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Effect of different exercise intensities on biomarkers of oxidant-antioxidant balance, inflammation, and muscle damage

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Abstract : We investigated the effect of different exercise intensities on biomarkers of oxidant-antioxidant balance, inflammation, and muscle damage. Eighteen healthy and untrained male subjects were enrolled.Subjects were randomly and equally assigned to a moderate-intensityexercise(MIE, 65%VO_2max) group(n=9) or a high-intensity exercise(HIE, 85%VO_2max) group(n=9).Blood samples were collectedimmediately pre-exercise, post-exercise, and 60min post-exercisetoexamine oxidant-antioxidant balance(d-ROMs; BAP), inflammation(CRP; fibrinogen), muscle damage(CK; LDH), and lactate. Serum d-ROMs and BAP levels were significantly increased post-exercise compared with pre-exercise levels in HIE group($p<0.05$). Lactate levels were significantly increased post-exercise compared pre-exercise levels in both the MIE and HIE groups($p<0.05$). In addition, post-exercise serum d-ROMs and plasma lactate levels were significantly higher in the HIE group than in the MIE group($p<0.05$). These results suggest that although relatively high-intensity exercises may increase oxidative stress levels in the body, they do not produce inflammatory response and/or muscle damage.

Keywords : exercise intensity; oxidative stress;inflammatory response; muscle damage

1. Introduction

In human breathing, oxygen in the atmosphere is inhaled and carbon dioxide is exhaled. Once inhaled, oxygen is the terminal electron acceptor in the mitochondrial electron transport chain. Oxygen is involved in various metabolic processes, and is an essential element in the generation of ATP, a source of energy. However, if during metabolism incomplete reduction-oxidation reaction occurs, some *in vivo* oxygen is modified to generate reactive oxygen species(ROS), which induce oxidative stress(OS) and could cause *in vivo*toxicity[1,2]. In other words, OS indicates the occurrence of ROS including non-radical derivatives of oxygen as well as oxygen radical and a severe imbalance among antioxidant defense systems. In a normally functioning aerobic organism, a balance exists between the production of

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prooxidants and antioxidant defense systems [1-3].

Several studies have demonstrated that regular exercise training increases antioxidant potential of the body to reduce OS level[4,5], whereas acute exercise induces the generation of ROS by increasing the rate of metabolism in mitochondria to, eventually, increase OS level[6,7]. In particular, the OS level increase during acute exercise was reported to be dependent on exercise intensity[8,9]. Specifically, in a study conducted with 15 healthy male adults, Roh et al. (2017) reported that when the subjects performed 60-min treadmill running to consume the same amount of energy(300kcal) at low(50%VO_2max), moderate(65%VO_2max), or high intensity (85%VO_2max), the greatest increase in serum ROS level was observed following the high-intensity trial[8]. Ramos et al. (2013) reported that after a single bout of swimming exercise, levels of plasma protein carbonylation (PC) and thiobarbituric acid reactive substances (TBARS), i.e., OS markers in serum, were significantly higher in a high-intensity swimming group than in a low-intensity group[9]. Suggestedmechanisms of ROS generation following acute exercise include increases in mitochondrial electron transport, catecholamines, and prostanoids due to increased oxygen uptake(VO_2) during exercise, and increased xanthine oxidase due to ischemia/reperfusion, among others[3,10,11], An increased OS level is also reportedly closely associated with damage to tissues such as skeletal muscle[12,13]. Tsakiris et al. (2006) measured changes in 8-hydroxy-2-deoxyguanosine(8-OHdG; a serum OS indicator) and creatine kinase(CK)(an indicator of muscle damage) in 10 basketball players before and after a 90-minute workout consisting of warm-up exercise(10min), technique drills(30min), a game(40min), and cool-down exercise(10min), and found that both 8-OHdG and CK increased significantly after exercise[14]. The finding is supported by Tauler et al. (2006), who measured the levels of malonaldehyde(MDA: an OS indicator) and CK, among others, in 9 men after 171.8km of mountain bike riding and observed significant increases in MDA and CK after exercise[15]. In addition, it has been suggested that tissue damage induced by high-intensity exercise may cause inflammation and additional ROS production[16,17].

However, limited research has been conducted to assess the interrelationships among *in vivo* OS levels according to intensity of acute exercise, inflammation, and muscle damage, or according to exercise levels of different intensity using derivatives of reactive oxygen metabolites(d-ROMs) and biological antioxidant potential(BAP) tests. Accordingly, the present study aimed to examine the effect of acute aerobic exercises of different intensity on d-ROMS and BAP levels in healthy male adults and to test whether the exercises induce inflammation and muscle damage.

2. Methods

2.1. Subjects

The subjects of the present study were 18 healthy males in their 20's who volunteered to participate in the study and did not have a relevant medical condition. All subjects were informed in detail of study purposes, methods, and procedures, and gave written consent, which included a clause that they could terminate participation in the study at any time. The 18 subjects were randomly assigned to either a moderate-intensity exercise(MIE) group or a high-intensity exercise(HIE) group, with n= 9 in each group(Table 1).

2.2. Anthropometric measurements

Anthropometric measurements included height, weight, body composition, and maximum oxygen uptake(VO_2max). VO_2max was measured on the treadmill(T150, Cosmed, Italy) at 1.7 mph and 10% grade using the

Table 1. Subject descriptive characteristics and anthropometric measures

Variables / Groups	MIE (n=9)	HIE (n=9)	p-value *
Age (years)	22.67±1.50	22.11±1.05	0.377
Height (cm)	177.89±5.56	178.11±5.44	0.933
Weight (kg)	72.22±5.47	72.70±8.18	0.886
BMI (kg/m^2)	22.84±1.67	22.90±2.34	0.954
Body fat (%)	19.07±6.12	18.73±3.90	0.892
VO_2max(ml/kg/min)	49.49±2.16	49.65±3.06	0.898

Data are presented as mean ± SD. MIE: moderate intensity exercise(65%VO_2max); HIE: high intensity exercise (85%VO_2max);BMI, body mass index; *p<0.05 as determined using the independent t-test

Bruce Protocol[18] that involves an increase of 0.8~0.9 mph and 2% grade every 3 min, and breath-by-breath type was applied using a gas analyzer(Quark CPET, Cosmed, Italy) and wireless heart rate analyzer(Polar H10, Polar, Finland). Height, weight, and body composition was measured using body composition analyzer(VENUS 5.5, JAWON MEDICAL, Korea) by using bioelectrical impedance analysis(BIA).

2.3. Treadmill running test

Subjects were instructed to perform treadmill running until 300kcal were consumed at 65% of VO_2max in the MIE group or 85% in the HIE group. The test was designed with reference to the treadmill running test at different intensity levels used in Roh et al. (2017)[8]. Specifically, while performing the test, subjects were assessed with a gas analyzer and running conditions were adjusted based on the VO_2 level displayed on the monitor. If a subject's VO_2 reached the corresponding exercise target, the slope and speed of the treadmill were adjusted to maintain VO_2 in the steady state. When the total energy consumption indicated by the gas analyzer reached 300 kcal, treadmill running was terminated.

2.4. Blood sampling and analyses

Using a 22-gauge needle, a serum separator tube(Becton Dickinson, Franklin Lakes, USA), and a Sod.Citrate tube(Becton Dickinson, Franklin Lakes, USA), 6 ml of blood was collected from the antecubital vein of each subject at the immediately pre-exercise(Pre), immediately post-exercise(Post), and 60min post-exercise(60min post). Collected blood samples were centrifuged for 15 minutes at 3000 rpm, and then were stored at -80 ° C until analysis. Blood lactate level was analyzed via a hand-portable lactate analyzer(Accutrend plus, Roche Diagnostics GmbH, Germany) using finger-stick blood, and all other serum variables were analyzed at Green Cross Lab. Specifically, serum d-ROMs and BAP were analyzed using colorimetry, and serum CK and lactatedehydrogenase(LDH) using the UV spectrophotometry(UVS) method. In addition, serum C-reactive protein(CRP) and plasma fibrinogen were analyzed via the turbidimetric immunoassay(TIA) method and an enzymatic assay, respectively.

2.5. Statistical analyses

Statistical analyses were conducted using SPSS version 24.0 for Windows. Data are presented as the mean ± standard deviation(SD) unless otherwise stated. For identifying differences in normally distributed results, two-way repeated measures ANOVA was employed. When significant group by time interactions occurred, simple main effects were

assessed using one-way ANOVA and independent *t*-tests. Levels of significance were set at 0.05.

3. Results

The serum d-ROMs and BAP levels are shown in Table 2. Following exercise, repeated measures ANOVA demonstrated a significant difference across the group by time interaction for serum d-ROMs(F=4.447) and BAP(F=3.838) levels(p<0.05). Serum d-ROMs and BAP levels were significantly increased post-exercise compared pre-exercise in HIE group(p<0.05). In addition, post-exercise serum d-ROMs levels were significantly higher in the HIE group than in the MIE group(p<0.05).

The serum CK and LDH levels are shown in Table 3. Serum CK(F=0.073) and LDH(F=2.705) levels were not significantly different between any groups or time points(p>0.05).

Theblood lactate, serum CRP, and plasma fibrinogen levels are shown in Table 4. Following exercise, repeated measures ANOVA demonstrated a significant difference across the group by time interaction for blood lactate(F=8.590) levels(p<0.05). Blood lactate levels were significantly increased post-exercise compared pre-exercise levels in both the MIE and HIE groups(p<0.05). In addition, post-exercise plasma lactate levels were significantly higher in the HIE group than in the MIE group(p<0.05). In contrast, serum CRP(F=1.406) and plasma fibrinogen(F=0.224) levels were not significantly different between any groups or time points(p>0.05).

Table 2. Changes in serum d-ROMs and BAP levels

Variables	Groups	Pre	Post	60min Post	*F*-value	
d-ROMs (CARR.U)	MIE	292.22±53.53	298.00±52.08	292.67±46.34	Group	2.147
	HIE	293.44±34.03	350.11±43.62+,#	324.33±44.29	Time	6.611
					G x T	4.447*
BAP (μmol/L)	MIE	2162.89±134.24	2290.78±179.58	2297.67±146.44	Group	1.055
	HIE	2094.56±153.45	2497.67±264.77+	2343.22±143.97	Time	15.075
					G x T	3.838*

Data are presented as mean ± SD. MIE: moderate intensity exercise(65%VO_2max); HIE: high intensity exercise(85%VO_2max);+p<0.05 vs. Pre; #p<0.05 vs. 65%; G x T; Group × Time interaction *p<0.05

Table 3. Changes in serum CK and LDH levels

Variables	Groups	Pre	Post	60min Post	*F*-value	
CK (U/L)	MIE	192.44±77.84	208.78±85.44	195.67±78.25	Group	0.008
	HIE	196.22±113.84	214.00±109.31	198.44±101.08	Time	16.276
					G x T	0.073
LDH (U/L)	MIE	310.00±42.12	315.22±41.70	306.44±34.50	Group	2.439
	HIE	321.78±30.89	356.00±30.82	328.89±41.77	Time	5.985
					G x T	2.705

Data are presented as mean ± SD. MIE: moderate intensity exercise(65%VO_2max); HIE: high intensity exercise(85%VO_2max)

Table 4. Changes in blood lactate, serum CRP, and plasma fibrinogen levels

Variables	Groups	Pre	Post	60min Post	*F*-value	
Lactate (mmol/L)	MIE	1.78±0.55	3.32±1.12+	1.82±0.25	Group	5.042
					Time	54.108
	HIE	1.66±0.42	5.21±1.60+,#	1.69±0.26	G x T	8.590*
CRP (mg/L)	MIE	0.42±0.39	0.43±0.40	0.41±0.38	Group	0.237
					Time	5.250
	HIE	0.53±0.65	0.58±0.73	0.53±0.66	G x T	1.406
Fibrinogen (mg/L)	MIE	216.22±45.02	223.11±43.83	211.00±38.87	Group	0.010
					Time	4.466
	HIE	214.11±36.75	219.11±36.33	211.44±38.15	G x T	0.224

Data are presented as mean ± SD.MIE: moderate intensity exercise(65%VO_2max); HIE: high intensity exercise(85%VO_2max);+$p<0.05$ vs. Pre; #$p<0.05$ vs. 65%; G x T; Group × Time interaction *$p<0.05$

4. Discussion

OS level in the body increases if more prooxidants are produced than antioxidant defense systems can handle, and regular exercise promotes antioxidant ability to reduce OS levels[19,20]. In contrast, acute high-intensity exercise induces generation of ROS by increasing the rate of metabolism in mitochondria, thereby eventually increasing the OS level[20,21]. The OS level in the body can be assessed using the d-ROMs test(i.e., a method to estimate the level of *in vivo* ROS by calculating the total amount of reactive oxygen metabolites in serum) and BAP test(i.e., a method to measure the serum level of antioxidants that can reduce Fe^{3+} to Fe^{2+})[22,23]. However, research that investigated changes in serum d-ROMs and BAP levels following acute exercise is limited. The present study aimed to examine the effect of acute aerobic exercises of different intensity on oxidant-antioxidant balance in the body and analyzed serum d-ROMs and BAP levels. The study findings showed that serum d-ROMs increased significantly after exercise in the HIE group, and that it was significantly higher in that group compared with MIE group at post-exercise assessment. These findings are supportive of several previous studies that reported significant increases in OS-related biomarkers in serum followingacute high intensity exercise[24,25], as well as a previous study that reported a high serum d-ROMs level following relatively high intensity exercise[26]. More specifically, Parker et al. (2014) reported a significantly higher d-ROMs level following exercise at 85% of VO_2max compared to that at 40%, a result consistent with the current finding[26]. In addition, the serum BAP finding in the present study showed that while there was no significant change in the MIE group, BAP increased significantly at post-exercise assessment in the HIE group. It is speculated that high intensity exercise increased OS level in the body, in turn activating antioxidant defense systems, and that BAP increased as a response to the activation. Parker et al. (2014) reported that BAP increased in proportion to exercise intensity(2,427±106 at 70% of VO_2max; 2,625±121 at 85%; 2,651±92 at 100%) from the level during steady state(2,015±57 μmol/L). The present study

finding on BAP is also supported by a previous report[27,28] that acute high intensity exercise increased not only the production of ROS in skeletal muscle, liver, and heart, but also the activation of antioxidant enzymes in various tissues, erythrocytes, and thrombocytes[27,28].

Exercise-induced muscle damage occurs episodically or over a longer term. The extent of damage can be estimated based on serum levels of CK and LDH released from muscle tissue by exercise[29,30,31]. Serum levels of these muscle enzymes are reported to increase after exercise not only in professional players who perform intense exercises but also in those who do not professionally train[31,32]. However, in the present study, neither CK nor LDH showed a significant difference between pre- and post-exercise levels, possibly because acute treadmill running may not have induced structural damage in muscle tissue. In short-distance or less intensive running, there is no significant change in membrane permeability, which is probably the reason for little change in serum CK and LDH(i.e., indices of muscle damage) in the present study; in contrast, competitive exercise performed for a longer time(e.g., marathon running) increases membrane permeability, leading to release of enzymes and an increase in serum levels. According to previous studies, muscle damage following exercise is more likely to occur during weight bearing exercise that can cause structural damage in muscle or in a situation inducing repetitive eccentric muscle contractions, such as down-slope running, in comparison to running on flat ground[31]. In a study that compared serum CK levels before and after treadmill running at zero slope, Lee (2003) observed an increasing trend, but the difference was not statistically significant, thus supporting the present study finding[33].

Lactate is a source of energy resynthesized to glucose in the liver and muscle, or converted again to pyruvate when necessary. It is primarily generated to satisfy the immediate need for energy, and is also used as a peripheral indicator of muscle fatigue caused by exercise[34]. The present study analyzed lactate to assess peripheral fatigue following aerobic exercise of different intensity, and found that lactate level was significantly higher after exercise, in addition to being significantly higher in the HIE group than in the MIE group at post-exercise assessment. These results support previous research findings that an increase in serum lactate level following exercise was dependent on exercise amount and intensity[35,36]. In contrast, serum CRP and plasma fibrinogen(i.e., indicators of inflammation) did not show statistically significant differences. Inflammation causes blood clotting, fever, and pain in response to damage in skeletal muscle and other tissue, or to an external agent. This defensive response is necessary to restore damaged tissue. Therefore, the lack of significant changes is believed to indicate that regardless of the intensity level, the exercises performed in the present study did not produce muscle damage.

5. Conclusion

This study confirmed that acute high-intensity aerobic exercise may increase OS level in the body, in response to which antioxidative defense systems may be activated. However, independent of those responses, an inflammatory response and/or muscle damage were not produced. Future research should examine weight bearing exercise or an exercise protocol that includes repetitive eccentric muscle contractions, such as down-slope running.

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References

1. Z. Radak, Z. Zhao, E. Koltai, H. Ohno, M. Atalay, "Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling" *Antioxid Redox Signal*, Vol.18, No.10 pp. 1208-1246, (2013).
2. M. Finkler, D. Lichtenberg, I. Pinchuk, "The relationship between oxidative stress and exercise" *J Basic ClinPhysiolPharmacol*, Vol.25, No.1 pp. 1-11, (2014).
3. K. Fisher-Wellman, H. K. Bell, R. J. Bloomer, "Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders" *Oxid Med Cell Longev*, Vol.2, No.1 pp. 43-51, (2009).
4. A. Pingitore, G. P. Lima, F. Mastorci, A. Quinones, G. Iervasi, C. Vassalle, "Exercise and oxidative stress: potential effects of antioxidant dietary strategies in sports" *Nutrition*, Vol.31, No.7-8 pp. 916-922, (2015).
5. M. Finkler, D. Lichtenberg, I. Pinchuk, "The relationship between oxidative stress and exercise" *J Basic ClinPhysiolPharmacol*, Vol.25, No.1 pp. 1-11, (2014).
6. S. K. Powers, W. B. Nelson, M. B. Hudson, "Exercise-induced oxidative stress in humans: cause and consequences. *Free RadicBiol Med*, Vol.51, No.5 pp. 942-950, (2011).
7. S. K. Powers, Z. Radak, L. L. Ji, "Exercise-induced oxidative stress: past, present and future" *J Physiol*, Vol.594, No.18 pp. 5081-5092, (2016).
8. H. T. Roh, S. Y. Cho, H. G. Yoon, W. Y. So, "Effect of Exercise Intensity on Neurotrophic Factors and Blood-Brain Barrier Permeability Induced by Oxidative-Nitrosative Stress in Male College Students" *Int J Sport NutrExercMetab*, Vol.27, No.3 pp. 239-246, (2017).
9. D. Ramos, E. G. Martins, D. Viana-Gomes, G. Casimiro-Lopes, V. P. Salerno, "Biomarkers of oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise" *ApplPhysiolNutrMetab*, Vol.38, No.5 pp. 507-511, (2013).
10. M. L. Urso, P. M. Clarkson, "Oxidative stress, exercise, and antioxidant supplementation" *Toxicology*, Vol.189, No.1-2 pp. 41-54, (2003).
11. L. M. Heunks, J. Viña, C. L. van Herwaarden, H. T. Folgering, A. Gimeno, P. N. Dekhuijzen, "Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease" *Am J Physiol*, Vol.277, No.6 pp. 1697-1704, (1999).
12. P. Castrogiovanni, R. Imbesi, "Oxidative stress and skeletal muscle in exercise" *Ital J AnatEmbryol*, Vol.117, No.2 pp. 107-117, (2012).
13. W. Aoi, Y. Naito, T. Yoshikawa, "Role of oxidative stress in impaired insulin signaling associated with exercise-induced muscle damage" *Free RadicBiol Med*, Vol.65, pp. 1265-1272, (2013).
14. S. Tsakiris, T. Parthimos, N. Parthimos, T. Tsakiris, K. H. Schulpis, "The beneficial effect of L-cysteine supplementation on DNA oxidation induced by forced training" *Pharmacol Res*, Vol.53, No.4 pp. 386-390, (2006).
15. P, Tauler, A. Sureda, N. Cases, A. Aguilo, J. A. Rodriguez-Marroyo, G. Villa, J. A. Tur, A. Pons, "Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage" *J NutrBiochem*, Vol.17, No.10 pp. 665-671, (2006).
16. L. L. Ji, S. Leichtweis, "Exercise and oxidative stress: Sources of free radicals

and their impact on antioxidant systems" *Age*, Vol.20, No.2 pp. 91-106, (1997).
17. M. Malaguti, C. Angeloni, S. Hrelia, "Polyphenols in exercise performance and prevention of exercise-induced muscle damage" *Oxid Med Cell Longev*, Vol.2013, pp. 1-9, (2013).
18. R. A. Bruce, J. R. Blackmon, J. W. Jones, G. Strait, "Exercising testing in adult normal subjects and cardiac patients" *Pediatrics*, Vol.32, No.4 pp. 742-756, (1963).
19. H. T. Roh, W. Y. So, "The effects of aerobic exercise training on oxidant-antioxidant balance, neurotrophic factor levels, and blood-brain barrier function in obese and non-obese men" *J Sport Health Sci*, Vol.6, No.4 pp. 447-453, (2017).
20. L. L. Ji, "Redox signaling in skeletal muscle: role of aging and exercise" *AdvPhysiolEduc*, Vol.39, No.4 pp. 352-359, (2015).
21. C. E. Cooper, N. B. Vollaard, T. Choueiri, M. T. Wilson, "Exercise, free radicals and oxidative stress" *BiochemSoc Trans*, Vol.30, No.2 pp. 280-285, (2002).
22. S. W. Kim, B. J. Kim, S. W. Youn, K. C. Park, C. H. Huh, "Evaluation of free oxygen radical and antioxidant capacity in alopecia areata" *J Dermatol*, Vol.37, No.8 pp. 762-764, (2010).
23. T. Fukui, K. Yamauchi, M. Maruyama, T. Yasuda, M. Kohno, Y. Abe, "Significance of measuring oxidative stress in lifestyle-related diseases from the viewpoint of correlation between d-ROMs and BAP in Japanese subjects" *Hypertens Res*, Vol.34, No.9 pp. 1041-1045, (2011).
24. A. L. Bessa, V. N. Oliveira, G. G. Agostini, R. J. Oliveira, A. C. Oliveira, G. E. White, G. D. Wells, D. N. Teixeira, F. S. Espindola, "Exercise Intensity and Recovery: Biomarkers of Injury, Inflammation, and Oxidative Stress" *J Strength Cond Res*, Vol.30, No.2 pp. 311-319, (2016).
25. J. S. Wang, Y. H. Huang, "Effects of exercise intensity on lymphocyte apoptosis induced by oxidative stress in men" *Eur J ApplPhysiol*, Vol.95, No.4 pp. 290-297, (2005).
26. L. Parker, T. A. McGuckin, A. S. Leicht, "Influence of exercise intensity on systemic oxidative stress and antioxidant capacity" *ClinPhysiolFunct Imaging*, Vol.34, No.5 pp. 377-383, (2014).
27. S. Y. Cho, *The effects of men and women's NADPH oxidase p22phox C242T polymorphism on endurance exercise performance and oxidative DNA damage in response to aerobic exercise training*, Yonsei University, (2009).
28. G. Bresciani, I. B. da Cruz, J. González-Gallego, "Manganese superoxide dismutase and oxidative stress modulation" *AdvClinChem*, Vol.68, pp. 87-130, (2015).
29. H. Gissel, T. Clausen, "Excitation-induced Ca2+ influx and skeletal muscle cell damage" *ActaPhysiolScand*, Vol.171, No.3 pp. 327-334, (2001).
30. P. M. Clarkson, M. J. Hubal, "Exercise-induced muscle damage in humans" *Am J Phys Med Rehabil*, Vol.81, No.11 pp. 52-69, (2002).
31. P. Brancaccio, N. Maffulli, F. M. Limongelli, "Creatine kinase monitoring in sport medicine" *Br Med Bull*, Vol.81-82, pp. 209-230, (2007).
32. S. H. Paik, I. Y. Paik, S. H. Suh, S. Y. Cho, H. T. Roh, "The Effects of Various Recovery Methods on Serum Inflammation, Muscle Damage and Fatigue Variables after a Game of Soccer Players in High School" *Journal of Sport and Leisure Studies*, Vol.56, No.1 pp. 987-995, (2014).
33. W. Y. Lee, *Skin Temperature and Body Fluid Changes During a Distance Running in Different Ambient Temperatures*, Kook-MIn University, (2003).
34. I. Y. Paik, *Exercise and Energy Metabolism*, Daehanmedia, (2006).

35. J. Finsterer, "Biomarkers of peripheral muscle fatigue during exercise" *BMC MusculoskeletDisord*, Vol.13, pp. 1-13, (2012).

36. M. Amann, S. K. Sidhu, J. C. Weavil, T. S. Mangum, M. Venturelli, "Autonomic responses to exercise: group III/IV muscle afferents and fatigue" *AutonNeurosci*, Vol.188, pp. 19-23, (2015).