

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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High-Genistin Isoflavone Supplementation Modulated Erythrocyte Antioxidant Enzymes and Increased Running Endurance in Rats Undergoing One Session of Exhausting Exercise – A pilot study

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Abstract: Genistein putatively acts as an antioxidant *in vitro*. To investigate the *in vivo* antioxidative activity of genistein, forty-eight male rats were divided into four groups and fed diets with or without 598 mg isoflavone extract per kg of diet for four weeks. On the final day of the study, twenty-four rats were exercised to exhaustion (22 meters/minute at 10% inclination on the treadmill) and then all the rats were sacrificed. The high-genistin isoflavone extract (HGI) diet significantly increased the running time (GE vs. CE: 54 vs. 48 min) and genistein concentrations in the plasma, liver, and gastrocnemius muscle (GE vs. GS: 730.3 vs. 348.5 ng/ml, 529.3 vs. 216.9, and 59.0 vs. 24.9 ng/g, respectively). Exercise doubled genistein concentrations in all tissues and significantly enhanced liver malondialdehyde (MDA). HGI supplementation did not prevent the increase of MDA; instead, it substantially increased MDA levels in muscle tissue (HGI vs. control: 0.46 vs. 0.29 mg/kg). HGI supplementation also maintained the activities of catalase and glutathione peroxidase (GPx) decreased due to exercise (GE vs. GS: 0.113 vs. 0.101 unit/g RBC protein and 0.412 vs. 0.403 $\mu\text{mol/s/mg}$ protein, respectively). It can be concluded that even though HGI modulates erythrocyte antioxidant enzymes against oxidative stress and increases endurance capacity, the supplemented level of HGI does not seem to be optimal for defending the liver and skeletal muscles against oxidative stress.

Key words: antioxidant enzymes, exercise, genistein, malondialdehyde, oxidative stress

Introduction

The role of exercise and physical activity in the prevention of chronic disease and promotion of optimal health has drawn the attention of the public (Singh, 1992). However, research on dietary intervention that protects body tissues from damage during vigorous exercise is in its infancy. This damage is mostly attributed to the sharply increased reactive oxygen species (ROS) in the body during exercise (Davies *et al.*, 1982 and Packer, 1997). Olinescu *et al.* (1995) reported that the increase of urinary excretion of peroxides demonstrated the presence of ROS during exercise. These highly reactive free radicals are known to cause damage to mitochondrial membranes and cytoplasmic structures through peroxidation of phospholipids, proteins, and nucleotides (Jenkins, 1993 and Packer, 1997). Fortunately, endogenous and exogenous antioxidant defense systems in the body can cope with ROS, including vitamin E, vitamin C, beta-carotene, and antioxidant enzymes (SOD, catalase, and GPx). However, an imbalance occurs when ROS, generated during exercise, overcome antioxidant defense systems, a state known as oxidative stress.

Generally, regular physical activity or participation in a sport will increase the total antioxidant capability of the body (Powers *et al.*, 1999). According to Ji (1995), the activities of antioxidant enzymes provide the first line of defense against ROS increase in the heart, liver, lung, blood platelets, and skeletal muscle, in order to cope with oxidative stress induced by acute or exhausting exercise. However, it is possible that acute or irregular participation in exercise will result in oxidative stress due to the elevated use of antioxidants during the defense against ROS. Therefore, more investigations have

focused on whether dietary antioxidant supplementation will boost the antioxidant defense systems and overcome oxidative stress. Goldfarb *et al.* (1994) found that rats fed a 250 IU vitamin E/kg diet for five weeks had lower thiobarbituric acid reactive substance (TBARS) and lipid peroxide levels in plasma and leg muscles after one hour of treadmill exercise, than rats fed a control diet. This finding suggested that antioxidant supplementation in humans and animals may be needed to protect tissues against ROS attack induced by exercise.

Since isoflavones from soy clearly exhibit antioxidant activity *in vitro* and *in vivo* (Kurzer and Xu, 1997) and Brandi (1997) surmised that isoflavones in soy might be responsible for the beneficial effect of lowering the incidence of diseases in Asians. There are many varieties of isoflavones found in soy protein. Genistein and daidzein, and their corresponding glucose conjugated forms, genistin and daidzin, account for the majority of isoflavones in soy.

Isoflavones have a chemical structure similar to estrogen, which has been reported to have a weak antioxidant activity because of the hydroxyl group on its "A" ring in the same location as in vitamin E (Tiidus, 1995). In addition, isoflavones are effective antioxidants, because their phenolic rings have multiple hydroxyl groups that reduce peroxyradicals by donating hydrogen atoms (Tikkanen *et al.*, 1998). With the highest antioxidant characteristic among isoflavones, genistein has been shown to prevent LDL oxidation initiated by oxidizing agents *in vitro* (Bakhit and Potter, 1995; Kapiotis *et al.*, 1997) and to protect microsomal lipid peroxidation induced by a Fe_2^+ -ADP complex (Jha *et al.*, 1985). Additionally, Cai and Wei (1996) have observed that dietary administration of genistein (50 and 250 PPM) for 30 days significantly increased

the activities of antioxidant enzymes in small intestine and skin of SENCAR mice.

Most prior studies have used *in vitro* measures to evaluate the antioxidative ability of isoflavones. However, it is not completely understood if isoflavones can defend against ROS or boost up total antioxidant defenses *in vivo*. In order to initiate the maximum impact of oxidative stress on rats, one single exhaustive exercise, without exercise training, was given to rats in an exercised group. We hypothesized that exercise would initiate oxidative stress in the rats, further resulting in changes of erythrocyte antioxidant enzymes and increased products of lipid peroxidation. We also hypothesized that four-week HGI supplementation would modulate activities of erythrocyte antioxidant enzymes and decrease products of lipid peroxidation in tissues after the rats were exercised on the treadmill. The objective of this study was to investigate the *in vivo* antioxidant ability of the isoflavones, especially genistein, in rats undergoing acute exhausting exercise. One high dose challenge was used in this pilot study to assist in determining the range of dose supplementation in a subsequent dose response study.

Materials and Methods

This study was approved by the Virginia Polytechnic Institute and State University Institutional Review Board.

Animals:Forty-eight one-year-old Sprague-Dawley male rats were purchased from Harlan Industries (Indianapolis, IN). The rats were housed at 25°C with a 12-hr light/dark cycle with free access to feed and water throughout the study. Upon delivery to the Virginia Tech animal research facility, the rats were acclimatized on a chow diet for one week.

Experimental design: The rats were randomly divided into four treatments of equal number. The four treatments were control diet and exercise (CE), control diet and sedentary (CS), high-genistin isoflavone extract (HGI) diet and exercise (GE), and HGI diet and sedentary (GS). All semipurified ingredients were purchased from ICN (ICN Pharmaceuticals Inc., Costa Mesa, CA). The control diet was prepared according to the formula of the American Institute of Nutrition (AIN 93 M). The HGI diet was identical to control diet except that 598 mg isoflavone extract per kg diet was added to replace an equal amount of cornstarch. The composition of isoflavones added in this diet was 84.4% genistein, 14.8% daidzein, and 0.8% glycitein. Each rat's feed intake was measured daily throughout the study. All the rats were given the experimental diets for 28 days.

On the final day of the study, all the rats in the CE and GE groups were exercised vigorously in one session until they were exhausted. The exercise protocol was 22 meters/minute at 10% inclination on a treadmill (Exer-4/8 treadmill, Columbus, OH). In order to familiarize the rats with the treadmill, they all were trained to walk on the treadmill before the final acute exercise session. The protocol for the walking training was ten meters/minute for five minutes, twice a week, for the first three weeks. The treadmill was equipped with an electric shocking grid on the rear barrier to motivate the rats to exercise. A rat was considered exhausted when it would not be prompted to run any more. The rats would stop running and when overturned would continue to lie on their backs disregarding gentle prods and electric shock. The running time of each rat in the CE and GE groups was recorded.

Sample collections: Immediately after acute exercise, all the

rats were anesthetized using a halogen and nitrogen gas mixture and the maximum possible volume of blood was drawn via the heart puncture. Blood samples were immediately kept in ice. Plasma and red blood cells (RBC) were harvested following centrifugation at 1000 x g for 20 minutes at 4°C. After blood collection, the rats were sacrificed by cervical dislocation and the liver and legs were both removed. In order to diminish tissue exposure to heat and air, whole liver and legs were immediately wrapped in aluminum foil and instantly frozen in liquid nitrogen. All samples, including the liver, plasma, RBC, and muscle were stored at -80°C until further analyses.

Biochemical measurements: Genistein concentrations in the plasma, gastrocnemius muscle, and liver were determined by a HPLC method, modified from methods of Wang and Murphy (1994) and Xu *et al.* (1994). *In vivo* thiobarbituric acid reactive substances (TBARS) method was used to measure MDA concentrations in the liver and gastrocnemius muscle tissues (Pikul *et al.*, 1989). Before the analysis, whole liver and muscle tissues were gently thawed on the ice. Subsequently, gastrocnemius muscle was collected from whole leg via the dissection. One gram of liver or muscle tissue, 5 ml of 5% trichloroacetic acid, and 200 µl of 0.15 % butylated hydroxytoluene were homogenized for one minute. Following homogenization, the mixture was spun at 17,000 x g for 15 minutes at 4°C. One ml of supernatant was filtered by using 9 cm GF/C filter paper and small funnels, and then was mixed with one ml of 0.67% thiobarbituric acid (TBA) solution. The absorbance was read on a spectrophotometer (532 nm) within 1 hour after incubation in a 95°C water bath for 40 minutes. The concentration of the MDA product was calculated by comparison with a standard curve established from different concentrations of tetraethoxypropane. RBCs were used to measure the activities of antioxidant enzymes, SOD, catalase, and GPx. The activities of antioxidant enzymes were standardized by erythrocyte protein. Superoxide dismutase (EC 1.15.1.1) was determined by the method of Xin *et al.* (1991). The activities of GPx (EC 1.11.1.9) and catalase (1.11.1.6) were determined according to the methods developed by Agergaard and Jenson (1982) and Aebi (1983), respectively.

Statistical analysis: A two-way ANOVA was used to compare the effects of exercise and diet, and their interactions on the means of MDA concentrations in tissues and activities of antioxidant enzymes in the rats (Sokal and Rohlf, 1995). The significant level was set at $p < 0.05$. When the interactions between exercise and dietary supplementation were statistically significant, Tukey's HSD procedure at the 0.05 level experimentwise was used to compare group means. A Student's t-test was used to contrast mean genistein concentrations between the GE and GS groups and the running time between the NE and GE groups (Sokal and Rohlf, 1995). The computer software program JMP (SAS Institute Inc., Cary, NC) was used for all computations.

Results

Body weight and running ability: At the time of sacrifice, mean body weights among the CE, CS, GE, and GS groups were not significantly different, 545.3, 549.9, 583.8, and 544.8 gm, respectively ($n = 12$, $SE = 12.7, 20.2, 15.6,$ and 16.6 gm). High-genistin isoflavone supplementation did not affect body weight gain or feed intake. The average running time of the rats in the GE group, 54 minutes ($n = 12$, $SE = 2$ minutes), was significantly longer than that of the rats in the CE group,

Table 1: Genistein concentrations in plasma, liver, and gastrocnemius muscle in rats

Diet	Exercise level	n	Plasma (ng/ml)		Liver (ng/g)		Muscle (ng/g)	
			Mean	SE	Mean	SE	Mean	SE
HGI	exercised	12	730.3 ^a	70.6	529.3 ^a	96.7	59.0 ^a	6.1
HGI	sedentary	12	348.5 ^b	41.0	216.9 ^b	33.9	24.9 ^b	4.5
control	exercised	12	0 ^c	0	0 ^c	0	0 ^c	0
control	sedentary	12	0 ^c	0	0 ^c	0	0 ^c	0

^{abc}Means with different letters were significantly different, using a student's t-test, $P < 0.05$.

Table 2: Mean liver concentration of malondialdehyde by dietary supplement and exercise

Diet	Exercise level	n	MDA (mg/kg)	
			Mean	SE
HGI	exercised	11 [*]	0.640 ^a	0.111
HGI	sedentary	12	0.311 ^b	0.044
control	exercised	12	0.449 ^{ab}	0.071
control	sedentary	12	0.366 ^{ab}	0.062

^{ab}Means followed by the same superscript were not significantly different at the 0.05 level of significance experimentwise (Tukey's HSD, Sokal and Rohlf, 1995). Exercise effect was significant, $P = 0.008$.

^{*}One sample was deleted from statistical analysis because it was an outlier.

which was 48 minutes ($n = 12$, $SE = 1.4$ minutes) ($P < 0.05$).

Genistein concentrations: High-genistein isoflavone supplementation led to significant ($P < 0.001$, 0.001 , and 0.002 , respectively) increases in genistein concentrations in plasma, liver, and gastrocnemius muscle in sedentary rats (Table 1). After the rats underwent one session of acute exhausting exercise on the treadmill, all genistein concentrations in plasma, liver, and gastrocnemius muscle were significantly higher than those were in the sedentary rats. Exercise more than doubled the concentrations of genistein in plasma, liver, and gastrocnemius muscle (109, 144, and 137% increase, respectively) as compared to sedentary HGI fed rats. There was no significant correlation between genistein concentration and other biochemical parameters.

Products of lipid peroxidation: Malondialdehyde is an index of the extent of lipid peroxidation. MDA concentration in the liver and gastrocnemius muscle were approximated by the TBARS method.

According to the results of a two-way ANOVA (Table 2), the interactions between exercise and diet were not significant ($P = 0.10$) and the main effects of exercise led to significant increase of MDA concentrations in the liver ($P = 0.008$), while the main effects of diet did not ($P = 0.37$). Multiple comparisons of the means suggested synergy of exercise and HGI supplementation on the increase of liver MDA, but the relationship was weak, with HGI and exercise explaining only $R^2 = 20\%$ of the variation in the liver MDA concentration.

The two-way ANOVA revealed no significant interaction effects between diets and exercise levels ($P = 0.47$) on MDA concentrations in the gastrocnemius muscle, and one session of acute exhausting exercise did not result in a significant augmentation of MDA concentrations in rats fed control diet and HGI diet ($P = 0.75$). Yet, genistein supplementation caused a slightly significant increase of MDA ($P = 0.044$) in both sedentary and exercised rats (Table 3). Mean MDA concentration was 0.454 mg/kg ($n = 24$, $SE = 0.049$ mg/kg) with HGI supplementation, and was 0.322 mg/kg ($n = 24$, SE

$= 0.040$ mg/kg) with no supplementation.

Activities of antioxidant enzymes: Two-way ANOVA analysis showed that one session of exercise significantly increased SOD activity in rats fed control and HGI diets ($P = 0.037$); however, HGI supplementation led to an inhibition of SOD activity in rats ($P = 0.031$) (Table 4). There was no significant interaction between exercise and dietary supplementation on SOD activity ($P = 0.19$).

The main effects of HGI supplementation and exercise on catalase activity (Table 5) were not significant ($P = 0.42$ and 0.18 , respectively), while the interactions between diet and exercise were significant ($P = 0.003$). Comparing means by Tukey's HSD procedure, one session of acute exhausting exercise significantly decreased catalase activities in the rats fed the control diet compared to those in sedentary rats, but exercise did not result in a reduction in erythrocyte catalase activities in rats fed the HGI diet.

The interactions between diet and exercise were significant ($P = 0.018$) in GPx activities of RBC, and the main effects of exercise were significant ($P = 0.038$), but the main effects of diet were not statistically significant ($P = 0.12$) (Table 6). The mean activity of GPx in rats that underwent one session of acute exhausting exercise was 0.353 $\mu\text{mol/s/mg}$ RBC protein ($n = 24$, $SE = 0.018$ $\mu\text{mol/s/mg}$ RBC protein), and was 0.415 $\mu\text{mol/s/mg}$ RBC protein ($n = 24$, $SE = 0.026$ $\mu\text{mol/s/mg}$ RBC protein) in sedentary rats. One session of acute exhausting exercise significantly decreased GPx activities in RBC in rats fed the control diet, based on multiple comparisons of means by Tukey's HSD procedure. However, GPx activities in the GE rats did not change with the acute exhausting exercise.

Discussion

This study assessed the effects of dietary high-genistein isoflavone supplementation on immediate post-exercise indices of tissue oxidative damage and modulations antioxidant enzymes (SOD, GPx, and catalase) in erythrocytes. It is the first study to examine the antioxidant potential of isoflavones using an *in vivo* animal exercise model. It was shown that significant accumulation of genistein in the plasma, skeletal muscle, and liver occurred after dietary HGI supplementation for four weeks but this did not increase the antioxidative capability of the liver and skeletal muscle in these rats. Nor did an increased genistein concentration in the liver provide significant protection against the oxidative stress due to acute exhausting exercise. However, the HGI supplementation significantly extended the rats' running time to reach exhaustion on the treadmill. Furthermore, in regard to the antioxidant enzymes in erythrocytes, HGI, while it significantly prevented the decrease of GPx and catalase activities by exercise, did not significantly influence the activities of GPx and catalase in the sedentary rats. In addition, the HGI supplementation significantly decreased erythrocyte SOD activity in both exercised and sedentary rats.

The major isoflavones in soy are the conjugated forms genistin and daidzin, which are glycosylated genistein and daidzein,

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Table 3: Malondialdehyde (MDA) concentrations in the gastrocnemius muscle

Diet	Exercise level	n	MDA (mg/kg)	
			Mean ^a	SE
HGI	exercised	12	0.441	0.052
HGI	sedentary	12	0.468	0.086
control	exercised	12	0.356	0.066
control	sedentary	12	0.228	0.046

^aThere was a statistical difference due to diets, P = 0.044 (Tukey's HSD, Sokal and Rohlf, 1995).

Table 4: The activities of superoxide dismutase (SOD) in RBC in rats

Diet	Exercise level	n	SOD (unit/mg RBC protein)	
			Mean	SE
HGI	exercised	12	0.091 ^{ab}	0.011
HGI	sedentary	12	0.080 ^a	0.013
control	exercised	12	0.138 ^b	0.016
control	sedentary	12	0.092 ^{ab}	0.012

^{ab}Means followed by the same superscript were not significantly different at the 0.05 level of significance experimentwise (Tukey's HSD, Sokal and Rohlf, 1995).

respectively. It has been suggested that conjugated isoflavones derived from foods could not be absorbed from the small intestine (Hollman, 1997). Hydrolysis of the mostly beta-glucosidic bonds that attach isoflavones to sugars by the gut microorganisms is necessary for absorption of genistin (Hollman 1997; King and Bursill, 1998; and Zhang *et al.*, 1999). Significantly increased genistein concentrations in the plasma, skeletal muscle, and liver in rats fed at 500 mg genistin containing compounds per kg diet indicated that isoflavones from food supplementation could lead to a significant accumulation of genistein in tissues. However, genistein concentrations varied among each of sedentary rats fed the HGI diet. It has been suggested that high inter-individual variation of isoflavone bioavailability in humans may be attributed to existence of gut bacteria, which are necessary for the degradation of conjugated isoflavones into aglycone forms for absorption (Xu *et al.*, 1994 and Wiseman, 1999). The current results support such a suggestion in rats. It may be that degradation of conjugated isoflavones in the colon possibly plays a role in their bioavailability in rats.

Genistein concentrations in the plasma, skeletal muscle, and liver of rats fed the HGI diet doubled after acute exhausting exercise. Exercise can significantly enhance antioxidants in tissues such as vitamin E in plasma and erythrocytes (Pincemail *et al.*, 1988 and Vasankari *et al.*, 1997) and vitamin C in plasma (Maxwell *et al.*, 1993). Pincemail *et al.* (1988) hypothesized that exercise moved vitamin E from other tissues into the plasma and that skeletal muscle utilized circulating vitamin E for protection against oxidative damage. Like vitamin E, genistein might be mobilized from some tissues other than the liver and skeletal muscle because both the above tissues and plasma had significantly higher genistein concentrations in exercised rats than in sedentary rats in this study. Chang *et al.* (2000) reported the presence of genistein in the prostate gland, testes, thyroid gland, and brain of rats fed a genistein diet. Therefore, it can be hypothesized that genistein may be transported from these tissues to the plasma, liver, and muscle tissue. In addition to mobilization from storage sites, acute exhausting exercise might also decrease the clearance of genistein from the liver. Like endogenous

estrogen, glucuronidated genistein is excreted through urine and bile and undergoes enterohepatic circulation (Kurzer and Xu, 1997). Several reports have noted an increase in plasma estrogen concentrations following acute exercise in women (Bonen *et al.*, 1981). It has been suggested that this increase is primarily due to the decreased metabolic clearance of estrogen as a direct consequence of exercise induced reduction in hepatic blood flow during exercise (Sutton *et al.*, 1990). Therefore, the increased genistein concentrations in the plasma, liver, and gastrocnemius muscle may be attributed to a movement of genistein from other tissues and low clearance from the liver.

Genistein also has a structure similar to estrogen and has been suggested to have estrogenic and anti-estrogenic responses in mammalian tissues (Kurzer and Xu, 1997). Estrogens were suggested to act as antioxidants in a manner similar to vitamin E to terminate peroxidation chain reactions (Burton and Ingold, 1989), however, they were reported to act as prooxidants and elevate plasma TBARS concentrations in rats when administered in the form of pharmacological contraceptives (Kose *et al.*, 1993 and Pizzichini *et al.*, 1993). In the present study, 500 mg genistin-containing compounds per kg diet from isoflavone extract given to rats may be too high to act as an antioxidant. Thus, a significantly increased MDA concentration in the gastrocnemius muscle in rats fed HGI diet means that genistein at high levels may act as a prooxidant rather than an antioxidant. Unpublished data from our lab showed that 399-ppm HGI supplementation led to a slightly lower plasma MDA concentration measured by the TBARS method than 598-ppm HGI supplementation in rats.

Oxidative stress induced by acute exercise can significantly elevate markers of tissue peroxidative damage such as MDA, and possibly elevate tissue antioxidant enzyme activities (Davies *et al.*, 1982; Ji, 1995; Sen, 1995; and Somani and Arroyo, 1995) because physical exercise promotes the production of ROS due to a substantial increase in oxygen consumption (Davies *et al.*, 1982; Packer, 1986; and Ayres *et al.*, 1998). The present study showed that exercise at 22 meters/min at 10% inclination for 1 hour significantly elevated MDA levels in the livers of rats fed control and HGI diets. However, one session of acute exhausting exercise did not result in a significant accumulation of MDA in the gastrocnemius muscle. Ji and Fu (1992) also observed that exhausting exercise (20 meters/min and 0% inclination) did not significantly elevate the MDA level in muscle, but significantly increased it in the liver. Therefore, acute exercise might not lead to lipid peroxidation in all tissues equally. It is plausible to speculate that products of lipid peroxidation may be transported from the muscle into the circulation, and possibly to the liver.

One session of acute exhaustive exercise significantly increased MDA levels in the liver; however, HGI supplementation did not diminish the increase of MDA. Although genistein has been strongly suggested as an antioxidant *in vitro* and *in vivo* (Kurzer and Xu, 1997), in this study, genistein did not perform as an antioxidant in the liver. Further research is necessary to explore whether there is a dose-dependent response of isoflavones on increased liver MDA initiated by exhaustive exercise.

Every antioxidant, including vitamin antioxidants, is a redox agent, protecting against ROS in some circumstances, but also promoting ROS generation in others (Herbert, 1996). Herbert (1994 and 1995) reported that antioxidant vitamin supplements at pharmacological levels might promote heart disease, cancer, and liver and kidney disease. The present study's use of genistin-containing compounds from isoflavone

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Table 5: The activity of catalase in RBC

Diet	Exercise level	n	Catalase (unit/g RBC protein)	
			Mean	SE
HGI	exercised	12	0.113 ^a	0.008
HGI	sedentary	12	0.101 ^{ab}	0.005
control	exercised	12	0.086 ^b	0.004
control	sedentary	12	0.116 ^a	0.009

^{a,b}Means followed by the same superscript were not significantly different at the 0.05 level of significance experimentwise (Tukey's HSD, Sokal and Rohlf, 1995).

Table 6: The activity of glutathione peroxidase (GPx) in RBC

Diet	Exercise level	n	GPx (imol/s/mg RBC protein)	
			Mean	SE
HGI	exercised	12	0.412 ^a	0.024
HGI	sedentary	12	0.403 ^{ab}	0.029
control	exercised	12	0.294 ^b	0.010
control	sedentary	12	0.428 ^a	0.043

^{a,b}Means followed by the same superscript were not significantly different at the 0.05 level of significance experimentwise (Tukey's HSD, Sokal and Rohlf, 1995).

extract in the diet may have been too high, thereby promoting ROS generations in tissues. The results of this study showed genistein not only enhanced MDA in the gastrocnemius muscle of exercised and sedentary rats, but also provided no significant protection against increased MDA in the liver due to exercise.

Although dietary HGI supplementation did not prevent MDA increase in the liver of rats run to exhaustion on the treadmill, the running time was significantly longer than rats fed the control diet. Because oxidative damage may occur with exercise, antioxidant administration has drawn much attention both in terms of preventing damage and in terms of affecting performance. Packer (1997) suggested that oxidative stress might play a role on the fatigue process, and antioxidant administration might reduce the fatigue, leading to an increase in performance. Yet, the reports from most studies showed that antioxidant supplementation exhibited no effect on performance in humans (Clarkson, 1995). Although antioxidant supplementation may not bolster the performance in humans, exogenous glutathione supplementation was found to increase swimming time in mice (Novelli *et al.*, 1991). Furthermore, Balakrishnan and Anuradha (1998) reported that exogenous glutathione influenced the endurance capacity of athletes. Studies conducted in our laboratory, demonstrated that isoflavones increased GSH concentration in the blood. In addition, Appelt and Reick (1999) observed that feeding rats 810-PPM soy isoflavones led to increased GSH concentration and decreased GSSG concentration in plasma. Therefore, isoflavones may increase endurance capacity in rats by their influence on glutathione concentrations. The present study suggests that HGI supplementation increases endurance capacity in one-year old rats. The further study is needed to investigate the effect of isoflavones on running endurance. It was reported that genistein may modulate antioxidant enzyme activities. Genistein, administered to female rats at 0.1 g/kg BW levels a day, inhibited the activity of glutathione reductase (GR), catalase, SOD, and GPx in RBC (Breinholt *et al.*, 1999). These researchers observed that the activities of antioxidant enzymes in RBC decreased concurrently with an increase in the antioxidant potential due to administered flavonoids. Others observed that genistein could suppress 12-O-tetradecanoylphorbol-13-acetate (TPA)-mediated H₂O₂

production *in vitro* and *in vivo* and inhibit superoxide anion formation by the xanthine-xanthine oxidase system (Wei *et al.*, 1993 and Wei *et al.*, 1995). Hence, Breinholt *et al.* (1999) hypothesized that antioxidant enzymes in RBC were down-regulated by genistein in response to an improved antioxidant status of the RBC due to the increase of high antioxidant potential from genistein supplementation. This study confirms the hypothesis of Breinholt *et al.* (1999), because it showed that HGI supplementation decreased SOD activities in RBC. Physical exercise can cause oxidative stress in erythrocytes because of increased generation of ROS, which may trigger antioxidant enzymes to enhance their activities and reduce ROS to safe compounds (Ji, 1995). However, the increase of activities of antioxidant enzymes by physical exercise is still controversial. A session of acute exhaustive exercise was shown to increase SOD activity, indicating increased superoxide production during exercise (Ji, 1993) in a number of biological tissues including heart (Ji, 1993), liver, (Alessio and Goldfarb, 1988; Ji *et al.*, 1988; and Ji *et al.*, 1990), lung (Reddy *et al.*, 1992), blood platelets (Buczynski *et al.*, 1991), skeletal muscle (Ji *et al.*, 1990 and Lavler *et al.*, 1993), and erythrocytes (Somani *et al.*, 1995). Ohno *et al.* (1986) and Kaczmarek *et al.* (1999), however, reported that physical exercise did not increase erythrocyte SOD activity in humans, and Ji *et al.* (1990) and Alessio and Goldfarb (1988) observed that one session of acute exercise did not increase SOD activity in muscle from rats. In contrast, most of the literature revealed no significant alternation in catalase activity with acute exercise (Ji *et al.*, 1990 and Meydani *et al.*, 1993), while studies conducted by Somani *et al.* (1995) and Kaczmarek *et al.* (1999) reported that erythrocyte catalase activity decreased after acute exercise. The effect of an acute session of exercise on GPx activity in various tissues has not been reported consistently in the literature (Ji, 1995). Erythrocyte GPx activity was slightly reduced after a brief (30 min) physical exercise in sedentary students (Ohno *et al.*, 1986), yet, Ji *et al.* (1990) observed that acute exercise did not affect GPx activity in the livers of rats. There is yet no clear explanation for these discrepancies (Ji, 1995). This study agrees with Somani *et al.* (1995) that erythrocyte catalase activities were significantly decreased by one session of acute exhausting exercise in the rats fed the control diet, while erythrocyte SOD activity significantly increased. In addition, RBC GPx activity was significantly decreased by exercise. In general, an increased generation of ROS during an acute session of strenuous exercise, causing the activation of antioxidant enzymes, remained the most viable explanation (Ji *et al.*, 1988; Ji *et al.*, 1990; and Ji, 1993). In the present study, only erythrocyte SOD in rats reflected the increased ROS production, because its activity was significantly enhanced by acute exercise. However, this explanation could not effectively account for the decrease of erythrocyte GPx and catalase activities in the study.

Erythrocyte SOD activity was significantly increased by acute exercise in the rats, while the activity of the GE rats was maintained by HGI administration at the same level as that of the NS rats. As Wei *et al.* (1993 and 1995) observed that genistein could suppress H₂O₂ production and inhibit superoxide formation, genistein may diminish superoxide anion generated by one session of acute exhaustive exercise. In regard to GPx and catalase activities in erythrocytes of exercised rats, HGI prevented the decrease of GPx and catalase activities due to acute exercise. Although the physiological justification in the decrease of enzymes' activities is not clear, the homeostasis of antioxidant defense is disturbed by oxidative stress due to one session of acute

exhaustive exercise. The HGI supplementation could prevent this disturbance of oxidative stress due to exercise, further leading to maintenance of a more reductive environment in erythrocytes. Therefore, we can speculate that HGI administration in the diet of the rats maintained redox status, because genistein acts as an antioxidant or exerts its estrogenic effect to modulate antioxidant enzyme activities. This study demonstrated that HGI supplementation modulated erythrocyte antioxidant enzyme activities in response to acute exhaustive exercise probably through either the antioxidative or estrogenic effect of genistein. Isoflavones also enhanced the endurance capacity in rats possibly by their influence on glutathione homeostasis. The increased genistein in the liver, skeletal muscle, and plasma may have been mobilized from other tissues such as the prostate, testes, thyroid gland, and brain, during exercise, or was due to reduced clearance from the liver. However, elevated genistein concentrations in the liver did not provide an extra protection against oxidative stress due to acute exhaustive exercise. The dose of HGI administered in this study might be too high, and consequently genistein may have acted as both an antioxidant and a prooxidant. It can be concluded that genistein may elevate antioxidant defense in erythrocytes against oxidative stress, and increase endurance capacity by its antioxidative effect. This may be either directly, or indirectly through its estrogenic effect. However, the amount of HGI used in this pilot study, may not be optimal to defend the liver and skeletal muscle against oxidative stress. Further study is necessary to explore the optimal dose for dietary genistein supplementation to produce a beneficial effect on antioxidant defense systems and to avoid the disadvantageous effect of increased MDA in skeletal muscle.

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Abbreviations Key: CE: Control diet and Exercise, MDA: Malondialdehyde, ROS: Reactive Oxygen Species, SOD: Superoxide dismutase,