged the need to breathe. In addition, BHT of trained divers were enlarge with the better oxygen conserving as diving experience (Robergs and Roberts, 1997) and (Schagatay and Andersson, 1998). In contrast, RHR, VLC, and VO_2max were not significantly changed after breath-holding training (p<.05) since the breath-holding training could enlarge diving reflexes and brain function without enlarged heart chamber that could increase stroke volume, decrease RHR, cardiac output, and respiratory muscle strength that could improve VLC and VO_2max (Robergs and Kateyian, 2003; Levitzky, 1991). In addition, Hct, RBC had no significant change (p>.05), since Hb and Hct that increased immediately after a maximal breath-holding series due to the spleen contraction would return towards the baseline after 10 minutes (Richardson et.al, 2003). However, this study found no WBC adaptation after training ($p<.05$), since an increased arterial pressure of CO_2 companied by an increased chemical drive would load diving reflexes and the brain that caused the need to breathe without loading the heart volume as endurance training that impacted the immune defense (Robergs and Kateyian, 2003; Sherwood, 2004).

As E+BH group, BHT was significantly higher after the 4th week of training ($p<.05$), since breath-holding training led to better oxygen conserving that enlarged BHT in trained divers. In addition, the combined endurance activity could load the heart volume without threaten the diving reflexes and the brain to improve BHT (Schagatay and Andersson, 1998; Sherwood, 2004). Significantly, RHR was lowered, while VLC and VO_2 max were improved after training ($p<.05$). As the reason, the dynamic endurance training could, reduced the sympathoadrenal drive that decrease RHR, increased oxidative capacity of the respiratory muscles that led to the increased VLC, elevated blood volume, stroke volume, and a better perfusion of blood in the active tissues that increased VO_2 max. In addition, the combined breath-holding training was the activity that loaded the diving reflexes and the brain could not threaten to improve cardiorespiratory condition (Viru, 1995; Powers, 2001; Plowman and Smith, 2002; Schagatay and Andersson, 1998; Sherwood, 2004). Significantly, Hct was lower but RBC was higher after training ($p<.05$), since the greater increased blood volume after endurance training resulting from more intense levels of training. This accomplished through an increase in both plasma and RBC volumes, but the elevated plasma volume was usually much greater than that of the RBC volume, thus Hct accually decreased. However, E+BH group had no significant difference in WBC ($p>.05$), since the breath-holding training activity that loaded the diving reflexes and the brain could not affect the immune system. In addition, the intensity of training protocol in this study was not enough to promote WBC change (Sherwood, 2004; Wilmore and Costill, 1988).

As comparing between groups, there was no significant different in BHT between E group and control group ($p>.05$). However, BHT of C and E groups were significantly lower than BH and E+BH groups ($p<.05$). In addition, BHT of BH group was significantly lower than E+BH group ($p<.05$). As the reason, endurance training that improved cardiorespiratory condition could not affect the diving reflexes and the brain with more experiences to tolerate the continual produced CO_2 and the need to breathe. However, improved cardiorespiratory condition affected by endurance training could support the combined breath-holding training to promote more bradycardia and arterial oxygen saturation that led to the better BHT during breath holding. Moreover, the cardiovascular responses to apnea in exercising human clearly delayed the development of hypoxemia by reducing a rate of up take from the main oxygen store, i.e. the lungs (Andersson and Schagatay, 1998; Lindholm, 2002).

As the cardiorespiratory condition, there was no significant different RHR, VLC, and VO_2max between C and BH group ($p>.05$). However, RHR, VLC, and VO_2max of C and BH groups were significantly different from E and E+BH groups ($p<.05$) without significant different RHR, VLC, and VO_2max between E and E+BH groups ($p>.05$). This finding implied that breath-holding training could not improve cardiorespiratory condition and still in the level as untrained. In contrast, endurance training with and without breath-holding training could improve cardiorespiratory condition (RHR, VLC, and VO_2max). As the reasons, the endurance training, with or without breath-holding training, could enlarge the heart dimensions, increase resting stroke volume, improve respiratory muscles and pulmonary diffusing capacity, increase a better perfusion of blood in active tissues, and improve RHR, VLC, VO_2max, without threat from the combined breath-holding training (Robergs and Kateyian, 2003; Levitzky, 1991; Boron and Boulpaep, 2005).

According to the results, there was no significant different Hct, RBC, and WBC between groups ($p>.05$), since Hct varied over or under normal was the disorder by abnormality of RBC. Moreover, RBC was the major factor contributing to blood viscosity. The condition that RBC dropped below the lower end of the range caused the low oxygen-carrying capacity. In contrast, higher RBC beyond the upper range raised the blood viscosity and decelerated blood flow (Marieb, 2001). As the results, there was no significant different WBC between groups ($p>.05$), since In contrast, moderate exercise training has either no effect on, or may stimulate, these immune parameters. the impact of exercise on immune defense depended on the intensity of the exercise (Mackinnon, 2000 and Sherwood, 2004) that activities assigned for this study were not intense and long enough to depress or to enhance white blood cell counts.

As the results, BHT related negatively to RHR since lowered RHR could elevate more bradycardia during breath holding that delayed the elevated CO_2, the depleted O_2, and decelerated the stimulation of chemical drive and prolonged BHT (Lindholm, 2002). Likewise, VLC and VO_2 max related negatively to RHR since endurance training could enhance the heart dimensions that increased the resting stroke volume and lowered RHR, but improved the respiratory muscles and pulmonary diffusing capacity and raised VLC. Accordingly, endurance training increased blood volumes, maximal cardiac outputs, and a better perfusion of blood in active tissues and improved VO_2max. The magnitude of the improvement depended on the type of program (Plowman and Smith, 2002). On the other hand, VLC related positively to VO_2 max since the variation of VLC typically depended on strength and endurance of the respiratory muscles that improved after endurance training that enhanced the pulmonary diffusing capacity associated the variation of gas exchange between the lungs and blood and then led to the variation of VO_2 max (Levitzky, 1991). Hct related negatively to BHT since during breath holding BHT depended on blood gas storage and oxygen conserving. According to individual with lower Hct, the splenic contraction during breath holding could increase more

RBC that promoted more oxygen conserving and prolonged BHT (Schagatay and Andersson, 1999). In addition, Hct related positively to WBC since the variation of WBC depended on the severity of physical exercise as same as the variation of Hct. The increased expansion of the plasma volume decreased Hct even though total RBC and total Hb masses also increased with endurance training. Accordingly, people who engaged in moderate exercise with a good aerobic codition always claimed about the lower WBC and fewer cold (Robergs and kateyian, 2003).

Conclusion

This study has concluded that endurance training alone cannot improve BHT, but the cardiorespiratory condition (RHR, VLC, VO_2 max, Hct, RBC). In contrast, breath-holding training alone cannot improve the cardiorespiratory condition, Hct, RBC, and WBC. Interestingly, the BHT of endurance training combined with breath-holding training can improve the cardiorespiratory condition, Hct, RBC, and promote the better BHT than breath-holding training alone. WBC counts were not affected by training method in this present study. This study also found that RHR related negatively to BHT, VLC and VO_2 max, while VLC related positively to VO_2 max. Finally, Hct related negatively to BHT but related positively to WBC.

Suggestion

This study has suggested that the better way to maintain or improve breath-holding ability is breath-holding training in the combination with endurance training, especially for swimmers and athletes who need to take advantage of breath-holding ability in water during competion. However, the practical breath-holding training program according to the training principle in air and under-water pressure should be the further studied. In addition, the practical endurance exercise-training intensity that affects the various immune defenses should also be further investigated.

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