Effect of age and fitness on vascular function and oxidative stress during acute inflammation

NCT03889158

Version: 6

Date: 12/05/2019

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
CV	Cardiovascular
FMD	Flow-mediated dilation
HR	Heart rate
IPL	Integrative Physiology Lab

1.0 Project Summary/Abstract

Acute and chronic inflammation both increase cardiovascular disease risk, especially with aging, which may be due to vascular dysfunction. Aging and inflammation also lead to increased oxidative stress, which impairs vascular function. During acute inflammation, endothelial function is altered differently in younger and older adults with decreases in endothelial function in younger, but not older adults. However, cardiorespiratory fitness is cardio-protective, impacting inflammation, vascular function, and oxidative stress. During acute inflammation, moderately fit older adults exhibit similar responses to younger adults, suggesting preserved endothelial reactivity. However, whether the protective mechanism is oxidative stress has not been confirmed. Furthermore, it is undetermined whether the vascular dysfunction is further propagated down the arterial tree during acute inflammation to the microvasculature. We will investigate these questions through 2 specific aims: 1) To determine if age and fitness moderate the vascular response to acute inflammation; 2) To determine if antioxidant administration eliminates vascular dysfunction during acute inflammation.

We will recruit 15 young low fit (18-35 years) and 30 older low or high fit (55-75 years) adults. An acute inflammatory bout will be induced by typhoid vaccination. The influence of oxidative stress will be assessed using an oral antioxidant. Baseline influences of the antioxidant on endothelial function will be assessed on Visit 1. Acute inflammation measures will be taken at baseline (Visit 2, prior to) and 24-hours after the vaccine (Visit 3). The antioxidant will be administered again on Visit 3.

We anticipate low fit older adults will have reduced endothelial responsiveness and microvascular reactivity compared to young and high fit older adults. Second, we anticipate the antioxidant will improve vascular function at baseline in low fit older adults. During acute inflammation, we anticipate improvement in vascular function to baseline levels in young and older high fit adults.

The results from this study will help to elucidate if fitness is a protective and preventive measure to ameliorate the detrimental cardiovascular response to acute inflammation. Thus, this study may provide health professionals with a behavioral intervention to reduce cardiovascular disease burden in the rapidly growing aging population.

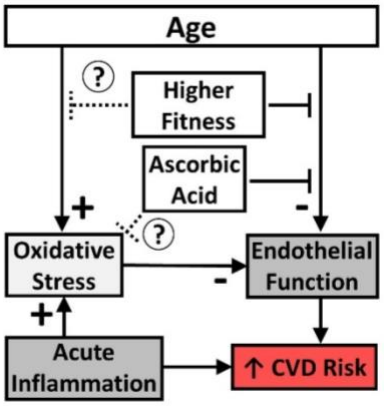
2.0 Background/Scientific Rationale

Acute inflammation increases CV risk [1–4] with a higher prevalence of older adults experiencing adverse CV events[3]. Aging is associated with immunosenescence[5,6], rendering older adults more susceptible to infection[7] and may contribute to the higher mortality and acute CV events following acute inflammation in the elderly[1,2,8]. Indeed, infectious disease hospitalization and the concomitant inflammatory insult leads to an 8-fold increased risk of acute coronary syndromes in older adults within 15 days after admission[9].

Increased CV risk with acute inflammation may also stem from detrimental changes in endothelial function[10–12] and arterial stiffness[13–15]. Indeed, our laboratory has shown that endothelial function during acute inflammation decreases in younger, but not in older, adults [16,17]. Moreover, acute inflammation results in concomitant increases in arterial stiffness, a predictor of CV events. These large artery impairments during acute inflammation may be further propagated down the arterial tree to the microvasculature, altering microvascular reactivity. During a severe acute inflammation model, peripheral near-infrared spectroscopy (NIRS) evaluation of microvascular reperfusion is altered earlier than other systemic variables[18]. NIRS may therefore serve as an early detector of altered peripheral perfusion during acute inflammation. As such, identifying means to restore vascular reactivity during acute inflammation to levels seen in young adults may help attenuate CV risk in older adults.

Higher cardiorespiratory fitness and habitual physical activity are protective for many aspects of CV health[19,20] and appear to also be protective during acute inflammation. Recent data suggests voluntary running is protective against acute inflammation and the concomitant microvascular dysfunction in a mouse-model of sepsis[21]. Furthermore, population-based data suggests inadequate exercise (<1.07 MET h/day) is associated with a doubling of the risk of mortality during severe

Figure 1.



acute inflammation (sepsis), independent of other risk factors[22]. Additionally, during acute inflammation, our group has shown moderately fit older adults 1) maintain vascular reactivity similar to that of younger adults[23], which may help protect maintenance of blood pressure and 2) do not experience increases in arterial stiffness as seen in low fit older adults.[15] These data suggest exercise has beneficial effects in clinical manifestations of inflammation and has shown potential cardioprotective effects during acute, low-grade inflammation.

The protective effect of fitness in older adults is potentially due to lower oxidative stress. Aging alone increases oxidative stress which reduces nitric oxide bioavailability, impairing baseline endothelial function (Figure 1). In older adults, habitual physical activity maintains endothelial function to similar levels as young healthy adults[24,25] and increases antioxidant defense by reducing NADPH oxidase and nuclear factor-κB (NFκB) activity[26]. Salsalate administration inhibits NFκB, reducing oxidative stress, and improving endothelial function[27]. Salsalate administration improves endothelial function in older, sedentary adults but not their trained counterparts or younger adults[28]. This supports the role of fitness in reducing oxidative stress with aging.

During *acute inflammation* in young adults, endothelium-independent vasodilation remains intact, indicating the change in endothelial function is likely due to reduced nitric oxide bioavailability and increased oxidative stress[11,12]. Increases in oxidative stress have been shown at 48 hours following a vaccination[10]. Additionally, ascorbic acid has partially resurrected forearm blood flow responses to acetylcholine during acute inflammation[11]. Since being physically active while aging maintains baseline levels of oxidative stress and endothelial function similar to that of young adults, changes in oxidative stress may explain the differing conduit endothelial responses between older low and high fit adults during acute inflammation. However, the mechanism of impairment has not been fully investigated. Antioxidant intake improves vascular function in older adults to similar levels of young adults, suggesting a role of oxidative stress[29,30]. Thus, ingestion of an antioxidant such as ascorbic acid may eliminate vascular dysfunction during acute inflammation.

To understand the vascular response to acute inflammation, an integrative approach must be applied to identify both the mechanism of vascular dysfunction and a potential therapeutic target to abrogate acute inflammation-induced vascular dysfunction. This study addresses both gaps of knowledge by investigating the role of oxidative stress and by recruiting participants based on training levels to effectively examine the effect of high fitness.

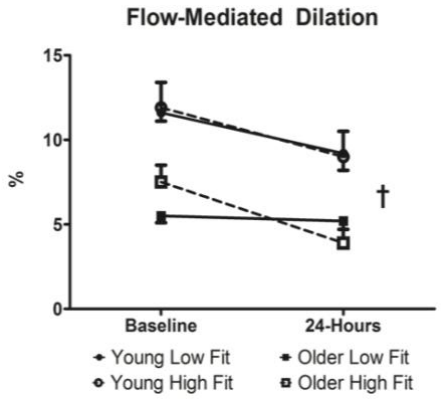
Relevance

CV disease is the leading cause of death in the United States and particularly burdens older individuals[31]. Aerobic exercise is recommended by the United States Physical Activity Guidelines, American Heart Association, and American College of Sports Medicine (ACSM) to improve quality of life, reduce risk of heart disease, diabetes, stroke, and osteoporosis, and improve immune function. If fitness moderates the effects of acute inflammation on vascular function, our study will provide insight into how physical activity and exercise interventions could reduce the risk of CV events during acute inflammation.

Preliminary Studies

Our laboratory has conducted a series of studies evaluating the effect of acute inflammation on cardiovascular function[15–17,32–34]. evaluated both cardiac and endothelial function during acute inflammation in younger and older adults[16,17,34]. These studies suggest age-associated differences in endothelial function, cardiac function, and blood pressure that may be detrimental to older adults[16,33,34]. We have shown that fitness, an indicator of chronic exercise, may exert protective effects. In older adults, high fitness protected from acute inflammation-induced arterial stiffening[15].

Figure 2



Recently our lab has shown that high fit older adults also have a similar endothelial response to acute inflammation as younger adults. The high fit older adults exhibit a greater reduction in flow-mediated dilation than older low fit adults. Fitness level did not influence the response in young adults (Figure 2)[23]. Additionally, we have collected pilot data on microvascular reactivity in young adults (n=16), showing no changes in tissue saturation index from baseline to 24h during acute inflammation in peak hyperemic response (13.7±3.9% to 13.8±2.9%) or reperfusion slope (1.84±0.52%/s to 1.83±0.49%/s).

3.0 Objectives/Aims

Aim 1: To determine if fitness moderates the vascular response to acute inflammation with aging. *We hypothesize that during acute inflammation* A) *high fit older adults will have similar endothelial and microvascular reactivity as young adults; B) high fit older adults will have greater endothelial and microvascular reactivity than low fit older adults.*

Aim 2: To determine if antioxidant administration eliminates vascular dysfunction during acute inflammation. *We hypothesize* A) *at baseline, ascorbic acid will improve endothelial function only in low fit older adults;* B) *during acute inflammation, ascorbic acid will improve vascular function to baseline values in young and older high fit adults.*

4.0 Eligibility

4.1 Inclusion Criteria

- Males and females willing to provide informed consent
- 18-35 or 55-75 years of age
- Non-smoker
- No antioxidant/vitamin supplementation
- No use of anti-inflammatory medication within last 2 weeks
- Aerobically trained (defined as performing aerobic exercise on ≥4 days/week, for ≥30 minutes, for at least the past 3 months AND a VO2max ≥75th age- and sex-specific percentile according to ACSM)

  OR

  Sedentary (defined as being involved in less than 30 minutes of moderately-intense physical activity per day, < 3 days/week AND a VO2max ≤ 50th age- and sex-specific percentile according to ACSM)

4.2 Exclusion Criteria

- Body mass index >35 kg/m^2
- Pregnancy, hormone replacement therapy, or peri-menopausal
- Known cardiovascular (i.e. atherosclerosis, uncontrolled hypertension, stroke, myocardial infarction, etc.), inflammatory (i.e. Crohn's disease, arthritis, etc.), or metabolic (i.e. Diabetes mellitus) disease
- Current use of >2 blood pressure medications, change in vasoactive medication type or dose within past 2 months.
- Regular use of medications to reduce inflammation (NSAIDS, aspirin, steroids, etc)
- Bleeding disorders
- Illness, other vaccination, or antioxidant use within 2 weeks prior to screening
- Typhoid vaccination within previous 2 years or prior adverse reaction
- VO2max in 51st – 74th age- and sex-specific percentile according to ACSM
- Non-English speaking participants

4.3 Excluded or Vulnerable Populations

- Pregnant women, women on hormone replacement therapy, and peri-menopausal women will be excluded due to the changes in hormonal concentrations, hemodynamics, and altered exercise capacity
- In addition, we exclude non-English speakers as the researchers must ensure the subjects completely understand the purposes and risks associated with our study and we do not have any Spanish speaking research personnel.

5.0 Subject Enrollment

Subjects will be recruited from the UIC campus and Chicago community using an IRB approved recruitment flyer, internet recruitment ad (i.e. UIC website, Craig's List, ResearchMatch, etc.), social media (i.e. Facebook, Twitter, etc.) and word of mouth including speaking engagements. Subjects will be asked to contact the IPL for further information about the study and subsequent screening for eligibility by either phone or e-mailing Elizabeth Schroeder.

After the screening is complete, participants will be asked if they are still interested in the study. If interested, their age, gender, and group (sedentary or active) will be kept by Elizabeth Schroeder. Once an individual of the same gender, similar age (within 2 years) and opposite group has been identified, participants will be contacted again for scheduling of visits. We have not had issues with this type of recruitment in the past.

If still interested, a packet will be sent (mail or email) to the potential subject with a copy of the informed consent and information regarding the first laboratory visit. All subjects will sign an institutionally approved informed consent prior to participation in the study during visit one.

6.0 Study Design and Procedures

Study site

All study procedures except the vaccination will be conducted in the Integrative Physiology Laboratory at the University of Illinois at Chicago located at 1640 W. Roosevelt Road Suite 158 in the Disability, Health and Social Policy building (DHSP). The typhoid vaccination will be administered by a registered nurse at the Clinical Research Center at UIC.

Design

A comparative, observational study design will be conducted with a group of 15 low fit young adults, 15 low fit older adults, and 15 high fit older adults, for a total of 45 research participants.

Subjects will participate in three study visits, with at least 72 hours between the first two visits, and exactly 24 hours between the last two visits. Menstruating females will be studied during the first 7 days of menses, or during the placebo phase if taking oral contraceptives. Participants will arrive to each visit having refrained from exercise, alcohol, and caffeine for 24h prior to each test day. Participants will be tested in the post-prandial (no food, only water) state (>10 hours) for all visits. (Figure 1)

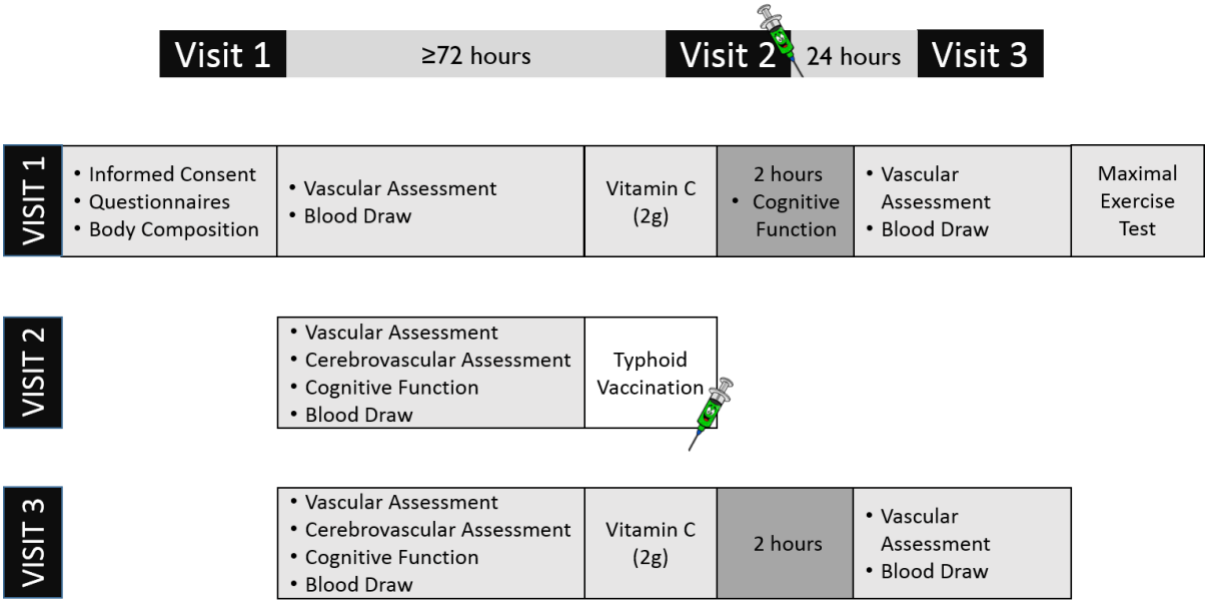


Figure 1. Study protocol schematic

Visit 1. Following informed consent, participants will complete a health history, food recall, and physical activity questionnaire and undergo anthropometric (height, weight, waist and hip circumference) and body composition measures (DEXA scan). For women of childbearing age, the first visit will include a urine pregnancy test using a test stick. Baseline influence of oxidative stress on endothelial and microvascular function will be assessed following supine rest and resting hemodynamics. Measures will include: 1) endothelial function, 2) microvascular function, 3) arterial stiffness, and 4) circulating markers of oxidative stress. Participants will then orally ingest 2g of ascorbic acid. This dose produces a physiological level of ascorbate in plasma[35], has been used in previous studies to improve endothelial function[35–39], and reaches optimal absorption after 2 hours[40,41]. During this time, an assessment of general cognitive function will be completed using the Montreal Cognitive Assessment (MOCA) and Symbol Digits Modality Test (SDMT), and be familiarized with a working memory cognitive function task (NBACK) by completing the task four times throughout the two hour period. Following 2 hours, all measures will be repeated to determine the influence of the antioxidant. The visit will finish with a treadmill exercise test to evaluate cardiorespiratory fitness (VO2peak).

Visit 2. At least 72 hours later, participants will arrive after an overnight fast (>8 h) and undergo supine baseline measures of: 1) endothelial function, 2) microvascular function, 3) arterial stiffness, 4) cerebrovascular function and 5) cognitive function, 6) heart function and 7) circulating markers of inflammation

and oxidative stress. The typhoid vaccination (Typhuim Vi, Sanofi Pasteur SA) will then be administered by a registered nurse. Acute inflammation is often induced in cardiovascular research by use of either the influenza or typhoid vaccination [10,11,13,23,33,34,42]. Vaccinations provide a safe and controlled inflammatory response, in which the typhoid vaccination has shown to increase hsCRP up to at least 32 hours post-vaccination[13].

Visit 3. Exactly 24-hours following Visit 2, participants will return to the lab under the same conditions. Visit 2 will be repeated. Following baseline measures, participants will consume 2g of ascorbic acid, as completed in Visit 1. Following 2 hours, measures of 1) endothelial function, 2) microvascular function, 3) arterial stiffness and 4) circulating markers of oxidative stress.will be repeated to determine the influence of the antioxidant.

All procedures are being completed for research purposes. The table below shows the estimated time commitment for each study visit and procedure.

	Visit 1	Visit 2	Visit 3
Informed Consent/ Questionnaires	30		
Anthropometrics	5		
DEXA	10		
Blood Draw	10	10	10
Rest/Preparation	10	10	10
Vascular Assessment			
Blood pressure	7	7	7
FMD/NIRS	13	13	13
Arterial stiffness (PWV)	10	10	10
Brain blood flow (TCD)		15	15
Carotid Flow		5	5
Heart Function		15	15
Cognitive: NBACK		12	
Vaccination		15	
Vit C	120		120
	NBACK Familiarization MOCA SDMT		Cognitive: NBACK
Blood Draw	10		10
Vascular Assessment			
Blood pressure	7		7
FMD/NIRS	13		13

Arterial stiffness (PWV)	10		10
Maximal Exercise Test	30		
TOTAL TIME (minutes)	285	112	245
Hours:	4.75	1.87	4.08

Measurements

DEXA Scan

We will determine percent body fat via a whole body scan by dual energy x-ray absorptiometry (GE, IDXA, Madison, Wi), which will be operated and calibrated using the manufacturer's stated guidelines. The duration of the scan will be less than 10 min and the subject will be exposed to a radiation dose of $\leq$0.3 mrem based upon the manufacturer's specifications and calculations from Standford Dosiometry, LLC RADAR Medical Procedure Radiation Dose Calculator. This amount of research protocol radiation exposure is minimal given the estimated effective equivalent dose is below the 100 mrem per year, a limit set by the Nuclear Regulatory Commission for "general public" exposure.

Venous blood samples

A blood sample will be collected at baseline during each visit (3 times) and following Vit C consumption (2 times). During the first visit, each blood sample will be 15 mL (approximately 1 tbsp). On visit 2 and 3, the baseline blood draw will be 25 mL (approximately 5 tsp). The final blood draw on visit 3 will be 15 mL (approximately 1 tbsp). At all time points, we will measure C-reactive protein and interleukin-6 for inflammatory markers, oxidized LDL as a marker of oxidative stress, total antioxidant capacity and ascorbate for antioxidant capacity, and endothelial extracellular vesicles, in which additional analyses on inflammatory markers may be performed. The baseline blood draw of visit 2 and 3 will also assess the cholesterol panel (Total cholesterol, HDL cholesterol, non-HDL cholesterol, triglycerides, LDL cholesterol, TC/HDL ratio) and glucose. The additional 10 mL during the baseline blood draw on visit 2 and 3 will be used for analyses of peripheral blood mononuclear cells, which provide valuable information about immune function. Immediately after acquisition of venous blood, plasma or serum will be separated by centrifugation (3000g at 4°C for 15 minutes), placed in aliquots, and stored at -80°C.

Blood pressure

Blood pressure will be measured in the arm using an automated microprocessor controlled ambulatory blood pressure monitor (Mobil-O-Graph 24 PWA, I.E.M, Stolberg Germany) in the supine position according to the guidelines of the American Heart Association. BP will be measured twice, one minute apart, and the average of the two values will use as resting BP. If the two BP measurements

differ by more than 5 mmHg, a third measurement will be taken. Cardiovascular variables such as derived ascending aortic BP waveform and central arterial indices (PWV and pulse wave analysis), stroke volume and cardiac output can be calculated from the pressure waveforms.

For central blood pressure, radial artery pressure waveforms will be attained in the supine position from a 10-second epoch using applanation tonometry and a high-fidelity strain gauge transducer (Millar Instruments, Houston, TX) [43]. A central aortic pressure waveform is reconstructed from the aforementioned radial artery pressure waveform using a generalized validated transfer function (SphygmoCor, AtCor Medical, Sydney, Australia). From this reconstructed waveform, aortic pressures, end-systolic pressure, and sub-endocardial viability index (a ratio of diastolic to systolic pressure-time intervals) will be obtained [43]. Only recordings receiving an in-device quality index $\geq$ 80% will be included in analysis [43].This index is derived from a weighted algorithm that examines average pulse height (average height of the pulse waveforms measured), pulse height variation (amount of variation in the height of the pulse waveforms measured), and the diastolic variation (an index of baseline pressure consistency during the measurement attained from the variation in the diastolic inflection point of the pulse waveform). Previous work shows that reproducibility of measures attained from this technique is high [44].

Pulse wave velocity (PWV)
Approximately 20-sec of pressure waveforms will be collected at the brachial, common carotid, and femoral arteries using a high-fidelity strain-gauge transducer. PWV will be calculated from the distances between measurement points and the measured time delay between proximal (carotid) and distal (femoral) waveforms (NIHem, Cardiovascular Engineering Inc.). The brachial waveforms will not be used for PWV calculations, but are necessary for calibrating carotid blood pressure (described below).

Carotid Blood Pressure
Carotid artery pressure waveforms obtained during the measurement of cf PWV will be ensemble averaged to construct a carotid pressure waveform. This carotid waveform is then calibrated to brachial waveforms and brachial mean and diastolic pressure. The resulting carotid systolic pressure is necessary for calibrating carotid stiffness measures (described below).

Flow-Mediated Dilation (FMD)
Brachial artery vasodilator function will be noninvasively measured through assessment of brachial artery dilatation using ultrasonography (Hitachi Aloka, Alpha 7 Japan). The brachial artery will be imaged in longitudinal section, 5–10 cm proximal to placement of a blood pressure cuff, just below the antecubital fossa, using a high frequency (5–13 MHz) linear array probe. Endothelium-

dependent, flow-mediated dilatation (FMD) of the brachial artery will be measured at baseline and again for 5 minutes following ischemic stimulus (inflation of a blood pressure cuff around the forearm to 250 mmHg for 5 minutes). After cuff deflation, resultant reactive hyperemia is calculated as the flow change from baseline and expressed as a percentage change in blood flow [45]. Analysis of the FMD will be carried out using a semi-automated edge detection software system. This system requires an ECG and will be measured by placing 3 electrodes on the torso of the subject. Arterial diameter response will be calculated as percentage change in brachial artery diameter from baseline and controlled for the shear stimulus created by the hyperemic flow. The computerized image analysis system with edge-detection software significantly attenuates observer error over manual methods and allows the detection of changes in endothelial function with substantially fewer subjects. This method conforms to the guidelines set out for the ultrasound measurement of endothelium-dependent FMD of the brachial artery [45].

Near-Infrared Spectroscopy (NIRS)
Tissue oxygenation saturation will be measured non-invasively by near-infrared spectroscopy (OxiplexTS, ISS Inc., Champaign, IL) on the forearm during the flow mediated dilation. The small NIRS sensor will be held in place with a flexible elastic band.

Transcranial Doppler
Cerebral blood flow velocity will be measured for 7 minutes in a resting state in the middle cerebral artery (MCA) using two 2-MHz transcranial Doppler ultrasound probe (TOCM Neurovision, MultiGon Industries, INC. Elmsford NY) fitted with a specific cushioned headpiece to hold the probe in place. The Doppler probe will be placed at the temporal window on the side of the head to insonate the vessels. The blood velocity wave form will be recorded continuously at 500 Hz and stored off-line for subsequent analysis of the systolic, diastolic and mean blood flow velocities. Brain blood velocity is influenced by carbon dioxide, thus, we will monitor end tidal CO_2 throughout this testing period. A 5-minute average at the end of the 7 minute period will be used for analyses.

Carotid Ultrasonography
Carotid stiffness:
Carotid stiffness is a common clinical marker and independent risk factor for future vascular events. Carotid stiffness will be assessed using high fidelity ultrasound (Hitachi Aloka Alpha 7, Japan) and high frequency (7-13 MHz) linear probe. Carotid artery distension will be assessed using wall-tracking and simultaneous measurement of blood velocity. Stiffness measures are generally calculated by adjusting carotid artery compliance for changes in carotid blood pressure. Measures of carotid stiffness will include carotid B-stiffness, Elastic modulus, and PWV-B using the following equations:
B-stiffness = $(\log P_1/P_0)/(D_1\text{-}D_0/D_0)$;
Elastic modulus = $(P_1/P_0)/((D_1\text{-}D_0)/D_0)$

PWV-B = (√((B-stiffness) x P_0)/2ρ)
where P_1 and P_0 are the highest (systolic) and lowest (diastolic) carotid pressures and D_1 and D_0 are the maximum (systolic) and minimum (diastolic) diameters.

Carotid Blood Flow:
Carotid blood flow will be measured on the neck using the same ultrasound probe described for carotid stiffness. Carotid artery diameter and blood velocity will be measured with the probe placed on the on the skin over the carotid artery with 60 degree insonation angle. Common carotid blood flow will be assessed approximately 1-2 cm proximal to the carotid bifurcation. Internal carotid blood flow will be measured just distal to the carotid bifurcation to obtain measures of carotid blood flow that feed directly into the middle cerebral arteries.

Cognitive Function

Montreal Cognitive Assessment (MOCA):
The MOCA is a tool that can be used to systematically and thoroughly assess global cognitive status. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The maximum score is 30, in which a score of 26 or greater is considered normal. The MOCA takes only 8-12 minutes to administer and is therefore practical to use repeatedly and routinely.

Symbol Digits Modality Test (SDMT):
The SDMT is a global test of cognitive impairment and is associated with selective attention and working memory. This test involves a visual substitution task, whereby the individual has 90 seconds to orally pair specific numbers with given geometric figures as quickly as possible. The test score is the number of correctly paired shapes/numbers.

N-back:
The N-back is a working memory task that lasts approximately 8 minutes. Participants will be presented with a series of digits one at a time. They will be instructed to press the 'right' response button if the number presented to them matches the digit that was presented 2 numbers previously. Participants will be instructed to hit the 'left' response button for every digit presented to them that does not match the number 2 digits prior. This test is widely used in cognitive psychology as a measure working memory and has been used previously following acute perturbations.

Heart function (cardiac echocardiography)
Cardiac output (Q), stroke volume (SV), and end systolic volume (ESV) will be assessed at rest using two-dimensional echocardiography via a Hitachi Aloka Alpha 7 system (Tokyo, Japan). With subjects in the left lateral position, measurements will be obtained using the four-chamber apical view. The interior of the left ventricle will be traced manually during both end systole and end

diastole. Volumes will be measured using Simpson's rule. Stroke volume will be calculated by subtracting EDV from ESV. Q will be calculated as HR multiplied by SV. Three beats will be measured and the average will be used in the analyses. Ejection fraction (EF) will be calculated from the ventricular volumes and expressed as a percentage of ESV to EDV. Mitral valve velocities will be obtained from the apical 4 chamber view, whereby E, A, and E/A will be measured. The slope of the inflow will also be determined. Tissue Doppler will also be performed to obtain E' with E/E' calculated. Lastly, speckle tracking will be performed using Aloka's tissue tracking software.

Maximal Oxygen Consumption (VO_2peak)
Cardiorespiratory fitness (VO2peak) will be measured using an incremental exercise test on a motorized treadmill following a Modified Balke protocol while analyzing expired gases (TrueOne, Parvo Medics, Sandy, UT) to determine maximal oxygen consumption. Due to differing fitness levels by design, participants will choose a comfortable walking/jogging pace to perform a 5-minute warm-up at 0% incline. The work rate will increase by 2.5% grade every 2 minutes up to 12.5%, after which speed will increase 0.5 mph/minute with constant grade until exhaustion. VO_2peak ($ml \cdot kg^{-1} \cdot min^{-1}$) will be based on highest recorded 20-second VO_2 value when two of three criteria are satisfied: 1) respiratory exchange ratio ≥1.10; 2) peak heart rate within 10 bpm of age-predicted maximum; or 3) peak rating of perceived exertion ≥17. Heart rate, blood pressure, and oxygen uptake will be monitored throughout the exercise and recovery.

For Specimen Collection Studies
Blood samples (15 ml each; 30 ml Visit 1, 15 ml Visit 2, 30 ml Visit 3) will be collected from participants following standard procedures. All study personnel collecting blood samples have been trained by UIC's CCTS/CRC staff. The samples will be analyzed for C-reactive protein and interleukin-6 for inflammatory markers, oxidized LDL as a marker of oxidative stress, and total antioxidant capacity and ascorbate for antioxidant capacity (in which additional analyses on inflammatory markers may be performed) following the specified ELISA procedures. Samples will be stored separate from the study data and be de-identified. The samples will be kept indefinitely for future analysis if specific consent is provided by participants. If consent is not provided, samples will be properly destroyed following study completion.

Studies involving use of product
Participants will receive a Typhoid Vi Polysaccharide Vaccine (Typhim Vi, Sanofi Pasteur SA). It will be administered intramuscularly at the deltoid (upper arm) of the non-dominant arm in a dose of 0.5 mL by a registered nurse. The product will be purchased from the manufacturer through UIC Investigational Pharmacy. The vaccination will be stored at 35° to 46° F in a temperature controlled refrigerator

in the Integrative Physiology Lab in the DHSP Building in a locked room in a controlled access suite. On-study personnel will have access. A log will be kept with date and subject number for each dose of the vaccine used. Adverse events will be immediately reported to the principal investigator, who will complete an Adverse Event Report Form. If it is deemed a severe adverse event, it will be reported to the IRB within 24 to 48 hours. Based on data from clinical trials with over 4,000 subjects, adverse reactions may consist of: local tenderness, pain, induration or erythema at the injection site; or, a systemic response of malaise, headache, myalgia, nausea, diarrhea, feverish, or vomiting. No severe or unusual side effects were observed and most adverse experiences were generally limited to 48 hours.

Participants will also receive 2g Vitamin C on the first and third visit. It will be administered orally. Supplementation will be ingested in front of laboratory personnel. This supplementation does not exceed daily recommendation and has been previously shown to acutely improve endothelial function[35–39]. The product will be purchased and stored in the IPL in the DHSP Building. On-study personnel will have access. A log will be kept with the date and subject number for each dose given. Adverse events will be immediately reported to the principal investigator, who will complete an Adverse Event Report Form. If it is deemed a severe adverse event, it will be reported to the IRB within 24 to 48 hours. There are no known risks associated with the administration of Vitamin C. The dosage of antioxidant vitamins are the same as those sold over the counter and safe. No side effects have been reported with this dose.

7.0 Expected Risks/Benefits

1. **Typhoid vaccine:** Mild reactions to the typhoid vaccine may occur, such as fever, headache, redness/swelling at the site of injection, nausea, or muscle pain. You may also faint or an allergic reaction may occur. The risk of serious harm or death is extremely small and serious problems from the typhoid vaccine are very rare. Participants with a history of adverse reactions to the typhoid vaccine, will be removed from the study.
2. **Health information:** Risk of loss of privacy or confidentiality of health information. To minimize the risk, all data will be stored coded.
3. **Questionnaires:** Participants may feel uncomfortable providing personal information in the questionnaires.
4. **Blood pressure:** Blood pressure will be measured using a cuff around the arm. Subjects may feel some discomfort when the cuff is inflated on the arm or may feel some tingling in the fingers.
5. **ECG (Heart rate):** Skin redness may appear where the electrodes are placed on the skin for the ECG.
6. **Pulse wave analysis and Pulse wave velocity:** No known risks.
7. **Ultrasound:** No known risks. All efforts will be made to maintain patient's modesty. The gel used during the ultrasound may feel cool and sticky and is hypoallergenic (not likely to cause an allergic reaction) and washes off easily.

8. **Fasting:** May cause mild discomfort and hunger pains. If participants develop any further symptoms due to fasting, we have snacks and drinks (water and juice) available. Participants will *not* have to perform the treadmill test in the fasted state, reducing risk.
9. **Flow-mediated dilation:** Subjects may feel uncomfortable (i.e. the arm may "fall asleep") when the blood pressure cuff is inflated for 5 minutes during flow-mediated dilation but this will subside when the cuff is released.
10. **Treadmill test (VO_2max):** The treadmill test may result in muscle soreness, feeling tired, or out of breath. The soreness should go away in a few days. Participants may also feel dizzy, faint, trip, fall, or sprain an ankle during the testing. Spotters will be used during the test. A mouthpiece will be worn to measure breathing so participants may feel uncomfortable. Participants may feel warm during the test. Temperature will be kept at a comfortable temperature between 72-75 degrees Fahrenheit. Additional fans will be provided, as needed, to keep the participant cool and comfortable. Participants will be instructed that they can stop the test at any time. Other rare, but serious, risks associated with a peak exercise test include: rapid or irregular heart rhythms, chest pain, heart attack, and very rarely death. All individuals assisting with the treadmill test are certified in cardiopulmonary resuscitation (CPR) and automated external defibrillator (AED) use. An AED is readily available, as well as a landline phone to call emergency services.
11. **Blood draw:** Subjects may experience mild pain, bleeding or bruising at the site of the blood draw. They may also faint or get a mild infection at the site of the draw.
12. **Near-infrared spectroscopy:** No known risks.
13. **Transcranial Doppler:** Participants may find the headpiece for the brain blood flow measurements uncomfortable and may develop a headache from wearing the head piece over a prolonged period of time (>45 min). The headpiece will be adjusted so that it is as comfortable as possible and the short time frame for wear (<15min) should minimize likelihood of a headache developing.
14. **Cognitive Function Tests:** No known risks.
15. **DEXA:** The DEXA scan involves radiation exposure: The duration of the scan will be less than 10 minutes. You will be exposed to a radiation dose of less than or equal to 0.3 mrem based upon manufacturer's specifications and pre-determined calculations. This amount of radiation exposure is minimal given the estimated effective equivalent dose is below 100 mrem per year, a limit set by the Nuclear Regulatory Commission for "general public" exposure.
16. **Vitamin C:** There are no known risks associated with the administration of the Vitamin C, as the dosage does not exceed the upper limit for dietary consumption. Additionally, any excess will be excreted out. No side effects have been reported with these doses.

Expected Benefits:

There will be no direct benefit to the subjects for participating in this research.

8.0 Data Collection and Management Procedures

Subject identification will be coded by numerical values. The subject number will be linked to a master key with subject identifiers. The master key, informed consent, health history questionnaires, and documents that provide subject identifiers will be stored separately in a locked cabinet located in the IPL, which is only accessible by key. Only study personnel will have access to this information. During data analyses, shall any abnormalities be discovered regarding the echocardiogram procedure, a letter will be sent to participants suggesting the participant contact their physician for follow-up.

Upon completion of the study, the master key will be destroyed. Subject demographics, such as height, weight, resting HR, and resting BP, will be recorded directly onto a coded data collection form. Data from each subject will be compiled, coded, and saved onto a computer hard drive that is only accessible to study personnel. Electronic data will be stored indefinitely following completion of the study. If the subject agrees to allow us to contact them for future research studies, we will store (by paper and electronically) their name, race, age, sex, height, weight, phone number and email address indefinitely following completion of the study in a separate document.

9.0 Data Analysis

Statistical data analysis will be conducted using IBM SPSS statistical analysis software. This study is a hypothesis generating study, designed to test the anticipated relationship of fitness with aging on vascular function and oxidative stress during an acute bout of inflammation, in generally healthy individuals. This study will provide preliminary data to conduct power analyses for future studies.

10.0 Quality Control and Quality Assurance

All raw data will be checked by Elizabeth Schroeder, as it is collected and all procedures of the protocol will be performed by trained personnel. Biweekly examination of the analyzed data will be performed by Dr. Fernhall to ensure high quality analysis is being performed.

11.0 Data and Safety Monitoring

In the event of an accident or injury occurring during the subject's visit, only emergency care (such as CPR and dialing 911) would be performed by study personnel until emergency medical personnel arrive. No other medical care will be provided. Minor cases, such as subject distress, will be communicated confidentially between the subject and the study personnel. The subject is free to withdraw from the study at any time without affecting future care at UIC. This will be addressed in the informed consent documentation and will be verbally reiterated to the subject on the orientation visit. The informed consent and study personnel will express to the subject the methods by which subject confidentiality will be maintained.

12.0 Statistical Considerations

The data will initially be examined for normality violations, entry errors, and missing values. Entry errors will be corrected by double-checking with original data files. Non-normally distributed data will have an appropriate transformation applied to achieve normality (i.e. log transformation, square root, etc.).

All analyses will be adjusted for age, sex, and body mass index, which are all factors known to influence hemodynamic parameters. Additionally, due to multiple tests, a correction will be applied for multiple comparisons (α divided by the number of tests). Significance level will be set at $\alpha=0.05$, with a Bonferroni correction used to correct for multiple comparisons.

Aim 1: The effects of acute inflammation on endothelial and microvascular function will be tested with a two-way group (3) by time (2) repeated measures ANOVA (V2 and V3 baseline). We expect older high fit adults to have similar vascular reactivity as young adults, i.e. decreased FMD and NIRS reperfusion, suggesting that fitness maintains vascular reactivity during an inflammatory insult with age. If no difference in the response to acute inflammation is seen between low and high fit older adults (not expected), this would indicate fitness is not protective with age during acute inflammation.

Aim 2: The effects of ascorbic acid ingestion will be tested with a two-way group (3) by time (2) repeated measures ANOVA to determine if the antioxidant caused changes at Visit 1 (baseline) or Visit 3 (acute inflammation). A two-way group (3) and time (3) repeated measures (V2 baseline, V3 before and after Vit C) ANOVA will determine if the antioxidant attenuated endothelial function during acute inflammation. At baseline, we expect ascorbic acid to improve vascular function, i.e. increase FMD and NIRS reperfusion, in older low fit adults, with no changes seen in young or high fit older adults. During acute inflammation, we expect ascorbic acid to restore vascular function to baseline in young and older high fit adults. If ascorbic acid does not alter vascular function during acute inflammation, this would indicate another mechanism other than oxidative stress is contributing to vascular dysfunction.

13.0 Regulatory Requirements

13.1 Informed Consent

Oral consent will be obtained by phone or email during the screening process. All subjects will receive an informed consent document to read and sign upon arrival to the IPL during the first visit. The informed consent will describe the protocol of the study, the intended risks, and the intended benefits of study participation. After reviewing the information in the informed consent, individuals may decline to participate. For those who are willing to participate in the study, they will sign the informed consent document and continue with the visit. The original signed informed

consent document will be stored in a locked cabinet that will be accessible to IPL members and a copy will be provided to the subject.

13.2 Subject Confidentiality

Subject identity will be numerically coded. All data recorded for that subject will be labeled and stored under that numeric code. All computers used to record data are password protected.

13.3 Unanticipated Problems

When an unanticipated problem is identified, the staff member identifying the adverse event will immediately report it to the principal investigator. The principal investigator will then contact the participant and an Adverse Event Report Form will be completed. If the principal investigator feels that a serious adverse event has occurred, a report of the serious adverse event will be forwarded to the UIC IRB within 24-48 hours of occurrence. If the event is not classified as a serious adverse event, it will be reported to the UIC IRB upon the next renewal.

14.0 References

1 Smeeth L, Ph D, Thomas SL, Ph D, Hall AJ, Ph D, *et al.* Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med* 2004; 351:2611–8.

2 Leinonen M, Saikku P. Evidence for infectious agents in cardiovascular disease and atherosclerosis. *Lancet Infect Dis* 2002; 2:11–17.

3 Meier CR, Jick SS, Derby LE, Vasilakis C, Jick H. Acute respiratory-tract infections and risk of first-time acute myocardial infarction. *Lancet* 1998; 351:1467–1471.

4 Corrales-Medina VF, Madjid M, Musher DM. Role of acute infection in triggering acute coronary syndromes. *Lancet Infect Dis* 2010; 10:83–92.

5 Kohut ML, Senchina DS. Reversing age-associated immunosenescence via exercise. *Exerc Immunol Rev* 2004; 10:6–41.

6 Aw D, Silva AB, Palmer DB. Immunosenescence: Emerging challenges for an ageing population. *Immunology* 2007; 120:435–446.

7 Pawelec G, Solana R, Remarque E, Mariani E. Impact of aging on innate immunity. *J Leukoc Biol* 1998; 64:703–712.

8 Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003; 289:179–186.

9 Corrales-Medina VF, Serpa J, Rueda AM, Giordano TP, Bozkurt B, Madjid M, *et al.* Acute bacterial pneumonia is associated with the occurrence of acute coronary syndromes. *Medicine (Baltimore)* 2009; 88:154–159.

10 Liuba P, Aburawi EH, Pesonen E, Andersson S, Truedsson L, Ylä-Herttuala S, *et al.* Residual adverse changes in arterial endothelial function and LDL oxidation after a mild systemic inflammation induced by influenza vaccination. *Ann Med* 2007; 39:392–399.

11 Clapp BR, Hingorani AD, Kharbanda RK, Mohamed-Ali V, Stephens JW, Vallance P, *et al.* Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc Res* 2004; 64:172–178.

12 Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, *et al.* Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 2000; 102:994–999.

13 Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, *et al.* Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation* 2005; 112:2193–2200.

14 Wallace SML, Mäki-Petäjä KM, Cheriyan J, Davidson EH, Cherry L, McEniery CM, *et al.* Simvastatin prevents inflammation-induced aortic stiffening and endothelial dysfunction. *Br J Clin Pharmacol* 2010; 70:799–806.

15 Jae SY, Yoon ES, Jung SJ, Jung SG, Park SH, Kim BS, *et al.* Effect of cardiorespiratory fitness on acute inflammation induced increases in arterial stiffness in older adults. *Eur J Appl Physiol* 2013; 113:2159–2166.

16 Lane-Cordova AD, Ranadive SM, Kappus RM, Cook MD, Phillips SA, Woods JA, *et al.* Aging, not age-associated inflammation, determines blood pressure and

endothelial responses to acute inflammation. *J Hypertens* 2016; 34:2402–2409.
17 Lane AD, Kappus RM, Bunsawat K, Ranadive SM, Yan H, Phillips S, *et al.* Age-related ventricular-vascular coupling during acute inflammation in humans: Effect of physical activity. *Eur J Prev Cardiol* 2015; 22:904–911.
18 Orbegozo D, Su F, Xie K, Rahmania L, Taccone FS, De Backer D, *et al.* Peripheral muscle near-infrared spectroscopy (NIRS) variables are altered early in septic shock. *Shock* 2018; 50:87–95.
19 Blair SN, Kampert JB, Kohl III HW, Barlow CE, Macera CA, Paffenbarger RS, *et al.* Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *J Am Med Assoc* 1996; 276:205–210.
20 Lee DC, Sui X, Church TS, Lavie CJ, Jackson AS, Blair SN. Changes in fitness and fatness on the development of cardiovascular disease risk factors: Hypertension, metabolic syndrome, and hypercholesterolemia. *J Am Coll Cardiol* 2012; 59:665–672.
21 Tyml K, Swarbreck S, Pape C, Secor D, Koropatnick J, Feng Q, *et al.* Voluntary running exercise protects against sepsis-induced early inflammatory and pro-coagulant responses in aged mice. *Crit Care* 2017; 21:1–12.
22 Williams PT. Inadequate exercise as a risk factor for sepsis mortality. *PLoS One* 2013; 8:21–23.
23 Schroeder EC, Lane-Cordova AD, Ranadive SM, Baynard T, Fernhall B. Influence of fitness and age on the endothelial response to acute inflammation. *Exp Physiol* 2018; 103:924–931.
24 Seals DR. Edward F. Adolph Distinguished Lecture: The remarkable anti-aging effects of aerobic exercise on systemic arteries. *J Appl Physiol* 2014; 117:425–439.
25 Eskurza I, Myerburgh LA, Kahn ZD, Seals DR. Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *J Physiol* 2005; 568:1057–1065.
26 Seals DR, Kaplon RE, Gioscia-Ryan RA, LaRocca TJ. You're Only as Old as Your Arteries: Translational Strategies for Preserving Vascular Endothelial Function with Aging. *Physiology* 2014; 29:250–264.
27 Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-{kappa}B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 2009; 119:1284–92.
28 Walker AE, Kaplon RE, Pierce GL, Nowlan MJ, Seals DR. Prevention of age-related endothelial dysfunction by habitual aerobic exercise in healthy humans: Possible role of nuclear factor-kB. *Clin Sci* 2014; 127:645–654.
29 Donato AJ, Uberoi A, Bailey DM, Wray D, Richardson RS. Exercise-induced brachial artery vasodilation: effects of antioxidants and exercise training in elderly men. *Am J Physiol Circ Physiol* 2010; 298:H671–H678.
30 Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol* 2004; 556:315–324.
31 National Center for Health Statistics. *Health, United States, 2016: With chartbook*

on long-term tredns in health. Hyattsville, MD: ; 2017.
32 Jae SY, Heffernan KS, Park SH, Jung SH, Yoon ES, Kim EJ, *et al.* Does an acute inflammatory response temporarily attenuate parasympathetic reactivation? *Clin Auton Res* 2010; 20:229–233.
33 Ranadive SM, Kappus RM, Cook MD, Yan H, Lane AD, Woods JA, *et al.* Effect of acute moderate exercise on induced inflammation and arterial function in older adults. *Exp Physiol* 2014; 99:729–739.
34 Lane-Cordova AD, Phillips SA, Baynard T, Woods JA, Motl RW, Fernhall B. Effects of ageing and physical activity on blood pressure and endothelial function during acute inflammation. *Exp Physiol* 2016; 101:962–971.
35 Levine GN, Frie B, Koulouis SN, Gerhard MD, Keaney JF, Vita JA. Ascorbic Acid Reverses Endothelial Vasomotor Dysfunction in Patients with Coronary Artery Disease. *Circulation* 1996; 93:1107–1113.
36 Wilkinson IB, Megson IL, MacCallum H, Sogo N, Cockcroft JR, Webb DJ. Oral vitamin C reduces arterial stiffness and platelet aggregation in humans. *J Cardiovasc Pharmacol* 1999; 34:690–693.
37 Williams MJ, Sutherland WH, McCormick MP, de Jong S a, McDonald JR, Walker RJ. Vitamin C improves endothelial dysfunction in renal allograft recipients. *Nephrol Dial Transplant* 2001; 16:1251–5.
38 Richards JC, Crecelius AR, Larson DG, Dinenno FA. Acute ascorbic acid ingestion increases skeletal muscle blood flow and oxygen consumption via local vasodilation during graded handgrip exercise in older adults. *Am J Physiol - Hear Circ Physiol* 2015; 309:H360–H368.
39 Raitakari OT, Adams MR, McCredie RJ, Griffiths KA, Stocker R, Celermajer DS. Oral vitamin C and endothelial function in smokers: Short-term improvement, but no sustained beneficial effect. *J Am Coll Cardiol* 2000; 35:1616–1621.
40 Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, *et al.* Vitamin C Pharmacokinetics : Implications for Oral and Intravenous Use. *Ann Intern Med* 2004; 140:533–537.
41 Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, *et al.* Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci* 1996; 93:3704–3709.
42 Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, *et al.* Acute systemic inflammation impairs endothelium-dependent dilation in humans. *Circulation* 2000; 102:994–999.
43 Sharman JE, Lim R, Qasem AM, Coombes JS, Burgess MI, Franco J, *et al.* Validation of a generalized transfer function to noninvasively derive central blood pressure during exercise. *Hypertension* 2006; 47:1203–1208.
44 Holland DJ, Sacre JW, McFarlane SJ, Coombes JS, Sharman JE. Pulse wave analysis is a reproducible technique for measuring central blood pressure during hemodynamic perturbations induced by exercise. *Am J Hypertens* 2008; 21:1100–1106.
45 Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, *et al.* Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. *J Am Coll Cardiol* 2002; 39:257–265.

46 Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, *et al.* Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Hear Circ Physiol* 2011; 300:H2-12.