

體育運動論文評論（生化營養組）（本試題共 8 頁）

注意：答案一律寫在答案卷上，否則不予計分

請詳細閱讀所附之論文後，作答下列各問題。作答方式一律須橫書。

- 1、請說明本論文的主要目的。(15%)
- 2、請詳述本論文的實驗方法及步驟。(30%)
- 3、請詳述本論文所得結果及討論。(45%)
- 4、本論文的優缺點為何？(10%)

Chronic Ginseng Consumption Attenuates Age-Associated Oxidative Stress in Rats¹

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ABSTRACT The antioxidant properties of North American ginseng (*Panax quinquefolium*) were investigated in young and old rats fed a ginseng-supplemented diet for 4 mo. Female Fischer 344 rats at 4 (Y, $n = 38$) or 22 (O, $n = 25$) mo of age were randomly divided into three groups and fed either a AIN-93G formula-based control diet (C) or a diet containing 0.5 g/kg (low dose, L) or 2.5 g/kg (high dose, H) dry ginseng powder for 4 mo. Oxidant generation, measured with 2',7'-dichlorofluorescein (DCFH), was significantly lowered with ginseng feeding in the homogenates of heart, soleus, and the deep portion of vastus lateralis muscle (DVL) ($P < 0.05$) in both Y and O rats, and the effects were dose dependent. Superoxide dismutase activity was elevated in heart and DVL of H rats, and in soleus of L rats ($P < 0.05$). H rats showed higher glutathione peroxidase activity in DVL and soleus muscle ($P < 0.05$), and elevated citrate synthase activity in the heart of both age groups and DVL of Y rats ($P < 0.05$). Neither the H nor L diet affected age-dependent lipid peroxidation in the heart or muscle, but protein carbonyl content was attenuated with the H diet in the heart ($P < 0.05$) and with both the L and H diets in DVL ($P < 0.01$). We conclude that ginseng supplementation can prevent age-associated increase in oxidant production and oxidative protein damage in rats. These protective effects are explained in part by elevated antioxidant enzyme activities in the various tissues. J. Nutr. 133: 3603-3609, 2003.

KEY WORDS: • antioxidant enzyme • free radical • ginseng • oxidative stress • rats

Reactive oxygen species (ROS)³ are generated ubiquitously in aerobic organisms (1). When these cytotoxic agents overwhelm the endogenous antioxidant defense system, serious oxidative stress and damage occur as reflected by the oxidative modification of macromolecules such as lipid, protein and DNA (2). ROS generation has important implications in the etiology of numerous diseases and in aging (3,4). Thus, it is critical that cells maintain optimal antioxidant defenses to reduce oxidative damage. Dietary supplementation and therapeutic use of antioxidants are emerging measures with which to prevent and treat oxidative stress-induced diseases (5,6).

Nature offers an abundance of antioxidant resources; most of these are found in fruits and vegetables and are known as phytochemicals (1,7). As one of the most popular dietary supplements, ginsengs, including *Panax C. A. Meyer* (Asian ginseng) and *Panax quinquefolius* L. (North American ginseng) have drawn attention worldwide for their broad and invaluable medicinal potential (8). Although the mechanism for ginseng's health-promoting effects is complex and largely unknown, it is believed that the primary active ingredients are composed of a mixture of saponin glycosides, known as gin-

senosides (8). Furthermore, a higher ginsenoside content is found in *Panax quinquefolius* (9,10).

Recent research indicates that ginseng has powerful antioxidant properties that may explain its antiaging and antineoplastic effects (11). Treatment with ginseng extract and dietary supplementation of ginseng have shown a variety of protective effects against oxidative damage in vitro and in vivo, ranging from isolated LDL oxidation and ischemic neuron dysfunction, to heart reperfusion injury and physical exercise (12-16). Furthermore, ginseng treatment reportedly increases longevity in rodents (17). Although a primary function of ginsenosides appears to be related to its free radical scavenging activity, some ginsenoside fractions have been shown to induce antioxidant enzyme cytosolic superoxide dismutase (CuZn SOD) via enhanced nuclear protein binding to its promoter (18,19). Because the majority of research was performed in vitro using Asian ginseng, the antioxidant functions of North American ginseng in vivo and the mechanisms of protection are largely unknown. Furthermore, there are controversies concerning whether high doses of phytochemical consumption including ginseng could have potentially adverse effects on the body (8).

Thus, the present study was conducted with the following purposes: 1) to examine whether dietary supplementation of North American ginseng could attenuate intracellular ROS production in the heart and various types of skeletal muscle of young and aged rats; 2) to examine whether ginseng supplementation could increase endogenous enzymatic antioxidant defense capacity in young and aged rats; 3) to determine whether ginseng could offer any protection against age-in-

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³ Abbreviations used: CS, citrate synthase; DCF, 2',7'-dichlorofluorescein; DCFH, 2',7'-dichlorofluorescein; DCFH-DA, DCFH-diacetate; DVL, deep portion of vastus lateralis muscle; ECL, enhanced chemiluminescence; GPX, glutathione peroxidase; H, high; L, low; MDA, malondialdehyde; O, old; RCD, reactive carbonyl derivative; ROS, reactive oxygen species; SOD, superoxide dismutase; SVL, superficial portion of vastus lateralis muscle; Y, young.

flicted tissue oxidative damage, marked by lipid peroxidation and protein oxidation; and 4) to assess whether any potential benefits of ginseng supplementation would be dose dependent.

MATERIALS AND METHODS

Animals. Female Fisher 344 rats at young (4 mo) and old (22 mo) age were obtained from the NIH rat colony (Indianapolis, IN) and used in this study. Upon arrival, the rats were fed a basal purified diet (see below) and caged individually in a temperature-controlled room (22°C) with a 12-h light-dark cycle, at the Animal Science Department of the University of Wisconsin-Madison. The animal treatment protocols were approved by the University of Wisconsin Research Animal Resource Center.

Diet. After a 2-d acclimation period, both young and old rats were randomly divided into three dietary groups: a control (C) diet based on the AIN-93 formula as reported previously (20), an AIN-93-based diet containing 0.5 g/kg dry ginseng powder (low dose, L) and an AIN-93-based diet containing 2.5 g/kg dry ginseng powder (high dose, H). The ginseng powder was supplied by Kaiser Farms (Wausau, WI) with a defined nutrient and ginsenoside (g/100 g wet weight): Rb1, 1.5; Rb2, 0.02; Rc, 1.67; Rd, 1.86; Re, 3.42; Rg1, 1.09; with total ginsenoside of 11.95 g/100 g. Rats were fed the three experimental diets for 4 mo. Tap water was available to rats during the entire daylight cycle. During the 4-mo period, diets were stored in a cold (0–4°C) room and transferred to the animal facility 2–3 times per week. Animals were weighed three times each week for the first 2 wk and daily food consumption was recorded to monitor the growth rate of rats and the potential effect of diet on food consumption. After 2 wk, the rats were weighed twice a week and food consumption was checked three times a week. The young and old rats were killed at the age of 8 and 26 mo, respectively.

Tissue preparation. The rats were decapitated and the heart, liver, kidney, deep (DVL) and superficial (SVL) portions of the vastus lateralis muscle, and soleus muscle were removed. One piece of each tissue was placed directly into an ice-cold buffer containing 130 mmol/L KCl, 5 mmol/L MgCl₂, 20 mmol/L NaH₂PO₄, 20 mmol/L Tris-HCl and 30 mmol/L glucose (pH 7.4) for measurement of oxidant production (see below). The rest of each tissue was freeze-clamped with aluminum tongs precooled in liquid nitrogen and stored at –80°C. The various tissues were minced and homogenized at 0–4°C with a motor-driven Potter-Elvehjem Teflon glass homogenizer. Homogenates were subjected to centrifugation at 9000 × g for 20 s. Cell debris and connective tissues in the pellets were discarded and the supernatants were stored at –80°C until further enzyme assays.

Determination of tissue oxidant production. 2',7'-Dichlorofluorescein (DCFH) was used as a fluorescent probe to measure the rate of oxidant production in the various fresh tissue homogenates according to LeBel et al. (21) with some modifications (22). Briefly, the assay buffer contained 130 mmol/L KCl, 5 mmol/L MgCl₂, 20 mmol/L NaH₂PO₄, 20 mmol/L Tris-HCl and 30 mmol/L glucose (pH 7.4), with a total volume of 3 mL. The assay was initiated with the addition of 5 μmol/L DCFH-diacetate (DCFH-DA) dissolved in 1.25 mmol/L methanol (5 μmol/L final concentration) and 50 μL (~1 mg protein) of tissue homogenate, and the mixture was incubated at 37°C for 15 min. This allowed DCFH-DA to be cleaved by intracellular esterase to derive free DCFH. The rate of oxidation from DCFH to 2',7'-dichlorofluorescein (DCF), which is indicative of oxidant production, was followed at an excitation wavelength of 488 nm and emission wavelength of 525 nm for 30 min using a Hitachi F-2000 fluorescence spectrometer. The rate was linear for at least 60 min at various concentrations of protein present, corrected for the autooxidation rate of DCFH. The unit of oxidant production was expressed as pmol DCF formed/(min · mg protein).

Antioxidant enzyme activity. SOD (EC 1.15.1.1) activity was measured using the method of Sun and Zigman (23), wherein one unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of epinephrine autooxidation to adrenochrome. Activity of glutathione peroxidase (GPX, EC 1.11.1.9) was determined according to Flohe and Gunzler (24) using H₂O₂ as substrate. Catalase (EC 1.11.1.6) activity was measured according to Aebi (25).

Citrate synthase (CS; EC 4.1.3.7) activity was measured according to Shepherd and Garland (26). Protein concentration in the tissue homogenates was determined by the Bio-Rad assay (Hercules, CA) using bovine serum albumin as the protein standard.

Lipid peroxidation and protein oxidation. Peroxidative damage to cellular lipid constituents was determined by measuring the malondialdehyde (MDA) concentration in butanol extracts according to Uchiyama and Mihara (27) with some modifications (28). Briefly, 10 mmol/L BHT and 200 mmol/L ferrous sulfate were included in the assay mixture. Sealed tubes were incubated for 15 min at 99°C. MDA concentration was calculated based on a standard curve using 1,1,3,3-tetraethoxypropane as a standard.

We employed two methods to assess protein oxidation in the various tissues. Heart and muscle carbonyl formation was measured using 2,4-dinitrophenylhydrazine (DNPH) as a reagent according to Levine et al. (29) with some modifications (22). Briefly, 200–300 mg of frozen tissue samples were homogenized at 0–4°C in 5 mmol/L potassium phosphate buffer (pH 7.4; weight:volume = 1:10) including 0.1% Triton X and the protease inhibitors leupeptin (1.0 mg/L), pepstatin (1.4 mg/L) and aprotinin (1.0 mg/L). The homogenate was centrifuged at 500 × g for 3 min and the 900-μL supernatant was transferred to a microfuge tube including 100 μL of 10⁶ streptomycin sulfate (in 50 mmol/L HEPES). The samples were vortexed vigorously and incubated at room temperature for 15 min before centrifugation at 6000 × g for 10 min at 4°C, and the supernatant was used. After reacting with 10 mmol/L DNPH:2 mol/L HCl in the dark, protein was precipitated with 20% trichloric acid followed by centrifugation at 14,000 × g for 10 min. The pellets were washed three times to remove excess DNPH, suspended in 6 mol/L guanidine HCl (in 20 mmol/L KH₂PO₄, pH 2.3), vortexed and allowed to dissolve overnight. The absorbance of the samples was measured at 366 nm. Carbonyl content was calculated using a molar absorption coefficient of 22,000 (mol/L)⁻¹cm⁻¹.

Reactive carbonyl derivatives (RCD) were measured with Western blot analysis using immunoprecipitation of protein-bound DNPH, by the method of Nakamura and Gota (30). Briefly, proteins precipitated with 20% (wt/v) trichloroacetic acid were suspended and incubated in a solution containing 10 mmol/L DNPH and 2 mol/L HCl for 1 h at 15°C. The resulting protein hydrazones were washed twice with 100% ethanol and centrifuged at 16,000 × g for 10 min. The pellets were then washed with acetone and centrifuged again at 16,000 × g for 10 min. The final precipitates (1 mg protein) were dissolved in 1 mL buffer containing 8 mol/L urea and incubated for 30 min at 37°C. SDS-PAGE in combination with an enhanced chemiluminescence (ECL) detection system was used to determine immunoreactive RCD content in the heart and DVL samples according to a previously described method (31). After electrophoresis, gels were equilibrated in transfer buffer (2.5 mmol/L Tris base, 19.2 mmol/L glycine) and transferred overnight at 20 V. Blots were blocked in 5% nonfat dry milk in PBS-T (80 mmol/L Na₂HPO₄, 20 mmol/L NaH₂PO₄, 100 mmol/L NaCl, 0.1% Tween-20) and treated with antibodies against RCD (Sigma Chemical, St. Louis, MO). Incubation with secondary antibodies and detection of signal was performed according to manufacturer's directions using an Amersham ECL detection system (Amersham, Arlington Heights, IL).

Statistical analysis. The data were analyzed using a two-way ANOVA. After a significant treatment effect (age or ginseng treatment) was found, Scheffé's post-hoc tests were performed to determine significance differences among means ($P < 0.05$).

RESULTS

Body weight and food consumption. The C, L and H groups of either young or old rats did not differ in body weight at the beginning of the study. Old rats were heavier than young rats throughout the experiment ($P < 0.01$). However, although 4 mo of low dose ginseng feeding did not affect body weight gain of either age group, high dose ginseng feeding significantly reduced body weight gain in both Y and O rats. In Y rats, body weight in the H group (219 ± 2.0 g) was 4.8% lower ($P < 0.01$) than that of the C group (230 ± 2.5 g). In

O rats, the H group (286 ± 9.7 g) had 11.7% ($P < 0.05$) lower body weight than the C group (324 ± 10.2 g).

Old rats had higher daily food consumption than young rats at the start ($P < 0.01$) and end ($P < 0.05$) of the experiment. When adjusted by body weight, food consumption per 100 g body weight was lower ($P < 0.01$) for O than for Y rats (data not shown). Ginseng treatment had no effect on food consumption in either Y or O rats. Thus, the effects of the H diet on body weight and other biochemical properties were not caused by a decrease in food consumption.

Oxidant production. Oxidant generation rate in the heart measured by DCF formation did not differ between Y and O rats fed the C diet (Fig. 1a). Both L- and H-fed rats had

decreased oxidant production ($P < 0.05$), expressed either per gram of wet weight or per milligram of protein (not shown), compared with their age-matched C counterparts.

Oxidant production in oxidative (type 1) muscle soleus was decreased in 26- vs. 4-mo-old rats ($P < 0.01$), whereas both Y and O rats fed the H diet had significantly lower DCF formation than their age-matched C rats (Fig. 1b). The reduction of DCF formation was 38% ($P < 0.05$) in Y rats and 18% ($P < 0.05$) in O rats. Oxidant production did not differ between L- and C-fed rats. In DVL muscle (Fig. 1c), no age difference in oxidant production existed in C-fed rats. H feeding decreased DCF formation by 18% ($P < 0.05$) in Y rats and by 24% ($P < 0.05$) in O rats. Neither L nor H feeding affected oxidant production in SVL muscle, which had only one third and one tenth of DCF formation rate compared with DVL and soleus, respectively (data not shown).

Antioxidant enzyme activities. Heart SOD activity was higher in O than in Y rats fed a C or L diet ($P < 0.01$), and was elevated by the H diet in Y rats ($P < 0.05$) (Fig. 2a). SOD activity in soleus muscle was increased in O compared with Y rats ($P < 0.05$, main effect) and with the L diet in both Y and O rats (Fig. 2b). In DVL (Fig. 2c), O rats had higher SOD activity than Y rats ($P < 0.05$, main effect), whereas the H diet increased SOD activity in Y rats. SOD activity in SVL was higher in O than in Y rats, but was unaltered with either the L or H diet (not shown).

GPX activity in the heart was increased with age ($P < 0.01$, Fig. 3a). However, it was not affected by ginseng treatment. Aged rats also had higher GPX activity than young rats in soleus ($P < 0.05$), and the H diet increased GPX activity by 16% ($P < 0.05$) in Y rats, but not O rats (Fig. 3b). In DVL of both Y and O rats, GPX activity was elevated only with the H diet ($P < 0.05$, Fig. 3c). GPX activity in SVL was higher in O than in Y rats ($P < 0.05$), but did not differ with diet (data not shown).

Catalase activity in the heart was not affected by age, but was significantly higher in soleus, DVL and SVL of O compared with Y rats (Table 1). Ginseng supplementation at low or high dose had no influence on catalase activity in any tissue.

Old rats had higher levels of mitochondrial enzyme marker CS activity in the heart regardless of diet (Fig. 4a). High dose, but not low dose ginseng treatment significantly increased CS activity in both Y and O rats. CS activity was not affected by animal age in three types of skeletal muscle. However, a significant increase in CS activity was observed with the L diet in DVL of Y rats (Fig. 4b).

Oxidative damage to lipid and protein. MDA concentration in the heart was significantly elevated with age ($P < 0.05$, Table 1). Ginseng treatment did not affect MDA levels in the heart, but L-fed rats showed lower MDA concentrations than C- and H-fed rats in DVL ($P < 0.05$). MDA concentrations in soleus and SVL were not measured due to the limited amount of tissue.

Protein carbonyl formation was more than twofold higher ($P < 0.01$) in the hearts of O compared with Y rats (Fig. 5a), and 65% higher ($P < 0.01$) in the DVL of O compared with Y rats (Fig. 5b). High dose, but not low dose ginseng treatment attenuated carbonyl levels in both Y and O hearts ($P < 0.05$). Further, carbonyl content was decreased with both the L and H diet in DVL of Y and O rats ($P < 0.01$). As revealed by the Western blot, the content of RCD was diminished in the heart of aged rats fed a ginseng-supplemented diet, and H rats had lower RCD levels than L and C rats (Fig. 6a). No difference in RCD was detected as a result of ginseng treatment in the heart of young rats (not shown). In DVL of aged rats (Fig. 6b), the H group appeared to have lower levels of RCD than the C

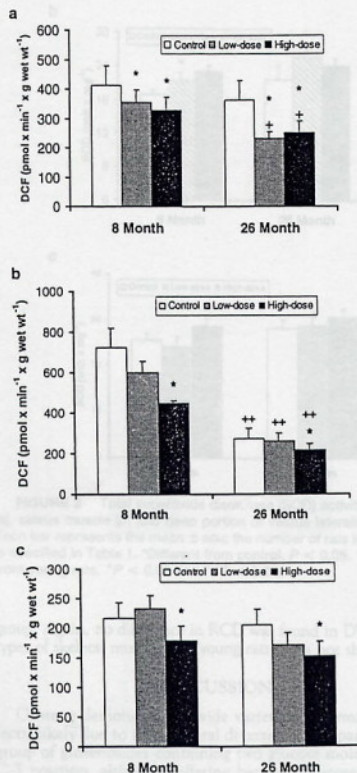


FIGURE 1 Oxidation rate of dichlorofluorescein (DCFH) to dichlorofluorescein (DCF) in the homogenate of rat heart (a), soleus muscle (b) and deep portion of vastus lateralis muscle (c). The assay buffer contained 130 mmol/L KCl, 5 mmol/L MgCl₂, 20 mmol/L NaH₂PO₄, 20 mmol/L Tris-HCl and 30 mmol/L glucose (pH 7.4) with 5 μ mol/L DCFH-diacetate dissolved in 1.25 mmol/L methanol. Each bar represents the mean \pm SEM; the number of rats in each group is specified in Table 1. *Different from control, $P < 0.05$. *Main effect of age, $P < 0.05$. **Different from young rats, $P < 0.01$.

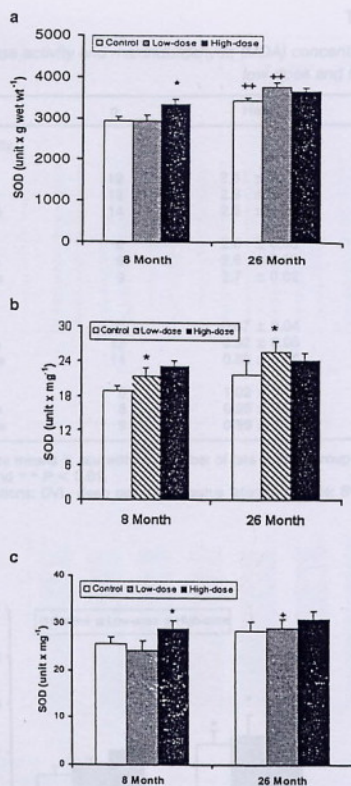


FIGURE 2 Total superoxide dismutase (SOD) activity in rat heart (a), soleus muscle (b) and deep portion of vastus lateralis muscle (c). Each bar represents the mean \pm SEM; the number of rats in each group is specified in Table 1. *Different from control, $P < 0.05$. **Different from young rats, $P < 0.05$ and $P < 0.01$.

group. Again, no difference in RCD was found in DVL or other types of skeletal muscle from young rats (data not shown).

DISCUSSION

Ginseng demonstrates a wide variety of pharmacologic effects, likely due to its structural diversity. Phytopanaxadiols, a group of ginsenosides containing two glucose moieties on the C-3 position, although differing between glucose and arabinose on C-20, such as Rb1, Rb2, Rc and Rd, are more abundant in North American ginseng than in Asia ginseng, and exhibit more potent antioxidant properties (8). Further, although ginsenosides as a whole appear to have free radical scavenging and metal ion chelating abilities, different fractions of phytopanaxadiols appear to exert their antioxidant function via different mechanisms. For example, Rb1 (containing four glucoses) was found to interact directly with hydroxyl radicals and protect ischemic neurons (14), whereas

Rh2 (containing three glucoses and an arabinose in pyranose form) has been shown to stimulate nuclear protein binding to gene regulatory sequences on the CuZn SOD promoter (18,19). Recently, Rh2 and Rh3 were found to modulate protein kinase C isoforms and differentiation of granulocytes (32). In the current study, we used a well-defined Wisconsin ginseng line that contained 12% ginsenosides; of this, 9.6% was in the form of phytopanaxadiol. We also applied two different dietary concentrations of ginseng to examine dose-response effects and potential adverse effects from overdose.

The most important finding of this study was that dietary ginseng supplementation could decrease intracellular ROS generation in the heart and oxidative type of skeletal muscles. The efficacy of ginseng treatment was dose dependent, especially in the muscle; attenuation of oxidant generation became significant only with a high concentration of ginseng in the

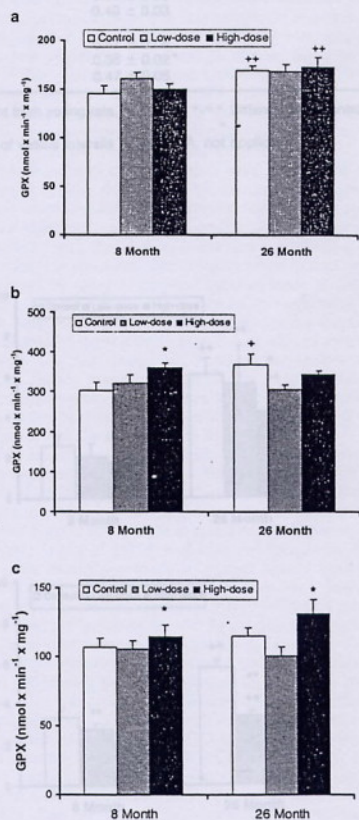


FIGURE 3 Glutathione peroxidase (GPX) activity in rat heart (a), soleus muscle (b) and deep portion of vastus lateralis muscle (c). Each bar represents the mean \pm SEM; the number of rats in each group is specified in Table 1. *Different from control, $P < 0.05$. **Different from young rats, $P < 0.05$ and $P < 0.01$.

TABLE 1

Catalase activity and malondialdehyde (MDA) concentration in young (4 mo old) and old (22 mo old) rats fed control, low dose and high dose ginseng diets

	<i>n</i>	Heart	Soleus	DVL	SVL
<i>(k × 10⁻²/mg)</i>					
Catalase activity					
Young					
Control	12	2.6 ± 0.04	2.0 ± 0.06	0.59 ± 0.04	0.59 ± 0.07
Low dose	12	2.9 ± 0.05	1.7 ± 0.06	0.53 ± 0.05	0.51 ± 0.08
High dose	14	2.6 ± 0.05	1.7 ± 0.06	0.66 ± 0.07	0.58 ± 0.08
Old					
Control	8	2.6 ± 0.05	2.5 ± 0.09**	0.92 ± 0.06**	0.91 ± 0.17*
Low dose	8	2.6 ± 0.09	2.4 ± 0.09**	0.78 ± 0.04**	0.69 ± 0.04*
High dose	9	2.7 ± 0.02	2.3 ± 0.08**	1.03 ± 0.06**	0.74 ± 0.11
<i>nmol/mg</i>					
MDA					
Young			NA		NA
Control	12	0.87 ± 0.04		0.49 ± 0.04	
Low dose	12	0.92 ± 0.05		0.39 ± 0.03+	
High dose	14	0.88 ± 0.05		0.49 ± 0.03	
Old					
Control	8	1.02 ± 0.05*		0.47 ± 0.04	
Low dose	8	0.95 ± 0.09		0.36 ± 0.02*	
High dose	9	0.99 ± 0.02*		0.47 ± 0.06	

1 Values are means ± SEM with the number of rats in each group as specified. * Different from young rats, $P < 0.05$. + Different from control, $P < 0.05$ and ++ $P < 0.01$.

2 Abbreviations: DVL, deep portion of vastus lateralis muscle; SVL, superficial portion of vastus lateralis muscle; NA, not applicable.

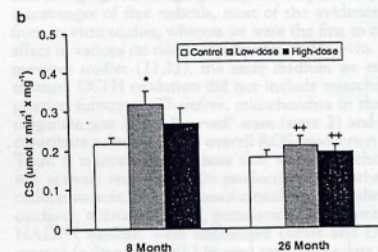
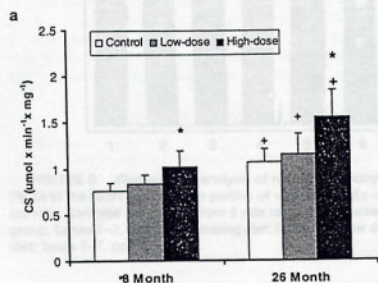


FIGURE 4 Citrate synthase (CS) activity in rat heart (a), and deep portion of vastus lateralis muscle (b). Each bar represents the mean ± SEM; the number of rats in each group is specified in Table 1. *Different from control, $P < 0.05$. + Different from young rats, $P < 0.05$ and ++ $P < 0.01$.

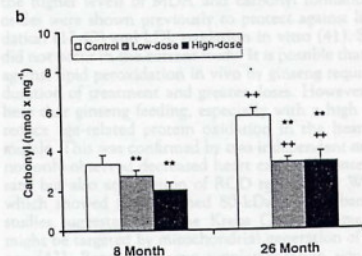
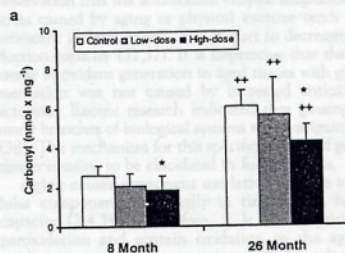


FIGURE 5 Protein carbonyl content in rat heart (a), and deep portion of vastus lateralis muscle (b). Each bar represents the mean ± SEM; the number of rats in each group is specified in Table 1. Different from control, $P < 0.05$ and ++ $P < 0.01$. + Different from young rats, $P < 0.05$ and ++ $P < 0.01$.

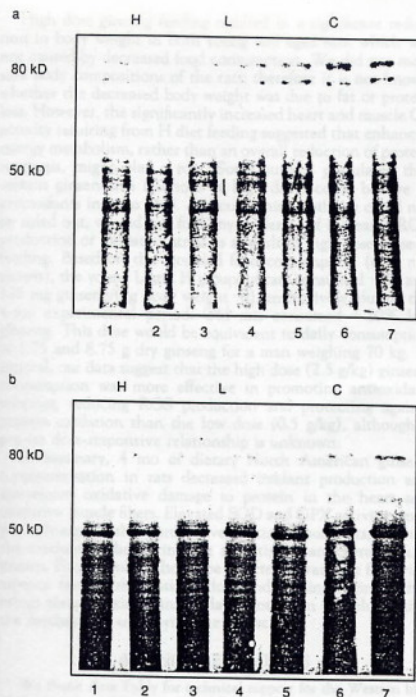


FIGURE 6 Western blot analysis of reactive carbonyl derivatives (RCD) in the heart (a) and deep portion of vastus lateralis muscle (b) of old rats. Samples are pooled from 5 rats randomly selected from each group. Lanes 1-2, high dose ginseng diet; lanes 3-4, low dose ginseng diet; lanes 5-7, control diet.

diet (2.5 g/kg). Although ginseng has long been reported to be a scavenger of free radicals, most of the evidence has come from *in vitro* studies, whereas we were the first to confirm this effect in various rat tissues rapidly obtained *in vivo*. Unlike our previous studies (22,33), the assay medium we employed to measure DCFH oxidation did not include mitochondrial respiration substrates. Therefore, mitochondria in the tissue homogenate was in the "starved" state (state 2) and should not contribute substantially to overall ROS production in the cell. Thus, it is interesting to note that when mitochondria were not actively respiring, ROS production from other potential cellular sources, such as cytosolic oxidases (including xanthine oxidase), microsomal P_{450} , peroxisomes and membrane-borne NADPH oxidase, were unchanged (heart and DVL) or decreased (soleus and SVL) in aged rats. These data support the view that the age-dependent increase in free radical generation is caused by deterioration of mitochondrial function (2,4). Nonetheless, regardless of animal age, ginseng supplementation attenuated ROS production in the various tissues.

DCFH oxidation rate in the tissue homogenate is deter-

mined by two opposing factors, the rate of oxidant generation and their removal by intracellular antioxidant systems (34). Although ginsenosides reportedly are an efficient scavenger of ROS, it is doubtful that dietary supplementation could result in a sufficient accumulation of ginsenosides within the tissue to intercept ROS and attenuate DCFH oxidation. A more plausible explanation would be that reduced ROS generation in ginseng-supplemented rats was due to modulation of endogenous antioxidant defense systems. Indeed, rats fed a ginseng-fortified diet generally demonstrated higher levels of SOD and GPX activities, although clear tissue-, age- and dose-specific differences were seen. In DVL and soleus muscle of young rats, SOD and GPX activities were significantly elevated with the H diet. These changes accompanied the decreased DCF oxidation in these two muscles. In contrast, no change in any antioxidant enzyme was observed in SVL, which had a low ROS production rate. Much curiosity remains concerning why ginseng feeding led to increased antioxidant enzyme activities. One potential mechanism was that certain structural motifs of phytoalexadiol (e.g., Rb2) are capable of interacting with specific gene regulatory sequences on antioxidant genes (e.g., CuZn SOD), thereby activating transcription (18,19). This interaction perhaps mimics cellular responses to oxidative stress, although without increasing oxidant production. Alternatively, ginsenosides or their derivatives may enhance the stability of mRNA for antioxidant enzymes.

Tissues from aged rats had higher basal antioxidant enzyme activities, a finding consistent with our previous observations (35) and those of others (36). However, among all tissues from aged rats examined, only DVL showed a significant increase in GPX activity with the H diet. These data agree with a general observation that the antioxidant enzyme adaptation to oxidative stress caused by aging or physical exercise tends to decline in senescent skeletal muscle, due in part to decreased signal transduction capacity (31,37). It is imperative that the dramatic decrease in oxidant generation in aged tissues with ginseng supplementation was not caused by increased antioxidant enzyme activities. Recent research indicated that ginseng could affect many branches of biological systems with intriguing implications (38). The mechanism for this specific efficacy of ginseng in aged tissues remains to be elucidated in future studies.

Aging causes prominent oxidative damage to various cellular components, especially in tissues with high oxidative capacity (2,4,39). Therefore, it is not surprising that lipid peroxidation and protein oxidation in the aged heart and oxidative muscle fibers were intensified, as demonstrated by the higher levels of MDA and carbonyl formation. Ginsenosides were shown previously to protect against lipid peroxidation (12,40) and LDL oxidation *in vitro* (41). Such effects did not occur in the current study. It is possible that protection against lipid peroxidation *in vivo* by ginseng requires a longer duration of treatment and greater doses. However, we report here that ginseng feeding, especially with a high dose, could reduce age-related protein oxidation in the heart and DVL muscle. This was confirmed by two independent methods. We not only observed decreased heart carbonyl content in H-fed rats, but also attenuation of RCD revealed by Western blot, which showed a diminished 80-kDa protein band. Previous studies suggested that the Krebs Cycle enzyme, aconitase, might be targeted by mitochondrial generation of ROS in old age (42). Because ginseng supplementation significantly increased mitochondrial enzyme marker CS activity and decreased ROS production without affecting antioxidant enzyme activity or lipid peroxidation in the aged heart, it seems that the protective effect of ginseng was mediated through some specific adaptive mechanisms in the mitochondria.

High dose ginseng feeding resulted in a significant reduction in body weight in both young and aged rats, which was not caused by decreased food consumption. We did not measure body compositions of the rats; therefore it is not known whether the decreased body weight was due to fat or protein loss. However, the significantly increased heart and muscle CS activity resulting from H diet feeding suggested that enhanced energy metabolism, rather than an overall reduction of protein synthesis, might play a role. Some authors postulated that certain ginsenoside fractions at high doses could behave as prooxidants in vitro (43). Although this possibility could not be ruled out, we did not find any evidence of increased ROS production or oxidative stress as a result of high dose ginseng feeding. Based on the recorded food consumption (data not shown), the young L and H groups of rats consumed ~25 and 125 mg ginseng/(kg body weight · d), respectively, during the 4-mo experimental period. Old rats consumed ~20% less ginseng. This dose would be equivalent to daily consumption of 1.75 and 8.75 g dry ginseng for a man weighing 70 kg. In general, our data suggest that the high dose (2.5 g/kg) ginseng consumption was more effective in promoting antioxidant enzymes, reducing ROS production and protecting against protein oxidation than the low dose (0.5 g/kg), although a precise dose-responsive relationship is unknown.

In summary, 4 mo of dietary North American ginseng supplementation in rats decreased oxidant production and age-related oxidative damage to protein in the heart and oxidative muscle fibers. Elevated SOD and GPX activities may partially explain these protective effects in young rats, whereas the mechanism benefiting the aged tissues are currently unknown. Future studies should be directed toward the following aspects: finding the optimal dose and feeding regimen that brings about maximal antioxidant protection and elucidating the mechanisms underlying the protection.

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LITERATURE CITED

- Halliwel, B. H. & Gutteridge, J.M.C. (1999) *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford, UK.
- Yu, B. P. (1994) Cellular defense against damage from reactive oxygen species. *Physiol. Rev.* 74: 139-162.
- Harman, D. (1996) Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 2: 298-300.
- Ames, B. N., Shigenaga, M. K. & Hagen, T. M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* 90: 7915-7921.
- Meydani, M. (2002) The Boyd Orr lecture. Nutrition, interventions in aging and age-associated disease. *Proc. Nutr. Soc.* 61: 165-171.
- Claycombe, K. J. & Meydani, S. N. (2001) Vitamin E and genome stability. *Mutat. Res.* 475: 37-44.
- Hertog, M. G. (1996) Epidemiological evidence on potential health properties of flavonoids. *Proc. Nutr. Soc.* 55: 385-397.
- Kitts, D. & Hu, C. (2000) Efficacy and safety of ginseng. *Public Health Nutr.* 3: 473-485.
- Chan, T.W.D., But, P.P.H., Cheng, S. W., Kwok, I.M.Y., Lau, F. W. & Xu, H. X. (2000) Differentiation and authentication of *Panax ginseng*, *Panax quinquefolius*, and ginseng products by using HPLC/MS. *Anal. Chem.* 72: 1281-1287.
- Wang, X., Sakuma, T., Asafu-Adjaye, E. & Shiu, G. K. (1999) Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. *Anal. Chem.* 71: 579-584.
- Kitts, D. D., Wijewickreme, A. N. & Hu, C. (2000) Antioxidant properties of a North American ginseng extract. *Mol. Cell. Biochem.* 203: 1-10.
- Zhang, D., Yasuda, T., Yu, Y., Zheng, P., Kawabata, T., Ma, Y. & Okada, S. (1996) Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. *Free Radic. Biol. Med.* 20: 145-150.
- Chan, P., Niu, C. S., Tomlinson, B., Hong, C. T., Chen, J. P., Hong, C. Y., Tsai, S. K. & Cheng, J. T. (1997) Effect of trilinolein on superoxide dismutase activity and left ventricular pressure in isolated rat hearts subjected to hypoxia and normoxic perfusion. *Pharmacology* 55: 252-258.
- Lim, J. H., Wen, T. C., Matsuda, S., Tanaka, J., Maeda, N., Peng, H., Aburaya, J., Ishihara, K. & Sakanaka, M. (1997) Protection of ischemic hippocampal neurons by ginsenoside Rb1, a main ingredient of ginseng root. *Neurosci. Res.* 28: 191-200.
- Voces, J., Alvarez, A. I., Vila, L., Ferrando, A., Cabral de Oliveira, C. & Prieto, J. G. (1999) Effects of administration of the standardized *Panax ginseng* extract G115 on hepatic antioxidant function after exhaustive exercise. *Comp. Biochem. Physiol. C* 123: 175-184.
- Jiang, F., DeSilva, S. & Turnbull, J. (2000) Beneficial effect of ginseng root in SOD-1 (G93A) transgenic mice. *J. Neurol. Sci.* 180: 52-54.
- Bittles, A. H., Fulder, S. J., Grant, E. C. & Nicholls, M. R. (1979) The effect of ginseng on lifespan and stress responses in mice. *Gerontology* 25: 125-131.
- Kim, Y. H., Park, K. H. & Rho, H. M. (1996) Transcriptional activation of the Cu, Zn-superoxide dismutase gene through the AP2 site by ginsenoside Rb2 extracted from a medicinal plant, *Panax ginseng*. *J. Biol. Chem.* 271: 4539-4543.
- Chang, M. S., Lee, S. G. & Rho, H. M. (1999) Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from *Panax ginseng*. *Phytother. Res.* 13: 641-644.
- Fiebig, R., Hollander, J. & Ji, L. L. (2001) Exercise down-regulates hepatic fatty acid synthase in streptozotocin-treated rats. *J. Nutr.* 131: 2252-2259.
- LeBel, C. P., Ischiropoulos, H. & Bondy, S. C. (1992) Evaluation of the probe 2',7'-dichlorofluorescein as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* 5: 227-231.
- Bejma, J. & Ji, L. L. (1999) Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J. Appl. Physiol.* 87: 465-470.
- Sun, M. & Zigman, S. (1978) An improved spectrophotometric assay for superoxide dismutase based on epinephrine autooxidation. *Anal. Biochem.* 90: 81-89.
- Flohe, L. & Gunzler, W. A. (1984) Assays of glutathione peroxidase. *Methods Enzymol.* 105: 114-121.
- Aebi, H. (1984) Catalase in vitro. *Methods Enzymol.* 105: 121-126.
- Shepherd, D. & Garland, P. B. (1969) Citrate synthase from rat liver. *Methods Enzymol.* 8: 11-16.
- Uchiyama, M. & Mihara, M. (1978) Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.* 86: 271-278.
- Leeuwenburgh, C., Chandwaney, R. & Ji, L. L. (1994) Aging and training induced alterations in glutathione and antioxidant enzymes in rat skeletal muscle. *Am. J. Physiol.* 267: R439-R445.
- Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S. & Stadtman, E. (1990) Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 186: 464-478.
- Nakamura, A. & Goto, S. (1996) Analysis of protein carbonyls with 2,4-dinitrophenyl hydrazine and its antibodies by immunoblot in two-dimensional gel electrophoresis. *J. Biochem.* 119: 768-774.
- Hollander, H., Bejma, J., Oikawara, T., Ohno, H. & Ji, L. L. (2000) Superoxide dismutase gene expression: fiber specific effects of aging. *Mech. Ageing Dev.* 116: 33-45.
- Kim, Y. S., Kim, D. S. & Kim, S. I. (1998) Ginsenoside Rh2 and Rh3 induce differentiation of HL-60 cells into granulocytes: modulation of protein kinase C isoforms during differentiation by ginsenoside Rh2. *Int. J. Biochem. Cell Biol.* 30: 327-338.
- Bejma, J., Ramirez, P. & Ji, L. L. (2000) Free radical generation and oxidative stress with aging and exercise: differential effects in the myocardium and liver. *Acta Physiol. Scand.* 169: 343-351.
- Reid, M. B., Haack, K. E., Franchek, K. M., Valberg, P. A., Kobzik, L. & West, M. S. (1992) Reactive oxygen in skeletal muscle I. Intracellular oxidant kinetics and fatigue in vitro. *J. Appl. Physiol.* 73: 1797-1804.
- Ji, L. L., Dillon, D. & Wu, E. (1990) Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. *Am. J. Physiol.* 258: R918-R923.
- Luhtala, T., Roecher, E. B., Pugh, T., Feuers, R. J. & Weindrich, R. (1994) Dietary restriction opposes age-related increases in rat skeletal muscle antioxidant enzyme activities. *J. Gerontol.* 49: B231-B238.
- Ji, L. L. (2002) Exercise-induced modulation of antioxidant defense. *Ann. N.Y. Acad. Sci.* 959: 82-92.
- Gillis, C. N. (1997) *Panax ginseng* pharmacology: a nitric oxide link? *Biochem. Pharmacol.* 54: 1-8.
- Sohal, R. S., Ku, H. H., Agarwal, S., Forster, M. J. & Lal, H. (1994) Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech. Ageing Dev.* 74: 121-133.
- Deng, H. W., Guan, Y. Y. & Kwan, C. Y. (1990) Effects of ginseng saponins on lipid peroxidation in liver and cardiac muscle homogenates. *Biochem. Arch.* 6: 359-365.
- Li, J., Huang, M., Teoh, H. & Man, R. Y. (1999) *Panax quinquefolius* saponins protect low density lipoproteins from oxidation. *Life Sci.* 64: 53-62.
- Yan, L. J. & Sohal, R. S. (2000) Prevention of flight activity prolongs the life span of the housefly, *Musca domestica*, and attenuates the age-associated oxidative damage to specific mitochondrial proteins. *Free Radic. Biol. Med.* 29: 1143-1150.
- Liu, Z. Q., Luo, X. Y., Liu, G. Z., Chen, Y. P., Wang, Z. C. & Sun, Y. X. (2003) In vitro study of the relationship between the structure of ginsenoside and its antioxidative or prooxidative activity in free radical induced hemolysis of human erythrocytes. *J. Agric. Food Chem.* 51: 2555-2558.