A randomized controlled crossover trial of the effect of ginseng consumption on the immune response to moderate exercise in healthy sedentary men

Patricia D. Biondo, Sarah J. Robbins, Jennifer D. Walsh, Linda J. McCargar, Vicki J. Harber, and Catherine J. Field

Abstract: Ginseng is a popular herbal remedy that is reputed to increase resistance to stress and improve immune function. Regular exercise results in acute physiologic stress that affects the immune response. This study was conducted to investigate the effects of daily consumption of a standardized ginsenoside-containing North American ginseng (*Panax quinquefolius*) extract on immune function before, during, and after a moderate-exercise protocol in healthy sedentary men. Ten healthy males were randomized to receive either ginseng (1125 mg·d^{-1}) or placebo for 35 days. After a 3 month washout period, subjects received the opposite treatment for another 35 days. An exercise test and blood collection were performed at the end of each treatment period. Immune parameters and blood hormone levels were measured before, during, and after the exercise stress protocol. Ginseng treatment reduced the peripheral blood concentration of CD8+ T cells and increased mitogen-stimulated T cell production of interleukin-2 ex vivo. Ginseng had no effect on total white blood cell counts; on concentrations of neutrophils, monocytes, or lymphocytes (CD3+, CD4+, CD16+, CD20+); on lymphocyte proliferation; or on neutrophil oxidative burst. Ginseng did not significantly affect exercise-induced changes in plasma concentrations of lactate, insulin, cortisol, or growth hormone. The consumption of ginseng for 5 weeks had a limited effect on the immune response to an acute exercise protocol.

Key words: ginseng, ginsenosides, immune system, exercise, randomized controlled trial, sedentary men.

Résumé : Le ginseng est un remède galénique populaire reconnu pour accroître la résistance au stress et améliorer la fonction immunitaire. La pratique régulière de l'activité physique suscite un stress physiologique qui conditionne la réponse immunitaire. Le but de l'étude est d'analyser l'effet de la consommation quotidienne de ginseng nord-américain (*Panax quinquefolius*), contenant une quantité donnée de ginsénoside, sur la fonction immunitaire avant, pendant et après une séance d'exercice physique d'intensité modérée réalisée par de jeunes hommes sédentaires en bonne santé. Dix hommes en bonne santé sont répartis aléatoirement dans deux groupes, l'un consommant du ginseng (1125 mg·d^{-1}) et l'autre, un placebo durant une durée de 35 jours. Après trois mois d'épuration, les sujets changent de groupe. À la fin de chaque période de traitement, on soumet les sujets à une épreuve d'effort et on fait des prélèvements d'échantillons sanguins. Avant, pendant et après l'épreuve d'effort, on évalue les paramètres immunitaires et on mesure les concentrations sanguines de quelques hormones. La consommation de ginseng diminue la concentration sanguine de lymphocytes T CD8+ et augmente la production ex vivo des lymphocytes T IL-2 activés par un mitogène. On n'observe aucun effet du ginseng sur le nombre de globules blancs, la concentration de neutrophiles, de monocytes et de lymphocytes (CD3+, CD4+, CD16+, CD20+), la prolifération des neutrophiles ou sur la stimulation du métabolisme oxydatif des neutrophiles. Le ginseng ne modifie pas significativement les concentrations plasmatiques de lactate, d'insuline, de cortisol et de l'hormone de croissance habituellement observées au cours d'un exercice physique. La consommation de ginseng durant une période de cinq semaines a un effet limité sur la réponse immunitaire observée au cours d'une séance d'exercice physique.

Mots-clés : ginseng, ginsénosides, système immunitaire, exercice physique, essai clinique aléatoire, hommes sédentaires.

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Introduction

Ginseng is a popular herbal remedy that has been used in Asian cultures for thousands of years to treat a variety of conditions. Ginseng is reputed to increase resistance to stress and to enhance immune function (reviewed by Kitts and Hu 2000) and physical performance (reviewed by Bucci 2000). Although the mechanisms underlying these effects of ginseng are unclear, there is an extensive amount of literature examining the effects of ginseng on the cardiovascular system, central nervous system, endocrine system, metabolism, and immune system (reviewed by Bahrke and Morgan 2000).

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The putative active compounds derived from the processing of ginseng root are the ginsenosides (saponin glycosides) and the water-soluble poly- and oligo-saccharides (Tan and Vanitha 2004). Both families of compounds have been shown to modulate parameters of the immune system in vitro and in vivo. When incubated with human peripheral blood mononuclear cells ex vivo or mouse splenocytes in vitro, ginsenoside preparations from the Asian species *Panax ginseng* have been shown to increase lymphocyte proliferation (Lee et al. 2004; Liu et al. 1995), increase interleukin (IL)-2 and IL-4 cytokine production, decrease interferon (IFN)-γ secretion (Lee et al. 2004), and alter the expression of CD25, CD45RO, and CD45RA on peripheral blood lymphocytes (Liu et al. 1995). The consumption of a *P. ginseng* ginsenoside preparation (G115, containing 4% *w*/*w* ginsenosides) by humans for 8–12 weeks increased phagocytic activity and chemotaxis of peripheral blood mononuclear cells (Scaglione et al. 1990), increased the number of total lymphocytes and T helper cells (Scaglione et al. 1990), increased antibody titres, increased natural killer (NK) cell activity, and decreased the incidence of influenza and the common cold (Scaglione et al. 1996). Recent randomized controlled trials have reported significant beneficial effects of the polysaccharide-rich North American ginseng extract COLD-fX on the incidence and severity of acute respiratory illnesses and colds (McElhaney et al. 2004; Predy et al. 2005). However, relatively few studies have focused on the potential immunomodulating effects of ginsenoside-containing extracts of North American ginseng (*Panax quinquefolius*) (Block and Mead 2003).

Although ginseng has been used by athletes as an ergogenic aid for many years, there is an absence of compelling evidence to support its efficacy for this purpose (Bahrke and Morgan 1994). Most of the studies performed have examined the potential effects of mainly whole-root ginseng extracts or standardized ginsenoside-containing extracts in well trained/fit individuals undergoing intense exercise. The effectiveness of the regular consumption of ginseng has not been clearly demonstrated; most studies have reported small (Kim et al. 2005) or no benefits (Dowling et al. 1996; Engels et al. 2001, 2003; Engels and Wirth 1997; Hsu et al. 2005; Kulaputana et al. 2007; Morris et al. 1996).

Regular moderate exercise, particularly by sedentary individuals, results in an acute physiologic stress that affects the immune response (Nieman 2003; Rowbottom and Green 2000). It has been reported that exercise in sedentary individuals increases the concentrations of CD8+ and CD4+ T cells, NK cells, and IL-2-receptor-positive cells in peripheral blood during or immediately after exercise (Ceddia et al. 1999; Gabriel et al. 1993; Rhind et al. 1996; Shore et al. 1999), increases resting concentrations of NK cells (Rhind et al. 1996; Shore et al. 1999), increases the ability of lymphocytes (Ceddia et al. 1999) and neutrophils (Rodriguez et al. 1991) to respond postexercise, and increases postexercise neutrophil phagocytic function (Rodriguez et al. 1991). Few ginseng trials have been performed in which untrained or sedentary individuals undergo moderate exercise. Recently, it was reported that consuming 1.35 $g \cdot d^{-1}$ *P. ginseng* (whole-root powder) improved endurance or time to exhaustion in a group of young untrained adults (Liang et al. 2005), suggesting that ginseng might have greater benefits in these individuals. This hypothesis is supported by the results of an exercise study performed in untrained rodents (Wang and Lee 1998). Our study was conducted to investigate the effects of the daily consumption of a standardized ginsenoside-containing North American ginseng (*P. quinquefolius*) extract on immune function before, during, and after acute physiologic stress (a moderate-exercise protocol).

Materials and methods

Subjects and study design

Healthy male university students between the ages of 18 and 35 years, with a BMI of 20–27 $kg \cdot m^{-2}$ and maximal oxygen consumption ($VO_{2\ max}$) ≤ 50 $mL \cdot kg^{-1} \cdot min^{-1}$, were recruited for the study. Exclusion criteria were smoking; inability to exercise; diabetes, thyroid, renal, or autoimmune disease; and the consumption of any medications or herbal supplements. Ethical approval for the study was obtained from the Human Ethics Review Committee of the Faculty of Agriculture, Forestry, and Home Economics at the University of Alberta, in Edmonton. Subjects were randomized to receive either ginseng or placebo for 35 days. After a 3 month washout period, subjects received the opposite treatment for 35 days. The 3 month washout period was chosen because certain effects of ginseng have been shown to subsist in people for up to 10 weeks after the cessation of treatment (Forgo and Schimert 1985). Dietary intake, physical activity, cardiovascular fitness, body composition, and resting energy expenditure were assessed at the end of each 35 d treatment period. An exercise stress protocol and blood collection were performed at the end of each treatment period, and several immune parameters and blood hormone levels (described below) were measured before, during, and after the exercise stress protocol.

Aerobic fitness assessment

During the screening phase, $VO_{2\ max}$, a measure of aerobic fitness, was determined to establish each participant's ventilatory threshold (VT) for the exercise stress protocol (Bhambhani and Singh 1985). The $VO_{2\ max}$ test was conducted on a Monark 818E cycle ergometer (Varberg, Sweden). Respiratory gases were continuously monitored and were averaged every 15 s on an automated metabolic measurement system (D-series Gas Exchange System, MedGraphics, Calif.). Subjects started pedaling at a resistance of 1.0 kiloponds (kp), and maintained a pedaling frequency of between 60 and 70 $r \cdot min^{-1}$. Every 2 min the resistance was increased by 0.5 kp; after VT was reached, resistance was increased by 0.5 kp every minute until $VO_{2\ max}$ was attained. VT was indicated by a decrease and plateau in the minute ventilataion to carbon dioxide production ratio (VE/VCO_2) before a systematic increase with increased power output and a respiratory exchange ratio of >1.05 (Bhambhani and Singh 1985). Attainment of $VO_{2\ max}$ was determined by a leveling or decrease in VO_2 with increasing workload (0.5 $kp \cdot min^{-1}$); a plateau in heart rate and (or) attainment of age-predicted maximum heart rate; a respiratory exchange ratio >1.1; and volitional fatigue (Thoden 1991).

Ginseng supplementation

North American whole-root ginseng extract powder (CNT

Table 1. Dietary and physical characteristics of 10 study participants after ginseng and placebo treatment.

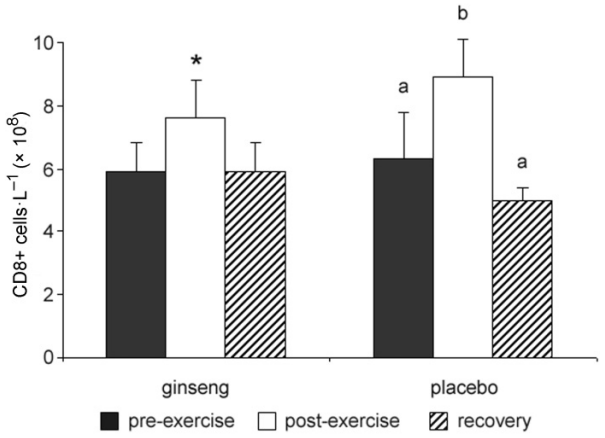
Parameter	Ginseng supplementation*	Placebo supplementation
Energy intake (kcal·d^{-1})	2514±103	2563±177
Carbohydrate intake (g·d^{-1})	359±14	377±33
Protein intake (g·d^{-1})	96±6	106±8
Fat intake (g·d^{-1})	78±8	65±5
Body weight (kg)	74.9±1.5	75.6±1.9
Body mass index (kg·m^{-2})	22.9±0.4	23.1±0.5
Percent body fat	15.6±1.7	16.0±1.9
$VO_{2\ max}$ (mL·kg^{-1}·min^{-1})	43.9±1.8	45.0±1.6
VT (mL·kg^{-1}·min^{-1})	29.8±1.8	29.6±1.0
REE (kcal·d^{-1})	1854±97	1887±87
Physical activity index†	7.6±0.3	7.9±0.4

Note: $VO_{2\ max}$, maximum oxygen consumption; VT, ventilatory threshold; REE, resting energy expenditure. Values are means ± SEM of 10 subjects, with the exception of the dietary intake values, which are the means ± SEM of 8 subjects.

*Randomized crossover design.

†Baecke et al. 1982.

Fig. 1. Effect of diet and exercise on the concentration of CD8+ cells in whole blood. Each bar represents the mean ± SEM (n = 10). Means that do not share a letter are different ($p < 0.05$). Asterisk (*) indicates a significant difference from the placebo postexercise value ($p < 0.05$).



2000) capsules and placebo capsules were provided by Chai-Na-Ta Corp. (Richmond, B.C.). CNT 2000 is produced using a proprietary process, and is standardized to contain 8% *w/v* ginsenosides from North American ginseng (Kitts et al. 2000). The recommended dosage for North American ginseng is 0.5–3.0 g·d^{-1} (Siegel 1979). Participants received 1125 mg of the ginsenoside-enriched extract daily, or placebo (cornstarch), taken in the form of 3 capsules per day. Participants were instructed to take 1 capsule 30 min before eating breakfast, lunch, and supper for the duration of the study. Compliance was monitored with weekly telephone calls, and unused capsules were collected and counted at the end of each study period.

Dietary intake

Dietary intake was assessed with 3 day food records, completed by each participant for 2 weekdays and 1 weekend day around the time of the exercise stress protocol. Food records were reviewed by study staff, who also confirmed the accuracy of the records with each participant. Records were analyzed, using Food Processor II for Windows (ESHA Research, Portland, Ore.), to estimate intakes of total energy and macronutrients.

Test day protocol

The Modified Baecke Questionnaire on Physical Activity (Baecke et al. 1982; Pols et al. 1995) was completed before aerobic assessment. Height and mass were measured, and body composition was determined using hydrodensitometry (Brodie et al. 1998). Residual lung volume was measured with the helium dilution technique, using SensorMedics 2450 Pulmonary Function Cart (Yorba Linda, Calif.). Underwater mass was measured with a computerized strain-gauge system, and percent body fat, fat mass, and fat-free mass were estimated using the equation of Siri (1956).

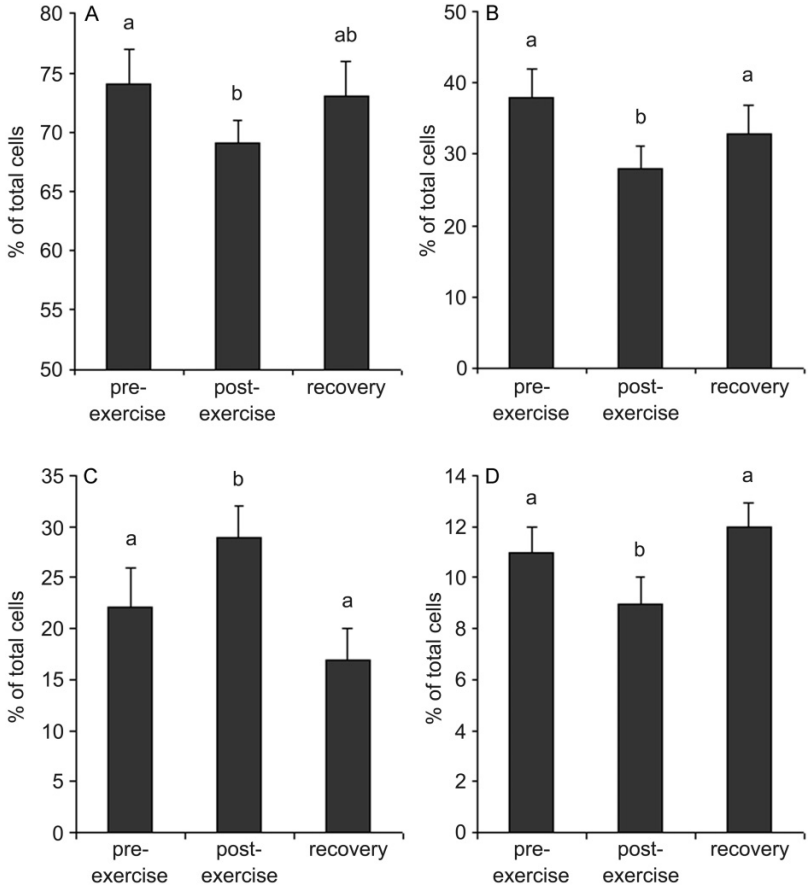
Resting energy expenditure was measured between 0700 and 0900 hours. Participants were in a fasting state and had refrained from intense physical activity for 24 h before the test. Oxygen consumption was measured with indirect calorimetry (Vmax 29N metabolic cart; SensorMedics), using the Weir equation (Weir 1949) to calculate energy expenditure (kcal·d^{-1}). The exercise stress protocol and blood collection were performed at either 1400 or 1530 hours, to control for the diurnal variation in serum cortisol (Thuma et al. 1995). Participants were asked to refrain from physical activity on the day of testing and to stop eating at least 2 hours before the exercise protocol. An intravenous catheter was inserted into the forearm of each subject before the start of the test, and venous blood samples (10 mL) were collected before (at –15 and 0 min), during (at 21 min), and after (at 36, 51, 66, and 96 min) exercise. Participants performed 36 min of continuous exercise, comprised of 6 min of warm-up at 1.5 kp and 50 r·min^{-1}, followed by 15 min at 80% VT, and then 15 min at 100% VT (60 to 70 r·min^{-1}) on a stationary bicycle. VT was determined from the $VO_{2\ max}$ of each participant. Respiratory gases were collected to monitor substrate oxidation and the respi-

Table 2. Effect of exercise on the concentrations of circulating white blood cells, neutrophils, lymphocytes, lymphocyte subsets, and monocytes.

Peripheral blood cell concentation	Pre-exercise (at –15 min)	Postexercise (at 36 min)	Recovery (at 66 min)	Exercise *p* value
Total white blood cells ($\times 10^9$ cells·L^{-1})	5.8±0.3a	7.9±0.4b	5.9±0.3a	<0.0001
Neutrophils ($\times 10^9$ cells·L^{-1})	3.2±0.2a	4.2±0.3b	3.6±0.3a	0.0002
Lymphocytes ($\times 10^9$ cells·L^{-1})	1.9±0.1a	2.8±0.2b	1.7±0.1c	<0.0001
CD3+ cells ($\times 10^8$ cells·L^{-1})	14.6±1.1a	19.0±1.2b	12.1±0.6c	<0.0001
CD4+ cells ($\times 10^8$ cells·L^{-1})	7.3±0.7a	7.5±0.7a	5.6±0.6b	0.0001
CD16+ cells ($\times 10^8$ cells·L^{-1})	4.3±0.5a	8.5±1.0b	2.8±0.4c	<0.0001
CD20+ cells ($\times 10^8$ cells·L^{-1})	2.1±0.2a	2.5±0.3b	2.0±0.2a	<0.0001
Monocytes ($\times 10^8$ cells·L^{-1})	4.3±0.3a	6.7±0.5b	4.5±0.2a	<0.0001

Note: Values are means ± SEM (n = 20). Where a significant effect of exercise was found, differences between timepoints were determined with least-squared means. Within a row, means that do not share a letter are different ($p < 0.05$).

Fig. 2. Effect of exercise on the relative percentage (% of total mononuclear cells) of (A) CD3+ cells, (B) CD4+ cells, (C) CD16+ cells, and (D) CD20+ cells in peripheral blood. Each bar represents the mean + SEM (n = 20). Where a significant effect of exercise was found by repeated-measures analysis, differences between pre-exercise, postexercise, and recovery values were determined with least-squared means. Means that do not share a letter are different ($p < 0.05$).



ratory exchange ratio. Analyzers were calibrated, with precision calibration gases, before, at the beginning of each 15 min bout, and after completion of the 36 min exercise bout.

Immune analysis

Whole blood (3 mL) was collected in EDTA-coated vacutainers at –15, 36, and 66 min, and complete blood cell counts were analyzed using a Coulter STKS instrument (Coulter Electronics Inc., Hialeh, Fla.) and manual differential. A 10 mL sample of blood was collected at –15, 36, and 66 min to estimate the effect of ginseng and exercise on the type of immune cells present, immune proliferation, and neutrophil function. Mononuclear lymphocyte phenotypes were identified with a direct immunofluorescence assay performed on an aliquot of whole blood, as described elsewhere (Pratt et al. 2002*a*). The absolute and relative amounts of each of the major mononuclear cell types were measured to help explain the changes that exercise or ginseng treatment have on immune function. Commercial monoclonal antibod-

ies, labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or biotin, were used to identify T cells (CD3-Biotin; Serotec, Raleigh, N.C.), T helper cells (CD4-FITC; Sigma-Aldrich Canada Ltd., Oakville, Ont.), T cytotoxic cells (CD8-PE; Sigma-Aldrich Canada Ltd.), monocytes (CD16-FITC; BD Pharmingen, Mississauga, Ont.), B cells (CD20-PE; Serotec), and antigen-mature (CD45RO-FITC; Serotec) and -naïve (CD45RA-Biotin; Serotec) T and B cells. All biotin-labeled antibodies had an additional 30 min incubation with strept-avidin-QR (quantum red; Sigma-Aldrich), and then all cells were fixed with PBS, containing 1% (*w*/*v*) paraformaldehyde (Anachemia Science, Montreal, Que.), and analyzed within 72 h by flow cytometry (FACScan; Becton Dickinson, Sunnyvale, Calif.), in accordance with relative fluorescence intensity, using CellQuest software (Becton Dickinson). Neutrophil function was estimated from the oxidation of dihydrorhodamine 123 (Molecular Probes, Eugene, Ore.) after the stimulation of whole blood with phorbol myristate acetate (3.2×10^3 nmol·L^{-1}; ICN Biomedicals Inc.), as described in detail elsewhere (Pratt et al. 2002*b*). From the remaining blood, peripheral mononuclear cells and plasma were isolated on a Ficoll gradient of Histopaque-1077 (Sigma-Aldrich Canada Ltd.), as described elsewhere (Field et al. 2000). The isolated cells were resuspended in complete culture medium (RPMI 1640; Invitrogen, Burlington, Ont.), containing 4% (*v*/*v*) fetal calf serum, 2.5% HEPES, 0.1% 2.5 μmol mercaptoethanol, and 1% antibiotic/antimycotic (Invitrogen). Cells were seeded in triplicate in 96-well microtiter plates at 200 000 cells per well, and were cultured with or without phytohemagglutinin (PHA; 5 ng·L^{-1}) for 48 and 72 h at 37 °C and 5% CO_2. Eighteen hours before harvesting the cells, each well was pulsed with 0.5 μCi [^{3}H]-thymidine (Amersham Biosciences, Baie D'UrfÕ, Que.). Stimulation indices were calculated as follows, where [^{3}H]-thymidine incorporation is expressed in decays per minute (decays·min^{-1}):

$$\frac{[^3\text{H}]\text{-thymidine incorporated by stimulated cells} - [^3\text{H}]\text{-thymidine incorporated by unstimulated cells}}{[^3\text{H}]\text{-thymidine incorporated by unstimulated cells}}$$

Cytokine production was measured as described elsewhere (Pratt et al. 2002*a*). Briefly, peripheral mononuclear cells (1×10^6 cells, isolated as above) were cultured in 1 mL of complete culture medium with or without PHA (5 ng·L^{-1}) for 48 h at 37 °C with 5% CO_2. The concentration of IL-2 was measured in the supernatants from the stimulated cells, using an OptEIA ELISA kit for human IL-2 (BD Pharmingen), in accordance with the manufacturer's instructions, and read immediately at 450 nm on a plate reader (Model EL309; Bio-Tek Instruments Inc., Winooski, Vt.).

Blood metabolite and hormone analysis

Lactate and hormone concentrations were measured in duplicate at all timepoints. Venous blood samples (7 mL) were analyzed for lactate and hormonal concentrations; 0.5 mL was deproteinized in 4% perchloric acid, set on ice for 5 min, centrifuged at 4 °C and 4000 r·min^{-1}, and then analyzed spectrophotometrically for lactate (Sigma Chemical Co. 1981). The remaining 6.5 mL of blood was allowed to clot at room temperature and was then centrifuged at 4 °C and 4000 r·min^{-1}, to obtain serum for hormone analysis. Serum was aliquotted and stored at –80 °C. Cortisol, growth hormone, and insulin concentrations were determined with radioimmunoassay, using commercial assay kits (Diagnostic Products Corporation, Los Angeles, Calif.).

Statistics

All statistical analyses were conducted using the SAS statistical program, version 8.1 (SAS Institute Inc. Cary, N.C.). Paired dependent *t* tests were conducted to compare the effects of ginseng and placebo on aerobic fitness, dietary intake, physical activity indices, and body composition. A crossover-design mixed-model repeated-measures analysis (Wang and Goonewardene 2004) was used to determine the effects of dietary treatment and exercise on peripheral blood cell counts, immune cell phenotypes, lymphocyte proliferation, cytokine production, neutrophil oxidative burst, and blood hormone levels measured before, during, and (or) after the exercise test. The appropriate covariance structure for each dataset was determined as per Wang and Goonewardene (2004). When a significant main effect (diet or exercise) or interaction effect (diet × exercise) was found, differences between groups were identified with lsmeans. When dietary treatment had no effect, diet groups were combined to report the effect of exercise on the measured parameters. For all measures, $p < 0.05$ was accepted as statistically significant. Results are presented as means ± standard errors of means.

Results

Participant characteristics

Fourteen healthy male university students (age, 25.1 ± 5.0 years) enrolled in the study. Ten subjects completed the trial and 4 withdrew for personal reasons. All participants reported that they were in good health before and throughout the study. Two subjects reported mild insomnia and hot flashes during the first few days of ginseng treatment; however, these side effects subsided after 1 week of treatment. Energy intake, body composition, $VO_{2\ max}$, VT, resting energy expenditure, and physical activity did not differ significantly between the treatments (Table 1). Compliance, determined from telephone calls and returned capsules, was deemed to be excellent. One of the 10 subjects returned 3 unused capsules at the end of the first treatment period, and another returned 1 unused capsule at the end of the second treatment period.

Table 3. Effect of exercise on [^{3}H]-thymidine incorporation by lymphocytes after 48 or 72 h stimulation with and without PHA (n = 20).

	Pre-exercise (at –15 min)	Postexercise (at 36 min)	Recovery (at 66 min)	Exercise p value
48 h UNS (decays·min^{-1})	1733±426a	2366±460a	3294±635b	0.007
48 h PHA (decays·min^{-1})	66627±10119	74415±11640	78904±11806	NS
48 h PHA (SI)	80±30	53±15	38±9	NS
72 h UNS (decays·min^{-1})	3638±736a	4464±736a	5775±843b	0.008
72 h PHA (decays·min^{-1})	162294±16163	172159±19501	177060±13607	NS
72 h PHA (SI)	76±16a	58±11ab	44±8b	0.043

Note: UNS, unstimulated; PHA, phytohemagglutinin; SI, stimulation index, calculated as $\frac{[^3\text{H}]\text{-thymidine incorporated by stimulated cells} - [^3\text{H}]\text{-thymidine incorporated by unstimulated cells}}{[^3\text{H}]\text{-thymidine incorporated by unstimulated cells}}$; NS, not significant. Values are means ± SEM (n = 20). Because ginseng had no significant effect, diet groups were pooled to study the effect of exercise on immune function. Where a significant effect of exercise was found, differences between pre-exercise, postexercise, and recovery values were determined with lsmeans. Within a row, means that do not share a letter are different ($p < 0.05$). No significant interactions between exercise and diet were found (p values not shown).

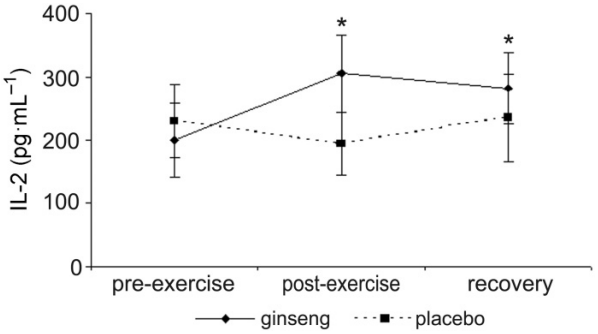
Peripheral blood cell concentrations and immune cell phenotypes

The concentration of CD8+ cells was significantly higher postexercise (at 36 min) during the placebo period than during the ginseng period (Fig. 1, $p < 0.05$). Ginseng had no effect on other peripheral blood cell concentrations or on the relative proportion of each phenotype, so the data were combined from the ginseng and placebo periods to determine the effect of a bout of exercise on the immune cells in peripheral blood (Table 2). Total white blood cells and the concentrations of circulating neutrophils, monocytes, total lymphocytes, CD3+, CD16+, and CD20+ cells were significantly higher immediately postexercise, and returned to pre-exercise levels or below during the recovery period (Table 2). The concentration of CD4+ cells was significantly lower at recovery (66 min) than at pre- and postexercise timepoints. All blood cell concentrations for all subjects were within normal reference ranges throughout the experiment. Immediately postexercise, the relative percentages of CD3+, CD4+, and CD20+ cells were lower ($p < 0.05$) than pre-exercise values, whereas the relative percentage of CD16+ cells was higher ($p < 0.05$) (Fig. 2). At recovery (66 min), the proportions of different immune cell types present in peripheral blood were not different from pre-exercise values. CD45RA+ and CD45RO+ cells were assayed at 36 min only. Neither ginseng nor placebo had any effect on the percentage of CD4+CD45RO+ cells (14 ± 3; n = 20 observations), CD4+CD45RA+ cells (15 ± 3; n = 16), CD8+CD45RO+ cells (8 ± 2; n = 18), or CD8+CD45RA+ cells (24 ± 4; n = 20), or on their absolute concentrations (data not shown).

Peripheral mononuclear cell [^{3}H]-thymidine incorporation

Ginseng had no effect on [^{3}H]-thymidine incorporation after 48 or 72 h in culture, so the data from the ginseng and placebo periods were combined. After 48 or 72 h in culture, unstimulated cells had a higher ($p < 0.05$) rate of [^{3}H]-thymidine incorporation at recovery (66 min) than at the pre-exercise timepoint (Table 3). After 72 h stimulation with PHA, the stimulation index (reflecting the rate of [^{3}H]-thymidine incorporation over baseline levels) was lower at recovery (66 min) than at the pre-exercise timepoint ($p < 0.05$; Table 3).

Fig. 3. Effect of ginseng and exercise on in vitro lymphocyte interleukin (IL)-2 production. Values are means ± SEM (n = 8 per treatment group). *Within the ginseng-treatment group, post-exercise and recovery IL-2 concentrations were significantly higher than pre-exercise concentrations (p = 0.002 and 0.014, respectively).



Lymphocyte IL-2 production

In vitro production of IL-2 was not detected in unstimulated lymphocytes (data not shown). Among PHA-stimulated cells, there was a significant interaction between dietary treatment and exercise (p = 0.013), such that during the ginseng-treatment period, IL-2 concentrations were higher immediately postexercise than they were pre-exercise (p = 0.002), and remained higher at recovery (p = 0.014; Fig. 3). IL-2 production did not change significantly throughout the exercise test during the placebo period.

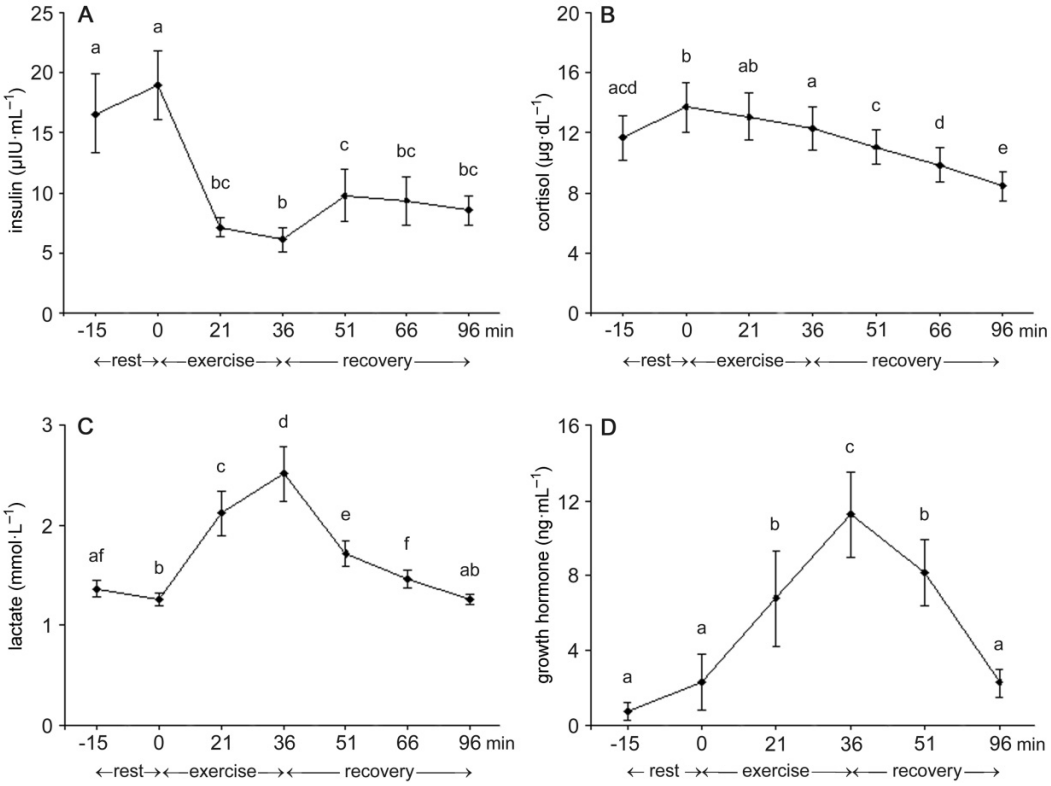
Neutrophil oxidative burst

The oxidative burst activity before (0 min) and after (5, 10, 15 min) phorbol myristate acetate stimulation was not significantly altered by either dietary treatment or exercise (data not shown).

Peripheral blood hormone levels

Ginseng consumption did not significantly affect the plasma concentrations of insulin, cortisol, growth hormone, or lactate during rest, exercise, or recovery, so the data were combined from the ginseng and placebo periods. Plasma insulin concentrations decreased during the exercise protocol and, at 96 min, remained lower than pre-exercise concentrations ($p < 0.05$; Fig. 4A). Plasma cortisol levels were highest immediately before exercise, and decreased

Fig. 4. Effect of exercise on plasma concentrations of (A) insulin, (B) cortisol, (C) lactate, and (D) growth hormone. Ginseng had no effect on the measured blood hormones or on lactate; therefore, values are means ± SEM (n = 20). Where a significant effect of exercise was found by repeated-measures analysis, differences between timepoints were determined with lsmeans. Means that do not share a letter are different ($p < 0.05$).



throughout the exercise test (Fig. 4B). Plasma lactate concentration rose throughout the exercise test, was highest at 36 min (immediately postexercise), and by 96 min had decreased to pre-exercise concentrations (Fig. 4C). Similarly, growth hormone levels rose during the exercise protocol, peaked immediately postexercise (36 min), and returned to pre-exercise levels by 96 min (Fig. 4D).

Discussion

This study is among the first to measure the effects of ginseng consumption on the immune response to an acute bout of exercise in sedentary adults. Similar to studies performed in trained athletes, we observed that ginseng had limited effects on the immune response to a moderate bout of acute exercise. The ginseng-induced effects on immune function were limited to changes in the concentration of peripheral blood CD8+ T cells and in vitro IL-2 production. Ginseng treatment attenuated the increase in peripheral blood CD8+ T cells that was observed immediately postexercise during the placebo period. Also, ginseng, but not placebo, increased PHA-stimulated IL-2 production immediately postexercise and at recovery. Several reports have indicated that acute exercise reduces IL-2 production (Espersen et al. 1990; Rhind et al. 1996; Tvede et al. 1993). Our results suggest that ginseng prevents this effect after moderate exercise. PHA is a polyclonal T cell mitogen (Lis and Sharon 1998), which stimulates both T helper 1 and CD8+ T cytotoxic cell populations to produce IL-2 (Gaffen and Liu 2004). We observed no differences in the concentration of CD4+ T cells between the ginseng and placebo periods, but saw a lower postexercise concentration of CD8+ T cells with ginseng than with placebo. Although there is no ideal ratio of T cell phenotypes, it is well established that the relative and absolute concentrations of the different T cell populations in blood can influence T cell function. In this case, changes in the relative concentration of CD8+ cells might have contributed to the higher production of PHA-stimulated IL-2 production immediately postexercise. The lower proportion of CD8+ cells in the blood after ginseng treatment is suggestive of a lower concentration of a T-suppressor cell subpopulation of CD8+ cells. It has been reported that T-suppressor cells inhibit IL-2 production by CD4+ cells (Filaci and Suciu-Foca 2002; Jiang and Chess 2004); therefore, the lower proportion of CD8+ cells after the ginseng treatment might have facilitated the higher production of IL-2 by CD4+ cells after PHA stimulation. However, there was no difference between treatments in the proliferative response to PHA, suggesting that further research is needed to determine the physiologic implications of the effect of ginseng on T-suppressor cells.

The physiologic significance of increased IL-2 production immediately postexercise and during recovery is not known. Epidemiologic studies have suggested that intense, prolonged exercise temporarily suppresses the immune system and increases one's susceptibility to infectious agents (Nieman 2003; Pedersen and Hoffman-Goetz 2000). IL-2 plays an important role in the anti-viral immune response

(Harari et al. 2006), and production is reportedly reduced during and after exercise (Espersen et al. 1990; Rhind et al. 1996; Tvede et al. 1993). Thus, it is possible that the higher IL-2 production after ginseng treatment reflects an increased ability of lymphocytes to respond to an immunologic challenge, and might reduce postexercise susceptibility to infection. However, more research is needed to follow-up on this finding, with a particular emphasis on other anti-viral immune responses (e.g., IFN-γ production, NK cell activity).

Despite the popularity of ginseng for its reputed ergogenic properties, few studies have identified significant benefits. And any reported benefits have been small, such as the change in lipid peroxidation and scavenger enzyme levels and the 1.5 min improvement in time to exhaustion (Kim et al. 2005). In the study by Kim et al. (2005), very high doses (6 g of ginsenoside-containing *P. ginseng* extract daily) and high-intensity exercise were administered. Engels et al. (2003) found that ginseng (400 mg·d^{-1} of G115, containing 4% *w*/*w* ginsenosides, for 8 weeks) had no effect on secretory immunoglobulin A levels in saliva before or after intense exercise. In healthy fit males, the daily administration of 3 g of a ginsenoside-containing North American ginseng extract for 8 weeks did not alter lactate threshold or aerobic performance (heart rate, exercise time, peak power output, or rate of fat oxidation) (Hsu et al. 2005). In healthy young women, consuming 400 mg·d^{-1} of *P. ginseng* (G115) for 8 weeks had no ergogenic benefits during short supramaximal exercise or in the recovery stage (Engels et al. 2001). A recent review reported that there was insufficient evidence to conclude that Siberian ginseng offered any ergogenic benefits during exercise that ranged in duration from 6–120 min (Goulet and Dionne 2005). Consistent with other studies performed after acute high-intensity (Kulaputana et al. 2007), moderate-intensity (Allen et al. 1998), or resistance exercise (Youl Kang et al. 2002), our study showed no significant effect of ginseng treatment on the plasma concentrations of insulin, cortisol, lactate, or growth hormone before or after exercise.

The effects of exercise on immune function that we found are consistent with previous reports. It is well documented that leukocyte and lymphocyte cell populations in peripheral blood increase significantly during exercise (Nehlsen-Cannarella 1998; Pedersen and Hoffman-Goetz 2000). Similarly, the decrease in lymphocyte concentrations (i.e., CD3+, CD4+, and CD16+ cells) during the recovery period to below pre-exercise levels has been well established (Pedersen and Hoffman-Goetz 2000). Such effects have been attributed to sustained cortisol production after intense exercise, because cortisol induces lymphopenia (Pedersen and Hoffman-Goetz 2000). However, in our study, cortisol levels declined throughout the exercise and recovery periods. It has been reported that cortisol levels increase with exercise of long duration (Pedersen and Hoffman-Goetz 2000), so it is possible that the moderate-exercise protocol used here was too short or of inadequate intensity to increase plasma cortisol concentrations, and that other mechanisms are responsible for the postexercise decrease in lymphocyte numbers. Consistent with the findings of our study, it has been reported that lymphocyte proliferation (estimated by the uptake of ^{3}H-thymidine after stimulation with a polyclonal T cell mitogen) decreases in the recovery period postexercise (Nieman 2000; Pedersen and Hoffman-Goetz 2000). This effect has been largely attributed to the decline in T cell numbers postexercise (Pedersen and Hoffman-Goetz 2000). We observed a decrease, below pre-exercise levels, in concentrations of T cells, primarily CD4+ T cells. Despite what would be predicted from moderate-intensity exercise studies (Pedersen and Hoffman-Goetz 2000), we did not observe a stimulatory effect of exercise on neutrophil oxidative burst activity. It is possible that the exercise intensity in our study (mean intensity of just less than 70% $\dot{V}O_{2\ max}$) was between moderate and heavy, which neither stimulated nor impaired neutrophil function.

Herbs have been used throughout history to enhance physical performance and immune function, but controlled clinical trials have only recently been conducted to verify these claims. Consumption of a standardized North American ginseng extract for a 5 weeks had a limited effect on the immune response to an acute-exercise protocol. Ginseng treatment reduced the concentration of peripheral blood CD8+ T cells and increased the mitogen-stimulated T cell production of IL-2 ex vivo. However, ginseng had no effect on other parameters of immune function (i.e., other leukocyte and lymphocyte cell concentrations, lymphocyte proliferation, or neutrophil oxidative burst).

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