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CHINESE HERBAL MEDICINE, PHYSICAL EXERCISE, AND VASCULAR FUNCTION

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CHINESE HERBAL MEDICINE, PHYSICAL EXERCISE, AND VASCULAR FUNCTION

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Dedication

To my wife, Yun-Ling, and to my family: my father, my mother, and my brother

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CHINESE HERBAL MEDICINE, PHYSICAL EXERCISE, AND VASCULAR FUNCTION

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Inflammation has been implicated the potential mechanism in pathogenesis of vascular dysfunction and acute unaccustomed exercise-induced inflammatory response was found associated with increase in central arterial stiffness. This dissertation first evaluated the significance of exercise-induced transient arterial stiffening by comparing different exercise models and we found downhill running exercise was able to elicit higher systemic inflammatory response than local eccentric resistance exercise, thus, prolonged the arterial stiffening effects in the following days. In addition, such stiffening effects did not result in significant changes in hemodynamic wave reflection on both exercise modes. Next, we studied the effects of short-term Chinese herb supplementation, *panax ginseng* and *salvia miltiorrhiza*, prior to downhill running exercise on preserving vascular functions. Measurements were compared in the herb and placebo groups by using a double-blinded randomized design. Our results demonstrated that the Chinese herb effectively attenuated the increase in arterial stiffness. Furthermore, a long-term supplementation with eccentric resistance exercise training also using double-blinded design was investigated to understand whether chronic eccentric training would result in elevated arterial stiffness and whether Chinese herb could also attenuate the adverse effects if any. Our data showed long-term weekly eccentric training did not impair vascular function and supplementation appeared to attenuate the hypertrophic effects

induced by exercise, but did not affect anti-oxidant status after intervention. In conclusion, we found the extent to which eccentric exercise induce arterial stiffening was inflammatory response-dependent, and *panax ginseng* and *salvia miltiorrhiza* supplementation appears to be effective in preserving vascular function in acute exercise model by lowering pro-inflammatory response following exercise, whereas the effects of long-term supplementation did not support our hypothesis, which requires more intervention studies to investigate its effectiveness and potential adverse effects on skeletal metabolism.

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Chapter I: Introduction

Cardiovascular disease (CVD) is the leading cause of deaths in most developed countries. More than one in three people have one or more types of cardiovascular diseases, including high blood pressure, coronary heart disease, heart failure, and stroke in the United States (4). Aging is unequivocally considered the dominant risk factor for CVD, not only because it is accompanied with changes in cardiovascular structure and function (141), but also it increases the time of cardiovascular structure exposed to multiple cardiovascular risk factors (190, 278). Currently, over 35 million people at 65 years of age or older exist in the United States, and this number will be expected to double by 2030. Accordingly, substantially greater CVD prevalence is anticipated in the future, which increases critical social and economic burden to the society (26, 190). The increase in arterial stiffness and endothelial dysfunction with advancing age are two most important clinical vascular changes that contribute to pathophysiology of CVD (236). Effective strategy and treatments to prevent and control these vascular dysfunctions deserve more attention by scientists and healthcare professionals.

It has been well recognized that chronic imbalances in endogenous pro-oxidants/ antioxidants contribute to cardiovascular disease development (76, 134, 292). In recent years, oxidative stress and inflammation have been closely linked with arterial stiffening and endothelial dysfunction (21, 58, 75). Antioxidant supplementation and regular physical activity have been identified as potential means in alleviating oxidative stress (133), yet the evidence supporting the efficacy of these interventions for disease prevention remains inconclusive. Short-term antioxidant supplementation, such as ascorbic acid (vitamin C) and/or α-tocopherol (vitamin E), appears to improve endothelial function in populations characterized by high oxidative stress (68, 71, 215). In contrast, chronic supplementation failed to improve vascular

function (71, 128) or rather increased oxidative stress (277) and mortality (13). Regular physical activity, especially aerobic exercise training, has been advocated for cardiovascular health because of the repeated increases in blood flow and shear stress, which contributes to the up-regulation of nitric oxide (NO) synthase, enhanced NO production, as well as improved anti-inflammatory function (133, 292). Indeed, aerobic exercise training can reverse arterial inflammation (157) and endothelial dysfunction (65) that occur with advancing aging. In contrast to aerobic exercise, resistance exercise training does not appear to be favorable for vascular health (180, 181).

A number of studies have shown that unaccustomed exercise accompanied with rigorous eccentric muscle contraction can trigger inflammatory responses characterized by leucocyte and cytokine release, resulting in excessive reactive oxygen species (ROS) production and consequent oxidative stress after exercise (161, 207, 209, 268). Notably, the increase in ROS is believed to be a possible cause of exercise-induced muscle damage as the mechanical stress produced on skeletal muscle during lengthening contraction (271) results in calcium leakage, inflammation response, and subsequent necrosis in muscle tissues (85). Thus, the available evidence indicates that muscle-damaging exercise is associated with inflammatory responses although it is not known how the increase in ROS production/oxidative stress would contribute to impaired vascular function. A recent study reported that muscle damage induced by eccentric resistance exercise produced transient unfavorable effects on macro-vascular function in humans (10), which was associated with the exercise-induced inflammation. This raises an important concern as resistance training program is increasingly prescribed for people with higher risk for cardiovascular diseases (133). Whether eccentric exercise involving higher inflammatory response would elicit more pronounced arterial stiffening effects is unknown. Further studies

elucidating this cause-effect relation are necessary. Although inflammatory responses induced after a single bout of exercise can be blunted with "repeat bout effect" of eccentric exercise (174), it is remains unknown whether long-term eccentric resistance exercise training that accumulate repeated inflammatory responses will result in increased arterial stiffness. Therefore, more research is needed to clarify the relation between exercise-induced inflammation and vascular function.

Chinese herbal medicine has been widely practiced for thousands of years in China and its surrounding countries. For CVD, herbal treatment has been used in patients with congestive heart failure, hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, and arrhythmia (169). In recent years, there is an increasing popularity among CVD patients pursuing complementary and alternative remedies in addition to or in lieu of conventional pharmacological treatments in the U.S. and other developed countries. As such, research efforts targeting effectiveness and mechanisms of Chinese medicine, particularly herbal medicine, have been increasing. Herbal formulas are typical prescriptions in Chinese medicine, which is characterized by adapting several types of herbs or minerals as a combination of multiple components that could attack different pathological targets in complex diseases. Such "cocktail" type of herb formulae design is often found to act synergistic manners on diseases with minimal adverse effects in vitro. Interestingly, recent studies (63, 292) suggested that antioxidant mixture, involving ascorbic acid, tocopherol, and α-lipoic acid, appears to be more efficacious in reducing age-related increases in oxidative stress and ameliorating the reduction in brachial artery vasodilation than a single antioxidant treatment (71, 292). Therefore, Chinese herb supplements or formulas may provide similar cocktail treatment effects on restoring redox state and diseases. Despite the fact that Chinese herbs are widely used in humans, scientific evidence supporting its

effects on vascular function remains lacking. Uncovering possible effects underlying cardiovascular protection afforded by Chinese herbs may enhance its clinical use and lead to new treatment strategies.

Chapter II: Statement of the Problem

- The increase in CVD prevalence in the aging society is becoming a more serious and critical issue from the societal and economical perspectives in many developed countries. Increasing efforts should be made in discovering new and effective strategies in preventing and treating CVD
- Herbal treatments for CVD have been practiced in the East for centuries and are gaining popularity in many western countries in recent years, yet its scientific evidence on therapeutic efficacy is still lacking.
- Eccentric exercise has been demonstrated to increase arterial stiffness and elevate the risk of developing CVD presumably through its impact of oxidative stress and inflammation. The association between exercise-induced oxidative stress, inflammation, and arterial stiffness remain unclear.
- The overall aims of these studies were to determine whether eccentric exercise resulting in a higher inducible inflammatory response would contribute to greater arterial stiffening effects, and whether supplementation using Chinese herbal medicine could ameliorate vascular dysfunction from acute and chronic perspectives.

Study 1 focused on investigating the association between eccentric exercise-induced inflammation and vascular function by employing 2 types of exercises that could elicit different levels of inflammation.

Study 1 Specific Aims. To determine whether aerobic-biased downhill running that involved greater muscle mass would contribute to more pronounced arterial stiffening than localized

eccentric resistance exercise and whether such arterial stiffening effect would further influence central hemodynamic measures.

Study 2 focused on investigating the preventive effects of acute Chinese herb supplement with *panax ginseng* and *salvia miltiorrhiza* prior to eccentric exercises on arterial stiffness and vascular reactivity by using a double-blinded randomized control design.

Study 2 Specific Aims. To determine whether herb supplementation of *panax ginseng* and *salvia miltiorrhiza* prior to exercise would reduce arterial stiffening and other dysfunctions in vascular measures.

Study 3 focused on determining the effects of a 12-week progressive eccentric resistance exercise training on vascular functions, and whether a long-term supplementation would ameliorate arterial stiffening effects elicited by exercise-induced inflammation and improve endothelial function by using a double-blinded randomized control design.

Study 3 Specific Aims. To determine the efficacy of short-term herb supplementation of *panax ginseng* and *salvia miltiorrhiza* in preserving arterial elasticity and vascular reactivity along with eccentric resistance exercise training.

Chapter III: Experimental Design

In order to accomplish the aims of this dissertation study, we first conduct a cross-sectional study investing whether arterial stiffening occurs more pronouncedly in whole-body downhill running than localized eccentric resistance exercise and whether such effects would be associated with changes in central hemodynamics after exercise. Subsequently, two studies utilizing a randomized, double-blinded, placebo-controlled, study design were conducted. The second study investigated whether a supplementation of Chinese herbs could acutely attenuate muscle damage, inflammatory response, central arterial stiffening and other vascular function changes that were induced by an acute bout of downhill running exercise. The third study explored whether a short-term supplementation of *panax ginseng* and *salvia miltiorrhiza* would enhance anti-oxidant and anti-inflammation effects, and improve vascular functions when they are administered with eccentric resistance exercise in older individuals.

Chapter IV: Study #1

Delayed Onset Vascular Stiffening Induced by Eccentric Exercises: Eccentric Resistance Exercise and Downhill Running

ABSTRACT

Eccentric exercise is known to induce muscle stiffening and soreness as well as unfavorable changes in macrovascular function. We tested the hypothesis that systemic eccentric exercise could evoke greater arterial stiffening than local eccentric resistance exercise. Twenty healthy young men were randomly assigned into either the downhill running (DR) group that performed a 30-min downhill running or the eccentric resistance exercise (RE) group that performed repeated eccentric muscle contractions. Muscle soreness and plasma creatine kinase concentrations increased similarly following exercise in both groups. Pulse wave velocity (PWV) increased significantly at 48 hr post-exercise in both groups and remained elevated at 72 hr in DR. C-reactive protein was elevated at 24 and 48 hr in DR, and 48 hr in RE. The increases in PWV were associated with the corresponding elevations in CRP in DR ($r=0.70$, $p<0.05$). There were no changes on arterial wave reflection measures. In conclusion, both systemic and localized eccentric exercise modes induced delayed onset vascular stiffening with more prolonged changes observed in downhill running. The effect on arterial stiffening was not accompanied by changes in arterial wave reflection but was associated with systemic inflammatory responses.

INTRODUCTION

Epidemiological studies have documented a strong, independent, and inverse relation between physical activity and cardiovascular mortality (135). Evidence from cross-sectional (262) and longitudinal (186, 237) studies indicates that habitual aerobic exercise is effective in preventing and reversing arterial stiffening that happens with advancing age (263). Resistance exercise training, in contrast, appears to exert unfavorable effects on arterial wall function at least in young adults (60, 180, 181). Resistance exercises involving eccentric muscle contractions induce muscle damage and lead to leukocyte infiltration and production of pro-inflammatory cytokines within local muscle tissue as well as into systemic circulation (95). Inflammation has been linked to vascular disease and cardiovascular events, and acute eccentric exercise may exert adverse influence on vascular health. Indeed, studies have shown that acute eccentric exercise impairs microcirculation (118, 119), augments vascular resistance (218), and reduces vascular reactivity (46, 82). We (10) and others (23) have demonstrated that acute eccentric exercise transiently induced unfavorable change in macrovascular function as evaluated by increased central arterial stiffness. However, studies in this area are still very limited.

Studies investigating the delayed onset muscle soreness showed that inflammatory responses after eccentric exercise are dependent on muscle groups and muscle mass recruited since eccentric-biased whole body endurance exercise produced higher inflammatory responses than local resistance exercises (207). Therefore, it is plausible to hypothesize that dynamic systemic eccentric exercise could evoke greater arterial stiffening than local eccentric resistance exercise. However, this hypothesis has not been tested. To do so, we have utilized whole-body downhill running and localized quadriceps resistance exercises to induce muscle soreness. It is not known whether such arterial stiffening effects would further impose central hemodynamic

burden following exercise. In this context, to address this question, we measured arterial wave reflection parameters as they are significant predictors of long-term cardiovascular mortality (286).

METHODS

Participants. A total of 20 apparently healthy young male adults (18-35 years old) were recruited from the neighboring area and randomly assigned into one of the eccentric exercise conditions after pre-screening and familiarization. Exclusions from the study participation were due to: (1) obesity (BMI>30 kg/m^2); (2) smoking within past six months; (3) hypertension (high blood pressure >140/90 mmHg) according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; (4) personal history of diabetes (fasting blood glucose >126 mg/dL), heart disease or other cardiovascular problems; (5) orthopedic injury that may prevent him or her from completing the exercise; or (6) the use of over-the-counter supplements or vitamins. Subjects must be sedentary or recreational active, but not participating in any type of resistance or endurance training. All participants gave written informed consent, and all procedure were reviewed and approved by the local Institutional Review Board.

Experimental design. Participants were randomly assigned to eccentric resistance exercise (RE) or downhill running exercise (DR) conditions. Prior to the main experiments, familiarization trials involving a set of submaximal inclined leg press exercise (8-10 repetitions without weights) or a 5-10 min walking exercise on treadmill (depending upon the group assignment) were performed. For the subjects assigned to RE, one-repetition maximum (1RM) strength of incline leg press was performed. The subjects completed 4-5 sets of leg press with a plate-loaded on the Cybex machine. Weight started low for the first set and progressively

increased. Strength was determined from muscular failure during the last set of 1 repetition. For the subjects assigned to DR, individual peak aerobic power (VO2peak) was determined using standard American College Sports Medicine protocol. After a 5-min warm-up on the treadmill, subjects walked or ran while treadmill slope was increased 1% every minute until the subjects could not continue the test. A mouthpiece and heart rate monitor were worn to collect expired air and assess heart rate throughout the test

All the tests were performed at the same time of day during morning hours for every participant. An overnight, 8 hour fast was required before the testing day. The measurements were taken at baseline, 90 minutes, 24 h, 48 h, and 72 h after an acute bout of respective eccentric exercise. Participants were asked to maintain their regular diet and lifestyles throughout all the testing sessions.

Eccentric resistance exercise. The subjects in the RE condition performed 6 sets of 10 maximal eccentric contractions on an incline leg press machine. Each subject was carefully instructed to maximally resist the movement with 2 legs using the knee extensors while lowering the weight (~3 seconds of muscle contraction). The weight was lifted to the starting position by the investigators, and 12 seconds of rests were given after each eccentric movement. The eccentric load was set at 120% of each subject's bilateral leg press 1RM. The amount of weight was reduced if participants were unable to complete the repetitions on the 3-second count at the calculated weight. Participants were allowed to rest for 2 minutes between the sets.

Downhill running exercise. Subjects warmed up for 5 min on a level grade at the speed that could elicit 75% of pre-determined individual VO_2peak (3). Each subject performed downhill running exercise on treadmill with the same speed at -10° of slope for 30 minutes. This protocol has been successfully adapted elsewhere to induce delayed onset muscle soreness (207).

All the subjects in the DR group were able to complete the protocol without discomfort during the exercise.

Muscle soreness. The subjects were asked to rate the perception of muscle soreness by using a Visual Analog Scale (VAS). A subjective scale ranged from 0 to 10 with 0 describing no soreness and 10 describing unbearable soreness (48).

Arterial stiffness. The subjects were instructed to rest quietly for 10 minutes prior to measurement. Arterial stiffness was measured using carotid-femoral pulse wave velocity (cfPWV), which was calculated from the traveling distance and foot-to-foot wave transit time between two arterial recording sites in the supine position (261). Doppler flowmeter (810B, Parks Medical Electronics, Aloha OR) connected to a physiological signaling processing system (MP36, Biopac, Goleta CA) was used to detect pulse waves on the carotid and femoral arteries.

Arterial wave reflection. Carotid pressure waveforms were measured noninvasively using pulse wave tonometer (SPT301, Millar, Houston TX) and calibrated by brachial blood pressure (261). Wave reflection parameters were derived from the calibrated pressure waveforms by using the validated triangulation method (286, 290) and analyzed by custom designed software written in Matlab (The Mathworks, Natick MA). Augmentation index normalized for heart rate of 75 bpm (AI_{75}), pressure amplitude of forward (Pf) and backward (Pb) waves, reflected wave transit time (RWTT), and characteristic impedance (Zc) were derived.

Blood sample. A blood sample (~10 mL) was acquired by venapuncture after an overnight 8 hour fast. Plasma and serum were centrifuged, aliquoted into microcentrifuge tubes, and stored at -80°C for later analysis. Blood concentrations of cholesterol, triglyceride, HbA1C were measured enzymatically. Serum creatine kinase (CK) and high sensitive C-reactive protein (CPR) were assessed in duplicate with the use of commercial ELISA kits.

Statistical Analyses. Descriptive statistics was used for the analyses of subject characteristics using SPSS statistical package (version 16.0; Chicago, IL). Dependent variables were analyzed separately within each treatment to determine the time effect using repeated measures ANOVA. A two-way mixed model ANOVA was used for analyses of time and treatment effects. Associations were determined by Pearson product-moment correlations. Significance was set *a priori* at $P<0.05$.

RESULTS

Selected subject characteristics are presented in Table 1. There were no significant differences in age, body mass index, heart rate, brachial blood pressure at rest, fasting lipid profiles, HbA1C between the two groups. Acute bout of systemic and localized eccentric exercises significantly increased muscle soreness at all the time points examined (Figure 1). There were no significant differences in muscle soreness between the groups. Creatine kinase, a marker of muscle damage, increased significantly at 24 hr, 48 hr, and 72 hr in both groups (Table 2). As shown in Table 2, two groups were not different in baseline central hemodynamics. Neither DR nor RE elicited significant changes in carotid artery pressure, AIx_{75}, Pf, Pb, RWTT and Zc. A measure of arterial stiffness, cfPWV, increased significantly at 48 and 72 hr post exercise in DR and at 48 hrs post exercise in RE (all $P<0.05$). When the PWV data were expressed in relative changes from the baseline, increases in PWV remained significant and no significant differences were observed between the groups (Figure 2).

CRP was significantly increased at 24 and 48 hrs post exercise in the DR group and 24 hr post exercise in the RE group. A positive relation ($r=0.70$, $P<0.05$) was found between changes in CRP and cfPWV at 48 hr post exercise in the DR group (Figure 3). No significant association was observed in the RE group.

DISCUSSION

The major findings of this study are as follows. First, both eccentric resistance exercise and downhill running produced similar levels of muscle soreness and delayed onset vascular stiffening (DOVS) as demonstrated by significant increases in cfPWV. Second, such arterial stiffening effects appeared to be more prolonged in downhill running group in spite of the fact that increases in creatine kinase were much smaller. Third, downhill running elicited more pronounced increases in CRP than eccentric resistance exercise. The elevation in CRP was correlated with the corresponding increases in cfPWV, indicating that systemic inflammation may have played a role in post exercise arterial stiffening. Fourth, a various measures of arterial wave reflection were not altered with either eccentric exercise, suggesting that eccentric exercise does not appear to modulate central hemodynamics.

Chronic inflammation has been studied in relation with vascular health (162). A systemic inflammatory marker, CRP, has been associated with the development of atherosclerosis (225, 226), arterial stiffness (171, 302) and future CVD events (225). The causal effects of acute inflammation on arterial stiffness have been investigated by using vaccination models (106, 280), in which the significant positive relations were observed between hs-CRP, interleukin-6, and cfPWV (280). Muscle fiber damages induced by eccentric muscle contractions are accompanied by a series of local and systemic inflammation (207). Such inflammation is likely to translate adverse effects to vasculature. Indeed, an acute bout of eccentric exercise has been shown to increase central arterial stiffness (10, 23) and impair endothelial-dependent vasodilation (46, 82). However, the relationship between exercise-induced inflammation and arterial stiffening remains unclear. Considering that exercise-induced inflammatory response is dependent on muscle mass recruited (207), we hypothesized that eccentric-biased whole-body

endurance exercise (i.e., downhill running) could evoke greater arterial stiffening than more localized eccentric resistance exercises. As hypothesized, serum CRP has a faster temporal change and a higher relative magnitude of increase following downhill running than those of eccentric resistance exercise. Additionally, increases in cfPWV following an acute bout of downhill running were significantly correlated with changes in hs-CRP from baseline, suggesting that acute systemic inflammation associated with muscle damage could also translate unfavorable effects to the vasculature. The increase in central arterial stiffness was associated with the degree of muscle damage (10) and soreness (23) in previous studies. In the present study, however, no such association was observed. The participants in current study were relatively sedentary (<1hr physical activity per week) and had lower aerobic and muscular fitness levels, which could partially explain why we found more pronounced effects in the present study. Indeed, individuals who have lower levels of physical activity or fitness tend to have higher hs-CRP (214, 269) and responded more drastically in arterial stiffening to an acute inflammation challenge (106).

Muscle damage induced by eccentric contractions has been well characterized by morphological changes in myofibrils and connective tissues followed by leucocyte infiltration, cytokine production and subsequent increase of reactive oxygen species (ROS) and inflammation (207), which introduce the potential underlying mechanism by which ROS induced by muscle damage leads to endothelial dysfunction and increases the arterial stiffness. Indeed, ROS production is believed to diminish nitric oxide bioavailability (80) and increase aortic stiffness (58). Recent studies showed that brachial flow-mediated vasodilation (FMD) was impaired following acute upper (46) and lower (82) body eccentric resistance exercises. However, reduced NO production cannot fully account for arterial stiffening effects induced by eccentric muscle

contractions as FMD can be dissociated from PWV (132). Blood pressure changes after exercise may also affect the measure of PWV, but this may not be the case since both eccentric exercises elicited no significant changes in brachial and carotid blood pressure in the present study. Future research is warranted to elucidate the precise mechanism underlying vascular stiffening.

Arterial wave reflection occurs when propagating pressure wave encounters bifurcations of conducting arteries, resistant microvasculature, and stiffening arteries (147). Eccentric exercise has been shown to cause arterial stiffening (10) and augment peripheral vascular resistance (218). Therefore, we sought to determine whether eccentric exercise would elicit changes in central hemodynamics and arterial wave reflections. With stiffening of the vasculature, reflected waves may appear earlier in the systole due to higher traveling velocity (or reduced RWTT), producing aortic pressure augmentation resulting in higher AI. Consistent with previous findings (23), neither eccentric resistance exercise nor downhill running elicited significant changes in AI_{75} in the present study. Unlike AI, the Pb is the time-independent backward wave reflection parameter providing information on arterial function coming back from the periphery to the central and has recently been shown as a significant predictor for cardiovascular mortalities (286). The magnitude and return time of the reflected wave did not change in either group despite the fact that Zc tended to increase after exercise in both groups. Our results indicate that eccentric exercise and the associated arterial stiffening did not produce any changes in arterial wave reflection parameters regardless of eccentric exercise modes. To our knowledge, this the first study to evaluate the association between eccentric exercise, muscle damage, and central hemodynamic changes.

In recent years, eccentric-biased exercise training has received more attention from clinical perspectives since this particular mode of exercise training requires lower

cardiopulmonary demand and induces less fatigue during exercise (98, 273). Additionally, it appears to exert more favorable metabolic and anti-inflammatory effects (64) and provide better functional advantages in increasing muscle strength for cardiac patients (177, 251). The clinical significance of our findings that eccentric exercise induces arterial stiffening is unclear as we conducted the present study in young healthy subjects with very low baseline arterial stiffness. It is also not clear whether arterial stiffening after an acute bout of eccentric exercise persists or disappears when eccentric exercises were performed repeatedly. Potentially, it may subside or disappear similar to the training effects observed in the delayed onset muscle soreness.

There are several other limitations of this study that need to be mentioned. First, a time control trial was not included in the present study. Second, we did not collect physical activity data during the follow-up period. However, subjects were reminded of the restrictions to maintain their usual lifestyle during the follow-up periods. Third, there is no proper or universally-accepted way to equate eccentric resistance exercise and downhill running. We have contemplated on this issue prior to the study initiation and decided to simply follow typical systemic and localized eccentric exercise modes that have been used in the literature. In this context, it is important to note that both exercise modes produced similar levels of muscle soreness ratings.

In conclusions, delayed onset vascular stiffening was observed after both localized (eccentric resistance exercise) and more systemic (downhill running) eccentric exercises. Such arterial stiffening was more prolonged after downhill running than resistance exercise although muscle soreness scales were similar and CK concentrations were smaller in downhill running. In the downhill running condition, arterial stiffening was associated with systemic inflammatory responses. These results suggest that eccentric exercises that result in muscle damage induce

delayed onset arterial stiffening effects, at least in part, through the heightened inflammatory responses.

Perspectives

Our current understanding of delayed onset vascular stiffening (DOVS) remains limited. In line with previous literature, DOVS was observed following different eccentric exercises in the present study; downhill running characterized with higher systemic circulation translated prolonged unfavorable effects on vasculature partially due to inflammation. All of which provided additional evidence for our understanding of DOVS. On the other hand, the clinical significance of DOVS is still unclear, but carotid wave reflection analysis in this study indicated central hemodynamic measures following exercise were not changed in young sedentary adults. Future study investigating its effects in older population and consequence of repeated eccentric exercise is needed.

Table IV.1 Selected subject characteristics

	Downhill Running (n=10)	Resistance Exercise (n=10)
Age, years	22±1	22±1
Height, cm	172±1	174±1
Body mass, kg	67±2	64±1
Body mass index, kg/m^2	23±1	21±1
Heart rate at rest, bpm	68±4	66±3
Systolic BP, mmHg	117±2	117±3
Diastolic BP, mmHg	67±3	69±2
Mean BP, mmHg	84±2	85±2
Triglyceride, mg/dL	80±11	80±17
Total cholesterol, mmol/L	4.5±0.3	3.9+0.3
HDL cholesterol, mmol/L	1.6±0.1	1.3±0.1
LDL cholesterol, mmol/L	2.4±0.2	2.1±0.3
HbA1C, %	5.1±0.3	5.3±0.1
Muscle strength, kg	-	242±20
VO_2peak, ml/kg/min	49.1±2.4	-

Data are mean±SEM. BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; HbA1C = hemoglobin A1C; VO_2peak = peak oxygen consumption.

Table IV.2 Central hemodynamics, arterial stiffness and biomarkers during downhill running (DR) and eccentric resistance exercise (RE)

	Pre	90min	24hr	48hr	72hr
Carotid systolic BP, mmHg					
DR	108±3	111±4	110±3	109±4	113±4
RE	114±4	109±4	113±3	112±4	113±4
Carotid diastolic BP, mmHg					
DR	65±3	65±3	67±3	67±2	66±2
RE	67±2	69±3	67±2	68±3	69±2
Carotid mean BP, mmHg					
DR	82±2	82±3	85±3	84±2	85±3
RE	85±3	85±2	85±3	86±3	86±3
Carotid AI_{75}, %					
DR	-2.9±9.1	4.6±9.9	4.4±8.0	8.5±6.1	2.5±8.5
RE	7.9±7.0	8.1±8.8	-3.5±9.5	-5.7±8.5	-1.8±7.7
Forward pressure, mmHg					
EE	39±3	42±3	38±2	38±3	42±3
RE	40±3	36±4	41±3	42±3	40±4
Backward pressure, mmHg					
DR	15±1	15±2	15±1	15±1	16±1
RE	17±1	14±1	17±1	14±1	16±2
RWTT, ms					
DR	293±38	284±49	303±55	244±28	309±44
RE	244±28	256±46	322±54	320±40	317±50
Zc, dyne/sec/cm^5					
DR	159±14	161±13	169±11	169±15	179±17
RE	160±16	169±22	180±16	185±15	165±18
cfPWV, cm/s					
DR	448±15	472±25	477±20	525±28*	521±31*
RE	453±18	457±16	495±15	512±26*	478±21
CRP, mg/dL					
DR	0.07±0.03	0.08±0.04	0.36±0.27*	0.33±0.20*	0.27±0.18
RE	0.04±0.01	0.05±0.01	0.11±0.06	0.13±0.07*	0.07±0.03
Creatine kinase, U/L					
DR	102±17	134±19	389±123*	221±66*	336±106*
RE	92±15	138±22	1322±682*	2355±1099*	13557±779*

Data are mean±SEM. BP = blood pressure; AI_{75} = augmentation index at hear rate of 75 beats/min; RWTT = reflected wave transit time; Zc = characteristic impedance; cfPWV = carotid-femoral pulse wave velocity; CRP = c-reactive protein.

Figure IV.1 Changes in muscle soreness scale following eccentric exercise

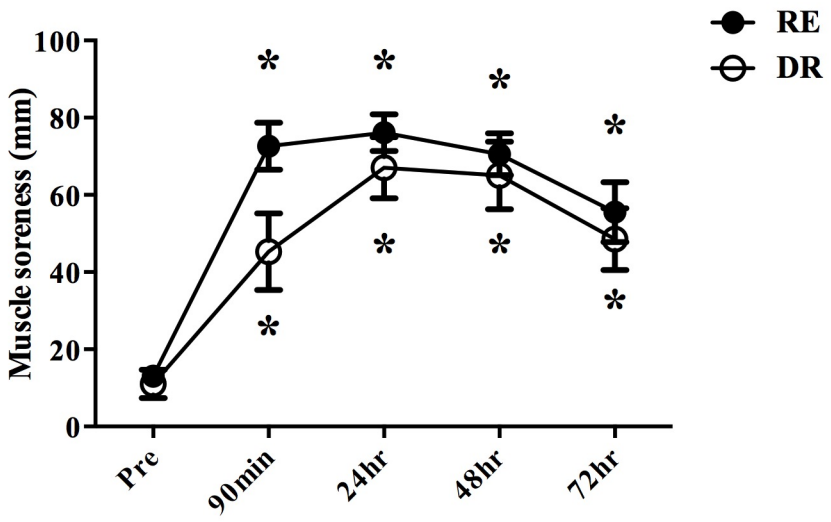


Figure IV.2 Changes in arterial stiffness as assessed by cfPWV with eccentric exercises

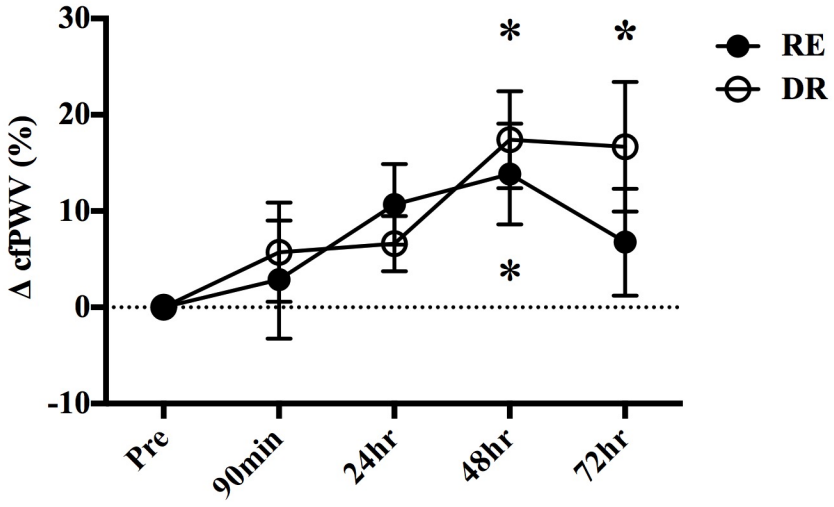
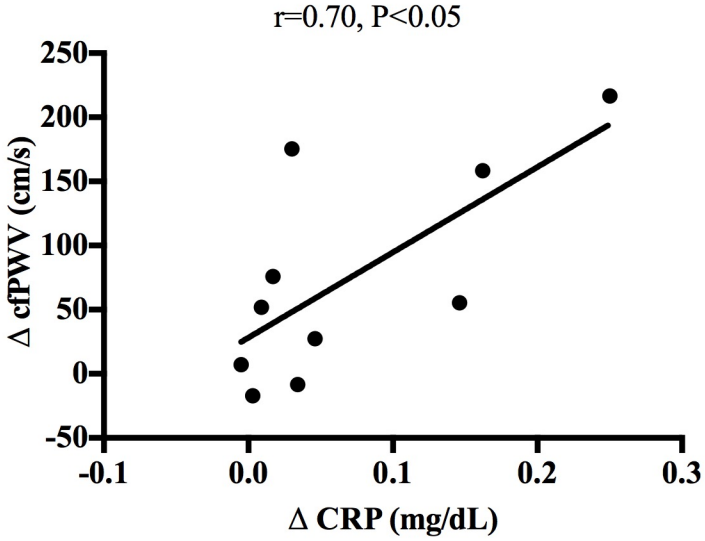


Figure IV.3 Association between changes in C-reactive protein (CRP) and carotid-femoral pulse wave velocity (cfPWV)



Chapter V: Study #2

Effects of Chinese Herb Supplementation on Delayed Onset Vascular Stiffening Induced by Acute Eccentric Exercise

ABSTRACT

Muscle damage induced by unaccustomed or eccentric exercise induces delayed onset vascular stiffening. We tested the hypothesis that a 7-day supplementation of *panax ginseng* and *salvia miltiorrhiza* prior to an acute eccentric exercise could reduce arterial stiffening. By using a double-blinded randomized design, subjects were assigned to either the Chinese herb (N=12) or the placebo group (N=11). Each subject performed a downhill running trial and a control trial (seated rest) and was followed for 3 days after each trial. Muscle soreness increased and active range of motion decreased 1-2 days after exercise similarly in both groups. Plasma creatine kinase concentration increased significantly at 24 hr in both groups but the magnitude of increase was attenuated in the herb group. Arterial stiffness as measured by pulse wave velocity increased significantly at 24 hr in the placebo group but such increase was absent in the herb group. Flow-mediated dilation, an index of endothelial-dependent vasodilation, did not change in either group. Plasma concentrations of CPR and IL-6 increased in the placebo groups but no such increases were observed in the herb group. There were no significant differences in tiobarbituric acid-reactive substances and tumor necrosis factor-α (TNF-α) levels between the groups. Changes in arterial stiffness induced by eccentric exercise were associated with the corresponding changes in IL-6 ($r= 0.46$, $P<0.05$). In conclusion, a short-term herb supplementation of *panax ginseng* and *salvia miltiorrhiza* ameliorated the delayed onset vascular

stiffening induced by acute downhill running exercise. Such effects were associated with the attenuation of oxidative stress and systemic inflammation.

INTRODUCTION

Muscle damage induced by unaccustomed or eccentric exercise is associated with increases in oxidative stress, inflammatory response, and delayed onset muscle soreness. (207). The increases in circulating pro-inflammatory cytokine and C-reactive protein (CRP) are the characteristic responses induced by eccentric exercise (22, 268). A growing body of evidence indicates that muscle damage may also exert adverse influences on vascular function a day or two days later on a similar time frame to muscle soreness. An impairment in microcirculation (118, 119), an increase in vascular resistance (218), and a reduction in vascular reactivity (46, 82) have been observed after acute eccentric exercise. We (10) and others (23) have demonstrated that acute eccentric exercise induced significant increases in central arterial stiffness and arterial stiffening after eccentric exercise was associated indicators of muscle damage (10).

In Chinese Medicine, Ginseng is one of the most commonly used herbs in over thousands of years (307). Ginsenosides, the major compounds of ginseng, and its metabolites are considered to exert protective effects on the vasculature, acting as a free radical scavenger (115) and increasing nitric oxide production and antioxidant effects (166). There have been a number of animal studies demonstrating that supplementation with either Asian ginseng (*panax ginseng C. A. Meyer*) or American ginseng (*panax quinquefolium L.*) could effectively protect against eccentric or strenuous exercise-induced muscle damage by attenuating CK release (25, 72) and inflammatory responses (306). Danshen (*salvia miltiorrhiza*) is another widely used Chinese medicinal herb with diverse pharmacological properties to improve circulation and blood stasis (89), including dilating coronary arteries, increasing coronary blood flow, and scavenging free radicals in ischemic diseases (109). Indeed danshen has been prescribed to treat angina pectoris,

hyperlipidemia, acute ischemic stroke (312), and coronary heart disease (44). The major compounds of *danshen,* Tanshinone IIA and salvianolical acid B, have been shown to suppress vasoconstrictor endothelin-1 production (264) and reduce the expression of vascular adhesion molecules in vitro (42, 265). *Panax ginseng* and *danshen* are often mixed in herb formulas in Chinese medicine, which is characterized by adapting several types of herbs or minerals as a combination of multiple components that could synergistically attack different pathological targets (159). Given that a mixture of *panax ginseng* and *danshen* has been proposed as a formula to treat cardiovascular disease in Chinese Medicine (159), we hypothesized that the supplementation with a combination of *panax ginseng* and *danshen* could preserve muscle cell permeability and exert protective effects on the vasculature following eccentric exercise.

METHODS

Participants. A total of 23 apparently healthy young male adults were recruited from the neighboring area. Exclusions from the study participation were due to: (1) obesity (BMI >30 kg/m^2); (2) smoking within past six months; (3) hypertension (high blood pressure >140/90 mmHg) according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; (4) personal history of diabetes (fasting blood glucose >126 mg/dL), heart disease or other cardiovascular problems; (5) orthopedic injury that may prevent him or her from completing the exercise; or (6) the use of over-the-counter supplements or vitamins. Subjects must have been sedentary or recreational active, but not been participating in any type of resistance or endurance training. All participants gave their written informed consents, and all procedure were reviewed and approved by local Institutional Review Board.

Experimental design. Subjects were assigned into either the Chinese herb supplement or the placebo group after pre-screening and familiarization. In each group, subjects underwent

two familiarization sessions followed by a pre-testing session that consists of the measurements of aerobic power and body composition and a control trial. Eccentric exercise trials took place following a 7-day supplementation. Subjects were asked to keep their regular diet and sedentary lifestyle throughout the testing sessions.

Aerobic power. Individual peak aerobic power (VO_2peak) was determined using standard American College Sports Medicine protocol. After a 5-min warm-up on the treadmill, subjects walked or ran while treadmill slope was increased 1% every minute until the subjects could not continue the test. A mouthpiece and heart rate monitor were worn to collect expired air and assess heart rate throughout the test. VO_2peak was used to set the exercise intensity during the eccentric exercise.

Body composition. Percent body fat was measured noninvasively by using a bioimpedance analyzer Inbody 2.0 (Biospace Co. Ltd., Seoul, South Korea). To avoid the hydration effects, the test was performed in the morning when subjects were fasted.

Supplement administration. Following the pre-testing sessions, subjects were asked to take 7 capsules in total of either herb or placebo capsules per day (in the morning and the afternoon). Herb supplement was prepared in capsules consisting of 250 mg of *panax ginseng* and 250 mg *salvia miltiorrhiza* extracts, whereas placebo capsule contained microcrystalline cellulose. Both capsules were identical in appearance. All supplement products were prepared by the Brion Research Institute, Sun Ten Pharmaceutical Co., LTD. Analyses of ginsenosides of *panax ginseng* as well as Salvianolic acid B and Tanshinone IIA in Radix *salvia miltiorrhiza* were performed by using high-performance liquid chromatography-electrospray mass (HPLC-MS) spectrometry method as previously described (50, 101). These chromatographic

quantification results of active compounds in herb supplement are shown in Table 1, Figures 1A and 1B).

Exercise protocol. Subjects were instructed to fast at least 8 hours and refrain from any strenuous exercise for at least 72 hours before the test. Experimental trial consisted of baseline measurements, downhill running (eccentric exercise) or seated rest (control), and measurements during the recovery period. In order to eliminate diurnal variation of inflammatory response to eccentric exercise, participants were asked to perform eccentric and control trial at the same time of day. Subjects warmed up for 5 min on a level grade at the speed that could elicit 75% of pre-determined individual VO_2peak (3). Each subject performed downhill running exercise on treadmill with the same speed at -10° of slope for 30 minutes. This protocol has been successfully adapted elsewhere to induce delayed onset muscle soreness (207).

Measurements. The measurements were made 30 minutes pre, 90 minutes post, 24h post, 48h post, and 72h post. Subjects were studied at the same time of day, during the morning hours to minimize the inconvenience of the 8-hour fast and to avoid diurnal effects.

Blood samples were collected to determine metabolic risk factors, markers of muscle damage, inflammation, and redox state. Serum CK was used as an indicator of muscle membrane permeability or muscle damage (233). Inflammatory markers (TNF-α, IL-6) as well as blood redox status marker, tiobarbituric acid-reactive substances (TBARS), were analyzed with the use of commercial ELISA kits only in the eccentric exercise condition.

Heart rate and blood pressure were measured in the supine position. Heart rate was measured using an ECG, and blood pressure was measured using an automatic blood pressure monitor (Omron HEM907).

Muscle soreness. Subjects were asked to rate the perception of muscle soreness by rating the soreness using a Visual Analog Scale (VAS) of 0-10 with 0 describing no soreness and 10 describing unbearable soreness immediately after a downhill running (48). In addition, active range of motion (AROM) was measured while the subjects was placed on bed in prone position with full knee extension and then moved both legs gradually to the flexion point where pain in quadriceps muscle groups was experienced. A manual goniometer was used to measure the knee angle difference from full extension to flexion point with the initiation of pain. This test was repeated three times, and the average was used for statistical analysis.

Arterial stiffness. Arterial stiffness was measured using carotid-femoral pulse wave velocity (cfPWV), which was calculated from the traveling distance and foot-to-foot wave transit time between two arterial recording sites in the supine position (261). Non-invasive pulse tonometer (SPT-301, Millar Inc. Houston TX) connected to a physiological signaling processing system (MP36, Biopac, Goleta CA) was used to detect pulse waves on the carotid and femoral arteries.

Vascular reactivity. Flow-mediated dilatation was measured noninvasively at the brachial artery using standardized procedure (52). Brachial artery diameter was measured using an ultrasound machine (Sonosite Ultrasound System; Bothell, WA) equipped with a high-resolution linear array transducer. A blood pressure cuff was placed on the forearm 3-5 cm distal to the antecubital fossa, and longitudinal images of the brachial artery were acquired 5-10 cm proximal to the antecubital fossa. After the acquisition of baseline measurement, the probe position was clearly marked to ensure that the image is acquired from the same location. The blood pressure cuff was inflated to 100 mmHg above resting systolic blood pressure for 5 minutes by using a customized rapid inflation system. After cuff deflation, ultrasound-derived measurements of

artery diameters were taken for 3 minutes. FMD was calculated by the following equation: (maximum diameter – baseline diameter) / baseline diameter × 100. All ultrasound images will be recorded and analyzed by the same investigator who was blinded to the groups.

Statistical Analyses. Descriptive statistics were used for the analyses of subject characteristics using SPSS statistical package (version 16.0; Chicago, IL). Dependent variables were analyzed within each treatment to determine the time effect using repeated measures ANOVA. A 2-way mixed model ANOVA was used for analyses of time and treatment effects. Bonferroni post-hoc analysis was performed when significance was achieved. Associations were determined by Spearman rank correlations. Significance was set *a priori* at $P<0.05$.

RESULTS

Selected subject characteristics are presented in Table 2.2. All the subjects were non-obese, normolipidemic, and normotensive. There was no significant difference in body composition, lipid profile, and baseline hemodynamic parameters between the placebo and the Chinese herb groups.

An acute bout of downhill running exercise increased muscle soreness significantly at 90 min, 24 hr and 48 hr post exercise (Figures 2.2A). As shown in Table 2.3, the magnitude of the increase in plasma CK concentration was significantly greater in the placebo group than in the herb group. Active range of motion (AROM) decreased significantly at 24 hr and 48 hr post exercise in both groups (Figures 2.2A and 2.2B). In the placebo group, the reduction in active range of motion remained significant at 72 hr post exercise.

An acute bout of eccentric exercise increased cfPWV at 24 hr post exercise in the placebo group (Figure 2.4). No such significant increase in cfPWV was observed in the herb group.

There were no changes in blood pressure in both groups (Table 2.3). As shown in Figure 2.5, there was no significant change in FMD.

Plasma CRP concentration increased significantly at 24 hr post eccentric exercise in both groups (Figure 2.4). Plasma TBARs and TNF-α concentrations did not change in either group. However, plasma IL-6 concentration increased significantly at 90 min after eccentric exercise in the placebo group and decreased at 24 hr post exercise in the herb group (Table 2.4).

The associations between increases in cfPWV and changes in selected biomarkers in combined groups are shown in Table 5. No significant associations were found between changes in CK, CRP, TBARs and cfPWV at any measured time points. Changes in IL-6 were associated with changes in cfPWV at 48hr post exercise ($r=0.46$, $P<0.05$). In addition, changes in TNF-α were associated with changes in cfPWV at 24 to 48hr after exercise ($r=0.57\sim0.60$, $P<0.05$).

DISCUSSION

The major findings of this study are as follows. Seven days of herb supplementation of *panax ginseng* and *salvia miltiorrhiza* prior to downhill running exercise did not affect muscle soreness or active range of motion but abolished the increase in arterial stiffness induced by muscle damage. This destiffening effect was independent of blood pressure changes as arterial pressure did not change in both groups. A lack of changes in arterial stiffness with the herb supplementation was in part associated with the attenuation of increases in inflammatory markers. These results suggest that the Chinese herb supplementation may be an effective strategy to minimize the delayed onset vascular stiffening.

The presence of elevated plasma CK has been recognized a marker of increased sarcolemma permeability or muscle damage resulted from unaccustomed exercise or eccentric muscle contractions (5, 8, 233). In the present study, an acute bout of downhill running exercise

increased plasma CK concentration significantly following eccentric exercise. The increase in plasma CK concentration was greater in the placebo group than in the herb group. Previous studies in animal models reported that North American ginseng, *panax ginseng* or *panax quinquefolus,* decreased plasma CK levels after eccentric exercise (24, 72) and preserved mitochondria integrity (25) and decreased macrophage infiltration (306) in skeletal muscle. One recent human study (113) also found that American ginseng decreased CK concentration at 72 hr post exercise when compared with the placebo control. Collectively, these results suggest that Chinese herb supplementation may have reduced the amount of muscle damage induced by eccentric exercise. However, such effects were not observed in muscle soreness scale or active range of motion.

Downhill running significantly increased arterial stiffness at 24 to 48 hr after the eccentric exercise in the placebo group. However, such arterial stiffening effects were abolished when the subjects were supplemented with Chinese herbs for 7 days before the unaccustomed exercise was performed. These effects were not related to changes in blood pressure because none of the blood pressure measures changed in either group. To our knowledge, this is the first study to demonstrate that Chinese herb supplementation effectively prevented the adverse effects of muscle damage on the vasculature.

The underlying mechanisms by which muscle damage induced from eccentric exercise resulted in increased arterial stiffness remain unclear. Arterial stiffening following eccentric exercise has been associated with subjective muscle soreness (23) and a marker of muscle damage (i.e., plasma CK) (10). We have previously found that the increase in arterial stiffness was significantly associated with the increase in CRP (Study 1). In the present study, plasma IL-6 levels increased significantly after eccentric exercise in the placebo group. Previous

studies using the vaccination model demonstrated that the acute inflammation induced by a vaccination increased IL-6 and CRP concentrations (106, 280) as well as the increase in arterial stiffness (302). These results suggest that arterial stiffening induced by muscle damage is associated with markers of muscle damage and/or systemic inflammation.

Consistent with this concept, we found that Chinese herb supplementation minimized the increases in IL-6 levels. Additionally, changes in arterial stiffness were significantly associated with the corresponding changes in plasma IL-6 concentrations in the present study. It is possible to speculate that the supplementation using *panax ginseng* and *salvia miltiorrhiza* reduced systemic inflammation induced by eccentric exercise and through such effect, minimized the arterial stiffening effects. Our present results using Chinese herbs as "cocktail" anti-oxidants are in accordance with Fisher et al. (77) who found that supplementation with the mixture of vitamins C and E attenuated the IL-6 mRNA expression and CRP response to long-duration muscle contractions in humans.

In addition to central arterial stiffness, endothelium-dependent vascular reactivity was also measured in this study. Eccentric resistance exercise has been shown to impair FMD and linked with the increase in reactive oxygen species and a subsequent reduction in NO (46, 82). In the present study, however, there were no significant reductions in FMD in either the placebo or herb group. The increase in oxidative stress and inflammation have been documented in association with endothelial dysfunction (62, 75), and anti-oxidant status could play a role in modulating vascular function after eccentric exercise (56). There were significant increases in inflammatory markers after eccentric exercise in the present study. The discrepancy between our findings and others on FMD could be attributed to the difference in exercise mode and measured time points. Acute resistance exercise (211, 275) has been shown to reduce FMD,

whereas FMD increases after an acute bout of aerobic exercise (93, 267). The present study utilized acute downhill running exercise as a mode of eccentric exercise. FMD is also known to display biphasic response after acute exercise in that FMD decreases immediately but increases 1 to 24 hr following exercise and reversed to the baseline in 48 hr (56). In the study (46) that found reductions in FMD after eccentric resistance exercise, measurements were made 45 min after exercise. We performed the measurement of FMD at 90 min post exercise. Additionally, the difference in subject characteristics may be another factor since FMD may respond differently following exercise in people of different physical fitness levels (56, 93).

There are a number of limitations in the present study that should be mentioned. First, the number of subjects studied is relatively small. Second, even though pharmacological compounds of *panax ginseng* and *salvia miltiorrhiza* were identified, it is unknown which component of the herb supplementation was effective. Additionally, pharmacological interactions between the compounds are also unknown.

In conclusion, a short-term Chinese herb supplementation incorporating *panax ginseng* and *salvia miltiorrhiza* was effective in ameliorating the delayed onset vascular stiffening induced by acute eccentric exercise, possibly via the reductions in oxidative stress and systemic inflammation.

Table V.1 Selected subject characteristics

	Placebo (n=11)	Herb (n=12)
Age, yr	24±1	26±5
Height, cm	173±1	174±3
Body mass, kg	68±2	68±3
BMI, kg/m^2	23±1	22±1
Body fat percentage, %	19±1	18±2
Waist-hip ratio	0.85±0.01	0.84±0.01
Heart rate, bpm	67±3	58±3
Systolic BP, mmHg	118±2	112±3
Diastolic BP, mmHg	66±2	62±2
VO_2peak, ml/kg/min	47±2	47±2
HDL cholesterol, mg/dL	53±2	53±3
LDL cholesterol, mg/dL	97±9	86±5
Total cholesterol, mg/dL	180±9	192±8
Triglyceride, mg/dL	78±9	58±8
HbA1C, %	5.4±0.1	5.4±0.1

Values are means±SEM. BMI=body mass index. BP=blood pressure, VO_2peak=peak oxygen consumption, HbA1c=glycosylated hemoglobin A1c.

Table V.2 Quantitative analyses of major compounds of the herb supplement

	Compound	Content (mg/g)
Panax ginseng	Rb1	2.24
	Re	1.13
	Rg1	1.47
Salvia miltiorrhiza	Salvianolia acid B	28.2
	Tanshinone IIA	0.6

Table V.3 Hemodynamic responses in control and eccentric exercise sessions

		Pre	90 min	24 hr	48 hr	72 hr
cfPWV, cm/s						
Placebo	Control	530±19	535±17	546±16	547±15	513±13
	Exercise	531±12	548±19	577±20*	570±24	552±22
Herb	Control	482±14	491±17	490±14	487±13	505±19
	Exercise	523±20	497±17	503±16	500±13	505±23
Heart rate, bpm						
Placebo	Control	63±4	58±3	62±3	66±3	65±3
	Exercise	62±3	69±4*	66±3	63±3	59±5
Herb	Control	57±3	54±1	56±3	59±4	55±3
	Exercise	56±2	65±4*	56±3	55±2	53±3
Systolic BP, mmHg						
Herb	Control	119±2	116±2	118±2	119±2	119±2
	Exercise	119±2	114±2	120±2	120±3	117±2
Placebo	Control	114±2	111±2	114±2	115±3	115±2
	Exercise	116±3	114±3	115±2	112±2	111±3
Diastolic BP, mmHg						
Placebo	Control	66±2	67±1	67±2	64±2	67±2
	Exercise	66±3	65±2	65±3	66±2	64±2
Herb	Control	63±3	63±1	62±2	62±1	62±1
	Exercise	63±3	61±2	62±1	63±3	60±1
Pulse pressure, mmHg						
Placebo	Control	52±2	49±1	51±2	55±2	53±2
	Exercise	53±2	48±2	55±2	53±2	53±1
Herb	Control	51±3	48±3	52±3	54±3	53±3
	Exercise	53±3	52±3	53±2	49±3	51±3

Values are means±SEM. cfPWV=carotid-femoral pulse wave velocity, BP=blood pressure.
*$P<0.05$ vs. Pre in the same condition.

Table V.4 Changes in muscle damage markers, inflammatory and oxidative stress markers in response to downhill running exercise in the placebo and herb group.

	Pre	90 min	24 hr	48 hr	72 hr
Placebo					
CRP, mg/dL					
Control	0.07±0.04	0.07±0.04	0.07±0.03	0.06±0.02	0.05±0.02
Exercise	0.08±0.02	0.11±0.05	0.15±0.05*	0.11±0.05	0.10±0.03
CK, U/L					
Control	94±10	98±8	89±8	87±9	90±11
Exercise	93±8	126±13	396±72$^{\dagger *}$	257±48$^{\dagger *}$	192±32*
Herb					
CRP, mg/dL					
Control	0.13±0.05	0.14±0.05	0.11±0.04	0.11±0.04	0.11±0.03
Exercise	0.10±0.03	0.09±0.03	0.15±0.03*	0.10±0.02	0.09±0.02
CK, U/L					
Control	110±12	108±9	119±17	109±11	113±16
Exercise	106±9	195±51*	291±35$^{\dagger *}$	204±26$^{\dagger *}$	151±20
TBARs, μM					
Placebo	7.1±1.1	8.1±1.5	7.9±1.0	6.5±1.0	-
Herb	7.0±1.0	6.6±0.8	8.1±0.9	5.7±0.6	-
IL-6, pg/ml					
Placebo	0.44±0.1	0.69±0.1*	0.32±0.1	0.46±0.1	-
Herb	0.50±0.2	0.45±0.1	0.23±0.1*	0.29±0.1	-
TNF-α, pg/ml					
Placebo	0.45±0.14	0.28±0.06	0.30±0.06	0.30±0.06	-
Herb	0.44±0.06	0.34±0.06	0.38±0.08	0.34±0.06	-

Values are means±SEM. TBARs, IL-6, and TNF-α were measured only during the eccentric exercise session. CRP=C-reactive protein; CK=creatine kinase; TBARs=thiobarbituric acid reactive substances; IL-6=interleukin-6; TNF-α=tumor necrosis factor-α. *P<0.05 vs. Pre in the same condition. †P<0.05 vs. Control or Placebo at the same time point.

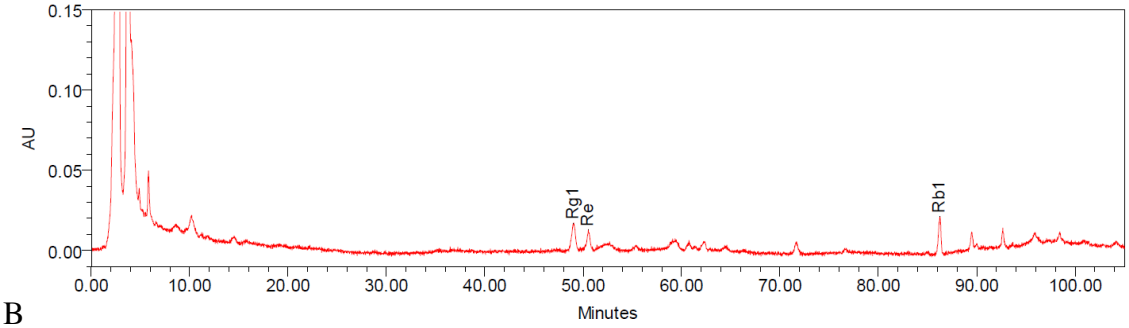
Table V.5 Associations between relative changes in arterial stiffness and selected biomarkers

	ΔcfPWV 24 hr	ΔcfPWV 48 hr
ΔCK	0.05	0.08
ΔCRP	0.17	0.21
ΔIL-6	0.11	0.46*
ΔTNF-α	0.60*	0.57*
ΔTBARs	0.09	0.36

*P<0.05. cfPWV=carotid-femoral pulse wave velocity, CK=creatine kinase, CRP=C-reactive protein, IL-6=interlukin-6, TNF-α=tumor necrosis factor-α, TBARs=thiobarbituric acid reactive substances

Figure V.1 High-performance thin-layer chromatography fingerprints of Panax ginseng (A) and Salvia miltiorrhiza (B).

A



B

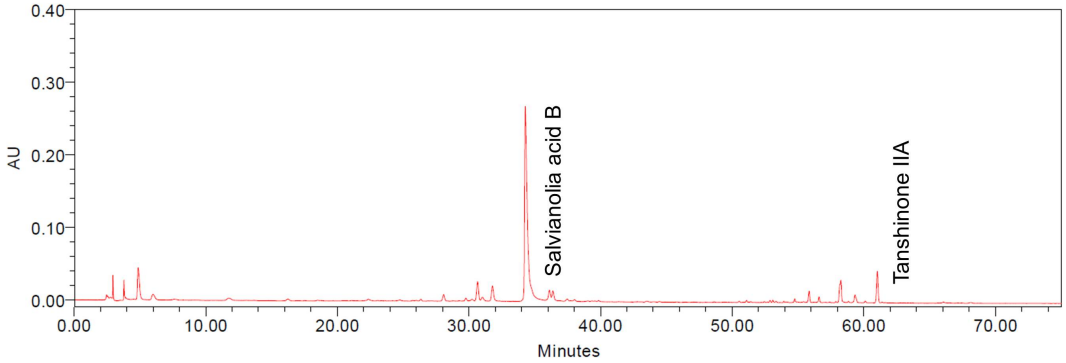
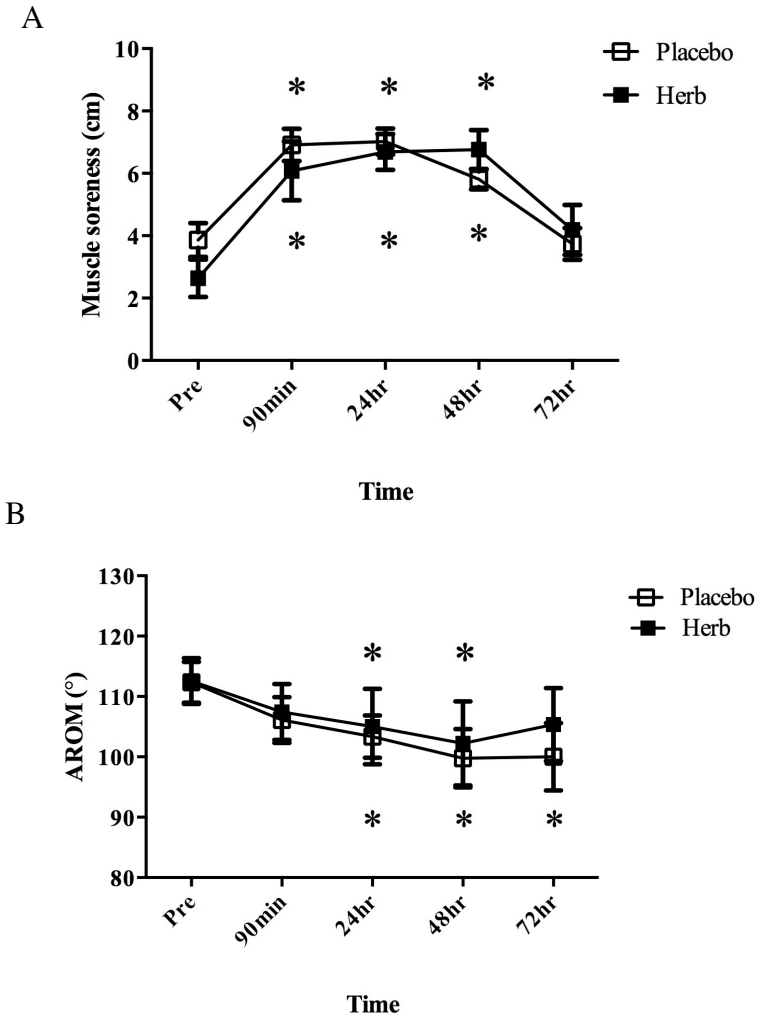
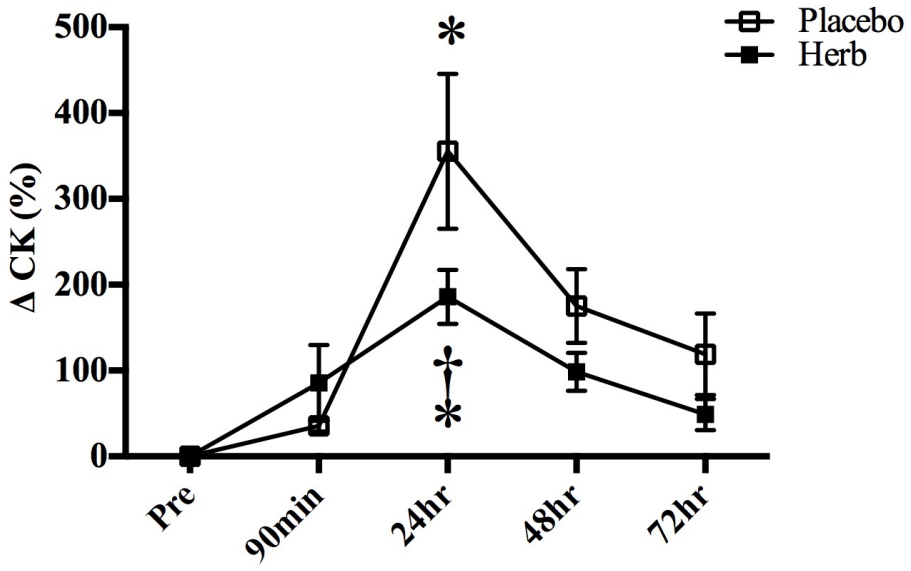


Figure V.2 Delayed onset muscle soreness (A) and active range of motion (AROM) (B) following downhill running exercise.



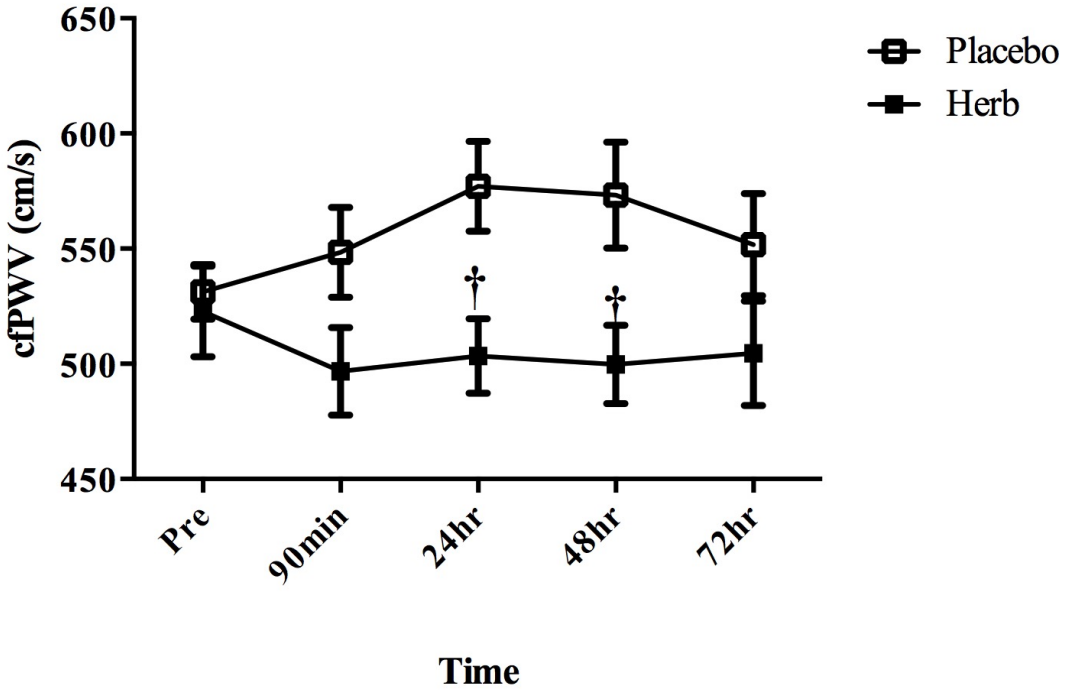
* $P<0.05$ vs. Pre in the same condition

Figure V.3 Relative changes in creatine kinase in response to the control and eccentric exercise sessions.



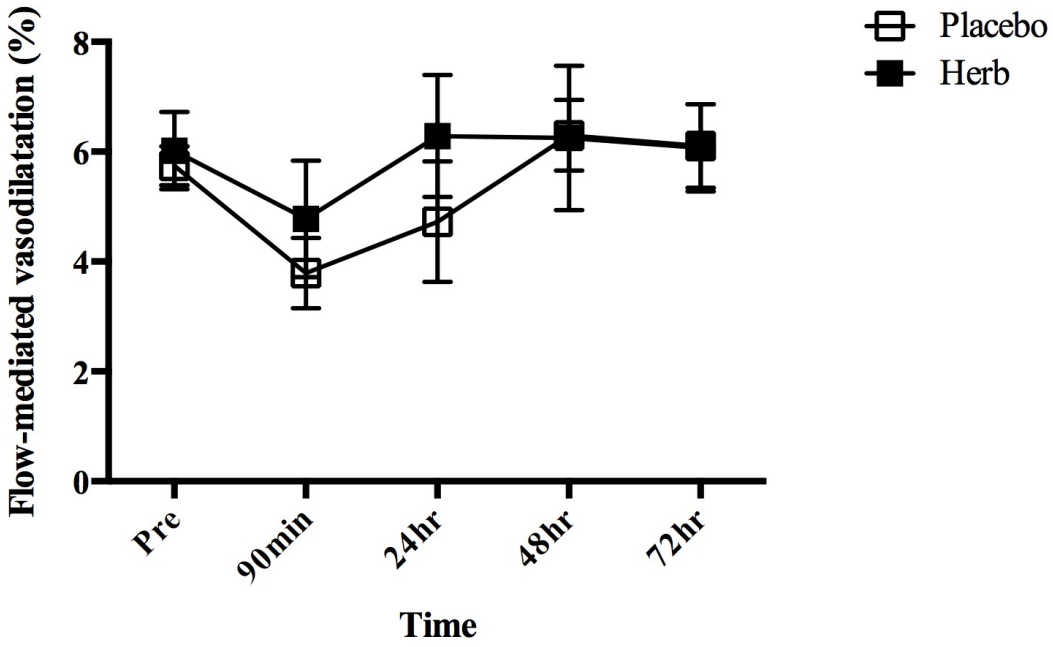
*P<0.05 vs. Pre in the same condition. †P<0.05 vs. Placebo at the same time point.

Figure V.4 Effects of herb supplementation on carotid-femoral pulse wave velocity (cfPWV).



†P<0.05 vs. Herb supplementation.

Figure V.5 Changes in flow-mediated vasodilatation in response to the eccentric exercise.



Chapter VI: Study #3

Long-term Chinese herb supplementation, eccentric resistance exercise training, and Vascular Function

ABSTRACT

Short-term herb supplementation of *panax ginseng* and *salvia miltiorrhiza* was demonstrated to ameliorate the delayed onset vascular stiffening induced by acute eccentric exercise in Study 1. In this study we sought to determine the effects long-term weekly progressive eccentric resistance exercise training on vascular functions, and whether herb supplementation would ameliorate the arterial stiffening effects elicited by exercise-induced inflammation and improve endothelial function. By using a double-blinded randomized design, older adults were recruited and assigned to either the Chinese herb (N=12) or the placebo group (N=11). After pre-training testing, all subjects underwent 12 weeks of unilateral eccentric-only exercise training on knee extensor and the follow-up testing was performed on 6^{th} and 12^{th} week. There were no treatment or time effects on creatine kinase, CRP, TNF-α , TBARs, and reduced GSH. However, serum IL-6 was significant elevated on 6^{th} week and return to baseline after training when two groups were combined. No significant difference was found on carotid-femoral pulse wave velocity, arterial compliance and flow-mediated vasodilation between and within groups. Isometric maximal voluntary contraction was found improved in both groups without significant change in knee extensor muscle mass. In conclusion, long-term progressive weekly eccentric resistance exercise did not contribute to increase in oxidative stress and inflammation, as well as chronic arterial stiffening or impaired vascular reactivity. Such training protocol significantly improved muscle quality (strength/muscle mass) without increase in muscle mass in older population. Long-term supplementation of *panax ginseng* and *salvia miltiorrhiza* did not exert significant affects on anti-oxidant status and vascular function.

INTRODUCTION

Cardiovascular disease is the number one cause of deaths in most developed countries. Cardiovascular diseases are a cluster of cardiovascular disorders affecting the heart and blood vessels. But the majority of cardiovascular diseases are arterial diseases (e.g., coronary artery disease, cerebrovascular disease, peripheral artery disease) and is attributed to vascular dysfunction. Among the vascular disorders, one that has received considerable attention in recent years is the stiffening of central elastic arteries. Regular aerobic exercise is one of the most effective lifestyle modifications to ameliorate and reverse arterial stiffness (263). In contrast, a number of studies (60, 180, 181) have reported that resistance-trained adults exhibit greater levels of arterial stiffness and strenuous resistance training intervention increases arterial stiffness. Eccentric muscle contractions during resistance exercise training may be one of the mechanisms underlying the adverse effects on vascular function since muscle damages induced by eccentric muscle contractions are associated with increased oxidative stress and inflammatory responses (207). Indeed acute bouts of eccentric exercises produce transient stiffening of central elastic arteries (10, 23). In the area of exercise prescription, eccentric exercise training has recently received increased attention since it requires lower cardiopulmonary demand and induces less fatigue during exercise compared with concentric training (98, 273). Additionally, eccentric exercise training is an effective exercise modality to improve muscle strength and muscle mass (219) and elicits anti-inflammatory effects (64) in older adults as well as in cardiac patients (177).

Panax ginseng and *salvia miltiorrhiza* are often adopted in herb formulas in Chinese medicine for treating cardiovascular disease because of its anti-oxidant and vasodilatory property. We have previously demonstrated a short-term supplementation with *panax ginseng*

and *salvia miltiorrhiza* prior to acute downhill running effectively prevented the increase in arterial stiffness induced by eccentric muscle contractions in study 2. Additionally, both *panax ginseng* and *salvia miltiorrhiza* have been shown to enhance the effects of exercise training on muscle function. Therefore, the primary aim of this study was to determine whether 12 weeks of supplementation with *panax ginseng* and *salvia miltiorrhiza* would prevent arterial stiffening induced by eccentric resistance exercise training. The secondary aim was to determine if the supplementation with *panax ginseng* and *salvia miltiorrhiza* would further enhance muscle hypertrophy and strength. We used a double-blind, placebo-controlled study design to address these two aims.

METHODS

Participants. A total of 24 middle-aged and older adults between the ages of 50-68 years were randomly assigned to either the placebo or the Chinese herb supplement groups. One subject in the placebo group did not complete the study due to personal reasons. Thus, eleven subjects were included in the placebo group and 12 subjects in the herb. Exclusion criteria from the study were 1) smoking within the past 6 months; 2) taking cardiovascular-acting drugs (including diuretics) and/or other medications that could influence our results; 3) orthopedic problems that would prohibit them from participating in exercise training; 4) alcohol consumption of >21 drinks/week; 5) peripheral vascular disease; 6) chronic heart failure; 7) coronary artery disease; 8) angina; 9) diabetes mellitus; 10) COPD and asthma and 11) lack of a signed physician's consent. All of the women were postmenopausal; premenopausal and perimenopausal women were excluded. Participants must have been sedentary for the past six months. All participants were required to gain physician's consent prior to being enrolled into this study and were subjected to a physical examination and treadmill exercise stress tests by a

licensed physician to ensure that the study was safe for them to participate. If the participants develop any symptoms other than fatigue, they were excluded from the study participation. Verbal and written explanations of procedure and its potential risks were provided to the subjects, and all the subjects gave their written informed consent. All procedures were reviewed and approved by Institutional Review Board of National Taiwan University Hospital.

Training protocol. Both groups underwent 12 weeks of progressive eccentric resistance exercise training (shown in Appendix). Eccentric leg extensor exercise was performed on each leg one session per week for 12 weeks. Isometric maximal voluntary contraction (MVC) was determined on a leg extensor machine equipped with a load cell. *Training intensity and repetition were progressively increased with the same rest interval between sets for safety consideration in older adults* (205). The frequency eccentric exercise was chosen beased on the previous study findning that once a week eccebtric exercise was sufficient to induce health-promoting effects (205). Each exercise session was closely supervised by an investigator. *Ad*herence to the exercise training was documented through the use of training logs.

Supplement administration. Subjects in the herb supplement group were required to take 1 dose (3 capsules) of supplement per day throughout 12 weeks of training. Supplements were given to subjects on a weekly basis, and subjects were asked not to consume additional supplements and maintain their regular diet throughout all testing session and intervention. Herb supplement was prepared in capsules consisting of 250mg of *panax ginseng* and 250mg *salvia miltiorrhiza* extracts. Placebo capsule contained microcrystalline cellulose. All supplement products were prepared one time together by the Brion Research Institute, Sun Ten Pharmaceutical Co., LTD. Analysis of ginsenosides of *panax ginseng* as well as Salvianolic acid

B and Tanshinone IIA in Radix *salvia miltiorrhiza* was performed by using high-performance liquid chromatography- electrospray mass (HPLC-MS) spectrometry method.

Testing protocol. Testing was conducted before and after the 12-week training intervention. Subjects did not receive any feedback about the results of the testing until the conclusion of the study. All post-training measurements were performed 24-48 h after the last exercise session to avoid the immediate effects of a single bout of exercise. In addition, pre- and post-training testing measurements were performed approximately at the same time in the morning for each subject. The subjects were instructed to arrive at the laboratory after a 12-hour overnight fast. If the participant was willing to take part in the study, the familiarization followed. The familiarization was implemented to ease anxiety during cardiovascular testing and to ensure subsequent accurate muscle strength test.

Dietary intake. In order to control for dietary effect on dependent variables, a dietary record was obtained for the three days prior to the experimental session. Each subject repeated the same diets prior to the pre- and post-measurements. Dietary record was analyzed by a registered dietician.

Body composition was measured noninvasively by dual-energy X-ray absorptiometry (Lunar DPX, GE Fairfield, CT).

Biochemical measurements. One small blood sample (~10mL) was obtained by venapuncture after an overnight 8 hour fast. Blood concentrations of cholesterol and triglycerides were analyzed enzymatically. HbA1c, alanine transaminase (ALT), aspartate transaminase (AST), and creatinine were analyzed using the commercially available kits. Biomarkers of muscle damage (creatine kinase), pro- and anti-inflammatory markers (CRP, TNF-α, IL-6) as

well as blood redox status (reduced glutathione (GSH), tiobarbituric acid-reactive substances (TBARS), were analyzed in duplicate with the use of commercial ELISA kits.

Heart rate and blood pressure were measured using an ECG (connected to Biopac MP36 data acquisition system) and an automatic blood pressure monitor (Omron HEM907) in the supine position.

Arterial stiffness and wave reflection were measured noninvasively in the supine position. Arterial stiffness was measured using carotid-femoral pulse wave velocity (cfPWV), which was calculated from the traveling distance and foot-to-foot wave transit time between two arterial recording sites in the supine position (261). Non-invasive pulse tonometer (SPT-301, Millar Inc. Houston TX) was connected to a physiological signaling processing system (MP36, Biopac, Goleta CA) and was used to detect pulse waves on the carotid and femoral arteries. Tonometric-obtained carotid arterial waveform was analyzed by custom designed software written in Matlab (The Mathworks, Natick MA) to calculate augmentation index (AI), an index of arterial wave reflection.

Carotid artery compliance was measured noninvasively in the supine position. Common carotid artery diameter was measured from the images derived from an ultrasound machine equipped with a high-resolution linear array transducer (Sonosite Ultrasound System; Bothell, WA). A longitudinal image of the cephalic portion of the common carotid artery was acquired 1-2 cm proximal to the carotid bulb. Carotid diameter image was analyzed using image analysis software (ImageJ, National Institute in Health, Bethesda MD). The combination of ultrasound imaging of a common carotid artery with simultaneous applanation tonometric-obtained arterial pressure waveforms from the contralateral artery permits noninvasive determination of arterial compliance.

Maximal voluntary contraction of each leg was measured on a customized leg extensor eccentric training machine, which is incorporated with a load cell to determine maximal isometric strength of leg extension at 90 degree of angle on each leg. The ratio of isometric strength (kg) and quadriceps muscle mass (kg) obtained from DEXA was computed as the indicator of muscle quality pre and post exercise intervention.

Statistical Analyses. Dependent variables were analyzed using repeated measures ANOVA. A 2-way mixed model ANOVA was used for analyses of time and treatment effects. Bonferroni post-hoc analysis was performed when significance was achieved. Associations were determined by Spearman rank correlations. Significance was set *a priori* at $P<0.05$ for all comparisons.

RESULTS

Selected subject characteristics were presented in Tables 3.1 and 3.2. All recruited subjects were apparently healthy, and no significant differences were observed in body composition, dietary intake, lipid profile, isometric muscle strength, as well as liver (ALT and AST) and kidney (creatinine) function parameters between the placebo and the herb group at baseline. There were no significant changes in most of the measured variables. There were no group differences in muscle damage, oxidative and inflammatory biomarkers at baseline. Eccentric exercise training did not elicit any significant changes in CK, CRP, TNF-α, TBARs, and reduced GSH throughout the intervention in both groups. As illustrated in Figure 3.1, maximal isometric leg strength and muscle quality increased significantly in both groups.

CfPWV and AI did not change significantly with eccentric resistance training in both groups. Moreover, there were no significant changes in blood pressure measures in both groups

(Table 3.3). For other vascular function measures, there were no time and treatment effects on changes in carotid arterial compliance or β-stiffness in both groups (Figure 3.2).

DISCUSSION

The major findings of this study are that weekly-performed progressive eccentric resistance exercise significantly improved muscle strength and muscle quality in older adults. However, eccentric resistance training was not associated with increased central arterial stiffness. Additionally, Chinese herb supplementation did not significantly affect indicators of muscle damage, inflammatory markers, muscle strength, or vascular function. These results suggest that Chinese herb supplementation does not appear to modulate muscular, vascular, and inflammatory adaptations to eccentric exercise training in older adults.

Resistance exercise has been advocated for older population to counteract sarcopenia with advancing age (1), yet there is a concern regarding the effects of resistance training on vascular health. For example, a recent meta-analysis showed that resistance training is associated with arterial stiffening (7), and a cessation of resistance training reversed the deterioration of central arterial compliance (181, 198). The underlying mechanisms by which resistance training elicits vascular stiffening are unknown. However, eccentric muscle contractions characterized by repeated mechanical damages and increased oxidative stress were emphasized. Previous studies using acute eccentric-only exercise models (10), including Study 1 and 2, demonstrated the association between muscle damage indicators and increased central arterial stiffness. In the present study, however, vascular stiffness was not affected by unilateral lower leg eccentric exercise training performed once a week, which could have produced less stress and inflammatory responses. Previous studies showing unfavorable effects on arterial stiffness used high-intensity whole-body resistance exercise protocol (179, 180, 198). Another possible

explanation could be the age range of recruited subjects. Arterial stiffness increases with advancing age. Most of the previous studies that have shown arterial stiffening effects of resistance training have used young subjects with reduced arterial stiffness values at baseline. In one of the studies, there was no significant decrease in central arterial compliance with strength training in middle-aged and older adults with low baseline arterial compliance (53) .In another study involving healthy postmenopausal women, 18 weeks of moderate resistance training program did not change AI (29). Moreover, twelve weeks of leg resistance training did not change aortic PWV in older men even though maximal muscular power was increased (168). Together with the present study, there may be a ceiling effect that older adults may not experience deleterious effects of resistance training on vascular wall function.

To our knowledge, this was the first study to investigate the effects of *panax ginseng* and *salvia miltiorrhiza* supplement intervention on vascular function. Chinese herb supplementation did not affect vascular function. In addition, short-term Chinese herb supplementation had no effects on muscle damage, inflammatory and oxidative stress biomarkers, as well as anti-oxidant status, suggesting that eccentric resistance exercise training did not contribute to chronic inflammation and increased oxidative stress induced by repetitive muscle damage; chronic herb supplementation did not increase anti-oxidant status with training. Plasma creatinine, alanine transaminase, and aspartate transaminase concentrations did not change significantly with supplementation, and no discomfort was reported by subjects during the intervention, suggesting a 12-week supplementation with this herb formula did not contribute to adverse effects on general indicators of liver and kidney functions. It is possible that a lack of effects of the Chinese herb formula might be related to the dosage. For a safety concern such as herb interactions, the

administrated dosage in currently study was relatively lower compared with previous human studies using only a single herb (e.g., *panax ginseng*) (113).

Important limitations of the present study should be emphasized here. First, no non-exercising control group was included in current study. Second, resistance exercise protocol was performed only once a week. This was chosen because such protocol was effective in increasing muscle strength (205) but may not have been frequent enough to elicit any changes in vascular function.

In conclusion, progressive weekly eccentric resistance exercise training did not result in arterial stiffening in older adults. In addition, long-term supplementation with *panax ginseng* and *salvia miltiorrhiza* does not appear to affect oxidative and anti-oxidant status, as well as vascular functions.

Table VI.1 Selected subject characteristics

	Placebo		Herb Supplement	
	Pre	**12 wk**	**Pre**	**12 wk**
Age, yrs	57±2	-	55±2	-
Male/Female, n	4/7	-	3/9	-
Height, cm	160±2		163±3	
Body mass, kg	62±4	62±4	58±3	58±3
BMI, kg/m^2	23±1	23±1	23±1	23±1
Body fat percentage, %	31±2	30±2	32±2	32±2
Leg strength, kg	47±5	54±6*	40±4	45±6*
Leg mass, kg	13±1	14±1	12±1	12±1
Calorie intake, kcal/day	1787±74	1775±118	1775±118	1679±117
Carbohydrate intake, %	54±2	52±2	51±2	51±2
Fat intake, %	32±1	33±2	33±1	33±1
Protein intake, %	14±1	15±1	16±1	15±1
Vitamin C intake, mg	122±14	115±11	107±19	85±10
Vitamin E intake, mg	5.1±0.4	4.4±0.9	3.8±0.5	4.3±0.4

Values are means±SEM. *$P<0.05$ vs. Pre. BMI=body mass index.

Table VI.2 Changes in blood concentrations of cardiovascular risk factors and inflammatory and oxidative stress markers with eccentric resistance exercise training.

	Placebo		Herb Supplement	
	Pre	**12 wk**	**Pre**	**12 wk**
Total cholesterol, mg/dl	229±10	225±8	234±11	242±9
LDL cholesterol, mg/dl	129±9	130±8	123±10	134±7
HDL cholesterol, mg/dl	62±4	57±4	68±4	64±4
Triglyceride, mg/dl	120±17	104±15	108±14	118±11
HbA1c, %	6.1±0.2	5.9±0.1	5.6±0.1	5.6±0.2
Alanine transaminase, IU/l	26±2	25±2	24±2	22±1
Aspartate transaminase, IU/l	26±4	27±5	22±5	20±2
Creatinine, mg/dl	0.78±0.05	0.75±0.03	0.78±0.05	0.78±0.06
CK, U/l	131±27	129±28	110±19	125±16
CRP, mg/dl	0.09±0.01	0.11±0.06	0.11±0.03	0.11±0.04
IL-6, pg/ml	0.39±0.08	0.46±0.08	0.41±0.08	0.42±0.09
TNF-α, pg/ml	0.47±0.03	0.50±0.02	0.45±0.02	0.48±0.02
TBARs, μM	9.6±0.7	11.4±1.7	13.5±1.4	16.7±2.1
Reduced GSH, μM	864±199	722±219	977±108	599±160

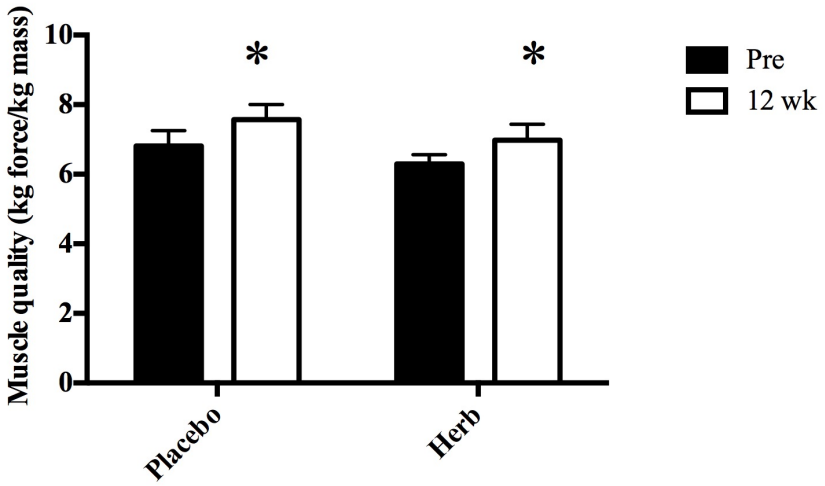
Values are means±SEM. HbA1c=glycosylated hemoglobin; CK=creatine kinase; CRP=c-reactive protein; IL-6=interleukin-6; TNF-α=tumor necrosis factor-α; TBARs= thiobarbituric acid reactive substances; GSH=glutathione.

Table VI.3 Changes in hemodynamic measures with eccentric resistance exercise training

	Placebo			Herb Supplement		
	Pre	**6 wk**	**12 wk**	**Pre**	**6 wk**	**12 wk**
cfPWV, cm/s	731±33	746±37	731±25	713±43	738±41	706±40
AI, %	26.8±0.05	23.6±0.06	24.8±0.03	27.9±0.03	26.3±0.03	26.7±0.03
Systolic BP, mmHg	119±4	116±3	116±4	109±3	110±3	107±4
Diastolic BP, mmHg	71±2	70±3	69±3	68±3	67±2	66±2
MAP, mmHg	87±3	85±2	84±3	81±3	81±2	80±3
PP, mmHg	48±3	46±3	48±3	41±2	43±3	41±2
Heart rate, bpm	60±3	61±3	62±2	62±2	62±2	61±1

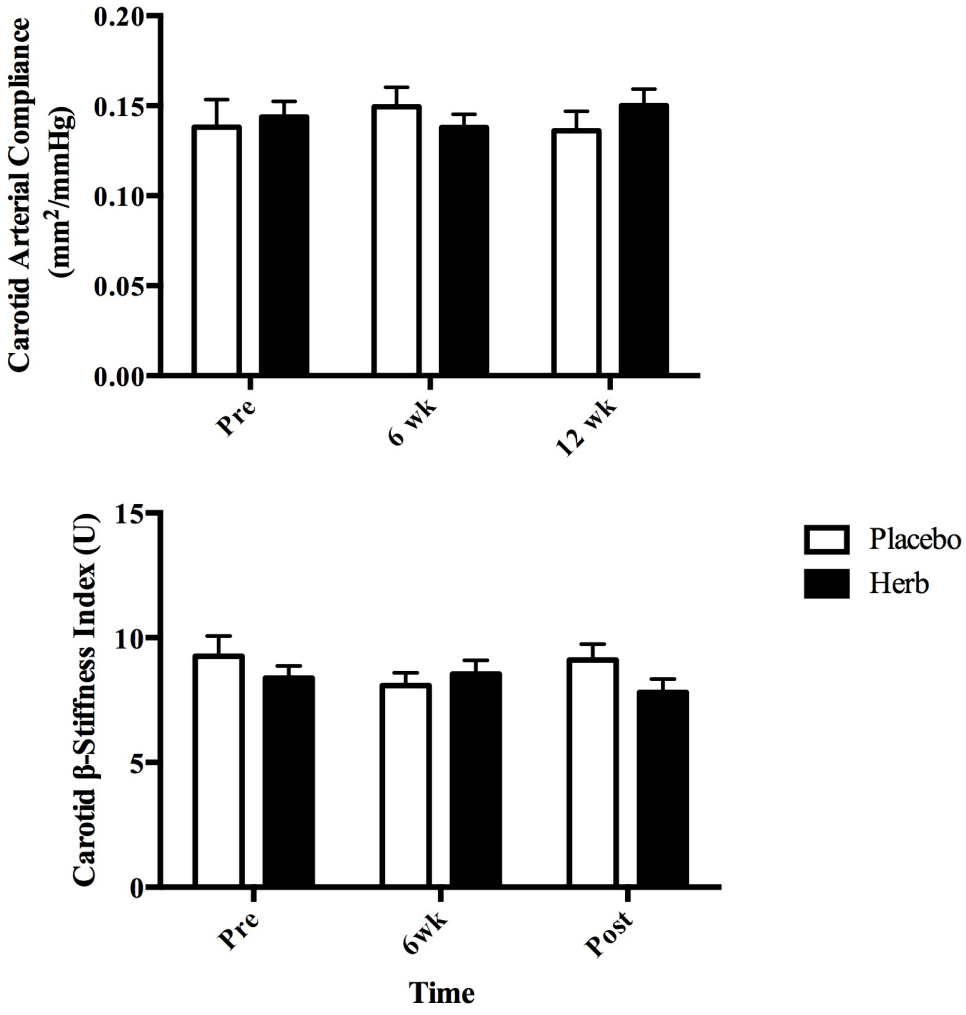
Values are means±SEM. cfPWV=carotid-femoral pulse wave velocity; AI=augmentation index; BP=blood pressure; MAP=mean arterial pressure; PP=pulse pressure

Figure VI.1 Changes in muscle quality after eccentric resistance training.



* $P<0.05$ vs. Pre.

Figure VI.2 Carotid arterial compliance and beta-stiffness index with exercise training intervention.



Chapter VII: Review of the Literature

VASCULAR DISEASE AND ITS UNDERLYING PATHOPHYSIOLOGY

Vascular remodeling and risk factors

Vascular morphological changes, such as fragmented and reduced elastin density, increased collagen concentration, advance glycation end-products (AGE) and vascular smooth muscle cell (VSMC) hypertrophy, play crucial roles in arterial stiffening with advancing age (79, 190, 236). Indeed, vascular extracellular matrix (ECM) remodeling is thought to be one of the most important steps in pathogenesis of vascular disease. In contrast to collagen, which is synthesized continuously throughout life (156), arterial elastin synthesis is negligible in adulthood (216). Repeated cyclic loading and unloading throughout the adult aging contribute to the fragmentation of elastin (87) and the formation of cross-links of AGE with collagen and elastin. Additionally, metalloproteinase protein expression is increased to cleave fragmented collagen and elastin in vasculature, leading to proliferation and hypertrophy of VSMC and calcification. As a result, arterial wall gets thickened and stiffer (156). The increase in arterial wall intima media thickness (IMT), aortic stiffness (as evidenced in aortic pulse wave velocity), and the augmentation of central blood pressure are believed to be the consequences of vascular remodeling and have been established to predict future cardiovascular outcomes in a variety of populations (146, 190, 234). Among these vascular changes, aortic stiffness has a particularly strong independent predictive value for all-cause and cardiovascular mortality, cardiovascular disease, coronary events, and strokes even after adjusting for traditional risk factors (146, 256) including brachial blood pressure (149), Framingham risk score (16), and carotid IMT (170).

Blood pressure is a well-known risk factor for CVD. It is established from cohort studies that high blood pressure predicts future CVD events and reduces life expectancy in healthy and diseased populations (78, 81, 276). Epidemiological studies demonstrate that the risk of developing CVD increases in proportion with blood pressure at all ages (117). A recent 20-year prospective study demonstrated that established CVD risk factors, other than blood pressure, have only modest effects on aortic stiffness and wave reflection (172) as the impact of traditional CVD risk factors on arterial stiffness and wave reflections is strongly dependent on age and it is largely driven by blood pressure (173).

However, the causal link between blood pressure and arterial stiffness has not been elucidated (30). The increase in arterial stiffness causes an early reflected wave in late systole and augments central pulse pressure. This is a generally accepted mechanism to explains age-related increases in blood pressure (148); however, this view was challenged recently by a meta-analysis study showing that the arrival of reflected wave did not contribute to the elevated blood pressure along with aging (9). An alternative emerging hypothesis is that elevated blood pressure stretches the arterial wall, leading to functional increases in arterial stiffness (31, 172, 173).

Oxidative stress, inflammation and endothelial dysfunction

Vascular endothelium situates at the interface of vessel wall and blood flow not only functioning as a protective barrier, but also possessing anticoagulatory properties and producing a numbers of autacoids that regulate vascular tone and homeostasis (17). Nitric oxide (NO), the endothelium-derived relaxant, plays a central role in control of vascular homeostasis by regulating vasomotion and vasoprotection. NO is generated from L-arginine by the action of endothelial NO synthase (eNOS) with the presence of co-factor, tetrahydrobiopterin, in the

endothelial cell. This endogenous NO diffuses to the vascular smooth muscle cells and activates guanylate cyclase leading to cGMP-mediated or endothelium-dependent vasodilation (14).

Endothelial dysfunction, characterized by reduced NO availability and increased release of pro-inflammatory cytokines, has been shown to be an independent predictor of future cardiovascular events (20, 36, 120, 176, 183, 192). In the vascular wall, ROS can be generated in the endothelium, adventitia, and vascular smooth muscle cell, and increased ROS production has been implicated in various pathologies, including hypertension, atherosclerosis, diabetes, and chronic kidney disease. Acute inflammation induced by a vaccine administration can cause a temporary increase in central arterial stiffness (280), and chronic inflammation as the result of excessive ROS formation in vascular wall induces growth, apoptosis, and migration of vascular smooth muscle cell, impaired endothelium-dependent relaxation, and modification of the extracellular matrix (80).

Free radicals

Free radicals are molecules with one or more unpaired electrons with short lifetime since they are very unstable and tend to pass electrons to other molecules. ROS is the major free radical category that is derived from oxygen, leading to lipid oxidation and peroxidation, protein oxidation, DNA damage, and apoptosis in cells (76). During aerobic metabolism, oxygen receives one electron at a time converting itself into a superoxide ion (O_2^-). In average, 2-5% of oxygen consumed is converted to O_2^- (76). The formation of O_2^- followed by Fenton and Haber-Weiss reactions, contributs to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-), as well as reactive nitrogen species (RNS), peroxynitrite ($ONOO^-$), hypochlorous acid, and lipid radical. All of them are known major pro-oxidants tightly regulated by antioxidants under normal conditions and act as second messengers to control vascular function and structure

(75). Under normal physiological conditions, ROS formation and elimination are balanced in the vascular wall. However, when antioxidant enzymes cannot counteract with oxidative enzyme, oxidative stress increases and more ROS are formed. It is well accepted that excessive free radicals likely to limit vasodilation and blood flow by reducing NO availability (134, 278) . However, there is growing evidence showing that some free radicals also play important roles in vasodilation (165, 224).

Oxidative enzymes

Nicotinamide adenine dinucleotide phosphate oxidase *(NAD(P)H oxidase system*) is the main source of ROS in vascular wall (80, 145). NAD(P)H oxidase was first identified in phagocyte to produce reactive oxygen-containing molecules that are anti-microbial (55). It is now believed that NAD(P)H exists in many other cell types, including vascular smooth muscle cells (12). NAD(P)H oxidase consists of the membrane subunits gp91phox and p22phox, cytosolic subunit p67phox, p47phox, and the small GTPase rac1 (289). Several homologues of gp91phox have been identified as NOX family where NOX1, NOX2, NOX4, and NOX5 are expressed in vascular cells and probably the major sources of ROS in cardiovascular system (238, 253). For example, NOX2 and NOX4 contribute to oxidative stress in human coronary disease and involved in the pathogenesis of atherosclerosis (246). Vascular NAD(P)H oxidase is regulated by humoral factors (growth factor, cytokines and vasoactive agents), mechanical factors (shear stress, stretch), and increased glucose and free-fatty acids (201). NAD(P)H oxidase can activate nuclear factor-kappa B (NF-kB) with mitochondria H_2O_2 production followed by up-regulated cytokine expression such as interleukin-6 (IL-6), C-reactive protein (CRP), tumor nuclear factor-α (TNF-α), and vascular adhesion markers (e.g., ICAM-1, VCAM-1), all of which are risk factors of impaired vascular function (14, 15). A recent cross-sectional study

demonstrated that the expressions of NAD(P)H oxidative stress and NF-kB expression in endothelial cell obtained from both artery and vein were increased with advancing aging in healthy (62) and obese (242) humans. Importantly, these increases were inversely related to brachial artery flow-mediated dilation (FMD), an established non-invasive vascular reactivity measure of endothelium-dependent vasodilatation in response to reactive hyperemia (62), suggesting that this signaling pathway may play a critical role in the development of vascular dysfunction.

Xanthine oxidase is not only expressed in vascular cell, but also circulates in the plasma, binding to extracellular matrix (289). Xanthine can produce O_2^- and H_2O_2 directly by catalyzing the oxidation of xanthine with the formation of uric acid (140), which have been implicated in endothelial dysfunction in hypertension. Experimental evidence in the hypertensive animal model indicates that elevated xanthine oxidase and ROS production are associated with increased vascular tone in arterioles (257). The mechanism by which xanthine oxidase induces increases in arteriolar tone is that circulating xanthine oxidase inhibits NO-dependent cGMP production in VSMCs (99). ET-1/ETA pathway is also involved according to the experiments using hypertensive animal models (279). Moreover, xanthine oxidase levels were found to be increased in patients with coronary diseases and related to aortic compliance (58). Treatment with xanthine oxidase inhibitor, allopurinol, lowers blood pressure significantly in people with established hypertension (114) as well as in adolescents with high blood pressure (74).

Endothelial Nitric Oxide Synthase, not only produces NO but also interacts with ROS. Under the condition of high oxidative stress, the increased ONOO- production resulting from the reaction of NO and O_2^- causes the oxidation of tetrahydrobiopterin, the co-factor of eNOS, leading to uncoupled eNOS (144). In the change of redox state, eNOS becomes a peroxynitrite

generator, leading to dramatic increase in oxidative stress, because accompanied peroxynitrite production is also detrimental to vascular function by contributing to lipid peroxidation and cell damage (80). In other words, excessive ROS production contributes to the reduction in NO availability and exacerbated oxidative stress in the vasculature. The increased levels of intracellular ROS influence redox-sensitive signaling pathways that induce vascular contractility, structural remodeling, fibrosis, and inflammation, leading to vascular remodeling and endothelial dysfunction (75).

HABITUAL PHYSICAL EXERCISE AND VASCULAR FUNCTION

Aerobic exercise

Habitual physical activity is beneficial for overall health in general and CVD in particular (272). Epidemiological studies have documented a strong, independent, and inverse relation between physical activity and cardiovascular mortality (135). Regular aerobic exercise has been advocated as the one of the effective exercise modes to ameliorate and reverse arterial stiffness (263) and to prevent age-related declines in endothelial function (235). Results from cross-sectional (185, 262) and interventional (186, 237) studies showed that aerobic exercise training is favorable for blood pressure control and attenuations in aortic stiffening in aged populations. Additionally, endothelium-dependent vasodilatation was found preserved and restored in older men who regularly performed aerobic exercise (59). Short-term aerobic exercise training at moderate intensity can improve endothelium-dependent vasodilatation in previously sedentary older men (59) and patients with metabolic syndrome (151). Thus, regular aerobic exercise appears to be highly effective in favorably influencing key vascular functions.

Resistance (strength) exercise

In contrast to the findings from aerobic exercise, previous studies in resistance exercise training are inconsistent as most studies (60, 180, 181), but not all (29, 217), found unfavorable effects on arterial wall structure and buffering function. It appears that higher intensity of resistance exercise training is associated with greater arterial stiffness (235) since moderate intensity did not deteriorate arterial stiffness (29, 217). Moreover, a cross-sectional study in rowers suggested that aerobic exercise could negate this stiffening effect of resistance training (51). Interestingly, this vasoprotective effect was only observed in the order of resistance exercise followed by the aerobic exercise (197). Current research evidence suggests that aerobic exercise should be incorporated into resistance exercise training program to prevent such stiffening effects, especially for those with cardiovascular concerns. In regard to the endothelial function, endothelial-dependent vasodilatory function was shown unaffected after an eight-week resistance exercise intervention in normotensive postmenopausal women (29)

Eccentric exercise

Muscle damage, as a result of unaccustomed physical activity or eccentric muscle contractions, can induce a series of inflammatory responses such as leukocyte infiltration and production of pro-inflammatory cytokines within local muscle tissue and into systemic circulation (95). Considering the fact that inflammation is closely linked to vascular disease and cardiovascular events, it is likely that eccentric exercise might not be beneficial to vascular health. Indeed, studies have shown that acute eccentric exercise could impair microcirculation (118, 119), augment vascular resistance (218) and increase blood pressure response during isometric exercise (178). We recently demonstrated that acute leg eccentric exercise transiently induced unfavorable change in macrovascular function and increased central arterial stiffness

(10). Interestingly, the increase in arterial stiffness was positively correlated with creatine kinase (CK), suggesting that increases in muscle damage biomarkers are associated the increase in arterial stiffness in response to eccentric exercise (10). Plasma cardiac troponins, the indicator of myocardium damage, is elevated following an acute downhill running in athletes (136) despite the fact that myocardial wall stress was considerably lower than that of flat course running. Collectively, these results indicate that acute eccentric exercise exerts unfavorable effects on arterial function.

Levels of inflammatory response induced by eccentric exercise are influenced by many factors, such as types of exercise (207), exercise intensity and duration (208, 268), age (268), sex (239), and training status (61) . In terms of eccentric exercise stimulus, downhill running (210, 245) appears to evoke greater plasma IL-1 and IL-6 production than eccentric resistance exercise on quadriceps (167). Downhill running also produces large increases in circulating neutrophil counts, CK, and myoglobin (Mb) even in well-trained runners (209). Although greater eccentric workload results in higher plasma CK and Mb (268), degree of muscle damage was not necessarily correlated with levels of inflammatory response (95). Some studies (18, 208, 222) suggested that humoral and cardiovascular component appeared to play more important roles in immune marker regulation. Indeed, exercise-associated increases in body temperature, stress hormone (221, 222), and blood flow (19) were associated with the induction of circulating cytokine release and redistribution. Accordingly, it is plausible to hypothesize that downhill running characterized by greater hemodynamic changes and systemic inflammatory response might induce higher arterial stiffening effects compared with eccentric resistance exercise. However, such hypotheses have not been addressed.

It has been well demonstrated that an acute bout of eccentric exercise can induce muscle damage and inflammation (174, 207). Acute eccentric exercise of nearly maximal contraction intensity performed prior to the eccentric exercise confers protective effects against subsequent bout of the same exercise (174). This adaptation, often referred to as the repeated bout effect, is characterized by a faster recovery of muscle strength and range of motion, and less development of swelling and muscle soreness (47) accompanied with lower level of responses in plasma CK, myoglobin, and delayed onset muscle soreness (DOMs) (40, 150, 244). The mechanisms underlying this protective effect in response to secondary damage are still inconclusive. However, the blunted inflammatory response has been proposed as one of the cellular mechanisms that contribute to this physiological adaptation (174). For example, circulating neutrophil and monocyte activation decrease in response to a repeated bout of eccentric exercise (212, 213, 244). Second bout of downhill running exercise induces a reduction in pro-inflammatory cytokines release such as macrophage chemotactic protein-1 (MCP-1), IL-6, IL7, IL-8, and an increase in IL-10, an anti-inflammatory cytokine. A recent study (110) demonstrated that NF-kB activation and related gene expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and IL-6 in peripheral blood mononuclear cells (PBMC) were attenuated after 8 weeks of eccentric resistance exercise training (245). In a long-term intervention study involving older adults, however, traditional regular exercise, including aerobic and resistance exercise, did not alter systemic inflammatory cytokine (11). Previous studies addressing repeated bout effect of eccentric exercise had looked mainly at muscular function. Whether such protective effects can also be observed in vascular function and whether young and older people adapt differently have not been addressed.

Recently, eccentric training has received increased attention from the clinical perspective since it requires lower cardiopulmonary demand to perform the task and induces less fatigue during exercise compared with concentric training (98, 273). Additionally, eccentric exercise appears to exert more favorable metabolic and anti-inflammatory effects (64). Perturbation of substrate oxidation and lipid profile evoked by an acute bout of eccentric exercise lasts for about 7 days was demonstrated (194, 204). Compared with concentric resistance exercise training, 8 weeks of eccentric resistance exercise training only one session per week was recently suggested more effectively improving lipid and lipoprotein profile on lean and young women (205). Therefore, eccentric exercise training of low frequency is sufficient to benefit traditional risk factors; however, whether these benefit translate into vascular function remains unclear. Moreover, for cardiac patients, eccentric endurance exercise provides better functional advantages in increasing muscle strength (251) though cardiovascular stress is comparable (177). Aging is associated with the impaired capacity to repair muscle damage (268). Reduced protective effect was conferred by an eccentric elbow flexor exercise in older but not in young people (150), suggesting that the efficacy of eccentric exercise may be influenced by the aging process. With advancing age, muscle strength declines progressively, and the magnitude of reductions in concentric and isometric strength is more pronounced than that in eccentric strength (274). The better preservation of eccentric strength is also observed in patients with chronic conditions (228). Therefore, eccentric exercise can be safely used to restore muscular function of people with limited capacity (229).

ANTIOXIDANTS SUPPLEMENTATION AND VASCULAR FUNCTION

Introduction

Antioxidants, such as ascorbic acid (vitamin C), tocopherol (vitamin) E or the mixture, have been extensively studied in the literature. However, the effects of antioxidant supplementation have been discouraging in many aspects, including muscle performance enhancement, blood oxidative stress attenuation, as well as cardiovascular health improvement (13, 86, 266). For example, some studies showed antioxidant supplementation reduces oxidative stress (49, 84), whereas others reported antioxidants induce pro-oxidant effects (277), or does not affect redox status (232, 266). Possible explanations for such divergent findings may include differences in type of antioxidant administrated, lifestyle history of subjects, oxidative stress biomarkers determined, as well as analysis method used (266). Moreover, the level of oxidative stress, the duration of the intervention and/or doses of antioxidants possibly explain such inconclusive findings (232). It should be noted that a growing body of evidence shows that antioxidant supplementation may not lead to additive health benefits along with regular exercise, another means that has been suggested to improve antioxidant capacity. On contrary, antioxidant supplement greatly negates the improvements induced by exercise training in humans (63, 86, 227, 293). For example, the ingestion of vitamin mixture (C and E) has been shown to prevent transient increase of oxidative stress after exercise and abolish subsequent gene expression and antioxidant defense (227), as well as blunt training-induced improvements in endothelial-independent function (294), suggesting that exercise-induced reactive oxygen species may play a crucial role that triggers adaptation during and after exercise.

Vitamins

Ascorbic acid (vitamin C) is one of most potent water-soluble extracellular antioxidant that can directly react with superoxide, hydroxyl radical, and singlet oxygen. Tocopherol

(vitamin E) is a lipid-soluble chain-breaking antioxidant that plays an important role in cell membrane because of its lipid peroxidation inhibition. Tocopherol can be regenerated by interacting with ascorbic acid after reacting with ROS (73).

Acute oral ascorbic acid supplementation has been reported to be effective in reducing augmentation index, an indirect measure of arterial stiffness, in Type II diabetic patients (188), but not in health young men (121, 188). In a study using peripheral artery pulse wave analysis to assess arterial stiffness, pretreatment with ascorbic acid also attenuated the increase in arterial stiffness induced by hyperglycemia (187). In addition, acute infusion with ascorbic acid improved cardiovagal baroreflex sensitivity in healthy old men (184). On contrary, studies directly imaging central large artery showed that a short-term moderate daily ascorbic acid supplementation did not affect large elastic artery compliance and central blood pressure in healthy population regardless of age (70). Likewise, acute infusion with ascorbic acid increased flow-mediated vasodilatation in healthy and sedentary old men but chronic oral supplementation did not contribute to the improvement in endothelial function regardless of training status and age (71). Therefore, acute ascorbic acid infusion appears to reduce ROS/oxidative stress, ameliorate arterial stiffness, and improve endothelial function in subjects characterized with high oxidative stress. However, the findings from chronic oral ascorbic acid supplementation on vascular function are largely equivocal. It may be due to different vascular function assessments adapted by different studies (peripheral artery-derived estimation vs. direct measures of central large artery compliance), and subject characteristics (ascorbic acid supplementation may be more likely to reduce large artery stiffness in populations with more severe pathophysiological vascular states) (70).

A large clinical trial study have documented that chronic treatment with tocopherol had no apparent effect on cardiovascular outcomes in patients at high risk for cardiovascular events (308). However, short-term supplementation in diabetic animals showed that tocopherol supplementation improved both endothelium-dependent and -independent function in mesenteric arteries (resistance arteries) though such effect was not observed in femoral artery (291). In humans, a double-blind, placebo-controlled, randomized study demonstrated that short-term daily oral supplementation with tocopherol improved both endothelial function in both the conduit and resistance vessels of young subjects with Type I diabetes (243).

Vitamin mixture of ascorbic acid and tocopherol also showed inconclusive findings. Short-term supplementation in children with hyperlipidemia (68) and essential hypertensive patients (215) improved endothelial function and arterial stiffness, whereas long-term supplementation did not contribute to any beneficial effects on endothelial function and arterial stiffness in patients with coronary artery diseases (128), healthy participants (314), or even impair myocardial perfusion and endothelial function in animals (277).

Accordingly, current literature regarding effects of vitamin supplementation on vascular function suggests that long-term oral vitamin treatment does not contribute to vascular benefits regardless of single or mixture supplements being used. Short-term supplementation is more likely to show positive effects, especially in patients with higher ROS/ oxidative stress potential. Recently, it was demonstrated that supplement incorporating ascorbic acid, tocopherol, and α-lipoic acid could better restore vasodilation in old subjects (63), indicating that such antioxidant "cocktail" may provide stronger antioxidant force against the aged-associated increase in oxidative stress.

Polyphenols

Polyphenols are the most abundant antioxidants in the diet as they are widely distributed in plant foods (252). To date, at least some polyphenols are reported to be favorable for cardiovascular health (97, 281). Epidemiological studies supported the beneficial effects of polyphenol consumption against cardiovascular diseases (6). The high level of flavonoids, catechins, tannins, and other polyphenolic compounds presented in vegetables, fruits, soy, tea as well as red wine are believed to contribute to their beneficial health effects (252). Polyphenols have been characterized by its antioxidant effects as polyphenols from red wine (193), grape juice (250), and olive oil (231) enhance antioxidant capacity of plasma and reduced circulating oxidized-LDL. In addition to antioxidant effects, both in vitro and in vivo studies indicate that polyphenols increase NO formation through enhanced eNOS expression (281, 282), increase other vasodilators, such as prostacyclin released from endothelial cells, (182), and inhibit ET-1 synthesis (123).

Hemodynamic effects of both acute and chronic administration of supplementation from polyphenol-rich foods or beverages, in the form of wine, tea or grape extracts, have been investigated in patients and animals. The endothelium-dependent vasodilation, as determined by FMD, improves after acute intake of red wine (94). This effect appears to be independent of alcohol as the increase in FMD was also observed with red wine without alcohol (94). One study using a meal containing high phenolic virgin olive oil, the major constituent of the Mediterranean diet showed that ischemia reactive hyperemia in postprandial state improved in hypercholesterolemic subjects (230), suggesting that endothelium-dependent vasodilatation improved at least in part by polyphenols. Moreover, a short-term intake of purple grape juice (250) and black tea (97) has also been shown to increase endothelium-dependent vasodilatation

significantly. To date, most of the available evidence supports the notion that polyphenol-rich diet can improve endothelial function. Interestingly, dietary polyphenols appear to have protective effects against lengthening contraction-induced muscle damage as studies demonstrating reduced tiobarbituric acid-reactive substances (TBAR) reaction and increase glutathione expression in animals (191), as well as improved strength recovery after eccentric exercise in humans (270). These studies suggest that polyphenols may also provide strong antioxidant effects on exercise-induced oxidative stress.

COMPLEMENTARY AND ALTERNATIVE MEDICINE AND VASCULAR HEALTH

Introduction

Complementary and alternative medicine (CAM), functionally defined as diagnosis, treatment and/ or prevention which complements mainstream medicine (69), is neither widely taught in medical school nor generally available in hospitals (67). CAM therapy has been gaining popularity in many Western countries. For example, there is a long-term trend in increasing use of CAM therapies in the United Sates (122) as the percentage of using at least one of the CAM therapies increased from 34% in 1990 to 42% in 1997 from a national telephone survey (66). Other counties have even higher annual prevalence in CAM therapies (69). CAM has emerged as a worldwide intervention that people use for health care in addition to or in lieu of conventional medicine. CAM therapies encompass diverse therapeutic and diagnostic approaches, such as Chinese herbal medicine, mind-body exercise, botanicals, and acupuncture, which are often used in conjunction with conventional medicine (163, 189). Herbal products and mind-body therapies are the two most commonly used CAM in the general population and in patients with CVD according to the National Health Interview Survey (NHIS) (303). It should be emphasized, however, that drug-herbal interactions have been reported in both in vitro and

animal studies (37). This raises an important concern that the interaction of CAM and conventional medicine might complicate or exacerbate existing disease states (259).

Yin/yang-oxidant/antioxidant theory

From the traditional Chinese medicine perspective, human body is functioning in a harmonious manner. Yin-yang theory is one of the unique healing systems and philosophy developed in Chinese medicine, which is characterized by the use of complementary opposite approaches to indicate how things function in relevance to each other (32). Under normal healthy conditions, yin and yang are in equilibrium of relative psychological and physiological balance, attaining the homeostasis as defined in the modern medical terms. Disturbance in this equilibrium may contribute to an excess or deficiency in either yin or yang and the development of subsequent disease. Yin, in Chinese character, represents cold, stillness, darkness, passivity, inwardness and downwardness, implying disease states such as cold limbs, fatigue, and lowered metabolic rates, whereas yang depicts heat, movement, brightness, activity, outwardness and superwardness, indicating diseases such as hyperactivity, hyperthyroidism, acute infections, fever and sweating (288).

"Tonifying action" with therapeutic effects similar to Western medicine characterizes the unique feature of the traditional Chinese medicine. By their pharmacological actions, Chinese herbs are generally classified into four categories: yin-nourishing, yang-invigorating, blood-enriching, and qi-invigorating actions; the blood-enriching and qi-invigorating medicines are grouped under yin and yang category. From the modern medicine perspective, yin-yang theory has been implicated to parasympathetic-sympathetic neural control in the body (206). Yin-yang balance was also suggested as an analogy to antioxidation-oxidation balance, in which yin is anti-oxidation and yang is oxidation. Indeed, yin-tonifying herbs have higher antioxidant

activity and polyphenol contents than yang-tonifying herbs based on the compound analysis (199). However, such concept is not fully supported as yang-invigorating herbs were found to possess even stronger free-radical scavenging activity than herbs of other categories in vitro (258, 304). This discrepancy might result from different criteria of herb selection and different methodology used between studies (131). Nevertheless, it has been suggested that both yin- and yang-tonifying herbs process antioxidant activity, and the effects on scavenging free radical might be associated with flavors (bitterness) (160). Additionally, yin-tonifying herbs play an immunoregulatory role whereas yang-tonifying herbs can enhance mitochondria ATP generation, which could act to preserve mitochondria integrity, maintain immune competence, and decelerate aging process (130).

CHINESE HERBS AND CARDIOVASCULAR DISEASES

Pharmacological effects of ginseng

Ginseng is a perennial herb of the Araliaceae family. It is considered as a tonic for restoration of strength or a panacea (240) and is considered a highly valued medicinal plant in the East. Asian ginseng (*panax ginseng C. A. Meyer*) and American ginseng (*panax quinquefolium L.*) are two most common ginsengs in the world; *Panax ginseng* has been widely used in Chinese Medicine over thousands of years (307). Ginsenosides, the major compounds of ginseng, have been demonstrated to exert protective effects that have been attributed to their antioxidant ability through increasing antioxidant enzymes (83) and acting as a free radical scavenger (115).

Ginsenoside is characterized by its 4-ring steroid-like structure with sugar moieties attached (saponin glycoside) and is amphipathic in nature that allow itself to interact with both hydrophilic and lipophilic molecules by its hydroxyl group (307). Based on the position, in

which sugar moieties attached to triterpene, ginsenosides are generally classified into protopanaxadiols (PPD) and protopanaxatriols (PPT). PPD groups consists of Ra1, Ra2, Ra3, Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3, whereas PPT groups includes Re, Rf, Rg1, Rg2, and Rh1(38, 166). To date, more than 40 different ginsenosides have been identified and isolated from the root of *panax ginseng*. Among them, ginsenoside Rb1, Rg1, Rg3, Re, and Rd are the most studied compounds that have been shown to contribute to vasorelaxation, antioxidation, and anti-inflammation (166). Of note, ginseng preparation and cultivated age play important roles in determining bioactive components and therapeutic effects of supplement (38). For example, unlike other forms of preparation, a single administration of red *panax ginseng* water extract elicits acute hypotensive effect in healthy males (91) whereas steamed ginseng (red ginseng) possesses different chemical constituents and pharmacological activities compared with air-dried ginseng (white ginseng) (203, 283). Ginsenoside content is >6 fold greater in ginseng root than that in ginseng body (57) and increases in accordance with age (38). Ginseng harvested at 4 years of age has significantly larger amount of ginsenoside content than 3 years of age (54) and was considered matured at 6 years of age (45).

Ginsenosides are thought to elicit vasorelaxation via the upregulation of NO (107, 108). Studies have found that the mixture of ginsenosides from PPT group produces greater cGMP in endothelial cells (116), and induce higher vasorelaxation (126) in the aortic ring than PPD ginsenosides *in vitro*. Purified Rg1 was found to elicit 5-fold greater cGMP than that of Rb1 at the same given concentration in animals (116). Although Rg3 is classified as a PPD, it is the most potent ginsenoside that mediates both endothelial-dependent (127) and -independent (126) vasorelaxation on a concentration-dependent fashion, possibly by activation of tetraethylammonium-sensitive K^+ channels in the endothelial cells. The potency of ginsenoside

Rg3 to induce endothelial-dependent relaxation was 3.4-fold greater than PPT ginsenoside and 79-fold greater than ginsenoside Rg1 (127). Notably, steam *panax ginseng* (red ginseng) contains a higher content of ginsenoside Rg3 than dried *panax ginseng* (white ginseng) according to the high-performance liquid chromatography analysis (203).

Although ginsenosides can exert vesorelaxation *in vitro* (41, 175), ginseng supplementation *in vivo* has not been quite successful in blood pressure reduction in both *panax ginseng* and *panax quinquefolium*. One early observational study in late 70s found that people became hypertensive after a 3-month use of *panax ginseng* (240), and subjects' blood pressure increased up to 150/96 mmHg from the initial 125/78 mmHg (241). It should be noted that subjects were not well characterized, and *panax ginseng* supplement was prepared differently among different subjects, which could have confounded their findings. In contrast, one later study found that chronic *panax ginseng* intake reduced blood pressure in patients with essential hypertension and white coat hypertension (92, 249), but not in healthy adults (28), which may not be surprising as improvement was more likely to occur in diseased populations. For *panax quinquefolius*, acute supplementation (247) does not appear to modulate blood pressure responses. Similarly, chronic intake (248) did not change blood pressure in hypertensive individuals. Taken together, existing evidence suggests *panax ginseng* and *panax quinquefolium* does not influence elevated blood pressure, and *panax ginseng* appears to be more efficacious in blood pressure reduction in hypertensive population, but not in healthy individuals.

Currently, only a few studies have evaluated effects of ginseng on arterial stiffness and endothelial function on humans. *Panax ginseng* has been shown to improve vascular endothelial dysfunction in hypertensive patients by demonstrating that individuals treated with *panax ginseng* for 24 months significantly improved in acetylcholine-induced vasodilation (254). Acute

intake of *panax ginseng* and isolated ginsenoside also improved augmentation index, an indicator of arterial wave reflection and aortic stiffness, in healthy individuals, whereas no effect was observed in the polysaccharside-only treated group (112). On contrary, chronic ginseng supplementation appears to have no beneficial effects on arterial stiffness after 3 months of intervention in individuals with hypertension (220).

Pharmacological effect of Danshen

Danshen (*salvia miltiorrhiza*) is a commonly used traditional Chinese medicine with diverse pharmacological properties to improve circulation and blood stasis (89). Danshen dilates coronary arteries, increase coronary blood flow, and scavenge free radicals in ischemic diseases (109). Danshen has been prescribed to treat angina pectoris, hyperlipidemia, acute ischemic stroke (312), and coronary heart disease (44). Chemical compounds from *salvia miltiorrhiza* extract have been identified and classified into 2 major categories: lipophilic and hydrophilic (89, 312). The lipophilic are diterpene compounds mainly consisting of tanshinone I, IIA, IIB, and cryptotanshinone; hydrophilic compounds are mainly composed of phenolic and polyphenolic acids, such as danshensu, protocatechuic aldehyde and salvianolic acid A, B. Among these compounds, tanshinone IIA, danshensu, and salvianolic acid B were most extensively studied (312) as described below.

Tanshinone IIA

Tanshinone IIA is the most abundant and major compound of lipophilic tanshionone that has been demonstrated to exert antihypertensive (35), anti-proliferative (111), anticancer (152) and antioxidant effects (164, 299). Tanshinone IIA has been shown to inhibit ET-1 production (264), vascular smooth muscle cell proliferation, and remodeling (111, 285), prevent the increase

in intracellular calcium concentration mediated by angiotensin II in myocytes (260), reduce the expression of vascular adhesion molecules (VCAM-1 and ICAM-1)(265), and increase endothelium-dependent vasodilation in isolated rat coronary arterioles presumably by modulating NO production and potassium channel activation (295). It was recently demonstrated that tanshinone IIA lowered blood pressure in hypertensive animals by augmenting NO production through alterations in eNOS gene expression (124). Collectively, tanshinone IIA appears to improve vascular function and exert antihypertensive effects by enhancing vasodilation via up-regulation of endothelial-derived relation factors. It should be noted, however, that such protective effects were only observed in diseased models, whereas treatments did not contribute to significant physiological changes on wild type controls (35).

Danshensu

Danshensu, 3-(3.4-dihydroxy-phenyl)-2-hydroxy-propionic acid, is another active component of Danshen. It improves microvascular function, suppress ROS formation, platelet aggregation and adhesion and protects myocardium against ischemia (43, 90, 284). High levels of homocysteine is known to cause endothelial dysfunction (33) and is associated with hypertension in older adults (255). Growing evidence based on in vitro studies is linking the protective effects of danshensu to homocystein metabolism and vascular function. For example, the deleterious effects of high homocysteine on endothelial injury were found to be attenuated by danshensu extracted from danshen (34). Additionally, danshensu can lower homocysteine levels (27) on a dose-dependent manner (34), and chronic danshensu treatment decreases serum homocystein, endothelin, and down-regulated TNF-α and ICAM-1 expression in the animal model of hyperhomocysteinmia (300). Similar to other compounds, danshensu was found to

induce vasorelaxation in isolated coronary artery by inhibiting Ca^{2+} influx in vascular smooth muscle cells (142).

Salvianolic acid B

Salvianolic acid B, a water soluble polyphenolic antioxidant, appears to lower cholesterol and inhibit LDL oxidation through its antioxidant function both in animal models (297) and in human endothelial cells (296). In vitro studies demonstrated that Salvianolic acid B induces vasorelaxation by activating the opening of calcium-activated potassium channels of porcine coronary artery smooth muscle cells via activation of guanylate cyclase (143). In addition, Salvianolic acid B attenuates VCAM-1 and ICAM-1 expression in TNF-α treated human aortic endothelial cells (42) and down-regulated plasminogen activator inhibitor-1 mRNA expression in human umbilical vein endothelial cells (313).

Sheng-Mai-San

Sheng-Mai-San (SMS) is a traditional Chinese medicine (TCM) compound that consists of three different herbal components; *Panax ginseng, Ophiopogon japonicus, and Fructus schisandra*. This formula has been used for more than 7 hundred years to treat cardiovascular diseases including coronary artery disease, angina pectoris, myocardial infarction, cerebral infarction, coronary heart disease, arrhythmia, myocarditis, shock, hypotension as well as Alzheimer's disease in Chinese medicine (39). SMS has also been used to treat damaged qi and body fluids resulting from summer heat and profuse sweating, as well as chronic coughing accompanied with symptoms such as dry throat, thirst, irritability, lethargy, shortness of breath, and a weak pulse (39). Besides *Panax ginseng* that is the chief herb used to reinforce the lungs and supplement qi in this formula, *Ophiopogon japonicus* extracted from lilyturf root is the

supplement for clearing the lungs and nourishing yin, and *Fructus schisandrae* (schisandra berry) is used to converge yin in the lungs (311).

Ophiopogon japonicus

Ophiopogon japonicus Ker Gawler (Liliaceae) is an evergreen perennial that has been identified as rich in steroidal saponins (287) and homoisoflavonoids (96). In vitro studies have shown that extracts from *Ophiopogon japonicus* have anti-inflammatory (137) and antithrombotic effects on endothelial cells (138, 139). Recently, homoisoflavonoids extracted from *Ophiopogon japonicus* were found to have antioxidant activity (158) and reduce chemokine release with dose-dependent manner in IL-4 and TNF-α-induced cell line models (102).

Fructus schisandrae

Fructus schisandrae is the main active ingredient isolated from the fruit of Schisandrae chinensis Baill. Lignans from *fructus schisandrae,* such as schisadrin A, B, C, and gomisin, are thought to be responsible for its pharmacological activity (200). *Fructus schisandrae* and its derivatives have been demonstrated to inhibit platelet activating factor release (153), inhibit iNOS expression (88), cause both endothelium-dependent and -independent vasorelaxation in rat thoracic aorta (202, 223), and react with estrogen receptor to induce NO-mediated vasodilation (155). It was also demonstrated that after 5 weeks of treatment, a group supplemented with aqueous extract form f*ructus schisandrae* exerted cardioprotective effects comparable to those of 17-β estradiol groups by significantly attenuating artery thickening, and lowering LDL-C and blood pressure in the balloon-induced carotid artery injury model (125).

SMS and antioxidant effects

Ischemia reperfusion injury on vasculature is associated with a variety of cardiovascular diseases, including stroke (2), myocardial infarction (315), and renal dysfunction (129). In

animal models, SMS have been demonstrated to prevent cerebral, myocardial, and renal oxidative damage induced by ischemia-reperfusion injury (103-105, 154) and reduce lipid peroxidation through its anti-oxidant effects on reducing the loss of glutathione peroxidase during ischemia (301, 305) and inhibition of tiobarbituric acid-reactive substances (TBAR) formation during reperfusion (105, 298, 301). SMS have also been shown to improve the antioxidant capacity against acute oxidative stress in vitro on C2C12 myoblasts (195) and prevented apoptosis PC-12 cell treated with amyloid-β peptide (196). Administration of SMS significantly attenuated myocardial and cerebrovascular dysfunction in heat failure (305) and stroke (298) models. Interestingly, components of SMS play synergistic and additive roles on antioxidant protection because hydroxyl radical scavenging activity of either single component or combinations of two was not as high as that of three components together (105).

In China, Shengmai injection and capsule, formulated on the basis of SMS, have been widely used to treat patients with coronary artery disease. There are accumulating evidence indicating that cardiac function (cardiac output, stroke volume, ejection fraction) (100, 309, 310) and vascular endothelial function (311) improve significantly in patients treated with Shengmai injection than those treated with conventional Western medicine (i.e., asprine, heparin and nitric ester drugs). These improvements were accompanied with reduced hs-CRP (309), angiotensin II, ET-1, as well as increased NO production (311) after 3 weeks of treatment in patient populations. In addition, SMS decoction also lowered mean arterial blood pressure, peripheral resistance, and improved ejection fraction in healthy adults (100).

SUMMARY

Vascular dysfunction is the most important event preceding the development of cardiovascular diseases. It is well established that chronic oxidative stress and ROS formation contribute to imbalanced redox state and reduced NO bioavailability in vasculature, leading to impaired endothelial function and increased arterial stiffness. Similar to antioxidant consumption, regular physical exercise, especially aerobic exercise, has been suggested an effective means to prevent age-related oxidative stress. However, eccentric exercise has been shown to induce unfavorable effects on vascular function, which was found to be associated with exercise-induced inflammation. Accumulating evidence indicates that antioxidant cocktail and polyphenol supplementation may provide more promising effects on restoring age-related increase in oxidative stress and vascular function.

Chinese herbal medicine has been practiced for thousands of years in China and its surrounding countries, and it is gaining popularity and widespread use in the West, yet pharmacological activity of many herbs remains unclear. With the increasing effort to integrate Chinese herb and conventional Western medicine, evidence is accumulating to suggest that Chinese herb possesses phenolic acids and polyphenol compounds that exert antioxidant effects to ameliorate vascular disorders. This is consistent with the Yin-yang philosophy of Chinese medicine, where disturbance in yang is adjusted to maintain homeostasis (199). *Panax ginseng* and *salvia miltiorrhiza* are two of most widely used Chinese herbs in treating CVD in Chinese medicine; Both *in vitro* and *in vivo* studies showed that they possess not only multi-antioxidant effects, but also regulate both vasodilators and vasoconstrictors (Table 1). Accordingly, it is reasonable to hypothesize that the combination of *panax ginseng* and *salvia miltiorrhiza* could provide strong vascular protective effects against exercise-induced inflammation and vascular dysfunction induced by eccentric exercise models.

Table VII.1 In vitro and in vivo pharmacological action of panax ginseng and salvia miltiorrhiza compounds

	eNOS/NO	Antiplatelet	FR scavenging	Antioxidant enzyme production	Adhesive molecule reduction/ anti-inflammatory	Ox-LDL reduction	ET-1 reduction	Ca2+ influx reduction
Panax ginseng	√		√	√	√	√		
Salvia miltiorrhiza								
Tan. IIA	√		√		√		√	√
Danshensu		√	√		√		√	√
Sal B	√	√		√	√	√		

Tan IIA: Tanshinone IIA; Sal B: Salvianolic acid B

Chapter VIII: Summary and Future Direction

Identifying risk factors for CVD, the worldwide leading cause of death, is crucial in reducing mortality and establishing effective lifestyle modification can help to improve disease prevention, thus lowering the cost for health-related care in the society. Vascular dysfunction manifested as an increase in arterial stiffness or impaired endothelium-dependent vasodilation is an important and independent risk factor for CVD; increased oxidative stress and inflammation is believed the underlying mechanism that closely linked with pathophysiology of vascular function impairment. Regular physical exercise and antioxidant consumption have been advocated to improve vascular function by its anti-inflammatory properties exerted on vasculature. In spite of this, a growing body of evidence showed muscle damage induced by accustomed exercise resulted in increased arterial stiffness, indicating the association between exercise-induced inflammation and impaired vascular function. However, currently our understanding of such phenomenon is limited and it is imperative to elucidate its clinical importance since eccentric exercise training has been recently advocated for the public due to other health benefits.

Herbal supplement is a commonly used intervention in complementary alternative medicine for treating CVD by its properties of anti-inflammatory effects, yet the scientific evidence to support its use is still lacking. Therefore, the purpose of this dissertation was to determine the association of eccentric-induced inflammation and vascular function, as well as efficacy of traditional Chinese herb supplementation in preserving vascular functions. In Study 1, we first tested the hypothesis acute downhill running exercise that could elicit higher inflammatory response may contribute to larger response in arterial stiffening effects than

local eccentric resistance exercise on lower legs. In addition, whether such transient arterial stiffening would affect hemodynamic wave reflection. Based on the results, we found that 1). downhill running exercise elicit prolonged arterial stiffening effects than eccentric resistance exercise; 2). the increase in central arterial stiffness was associated with systemic inflammatory marker, CRP; 3). the transient increase in central arterial stiffness did not lead to additional hemodynamic burden in terms of wave reflection analysis.

Based on the findings from Study 1, we sought to determine the efficacy of Chinese herbal supplementation (*panax ginseng* and *salvia miltiorrhiza*) in preserving vascular function following acute downhill running exercise in Study 2, as well as along with chronic eccentric resistance exercise training intervention in Study 3. In Study 2, we found that 1). acute downhill running did result in similar transient arterial stiffening effects following exercise. 2). supplementation with *panax ginseng* and *salvia miltiorrhiza* prior to exercise did ameliorate central arterial stiffness following exercise, possibly by attenuating the level of muscle permeability (CK release), and pro-inflammatory IL-6 release from damaged muscle tissue or immune cells. The salient findings of Study 2 provide scientific evidence of efficacy and effectiveness of such herbal formula in protecting vascular function from eccentric exercise model. By using a double-blinded randomized design in Study3, we further sought to determine whether chronic weekly eccentric resistance training would contribute to adverse effects on vascular functions and whether daily supplementation of *panax ginseng* and *salvia miltiorrhiza* along with training would attenuate the stiffening effect. We found that 1). the progressive and weekly eccentric resistance exercise protocol used in Study 3 did not contribute to elevated arterial stiffness along with training; 2). long-term supplementation appear to attenuate the hypertrophic effect induced exercise training when compared with the

placebo group on relative changes in muscle mass; 3). long-term supplementation did to affect anti-oxidant status, creatinine, alanine transaminase, and aspartate transaminase, suggesting chronic intake of our supplementation dosage in Study 3 did not elicit side-effects on general indicators of liver and kidney functions, as well as affect anti-oxidant status in the body.

In this dissertation, eccentric exercise was used as a stimulus to induced acute inflammation and we demonstrated greater systemic inflammation resulted from exercise contribute to prolonged arterial stiffening. Furthermore, we found *panax ginseng* and *salvia miltiorrhiza* prior to exercise effectively modified such unfavorable effects of acute inflammation. Future studies directed at seeking the efficacy of different supplementation dosage and timing on protecting vascular function by using different inflammation models would provide additional scientific evidence on its use in herbal products.

Appendix: Progressive Eccentric Resistance Exercise Training Protocol

	Set	Repetition	Intensity	Interval
1	3	10	10%MVC	2 minutes
2	3	10	20%MVC	2 minutes
3	3	10	40%MVC	2minutes
4	6	10	40%MVC	2 minutes
5	3	10	60%MVC	2 minutes
6	6	10	60%MVC	2 minutes
7	2	10	80%MVC	2 minutes
8	4	10	80%MVC	2 minutes
9	6	10	80%MVC	2 minutes
10	2	10	100%MVC	2 minutes
11	4	10	100%MVC	2 minutes
12	6	10	100%MVC	2 minutes

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Vita

Hsin-Fu Lin, also known as Thomas, was born in Chunghwa city in Taiwan. After graduating Ming-Dao High School in 1994, he entered National College of Physical Education (known as National Taiwan University of Sports at present) and graduated in June 1998 with a Bachelor of Science degree in Athletic Training. In September of 1998, Hsin-Fu entered National Taiwan Normal University to work in Exercise Physiology Lab under the guidance of Dr. Jung-Charng Lin. He received a Master of Education degree in Physical Education with an emphasis on Exercise Physiology in June of 2000. In September of 2000, he continued to start the Ph.D. program in the same institute also under Dr. Lin. With the encouragement of Dr. Lin, Hsin-Fu succeeded on the exam and earned the Scholarship to Study Abroad from the Ministry of Education of Taiwan in 2003 and started to pursue a Ph.D. degree at the University of Maryland in College Park in 2004. With a greater interest in cardiovascular physiology on aging, Hsin-Fu went to work with Dr. Hirofumi Tanaka at the University of Wisconsin-Madison in 2005 and moved with Dr. Tanaka to The University of Texas at Austin and enrolled in the Ph.D. program. After finishing coursework, Hsin-Fu went back to Taiwan and served as Assistant Professor in National Taiwan University. Meanwhile, he finished data collection and dissertation writing in Taiwan under the guidance of Dr. Tanaka. His research has been funded by Ministry of Science and Technology and National Taiwan University in Taiwan. His work has been presented at the American College of Sports Medicine, Taiwan Society of Exercise Physiology and Fitness, Pulse of Asia, and National Strength and Conditioning Association conference. He has contributed to publications appearing in *Journal of Human Hypertension* and *Journal of Sports Medicine and Fitness,* as well as several peer-reviewed Chinese journals in Taiwan.

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