Age related changes in venoconstriction: implications for understanding the pathophysiology of diastolic heart failure and syncope

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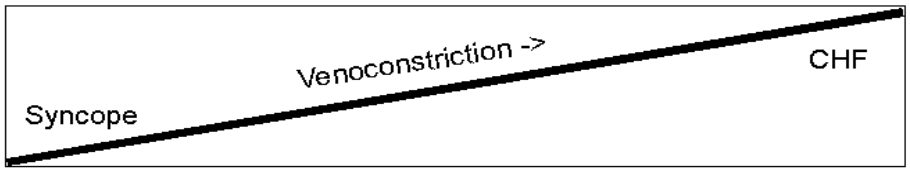
A. Background and significance

The elderly population is particularly vulnerable to disorders such as congestive heart failure (CHF) and syncope. The incidence and prevalence of CHF are strikingly age-dependent, with prevalence rates in adults over 80 years of age approaching 10%. CHF is the leading indication for hospitalization and the most costly diagnosis ($10 billion/year in 1989) in the Medicare population. Older patients are more prone to develop DHF; it is estimated that up to 50% of cases in those older than 65 occur in the setting of normal systolic function (1). Syncope also has marked increase in prevalence with age, leading to enormous expenditure on diagnostic testing and hospital stays as well as severe morbidity from complications such as hip fractures.

Veins serve an important yet often overlooked variable blood storage function. The large capacity of these vessels permits this low-pressure reservoir to contain as much as 85% of the systemic blood volume based on data from nonhuman species; approximately seventy percent of this is in the systemic veins and 30% in the heart and lungs (2). The pronounced capacity of this reservoir implies that relatively small volume changes in the peripheral veins are followed by substantial alterations in central blood volume. Reflex alterations in venous vasomotor tone provide a rapidly acting mechanism for compensatory redistribution of the blood volume. For example, venoconstriction acts to partially restore a normal cardiac preload during the assumption of upright posture. Impaired venoconstriction in systemic and/or splanchnic beds has been implicated as a precipitating factor for neuromediated syncope (3). By contrast, increased venoconstriction could be very important in the pathogenesis of the clinical syndrome of DHF. Several studies over the past five decades have demonstrated increased venous tone in patients with CHF. An increase in central blood volume from venoconstriction with a concomitant reduction in peripheral venous capacitance could result in an increased preload and provide an explanation for the higher filling pressures characteristic of DHF. Computer modeling of acute pulmonary edema indicates that the main determinant of elevated pulmonary venous pressure is not the hemodynamic effects of systolic or diastolic dysfunction, but likely increased central volume secondary to venoconstriction (4). If venoconstriction were demonstrated as an important pathophysiologic contributor, disorders such as syncope and DHF could then be seen as part of a continuum as shown below:



Physiologic differences in venous capacitance, venous tone and possibly venous responses to sympathetic stimulus with age have been described and support the role of the venous system in the genesis of the aforementioned syndromes. Elderly patients demonstrate generalized reduction in baroreceptor response in response to orthostatic stress. Recent data suggest that the abnormality lies not in impaired efferent function of baroreceptors but in decreased venous pooling in non-compliant veins (5). The reduction in peripheral venous capacitance results in a smaller decrease in central volume with upright posture and less signal to the baroreceptors, resulting in a reduced baroreflex response. Secondarily, the autonomic control of the cardiovascular system regulated by both the α- and β-

adrenergic receptors changes with age. While the density of β-receptors appears unaltered, the inotropic response to catecholamines was diminished with age both clinically and in studies of human myocardium. The ability of phenylephrine (α 1-agonist) to raise blood pressure is unaltered by aging in human subjects (6). The concentration of locally infused norepinephrine needed to produce 50% constriction of superficial veins has been reported to be equal between healthy young and elderly normals; however, the study was not adequately powered to detect such a difference (7). The combination of decreased venodilatory β 2 activity and preserved constrictive α 1-receptors would conceptually produce an increase in resting venous tone. Additionally, an increase in basal plasma norepinephrine with aging has been described (8). These factors could be an explanation for the reported large (~ 45%) decrease in venous compliance of the calf with age (9). Increased venous tone in healthy elderly subjects has been demonstrated in at least one trial (10). Whether or not venous response to *acute* sympathetic stimulus differs between young and elderly persons is currently unknown.

Techniques exist for the examination of superficial venous response and also a more global measurement of the response in an entire limb – each has limitations. Measurement of generalized venoconstriction carries the attendant risks of systemic sympathetic stimulus, often causing stress and/or pain, and in populations with coronary artery disease, etc. theoretically increasing risk for myocardial infarction. It is impossible to determine a maximal response to stimulus, and it is also difficult to separate out the effects of reflexes, such as the baroreceptor system, from direct constriction of the venous bed measured. Measurement of superficial venous response to locally infused sympathomimetic may be confounded by such factors as ambient temperature, and difficulty exists when attempting to generalize findings in a single vein to an entire venous bed – little is known about differences in adrenergic receptor populations in different vein subpopulations.

Strain-gauge plethysmography has been used to study the properties of the human venous system since 1953. It involves placing distensible silastic tubing filled with mercury around a limb; voltage changes across the internal resistance are proportional to the change in circumference divided by original circumference (strain). Whitney (11) showed in 1953 that the percentage change in volume of a limb could be determined by the following equation:

$$\delta V = 2\delta C/C * 100$$

where C is limb circumference and V is volume. Since approximately 97% of the vascular capacitance in an extremity is found in veins, alterations in leg volume in response to an applied venous congestion pressure can therefore be used as an index of change in venous volume in the measured limb.

Linear variable differential transformer (LVDT) techniques were developed in the early 1980's as a method of directly measuring the response of superficial veins to stimuli. The LVDT apparatus consists of a movable metallic rod lightly contacting the apex of a vein on the dorsum of the hand. The free-sliding core is placed within a metal cylinder which produces voltage changes in the system linearly proportional to the displacement of the core from a zero point. In this manner, the distension or contraction of the subject vein (which would produce movement of the core and thereby a measurable voltage change) can be determined in response to local or systemic stimuli.

Cold-pressor testing involves placing ice-cold water on parts of the human body to produce sympathetic stimulus. The test has been shown to produce an increase in sympathetic outflow as evidenced by increases in mean arterial pressure and heart rate (12,13) as well as plasma catecholamines (13). The use of hand or foot immersion likely produces less (possibly confounding) increase in vagal tone than the use of the forehead (12). Cold pressor stimulus produces greater relative increase in norephinephrine than epinephrine, especially when compared with mental arithmetic stress as sympathetic stimulus (13). Data suggest that the main effect of cold-pressor testing is an α-adrenergic stimulus. While both α- and β-adrenergic stimuli have been shown to augment venous return, animal studies imply that active changes in systemic venous capacity are mostly due to α-adrenergic mechanisms (14). For these reasons, limb cold-pressor testing is an appropriate non-pharmacological manner of examining venoconstriction in response to a sympathetic stimulus.

The purpose of our study is to establish a simple, relatively non-invasive method to evaluate venoconstriction in response to sympathetic stimulus. The technique will then be used to determine if venoconstriction changes with normal aging. We propose to quantify the constriction of leg veins in healthy young and elderly subjects by performing calf venous occlusion strain-gauge plethysmography at rest and after sympathetic stimulation via cold-pressor test. Prior studies show that the venous pressure-volume relationship is linear at physiologic pressures, with equal slope (i.e. compliance) under control and mental-arithmetic stress conditions in healthy young subjects (15). We expect the same finding in response to cold pressor stimulus in both of our study groups.

Little is known about the receptor populations in different venous beds, or how a particular response to stimulus in a superficial vein might be related to more global change (e.g. in all veins in a human limb). The locally infused concentration of phenylephrine required to produce 50% constriction of a dorsal hand vein has been significantly correlated with the systemic dose producing an elevation in mean arterial pressure (MAP) of 20 mm Hg (16). However, it is unclear if such an elevation in MAP is also reflective of the changes occurring in the venous system in a more global fashion – blood pressure is dependent not only on vascular adrenergic receptor stimulation, but on many other factors such as circulatory volume, cardiac contractility, and heart rate. Both LVDT technique and plethysmography have been used to characterize venous response to drugs and other stimuli such as mental arithmetic stress, cold pressor, tilt-table testing, etc., but the two techniques have never been compared directly. One early study shows that the vast majority of venomotor response in a limb occurs within the cutaneous veins (17) – implying that LVDT measurements of superficial venoconstriction might correlate well with plethysmographic determination of venoconstriction. However, while intrasubject variability is low with LVDT, intersubject variability is often high with coefficients of variation greater than 50% in subjects with similar characteristics.

B. Study Design and Statistical Analysis

The study will be a cohort analysis of two groups of human subjects: 1) age 20-35 and 2) aged greater than 60 years; each subject will have measured, in sequence: 1) Calf venous pressure-volume curves at rest and following cold pressor stress, 2) Measurement of ED_{50} for norepinephrine in a superficial hand vein, and 3) Measurement of superficial hand vein constriction following cold pressor stress. The primary goal of the study is to determine the difference, if any, of venoconstriction following acute sympathetic stimulus between healthy young and elderly subjects. A secondary goal of the experiments is to examine any correlation between measurements gained from venous plethysmography/cold pressor with those acquired through a well-established local sympathomimetic infusion protocol and measurement of superficial venoconstriction with LVDT. Cold pressor testing will also be performed with LVDT measurement and the results will be correlated with those from the other two protocols.

C. Study Procedures

Calf venous pressure-volume curves before and after cold pressor stress: The midpoint of the calf will be elevated five cm above the level of the right atrium with foam cushions to support and prevent undue movement of the limb. A mercury-in-silastic strain gauge will be applied at the maximal circumference of the calf ~ 10 cm distal to the knee to measure increases in circumference during rapid venous occlusion by a pneumatic low-pressure cuff. The cuff will be attached to a cuff inflator [D.E. Hokanson E-10 Rapid Cuff Inflator, Issaquah, Wash.] with an air source [D.E. Hokanson G101 Air Source, Issaquah, Wash.] and placed around the upper thigh. The cuff pressure will be varied from 20 to 30 mm Hg in 5-mm Hg increments. The strain gauge displacement, which represents the volume change in the measured limb, will be plotted as a function of pressure. The volume changes as determined by the initial point of equilibration after the first 30 seconds of each pressure will used, as this is an adequate time for completion of the volume change component and secondary increases in strain gauge length after

60-90 seconds reflect transcapillary fluid flux [18]. Our preliminary data corroborates prior findings of equivalent venous compliance at baseline and with sympathetic stimulus.

Since standard strain gauge plethysmography does not actually measure either relative or absolute venous volume in the limb but rather relative percent changes of volume/100 ml of forearm circumference, resting volumes within the same patient cannot be compared. This limitation is significant when construction of pressure volume curves is attempted during a physiologic stimulus. To overcome this limitation, during the measurement of venous pressure volume curves the strain gauge will not removed from the patients arm between the measurements in the control period and during cold pressor and the strain gauge will not be recalibrated.

Cold pressor test: The subject's left hand will be immersed in ice water (temperature 0-2°C) to the wrist for 60 seconds (8). Following this stimulus, pressure-volume curves will be constructed a second time in the same manner as described above. The norepinephrine infusion portion of the protocol is expected to take approximately one hour; the intervening time should be sufficient to allow return of the systemic sympathetic tone to baseline prior to repeating cold pressor for LVDT measurements.

Venous distensibility (VD) using the dorsal hand-vein technique: Measures of VD will be done using dorsal hand vein measures according to the technique described by Aellig with LVDT (19). The arm to be studied is placed on a rigid support sloping upwards at an angle of 30^0 from the horizontal, allowing complete emptying of the superficial veins. A suitable vein on the dorsum of the hand is chosen after a sphygmomanometer cuff on the upper arm is inflated to 60mm Hg. Saline is infused through a 25G needle inserted into the vein 10 mm distal to the site of measurement for 30 minutes at 24 ml/h in order to maintain patency of the needle and allow the vein to restore its tone after the needle insertion. The LVDT [Model 100 MHR, Schaevitz, Pensauken, NJ], supported by a flexible jointed metal tube locked in place, is mounted on the back of the hand with its central aperture over the vein under investigation. The LVDT signal is sent to an ATA 2001 LVDT signal conditioner [Schaevitz Sensors, Pensauken, NJ] and recorded on a data acquisition program [Ponemah, Gould Instruments]. Recordings of the position of the core situated over the summit of the vein are made before and after inflation of the sphygmomanometer cuff to 60 mmHg. The difference between the 2 positions of the core is a measure of VD; the position at 60 mm Hg is considered as baseline maximal VD. After setup is completed, a norepinephrine infusion is started at 0.125 ng/min through the 25G needle. The IV infusion rate is kept constant by varying the relative rates of the saline and drug infusions. Administration of the locally infused drug will proceed as following:

- Norepinephrine will be administered in incremental doses, by doubling the infusion rate or the concentration of the drug. For each dose, blood pressure and heart rate will be recorded. After 3 minutes of each infusion period, the sphygmomanometer cuff will be inflated to 60 mmHg for two minutes in order to assess the effect of the drug on the VD. Venoconstriction will be calculated as follows:

  $$VC = (VD_{baseline} - VD_{drug\ infusion})/100$$

- The norepinephrine infusion will start at 0.125 ng/min and increase every five minutes until a 70% vasoconstriction is obtained, with a maximum concentration infused of 1024 ng/min. This maximum concentration has shown to have no effect on systemic blood pressure or forearm blood flow.

As the ambient environmental temperature might be expected to produce significant alterations in resting superficial venous tone (as such vessels are important in thermoregulation), the temperature of the laboratory will be controlled at 20-22 °C and monitored via digital thermometer during experimental procedures.

D. Data Analyses and Anticipated Findings

Venous pressure-volume curves for the calf will be constructed with the x-axis being occluding pressure and the y-axis being change in calf volume. Calf volume will be determined by measuring the changes in calf circumference with strain-gauge plethysmography [11]. The change in calf venous pressure-volume curves at rest and after cold pressor stimulus between healthy young and elderly subjects will be compared by an analysis of covariance (ANCOVA) with repeated measures. This type of analysis assumes that the venous pressure-volume curves pre and post cold pressor test are linear and parallel. Since the slope of these curves, the venous compliance, is equal at rest and after sympathetic stimulus (15), the difference in the y-intercepts of the two curves is representative of the venoconstriction produced by cold pressor.

The results of LVDT trials are generally reported as the ED_{50}, or the dose of locally infused sympathomimetic needed to produce 50% constriction of the measured vein. The dose-response curve for norepinephrine measured in percent venoconstriction vs. log dose is sigmoidal, and is best fit via a four-parameter logistic equation in order to determine the E_{max}, or the maximal effect of the infused drug, and the ED_{50}.

Power Calculation: Based on our preliminary data alone (on five subjects), 260 subjects would be required to determine a 20% difference in venoconstriction between young and elderly subjects. An examination of the literature to date, however, indicates that mental stress induces a 13.5% decrease in venous volume ; given that norepineprine levels produced are higher at one minute with cold pressor than mental stress (13) the venoconstriction produced at this time might be expected to be similar or perhaps slightly more with cold pressor. A standardization paper for strain-gauge plethysmography indicates that the standard deviation of venous distensibility is approximately 25% of the total distensibility. A sample size calculation was made assuming 80% power and α-error of 0.05. If we assume that a 20% difference in venoconstriction between young and elderly subjects would be clinically meaningful, then the difference in venous capacitance between groups would be 2.7% units (=13.8% - [13.8%*0.8]). Based on a power calculation employing an unpaired t-test for group means we would need 52 subjects to show a significant difference in venoconstriction. Accounting for dropouts we plan to recruit 60 subjects – 30 young (20-35 years of age) and 30 elderly (60 or more years of age).

Correlation calculation: Correlation calculations (Pearson's correlation coefficients), after determining the E_{max} and ED_{50} for norepinephrine as described above, will be performed to examine three relationships:

1) ED_{50} of norepinephrine (LVDT) and venoconstriction by cold pressor (calf strain-gauge plethysmography)
2) ED_{50} of norepinephrine (LVDT) and superficial venoconstriction by cold pressor (LVDT)
3) Venoconstriction by cold pressor (calf strain-gauge plethysmography) and superficial venoconstriction by cold pressor (LVDT)

E. Study Drugs

Local infusion of norepinephrine will proceed as described above. Norepinephrine is a naturally occurring catecholamine released from sympathetic nerve endings

F. Medical Devices

CBM-700, Noninvasive beat to beat blood pressure monitor. This is an FDA approved device for recording beat to beat blood pressure using the technique of arterial tonometry. Applanation of the curved surface of the artery, balancing circumferential stresses in its wall, allows accurate registration of pressure waveforms. The accuracy of the tonometer compared to simultaneous intra-arterial recording has been validated in humans.

Linear variable differential transformer: Consists of a metal cylinder approximately 2 cm in diameter and 5 cm in length with a central aperture. The cylinder contains a central coil energized by

alternating current; two outer coils are connected in serial opposition so that the total voltage in the system is zero. A free-sliding steel core is placed within the cylinder; the displacement of the cylinder away from the zero point produces a linearly-proportional voltage. The voltage signal is amplified and changed to direct current by a signal conditioner. The device is factory-calibrated to produce a signal of one volt per two mm of displacement.

G. Study Questionnaires

No questionnaires will be administered during the study.

H. Study Subjects

Potential research subjects will include members of the Washington Heights community and New York Presbyterian Hospital outpatients and/or staff.

Inclusion criteria: Subjects will be non-smoking and aged 20-35 years, free from medical disorders and the use of any medications known or suspected to affect autonomic and/or vascular tone. A limited medical history and physical examination will be performed, and blood pressure, EKG, fasting blood glucose, creatinine, and TSH will be checked prior to study participation.

Exclusion criteria: Medical diagnosis, as determined from clinical history and/or physical examination) including hypertension, congestive heart failure, renal insufficiency, or endocrinopathies that possibly affect vascular tone such as thyroid disease, adrenal disorders, and diabetes mellitus. Smoking, illicit drug use, use of any medication known or suspected to affect autonomic and/or vascular tone. History of prior myocardial infarction, arrythmia, or syncope. History of peripheral or central arterial disease, including conditions such as Raynaud's phenomenon.

Subjects will refrain from drinking caffeine- or alcohol-containing beverages for 12 hours prior to the study.

I. Recruitment of Subjects

Recruitment will not be based on gender, race, or socioeconomic status. No data currently exists on the alteration of venous responses by any of these factors, and our study will not be powered to determine such differences. Subjects will be made aware of the study primarily through flyers, word-of-mouth, and possibly e-mail. Prior to enrolling an individual subject in the study, the patient's primary care provider will be approached to determine if the patient is appropriate for participation. Informed consent will be obtained from all subjects prior to their enrollment. Members of the research team will be available to answer questions and provide further information regarding the study on request.

J. Confidentiality of Study Data

Any information obtained during this study and identified with the patient will remain confidential. All subjects enrolled in the study will be given a unique identifier, which will be used for all further evaluations. All the data will be stored on a mainframe network computer, which can only be retrieved by members of the research team through personalized logon codes

K. Potential Conflict of Interest

No researchers in this study have proprietary or other financial/material interest in the equipment or procedures used.

L. Location of Study

The study will be performed in the Clinical Cardiovascular Research Laboratory for the Elderly lab at the Allen Pavilion of New York Presbyterian Hospital and the Irving Center for Clinical Research on the 10th floor of the Harkness Pavilion at the Columbia Campus of New York Presbyterian Hospital.

M. Potential Risks

Given the general good health of subjects and the exclusion criteria as listed above, risks are expected to be minimal in this protocol. The cold pressor test is associated with mild increases in blood pressure and heart rate, which are transient and resolve within five minutes after removal of the subject's hand from the ice water in both young and elderly healthy subjects. Hand cold pressor testing has been performed without adverse effects in subjects with average age of 84 years (8). The test may be associated with local discomfort and pain in the immersed hand. If discomfort or pain is present, subjects will be allowed to remove the hand before one minute has elapsed. Pain rapidly resolves upon withdrawal from the ice water. Subjects' heart rate and blood pressure will be monitored continuously during and after cold pressor stimulus.

Locally infused norepinephrine has been shown to have no effect on resting blood pressure or heart rate under experimental conditions. Risks of the infusion protocol are expected to be limited to those inherent to intravenous catheters (bleeding, pain, and infection).

A physician will be present during all testing protocols.

N. Potential Benefits

Subjects will not benefit personally from this study. Any information gained during evaluation will be shared with subjects upon request.

O. Alternatives

Alternatives to participating in this study would be not to participate in this study.

P. Compensation to Subjects

The protocol is estimated to take approximately two hours from start to finish. Some discomfort is anticipated from intravenous catheter placement and from cold pressor testing. Accordingly, subjects will be compensated $100.00 for their time and effort and will be given $20.00 to cover travel expenses.

Q. Minors as Research Subjects

No minors will be involved in the study.

R. Radiation or Radioactive Substances

No radiation or radioactive substances will be administered in the study.

S. Bibliography

1. Senni M, Redfield MM. *Congestive heart failure in elderly patients.* Mayo Clin Proc (1997), 72, pp. 453-460.
2. Pang CY. *Measurement of body venous tone.* Journal of Pharmacological and Toxicological Methods (2000) 44, pp. 341-360.
3. Thomson HL, Atherton JJ et al. Failure of reflex venoconstriction during exercise in patients with vasovagal syncope. Circulation (1996), 93, pp. 953-959.

4. Burkhoff D, Tyberg JV. Why does pulmonary venous pressure rise after onset of LV dysfunction: a theoretical analysis. American Journal of Physiology (1993), 265(5 Pt 2), pp. H1819-1828.
5. Olsen H, Vernersson E, Lanne T. Cardiovascular response to acute hypovolemia in relation to age: implications for orthostasis and hemorrhage. Am J Physiol (2000) 278, pp. H222-232.
6. Klein C, Gerber JG et al. The effect of age on the sensitivity of the ∝1-adrenoceptor to phenylephrine and prazosin. Clin Pharmacol Ther (1990) 47, pp. 535-539.
7. Martin SA, Alexieva S, Carruthers SG. *The influence of age on dorsal hand vein responsiveness to norepinephrine.* Clin Pharmacol Ther (1986) 40, pp. 257-260.
8. Pascualy M, Petrie EC et al. Effects of advanced aging on plasma catecholamine responses to the cold pressor test. Neurobiology of Aging (1999) 20, pp. 637-642.
9. Olsen H, Lanne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. Am J Physiol (Heart Circ Physiol) (1998) 44, pp. H878-886.
10. Gascho JA, Fanelli C, Zelis R. Aging reduces venous distensibility and the venodilatory response to nitroglycerin in normal subjects. Am J Cardiol (1989) 63, pp. 1267-1270.
11. Whitney R. *The measurement of volume changes in human limbs.* Journal of Physiology (1953) 121, pp. 1-27.
12. P. Saab, M. Llabre et al. The cold pressor test: vascular and myocardial response patterns and their stability. Psychophysiology (1993) 30, pp. 366-373.
13. LeBlanc J, Cote J et al. Plasma catecholamines and cardiovascular responses to cold and mental activity. J Appl Physiol (1979), 47, pp. 1207-1211.
14. Robinson MB, Dante E et al. Volume-pressure analysis of reflex changes in forearm venous function. Circulation (1989) 80: pp. 99-105.
15. K. Shigemi, M. Brunner et al. α- and β-adrenergic mechanisms in the control of vascular capacitance by the carotid sinus baroreflex system. Am J Physiol (1994) 36, pp. H201-210.
16. Robinson MB, Dante E et al. Volume-pressure analysis of reflex changes in forearm venous function. Circulation (1989) 80: pp. 99-105.
17. Vincent J, Blaschke TF, Hoffman BB. Vascular reactivity to phenylephrine and angiotensin II: comparison of direct venous and systemic vascular responses. Clin Pharmacol Ther (1992) 51, pp. 68-75.
18. Zelis R, Mason DT. Comparison of the reflex reactivity of skin and muscle veins in the human forearm. Journal of Clin Invest (1969), 48, pp. 1870-1877.
19. Gamble J, Gartside I et al. A reassessment of mercury in silastic strain gauge plethysmography for microvascular permeability assessment in man. Journal of Physiology (1993) 464, pp. 407-22.
20. H. Aellig. A new technique for recording compliance of human hand veins. J. Clin. Pharmacology (1981), 11, pp. 237-43.