AGE SUPPLEMENTATION MODULATES IL-6, IL-10 AND HSP27 RESPONSE IN HEALTHY MEN: EFFECT ON THE MUSCLE STRENGTH

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SUMMARY

Aged garlic extract (AGE) is a dietary supplement and is reported have numerous health benefits including protection against oxidative stress, inflammatory, and dyslipidemia. The purpose of this investigation was to test the effects of AGE on the serum levels of interleukin-6 (IL-6), interleukin-10 (IL-10), heat shock protein 27 (HSP27), testosterone, cortisol, total antioxidant status (TAS), total oxidant status (TOS) as well as exercise performance parameters. Sedentary healthy men (n=6) participated in this study. After baseline cycling performance and isokinetic muscle contraction testing, the subjects ingested AGE (5 ml) for 10 days. At the end of this period, venous blood samples were obtained in the morning prior to and 10 days after AGE supplementation, followed by another sample 10 days after the supplementation (wash out) period. The serum was analysed for IL-6, IL-10, HSP27, cortisol, testosterone, total antioxidant status (TAS), and total oxidant status (TOS). The serum samples were analysed and physical performance evaluated at the beginning of the study, end of AGE supplementation and at the end of the washout period. Cycling performance parameters were not affected by AGE supplementation, whereas isokinetic muscle strength properties improved in the post-supplementation period. AGE upregulated serum level of the IL-6, IL-10 and HSP27 response in the post-supplementation period. However, serum level of the cortisol, testosterone, total antioxidant status (TAS), total oxidant status (TOS) were not affected by AGE supplementation. Therefore, AGE supplementation enhanced isokinetic knee muscle work parameters and it may play a protective role against the oxidative stress and apoptosis by upregulating the levels of HSP27, IL-10 and IL-6.

Key words: Aged garlic extract, Oxidative stress, Antioxidants, Muscle strength

Introduction

Aged garlic extract (AGE) is manufactured from organically grown garlic cloves (*Allium sativum* L.) that are sliced and soaked in an aqueous ethanol solution and extracted and aged up to 20 mo. Numerous compounds have been detected in AGE that have the potential to affect immunity, including the lectin family, which is known to interact with pathogen recognition receptors on immune cell surfaces (Huysamen, Brown 2009; Kingeter, Lin 2012). Previous studies have demonstrated that AGE reduces lipid cholesterol, oxidative stress, blood pressure, and improves endothelial function (Moriyama et al. 2011, Ried et al. 2010, Lee et al. 2009)

IL-6 is a key cytokine featuring redundancy and pleiotropic activity (Kishimoto, 1989). It plays a central role in host defence against environmental

stress such as infection and injury. Also, IL-6 acts as a major proinflammatory mediator for the induction of the acute phase response (Fattori et al., 1994), leading to a wide range of local and systemic changes including fever, leucocytes recruitment and activation, hepatic regeneration and hemodynamic effects. Considering the key role of IL-6 in mediating the acute phase response, its value as a prognostic biomarker in sepsis and various acute organ injuries has been extensively investigated in clinical and experimental studies. IL-10 is known to be a pleiotropic and potent anti-inflammatory and immunosuppressive cytokine. The expression of IL-10 is controlled on the transcriptional and post-transcriptional levels. Both direct (e.g., inflammation of the CNS through trauma, neurosurgery, or increased intra-brain pressure) and indirect (e.g., bacteremia) activation of the stress axis promotes IL-10 secretion. Oxidative stress in kid-

neys was associated with decreased IL-10 concentration. IL-10 has an important role in the prevention of infection-related tissue damage (Arimoto et al. 2007). Increased IL-10 concentrations have been shown to protect against damage to tissues, including diabetic wounds, ischemic stroke, and myocardial remodeling (Kanazawa et al., 2015; Kant et al., 2014;). Hsp27 is ubiquitously expressed and involved in the regulation of main cellular physiologic functions (Mymrikov et al., 2011) as inhibition of apoptosis, protection against oxidative stress, binding erroneously folded proteins for transfer to ATP-dependent chaperones and to the proteasome for further degradation and the regulation of the cytoskeleton (Mymrikov et al., 2011).

Many studies have already been shown the association of cardiovascular functions of aged garlic have cardiovascular protective effects on many types of cardiovascular disorders (Ried et al., 2016; Pérez-Torres et al., 2016; Avula et al., 2014; Takashima et al., 2017). However, the effects of AGE on pro-inflammatory cytokine (IL -6, IL-10) and HSP response as well as physical performance parameters have not been clearly defined in humans

Methods

Participants and procedure

Six sedentary healthy male volunteers (Mean age 30.2 ± 2.9 year, height 175.6 ± 2.8 cm) participated in this study. The study complied with the principles of the Declaration of Helsinki and was approved by the School of Medicine Balikesir University Ethics committee and informed written consent was obtained from each participant (Ethic prot. number 2014/61). The participants who informed that they were taking any medicine, had any medical history of disease, taking any antioxidant or other dietary supplementation, had a history of allergy to supplementation, or any injuries were excluded from the study. Body composition parameters namely BMI, percentage of fat percent, fat mass and fat free mass measured by using the Tanita BC418 MA (Tanita Corporation, Japan).

Physical performance test

Isokinetic knee extensor and flexor muscles performance assessments were assessed using

by isokinetic dynamometer (Isomed 2000 Basic). Participants performed a maximal incremental test on acycle Simulator (Tacx Vortex T1960, Tacx,

Wassenaar, Netherlands) that included a 5-min warm-up at a PO corresponding to 100 W, followed by increments of 30 W every 3 min until voluntary exhaustion as judged by the participants not being able to maintain the pedal frequency between 80–90 revolutions (rpm) was reached. Then, they were performed to cycle a distance of 10 km with free RPM, and were instructed to complete this distance in the shortest possible time.

Biochemical Analysis

Plasma levels of IL-6, IL10 and HSP27 level (eBioscience, Austria) were determined

by enzyme-linked immuno-sorbent assay (ELISA) using on a diagnostic instrument (BioTek, ELx 800, U.S.A). The levels of testosterone and cortisol hormone were measured on diagnostic instrument. Total antioxidant (TAS) and oxidative (TOS) activity was measured by spectrophotometry.

AGE supplementation

The supplementation with AGE was carried out essentially as described previously by Dillon et al. (Dillon et al., 2002). The AGE is prepared by soaking sliced raw garlic (*A. Sativum*) in 15–20% aqueous ethanol for up to 20 min at room temperature. The extract is then filtered and concentrated under reduced pressure at low temperature. The content of water-soluble compounds is relatively high, whereas that of oil soluble compounds is low. After the baseline test, subjects consumed 5 mL of AGE (taken in a small volume of fruit juice) daily for 10 d between 0700 and 0900 h; otherwise, subjects followed their usual diet and lifestyle, excluding alcohol intake. This dose of AGE is that recommended as a dietary supplement by the manufacturers and was used in previous study, which showed inhibition of ADP-induced platelet aggregation (Vang et al. 2011).

Results

AGE upregulated serum level of the IL-6, IL-10 and HSP27 response in the post-supplementation period ($p=0.05$, $p=0.04$, $p=0.001$, respectively, Table 3). However, these levels of the interleukins were declined in the washout period of the experiment. The serum level of the cortisol, testosterone, total antioxidant status (TAS), total oxidant status (TOS) were not affected by AGE supplementation ($p > 0.05$). Isokinetic knee extensor and flexor muscle function of the peak work, peak work/

body weight, average work, total work parameters were increased in the post-supplementation at 60 and 180°/sec velocity ($p \leq 0.001$, table 2). Whereas, cycling performance parameters were not affected by AGE supplementation. Interestingly, ten day garlic administration significantly elevated the body weight ($p=0.011$), BMI ($p=0.012$), fat percent ($p=0.013$), fat mass ($p=0.003$) in the washout with compared the post supplementation period, however AGE supplementation had no effect on fat free mass (Table 1).

Discussion

Our primary findings indicate that 10 days of dietary garlic supplementation significantly upregulated plasma level of IL-6, IL-10 and HSP27 response in the post-supplementation, also affected on isokinetic knee concentric extensor and flexor muscle work properties in both velocity at 60 and 180°/sec in sedentary human subject. This is the first study investigating the effects of AGE supplementation on IL levels, HSP response and isokinetic muscle contractile properties in healthy human subjects. Therefore, these results are novel and to the best of our knowledge, no data exists concerning the acute effect of AGE administration on these parameters. In literature, limited studies have been reported on anti-fatigue effect using garlic supplementation in animal or patients (Ushijima et al., 1997). According to this study results have shown that AGE significantly improved the running time in rat (Ushijima et al., 1997). Also, one study has been reported that six week AGE oil supplementation reduced the heart rate at peak exercise and the work load of the heart in cardiac patients (Verma et al., 2005).

We also found that AGE upregulated IL-6–10 and HSP response in the post-supplementation period in this study. As far as we are aware this finding has not been reported before and IL-6 and IL-10 is a cytokine and acts on a variety of tissues, and exhibits both pro-and anti-inflammatory prop-

erties (Starkie et al., 2003). In our study, the plasma level of the IL-6, IL-10 indicates a physiological level, although these levels were upregulated in the post-supplementation period. It has been well documented that the exercise induces plasma IL-6 levels which exerts inflammatory properties (Ostrowski et al., 1999) and also plays an anti-inflammatory roles within the body (Villarino et al., 2004). Additionally, IL-10 has an important role in the prevention of infection-related tissue damage (Arimoto et al. 2007). Increased IL-10 concentration has been shown to protect against damage to tissues, including diabetic wounds, ischemic stroke, and myocardial remodeling (Kanazawa et al., 2015; Kant et al., 2014). Furthermore, Hsp27 is also ubiquitously expressed and involved in the regulation of main cellular physiologic functions (Mymrikov et al., 2011) as inhibition of apoptosis, protection against oxidative stress, binding erroneously folded proteins for transfer to ATP-dependent chaperones and to the proteasome for further degradation and the regulation of the cytoskeleton (Mymrikov et al., 2011).

Conclusion

AGE enhanced muscle work properties in both flexor and extensor muscle group than the torque values in untrained healthy human knee. Furthermore, AGE was increased the, IL-6, IL-10 and HSP27 level in the post-supplementation period. Therefore, AGE supplementation, it may be played a protective role against the oxidative stress and apoptosis with up regulated the level of some of the interleukins and HSP27 in this study. Limitations to the present study include: 1) daily nutrition level and metabolism was not followed and may have had a significant effect on the muscle strength values. 2) This was a pilot study in which six participated in the study and it will need to be investigated in future studies.

TABLES

Table 1. Effect of garlic supplementation on physical characteristics of the subjects.

	<i>Pre-Supp Test 1</i>	<i>Post-Supp Test 2</i>	<i>Washout Test 3</i>	<i>F</i>	<i>P</i>	<i>Significant difference</i>
Weight (kg)	73.86 (5.52)	73.76 (5.68)	75.10 (5.85)	8.48	0.011	Test 2-3
Body Mass Index (kg/m ²)	24 (1.92)	23.98 (1.94)	24.52 (1.95)	8.08	0.012	Test 2-3
Fat Percent (%)	11.18 (3.95)	12.10 (3.70)	14.02 (3.17)	7.80	0.013	Test1-3
Fat Mass (kg)	9.02 (3.71)	9.70 (3.58)	11.22 (3.41)	13.80	0.003	Test 1-3
Fat Free Mass	64.8 (5.51)	64.1 (5.69)	63.8 (6.12)	1.44	0.290	

Values are expressed as mean (SE), (n=6). A significant difference was found by repeated measures by ANOVA. Significant differences were observed between post-supplementation and washout peri-

od in experimental sessions.*P<0.05, **P<0.001. Pre-Supp: Pre-supplementation, Post-Supp: Post supplementation.

Table 2. Effect of garlic supplementation on knee extensor and flexor muscle function of the subjects at 60 and 180° /sec velocity

<i>Extensor muscle function</i>						
	<i>Pre-Supp Test 1</i>	<i>Post-Supp Test 2</i>	<i>Washout Test 3</i>	<i>F</i>	<i>P</i>	<i>Significant difference</i>
Peak work (J)	132.8 (8.32)	110.4 (6.02)	108 (5.82)	7.74	0.01	1-2, 1-3
Peak work/weight (J/kg)	1.81 (0.30)	1.52 (0.27)	1.47 (0.31)	9.91	0.007	1-2,1-3
Average work (J)	124.4 (7.90)	100 (6.67)	97.5 (6.57)	10.55	0.006	1-2
Total work (J)	746.8(47.55)	599.2(40.18)	584.8 (39.5)	10.50	0.006	1-2
<i>Flexor muscle function</i>						
Peak work (J)	90 (5.85)	119.6 (17.25)	119.6 (6.32)	63.94	0.000	1-2, 1-3
Peak work/weight (J/kg)	1.30 (0.25)	1.66 (0.40)	1.62 (0.35)	11.75	0.004	2-3
Average work (J)	124.4 (7.90)	100 (6.67)	97.58 (6.57)	29.92	0.002	1-2, 1-3
Total work (J)	463.8 (33.23)	639 .8 (39.4)	646 (29.68)	28.74	0.000	1-2, 1-3

<i>Extensor muscle function</i>						
	<i>Pre-Supp Test 1</i>	<i>Post-Supp Test 2</i>	<i>Washout Test 3</i>	<i>F</i>	<i>P</i>	<i>Significant difference</i>
Peak work (J)	182.2 (8.06)	160.6 (4.68)	157.2 (6.31)	18.92	0.001	1-2, 1-3
Peak work/weight (J/kg)	2.50 (0.38)	2.21(0.36)	2.13 (0.40)	26.42	0.000	1-2,1-3, 2-3
Average work (J)	170.5 (7.4)	149.5 (3.4)	146.7 (5.9)	13.36	0.003	1-2, 1-3
Total work (J)	1022 (45)	897 (20)	880 (34)	13.16	0.003	1-2
<i>Flexor muscle function</i>						
Peak work (J)	125.8 (7.2)	157.8 (8.5)	153.6 (10.4)	50.5	0.000	1-2, 1-3
Peak work/weight (J/kg)	1.74 (0.40)	2.17 (0.42)	2.08 (0.44)	22.97	0.009	1-2,1-3
Average work (J)	116.4 (6.4)	147.9 (15.8)	142.7 (9.7)	23.13	0.009	1-2, 2-3,3-1
Total work (J)	698 (38.2)	886 (42.5)	856 (58.5)	23.94	0.008	1-2, 1-3 2-1, 3-1

Values are expressed as mean (SE) (n=6). A significant increase in the post-supplementation period according to Pre-Supp P<0.05, P<0.001, anal-

ysed by repeated measures by ANOVA. Pre-Supp: Pre-supplementation, Post-Supp: Post supplementation.

Table 3. Effect of AGE supplementation on biochemical parameters

	Pre-Supp (Test 1)	Post-Supp (Test 2)	Washout (Test 3)	F	P	Significant difference
IL10 (pg/ml)	3.1 (0)	60.2 (37.69)	44.1 (16.66)	2.49	0.04	Test 1-2
IL6 (pg/ml)	5.66 (0.15)	6.14 (0.35)	5.51 (0.64)	11.11	0.05	Test 2-3
HSP27 (pg/ml)	1549.7 (472.2)	5263.1 (800)	724.3 (120.15)	11.11	0.001	Test 1-2 Test 2-3
Testosterone (pg/ml)	751 (115.7)	614.6 (115.2)	616.4 (88.6)	0.55	0.58	
Kortizol (pg/ml)	10426 (174)	10127 (455)	9560 (784)	0.77	0.47	
Total Antioksidan seviye (mmol/L)	1.7 (0.76)	1.82 (0.08)	1.75 (0.11)	0.43	0.65	
Total Oksidatif seviye (mmol/L)	6.36 (0.41)	7.27 (0.43)	6.51 (0.38)	1.26	0.31	

Values are expressed as mean (SE) (n=6). A significant increase in the post-supplementation period according to Pre-Supp $P < 0.05$, $P < 0.001$, analysed by repeated measures by ANOVA. Pre-Supp: Pre-supplementation, Post-Supp: Post supplementation.

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