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ORIGINAL ARTICLE

The combinatory effects of combined training (endurance—resistance) and garlic supplementation on oxidative stress and antioxidant adaptations in untrained boys

Effet des exercices combinés (de résistance et d'endurance) et des compléments alimentaires à base d'ail sur l'indice du stress oxydatif et de la défense antioxydante chez les garçons inactifs

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MOTS CLÉS
Ail ;
Stress oxydatif ;
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Summary
Objectives. — Physical activity and exercise have been regarded as beneficial and therapeutic strategies in the prevention, treatment, and rehabilitation of a variety of medical disorders and diseases, even before advanced medical therapies are prescribed. The aim of this study was to characterize the oxidative stress and antioxidant defense adaptations in untrained boys after a regimen of combined training alone or together with garlic administration.
Methods. — In the present quisi-experimental study, 46 untrained volunteer boys were randomly assigned into 4 groups: garlic + train ($n = 13$), placebo + train ($n = 13$), garlic ($n = 10$), and control ($n = 10$). The combined training protocol comprised of endurance training (60—80% HR_{max}, running) and resistance training (40—85% 1RM, weight training) for 8 weeks. Capsules containing 250 mg garlic were administered on a daily basis in the garlic and train + garlic groups for 8 weeks. The two-way ANOVA test was employed to determine within-group and between-group differences. The Least Significant Difference (LSD) post hoc test was performed to locate the differences. Data were analyzed by SPSS v.20 with a significance level of $P < 0.05$.

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Results. — Malondialdehyde (MDA) level, as a marker of oxidative stress, decreased significantly in the train + placebo ($P = 0.01$), train + garlic ($P < 0.001$), and garlic ($P < 0.001$) groups, whilst no change occured in the control ($P = 0.33$) group. The index of total antioxidant capacity (TAC) significantly increased in the train + placebo ($P = 0.02$), train + garlic ($P = 0.01$), and garlic ($P = 0.005$) groups with no change in the control group ($P = 0.90$). The two indices TAC ($P = 0.001$) and serum MDA ($P = 0.001$) had significant differences between four groups. The LSD post hoc test showed significant differences between garlic and control and between train + garlic and control groups for both MDA and TAC.
Conclusion. — the present research suggests that resistance and endurance training synergistically improve the defense against oxidative stress, and that this effect is even enhanced by garlic antioxidant supplement.

KEYWORDS
Garlic;
Oxidative Stress;
Antioxidant defense

Résumé
Contexte et objectif. — Le sport et les activités physiques forment un moyen utile et efficace pour la prévention, le traitement et la réhabilitation d'un grand nombre des maladies et désordres de santé ; ils sont même conseillés en amont de la prescription des méthodes médicales avancées. La présente recherche a pour but d'étudier l'effet des exercices combinés (de résistance et d'endurance) et de la consommation des compléments alimentaires à base d'ail sur l'indice du stress oxydatif et de la défense antioxydante chez les garçons inactifs.
Méthodes. — Quarante-six garçons inactifs ont participé volontairement à la présente recherche quasi expérimentale. Ils sont répartis en quatre groupes de façon aléatoire : exercices et compléments alimentaires (13 personnes), exercices et placebo (13 personnes), compléments alimentaires (10 personnes) et contrôle (10 personnes).
Le protocole des exercices combinés. — La combinaison des exercices en endurance (courir à l'intensité de 60 % à 80 % de la fréquence cardiaque maximale) et des exercices de résistance (travailler avec des poids à l'intensité de 40 % à 85 % RM1) a été réalisée pendant 8 semaines. 250 mg de capsules d'ail et de placebos ont été consommés quotidiennement dans les deux groupes de « ail » et de « exercices + ail » pendant 8 semaines. Nous avons employé le test *t* pour échantillons appariés et Anova unidirectionnelle pour déterminer les différences entre les groupes et dans les groupes ; pour déterminer la différence, nous avons eu recours à l'analyse post hoc LSD (test de la plus petite différence). Les données sont analysées par le logiciel SPSS, version 20, à seuil de signification de $p < 0{,}05$.
Découvertes. — Le niveau de malonedialdéhyde (MDA) comme l'indice du stress oxydatif présente une baisse significative dans les groupes « exercices + placebo » ($p = 0{,}01$), « exercices + ail » ($p < 0{,}001$) et « ail » ($p < 0{,}001$) ; il reste invariable pour le groupe de « contrôle » ($p = 0{,}35$). Tandis que l'indice de capacité d'antioxydant total (TAC) présente une croissance significative dans les groupes « exercices + placebo » ($p = 0{,}02$), « exercices + ail » ($p = 0{,}02$) et « ail » ($p = 0{,}006$), il reste stable pour le groupe de « contrôle » ($p = 0{,}90$). Les deux indices TAC ($p < 0{,}001$) et MDA ($p = 0{,}001$) présentent une différence significative entre les quatre groupes. L'analyse post hoc LSD a montré une différence significative entre les groupes de « contrôle » et « ail » ainsi qu'entre les groupes « exercices + ail » et « contrôle » pour les deux indices de TAC et MDA.
Conclusion. — La présente recherche suggère que l'entraînement en résistance et en endurance améliore de manière synergique la défense contre le stress oxydatif, et que cet effet est même renforcé par le supplément antioxydant à l'ail.

1. Introduction

The reactive oxygen species (ROS) may be increasingly generated in skeletal muscles, the liver, and the heart and may result in stress oxidative conditions. Nevertheless, regular and moderate exercises attenuate oxidative stress through antioxidant defense augmentation and diabetes reduction phenomena. Human body oxygen consumption in response to endurance training is increased by 10 to 20 times systematically. The accretion is greater in muscles and reaches to 100—200 times more than the time at rest [1]. Considering the antioxidant status at the time of increased ROS production in the body, malondialdehyde (MDA) and total antioxidant capacity (TAC) stand as the main stress oxidative markers to measure [2].

Allium sativum, known under the general name garlic, is a member of Alliacea family. The unique organosulfure compounds in the garlic determine the fragrance, taste, and

potent biological benefits of the plant. The fresh garlic bean contains large amounts of gamaglutamin systein, alliin, and oxidation products. However, excessive garlic consumption (more than 1000 mg/kg body weight, based on the World Health Organization) may have side-effects such as strong garlic-scented breath, vomiting, flux, indigestion or hypersensitive reactions, or blood coagulation disorders [3].

Recently, there has been extensive interest in the short-term and long-term benefits of herbal products along with regular exercise training (resistance or endurance) on inflammatory/oxidative conditions in various statuses [4—10]. Koseoglu et al. reported an improvement in cell membrane damage indices and serum TAC increase after short-term supplementation of garlic in healthy subjects [7]. Also, Morihara et al. showed stress oxidative attenuation in Wistar rats after a short-term administration of garlic and endurance training [8]. In addition, Seo et al. highlighted the effect of 12 weeks of regular endurance and resistance training and 80 mg/daily garlic administration on MDA in menopausal women [10]. Moreover, as reported by Norouzian et al., a significant increase occurred in serum TAC and MDA after eccentric exercise in young girls [11].

The biological effects of potent oxidant components in the human body are controlled by antioxidant agents. The disruptions in organ functions may be related to the reactions between ROS and cell membranes. It has been documented that cell membrane lipids are the main targets for oxygen radicals. Some reports support the notion that peroxides play a vital role in the atherosclerosis progression. Human body tissues, e.g., erythrocytes, include significant antioxidants as glutathione peroxides and glutathione. Thus, intrinsic erythrocytes are resistant to oxidative injuries [12].

Thus, regarding the mentioned investigations, it is obvious that the training protocols utilized in previous studies were endurance, resistance, or interval trainings. To the best knowledge of the current researchers, the effects of combined training along with garlic on stress oxidative markers have not been explored yet. Thus, given the lack of studies on complementary effect of garlic supplement along with combined training (endurance-resistance), and in order to elucidate the impact of combined training alone on antioxidant markers, the researchers decided to follow a two-fold purpose: Whether eight weeks of combined training (endurance-resistance) and garlic administration can significantly change oxidative stress and antioxidant defiance in untrained boys and whether there are any significant differences related to training and supplementation between garlic + train, placebo + train, garlic, and control groups.

2. Materials and methods

2.1. The study design

In this quasi-experimental study, the statistical population included all the boys in Zahedan Youth Detention Center (age range: 15—25 years). From this population, 46 boys volunteered to participate in the research and were assigned randomly into four groups ($n = 13$ in each training group and $n = 10$ as per the garlic and control groups). The participants were first presented with the aims and process of the research and were subsequently asked to fill out and sign the informed consent form. In order to avoid any interrupting factors that might affect the study outcomes, the participants were checked in terms of well-being, medication use, and suffering from diseases before the study initiated. A medical health questionnaire was filled out by a physician for each participant. Subjects in the control and garlic groups were asked not to take part in any physical activity during the intervention period. The study's protocol was reviewed by the Research Ethics Committee of Sport Sciences Research Institute and was approved concerning compliance with Ethical Standards in Research of the Iranian Ministry of Science, Research and Technology with the identifier IR.SSRI.REC.1395.123.

2.2. The supplement intake procedure

Subjects in the garlic and train + garlic groups received 250 mg (the quantity used may be generalized to realistic every day life) garlic capsules (produced by Amin Co., Isfahan) on a daily basis, while the train + placebo group received 250 mg starch powder capsules (produced by Amin Co., Isfahan) in the same manner for 8 weeks, 7 days/week, after breakfast with a glass of water. All the garlic and placebo capsules were distributed by the same person.

2.3. The combined training protocol

Eight weeks of combined training included resistance and endurance exercises three times/week. Each training session started with a 10-min general warm-up followed by resistance and endurance exercise and a 5-min cool-down at the end of each session [13].

2.4. The endurance training protocol

The endurance-training regimen was administered according to the protocol presented in Table 1 [14], and the intensity was monitored in terms of maximum heart rate using heart rate Telemetry (Polar, Finland).

2.5. The resistance training protocol

The resistance training program included bench press, legs, shoulders, squat, and armpit, which were performed in a circular manner. In the first week of training, exercises with

Table 1 Endurance training protocol.

Week	Total time, each session (min)	Intensity (HR_{max}%)
First	15	60—65
Second	15	60—65
Third	20	65—70
Fourth	20	65—70
Fifth	25	70—75
Sixth	25	70—75
Seventh	30	75—80
Eighth	30	75—80

Table 2 Comparison of demographic characteristics at baseline.

Variables	Garlic + train M ± SD	Placebo + train M ± SD	Garlic M ± SD	Control M ± SD	P value (One-way ANOVA)
Age (year)	2.29 ± 18.62	1.77 ± 18.84	1.64 ± 17.60	1.60 ± 20.10	0.13
Height (m)	6 ± 165.64	6.66 ± 161.54	6.98 ± 163.40	6.17 ± 167.10	0.23
Weight (kg)	5.21 ± 56.17	9.78 ± 61.77	8.01 ± 57.53	7.61 ± 57.53	0.34

Data are shown as mean ± standard deviation; ANOVA: analysis of variance.

Table 3 Indicators changes in four groups according to Two-ways ANOVA test.

Variables	Change source		
	Time × group effect (*P* value)	Group effect (*P* value)	Time effect (*P* value)
Malondialdehyde (MDA) (nmol/ml)	*0.001	*0.002	*0.01
Total antioxidant capacity (TAC) (μ/ml)	*0.001	*0.03	*0.001

* p<0.05 is significant.

40% to 50% one repetition maximum (1RM) were carried out. The exercises increased from 50% to 55%, 55% to 60%, 60% to 65%, 65% to 70%, 70% to 75%, 75% to 80%, and 80% to 85% 1RM for the second, third, fourth, fifth, sixth, seventh, and eighth weeks, respectively [15].

2.6. Biochemical analyzing

Blood samples were collected after 10 h in fasting state at 7 a.m. the day before the protocol started in the pre-test stage and 48 h after the last training session at the end of the 8-week intervention in the post-test stage. Blood was taken from the right arm of each participant while the subject was sitting in a relaxed manner. About 5 ml of blood was taken and the serum was separated immediately using a centrifugation speed of 3000 rpm for 5 minutes. The blood samples were kept at − 70°C for further analysis. The selected antioxidant and oxidative stress indicators (MDA and TAC) were measured in a specialized laboratory using ELISA method. The MDA ELISA kit made in China-USA with a sensitivity of 0.22 nmol/ml, and the Human TAC kit is made in China-USA with a sensitivity of 0.03 unit/ml.

2.7. The statistical analysis

After the normal distribution of the data confirmed, using the Kolmogorov—Smirnov test, the mean within-group changes of the variables were assessed using paired t-test. To analyze between-group changes of variables (pre-post-test), one-way ANOVA was applied. Data were analyzed by SPSS version 20 and the significance level was set at $P < 0.05$.

3. Results

According to Kolmogorov—Smirnov test, the data were distributed normally. The demographic characteristics of participants are summarized in Table 2. There was no significant difference in pre-test values of demographic characteristics between groups. Comparison of between-group and within-group differences using two-way Anova displayed in Table 3 which shows there is a time effect for both indicators, as well as group effect while time × group effect is also significant (Table 3). Based on the results of LSD post-hoc test, MDA mean changes decreased significantly compared to pre test in the train + garlic ($P < 0.001$), (−13.09%), garlic ($P = 0.001$), (− 20.58%), and train + placebo ($P = 0.01$), (− 8.06%) groups, whereas no difference was found in the control group ($P = 0.33$), (4.34%). Parallel to this result, TAC mean changes increased significantly in the train + garlic ($P = 0.01$), (19.29%), garlic ($P = 0.005$), (23.91%), and train + placebo ($P = 0.02$), (17.01%) groups, while no difference occured in the control ($P = 0.90$), (− 0.5%) group (Figs. 1 and 2). We can speculate from percentage changes (%) of mentioned indices that however the effect of intervention is significative but the magnitude is not very powerful.

The results of LSD post hoc test for between group changes showed that mean MDA changes in the garlic group ($p = 0.04$) was significantly lower than control, moreover MDA decreased significantly in train + garlic group ($p = 0.03$) compared to control. In addition, the mean changes in TAC was significantly higher in garlic ($p = 0.01$) compared to control and TAC was higher in train + garlic ($P = 0.01$) rather than control.

4. Discussion

The results of this randomized trial demonstrated that combined training and garlic administration were beneficial in reducing oxidative stress, as the experimental groups train + garlic, train + placebo, and garlic had reduced MDA and increased TAC levels. Our results also indicated that there was a significant difference between train + garlic and control as well as garlic and control groups in terms of both MDA and TAC.

The related literature reports a lower MDA level after garlic consumption and exercise training [10,16—20]. In

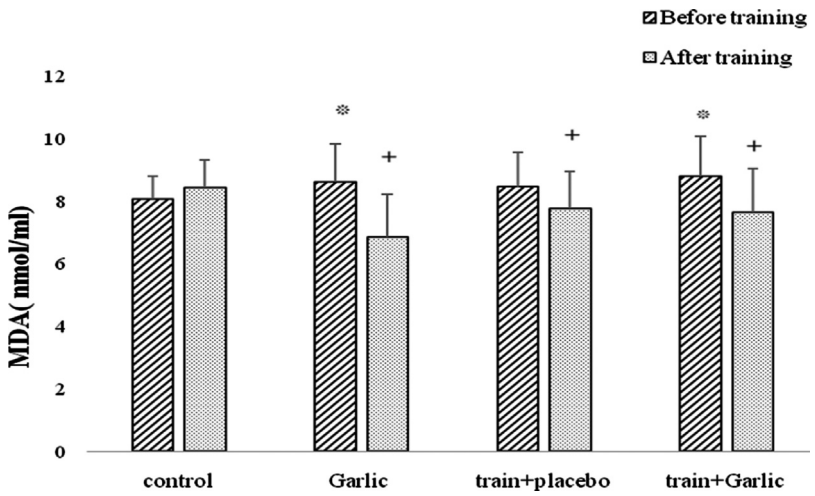

Figure 1 Comparison of mean malondialdehyde (MDA) between groups *significant difference with control group + significant difference with pre test levels.

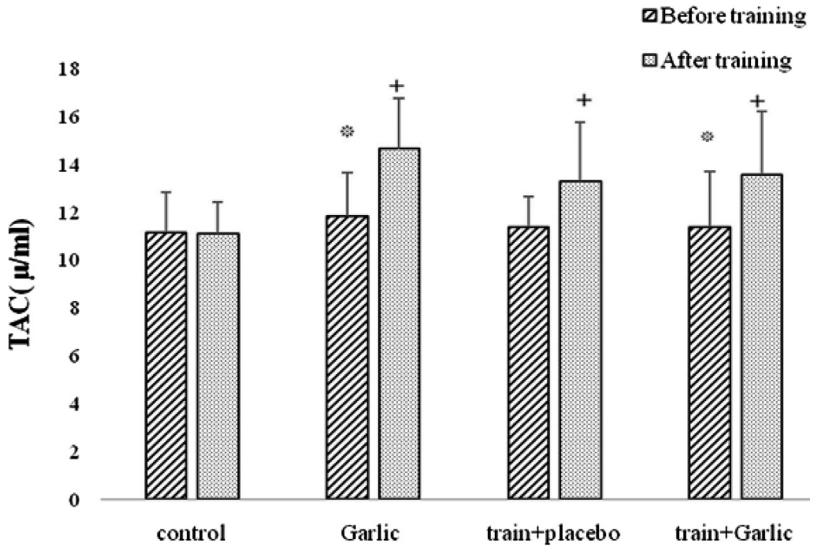

Figure 2 Comparison of mean total antioxidant capacity (TAC) between groups. *significant difference with control group. + significant difference with pre test levels.

Radak et al.'s study, MDA level decreased significantly after aerobic and anaerobic training together with garlic administration. Moreover, Rostami et al. showed that 8 weeks of resistance and endurance training with or without garlic supplementation improved MDA contents in metabolic syndrome Wistar rats. Nevertheless, endurance training + garlic was more effective than resistance training + garlic on MDA levels. Also, in another study, Seo et al. reported that 12 weeks of regular endurance and resistance training and 80 mg/daily garlic administration decreased serum MDA concentrations in menopausal women. In addition, a research performed by Cakir et al. exhibited the effectiveness of long-term (6 weeks) resistance training on MDA contents. The ischemia-reperfusion process exacerbates active oxygen specious production and counter the muscle to damage as a result of tissue membrane unsaturated lipids [21].

On the contrary, adaptation to regular training improves lipid peroxidation indices levels [22,23]. This is probably the underlying mechanism of MDA reduction in training groups. On the other hand, in vivo studies including intensive acute and chronic [25,26] sprinting protocols, significantly increase serum MDA levels. This increment may be due to the short duration and acute nature as well as the speedy and high-intensity exercise protocol in studies with opposing findings. Another investigation similar to the present protocol duration (8 weeks) demonstrated that the endurance training alone increased serum MDA level [23]. Different training protocols (combined vs. endurance) and subjects underlied the diversity in results. Researchers have shown that intensive exercise training elevates serum MDA level as a lipid peroxidation index [24], while the intensity used in the mentioned training was not high, hence improved MDA level. It should be noted that regular, moderate-intensity exercise may be a tool to amplify the antioxidant defense system [23,26]. Moreover, no change in serum MDA in response to short-term and long-term training and 14 days

of garlic supplementation was observed in cardiac patients (40—50 years) in two investigations [27,22]. Dissimilarity in training protocols, duration of garlic consumption, and participants' health state underlied the inconsistencies. Here, we explored both endurance and resistance training mechanisms on oxidative stress markers.

In relation to TAC, parallel to MDA results, TAC-associated benefits were also observed in the experimental groups (MDA level decreased and TAC level increased). The combined training and garlic administration regimen is an anti-oxidative maneuver, which explains the homologous adaptation of enzymatic antioxidant defense against oxidative stress created in the body. Our results are consistent with findings that show a significant enhancement in the circulation TAC contents after aerobic training alone or along with garlic administration [16—20,28—31], resistance training alone or together with garlic consumption [17,29], and interval training [16], where the basal oxidative stress is reduced and organism antioxidant capacities are fortified to perform physical activity for a longer time. Rostami et al. reported that eight weeks of resistance and endurance training with or without garlic (500 mg/kg) increased TAC concentrations in syndrome metabolic Wistar rats. Of course, the endurance training + garlic was more effective on TAC than resistance training + garlic. It has been suggested that tissues probably grow a typical adaptation to counteract oxidative stress due to MDA addition during two months of training, such that the TAC level increases significantly [32].

In contrast to the present findings, some experimental studies have indicated no change [22,27] or reduction [24] in TAC levels. The reason for these discrepancies with Bloomer et al.'s and Williams et al.'s findings may be related to subjects and diseases, where the more severe the disease, the lower the basal antioxidative power [22]; moreover, the garlic consumption duration in both studies are different from that of the current study (8 weeks vs. 14 days). Besides, in a study by Liu et al., TAC decreased while MDA increased after one week of high-volume resistance training. The compressed training duration, protocol differences (combined training in the present study vs. resistance training in Liu et al.'s), and non-administration of garlic may be the reasons for contradiction. Okada et al. implemented a detailed study on the antioxidant activity of allicin, one of the main thiosulfinates in the garlic, indicating that the antioxidant activities of allicin may be generated by the inhibition of peroxyl radical chain and the transference of allylic peroxides from the main substance. In other words, allylic hydrogen atom of the allicin is responsible for the antioxidant activity.

Based on the literature, it has been stated that the ROS production process during the endurance training is different from ROS production paths in the resistance training. The underlying reasons may be the inflammation related to cell membrane damage existing in the resistance training on the one hand and the ischemia process accelerating the free atoms' formation on the other hand [24,33]. Thus, the present researchers suggest the combinatory effects of resistance and endurance training as an optimum protocol for oxidative stress improvement and even its combination with garlic antioxidant supplement in order to make faster adaptations in respiratory chain proteins and probably inhibition of electron leakage in an indirect manner. However, in the present prob the direct role of protection by Galric on ROS production and tissues and mitochondrial respiratory rates of NADH and $FADH_2$ were not determined according to some limitations. Although some documents previously demonstrated the protective effect of garlic on mitochondria electron leakage is through removing excess H_2O_2 and reducing total ROS [34—36]. As mentioned earlier, serum TAC and MDA improved more in the train + garlic and garlic groups than the control group. Meanwhile, no differences were found between the experimental groups since in all the three experimental conditions, there was at last one antioxidant factor (training or supplement).

5. Conclusion

The eight-week combined training (endurance-resistance) per se or together with garlic supplementation improved TAC and MDA levels in untrained boys. These findings demonstrate the efficient role of combined training and the antioxidant supplementation of garlic in establishing antioxidant balance in untrained boys.

Disclosure of interest

The authors declare that they have no competing interest.

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