

Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats

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BACKGROUND/OBJECTIVES: Isoflavones are widely believed to be beneficial to human health, in relation to their antioxidant potentials. Exercise can cause an imbalance between reactive oxygen species (ROS) and antioxidants. This study was conducted in order to investigate the ability of isoflavones in amelioration of oxidative stress induced by exercise.

MATERIALS/METHODS: Male Sprague-Dawley rats were assigned to one of four groups: isoflavone-free with no exercise (CON-sd), isoflavone-free with exercise (CON-ex), isoflavone-supplemented with no exercise (ISF-sd), and isoflavone-supplemented with exercise (ISF-ex). Animals exercised on the treadmill for 30 minutes per day, five days per week. TBARS as a marker of oxidative stress and antioxidant enzyme activity, including SOD, GSH-px, and catalase were determined in liver tissue. Serum lipid profile was also examined.

RESULTS: A significant effect of isoflavone alone was observed on abdominal fat pad mass. ISF-ex had significantly less abdominal fat pad than CON-ex. Both exercise and isoflavone treatment had significant effects on lowering plasma triglyceride (TG), thus, the ISF-ex group had a significantly lower TG level than the CON-sd group, by 30.9%. However, no differences were observed in plasma cholesterol, HDL-C, and cholesterol/HDL-C ratio. Exercise, isoflavone, and exercise-isoflavone interaction effects were significant on thiobarbituric acid reactive substances (TBARS) ($P = 0.001$, 0.002 , and 0.005 , respectively). The CON-ex group showed a higher TBARS level than the other three groups. By contrast, in the ISF-ex group, TBARS was restored to the level of the ISF-sd or CON-sd group. Isoflavone had a significant effect on superoxide dismutase (SOD) ($P = 0.022$) and catalase activities ($P = 0.049$). Significantly higher SOD and catalase activities were observed in ISF-ex than CON-ex. SOD and catalase activities showed an inverse pattern of TBARS. Taken together, isoflavones increased the activities of SOD and catalase with concomitant decreases in TBARS, indicative of decreased oxidative stress.

CONCLUSIONS: Isoflavone supplementation enhances antioxidant action with attenuation of exercise-induced oxidative stress, as measured by decreases in TBARS, and inhibits body fat accumulation and plasma TG increase. Antioxidative effects ascribed to isoflavones may be partially exerted via enhancement of antioxidant enzyme activities.

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INTRODUCTION

Chronic exposure to oxidative stress is known to exert deleterious effects on health with accumulating oxidative damage throughout a lifetime. Formation of reactive oxygen species occurs during normal physiological processes by both nonenzymatic and enzymatic sources, causing continuous damage to lipids, proteins, and nucleic acids [1]. Oxidative modification of these molecules by ROS plays a pivotal role in a wide range of common diseases and age-related degenerative conditions [2]. Consequently, increases in antioxidative capacity are believed to be protective against this oxidative damage. Isoflavones in soy products have recently received attention as one of the phytochemicals with diverse properties [3]. Isoflavones have

been linked to decreased risk of cardiovascular disease, osteoporosis, endocrine-responsive cancer (eg breast, prostate, and colon cancer), and menopausal symptoms, due in part to their possible antioxidant activities [4]. Research on the antioxidant action of isoflavones suggests free radical scavenging ability, ability to reduce low-density lipoprotein (LDL) and DNA susceptibility to oxidative stress and ability to boost the activity and expression of antioxidant enzymes [5].

Antioxidant activity of phenolic compounds can be direct, through their activity as free radical scavengers, or indirect as modulators of intracellular pro- and anti-oxidant enzymes [6]. Genistein, a typical soy isoflavone, acts directly as an antioxidant via hydrogen atom donation from the hydroxyl group attached to the benzene ring, thereby protecting against oxidative

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damage [7, 8]. Daidzein-treated rats were reported to show significantly elevated catalase and SOD activities, and treatment with genistein caused elevation of SOD activity to a lesser degree [9]. Mice supplemented with soy isolate containing 400 mg/g isoflavone aglycones (226 mg/g genistein and 174 mg/g daidzein) had significantly lower hepatic malondialdehyde (MDA) and conjugated dienes. SOD and catalase activities increased in the liver by the soy isolate, but not glutathione peroxidase (GSH-px) activity [10].

Exercise is a well-accepted model for induction of oxidative stress. Cells continuously produce free radicals and ROS during the metabolic process [11]. These radicals are neutralized by an elaborate antioxidant defense system, an integrated array of antioxidant enzymes and free radical scavengers [12]. Physical exercise might increase oxygen consumption for energy generation, which is accompanied by an increase in production of reactive oxygen species. Excessive ROS production may cause oxidative stress and lead to extensive cell and tissue damage [13]. Accordingly, there is a great deal of interest in antioxidant potential against exercise-induced damage.

The current study was conducted in order to examine the effects of isoflavone supplementation and exercise on oxidative stress in experimental animals. Using a rat model of treadmill running, the current study investigated the possible effects of isoflavone supplementation on the antioxidant action by evaluating TBARS as a marker of oxidative stress and antioxidant enzyme activity, including SOD, GSH-px, and catalase. In addition, its effects on the blood lipid profile and body fat accumulation were also examined.

MATERIALS AND METHODS

Animal care and experimental design

Twenty four Sprague-Dawley (SD) rats aged four weeks old were individually housed in an environmentally clean room, with $22 \pm 3^\circ\text{C}$. The rats were randomly assigned to one of four treatment groups of six rats each, receiving isoflavone or/and exercise treatment for five weeks. The CON-sd group was given an isoflavone-free diet with no exercise, and the CON-ex group, an isoflavone-free diet with exercise. The ISF-sd group was fed a diet containing isoflavone with no exercise, and the ISF-ex group, a diet containing isoflavone with exercise. Diets were similar except for supplementation with or without isoflavone. All diets contained, by weight, 20% protein, 15% fat (coin oil), and 1% cholesterol, based on AIN-93G for rodent. Isoflavone supplemented diet contained 0.5% isoflavone-rich soy isolate. Isoflavone-rich soy isolate with isoflavone content of 33.4% (daidzein 6.6 mg/g, genistein 2.6 mg/g, glycitein 4.7 mg/g, daidzin 166.3 mg/g, genistin 33.7 mg/g, and glycitin 120.3 mg/g) was generously donated by Bioland (Ansan, Korea). The animals were given free access to tap water and their respective diet. Feed consumption was measured daily, and the body weights were recorded weekly.

Exercise was performed on a motor-driven rodent treadmill (Daedong Co, Korea) equipped with an electric shock grid on the rear barrier to provide exercise motivation to the animals. Animals in exercise groups ran on the treadmill for 30 min per day, five days per week, at the speed of 18-20 m/min up on

a 0% degree slope. The time of daily exercise and the running speed was gradually increased during the first week, from 10 m/min for 10 min to 20 m/min for 30 min. Following this adaptation period, animals were made to run under constant conditions until the end of the experimental period. All procedures were approved by the University Laboratory Animal Care committee (R2014-013).

Preparation of samples

At the end of the experimental period of five weeks, all animals were sacrificed by exsanguination. The exercise treated groups were sacrificed 24 h after the last exercise in order to prevent any acute exercise effect. Blood samples were collected into EDTA-coated tubes and plasma was separated by centrifugation at $1500 \times g$ for 20 minutes at 4°C . Plasma samples were stored at -20°C until the time of analysis. The liver was removed and washed several times with ice cold 0.1 M phosphate buffered saline. A 10% liver tissue homogenate was prepared at 4°C in cold phosphate buffer (50 mM, pH 7.4) containing 0.25 M sucrose and 0.5 mM EDTA using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at $10,000 \times g$ for 15 minutes at 4°C . Part of the collected supernatant was stored at -70°C for the TBARS assay. After further centrifuging the remaining supernatant, the precipitate was collected and resuspended for determination of catalase activity. The supernatant was further centrifuged at $100,000 \times g$ for 1 hour at 4°C , and the resulting supernatant was collected for measurement of SOD and GSH-px activity. Protein concentrations in suspension and supernatants were measured using the Buret method using a commercial kit (Asan Pharmaceuticals, Korea).

Determination of plasma lipids

Concentrations of triglyceride, cholesterol, and HDL-C were determined in plasma using a commercially available enzymatic analysis kit (Asan Pharmaceuticals, Korea). Triglyceride was measured enzymatically according to the lipase-glycerol phosphate method. The cholesterol kit was based on the cholesterol oxidase method. HDL-C was measured following separation of the HDL fraction using the heparin-manganese precipitation procedure.

Determination of TBARS

TBARS in liver tissue was determined according to the method of Ohkawa *et al.* [14] with slight modification. A mixture of tissue sample, 8.1% sodium dodecyl sulfate, 20% acetic acid solution and 0.8% thiobarbituric acid was incubated for 60 min. After cooling, a mixture of n-butanol and pyridine was added, followed by centrifugation at 4000 rpm for 10 min. The upper organic layer was collected and the absorbance was read at 532 nm. The quantification of TBARS was determined by comparing the absorption to the standard curve of MDA equivalents generated by hydrolysis of 1,1,3,3-tetramethoxypropane and expressed nM MDA/g tissue.

Determination of antioxidant enzymes

GSH-px activity was measured spectrophotometrically using H_2O_2 as peroxide, according to the method of Flohé and Günzler

[15]. Activity was assayed as NADPH decreased per minute by a reaction coupled with glutathione reductase, by monitoring spectrophotometrically the change in absorbance at 340 nm. Catalase activity was determined spectrophotometrically according to the Aebi procedure, with a slight modification [16]. The decomposition of peroxide was followed by the decrease in absorbance at 240 nm. SOD activity was assayed using the Fidanza method [17], using reduction of superoxide-mediated nitroblue tetrazolium. The ability of enzyme to inhibit the reduction of nitroblue tetrazolium was used as a measure of SOD activity, spectrophotometrically at 560 nm. One unit of enzyme was defined as the amount inhibiting the reduction of nitroblue tetrazolium by 50% and expressed as units per milligram protein.

Statistical analysis

Statistical analysis was performed using SAS 9.3 software. All data were expressed as mean and stand error of mean ($M \pm SE$). The effects of isoflavone supplementation on oxidative stress and antioxidant enzyme activities were analyzed by two-way analysis of variance (2-way ANOVA), with isoflavone supplementation and exercise as factors. For relevant comparisons, the data were further analyzed to determine any significant main effects or interactions. Differences between means of experimental groups were evaluated using Duncan's multiple range test, following one-way ANOVA. A P value of less than 0.05 was considered statistically significant.

RESULTS

Body weight and abdominal fat pad mass

The changes of body weight and abdominal fat pad weight are shown in Table 1. Four groups of rats started with similar initial body weight. No difference in final body weight was observed among the groups at the end of the five week experimental period. Both exercise and isoflavone treatments had no effect on weight changes. Although exercise had no effect, a significant effect of isoflavone alone was observed on abdominal fat pad mass (7.15 ± 0.37 vs 8.52 ± 0.49 g, $P = 0.039$). After correcting for body weight, this effect remained on abdominal fat mass per 100 g body weight (1.82 ± 0.07 vs 2.13 ± 0.10 g/100g BW, $P = 0.021$). Significantly lower abdominal fat pad mass was observed in isoflavone supplemented groups compared with those of isoflavone-free groups. Both ISF-ex and CON-ex rats exercised on a treadmill, however, ISF-ex rats had significantly less abdominal fat pad than CON-ex rats.

Plasma lipid variables

Plasma lipid levels are shown in Table 2. Both exercise and isoflavone treatment had significant effects on plasma TG level ($P = 0.030$ and $P = 0.045$, respectively), with a lower TG level in exercised groups than in sedentary groups (102.60 ± 5.91 vs 125.09 ± 7.99 mg/dl) and in isoflavone supplemented groups than in isoflavone-free groups (103.45 ± 7.03 vs 124.16 ± 7.12 mg/dl). Combination of exercise and isoflavone inhibited the increase in plasma TG, thus, a significantly lower TG level was

Table 1. Changes in body weight and fat accumulation by isoflavone supplementation in exercised or non-exercised rats.

Group	Initial body weight (g)	Final body weight (g)	Abdominal fat pad (g)	Abdominal fat pad ratio (g/100g BW)
CON-sd	172.72 ± 3.05^{NS}	392.94 ± 11.50^{NS}	8.34 ± 0.62^{ab}	2.12 ± 0.14^a
ISF-sd	172.25 ± 2.44	404.72 ± 13.25	7.82 ± 0.61^{ab}	1.92 ± 0.11^{ab}
CON-ex	171.62 ± 2.91	395.49 ± 13.41	8.79 ± 0.89^b	2.13 ± 0.14^a
ISF-ex	171.82 ± 2.45	379.94 ± 9.83	6.48 ± 0.26^b	1.71 ± 0.07^b
Effect (P value)*				
Exercise	0.923	0.369	0.375	0.351
Isoflavone	0.894	0.878	0.039	0.021
Interaction	0.952	0.272	0.153	0.359

Values are presented as mean \pm SE.

* P values were determined by two-way ANOVA. Values in columns with uncommon superscript letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

Table 2. Changes in plasma lipids by isoflavone supplementation in exercised or non-exercised rats.

Group	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Cholesterol/HDL-C ratio
CON-sd	132.02 ± 11.71^a	70.88 ± 7.73^{NS}	31.75 ± 3.51^{NS}	2.29 ± 0.23^{NS}
ISF-sd	118.17 ± 11.22^{ab}	69.65 ± 6.50	32.29 ± 1.32	2.37 ± 0.28
CON-ex	116.30 ± 7.75^{ab}	65.03 ± 5.79	31.60 ± 2.46	2.32 ± 0.33
ISF-ex	91.19 ± 5.56^b	67.37 ± 4.18	30.28 ± 2.95	2.30 ± 0.22
Effect (P value)*				
Exercise	0.030	0.524	0.692	0.943
Isoflavone	0.045	0.930	0.886	0.891
Interaction	0.545	0.778	0.733	0.852

Values are presented as mean \pm SE.

* P values were determined by two-way ANOVA. Values in columns with uncommon superscript letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

observed for the ISF-ex group than for the CON-sd group, by 30.9%. However, plasma cholesterol, HDL-C, and cholesterol/HDL-C ratio were not affected by exercise or isoflavone treatment. No differences in those variables were observed among groups.

Thiobarbituric acid reactive substances in liver

Values of TBARS in liver are shown in Fig. 1. Exercise, isoflavone, and exercise-isoflavone interaction had significant effects on TBARS ($P = 0.001$, $P = 0.002$ and $P = 0.005$, respectively). As a result, significantly greater TBARS was observed in exercise treated groups than in sedentary groups (0.23 ± 0.04 vs 0.13 ± 0.01 nM/g liver) and higher values were observed in the isoflavone-free groups (0.23 ± 0.04 nM/g liver) compared with isoflavone supplemented groups (0.13 ± 0.01 nM/g liver). Combination of exercise and isoflavone-free treatment resulted in an increase of TBARS, with the CON-ex group showing a higher TBARS level than the other three groups. By contrast, in the ISF-ex group, TBARS was restored to the level of the ISF-sd group.

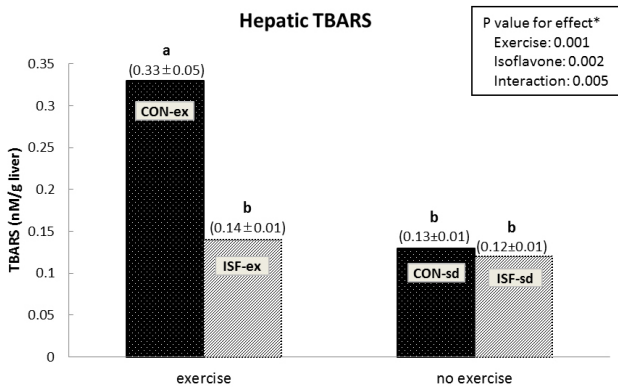


Fig. 1. Changes in hepatic TBARS by isoflavone supplementation in exercised or non-exercised rats. Values are presented as mean ± SE. *P values were determined by two-way ANOVA. Values marked with uncommon letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

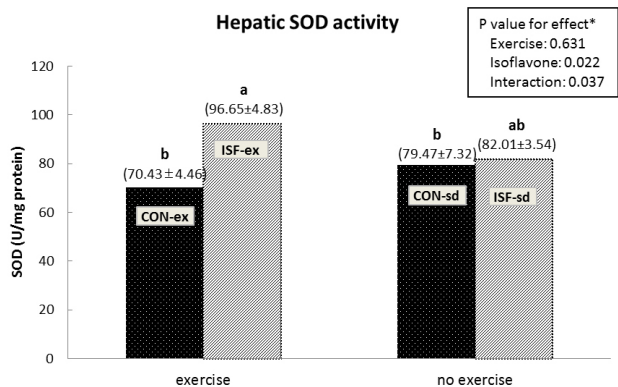


Fig. 2. Changes in hepatic superoxide dismutase activity by isoflavone supplementation in exercised or non-exercised rats. Values are presented as mean ± SE. *P values were determined by two-way ANOVA. Values marked with uncommon letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

Activities of antioxidant enzymes

The activities of SOD, catalase, and GSH-px, respectively, are illustrated in Fig. 2-4. Isoflavone and exercise-isoflavone interaction had significant effects on SOD activity (Fig. 2). Isoflavone supplementation resulted in a significant increase in SOD activity when compared with non-supplementation (87.86 ± 3.61 vs 74.95 ± 4.31 U/mg protein). Significantly higher SOD activity was observed in the ISF-ex group than in the CON-ex and CON-sd groups.

Isoflavone alone had a significant effect on catalase activity (Fig. 3). No effect of exercise or exercise-isoflavone interaction on that variable was found. Catalase activity was significantly higher in isoflavone supplemented groups than in isoflavone-free groups (1.10 ± 0.11 vs 0.85 ± 0.09 U/mg protein). Significantly higher catalase activity was observed in the ISF-ex group than in the CON-ex group. However, the activity of GSH-px was unaffected by exercise or isoflavone treatment (Fig. 4). No difference in activity of GSH-px was observed among groups.

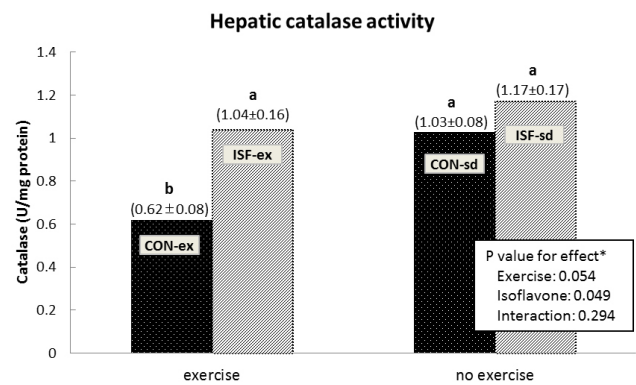


Fig. 3. Changes in hepatic catalase activity by isoflavone supplementation in exercised or non-exercised rats. Values are presented as mean ± SE. *P values were determined by two-way ANOVA. Values marked with uncommon letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

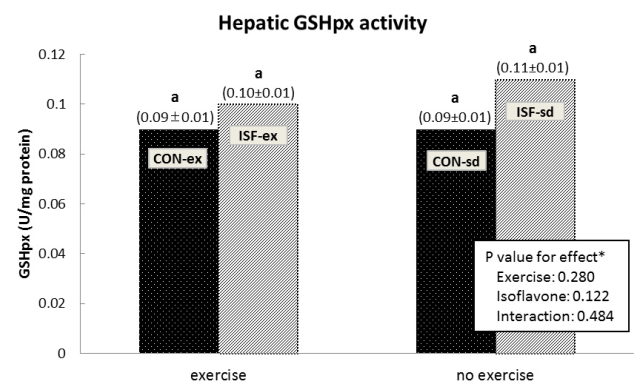


Fig. 4. Changes in hepatic glutathione peroxidase activity by isoflavone supplementation in exercised or non-exercised rats. Values are presented as mean ± SE. *P values were determined by two-way ANOVA. Values marked with uncommon letters are significantly different by Duncan's multiple range test ($P < 0.05$). CON-sd, isoflavone-free diet with no exercise; CON-ex, isoflavone-free diet with exercise; ISF-sd, isoflavone-supplemented diet with no exercise; ISF-ex, isoflavone-supplemented diet with exercise.

DISCUSSION

In recent years, ROS and antioxidant usage have received significant attention because aberrant production or regulation of ROS activity has been shown to contribute to development of some prevalent diseases and conditions [18]. Dietary flavonoids appear to play a role in prevention of a number of chronic diseases, such as cancer and cardiovascular disease, with a particular focus on isoflavones [4]. Isoflavone, a putative health beneficial component in soy along with amino acid composition and fiber, may contribute directly to the antioxidant defense systems in the body by scavenging ROS or indirectly by interacting with other antioxidant defense systems, such as enhancement of glutathione synthesis and sparing of vitamins C and E [19].

Isoflavones are found in vegetables and fruits in a biologically inactive glycoside form. The two major isoflavones, genistein and daidzein, are present in soy as β -D-glycosides, genistin and daidzin. These glycoside forms are biologically inactive. After ingestion, β -glucosidases in the intestinal wall hydrolyze the glycosides, resulting in conversion to their corresponding bioactive aglycones, genistein and daidzein. Only these aglycone forms are absorbed and are therefore biologically active. Genistein is further metabolized to p-ethyl phenol, and daidzein to equol and O-demethylangolensin [20].

Abnormal lipid levels contribute to the risk of cardiovascular disease. Isoflavones have been shown to be beneficial to plasma lipids level [21,22]. Diet with 0.2% soy isoflavone rich powder decreased the levels of serum cholesterol and TG in SD rats [23]. In rats fed a diet containing 0.37 g/kg isoflavone, although plasma TG level declined, no decrease in plasma cholesterol was observed [24]. In the current study, there were no changes in plasma lipids except for TG. Addition of isoflavone or exercise alone showed significant TG lowering effects, by 16.7% and 18.1%, respectively. In comparison with CON-sd, ISF-ex showed a lower TG level by 30.9%.

The abdominal fat pad weight showed a significant decrease of 16.1% with addition of isoflavone. This effect remained when corrected for body weight. The results indicate that isoflavone had an inhibiting effect on body fat accumulation. Ovariectomy is known to lead to body weight gain and fat deposition. Six-week intervention of exercise or isoflavone alone resulted in partial inhibition of body fat gain in ovariectomized mice, and the combined intervention completely restored fat mass to the sham level [25]. This finding suggests that abdominal fat gain may be attenuated with soy isoflavones, in agreement with the current results.

Antioxidant usage is a very attractive topic because excess ROS is now recognized to be associated with some prevalent diseases, as well as with the normal aging process [18, 26]. During normal cellular activities, various metabolic processes inside of cells produce free radicals and ROS. Production of free radicals is increased by physical activity in several ways. Therefore, exercise can cause an imbalance between ROS and antioxidants, referred to as oxidative stress. ROS, when present in a high enough concentration, can affect cellular components, especially lipids, which initiate a chain reaction called lipid peroxidation [11,18]. The accumulation of lipid peroxidation

products provides the most common biochemical marker of oxidative stress. The TBARS assay is the most widely used for assessment of lipid peroxidation, although it is somewhat controversial due to lack of specificity [11,26].

TBARS level was significantly the highest in the CON-ex group, which resulted from the combined effect of exercise and isoflavone-free diet. Exercise-induced TBARS formation in liver was inhibited in ISF-ex, in which the TBARS level returned to close to that of CON-sd or ISF-sd that did not exercise. Reflecting on this significant effect of exercise and isoflavone treatments on TBARS, it is supposed that regular exercise could protect tissues from oxidative stress with isoflavone supplementation, while isoflavone-free would augment the oxidative stress induced by exercise. Isoflavones are found to be protective antioxidants, which reduce the formation of radicals and ROS by decomposition of hydrogen peroxide without generating radicals, by quenching active singlet oxygen, and by trapping and quenching radicals before they reach a cellular target. Isoflavones are heterocyclic phenols and the activity of phenolics is based on their ability to donate a hydrogen atom to a free radical [27]. Due to its powerful radical scavenging capacity, genistein showed a more potent antioxidant activity than ascorbate and α -tocopherol in protecting cells against oxidative stress [7,8]. Treatment with isoflavones of 2.5 mg/kg BW caused a significant decrease in TBARS in plasma, liver, and brain by 33%, 18%, and 12%, respectively. The same results were also observed in plasma and tissues of rabbit under cypermethrin-induced oxidative stress, showing a decrease of TBARS with isoflavones [4,21]. These findings suggest that isoflavone supplementation may be needed to protect tissue against TBARS attack.

Formation of ROS is a natural consequence of aerobic metabolism and is integral for maintenance of tissue oxygen homeostasis. Potentially damaging oxidative stress can be generated by excess ROS, which are kept in check by endogenous cellular antioxidant mechanisms. Oxidative stress-related enzymes include SOD as well as catalase and GSH-px [28, 29]. In the current study, SOD and catalase activities showed an inverse pattern of TBARS. The CON-ex group, which had the highest TBARS level, showed the lowest activities of SOD and catalase. By contrast, the ISF-ex group with a low TBARS level, compared with CON-ex, had a significantly higher SOD and catalase activity. As above, the pattern of antioxidant enzymes activities appeared to be linked to that of TBARS. These findings regarding the effect of isoflavone on enzyme activities imply that isoflavone supplementation may result in up-regulation of SOD and catalase activity, leading to counteracting the oxidative stress. The lowest enzyme activities in the CON-ex group appeared to be related to a condition of elevated oxidative stress. Regular exercise was reported to result in lower SOD activity compared to the sedentary group, suggesting that SOD may be inactivated by ROS at the posttranscriptional level [30].

Soy isoflavone supplementation effect was reported against exercise-induced oxidative stress in ovariectomized SD rats. Exercise with isoflavone supplementation induced a significant increase in FRAP (ferric reducing antioxidant power) values with no differences in SOD activity and DNA damage, while exercise markedly affected SOD activity and DNA tail length with no

change in FRAP. It was concluded that isoflavone offered protection from exercise-induced oxidative stress [31]. Antioxidant activity of phenolic compounds can be direct, through their activity as free radical scavengers, or indirect as modulators of intracellular pro- and anti-oxidant enzymes [6]. Diabetes mellitus (DM) is a disease involving high oxidative stress, and lipid peroxide or conjugated diene are elevated in diabetes [32]. Oxidative damage in the liver was observed in streptozotocin (STZ)-induced diabetes mice, with a 50% increase in ROS and 30% in MDA. Treatment with genistein reversed hepatic ROS increase and reduced the MDA increase. Reduced hepatic GSH-px and catalase activities were restored by treatment with isoflavone [33]. This effect might be due to the direct scavenging activity of the genistein phenolic ring, activation of antioxidant defense gene transcription, and modulation of ROS producing enzyme expression [34]. Also, in STZ-induced diabetic SD rats, the activities of SOD, catalase, and GSH-px were decreased, and TBARS was increased. These results were reversed with supplementation of genistein or isolated soy protein [35]. Exposure to radiation also increases the oxidative burden; when it is severely increased, the endogenous antioxidant defense mechanism cannot cope with this increased stress. Male Wistar rats received oral administration of soy isoflavones (60 mg/kg BW) for 21 days, and were then exposed to gamma irradiation. Pretreatment with isoflavones resulted in significant reduction of lipid peroxide and enhanced the activity of SOD, catalase, and GST, in liver and erythrocytes, when compared with animals who received no pretreatment. These findings suggest that pretreatment with isoflavones prior to irradiation prepares the animals to sustain oxidative stress and thus inhibits radiation-induced cellular damage [36].

The increase in ROS production is usually protected by the antioxidant defense system, and its efficiency in counterbalancing ROS production determines the level of cell damage. Many studies have sought to determine whether antioxidant supplements would benefit the condition of oxidative stress, such as exercise, diabetes mellitus, or exposure to radiation or chemicals. The studies were conducted in varied research models with different oxidative stress conditions or different supplement types, therefore, the results produced were varied. Although further studies need to be conducted in order to clarify whether exercise increases the need for additional antioxidants, oxidative damage might be prevented by optimizing dietary antioxidants, particularly by isoflavone supplementation.

The current study demonstrates that isoflavones exert a beneficial effect on abdominal fat pad weight and plasma TG level. Exercise and isoflavone-free diet had an interactive effect on oxidative stress induction as indicated by an increase in TBARS. However, TBARS production elevated by exercise was inhibited with isoflavone. By contrast, the activities of SOD and catalase that were decreased by exercise were increased with isoflavone, leading to counteracting the oxidative stress. Taken together, isoflavones increased the activities of SOD and catalase with concomitant decreases in TBARS, indicative of decreased oxidative stress. The results presented here suggest that supplementation with soy isoflavones enhances antioxidative function and prevents lipid peroxidation, possibly through activation of the antioxidant enzymes.

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REFERENCES

1. Radák Z, Kaneko T, Tahara S, Nakamoto H, Ohno H, Sasvári M, Nyakas C, Goto S. The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes. *Free Radic Biol Med* 1999;27:69-74.
2. Borek C. Antioxidant health effects of aged garlic extract. *J Nutr* 2001;131:1010S-1015S.
3. Alessio HM, Hagerman AE, Romanello M, Carando S, Threlkeld MS, Rogers J, Dimitrova Y, Muhammed S, Wiley RL. Consumption of green tea protects rats from exercise-induced oxidative stress in kidney and liver. *Nutr Res* 2002;22:1177-88.
4. Yousef MI, Kamel KI, Esmail AM, Baghdadi HH. Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. *Food Chem Toxicol* 2004;42:1497-503.
5. Erba D, Casiraghi MC, Martinez-Conesa C, Goi G, Massaccesi L. Isoflavone supplementation reduces DNA oxidative damage and increases O- β -N-acetyl-D-glucosaminidase activity in healthy women. *Nutr Res* 2012;32:233-40.
6. Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. *Arch Biochem Biophys* 2008; 476:102-6.
7. Sawa T, Nakao M, Akaike T, Ono K, Maeda H. Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effect of vegetables. *J Agric Food Chem* 1999;47:397-402.
8. Sierens J, Hartley JA, Campbell MJ, Leatham AJ, Woodside JV. In vitro isoflavone supplementation reduces hydrogen peroxide-induced DNA damage in sperm. *Teratog Carcinog Mutagen* 2002;22: 227-34.
9. Banz W, Hauck S, Gename B, Winters T, Bartke A. Soy isoflavones modify liver free radical scavenger systems and liver parameters in Sprague-Dawley rats. *J Med Food* 2004;7:477-81.
10. Ibrahim WH, Habib HM, Chow CK, Bruckner GG. Isoflavone-rich soy isolate reduces lipid peroxidation in mouse liver. *Int J Vitam Nutr Res* 2008;78:217-22.
11. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 2003;189:41-54.
12. Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, Padovani CR, Cicogna AC, Novelli EL. Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. *Food Chem Toxicol* 2006;44:1167-72.
13. Qiao D, Hou L, Liu X. Influence of intermittent anaerobic exercise on mouse physical endurance and antioxidant components. *Br J Sports Med* 2006;40:214-8.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
15. Flohè L, Günzler WA. Assays of glutathione peroxidase. *Methods Enzymol* 1984;105:114-20.
16. Aebi H. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. Weinheim: Academic Press; 1974. p.673-84.
17. Fidanza F. Erythrocyte superoxide dismutase. In: *Nutritional status assessment: a manual for population studies*. New York (NY):

- Chapman & Hall; 1991. p.398-401.
18. Seifried HE, Anderson DE, Fisher EJ, Milner JA. A review of the interaction among dietary antioxidants and reactive oxygen species. *J Nutr Biochem* 2007;18:567-79.
 19. Chen CY, Bakhiet RM, Hart V, Holtzman G. Isoflavones improve plasma homocysteine status and antioxidant defense system in healthy young men at rest but do not ameliorate oxidative stress induced by 80% VO₂pk exercise. *Ann Nutr Metab* 2005;49:33-41.
 20. Cederroth CR, Nef S. Soy, phytoestrogens and metabolism: a review. *Mol Cell Endocrinol* 2009;304:30-42.
 21. el-Demerdash FM, Yousef MI, Al-Salhen KS. Protective effects of isoflavone on some biochemical parameters affected by cypermethrin in male rabbits. *J Environ Sci Health B* 2003;38:365-78.
 22. Weggemans RM, Trautwein EA. Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a meta-analysis. *Eur J Clin Nutr* 2003;57:940-6.
 23. Kawakami Y, Tsurugasaki W, Yoshida Y, Igarashi Y, Nakamura S, Osada K. Regulative actions of dietary soy isoflavone on biological antioxidative system and lipid metabolism in rats. *J Agric Food Chem* 2004;52:1764-8.
 24. Demonty I, Lamarche B, Deshaies Y, Jacques H. Role of soy isoflavones in the hypotriglyceridemic effect of soy protein in the rat. *J Nutr Biochem* 2002;13:671-7.
 25. Sohn E, Deyhim F, Devareddy L, Arjmandi BH. Soy isoflavones attenuate estrogen-deficient-induced increases in abdominal fat in the hamster. *Nutr Res* 2004;24:1023-9.
 26. Nelson SK, Bose SK, Grunwald GK, Myhill P, McCord JM. The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy. *Free Radic Biol Med* 2006;40:341-7.
 27. Pszczola DE, Katz F, Giese J. Research trends in healthful foods. *Food Technol* 2000;54:45-52.
 28. Castro L, Freeman BA. Reactive oxygen species in human health and disease. *Nutrition* 2001;17:161, 163-5.
 29. McCord JM. The evolution of free radicals and oxidative stress. *Am J Med* 2000;108:652-9.
 30. Itoh M, Oh-Ishi S, Hatao H, Leeuwenburgh C, Selman C, Ohno H, Kizaki T, Nakamura H, Matsuoka T. Effects of dietary calcium restriction and acute exercise on the antioxidant enzyme system and oxidative stress in rat diaphragm. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R33-8.
 31. Oh HY, Lim S, Lee JM, Kim DY, Ann ES, Yoon S. A combination of soy isoflavone supplementation and exercise improves lipid profiles and protects antioxidant defense-systems against exercise-induced oxidative stress in ovariectomized rats. *Biofactors* 2007;29: 175-85.
 32. Hsu CS, Chiu WC, Yeh SL. Effects of soy isoflavone supplementation on plasma glucose, lipids, and antioxidant enzyme activities in streptozotocin-induced diabetic rats. *Nutr Res* 2003;23:67-75.
 33. Valsecchi AE, Franchi S, Panerai AE, Rossi A, Sacerdote P, Colleoni M. The soy isoflavone genistein reverses oxidative and inflammatory state, neuropathic pain, neurotrophic and vasculature deficits in diabetes mouse model. *Eur J Pharmacol* 2011;650:694-702.
 34. McCarty MF. Isoflavones made simple - genistein's agonist activity for the beta-type estrogen receptor mediates their health benefits. *Med Hypotheses* 2006;66:1093-114.
 35. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. *Life Sci* 2006;79:1578-84.
 36. Dixit AK, Bhatnagar D, Kumar V, Chawla D, Fakhruddin K, Bhatnagar D. Antioxidant potential and radioprotective effect of soy isoflavone against gamma irradiation induced oxidative stress. *J Funct Foods* 2012;4:197-206.