

ORIGINAL ARTICLE

Effects of combined training on blood d-ROM, BAP, glucose, triglyceride levels and body-composition

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ABSTRACT

BACKGROUND: Effects of combined aerobic-resistance training on blood derivatives of reactive oxygen metabolites (d-ROM), biological antioxidant potential (BAP), glucose and triglyceride (TG) levels are unclear. Thus, this study examined the effects of combined training on blood d-ROM, BAP, glucose, TG levels, and body composition in adult men.**METHODS:** Forty-two healthy adult men (mean±SD, age: 23.45±1.38 years; height: 173.71±4.87 cm; body weight: 69.26±5.15 kg; fat%: 14.30±3.49%) were randomly assigned to combined aerobic-resistance exercise (N.=22) and aerobic exercise (N.=20) groups. Combined training (CT) and aerobic training (AT) were performed 4 times a week for 12 weeks. The CT and AT subjects were tested for body composition and physical fitness at 0 week and 12 weeks. Blood samples were collected at 0 week and 12 weeks, and analysis for d-ROM, BAP, glucose, and TG levels was conducted.**RESULTS:** The d-ROM levels after 12 weeks were significantly lower in the AT group than in the CT group. The BAP levels after 12 weeks were significantly higher than in 0 week for both groups. Moreover, the TG levels after 12 weeks were significantly lower in the AT group than in the CT group. Lean mass and waist-to-hip ratio (WHR) after 12 weeks were significantly higher in the CT group than in the AT group, respectively. Furthermore, the fat percentage after 12 weeks was significantly lower than in 0 week in both groups.**CONCLUSIONS:** To summarize, CT during 12 weeks is effective in increasing lean mass, BAP levels, and reducing WHR and TG levels. Furthermore, AT during 12 weeks was effective in reducing free radicals by decreasing d-ROM and TG levels while increasing BAP levels.*(Cite this article as: Kim CG, Zhang HX, Lee JS. Effects of combined training on blood d-ROM, BAP, glucose, triglyceride levels and body-composition. Gazz Med Ital - Arch Sci Med 2019;178:625-31. DOI: 10.23736/S0393-3660.18.03914-1)***KEY WORDS:** Exercise; Triglycerides; Body-composition.

The human body produces energy from mitochondria to maintain life, oxygen is needed for this. This process transforms oxygen into reactive oxygen species (ROS) such as free radicals, the human body maintains a balance of antioxidant system such as antioxidant enzymes and ROS, thereby minimizing the damage caused by ROS. However, excessively produced free radicals cause glucose uptake, deformation of proteins, deoxyribonucleic acid (DNA), and pro-

moties aging.¹⁻⁵ In addition, excessive production of free radicals is causing functional decrease of cells by acting as a toxic substance,⁶ that direct or indirect cause of various degenerative diseases such as hypertension, atherosclerosis.⁷⁻⁹ The ROS can be caused by various diseases, exercise, stress, smoking,^{4, 10} and in particular, strenuous exercise is known to promote the production of ROS.¹¹⁻¹⁵

Numerous studies have examined the effects

of aerobic and resistance training on ROS and antioxidant activity.^{13, 16-19} Several studies have shown that acute aerobic exercise increases ROS, and most previous studies have concluded that the ROS and the antioxidant capacity were found to be more active at higher exercise intensity, and strenuous exercise may have a negative impact on health maintenance and promotion.^{12, 14, 15, 20} Also, another study have reported regular aerobic training reduce oxidative stress.²¹ ROS is closely related to protein damage, lipid peroxidation and glucose uptake.^{1, 2} According to Vincent and colleagues, have reported resistance training during six months lowered lipid peroxidation levels following acute aerobic training in elderly.¹⁹ Like this, both aerobic and resistance training were effective in improving the antioxidant system. In addition, both aerobic and resistance training benefits from improved body composition, such as reduced body fat percent and concentrations of triglyceride (TG), glucose.²²⁻²⁴ However, there is still controversy regarding the combined training. In addition, study on effects is very limited that the effects on the levels of ROS and antioxidant capacity in combined aerobic-resistance training during 12 weeks.

Therefore, the purpose of this study was to examine effects of 12 weeks combined training on blood derivatives of reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP) glucose, triglyceride (TG) levels and body-composition in adult men.

Materials and methods

Participants

Forty-two healthy individuals without any medical problems were recruited from Gachon University and were randomly assigned to a combined exercise group (N.=22) (mean±SD, age: 23.14±1.04 years; height: 172.09±4.73 cm; body weight: 67.32±2.87 kg; fat%: 14.28±3.99%) or an aerobic exercise group (N.=20) (mean±SD, age: 23.80±1.64 years; height: 175.50±4.48 cm; body weight: 71.40±6.23 kg; fat%: 14.32±2.96%). This study was approved by the Institutional Review Board (IRB) of Gachon University, South Korea. Following their

completion of a health screening questionnaire, written informed consent was obtained from all participants after the study's purpose and procedures were explained.

Study design

On day 1, the participants were told about the purpose and procedures of the experiment. On day 2, body composition, blood pressure, and physical fitness factors were measured, and blood samples were collected at rest. Exercise intensity was calculated the next day. For the next twelve weeks the subjects performed either combined aerobic-resistance or aerobic training four times a week. After the 12 weeks, body composition and physical fitness factors were measured, and blood samples were collected. All testing was performed at the same time of day for each subject.

Combined exercise

In order to evaluate for aerobic training intensity, this study used a breath-by-breath respiratory gas analyzer system (Quark b2, COSMED Co., Ltd., Italy). The Bruce protocol was performed on a treadmill,²⁵ starting with a 10% slope at 1.7 mph; every 3 minutes, the slope and speed were increased by 2% and 0.8 mph. The exercise was stopped for any of the following reasons: exhaustion, respiratory exchange ratio > 1.10,²⁶ participants asking to stop due to cardiovascular event or fatigue, > 90% HRmax (220-age), or rating of perceived exertion > 17.

Combined training group was performed by constructing resistance and aerobic training at every other day. In combined training, aerobic training intensity used the heart rate reserve (HRR) method: target heart rate (THR) = [(HRmax - HRrest) × 45 ~ 75% intensity] + HRrest, and HRmax = 220 - age.^{27, 28} Aerobic training sessions lasted 60 mins and were composed of a warm-up of 5 mins of walking at a training intensity of 30 ~ 40% of the participant's HRR, 5 mins of stretching exercises, 40 mins of running at an intensity of 45 ~ 55% HRR, a cool-down of 5 mins of walking at an intensity of 30 ~ 40% HRR, and 5 mins of stretching. During the 12 weeks, training intensity started at an initial 45~55% of the participants' HRR and was

increased by 10% every 4 weeks. Training intensity was monitored by a wireless heart rate system (Bodypro M100, Du-sung Technology Co., Korea).

A one repetition maximum (1-RM) test was conducted to establish the intensity of resistance exercise at 0 week, 4 weeks and 8 weeks.²⁹ The 1-RM was performed for a total of 3 sets with a reasonable rest interval between sets of 1 min and 30 secs.²⁰ Combined exercise resistance training sessions consisted of 60 mins beginning with a warm-up of 5 mins of walking at an exercise intensity of 30 ~ 40% of the participant's HRR on a treadmill and 5 mins of stretching followed by 40 mins of resistance training consisting of chest presses, shoulder presses, lat pull-downs, leg curls, leg extensions, leg presses, and dead lifts. The sessions ended with a cool-down of 5 mins of walking at a training intensity of 30 ~ 40% HRR and 5 min of stretching. Resistance training was performed at 50% (initially) of 1-RM for 3 sets of 12 repetitions at 90 second intervals for each exercise. Training intensity began at the initial 50% and was increased by 10% every 4 weeks. The aerobic training group followed the same aerobic training regimen as the combined training group did.

Blood analysis

The subjects refrained from eating food and drinking alcohol for 12 hours before being tested and avoided physical activity or exercise on the day of the assessment. Blood samples were collected from a finger-tip capillary at rest. The d-ROM test was conducted with plasma using the international scientific community certified oxidative stress analyzing system (FRAS 4 Evolve, H & D Co., Italy) according to the instructions in the manual.³⁰ The d-ROM levels were measured in U.CARR., where 1 U.CARR. corresponded to 0.08 mg/dL hydrogen peroxide. Based on the manufacturer's instructions, values were classified as follows: very high oxidative stress, above 500 U.CARR; high oxidative stress, from 401 to 500 U.CARR; slight oxidative stress, from 321 to 340 U.CARR; borderline stress, from 301 to 320 U.CARR; and normal, from 250 to 300 U.CARR. Plasma BAP levels were measured using a free radical electric evaluator (FRAS 4

Evolve, H & D Co., Italy) according to the manual,^{31, 32} and units were expressed as $\mu\text{mol/L}$. The manufacturer's instructions showed the following classifications: optimum range, from 2201 to 4000 $\mu\text{mol/L}$; borderline, from 2001 to 2200 $\mu\text{mol/L}$; moderate shortage, from 1801 to 2000 $\mu\text{mol/L}$; shortage, from 1601 to 1800 $\mu\text{mol/L}$; severe shortage, from 1401 to 1600 $\mu\text{mol/L}$; and very severe shortage, less than 1400 $\mu\text{mol/L}$. The glucose and TG levels were automatically analyzed using the Accutrend Plus (Roche Diagnostik, Germany) and were measured in mmol/L.

Body composition

The height, body weight, lean mass, fat percentage, and WHR were measured with a scale and bio-impedance analyzer (BSM340, Biospace Ltd., Korea; Inbody 720, Biospace Ltd., South Korea) while participants were dressed in short-sleeved cotton shirts and cotton shorts. The bio-impedance analyzer uses impedance and reactance values by segment and at multiple frequencies; it has high accuracy with a high correlation coefficient of 0.98 with DEXA, the standard equipment for body composition analysis.³³

Statistical analysis

Statistical analysis was performed using Statistical Packaged for Social Science (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA). All data were expressed as mean $\pm$ standard deviation. Normality of distribution of all outcome variables was verified using a Kolmogorov-Smirnov test. A mixed-design analysis ("2 group" $\times$ "2 time") of variance was used to analyzing the effects of combined and aerobic training on each continuous dependent variable. Partial eta-squared (η^2) was calculated as measures of effect size ES: partial Eta squared of the interaction). The ES was classified as follows: small <0.05, medium 0.06-0.13, and large >0.14.³⁴ The level of statistical significance was set at $P < 0.05$.

Results

The results of the d-ROM and BAP levels are shown in Table I. The d-ROM levels of the com-

TABLE I.—Comparison of d-ROM and BAP levels between combined and aerobic exercise (mean±SD).

Variables	Group	Before	After	Significant level (P)	F	ES
d-ROM (U.Carr.)	CE	283.55±18.89	296.55±47.67	Group: 0.001*	40.465	0.053
	AE	239.30±30.27	230.60±25.30	Time: 0.676	0.177	0.004
BAP (μmol/L)	CE	2271.82±195.60	2453.27±198.67	GroupxTime: 0.040*	4.501	0.101
	AE	2375.70±204.96	2493.65±164.29	Group: 0.146	2.200	0.052
				Time: 0.001*	19.494	0.328
				GroupxTime: 0.335	0.877	0.021

ES was classified as follows: small <0.05; medium 0.06-0.13; large >0.14.

ES: effect size; CE: combined exercise; AE: aerobic exercise; d-ROM: derivatives of reactive oxygen metabolites; BAP: biological antioxidant potential.

*P<0.05.

TABLE II.—Comparison of glucose and triglyceride levels between combined and aerobic exercise (mean±SD).

Variables	Group	Before	After	Significant level (P)	F	ES
Glucose (mmol/L)	CE	91.82±10.36	91.18±5.10	Group: 0.010*	7.277	0.007
	AE	91.80±5.29	89.30±3.92	Time: 0.375	0.806	0.054
Triglyceride (mmol/L)	CE	115.82±16.58	113.95±14.68	GroupxTime: 0.596	0.286	0.020
	AE	2375.70±204.96	2493.65±164.29	Group: 0.754	0.100	0.015
				Time: 0.001*	49.113	0.311
				GroupxTime: 0.001*	113.424	0.044

ES was classified as follows: small <0.05; medium 0.06-0.13; large >0.14.

ES: effect size; CE: combined exercise; AE: aerobic exercise.

*P<0.05.

TABLE III.—Comparison of body-composition between combined and aerobic exercise (mean±SD).

Variables	Group	Before	After	Significant level (p)	F	ES
Body weight (kg)	CE	67.32±2.87	67.62±3.37	Group: 0.010*	7.277	0.115
	AE	71.40±6.23	70.60±6.86	Time: 0.375	0.806	0.043
Lean mass (kg)	CE	32.60±1.83	33.71±1.85	GroupxTime: 0.596	0.286	0.184
	AE	33.51±2.51	33.27±2.52	Group: 0.754	0.100	0.003
				Time: 0.001*	49.113	0.561
Fat% (%)	CE	14.28±3.99	13.23±3.81	GroupxTime: 0.001*	113.424	0.754
	AE	14.32±2.96	13.68±2.60	Group: 0.818	0.054	0.001
				Time: 0.001*	52.904	0.569
WHR	CE	0.79±.03	0.78±.03	GroupxTime: 0.085	3.114	0.072
	AE	0.81±.03	0.81±.03	Group: 0.009	7.574	0.159
				Time: 0.001*	101.793	0.718
				GroupxTime: 0.001*	25.832	0.392

ES was classified as follows: small <0.05; medium 0.06-0.13; large >0.14.

ES: effect size; CE: combined exercise; AE: aerobic exercise; WHR: waist to hip ratio.

*P<0.05.

combined training group at 0 week and 12 weeks were 283.55±18.89 U.CARR and 296.55±47.67 U.CARR, and of the aerobic training group at 0 week and 12 weeks were 239.30±30.27 U.CARR and 230.60±25.30 U.CARR, respectively. The d-ROM levels showed an interaction effect between group and time (F=4.501, P<0.05) and showed the main effect by group (F=40.465, P<0.05). The BAP levels of the

combined training group at 0 week and 12 weeks were 2271.82±195.60 μmol/L and 2453.27±198.67 μmol/L, and those of the aerobic training group at 0 week and 12 weeks were 2375.70±204.96 μmol/L and 2493.65±164.29 μmol/L, respectively. The BAP levels showed no interaction effect and showed the main effect by time (F=19.494, P<0.05).

The results of glucose and TG levels are

shown in Table II. The glucose levels showed no interaction effect, and showed the main effect by time ($F=19.494$, $P<0.05$). Additionally, the TG levels showed no interaction effect and exhibited the main effect by time ($F=18.093$, $P<0.05$).

The results for body composition are shown in Table III. The body weight and fat percentage showed no interaction effect, but showed the main effect by time (respectively, $F=7.277$, $P<0.05$; $F=52.904$, $P<0.05$). The interaction effects between group and time for lean mass were significant ($F=113.424$, $P<0.05$) and within the main effect of time ($F=49.113$, $P<0.05$), while the WHR showed the interaction effect between group and time ($F=25.832$, $P<0.05$) and the main effect by group ($F=101.793$, $P<0.05$).

Discussion

ROS have a chemical affinity for reacting with cells and causing structural and functional cellular changes and cell membrane damage. They promote aging and can become the cause of various diseases.⁴⁻⁸ However, the human body normally minimizes the damage caused by ROS that activate antioxidant enzymes. In this regard, several studies have reported that antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase increase with excessive ROS production due to exercise.^{17-19, 21} In particular, researchers have observed that the activity of antioxidant enzyme was clearly increased at 60 minutes postexercise while was no change at pre-exercise.^{13, 35} In a study of acute exercise, protein carbonyls values were greater 24 hours after squatting than after cycling.¹⁸

Aim of our study was to investigate the effects of long-term training types on d-ROM and BAP levels. We found that d-ROM levels after 12 weeks were significantly lowered by aerobic training in comparison to combined aerobic-resistance training. Meanwhile, the BAP levels after 12 weeks were significantly increased by both types of training. Several studies have reported that prolonged aerobic training is effective in reducing ROS, which is consistent with our study.^{4, 21} Other studies have reported that

resistance training during 6 months is effective in reducing ROS in the elderly.¹⁹ However, this study confirmed that BAP levels were increased by resistance training while ROS levels were unchanged. This can be attributed to either differences in subjects or the limited number of training sessions.

Limitations of the study

This study has the limitation of defining change in oxidative stress by reviewing change in d-ROM and BAP levels. Therefore, in future studies, to clearly demonstrate the effects of training type on oxidative stress, it will be necessary to analyze the concentration of GPX, SOD or malondialdehyde to better test the effects of exercise form on active oxygen species.

In addition, this study examined the effects of aerobic and resistance training on glucose and TG levels. After 12 weeks, both groups of trainees showed no difference in glucose, but the levels of triglyceride of the aerobic training group showed significant decreases compared with those of the combined training group. Previous studies have shown that prolonged aerobic training is effective in reducing TG,^{36, 37} and other studies have reported that 12 weeks of combined aerobic-resistance training was effective in reducing TG.^{33, 38} Body fat reduction is associated with a decrease in triglycerides. During this study, both combined and aerobic training were shown to be effective in decreasing body fat mass, and In this study, the long-term combined training significantly increased lean mass. This is consistent with previous studies and is a result of the combined training being beneficial to the improvement of body composition.^{30, 35} A decrease in body fat mass seems to have a positive effect on triglyceride reduction. That this study revealed differences in glucose levels between the aerobic and resistance training groups is thought to be due to the participants being physically healthy people.

Conclusions

To summarize, combined aerobic-resistance training during 12 weeks was effective in increasing lean mass, BAP levels, and reducing WHR and

TG levels. Furthermore, aerobic training during 12 weeks was effective in reducing free radicals by decreasing d-ROM and TG levels and increasing BAP levels, showing that aerobic training provides more benefits to the antioxidant system.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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