ORIGINAL PAPER

Responses of m-GOT and humeral immune biomarkers to exhaustive endurance running and antioxidant supplementation in elite distance runners

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(Received: 2011/1/23, Revised: 2011/3/15, Published online: 2011/3/31)

Abstract

This investigation examined the responses of mitochondrial aspartate aminotransferase (m-GOT) and immunoglobulins (IgA, IgG, IgM) to exhaustive endurance running and to antioxidants supplementation. Twelve female elite distance runners were divided into two groups (EG, CG) and participated in treadmill running test. EG was supplied with antioxidants for 12 weeks. Blood samples were collected before (Pre-test) and after (Post-test) 12 weeks at baseline (T1), exhaustion (T2), 30 minutes after exhaustion (T3) and 60 minutes after exhaustion (T4). m-GOT was not changed significantly, which suggested that the mitochondrial function of elite distance runners might not be hurt easily. There were significant differences of IgA, IgG and IgM within groups. In the Pre-test, EG and CG showed a significantly higher level in T2 than in T3. In the Post-test, EG didn't reveal such similarity but, compared to EG, CG showed a significantly higher level in T2 than in T3. These results suggest that antioxidant supplementation may have some beneficial effects on the humoral immune responses of elite distance runners. Correlation, factor- and regression-analysis showed that IgG has a significant relationship with all variables. These findings suggest that IgG may play important roles not only in maintaining of mitochondrial function but also in mediating immune responses.

Keywords: treadmill running test, mitochondrial damage, immunoglobulins

INTRODUCTION

Human beings require oxygen to generate energy in mitochondria in their cells. For this, it is often forgotten that oxygen can also be changed into toxic agents, such as reactive oxygen species (ROS) [1]. ROS not only mediate tissue injury but also regulate metabolism, signal transduction, and, ultimately, tissue function [2]. That is, ironically, various ROS-mediated actions actually protect cells against ROS-induced oxidative stress and re-establish or maintain "redox balance", also termed "redox homeostasis" [3].

Nonetheless, overproduction of ROS results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. In contrast, the beneficial effects of ROS and RNS (reactive nitrogen species, e.g. nitric oxide) occur at low/moderate concentrations and involve cellular responses to noxia, for example, defense against infectious agents, functioning in a number of cellular signaling pathways, and

the induction of a mitogenic response [3]. That is, free radicals play crucial roles not only in maintaining health and mediating disease but also in maintaining normal biological functions.

Therefore, many studies have been performed for the purpose of determining the mechanism of ROS generation [4,5], the method of decreasing the exercise-induced oxidative stress [6], and a way of attenuating ROS toxicity [7].

Aerobes survive in the presence of ROS since they have evolved antioxidant defenses [8]. Given that ROS may act as toxins, mediators, and modulators of inflammatory gene activation, efforts have focused on the investigation of antioxidant molecules as potential therapeutic agents [9]. Antioxidants are defined as substances that, when present at low concentrations compared to those of an oxidizable substrate, significantly delay or prevent oxidation of that substrate [10]. Antioxidants work by preventing the formation of new free radical species, by converting existing free radicals into less harmful molecules, and by preventing chain reactions [11].

Overload training performed by well-trained endurance

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athletes increases oxidative stress through the accumulative effect of repeated high-intensity exercise [6]. Oxidative stress refers to the cellular injuries and pathological changes that occur when there is an imbalance favoring oxidants over antioxidants within a living organism [11]. Athletes who maintain a very high volume of exercise, as a result of greater production of ROS, may be particularly susceptible to oxidative damage [12]. Oxidative damage of biomolecules is postulated to be a major causal factor of various physiological function disorders [13].

Therefore, exhaustive endurance exercise practiced by elite middle to long distance runners is likely to trigger oxidative stress. Consequently, many studies have focused on the relationship between exercise, antioxidants, and oxidative stress [14,15]. Further, the actual methods of reducing oxidative stress caused by exhaustive endurance exercise must involve supplementation with antioxidants. The potential of dietary antioxidants as an endogenous defense against lipid peroxides produced during exercise has received increasing attention in recent years. However, the results of human studies on the effects of supplementation with antioxidant vitamins on lipid peroxidation or enzyme muscle damage are controversial [16].

Meanwhile, moderate physical activity, when performed on a regular basis, presents a number of benefits to the whole organism, especially regarding immune system function [17], increased oxygen consumption, and energy expenditure during physical work [18]. Free radical production is increased during exercise, and oxidative damage occurs in several tissues [19]. That is, free radicals generated during exhaustive exercise cause subsequent oxidative damage to cells. This kind of tissue injury may be accompanied by immune responses. An increased systemic concentration of stress hormones as well as some cytokines may contribute to the depression of immune cell function typically observed after prolonged exercise [20]. These studies suggest that there are some interactions between exercise-induced oxidative stress and immune responses.

Immunoglobulins play important roles in humoral immunity. Humoral immunity, in particular secretion of neutralizing antibodies, is of central importance to protecting the body against acutely cytopathic viruses, whereas noncytopathic viruses have found ways to coexist with the immune system to avoid antibody-mediated elimination. Secreted protective antibodies of humoral memory provide an efficient line of defense against reinfection [21]. Immunoglobulin A (IgA) plays a central role in local immunity [22]. Immunoglobulin G (IgG) is the most abundant of the immunoglobulins in serum and is the most important antibody component [23].

IgG also attaches promptly to surface receptors on macrophages (or phagocyte). Intense endurance exercise suppresses salivary immunoglobulins. This exercise-induced decrease is specific for the secretory antibodies IgA and IgM [24]. IgA and IgG concentrations were correlated positively with serum IL-6 levels, which is a marker of inflammation [25].

Mitochondria consume about 90 percent of the oxygen used by the body and are a particularly rich source of ROS [26]. That is, mitochondria are an active and continuous source of ROS during respiration. Increased production of ROS during endurance training is the result of augmented electron transport through the respiratory chains, which makes the mitochondria a potential target for oxidative damage [27]. In addition to the well-established role of mitochondria in energy metabolism, regulation of cell death has recently emerged as a second major function of these organelles [28]. m-GOT, a general marker for cell damage [29], is located in the mitochondrial matrix and is useful for investigating the permeability of the mitochondrial inner membrane [30]. When mitochondria injury becomes serious, the rate of m-GOT that flows out into the serum is increased [31].

Therefore, the objective of this study was to investigate the relationships between exhaustive endurance exercise, antioxidant supplementation, humoral immune response, and mitochondrial damage in well-trained distance runners.

METHODS AND MATERIALS

Human subjects

Twelve healthy young women, who ran for an average of nearly 20 hours per week, and were thus well-trained distance runners (age 17.75 ± 0.62 years; body weight 51.42 ± 2.02 kg; height 165.71 ± 3.36 cm; athletic career 5.12 ± 0.71 years; HR_{rest} 48.67 ± 3.47 beats/min; HR_{max} 189.67 ± 2.71 beats/min) volunteered to participate in the present study. All subjects signed a written informed consent form. Twelve subjects were randomly divided into two groups (experimental group, EG, $n = 6$; comparative group, CG, $n = 6$). EG was supplied by oral administration of antioxidants (671.14 mg α -tocopherol; 400 mg Vitamin C) every day for 12 weeks. CG was not given any supplemental antioxidants. All subjects abstained from alcohol and caffeine consumption for at least 24 hours, and did not exercise for the 24 hours prior to testing.

Experimental exercise protocol

In the present study, all subjects carried out experimental

exercise (incremental treadmill endurance test) before (Pre-test) and after (Post-test) 12 weeks of antioxidant ingestion. Experimental exercise protocol was the Modified Bruce Treadmill Protocol, in which each level consisted of 6 minutes instead of 3 minutes. All subjects practiced the experimental exercise protocol until exhaustion. In this study, the average exercise time was 26 minutes 47 seconds.

Blood sampling

Blood samples were carried out before and after 12 weeks of antioxidant ingestion. Peripheral blood samples were drawn from an antecubital vein at 10:00 a.m. (rest, T1) after an overnight fast, immediately after exhaustion (T2), 30 minutes after exhaustion (T3), and 60 minutes after exhaustion (T4) in a seated position. Blood was placed into a centrifuge tube and allowed to clot to obtain the serum. The serum was separated by centrifugation at 3000 rpm for 15 minutes. Separated serum was quickly frozen and stored at -75 °C until being used.

Analysis of mitochondrial aspartate aminotransferase (m-GOT)

There is accumulating evidence supporting a direct link between mitochondria, oxidative stress, and cell death [32, 33]. For the study on the effects of antioxidants and exhaustive endurance exercise on mitochondria, m-GOT was measured by the Protease method.

Analysis of immunoglobulins

For the study on the effects of antioxidants and exhaustive endurance exercise on the immune response, immunoglobulins (IgA, IgG, and IgM) were measured by the Nephelometry method.

Statistical analysis

Results for each experimental group are reported as means $\pm$ SD. All variables in each of the experimental groups were analyzed using the repeated measures analysis of variance (ANOVA) function within the SPSS statistical package, with group type (EG and CG), measurement period (Pre- and Post-test), and measurement time (T1, T2, T3, T4) as main factors. When a significant F value was obtained, LSD post hoc analysis was performed to determine the specific differences. Baseline characteristics and cumulative balance of all variables of the two groups were compared using

independent sample t-tests. Significant main effects or interactions were further investigated using paired-sample t-tests to locate differences. Correlation coefficients were calculated over each variable's whole data. Factor analysis was used to identify the best fit among the variables. Multiple regression analysis was used to correlate the measured parameters. P values < .05 were considered statistically significant.

RESULTS

m-GOT

In the present study, 12 weeks of antioxidant supplementation to elite middle to long distance runners did not cause a difference in m-GOT between EG and CG. Changes in m-GOT levels as the result of antioxidant supplementation and exhaustive endurance exercise are shown in Table 1. Multivariate analysis of m-GOT is shown in Table 2. m-GOT levels revealed no significant differences according to the measurement period [(Pre-test, $F = 1.172$, $p = .304$), (Post-test, $F = 1.522$, $p = .246$)] and measurement time [(Pre-test, $F = .309$, $p = .818$), (Post-test, $F = 1.645$, $p = .200$)]. The analysis results of the measurement times of m-GOT within the groups followed by LSD's multiple range test are shown in Table 1.

IgA

Changes in IgA levels as the result of antioxidant supplementation and exhaustive endurance exercise in well-trained distance runners are shown in Table 1. Multivariate analysis revealed no significant difference between the EG and CG groups <Table 2>. However, there were significant differences according to the measurement time of each group (Pre-test, $F = 7.926$, $p = .001$; Post-test, $F = 3.387$, $p = .031$). According to the tests, within subjects contrasts, in the Pre-test of EG, T2 level was higher than that of T3 ($F = 42.454$, $p = .001$). However, there were no significant differences in the Post-test of EG.

Moreover, IgA levels in CG revealed significant differences between the Pre- and Post-tests. In the Pre-test, the T2 level was higher than those of T1 ($p = .043$), T3 ($p = .01$), and T4 ($p = .016$). In the Post-test of CG, the T2 level was higher than that of T3 ($p = .040$) <Table 1>.

IgG

IgG levels are shown in Table 1. Multivariate analysis for

Table 1. Levels of m-GOT, IgA, IgG, IgM as the result of antioxidant supplementation and exhaustive endurance exercise

Variables	MPs	Group	T1	T2	T3	T4
m-GOT (U/L)	Pre	EG	3.33 ± 1.03	3.83 ± .41	3.83 ± .41	3.67 ± .82
m-GOT (U/L)	Pre	CG	3.50 ± .84	3.50 ± .84	3.17 ± .75	3.33 ± .52
m-GOT (U/L)	Post	EG	4.33 ± .52	4.12 ± .41	4.33 ± .52	4.33 ± .52
m-GOT (U/L)	Post	CG	4.17 ± .41	3.83 ± .75	4.33 ± .52	3.67 ± .82
IgA (mg/dl)	Pre	EG	209.33 ± 61.10	231.33 ± 57.74	205.83 ± 49.94***	217.67 ± 66.16
IgA (mg/dl)	Pre	CG	256.83 ± 103.35***	279.17 ± 113.71	254.67 ± 103.80***	247.17 ± 100.89***
IgA (mg/dl)	Post	EG	243.50 ± 83.55	272.00 ± 77.05	237.33 ± 74.28	238.83 ± 85.08
IgA (mg/dl)	Post	CG	253.67 ± 91.79	301.67 ± 147.70	249.67 ± 105.34*	242.83 ± 89.77
IgG (mg/dl)	Pre	EG	1588.33 ± 326.52**	1751.67 ± 273.96	1566.67 ± 232.09**	1645.00 ± 293.31
IgG (mg/dl)	Pre	CG	1352.50 ± 230.34***	1466.67 ± 236.11	1298.33 ± 203.71***	1307.50 ± 258.68***
IgG (mg/dl)	Post	EG	1751.67 ± 663.91	2028.33 ± 691.01	1703.33 ± 624.84	1648.33 ± 293.35
IgG (mg/dl)	Post	CG	1311.50 ± 315.44	1541.67 ± 302.29	1325.00 ± 195.01*	1281.67 ± 161.54
IgG (mg/dl)	Pre	EG	217.00 ± 114.45	229.67 ± 107.41	210.33 ± 102.68**	215.67 ± 124.57
IgG (mg/dl)	Pre	CG	238.83 ± 89.77	251.67 ± 86.51	227.50 ± 81.08**	221.17 ± 75.88**
IgG (mg/dl)	Post	EG	232.00 ± 88.32	270.00 ± 112.17	219.50 ± 73.46	224.17 ± 91.76
IgG (mg/dl)	Post	CG	245.67 ± 112.95	269.17 ± 110.71	227.67 ± 91.24*	223.33 ± 90.81

All values are means ± SD. MPs = measurement periods; Pre = Pre-test; Post = Post-test; T1 = rest situation; T2 = exhaustion; T3 = 30 minutes after exhaustion; T4 = 60 minutes after exhaustion

* $p < .05$ vs T2, ** $p < .01$ vs T2, *** $p < .001$ vs T2

IgG revealed no significant difference between EG and CG <Table 2>. However, there were significant differences according to the measurement times of the groups. That is, there were significant differences in the Pre-test of the groups ($F = 14.121$, $p = .001$). However, there were no significant differences in the Post-test of groups ($F = 2.825$, $p = .055$). Pair-wise comparisons revealed significant differences in the Pre-test of EG ($F = 5.556$, $p = .008$). The T2 level of EG in the Pre-test was higher than those of T1 ($p = .017$) and T3 ($p = .006$). IgG levels of EG in the Post-test showed no significant differences according to the measurement time ($F = 1.115$, $p = .374$). Pair-wise comparisons revealed significant differences between the Pre- ($F = 12.441$, $p = .001$) and Post-tests ($F = 3.346$, $p = .048$) of CG. The T2 level of CG in the Pre-test was higher than those of T1 ($p = .024$), T3 ($p = .002$), and T4 ($p = .001$). The T2 level of CG in Post-test was higher than that of T3 ($p = .025$) <Table 1>.

IgM

IgM levels are shown in Table 1. Multivariate analysis for IgM revealed no significant difference between EG and CG <Table 2>. However, there were significant differences according to the measurement times of the groups (Pre-test, $F = 9.066$, $p = .001$; Post-test, $F = 4.641$, $p = .009$). According to the tests, within subjects contrasts, in the Pre-test of EG, T2 level was higher than that of T3 ($F = 22.822$, $p = .005$). However, there were no significant differences in the Post-test

of EG ($F = 1.733$, $p = .203$). Further, there were significant differences between the Pre- ($F = 8.548$, $p = .002$) and Post-tests ($F = 11.649$, $p = .019$) of CG. In Pre-test, the T2 level was significantly higher than those of T3 ($p = .007$) and T4 ($p = .009$). In the Post-test, the T2 level was also significantly higher than that of T3 ($p = .019$) <Table 1>.

Correlation between measurement periods and variables

Pearson correlations between the groups and variables are shown in Table 3. IgG of EG revealed a strong positive relationship to all variables, except IgM of CG. A significant inverse relationship ($r = -0.29$, $p < .05$) was present between IgG of EG and IgM of CG. IgG of CG also showed a significant positive relationship to all variables except m-GOT of CG and IgA of EG. m-GOT of EG showed a significant positive correlation to m-GOT of CG ($r = 0.33$, $p < .05$), to IgG of EG ($r = 0.36$, $p < .05$), and IgG of CG ($r = 0.35$, $p < .05$). However, there was no significant relationship between m-GOT of EG and other variables. m-GOT of CG had a significant positive relationship to IgA of EG ($r = 0.41$, $p < .05$) and IgG of EG ($r = 0.35$, $p < .05$). IgA of EG had a significant positive relationship to IgG of EG ($r = 0.30$, $p < .05$) and IgM of EG ($r = 0.52$, $p < .01$), and had a strong inverse correlation to IgA of CG ($r = -0.35$, $p < .05$). IgA of CG showed a significant positive relationship to IgG of EG ($r = 0.68$, $p < .01$) and IgG of CG ($r = 0.61$, $p < .01$), but had no significant correlation to IgM of each group.

Table 2. Multivariate analysis results for m-GOT, IgA, IgG, and IgM

Source	MPs		SS	Df	MS	F
m-GOT	Pre	MTs	.396	3	0.132	0.309
m-GOT	Pre	Group	1.021	1	1.021	1.172
m-GOT	Pre	MTs*Group	1.063	3	0.354	0.831
m-GOT	Pre	Error(MTs)	12.792	30	0.426	
m-GOT	Post	MTs	1.063	3	0.354	1.645
m-GOT	Post	Group	1.021	1	1.021	1.522
m-GOT	Post	MTs*Group	0.729	3	0.243	1.129
m-GOT	Post	Error(MTs)	6.458	30	0.215	
IgA	Pre	MTs	4952.667	3	1650.889	7.926***
IgA	Pre	Group	22620.083	1	22620.083	0.790
IgA	Pre	MTs*Group	777.583	3	259.194	1.244
IgA	Pre	Error(MTs)	6248.250	30	208.275	
IgA	Post	MTs	16649.562	3	5549.854	3.387*
IgA	Post	Group	2366.021	1	2366.021	0.073
IgA	Post	MTs*Group	1088.729	3	362.910	0.221
IgA	Post	Error(MTs)	49160.458	30	1638.682	
IgG	Pre	MTs	214545.833	3	71515.278	14.121***
IgG	Pre	Group	952033.333	1	952033.333	3.745
IgG	Pre	MTs*Group	16220.833	3	5406.944	1.068
IgG	Pre	Error(MTs)	151933.333	30	5064.444	
IgG	Post	MTs	741374.229	3	247124.743	2.825
IgG	Post	Group	2096270.021	1	2096270.021	3.727
IgG	Post	MTs*Group	28245.062	3	9415.021	0.108
IgG	Post	Error(MTs)	2623981.458	30	87466.049	
IgM	Pre	MTs	3906.563	3	1302.188	9.066***
IgM	Pre	Group	3316.688	1	3316.688	0.085
IgM	Pre	MTs*Group	540.229	3	180.076	1.254
IgM	Pre	Error(MTs)	4308.958	30	143.632	
IgM	Post	MTs	16867.062	3	5622.354	4.641**
IgM	Post	Group	305.021	1	305.021	0.009
IgM	Post	MTs*Group	459.562	3	153.187	0.126
IgM	Post	Error(MTs)	36340.125	30	1211.337	

MPs = measurement periods; MTs = measurement times; Pre = Pre-test; Post = Post-test

* $p < .05$, ** $p < .01$, *** $p < .001$

Correlations for variables

Relationship analysis of the pooled data [both groups (EG, CG), both periods (Pre-test, Post-test), and four measurements (T1, T2, T3, T4) combined] showed that IgG was significantly correlated to all variables [m-GOT ($r = 0.34$, $p < .01$); IgA ($r = 0.26$, $p < .01$); IgM ($r = 0.25$, $p < .05$)]. m-GOT showed a significant positive relationship to IgA ($r = 0.26$, $p < .05$); IgG ($r = 0.34$, $p < .01$), but there was no significant relationship between m-GOT and IgM ($r = -0.06$, $p > .05$). IgA revealed a significant correlation to m-GOT ($r = 0.26$, $p < .05$) and IgG ($r = 0.26$, $p < .01$) but no relationship to IgM ($r = 0.06$, $p > .05$). IgM showed a significant positive

Table 3. Correlation between measurement periods and variables

	m-GOT (EG)	m-GOT (CG)	IgA (EG)	IgA (CG)	IgG (EG)	IgG (CG)	IgM (EG)
m-GOT(CG)	0.33*						
IgA(EG)	0.14	-0.07					
IgA(CG)	0.24	0.41*	-0.35*				
IgG(EG)	0.36*	0.35*	0.30*	0.68**			
IgG(CG)	0.35*	0.21	-0.13	0.61**	0.40**		
IgM(EG)	0.16	-0.02	0.52**	-0.07	0.30*	0.30*	
IgM(CG)	0.12	-0.26	-0.23	-0.28	-0.29*	0.30*	-0.04

* $p < .05$, ** $p < .01$

Table 4. Correlations between variables

	m-GOT	IgA	IgG
IgA	0.26*		
IgG	0.34**	0.26**	
IgM	-0.06	0.06	0.25*

* $p < .05$, ** $p < .01$

Table 5. Results of principal component analysis

Component	IE			ESSL			RSSL		
	T	V%	C%	T	V%	C%	T	V%	C%
IgG	1.61	40.34	40.34	1.61	40.34	40.34	1.54	38.51	38.51
IgA	1.07	26.83	67.17	1.07	26.83	67.17	1.15	28.65	67.17
IgM	0.76	19.08	86.25						
m-GOT	0.55	13.75	100						

IE = Initial Eigenvalues; ESSL = Extraction Sums of Squared Loadings; RSSL = Rotation Sums of Squared Loadings; T = Total; V% = % of Variance; C% = Cumulative %

relationship to IgG ($r = 0.25$, $p < .05$) but no significant correlation to m-GOT ($r = -0.06$, $p > .05$) and IgA ($r = 0.06$, $p > .05$) <Table 4>.

Factor Analysis

As presented in Table 5, the results of principal component analysis showed two factors that initial the eigen value was over 1. IgG, the first factor, showed an initial eigen value of 1.613 and 40.336 % (1.613/4×100) variance. IgA, the second factor, showed an initial eigen value of 1.073 and 26.832 % (1.073/4×100) variance. The total cumulative percent of these two factors was 67.168 %.

Multiple regression analysis

Table 6 shows the results of the multiple regression analysis. The adjusted coefficient of variations for this model regarding m-GOT (dependant variable) was 16.9 % ($R^2 = 0.169$, $F = 6.252$, $p < .001$). Stepwise linear multiple regression analysis showed that the significant and reliable factor independently

Table 6. Results of the stepwise linear multiple regression analysis

Dependent Variables		Unstandardized Coefficients		Standardized Coefficients	t	R ²	F
		B	SE	Beta			
m-GOT	(Constant)	2.821	0.322		8.758***	0.169	6.252***
m-GOT	IgG	0.001	0.000	0.331	3.261**	0.169	6.252***
m-GOT	IgA	0.002	0.001	0.182	1.853	0.169	6.252***
m-GOT	IgM	-0.001	0.001	-0.151	-1.544	0.169	6.252***
IgA	(Constant)	84.310	54.355		1.551	0.102	5.261**
IgA	m-GOT	23.949	12.922	0.197	1.853	0.102	5.261**
IgA	IgG	0.042	0.024	0.189	1.722	0.102	5.261**
IgA	IgM	0.023	0.099	0.024	0.232	0.102	5.261**
IgG	(Constant)	432.566	229.941		1.881	0.213	8.291***
IgG	m-GOT	172.930	53.028	0.313	3.261**	0.213	8.291***
IgG	IgM	1.108	0.405	0.255	2.739**	0.213	8.291***
IgG	IgA	0.752	0.437	0.165	1.722	0.213	8.291***
IgM	(Constant)	202.739	54.084		3.749***	0.084	4.241*
IgM	IgG	0.068	0.025	0.296	2.739**	0.084	4.241*
IgM	m-GOT	-21.162	13.703	-0.167	-1.544	0.084	4.241*
IgM	IgA	0.025	0.110	0.024	0.232	0.084	4.241*

SE = Standard Error; * $p < .05$, ** $p < .01$, *** $p < .001$

associated with m-GOT was IgG ($t = 3.261$, $p < .01$).

The adjusted coefficient of variations for this model regarding IgA (dependant variable) was 10.2 % ($R^2 = 0.102$, $F = 5.261$, $p < 0.01$). However, stepwise linear multiple regression analysis showed that there were no significant and reliable factors independently associated with IgA. The adjusted coefficient of variations for this model regarding IgG (dependant variable) was 21.3 % ($R^2 = 0.213$, $F = 8.291$, $p < .001$). Stepwise linear multiple regression analysis showed that the significant and reliable factors independently associated with IgG were m-GOT ($t = 3.261$, $p < .01$) and IgM ($t = 2.739$, $p < .01$).

The adjusted coefficient of variations for this model regarding IgM (dependant variable) was 8.4 % ($R^2 = 0.083$, $F = 4.241$, $p < .05$). Stepwise linear multiple regression analysis showed that the significant and reliable factor independently associated with IgM was IgG ($t = 2.739$, $p < .01$).

DISCUSSION

Mitochondrial function is related with indices of oxidative damage and nitric oxide production, scavenger levels, and mitochondrial ROS production rates [34]. The resting energy expenditure for skeletal muscle is very moderate and is around 13 kcal/kg organ mass/day [35], which, during heavy exercise, results in a large increase in energy turnover (> 100 -fold) and introduces a major energetic challenge with massive oxygen flow to mitochondria [36]. Therefore, it is not surprising that

mitochondrial generation of ROS is significantly enhanced during exercise [37]. The generation of ROS in mitochondria acts as a redox signal in triggering cellular events, such as apoptosis, proliferation, and senescence [38].

Maintenance of long-term antibody responses is critical for protective immunity against many pathogens [39]. Although exercise has been recognized to provoke upregulation of antibodies, the mechanism has not yet been explained [5].

The present investigation demonstrated that the level of m-GOT, a general marker of cell damage, did not significantly change in response to acute exhaustive running and long-term antioxidant supplementation. This result was different from a report that observed that untrained healthy male volunteers experience significant increases in m-GOT activity after exercise [40]. The present study suggests that, during exhaustive endurance exercise, the mitochondrial function of elite middle to long distance runners might not be easily affected. Therefore, well-trained distance runners who have are well-trained in high intensity endurance exercise may have excellent capacity of maintaining mitochondrial function, which can help delay fatigue, prolong training time of muscles, prevent muscle damage from exhaustive running, and decrease the incidence of oxidative stress-associated diseases. At this point, the present study was in accordance with a report that animals frequently exposed to exercise (chronic training) show less oxidative damage after exhaustive exercise than untrained animals, and that chronic training may result in the upregulation of endogenous antioxidant enzymes such as mitochondrial superoxide dismutase (MnSOD),

glutathione peroxidase, and γ -glutamylcysteine synthetase [41].

Since exercise produces enhanced ROS, and enhanced ROS production can result in impaired cell function, and impaired cell function can result from mitochondrial dysfunction [34], and mitochondrial dysfunction can result in impaired ATP production of autoxidizable electron carriers, it is important for distance runners to maintain their mitochondrial function under oxidative stress generated during exhaustive endurance exercise. Therefore, the result of this study was also in accordance with a previous report that found that physical training strengthens antioxidant defense mechanisms in several tissues, including the skeletal muscle [42].

Although the present study did not observe changes in mitochondrial damage, a previous study reported that acute exhaustive endurance exercise performed by middle to long distance runners results in significant elevation of serum malondialdehyde [43], a biomarker of cell membrane injury [44]. This suggests that, during exhaustive endurance running by well-trained distance runners, there may be varying levels of cell membrane damage and mitochondrial injury, and that a single bout of exhaustive endurance exercise by elite distance runners may elicit cell membrane injury without damaging mitochondrial function. The present study demonstrated that acute exhaustive endurance exercise performed by well-trained distance runners did not easily trigger damage to the mitochondrial membrane. Thus, this study was in accordance with the opinion that the role of mitochondria in the formation of free radicals in exercise should be reconsidered and perhaps alternative sources of ROS identified [14].

Meanwhile, according to multivariate analysis regarding immunoglobulins (IgA, IgG, and IgM), there was no significant difference between EG and CG. However, there was a significant difference according to measurement time between the groups <Table 1, 2>. Results of analysis according to measurement time between the groups demonstrated that, before long-term antioxidant supplementation, EG showed a general tendency of higher T2 than T3, but after 12 weeks of antioxidant supplementation, EG did not reveal the same tendency. Further, CG also showed a general tendency of higher T2 than T3 in the Pre-test, but compared to EG, CG showed a similar tendency of higher T2 than T3 in the Post-test <Table 1>. These results suggest that 12 weeks of antioxidant supplementation can result in reinforcement of the antioxidant defense system in elite distance runners, which has a beneficial effect on their humoral immune responses.

Although intense physical exercise has been shown to be associated with immunosuppression and increased rate of infection, some athletes can withstand intense training periods

without health problems while others are prone to infections [45]. Thus, the present study also suggests that other factors may interfere with immunoregulation, and that exercise training increases the resistance against oxidative stress, providing enhanced protection [46]. At this point, the present study might be in accordance with a report that found that regular exercise plays a preventive role against lifestyle-dependent diseases, and that the molecular mechanism behind this favorable effect could be linked to redox homeostasis, a free radical-related adaptive mechanism [47].

Meanwhile, the correlation between measurement periods and variables (m-GOT, IgA, IgG, and IgM) showed a meaningful result that IgG in EG was significantly correlated with all variables <Table 3>. Moreover, IgG of EG showed inverse correlation to IgM of CG. This result was interesting in that IgG of EG was positively correlated with other variables. The mechanism responsible for this difference is unknown, but this finding suggests that IgG may play a crucial role in sustaining antigen-specific immunoglobulin levels in the serum and in continuously producing antibody.

Correlation between variables (m-GOT, IgA, IgG, and IgM) revealed that IgG was significantly correlated with all variables. Factor analysis showed similar results. That is, IgG, the first factor, revealed an initial eigen value of 1.613 and 40.336 % variance. Multiple regression analysis also revealed a similar tendency that IgG was significantly correlated with other variables.

In summary, although the exact mechanisms involved in these relationships are not completely clear, these findings suggest that antioxidant supplementation may have some beneficial effects on the humoral immune responses in elite distance runners, and that IgG may play important roles not only in maintaining mitochondrial function but also in mediating immune responses.

REFERENCES

- [1] Jorge Rodríguez, Claudio Olea-Azar, Cristina Cavieres, Ester Norambuenab, Tomás Delgado-Castro, Jorge Soto-Delgado, Ramiro Araya-Maturana. Antioxidant properties and free radical-scavenging reactivity of a family of hydroxynaphthalenones and dihydroxyanthracenones. *Bioorganic & Medicinal Chemistry*. 2007;15(22):7058-65.
- [2] Marczin N, El-Habashi N, Hoare GS, Bundy RE, Yacoub M. Antioxidants in myocardial ischemia-reperfusion injury: therapeutic potential and basic mechanisms. *Arch Biochem Biophys* 2003;420:222-36.
- [3] Valko M, Leibfriz D, Moncol J, Cronin MT, Mazur M,

- Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;39(1):44-84.
- [4] Jackson MJ. Free radicals generated by contracting muscle: By-products of metabolism or key regulators of muscle function. *Free Radical Biology and Medicine.* 2008;44(2):132-41.
- [5] Suzuki K, Tagami K. Voluntary wheel-running exercise enhances antigen-specific antibody-producing splenic B cell response and prolongs IgG half-life in the blood. *Eur J Appl Physiol.* 2005;94(5-6):514-9.
- [6] Volvaard NB, Cooper CE, Shearman JP. Exercise-induced oxidative stress in overload training and tapering. *Med Sci Sports Exerc.* 2006;38(7):1335-41.
- [7] McKenna MJ, Medved I, Goodman CA, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC, Sostaric S, Gong X. N-acetylcysteine attenuates the decline in muscle Na⁺,K⁺-pump activity and delays fatigue during prolonged exercise in humans. *J Physiol.* 2006;576(1):279-88.
- [8] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine.* Ed 4. New York, USA. Oxford University Press. 2007, p.79.
- [9] Conner EM, Grisham MB. Inflammation, free radicals and antioxidants. *Nutrition.* 1996;12:274-7.
- [10] Barry Halliwell. Antioxidant characterization methodology and mechanism. *Biochemical Pharmacology.* 1995;49(10):1341-8.
- [11] Soffler C. Oxidative stress. *Vet Clin North Am Equine Pract.* 2007;23(1):135-57.
- [12] Knez WL, Jenkins DG, Coombes JS. Oxidative stress in half and full Ironman triathletes. *Med Sci Sports Exerc.* 2007;39(2):283-8.
- [13] Lia XM, Mab YL, Liuc XJ. Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology.* 2007;111(3):504-11.
- [14] Gomez-Cabrera MC, Domenech E, Viña J. Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. *Free Radical Biology and Medicine.* 2008;44(2):126-31.
- [15] Peake JM, Suzuki K, Coombes JS. The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. *The Journal of Nutritional Biochemistry.* 2007;18(6):357-71.
- [16] Schulpis KH, Moukas M, Parthimos T, Tsakiris T, Parthimos N, Tsakiris S. The effect of α -Tocopherol supplementation on training-induced elevation of S100B protein in sera of basketball players. *Clinical Biochemistry.* 2007;40(2):900-6.
- [17] Silveira EM, Rodrigues MF, Krause MS, Vianna Tauler P, Aguilo A, Guix P, Jimenez F, Villa G, Tur JA, Cordova A, Pons A. Pre-exercise antioxidant enzyme activities determine the antioxidant enzyme erythrocyte response to exercise. *J Sports Sci.* 2005;23(1):5-13.
- [18] Vesovic D, Borjanovic S, Markovic S, Vidakovic A. Strenuous exercise and action of antioxidant enzymes. *Med Lav.* 2002;93(6):540-50.
- [19] Tauler P, Aguilo A, Guix P, Jimenez F, Villa G, Tur JA, Cordova A, Pons A. Pre-exercise antioxidant enzyme activities determine the antioxidant enzyme erythrocyte response to exercise. *J Sports Sci.* 2005;23(1):5-13.
- [20] Davison G, Gleeson M. The effect of 2 weeks vitamin C supplementation on immunoendocrine responses to 2.5 h cycling exercise in man. *Eur J Appl Physiol.* 2006;99(6):707-8.
- [21] Dörner T, Radbruch A. Antibodies and B cell memory in viral immunity. *Immunity.* 2007;27(3):384-92.
- [22] Kim JC, Kim MG, Sung YG, Han IS. *Immunology at a Glance.* Seoul, S. Korea. E-Public. 2006, p.27.
- [23] Paul WE. *Fundamental Immunology.* New York, USA. Raven Press. 1993, p.865-85.
- [24] Mackinnon LT, Chick TW, van As A, Tomasi TB. The effect of exercise on secretory and natural immunity. *Adv Exp Med Biol.* 1987;216A:869-76.
- [25] Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, Fernandez-Merino C, Vidal C. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin Exp Immunol.* 2008;151(1):42-50.
- [26] Alam K, Moinuddin, Jabeen S. Immunogenicity of mitochondrial DNA modified by hydroxyl radical. *Cell Immunol.* 2007;247(1):12-7.
- [27] Delchev SD, Georgieva KN, Koeva YA, Atanassova PK. Bcl-2/Bax ratio, mitochondrial membranes and aerobic enzyme activity in cardiomyocytes of rats after submaximal training. *Folia Med(Plovdiv).* 2006;48(2):50-6.
- [28] Orrenius S. Reactive oxygen species in mitochondria-mediated cell death. *Drug Metab Rev.* 2007;39(2-3):443-55.
- [29] Ryo K, Yamada H, Nakagawa Y, Tai Y, Obara K, Inoue H, Mishima K, Saito I. Possible involvement of oxidative stress in salivary gland of patients with Sjogren's syndrome. *Pathobiology.* 2006;73(5):252-60.
- [30] Aoki Y, Tanigawa K, Itoh H. Transmigration of mitochondrial GOT in cholestatic liver injury. *J Toxicol*

- Sci. 1986;11(3):145-54.
- [31] Lee GN, Lee JS. Clinical Pathologic File. Seoul, S. Korea. Medicine Press. 1996, p.467.
- [32] Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis. 2007;12(5):913-22.
- [33] Vázquez-Memije ME, Capin R, Tolosa A, El-Hafidi M. Analysis of age-associated changes in mitochondrial free radical generation by rat testis. Mol Cell Biochem. 2008;307(1-2):23-30.
- [34] Venditti P, Bari A, Di Stefano L, Di Meo S. Role of mitochondria in exercise-induced oxidative stress in skeletal muscle from hyperthyroid rats. Arch Biochem Biophys. 2007;463(1):12-8.
- [35] Kinney JM, Tucker HN. Energy metabolism. New York, USA. Raven Press. 1991, p.19-59
- [36] Sahlin K, Tonkonogi M, Soderlund K. Energy supply and muscle fatigue in humans. Acta Physiol. 1998;162:261-6.
- [37] Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun. 1982;107:1198-205.
- [38] Chen CL, Zhang L, Yeh A, Chen CA, Green-Church KB, Zweier JL, Chen YR. Site-specific S-glutathiolation of mitochondrial NADH ubiquinone reductase. Biochemistry. 2007;46(19):5754-65.
- [39] Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007;357(19):1903-15.
- [40] Ohno H, Watanabe H, Kishihara C, Taniguchi N, Takakuwa E. Effect of physical exercise on the activity of GOT isozyme in human plasma. Tohoku J Exp Med. 1978;126(4):371-6.
- [41] Salminen A, Vihko V. Lipid peroxidation in exercise myopathy. Exp Mol Pathol. 1983;38:380-8.
- [42] Chandan KS, Lester Packer, Osmo Hänninen. Handbook of Oxidants and Antioxidants in Exercise. Oxford, UK. Elsevier Press. 2000, p.297-320.
- [43] Oh BS. Effects of antioxidant supplementation on cell membrane and anti-inflammatory immunity in middle to long distance runners. KAHPERD. 1999;38(4):387-400.
- [44] Orhan H, van Holland B, Krab B, Moeken J, Vermeulen NP, Hollander P, Meerman JH. Evaluation of a multi-parameter biomarker set for oxidative damage in man: increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise. Free Radic. 2004;38:1269-79.
- [45] König D, Weinstock C, Keul J, Northoff H, Berg A. Zinc, iron, and magnesium status in athletes--influence on the regulation of exercise-induced stress and immune function. Exerc Immunol Rev. 1998;4:2-21.
- [46] Radak Z, Naito H, Kaneko T, Tahara S, Nakamoto H, Takahashi R, Cardozo-Pelaez F, Goto S. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. Pflugers Arch. 2002;445: 273-8.
- [47] Zsolt Radak, Hae Young Chung, Sataro Goto. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radical Biology and Medicine. 2008; 44(2):153-9.