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Cardiovascular regulation in women with vasovagal syncope

With special reference to the venous system

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To Lea, Isak and Jakob.

Som av en händelse kom det igår fram en man till mig och talade om slumpen. [...] Han sa att han hade gått in på denna bar och kommit fram och pratat med mig av en slump. Jag blev givetvis ställd, som jag förmodar att de flesta skulle bli, av ett sådant påstående. "En slump", sa jag, "menar du att det finns flera slump?!"

Christer Johansson

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Abstract

Although vasovagal syncope (VVS) is a common clinical condition the mechanisms behind VVS remain elusive. Upright posture is the major trigger of VVS and lower limb blood pooling affecting cardiac output has been proposed as a major determinant. The overall aim of this thesis was twofold. First, to develop new methodology for calculating limb venous compliance. Second, to study lower limb venous volume load and cardiovascular responses during hypovolemic circulatory stress caused by lower body negative pressure (LBNP) in healthy women and women with VVS, emphasizing compensatory mechanisms to maintain central blood volume.

Net fluid filtration was associated with an underestimation of venous compliance. This could be accounted for with a correction model. Further, a new venous wall model made it possible to adopt the venous pressure-volume curve through the entire pressure range and thus provide a valid characterization of venous compliance.

Calf blood pooling was similar between the groups and was not associated with tolerance to hypovolemic circulatory stress. Venous compliance was reduced at low venous pressures in VVS and correlated with decreased tolerance to circulatory stress. VVS women displayed attenuated sympathetic vasoconstrictor responses during graded circulatory stress, and mobilization of arm capacitance blood as well as capillary fluid absorption from extra- to intravascular space were reduced. Accordingly, more pronounced reductions in cardiac output were found in VVS. Thus, reduced compensatory mechanisms to maintain cardiac output could contribute to the pathogenesis of orthostatic VVS.

In healthy women, rapid pooling in the lower limb was associated with higher tolerance to circulatory stress and more efficient cardiovascular responses, in part due to speed-dependent baroreflex-mediated sympathetic activation. In VVS however, rapid lower limb blood pooling was associated with lower tolerance and deficient cardiovascular responses. No speed-dependent baroreflex-mediated sympathetic activation was found in VVS, indicating well-defined differences in cardiovascular regulation already in the initial responses to orthostatic stress.

Abstract

Populärvetenskaplig sammanfattning

Synkope (svimning) är vanligt förekommande och ligger bakom ca 3-5% av besöken på akutmottagningar. Vasovagal synkope (VVS) är den vanligaste orsaken och drabbar främst yngre kvinnor. Upprepade episoder av VVS leder till en kraftigt försämrad livskvalité och de farmakologiska behandlingsalternativen är idag begränsade. Detta beror till stor del på att de bakomliggande orsakerna inte är klarlagda. VVS karaktäriseras av ett plötsligt blodtrycksfall vilket påverkar blodflödet till hjärnan med svimning som följd. Då VVS vanligtvis inträffar vid stående (ortostatisk stress) har fokus framförallt varit riktat på den venösa blodansamlingen som sker i nedre delen av kroppen i samband med stående vilket leder till en minskad central blodvolym (central hypovolemi) som påverkar möjligheten att bibehålla ett adekvat blodtryck.

Vid ortostatisk stress initieras en serie av olika kardiovaskulära försvarsmekanismer som har som mål att upprätthålla ett stabilt blodtryck. En avgörande faktor är att när blodtrycket sjunker i centrala artärer avlastas baroreceptorer som i sin tur aktiverar det sympatiska nervsystemet. Aktiveringen påverkar direkt hjärtat genom ökad hjärtfrekvens samtidigt som det sker en kärlsammandragning (vasokonstriktion) i artärsystemet med ett minskat blodflöde i perifera vävnader som följd. På grund av detta minskar trycket i perifera vener vilka återfjädrar och venblod mobiliseras från den perifera till den centrala cirkulationen. Sammanfattningsvis leder det till ett ökat venöst återflöde till hjärtat vilket innebär att hjärtat får en större blodvolym att pumpa och att blodtrycket kan bibehållas. Genom kärlsammandragningen initieras även en process som leder till att vätska förflyttas från vävnad till blodbana via kapillär absorption vilket bidrar till ökad cirkulerande blodvolym. Sammantaget kan mobilisering av venblod och absorption av vätska öka den effektivt cirkulerande blodvolymen med ungefär 1 liter efter cirka 10 min uttalad central hypovolemi.

Den venösa sidan av det kardiovaskulära systemet kan liknas vid en stor blodreservoar och innehåller ca 70% av den totala blodvolymen. Denna reservoar spelar en stor roll för kontrollen av blodvolymen och är utformad att reglera inflödet av blod till hjärtat vid olika

kardiovaskulära påfrestningar. En effektiv mobilisering av perifert blod är beroende av det sympatiska nervsystemet med dess kärlsammandragning i artärsystemet samt att venerna har en hög eftergivlighet (compliance) vid låga ventryck. Det medför nämligen att även små tryckförändringar i perifera vener leder till en betydande mobilisering av blod samt ökning av den centrala blodvolymen.

I avhandlingen studeras dels metodologiska aspekter gällande karaktärisering av venväggen (compliance), dels kardiovaskulära försvarsmekanismer inriktade på att bevara den centrala blodvolymen vid ortostatisk stress hos friska kvinnor och kvinnor med VVS. Med hjälp av ett undertryck applicerat kring underkroppen (lower body negative pressure, LBNP) skapades en experimentell minskning av den centrala blodvolymen. LBNP leder till en vidgning av vener och ansamling av blod i nedre delen av kroppen och användes som modell för ortostatisk cirkulatorisk stress.

Delarbete I och II studerade venös ocklusionspletysmografi (VOP) som metod för mätning av venös compliance. VOP mäter förändringar i volym i extremiteterna genom applicering av ett tryck runt början på extremiteten. Det pålagda trycket medför att blod ansamlas i venerna (kapacitanssvar) distalt om området där trycket applicerats. Utöver det kommer vätska filtreras från blod till vävnad (kapillär vätskefiltration). Båda dessa processer leder fram till en tryck/volym-kurva genom vilken venös compliance beräknas. Nuvarande tekniker särskiljer inte kapacitanssvar från kapillär vätskefiltration vilket kan leda till feltolkningar. Genom att en metod utvecklades som korregerar den additiva effekt som vätskefiltrationen har på volymförändringen kunde vi påvisa att venös compliance underskattades, framförallt i de högre tryckområdena, om inte vätskefiltrationen subtraherades från volymkurvan. Vidare utvecklades en ny venväggsmodell som jämfört med tidigare rådande modell visade en bättre kurvanpassning av den venösa tryck/volym-kurvan. Den nya venväggsmodellen bidrog således till en säkrare beräkning av venös compliance.

Delarbete II visade också att kvinnor med VVS har lägre venös compliance vid låga venösa tryck jämfört med friska kvinnor. Ingen skillnad i ansamlingen av blod i de nedre extremiteterna hittades och den maximala toleransen för cirkulatorisk stress (LBNP-tolerans) var

inte relaterad till mängden blod som ansamlades i samband med LBNP. Däremot var venös compliance vid låga ventryck associerat med LBNP-tolerans där individer med lägre compliance uppvisade en lägre LBNP-tolerans.

Delarbete III studerade kompensatoriska försvarsmekanismer för upprätthållandet av den centrala blodvolymen vid ortostatisk stress. Kvinnor med VVS uppvisade en reducerad kapacitet till sympatogen kärlsammandragning av artärsystemet jämfört med friska kvinnor vid cirkulatorisk stress. Vidare påvisades en minskad mobilisering av perifert venblod till den centrala cirkulationen samt en lägre absorption av vätska från vävnad till blod. Hjärtminutvolymen minskade kraftigare hos VVS. Sammantaget indikerar detta att kvinnor med VVS har mindre effektiva kompensatoriska mekanismer för bibehållande av central blodvolym vid ortostatisk stress.

Delarbete IV belyste hur det kardiovaskulära svaret påverkas av den tid det tar för blodansamlingen i nedre extremiteterna att utvecklas i samband med LBNP. Hos friska kvinnor var en snabbare blodansamling associerad med högre LBNP-tolerans och effektivare kardiovaskulär reglering, vilket delvis kan förklaras av en hastighetsberoende baroreflex-aktivering där snabbare blodansamling leder till kraftigare stimulus med bland annat ökad arteriell kärlsammandragning. Hos kvinnor med VVS däremot var en snabbare blodansamling associerad med lägre LBNP-tolerans och ineffektivare kardiovaskulär reglering. Ingen hastighetsberoende baroreflex-aktivering kunde urskiljas. Fynden påvisar skillnader i den kardiovaskulära regleringen redan i det initiala svaret vid ortostatisk stress. Ytterligare studier av dessa tidigare ej kända skillnader kan leda till ökad förståelse av patofysiologin bakom VVS, såväl som ökad förståelse för individuella skillnader i ortostatisk tolerans hos friska individer.

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numbers.

- I. **Skoog J**, Zachrisson H, Lindenberger M, Ekman M, Ewermand L, Lanne T. Calf venous compliance measured by venous occlusion plethysmography: Methodological aspects. *Eur J Appl Physiol*. 2015 Feb;115(2):245-56.
- II. **Skoog J**, Lindenberger M, Ekman M, Holmberg B, Zachrisson H, Lanne T. Reduced venous compliance – an important determinant for orthostatic intolerance in women with vasovagal syncope. *Am J Physiol Regul Integr Comp Physiol*. 2016 Feb 1;310(3):R253-61.
- III. **Skoog J**, Zachrisson H, Lanne T, Lindenberger M. Reduced compensatory responses to maintain central blood volume during hypovolemic stress in women with vasovagal syncope. *Submitted*.
- IV. **Skoog J**, Zachrisson H, Lanne T, Lindenberger M. Slower lower limb blood pooling increases orthostatic tolerance in women with vasovagal syncope. *Submitted*.

List of papers

Abbreviations

ACh	Acetylcholine
ANP	Atrial natriuretic peptide
AVP	Arginine vasopressin
BMI	Body mass index
BP	Blood pressure
$\beta_0, \beta_1, \beta_2$	Quadratic regression model parameters for calculation of venous compliance
C_{calf}	Calf venous compliance
CBF	Calf blood flow
CBP	Calf blood pooling
CFC	Capillary filtration coefficient
CO	Cardiac output
CPT	Cold pressor test
CVR	Calf vascular resistance
DBP	Diastolic blood pressure
ΔP	Change in pressure
ΔV	Change in volume
FBF	Forearm blood flow
FVR	Forearm vascular resistance
HR	Heart rate
HUT	Head-up tilt
IPAQ	International Physical Activity Questionnaire
LBNP	Lower body negative pressure
LTI	Lower body negative pressure tolerance index
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
n_0, n_d, R_{max}	Venous wall model parameters for calculation of venous compliance
NE	Norepinephrine
PCM	Physical contra maneuvers
P-NE	Plasma norepinephrine
Pooling _{time}	Time (sec) from LBNP onset to the development of 50% of calf blood pooling
PP	Pulse pressure
QRE	Quadratic regression model
RAAS	Renin-Angiotensin-Aldosterone System

Abbreviations

RAP	Right atrial pressure
SBP	Systolic blood pressure
TPR	Total peripheral resistance
SV	Stroke volume
VWM	Venous wall model
VOP	Venous occlusion plethysmography
VTI	Velocity time integral
VVS	Vasovagal syncope

Introduction

Syncope is defined as transient loss of consciousness due to a temporary cerebral hypoperfusion, and characterized by a rapid onset, short duration and a spontaneous recovery (1). Syncope is a common problem in the general population with a lifetime cumulative incidence between 25 and 50% (2,3). Women are often reported to have higher incidence compared to men and several studies indicate a bimodal age distribution with a first peak in adolescence and a second peak in older age (2-4).

Several different factors may be involved in the pathophysiology of syncope. However, the ESC classification provides three principal etiologies (1): 1) reflex (neurally mediated) syncope, 2) syncope due to orthostatic hypotension, 3) cardiac syncope (cardiovascular). Overall, reflex syncope is the most frequent cause of syncope and vasovagal syncope (VVS) is by far the most common form within this subset. Although VVS could be initiated via central triggers (emotions and/or pain) the most common trigger is quiet standing (5). The increased susceptibility towards VVS during upright posture is often attributed to the fact that the force of gravity induces a powerful challenge to human blood pressure control. However, during most circumstances blood pressure homeostasis is preserved due to activation of the autonomic nervous system which facilitates a well-adjusted neural and humoral circulatory control. Although the term “vasovagal” was introduced as early as 1932 by Sir Thomas Lewis (6) in order to empathize the contribution of both vasodilation of arteries and bradycardia, the pathophysiology of VVS remains elusive.

This thesis presents integrative studies investigating vascular wall function and autonomic cardiovascular regulation in young women with vasovagal syncope. Focus is set on mechanisms responsible for circulatory adaptations in response to orthostatic shifts in blood volume.

Background

Cardiovascular responses to orthostatic stress

The adoption of upright posture provokes blood pressure homeostasis in two principle ways. Firstly, gravity causes cerebral perfusion pressure to be approximately 20 mmHg lower than at the level of the heart (7,8). Secondly, changes in transmural pressure cause a progressive pooling of 500-1000 ml blood in the splanchnic and the lower extremities' venous capacitance system (9). This represents a central decrease in volume of approximately 25-30% (5). Moreover, the increase in transmural capillary pressure in the dependent circulation results in a prominent filtration of plasma fluid into the extravascular tissue. During 5 min of quiet standing the decrease in plasma volume can be as much as 450 ml (10). This redistribution of blood decreases venous return, cardiac output (CO) and blood pressure (BP) and continued maintenance of upright posture necessitates the interaction between several cardiovascular regulatory systems (11).

Initial adjustments to orthostatic stress are essentially mediated by neural pathways of the autonomic nervous system, involving arterial baroreceptors and cardiopulmonary receptors (12). Arterial (high pressure) baroreceptors are mechanoreceptors, located in the adventitia of the aortic arch and carotid sinuses. These mechanoreceptors are tethered to the surrounding structure and sensitive to distortion of the vessel wall. However, since changes in distortion and pressure are closely related, the arterial baroreflexes responds to beat-to-beat changes in BP by altering autonomic neural outflow to maintain cardiovascular homeostasis (13). Afferents from the carotid sinuses are transmitted via the carotid sinus nerve and then the glossopharyngeal nerve whereas the corresponding signals from the aortic arch are conveyed via branches of the vagus nerve. The afferent signals converge to a large extent within the nucleus tractus solitarii in the medulla oblongata where integration with other incoming baroreceptor information occurs (14). All baroreceptors function as sensors in a negative feedback system, i.e., in the event of a sudden elevation of BP the baroreceptors are stretched and this induces an increase in neuronal firing with a concomitant increase in parasympathetic activity and decrease in sympathetic activity.

Conversely, the arterial baroreceptors become unloaded during BP reductions and the decrease in neuronal firing evokes an almost instantaneous decrease in parasympathetic activity with reduced release of acetylcholine (ACh) from postganglionic parasympathetic fibers to the SA node and AV node. The sympathetic activity increase 5 to 10 sec later and is primarily mediated by norepinephrine (NE) from postganglionic sympathetic fibers innervating the blood vessel wall, SA node, atria and the ventricle (12). The early response to orthostatic stress is thus characterized by tachycardia (parasympathetic withdrawal) and arterial vasoconstriction (sympathetic activation).

The cardiopulmonary (low-pressure) receptors are a group of mechanoreceptors located in the heart, pulmonary artery and the junction of the atria with their corresponding veins. They act in concert with the arterial baroreceptors and decrease their neuronal firing in response to decreases in transmural pressure within their location (15). When stimulated, the low-pressure receptors mainly act by alter peripheral vascular resistance; the effect on heart rate (HR) is generally minor (12). However, the importance of cardiopulmonary baroreceptors in the initial reflex adjustment to orthostatic stress is unclear. Cardiopulmonary baroreflexes have been shown to potentiate the action of the arterial reflexes (16), although cardiopulmonary denervation in humans does not appear to be followed by any obvious deficit in blood pressure regulation (17).

Nevertheless, intact arterial baroreflexes are a key mechanism in the control of BP during orthostatic stress (18). Vagally mediated tachycardia has been suggested to protect against reduction in CO and subsequent hypotension during the initial seconds of an orthostatic transition (19). However, the importance of reflex tachycardia on hemodynamic stability for extended durations of orthostasis is less clear (20). One reason could be that the effect of increased HR only seems to have an initial transient effect on CO since right atrial pressure falls (RAP) towards 0 mmHg upon standing. A further increase in HR would not be able to increase CO without also lowering RAP. This implicates that the possibility to increase CO is limited since any further decrease in RAP leads to a negative transmural pressure across the large veins supplying the atrium and a

consequently vessel collapse, hampering any increment in CO (12). There are several clinical observations supporting this view. Wiessler showed that administration of atropine in the upright position increased HR but had marginal effects on BP as well as CO, and was unable to prevent impending VVS (21,22). Further, patients with cardiac transplants have intact BP control during orthostasis without any increase in HR, while patients with sympathetic vasomotor lesions display pronounced orthostatic hypotension despite intact vagal reflex tachycardia (20,23).

This implies that peripheral vasoconstriction is crucial for maintaining BP during orthostatic stress and that the venous blood reservoir is an important determinant because the heart cannot pump blood that it does not receive (12,20). Arterial vasoconstriction reduce blood flow to the venous section and decrease peripheral venous pressure, leading to passive elastic recoil of pooled venous blood from the lower limbs and splanchnic vasculature to the central circulation (24). Active venoconstriction within the splanchnic circulation may also help to counteract the loss of central blood volume (11). Further, the slower but continuous net capillary absorption of extra-vascular fluid is also dependent on a reflex decline in capillary pressure due to adjustment of pre- and postcapillary resistance (25,26) and act as a powerful mechanism to increase plasma volume (27-30). Thus, the combined effect of mobilizing venous capacitance blood and net capillary absorption serve as important factors for preserving venous return and act momentarily to maintain cardiovascular homeostasis (24,28,29,31).

Continued orthostatic stress also activates a series of neurohormonal changes that reinforce the actions of the cardiovascular reflexes. In parallel with the sustained increase in NE, a transient increase in epinephrine, activation of the renin-angiotensin-aldosterone system (RAAS) and releases of arginine vasopressin (AVP) have been noted (32). These additional responses exert numerous effects to maintain cardiovascular homeostasis, e.g., via direct vasoconstriction at the level of the vascular smooth muscle and by increasing tubular Na⁺ reabsorption in the kidneys to minimize loss of body water (17,32).

Pathophysiology of vasovagal syncope

VVS is generally triggered by emotional or orthostatic stimuli (33). Emotional VVS are thought to act via central, non-baroreflex pathways, while orthostatic VVS is closely related to the function of arterial baroreceptors as well as to cardiac and pulmonary receptors (34,35). Thus, different trigger mechanisms seem to be involved in the two types. In this thesis, focus is set on orthostatic VVS.

Based on the Sharpey-Schafer model (36), it has been suggested that the main conditions leading to VVS in the event of orthostatic stress is a reflex increase in sympathetic tone to the heart causing vigorous contraction of the volume-depleted ventricle (37-39). This is thought to stimulate ventricular afferents in the left ventricle which might trigger a paradoxical withdrawal of peripheral sympathetic tone and increase vagal tone, leading to vasodilatation and bradycardia. This is the ventricular theory (40). Although the proposed afferent pathway has been demonstrated in cats (41), several observations in humans have challenged the universality of this theory. First, P-NE levels have been found to be normal or decreased preceding VVS (42-45) and reduced maximal increase in MSNA has been found in patients developing VVS (44,46). Further, echocardiographic measurements during head up tilt (HUT) have not reliably demonstrated decreased left ventricular size or volume before the onset of syncope (47,48). Second, there is evidence that VVS can be evoked in patients with cardiac transplantations, i.e., when the heart has undergone major efferent and afferent denervation (49). Thus, the universality of the ventricular theory has been widely challenged and the knowledge of the afferent pathways in VVS are still limited (40).

The efferent pathways of the reflex is better characterized since this involves variables that can be measured more directly (7). In all syncope cases there is a drop in BP and often HR. VVS is usually defined by three subtypes based on the efferent pathway; cardioinhibitory, vasodepressor or mixed type (1). The decrease in HR is caused by increased vagal stimulus to the sinus node and characterized as the cardioinhibitory part of the reflex. However, even without any substantial drop in HR, BP can decrease enough to cause VVS due to reductions in peripheral resistance (50). This has for

example been the major challenge for pacemaker therapy (51-53). The decrease in peripheral resistance is conventionally characterized as the vasodepressor part of the reflex. Profound vasodilation in the forearm has been observed during syncope (54-56) and earlier studies have suggested that vasodilation mainly resulted from a sympathetic withdrawal (44,57-59), leading to decreased peripheral resistance and increased blood pooling in the venous system, all contributing to the low arterial pressure. Since loss of sympathetic tone has been observed during fully developed syncope it was assumed that reduced sympathetic activity might play a causal role in VVS. The two mechanisms (cardioinhibitory and vasodepressor) do not always act exclusive, so the mixed type is used if both mechanisms are present (1).

However, the traditional concept of the vasodepressor explanation has been challenged during the last years. Vaddadi et al. (60) observed that only 1 out of 10 VVS patients demonstrated an abrupt cessation of MSNA at the onset of hemodynamic collapse. In accordance, Cooke et al. (61) noted that MSNA was maintained throughout cardiovascular compromise in 40% of the healthy individuals during LBNP. This challenge the notion that sympathetic withdrawal is the final trigger resulting in hypotension. In parallel, a recent study from Fu et al. (62) showed that when MSNA withdrawal occurred, it was a late event, observed after the onset of hypotension. It is currently debated whether vasodilation is the dominant hypotensive mechanism preceding VVS, and recent studies have suggested that reduction in CO, rather than vasodilation, may be the primary cause of the hypotension (62-67). For example, Jardine et al. (68) reported that although all subjects showed an initial decrease in CO during HUT, patients who became hypotensive demonstrated a further decline in CO. Subsequent studies further indicated that the marked hypotension in VVS patients during HUT-induced syncope might be CO-mediated, without clear evidence of sympathetic inhibition (64,66). Recently, Fu et al. (62) observed that all individuals reaching presyncope showed a moderate to severe fall in CO 1-2 min before syncope and the authors suggested that the fall in CO could be driven by a decrease in HR and/or a decrease in SV.

Lower or earlier decreases in SV during LBNP have been linked to orthostatic intolerance in healthy individuals and syncope patients (66,68-71). Excessive venous blood pooling in the lower body and reduced venous return is thought to be responsible for the decrease in SV during orthostatic stress. Increased calf venous pooling has been reported in subjects prone to syncope (72), while others have argued against the importance of blood pooling in the lower limb (73,74). Increased blood pooling in the splanchnic beds, possibly leading to increased central hypovolemia has also been detected in VVS patients (75). The central hypovolemia that occurs during orthostatic stress is compensated by mobilization of blood from peripheral capacitance vessel towards the central circulation, as well as by net capillary fluid absorption from tissue to blood in order to increase venous return to the heart and thus defend central blood volume (24,28,31,76). However, compensatory mechanisms have not been studied in VVS.

Furthermore, studies in healthy subjects without a history of VVS have suggested that not just the pooled volume, but also the rate by which the hypovolemic stimulus is instituted can affect the responses to orthostatic stress (27,77). Despite a similar capacitance response, Lindenberger and Länne (77) found a much slower lower limb blood pooling in otherwise healthy women experiencing vasovagal reactions during moderate levels of LBNP compared to hemodynamic stable women. In analogy, greater increases in MSNA during HUT with a rapid tilt have suggested a speed-dependent sympathetic activation (78). The importance of initial blood pooling time as well as its effects on cardiovascular regulation and orthostatic tolerance in patients with VVS are unknown.

Measurements of venous compliance

Due to its great compliance, the venous system harbors roughly 70% of the systemic blood volume and is designed to maintain a steady venous return to the heart during various conditions (12). However, this specific feature makes humans vulnerable to hydrostatic venous blood pooling. The curvilinear venous pressure-volume curve reflects these properties and is characterized by a compliant part at low venous pressures and a stiffer part at high venous pressures (12,24). The stiffer part at high pressures is crucial for minimizing gravity-induced venous

blood pooling in the lower limb, while the compliant part at low pressures permits large translocations of venous blood in response to only small changes in pressure to preserve cardiovascular homeostasis during, e.g., central hypovolemia. In analogy, greater leg venous compliance has been suggested to be associated with orthostatic intolerance due to greater reductions in venous return and stroke volume (79-81), although the results are inconclusive (82). Furthermore, Freeman et al. (83) reported lower venous compliance in patients with idiopathic orthostatic intolerance. Lower venous compliance could lead to reduced mobilization of capacitance blood, further aggravating the reduction in venous return and trigger the vasovagal reaction (66,77).

Venous occlusion plethysmography (VOP) is a method to study human vascular physiology *in vivo* and the technique has been widely used for measurements of changes in tissue volume since it was introduced in 1953 by Whitney (84). The underlying principle of VOP is that when venous drainage is interrupted, through inflation of a collecting cuff, the arterial inflow is unchanged and blood can enter the occluded segment but cannot escape (85). VOP is the gold standard method for evaluating venous compliance and thus an important tool within physiological research. The frequently applied technique (83,86-89) outlined by Halliwill et al. (90) uses the venous pressure-volume relationship during 1 min of linear cuff deflation (60 to 10 mmHg) after venous stasis of 4 to 8 min. However, it remains unknown if the applied cuff pressure accurately reflects venous pressure in the lower limb. Moreover, the approach evaluates venous compliance based on the total volume increase during venous stasis without separating net fluid filtration and venous capacitance response. According to previous studies (87,90), it has been argued that the rapid decrease in cuff pressure of 1 mmHg/s minimizes the possibility for the fluid filtrate to re-enter the circulation and thus affect the pressure-volume relationship. Yet, fluid filtration seems to affect venous compliance when transmural pressure is increased by lower body negative pressure (91). The conflicting results may partly be due to differences in study design but it emphasizes the importance to validate the influence of fluid filtration on venous compliance during VOP measurements.

Background

Further, a quadratic regression equation is used to model the pressure-volume relation (90). The major shortcoming is that the curvilinear pressure-volume relation is fitted to a strict mathematical parabolic function. With this approach, venous compliance is bound to become negative at a pressure within or very close to the applied physiological pressure range, precluding a valid interpretation (86-88,90,92,93). Due to the form of the venous pressure-volume curve it appears to be of great importance to model the whole curve accurately, especially since gravitational forces could increase venous pressure well above 60 mmHg during prolonged standing.

Aims

- To study the effect of net fluid filtration on VOP measurements, and to develop a method that permits correction of the effect of net fluid filtration on volume increase during VOP.
- To develop a new model for the characterization of the venous pressure-volume relation and the calculation of venous compliance
- To assess calf venous compliance and capacitance response as well as to determine the association between both venous compliance and capacitance to maximal circulatory stress tolerance in women with VVS.
- To study cardiovascular responses to hypovolemic circulatory stress in women with VVS, emphasizing compensatory responses in order to defend central blood volume.
- To assess the relation between initial blood pooling time and hemodynamic responses to maximal circulatory stress tolerance with aid of lower body negative pressure in women with VVS.

Aims

Materials

Ethical approval

The studies were approved by the Regional Ethical Review Board in Linköping, Sweden. Each subjects have signed a written informed consent in accordance with the declaration of Helsinki.

Healthy subjects

A total of 25 healthy subjects were studied and divided into two groups; 10 young men (21.6 ± 0.6 years) and 15 young women (22.8 ± 0.8 years). Subjects were recruited by means of advertising at Linköping University. All subjects were healthy, without any history of cardiovascular disease, non-smokers and not taking any medication. Further, none of the young women had any history of syncope and all experiments were conducted during the follicular phase of the menstrual cycle (day 1-10). Physical activity, evaluated from the International Physical Activity Questionnaire (IPAQ-short), was overall moderate to high in both young men and women. Table 1 shows the demographic values in young men and women at rest. All healthy subjects were studied in paper I. The young women were also studied in paper II, III and IV.

Women with vasovagal syncope

15 women (25.5 ± 1.3 years) had prior to the study been examined at the Department of Clinical Physiology in Linköping for recurrent syncope in daily life and diagnosed with VVS by means of a positive head-up tilt test (HUT) using the Italian protocol (94). HUT was considered positive when syncope was reproduced in association with hypotension, bradycardia, or both in accordance with ESC guidelines (1). Women with a cardiovascular and/or neurological disease were excluded. Three of the VVS women (20%) had weekly, four (27%) monthly, and eight (53%) yearly problems due to vasovagal reactions. The mean syncope history was 7.0 ± 1.0 years. All VVS women were non-smokers and were not taking any medication, with the exception of 7 using different contraceptives. All VVS women were scheduled within the follicular phase of their menstrual cycle or in the low-

hormone phase in contraceptive-users (day 1-10). Physical activity, evaluated from the International Physical Activity Questionnaire (IPAQ-short), was overall moderate to high in VVS women. VVS women were studied in paper II, III and, IV. Table 1 shows the demographic values in VVS at rest.

Table 1. Demographic resting values.

	Healthy men	Healthy women	VVS women
<i>n</i>	10	15	15
Age, yr	21.6±0.6	22.8±0.8	25.5±1.3
Height, cm	185±1.9***	167±1.4	164±1.9
Weight, kg	76±2.3**	64±2.9	62±2.5
BMI, kg/m ²	22.1±0.6	22.9±1	22.7±0.8
Calf circumference, cm	36±0.9	36±0.7	36±0.8
HR, beats/min	62±3.2	67±2.6	66±2.7
SBP, mmHg	122±2.8*	111±3	104±3.9
DBP, mmHg	58±2.1*	65±1.9	63±2.2
MAP, mmHg	80±2.1	82±2.4	76±3.9
CBF, ml/100ml/min		3.9±0.3##	2.6±0.2
CVR, CVR units		22±1.2##	34±3.4
P-NE, nmol/L		0.75±0.07##	1.06±0.08
IPAQ activity			
Low, n (%)	0 (0)	1 (7)	2 (13)
Moderate, n (%)	7 (70)	9 (60)	6 (40)
High, n (%)	3 (30)	5 (33)	7 (47)

BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; CBF, calf blood flow; CVR, calf vascular resistance; P-NE, plasma norepinephrine; IPAQ, International Physical Activity Questionnaire. Mean ± SE. *Healthy men vs. Healthy women; # Healthy women vs. VVS women. **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ##*P* < 0.01.

Methods

Venous occlusion plethysmography (VOP)

Changes in lower limb volume ($\text{ml} \cdot 100\text{ml}^{-1}$) were measured with strain gauge plethysmography. All recordings were performed in a temperature stable room (25°C). Subjects were placed in the supine position with an acclimatization period of at least 15 min with the right leg slightly elevated and supported at the ankle. A strain gauge was applied at the maximal calf circumference and manually calibrated. A cone-shaped, 22-cm-wide thigh cuff was placed on the thigh proximal to the knee on the right leg. The thigh cuff was within one sec inflated to the appropriate pressure using a cuff inflator (Bergenheim, Elektromedicin, Göteborg, Sweden). After 4 or 8 min, the cuff pressure was reduced with a rate of 1 mmHg/s by means of a custom-built device, enabling a linear pressure decrease (95). All data were recorded, stored and analyzed using the PeriVasc Software (Ekman Biomedical Data AB, Göteborg, Sweden).

Calf capacitance response and net fluid filtration

The inflation of cuff pressure evokes a rapid increase of calf volume, representing the maximum volume stored in the veins at the given pressure (capacitance response, $\text{ml} \cdot 100\text{ml}^{-1}$), followed by a slower increase caused by net capillary fluid filtration ($\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$) into the extravascular space. Previous studies have shown that the capacitance response is completed within 3-4 min when cuff pressure is elevated to 60 mmHg (90,96). Thus, capillary fluid filtration was calculated as the slope of the volume curve between 4 and 8 min. The capacitance response was obtained by a backward extrapolation of the filtration slope to the onset of VOP (30) (figure 1). The total capillary filtration ($\text{ml} \cdot 100 \text{ ml}^{-1}$) was calculated as the value of the filtration slope times the duration of venous stasis (8 min). The total calf volume increase ($\text{ml} \cdot 100 \text{ ml}^{-1}$) was determined as the capacitance response + total capillary fluid filtration.

Figure 1. Representative tracing of calf volume changes in a 21-year-old healthy woman during VOP. A: Representative tracing of VOP recordings in the calf at 60 mmHg during 8 min with a subsequent reduction of 1 mmHg/s in cuff pressure. Initial rapid volume increase represents the capacitance response. The later slower increase in volume represents net fluid filtration.

Venous compliance

Venous compliance is defined as a change in volume (ΔV) generated by a change in venous transmural pressure (ΔP), i.e., venous compliance ($\Delta V / \Delta P$) denotes the slope of the tangent to any point along the pressure-volume relationship (12).

Quadratic regression equation (QRE). Venous compliance is commonly calculated with the model developed by Halliwill et al. (90), i.e., the characteristics of the pressure-volume curve is described by a QRE.

$$\Delta \text{ volume} = \beta_0 + \beta_1 \cdot (\text{cuff pressure}) + \beta_2 \cdot (\text{cuff pressure})^2 \quad (\text{Eq.1})$$

β_0 is the y-intercept and β_1 together with β_2 are characteristics of the slope generated by the pressure-volume curve. Calf venous compliance (C_{calf}) is defined as the first derivative of the pressure-volume curve, creating a linear pressure-compliance curve:

$$C_{\text{calf}} = \beta_1 + 2 \cdot \beta_2 \cdot (\text{cuff pressure}) \quad (\text{Eq.2})$$

By using Eq. 2 C_{calf} was calculated for pressures between 10 and 60 mmHg.

The QRE was used in paper I. However, the QRE is a strict mathematical function and with this approach venous compliance is bound to become negative at a pressure within or very close to the applied physiological pressure range, precluding a valid interpretation of compliance at high venous pressures (86-88,90,92,93). Therefore, we developed a new venous wall model (VWM), for characterization of the venous pressure-volume curve and compliance (see Results).

Capillary filtration coefficient

The capillary filtration coefficient (CFC, $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$) in the calf was calculated from the net fluid filtration induced by a fixed increase in transcapillary hydrostatic pressure and defined as:

$$\text{CFC} = \text{net fluid filtration} / \text{cuff pressure}$$

Intravenous pressure at rest was subtracted from cuff pressure and it was further assumed in the CFC calculation that 80% of the cuff pressure was transmitted to the capillary bed (97).

Blood flow

Calf blood flow (CBF, $\text{ml} \cdot 100\text{ml}^{-1} \cdot \text{min}^{-1}$) was measured by VOP as the slope of the volume change initiated by the rapid increase in thigh cuff pressure over a period of 6 sec (98). Calf vascular resistance (CVR) was calculated as mean arterial pressure (MAP) divided by calf blood flow.

Intravenous pressure

A 20-gauge venous catheter, connected to a pressure transducer (Duet Multi P, Medtronic, Skovlunde, Denmark), was inserted in a dorsal foot vein on the same leg as used for VOP measurements to facilitate simultaneous recordings of intravenous pressure and cuff pressure.

Lower body negative pressure (LBNP)

Application of LBNP redistributes blood from the upper body to the lower extremities, leading to a central hypovolemia (99) (figure 2A). Due to restriction of the muscle fascia envelope, approximately 80% of the externally applied negative pressure is transmitted to the underlying muscle tissue. This results in an increase in the transmural pressure over the vessel wall, followed by a vessel dilatation and a concomitant blood pooling (100). The LBNP method is a commonly used model to study cardiovascular responses to hemorrhage and orthostatic stress (46,77,99,101). In comparison with other techniques used to model orthostatic stress, such as HUT, the LBNP method has some major advantages. LBNP is applied in the supine position which facilitates physiological recordings and minimizes the possibility of movement artifacts. Further, the applied negative pressure is easy to define and can easily be adjusted. LBNP of 40-50 mmHg corresponds to HUT of 70° and results in a similar degree of central hypovolemia with cardiac output reduction (25%) and blood pooling in the pelvic region as well as the lower limb. However, HUT and LBNP differ in the sense that blood in the splanchnic reservoir increases during HUT whereas LBNP induce a decrease (102).

All recordings were performed in a temperature stable room (25°C). Each subject assumed a supine position in the LBNP chamber, hermetically sealed at the level of the iliac crest. The negative pressure in the LBNP chamber was generated by a vacuum source, constantly measured by a manometer (DT-XX disposable transducer, Viggo spectramed, Helsingborg, Sweden) and held constant by a rheostat. The LBNP protocol consisted of two experiments. Firstly, after at least 20 min of rest the LBNP chamber pressure was reduced by 30 mmHg during 8 min (LBNP₃₀). Secondly, after at least 20 min of rest a LBNP stress test was conducted in which the LBNP chamber pressure was reduced by 20 mmHg for 4 min and subsequent reductions in pressure of 10 mmHg every 4 min (LBNP_{stress}) (Figure 2B). The test was terminated according to the following criteria: 1) after completion of 4 min LBNP of 70 mmHg; 2) at the onset of presyncopal signs or symptoms (decrease in systolic blood pressure ≥ 25 mmHg between adjacent 1-min readings, a decrease in diastolic blood pressure of ≥ 15 mmHg between adjacent 1-min readings, a sudden decrease in heart

rate ≥ 15 bmp, nausea, pallor, profuse sweating, dizziness); 3) at the subjects' request. LBNP tolerance was calculated as LBNP tolerance index (LTI) (103).

Figure 2. Lower body negative pressure. **A:** Schematic drawing of the LBNP chamber. **B:** Experimental protocol of single step (I) and graded (II) LBNP.

Changes in calf and arm volume

Changes in tissue volume ($\text{ml} \cdot 100\text{ml}^{-1}$) were evaluated with strain gauge plethysmography during LBNP (Hokanson EC-6, D.E. Hokanson, Bellevue, WA). A strain gauge was placed at the maximal circumference of the right calf and the leg was slightly elevated with the heel resting on a foot support with the lowest part of the calf approximately 2 cm above the floor of the LBNP chamber. A strain gauge was also placed at the maximal circumference of the right upper arm, which rested comfortably on a support at the level of the heart.

Calf capacitance response and net fluid filtration

As shown in figure 3, the onset of LBNP evokes a rapid increase in calf volume representing the venous capacitance response (blood pooling), followed by a slower increase caused by capillary fluid filtration ($\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$) into the extravascular space and finally a rapid decrease in calf volume at the cessation of LBNP. Previous studies have shown that the capacitance response is completed within 3 min (30,96). Thus, capillary fluid filtration was calculated as the slope of the volume curve between 3 and 8 min. The capacitance response ($\text{ml} \cdot 100 \text{ ml}^{-1}$) was obtained by a backward extrapolation of the filtration slope to the onset of VOP (30).

Figure 3. Original tracing illustrating calf volume changes in a 31-year-old healthy woman during 8 min of lower body negative pressure (LBNP) 30 mmHg. The initial rapid increase in volume represents blood pooling (calf capacitance response) and the slower increase reflects net fluid filtration (dotted line). Net fluid filtration is a continuous process and the capacitance response was calculated by extrapolating the slope of net fluid filtration to the onset of LBNP.

Further, the time (sec) from LBNP onset to the development of 50% of calf blood pooling ($\text{pooling}_{\text{time}}$) was defined in each subject. To evaluate the main determinants of $\text{pooling}_{\text{time}}$, five parameters were identified and divided in static components (i.e. resting factors); blood flow at rest (I) and venous compliance (II), as well as dynamic components (i.e. factors affected by LBNP-induced baroreceptor unloading); blood flow during LBNP (III), increase in vascular resistance (IV) and the LBNP-induced blood pooling (V).

Arm capacitance response and net fluid absorption

As shown in figure 4, the onset of LBNP evokes a rapid decrease in the upper arm volume (I), representing the maximal blood volume mobilized from the veins (capacitance response), followed by a slower but steady reduction in volume during the LBNP procedure (II), representing transcapillary fluid absorption from extra- to intravascular space. After completion of LBNP there was a rapid increase in volume (III), signifying a regain of regional blood. This phase was followed by a slow capillary filtration from intra- to extravascular space, gradually restoring the fluid volume (IV). Finally, the clear-cut demarcation between the rapidly regain of blood and the slower filtration provided a marker for the total fluid absorption during LBNP (V).

Figure 4. Original tracing illustrating compensatory volume changes in the upper arm of a 27-year-old healthy woman during LBNP 30 mmHg. The initial rapid decline in tissue volume reflects mobilization of peripheral blood towards the central circulation (capacitance response (I)), whereas the following slower, but continuous, decline represents capillary fluid absorption (II). At LBNP termination there is a rapid regain of blood (III), followed by a slow capillary filtration from intra- to extravascular space, gradually restoring the fluid volume (IV). Finally, the clear-cut demarcation between the rapidly regain of blood and the slower filtration after LBNP termination represents the total fluid absorption during LBNP (V).

This interpretation has previously been validated with the use of technetium-marked erythrocytes and it has been shown that the arm capacitance response is fully developed within the first 2 min after onset of LBNP (29,30). During LBNP₃₀, arm capacitance response and net capillary fluid absorption were evaluated according to the technique described in detail by Lindenberger et al. (28). During LBNP_{stress}, arm capacitance response was evaluated as the maximal volume decrease after 2 min at each LBNP level.

Hemodynamic measurements

Heart rate and blood pressure at rest was measured noninvasively using a semiautomatic blood pressure cuff positioned over the brachial artery on the upper arm (Dinamap Pro 200 Monitor; Criticon, Tampa, FL; USA). Heart rate and blood pressure during the LBNP protocol were monitored non-invasively, beat-by-beat (Finometer® Midi, Finapres Medical Systems, Amsterdam, the Netherlands). Aortic outflow was measured from the suprasternal view (jugulum) using a Vivid E-9 ultrasound scanner (GE Healthcare, Wauwatosa, WI, USA) with a non-imaging 2.5 MHz Doppler probe. All measurements were conducted at the same phase in the respiratory cycle (expiration). The recordings were carried out just prior to the start of the LBNP_{stress} and then between minute 2 and 3 at each LBNP level, allowing time for new equilibrium. Two subsequent aortic outflow measurements, whereby the probe was displaced in between, were conducted at each point of measure in order to optimize the detection of the peak velocity integral. The same individual conducted all measurements for both the VVS group and the control group. During the aortic flow measurements, the valvular velocity time integral (VTI) was analyzed during three consecutive heart beats, and stroke volume (SV, mL) was calculated as the sub-valvular area $\times$ VTI. Cardiac output (CO, L $\cdot$ min⁻¹) was measured as the product of heart rate (HR, beats $\cdot$ min⁻¹) and SV. Total peripheral resistance (TPR, TPR-units) was calculated as the ratio between mean arterial pressure (MAP, mmHg) and CO.

Heart rate variability (HRV) analyses (frequency domains) were conducted from continuous ECG recordings, using commercial HRV software (SphygmoCor®, AtCor Medical Pty Ltd, West Ryde, Australia), which assessed low frequency (LF), high frequency (HF),

LF/HF ratio, as well as total power. At rest and LBNP₃₀, the analysis included a division of the power spectrum into LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz) bands expressed in normalized units (104).

Cold pressor test

Cold pressor test (CPT) was performed in the supine position after at least 10 minutes of rest. The right foot was immersed into a water bath of 5° C (checked with a digital thermometer just prior to the test) for 120 sec. Subjects were instructed to relax, maintain normal breathing and to avoid isometric muscular contraction throughout the test. Heart rate and blood pressure at rest and during CPT (at 40, 80 and 120 sec) were measured noninvasively using a semiautomatic blood pressure cuff positioned over the brachial artery on the left upper arm (Dinamap Pro 200 Monitor; Criticon, Tampa, FL; US). Forearm blood flow (FBF) was measured by standard venous occlusion plethysmography (Hokanson EC-6, D.E. Hokanson, Bellevue, WA) repeatedly at baseline (x6) and 40, 80, and 120 sec (each x2) after the initiation of the CPT, with the right arm at heart level and a strain-gauge at the maximal forearm circumference. BP and forearm blood flow were measured simultaneously, and mean forearm vascular resistance was calculated as mean arterial pressure (MAP) divided by mean FBF at baseline and CPT.

Blood samples

Assessment of plasma levels of norepinephrine (P-NE, nmol·L⁻¹) was conducted during LBNP_{stress}. Antecubital venous blood was sampled before, after 3 min of LBNP 30 mmHg and at presyncope (or completion of the test). Blood samples were promptly placed on ice, centrifuged within 20 min and stored in a -70°C freezer. Subsequent analysis of P-NE was performed with HPLC technique.

Statistics

Continuous variables with normal distribution are expressed as mean and standard error. For these variables parametric tests were used. Differences between two groups or within groups were tested by unpaired or paired *t*-tests with Bonferroni correction for multiple

measurements when appropriate (Paper I-IV). Repeated measure ANOVA with Bonferroni correction for multiple comparisons was used to assess between and within-group differences in calf and arm volume changes, venous compliance as well as hemodynamic responses during LBNP and CPT (Paper I-IV). To compare the models' ability (QRE and VWM) to fit the experimentally induced pressure-volume curve, as well as to compare pressure-compliance curves between the two models repeated measure ANOVA with Bonferroni correction were also used (Paper II). Non-normal distributed continuous variables are expressed as median with interquartile range (25th-75th percentiles). For these variables nonparametric tests were used. Differences between two groups were tested by Mann-Whitney U-test and Wilcoxon matched pair test were used to detect changes within groups (Paper II-III). Simple linear regression was applied to assess association between intravenous pressure and cuff pressure (Paper I). Simple linear regression was also applied to assess association between compliance/capacitance response/pooling_{time} and LBNP-tolerance as well as between pooling_{time} and hemodynamic responses during LBNP (Paper II-IV). Multiple linear regression was used to evaluate the determinants of pooling_{time} in healthy women and women with VVS (Paper VI). *P*-values < 0.05 were considered statistically significant. Statistical analyses were carried out using SPSS 22.0 and 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Methodological aspects of VOP (Paper I-II)

Intravenous pressure

Intravenous pressure was 8.5 ± 0.5 mmHg at rest and cuff pressure reached 100% transmission, i.e., 60 mmHg after 3-4 min (184 ± 18 s) of venous occlusion. The rapid reduction in cuff pressure during the deflation phase correlated well with intravenous pressure reduction ($r = 0.992$, $P < 0.001$, figure 5).

Figure 5. Relationship between intravenous pressure and cuff pressure during cuff deflation at 1mmHg/s. All the individual data is displayed. The average regression is shown as a thick solid line ($r = 0.992$, $P < 0.001$).

Correction of net fluid filtration

The correction of net fluid filtration was implemented during the entire VOP measurement and could be divided into three phases. Firstly, from onset of VOP to 4 min, net filtration flow was taken to linearly increase from zero to the value evaluated between 4 and 8 min. Secondly, from 4 to 8 min, net filtration flow was taken to be constant. Thirdly, during the deflation phase, net filtration flow was only assumed to occur when cuff pressure exceeded the intravenous pressure at rest. Net filtration flow was also considered to be in

proportion to the transmural pressure, i.e., when the transmural pressure decrease during the deflation phase, net filtration flow was assumed to display a proportional decrease. These phases were integrated to form an accumulated filtration volume, and the filtrated volume was then evaluated and subtracted from the original volume curve (figure 6).

Figure 6. Representative tracing of calf volume changes in a 21-year-old healthy woman during VOP. VOP recording in the calf at 60 mmHg during 8 min with a subsequent reduction of 1 mmHg/s in cuff pressure. The black curve shows the initial rapid volume increase (capacitance response) with a following slower increase in volume (net fluid filtration). The red dashed curve shows the same recording with correction of net fluid filtration. At the end of the deflation phase the uncorrected volume curve was markedly increased, whereas the corrected volume curve was close to the original baseline level.

Total calf volume increase (venous capacitance response + total filtration) was comparable between men and women. However, total net fluid filtration was higher in women ($P < 0.01$) and showed a higher percentage contribution to the total calf volume increase; being 36% in women and 25% in men ($P < 0.01$).

The impact of net fluid filtration on calf venous compliance (C_{calf}) in women and men during both 4 and 8 min VOP was studied by using

the developed correction model. Overall, C_{calf} was underestimated when fluid filtration was not accounted for, reflected by significant differences in β_1 , β_2 and the slope of the pressure-compliance curve (all $P < 0.01$). The most obvious differences (%) between the uncorrected and corrected measurements of C_{calf} were found at pressures > 30 mmHg with greater difference in C_{calf} in women than men during 8 min VOP ($P < 0.01$, figure 7). No difference in C_{calf} was found between the corrected 4 and 8 min trial.

Figure 7. Differences (%) between uncorrected and corrected values of C_{calf} for pressures from 10 to 50 mmHg in women during 8 min VOP and men during 8 and 4 min VOP. The most obvious differences in C_{calf} were found at pressures > 30 mmHg, with C_{calf} being more affected in women than men during 8 min VOP (Interaction, $P < 0.05$). No differences were found between VOP of 8 and 4 min in men. * $P < 0.05$, *** $P < 0.001$ women 8 min VOP vs. men 8 min VOP.

Venous wall model

The VWM was constructed as a 3 parameter model that describes the relationship between transmural pressure, vessel wall stiffness and vessel radius. Below follows a brief overview of the VWM. A more detailed description is given in paper II. The model parameters of a fictitious vein/venous system per unit length with a radius = 1 [a.u] (a.u = arbitrary unit) for pressure = 0 [mmHg] were defined as:

n_0 [a.u] The initial stiffness of the vessel wall at pressure = 0 mmHg, hence the radius = 1.

Results

nd [a.u]	The initial increase in vessel wall stiffness as a function of radius extension/increase.
$Rmax$ [a.u]	The maximal radius/circumference, obtained by letting the wall stiffness approach infinity as the radius approaches $Rmax$.

Firstly, by using a given set of model parameters (n_0 , nd and $Rmax$) a pressure-radius relation is computed by the VWM. Secondly, in accordance to measurement of volume changes with strain gauge plethysmography (84) the pressure-radius relation is converted to a pressure-volume relation, and by altering the model parameters (n_0 , nd and $Rmax$) the shape of the curvilinear venous pressure-volume relation can be adjusted (figure 8). Thirdly, a numerical algorithm is required to find the optimal model parameters and the Downhill Simplex algorithm with least square deviation from raw data as an error function was used for the parameter identification.

Figure 8. Influence of model parameters on the venous wall model. Effects of model parameters on the venous pressure-volume relation. The pressure-volume relation with parameters $n_0 = 350$, $nd = 50$ and $Rmax = 1.1$ is used as reference value (red straight line). The effects of decrease n_0 from 350 to 250 (blue dotted and dashed line), the effects of increase nd from 50 to 150 (black dotted line) and the effects of increase $Rmax$ from 1.1 to 1.15 (green dashed line) are illustrated.

Fourthly, after optimal parameter identification, C_{calf} was calculated as the derivative of the pressure-volume curve generated by the VWM for pressures between 10 and 60 mmHg.

Figure 9. Comparison between QRE and VWM. **A:** Representative tracing of the QRE (dashed blue) fit to the experimentally induced pressure-volume raw data (black). **B:** Representative tracing of the VWM (dashed red) fit to the same pressure-volume raw data (black) as above. **C:** Deviation from pressure-volume raw data. The VWM (red) showed a smaller deviation from the pressure-volume raw data compared to the QRE (dotted blue) (Interaction, $P < 0.001$, VWM vs. QRE, $P < 0.001$). **D:** Pressure-compliance curves. The slope of the pressure-compliance curve was steeper at high venous pressures when calculated with the QRE (dashed blue) and calf venous compliance was underestimated (Interaction, $P < 0.05$). * $P < 0.05$, *** $P < 0.001$ VWM vs. QRE.

Comparing the representative original curve of the quadratic regression equation (QRE) and the venous wall model (VWM) fit to the same venous pressure-volume curve showed that the QRE reached its vertex at approximately 50 mmHg, while the VWM was able to adopt the curvilinear venous pressure-volume relation during the entire pressure range (figure 9A-B). In accordance, VWM demonstrated a significantly better fit to the experimentally induced pressure-volume curve compared to QRE ($P < 0.001$, figure 9C), and C_{calf} was underestimated with the QRE (Figure 9D). C_{calf} became negative at the highest pressures when calculated with the QRE. This was demonstrated in 3 subjects (10%) at 55 mmHg and in 22 subjects (73%) at 60 mmHg. No negative values of C_{calf} were generated with the VWM.

LBNP tolerance and cardiovascular responses (Paper II-IV)

No participant chose to terminate the LBNP protocol in advance at their own request. All VVS terminated the LBNP_{stress} protocol due to presyncopal signs or symptoms. Thirteen of the controls terminated the LBNP_{stress} protocol due to presyncopal signs, i.e., two of the controls passed the entire protocol without showing signs of presyncope. In the VVS group, one woman could not participate in the LBNP experiment and one woman developed signs of syncope within the first minutes of LBNP 20 mmHg and no LBNP tolerance index (LTI) could be calculated. LTI was reduced in VVS compared to controls ($P < 0.001$).

Hemodynamic responses

Figure 10A-F presents cardiovascular responses during LBNP_{stress}. Systolic blood pressure (SBP) decreased with increasing LBNP in both controls and VVS ($P < 0.05$). Diastolic blood pressure (DBP) and mean arterial pressure (MAP) displayed an overall stable pattern with no systematic differences between the groups. Pulse pressure (PP) declined in both groups ($P < 0.05$), with VVS presenting with significant lower PP ($P < 0.05$). Heart rate (HR) showed a similar initial increase in both controls and VVS ($P < 0.05$). Stroke volume (SV) as well as cardiac output (CO) decreased rapidly in both groups ($P < 0.05$), with VVS displaying more pronounced decreases than controls (both $P < 0.05$). Although total peripheral resistance (TPR)

increased in both groups only controls displayed a significant increase in TPR ($P < 0.05$).

Figure 10. Cardiovascular responses to LBNP_{stress} in controls (black square) and VVS (white circle). **A:** Systolic blood pressure (SBP) and diastolic blood pressure (DBP). **B:** mean arterial pressure (MAP) and pulse pressure (PP). **C:** heart rate (HR). **D:** stroke volume (SV). **E:** cardiac output (CO). **F:** total peripheral resistance (TPR). In controls, the number of subjects is 15 for each data point at LBNP 0, 20, 30, and 40 mmHg. Due to presyncope the number decreased to 11 at 50 mmHg, 9 at 60 mmHg and 2 at 70 mmHg. In VVS, the number of subjects is 13 for each data point at LBNP 0, 20 and 30 mmHg. Due to presyncope the number decreased to 8 at 40 mmHg and 3 at 50 mmHg. Analyses were performed between LBNP interval 0-40 mmHg. # $P < 0.05$ compared with baseline within the group; * $P < 0.05$ control vs. VVS.

The maximal cardiovascular responses (%) at approximately 2 min before presyncope or completion of LBNP_{stress} is displayed in table 2. VVS demonstrated a lower increase in HR ($P < 0.001$), TPR ($P < 0.05$) and P-NE ($P < 0.01$).

Table 2. Maximal cardiovascular responses evoked by LBNP_{stress}.

Group	MAP (%)	HR (%)	TPR (%)	P-NE (%)
Controls	97±2	160±5***	150±10*	306±31**
VVS	97±1	130±6	126±7	174±19

Percentage of resting values. Mean ± SE. Controls vs. VVS * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Figure 11 shows changes (%) in P-NE during LBNP_{stress}. P-NE increased as the hypovolemic stimuli increased ($P < 0.001$). However, this was less pronounced in VVS compared to controls ($P < 0.01$).

Figure 11. P-NE responses during LBNP_{stress} in controls (black square) and VVS (white circle). P-NE increased (%) with increasing hypovolemia ($P < 0.001$) but VVS women displayed an attenuated P-NE response compared to controls. ** $P < 0.01$ controls vs. VVS.

Table 3 displays HRV data during rest and LBNP₃₀. LF and LF/HF ratio increased (both $P < 0.05$), while HF ($P < 0.05$) and total power decreased ($P < 0.01$) in both groups in response to LBNP. There were no differences in HRV parameters between the groups.

Mobilization of venous blood and fluid absorption

Mobilization of arm capacitance blood was similar between controls and VVS at lower levels of LBNP_{stress}. However, during higher levels VVS presented with reduced mobilization of arm venous capacitance blood (40 mmHg, $P < 0.05$; 50 mmHg, $P < 0.05$, figure 12A).

Table 3. Heart rate variability before LBNP onset and during LBNP of 30 mmHg.

		LF, nu	HF, nu	LF/HF ratio	TOT ms ²
Controls	LBNP 0	54	46	1.16	5390
		(39-65)	(35-61)	(0.69-1.84)	(1920-7003)
	LBNP 30	71 [#]	29 [#]	1.43 [#]	1716 ^{##}
		(51-78)	(21-49)	(1.04-4.00)	(851-2552)
VVS	LBNP 0	57	43	1.33	3471
		(39-76)	(24-61)	(0.65-3.25)	(1537-6020)
	LBNP 30	70 [#]	30 [#]	2.43 [#]	1253 ^{##}
		(61-85)	(15-39)	(1.60-5.72)	(1101-1720)

Data are shown as median and 25th – 75th percentiles. LF and HF; low and high frequencies expressed in normalized units (nu). TOT, total power (ms²). [#] $P < 0.05$, ^{##} $P < 0.01$ compared with baseline within the group.

Further, the net capillary fluid absorption was reduced by almost 40 % in VVS ($P < 0.05$, figure 12B).

Figure 12. Arm capacitance responses during LBNP_{stress} and net capillary fluid absorption in response to LBNP₃₀ in controls (black) and VVS (white). **A:** VVS displayed a reduced mobilization of capacitance blood during LBNP_{stress} of 40 mmHg and 50 mmHg. **B:** Net capillary fluid absorption was reduced in VVS. * $P < 0.05$ controls vs. VVS.

Calf volume responses (Paper II-IV)

Venous capacitance response, net fluid filtration and LBNP tolerance

Calf venous capacitance response was comparable between VVS and controls during both VOP and single step LBNP of 30 mmHg. Further, no group differences in capacitance response were found at any LBNP level during LBNP_{stress} (figure 13).

Figure 13. Calf capacitance response during LBNP_{stress} in controls (black bars) and VVS (white bars). No differences were found between the groups.

Similarly, net capillary fluid filtration and CFC showed no significant differences. No correlation was found between the VOP induced calf capacitance response and LTI ($r = 0.204$, $P = 0.30$).

Venous compliance and LBNP tolerance

VWM was used in the following comparison of the pressure-volume and pressure-compliance curves between VVS and controls. Figure 14A shows the pressure-volume relationship in VVS and controls during VOP, with VVS presenting a significantly less steep curve (Interaction, $P < 0.05$). Figure 14B shows the corresponding C_{calf} curves, with reduced C_{calf} in VVS compared to controls (Interaction, $P < 0.05$; VVS vs. control, $P < 0.05$). The differences between the groups were most pronounced in the low pressure range up to 20 mmHg ($P < 0.05$).

Figure 14. Pressure-volume and pressure-compliance relation in VVS and controls. **A:** The volume curve was less steep in VVS (white circle) compared to controls (black square) (Interaction, $P < 0.05$). **B:** Venous compliance was significantly lower in VVS (Interaction, $P < 0.05$; VVS vs. control, $P < 0.05$). VVS vs. control, * $P < 0.05$, ** $P < 0.01$.

Figure 15 shows the relationship between LTI and C_{calf} (at 20 mmHg) in all subjects pooled. C_{calf} at 20 mmHg was positively correlated to LTI ($r = 0.459$, $P < 0.05$).

Figure 15. Correlations between LTI and calf venous compliance. Calf venous compliance at 20 mmHg (VVS, white circle; controls, black square) correlated positively to LTI ($r = 0.459$, $P < 0.05$).

Blood pooling time and LBNP tolerance

Figure 16A-B shows the correlation between pooling_{time} and LTI.

Figure 16. Correlation between LBNP tolerance (LTI) and pooling_{time} in controls (black square) and VVS (white circle). A: shorter pooling_{time} correlated to a higher LBNP tolerance in controls ($r = -0.550$, $P < 0.05$). **B:** shorter pooling_{time} correlated to a lower LBNP tolerance in VVS ($r = 0.821$, $P < 0.001$, control vs. VVS, $P < 0.001$).

In controls, shorter pooling_{time} correlated with higher LBNP tolerance ($r = -0.550$, $P < 0.05$, figure 16A). Conversely, in VVS, shorter pooling_{time} correlated with lower LBNP tolerance ($r = 0.821$, $P < 0.001$, figure 16B), revealing a reversed relationship between pooling_{time} and maximal LBNP tolerance ($P < 0.001$). Despite this, no overall group differences in pooling_{time} were seen during LBNP, being 27 ± 3 sec in controls and 27 ± 2 sec in VVS ($P = 0.91$). Figure 17A-F depicts the correlation between pooling_{time} and changes (%) in SV, CO and PP during LBNP_{stress} at 30 mmHg, further examining the impact of pooling_{time} on hemodynamic stability. In controls, shorter pooling_{time} correlated to a better maintained SV ($r = -0.698$, $P < 0.01$, figure 17A) and CO ($r = -0.563$, $P < 0.05$, figure 17B), with similar trend with PP ($r = -0.468$, $P = 0.078$, figure 17C). VVS showed the reversed pattern compared to the control group; shorter pooling_{time} correlated with a greater decline in SV ($r = 0.611$, $P < 0.05$, figure 17D), and a tendency towards a greater decline was found in both CO ($r = 0.511$, $P = 0.109$, figure 17E), and PP ($r = 0.457$, $P = 0.117$, figure 17F). Accordingly, the slopes of the regression lines were significantly different between controls and VVS (SV, $P < 0.001$; CO, $P < 0.05$; PP, $P < 0.05$, figure 17A-F).

Figure 17. Correlations between hemodynamic responses during LBNP_{stress} and pooling_{time} in controls (black square) and VVS (white circle). **A:** In controls, shorter pooling_{time} correlated to a better preservation of stroke volume (SV) ($r = -0.698$, $P < 0.01$), **B:** cardiac output (CO) ($r = -0.563$, $P < 0.05$) and **C:** there was a tendency to a better preservation of pulse pressure (PP) ($r = -0.468$, $P = 0.078$). **D:** In VVS, shorter pooling_{time} correlated to a greater decline in stroke volume (SV) ($r = 0.611$, $P < 0.05$, control vs. VVS, $P < 0.001$) and **E:** there was a tendency to a greater decline in both cardiac output (CO) ($r = 0.511$, $P = 0.11$, control vs. VVS, $P < 0.01$) and **F:** pulse pressure (PP) ($r = 0.457$, $P = 0.12$, control vs. VVS, $P < 0.05$).

Multiple linear regression was performed to identify the determinants of pooling_{time} in controls and VVS, see Methods section (Table 4). The models explained 81% of the variance regarding pooling_{time} in controls ($P < 0.001$) and 86 % in VVS ($P < 0.001$). In controls, only dynamic factors, i.e., CVR_{LBNP} ($P < 0.001$), CBF_{LBNP} ($P < 0.001$) and CBP_{LBNP} ($P < 0.01$) contributed significantly to the model. In VVS, only static factors, i.e., CBF_{rest} ($P < 0.001$) and C_{rest} ($P < 0.05$) contributed significantly to the model.

Table 4. Multiple linear regression for prediction of pooling_{time} during LBNP of 30 mmHg.

Variables	Control			VVS		
	Adj. R^2	B	$SE\ B$	Adj. R^2	B	$SE\ B$
CBF_{rest}					-7.53***	0.92
$Ccalf_{rest}$					1.70*	0.64
CBP_{LBNP}		7.68**	1.95			
CBF_{LBNP}		-13.4***	2.20			
CVR_{LBNP}		-0.15***	0.03			
Model	0.809***			0.859***		

Adj. R^2 denotes the adjusted proportion of the variance explained by the model. B denotes the unstandardized coefficient, $SE\ B$ the standard error of the unstandardized coefficient. CBF_{rest} , calf blood flow at rest; $Ccalf_{rest}$, calf venous compliance at rest (30 mmHg); CBF_{LBNP} , calf blood flow after 30 sec of LBNP; CVR_{LBNP} , maximal increase (%) in calf vascular resistance during the initial 30 sec of LBNP.

Cold pressor test (Paper IV)

Figure 18A-C shows hemodynamic responses (%) during cold pressor test (CPT). CPT induced similar increases in MAP ($P < 0.001$, control vs. VVS, $P = 0.85$, figure 18A) and HR ($P < 0.001$, control vs. VVS, $P = 0.76$, figure 18B). FVR increased in controls while VVS displayed a successive decrease during CPT (Interaction, $P < 0.05$, control vs. VVS, $P < 0.05$, figure 18C).

Figure 18. Hemodynamic responses to cold pressor test in controls (black square) and VVS (white circle). **A:** mean arterial pressure (MAP) and **B:** heart rate (HR) increased in both groups (both $P < 0.001$). **C:** forearm vascular resistance (FVR) was significantly attenuated in VVS (Interaction, $P < 0.05$, control vs. VVS, $P < 0.05$).

Discussion

Methodological aspects of VOP

Due to its great compliance, the venous system harbors roughly 70% of the systemic blood volume and is designed to maintain a steady venous return to the heart during various conditions (12). At low transmural pressures the pressure-volume curve is steep, indicating a high compliance which means that already a small increase in transmural pressure is followed by a relatively large change in volume. At higher pressures the pressure-volume curve is flatter and thus compliance is lower (24). The expansion of the veins at low transmural pressures (< 10 mmHg) is also related to changes in geometry, i.e., a transformation from collapsed to elliptical to a circular cross section (105). At higher pressures, the volume changes are dependent of the elastic properties of the venous wall. The venous wall consists of elastin, collagen and smooth muscle in which the highly elastic elastin fibers are the first to be engaged during tension of the vessel, followed by a gradual recruitment of the collagen fibers which act to stiffen the venous wall and restrict further increase of the circumference (12,24). As such, the venous pressure-volume curve is characterized by two functional features, i.e., to enable large translocations of blood from peripheral capacitance veins at low venous pressures in response to small pressure changes and thereby increase cardiac filling, e.g., during acute hypovolemia, as well as to minimize venous pooling and the concomitant central hypovolemia at high venous pressures during upright posture (12,24).

VOP is the method of choice for evaluating whole limb venous compliance and thus an important tool within physiological research. However, there are several techniques in use. Earlier approaches where cuff pressure is raised in a stepwise manner (106) or as a single step (107) all share some inherent problems thoroughly discussed by Halliwill et al. (90) and Monahan et al. (87). In order to overcome these methodological limitations Halliwill et al. (90) developed a technique in which venous compliance is calculated from the pressure-volume relationship during 1 min of linear cuff deflation (60 to 10 mmHg) after venous stasis of 4 to 8 min. However, the application of

this technique in the lower limb relies on two not previously validated assumptions: first, that applied thigh cuff pressure is equal to venous pressure; second, that venous compliance is not affected by net fluid filtration. Further, a quadratic regression equation is used to model the pressure-volume relation (90). The major limitation is that the curvilinear pressure-volume relation is fitted to a strict mathematical parabolic function, which in the majority of cases reaches its vertex within the investigated high pressure range and thereby precluding a valid interpretation. In addition to orthostatic intolerance, several conditions in humans, e.g., diabetes mellitus, hypertension, chronic venous insufficiency as well as aging and gender affect limb venous compliance (91,100,108,109). Thus, a valid characterization of venous compliance throughout the entire pressure range may be of great importance for further understanding of both physiological and pathophysiological aspects of venous function.

Intravenous pressure and correction of net fluid filtration

The increase in venous pressure showed 100% transmission and invasively measured venous pressure correlated well with thigh cuff pressure during the rapid decrease of 1 mmHg/s in cuff pressure (figure 5). However, the lowest cuff pressure, i.e., 10 mmHg, needs to be interpreted with caution since the variance of the intravenous pressure is greatest in this pressure region.

Previous studies have shown that net fluid filtration into extravascular tissue increases the measured calf volume substantially during VOP, lower body negative pressure (LBNP) and quiet standing (10,30,91,96,110). The effect of net fluid filtration may be especially important to consider because there is compelling evidence that filtration and/or CFC differs among different population groups, e.g., increased net fluid filtration has been shown in patients with postural orthostatic tachycardia syndrome (74) as well as in young women compared to age-matched men (91), whereas reduced net fluid filtration has been seen in aging women (111) and patients with diabetes (76,112). In accordance we found net fluid filtration to be higher in women compared to men during VOP and the contribution of fluid filtration to the total calf volume increase was greater in women.

To examine the effect of net fluid filtration on the pressure-volume relation during VOP, Halliwill et al. (90) calculated C_{calf} from a shorter period (4 min) and a longer period (8 min) of venous stasis. Since no differences in C_{calf} were found they argued that the rapid decrease of 1 mmHg/s in cuff pressure minimizes the possibility for the interstitial fluid to re-enter the circulation (87,90). However, this reasoning ignores the fact that a more prominent filtration ought to occur in the high pressure zone during the deflation phase, leading to an upward shift of the volume curve and thus a change in the volume slope from which C_{calf} is calculated. Accordingly, we showed that C_{calf} was underestimated by more than 20% at higher transmural pressures if the effect of fluid filtration was not accounted for (figure 7). Since the effect of net fluid filtration on C_{calf} was unchanged between 4 and 8 min VOP we conclude that the main impact of fluid filtration could be attributed to the deflation phase. This seems reasonable because a complete pressure transmission is generally reached within 3-4 min, which implies that net fluid filtration is fully developed at the beginning of the deflation phase during both 4 and 8 min VOP. Since fluid filtration is pressure dependent, the effect of net fluid filtration on the volume curve will decrease as the cuff pressure decrease in a similar manner for both protocols, i.e., net fluid filtration will have the same effect on the slope of volume curve during the deflation phase for both 4 and 8 min VOP. Moreover, the significantly increased net fluid filtration in women led to a more pronounced underestimation of C_{calf} in women compared to men (figure 7). It therefore seems that the correction model could be a useful tool when C_{calf} is evaluated in groups with possible differences in net fluid filtration.

Venous wall model

The venous pressure-volume curve is curvilinear and characterized by a compliant part at low venous pressures and a stiffer part at high venous pressures (12,24). Present assessments commonly use a mathematically derived quadratic regression equation (QRE) to model the venous pressure-volume curve and the first derivative of this equation to characterize venous compliance. With this approach, venous compliance is bound to become negative at a pressure within or very close to the applied physiological pressure range, precluding a valid interpretation (86-88,90,92,93). In accordance, we found that 10% of the subjects at 55 mmHg and almost 75% of the subjects at 60

mmHg displayed a negative C_{calf} with the QRE. Thus, the major limitation with the QRE is located at the high pressure zone and could likely be explained by the mathematically applied quadratic function which in the majority of cases reaches its vertex within the investigated pressure range (Figure 9A). Due to the form of the venous pressure-volume curve it appears to be of great importance to model the whole curve accurately, especially since gravitational forces could increase venous pressure well above 60 mmHg during prolonged standing.

The venous wall model (VWM), which is based on physiological features of the venous vessel wall, was able to adopt the curvilinear form of the venous pressure-volume curve and the overall adjustment to the experimentally induced pressure-volume curve was significantly better compared to the QRE (figure 9A-C). As expected, the major differences were found at the high pressure range where the QRE was unable to model the curvilinear form of the pressure-volume curve. However, also at the low and mid-section of the pressure-volume curve (15 to 40 mmHg) VWM provided a lower mean deviance from the experimentally induced pressure-volume curve (figure 9C). Compared to the VWM, C_{calf} was significantly underestimated at the high pressure zone when calculated by the QRE (figure 9D). Thus, the ability of the VWM to characterize venous compliance throughout the entire pressure range may be of great importance for further understanding of venous function.

LBNP tolerance and cardiovascular responses

In this thesis LBNP was used to create experimental orthostatic stress in healthy women and women with VVS. LBNP induce central hypovolemia with concomitant unloading of baroreceptor and is a widely used technique to simulate and quantify orthostatic stress and tolerance (46,77,101). There are nonetheless some differences between LBNP and standing (or HUT which is used in clinical settings). Quiet standing or HUT, but not LBNP, is associated with increased pooling in the splanchnic area (102) as well as gravity induced changes in transmural carotid pressure which may be of importance for unloading of the carotid baroreflexes (17). However, LBNP of 40 to 50 mmHg results in a comparable shift in central blood

volume as HUT to 70° (102), and cardiovascular responses to LBNP, standing, and HUT seems to be both qualitatively and quantitatively similar (113,114).

Hemodynamic responses

Consistent with the history of frequent episodes of syncope in VVS women they displayed a markedly reduced LBNP tolerance index (LTI) compared to controls, i.e., women with VVS had lower orthostatic tolerance. VVS is frequently triggered by orthostatic stress which cause a gravitational displacement of blood from the upper to the lower body (115). As a result, stroke volume (SV) is reduced and this affects both cardiac output (CO) and mean arterial pressure (MAP). In order to preserve a sufficient CO, peripheral vasoconstriction and an increase in heart rate (HR) are induced by sympathetic activation (12,116). We found that graded LBNP was associated with a more rapid and prominent decrease in CO in VVS (figure 10), supporting the notion of CO as an important factor in the vasovagal reaction (62,66). Earlier studies have indicated that VVS is likely to occur when CO is about half of its baseline value. Present findings of an approximate reduction of 50% in CO at presyncope in both VVS and controls (figure 10) are in agreement with these data (66). The reasons for the more pronounced decrease in CO in VVS are uncertain. However, our findings of similar initial increases in HR but earlier and greater reductions in SV indicates changes in venous return as a key determinant. Lower or earlier decreases in SV during LBNP have previously been linked to orthostatic intolerance in athletes, healthy young men and women and syncope patients (66,68-70). Changes in SV seem also to affect pulse pressure (PP) (117). In accordance, the decrease in PP was more pronounced in VVS. From a mechanistic perspective this may be disadvantageous since PP is an important determinant of cerebral blood flow pulsatility, which is thought to maintain cerebral perfusion in times of reduced mean cerebral blood flow (118).

LBNP induced similar changes in HRV parameters in both groups indicating normal cardiac autonomic modulation in VVS, at least during low to moderate hypovolemic stress (table 3) (119,120). However, during extended durations of orthostatic stress adequate sympathetic vasoconstriction, rather than reflex tachycardia, has been

suggested as the main determinant for maintaining BP, and higher LBNP tolerance has previously been associated to an enhanced sympathetic reserve (12,121). Further, Fu et al. (101) demonstrated that the reserve capacity for sympathetic mediated vasoconstriction seems to have an individual maximal range. The present results of an attenuated P-NE development and decreased TPR reinforce the image of a dysfunctional sympathetic regulation and/or activation in VVS with a lesser reserve during orthostatic stress (44,46,122-124) (figure 11). The origin of the diminished vasoconstrictor reserve remains unclear, but our findings of an increased resting P-NE in VVS provide indirect support for the view that VVS use a larger fraction of their reserve during normal conditions (101,125). However, another possibility may be a reduced adrenergic vasoconstrictor range in VVS (44,126). The reason for the discrepancy is not clear but one explanation could be an earlier saturation of postsynaptic adrenergic receptors as a consequence of the elevated resting state (101). Abnormalities in sympathetic nerve proteins that affects NE synthesis and reuptake might also be of importance (126). The results of P-NE should, however, be interpreted with some caution. Although P-NE is a useful marker of average sympathetic response (127), it only provides an indirect estimate of sympathetic nerve activity (128) and the levels of P-NE during orthostatic stress is mediated of both reductions of NE clearance and increases of NE spillover into plasma (32,129).

Mobilization of venous blood and fluid absorption

During graded hypovolemic stress VVS presented with reduced mobilization of peripheral venous capacitance blood as the hypovolemic stimulus increased (figure 12A). LBNP induces unloading of baroreceptors with a concomitant sympathetic activation (99) and since active venous constriction in the musculocutaneous circulations of the limbs appears to have a minor impact on venous capacitance control during baroreflex activation it seems that passive mechanisms are of greater importance (12,130). Passive translocation of capacitance blood is to a large extent determined by two factors. First, a decrease in transmural pressure over the venous wall as a result of resistance vessel constriction. Second, compliant venous walls (high venous compliance) facilitating passive recoil of blood to the heart. A possible explanation for the reduced capacitance response at

increasing LBNP levels may be found in the attenuated P-NE increase in VVS (figure 11). Activation of α -receptors by NE in resistance vessels is crucial for proper vasoconstriction during orthostatic stress and as such a major determinant for passive translocation of venous capacitance blood (12). The present findings supports the hypothesis of a reduced vasoconstrictor reserve (125) in VVS and extends the implications of these findings by demonstrating diminished mobilization of venous capacitance blood towards the central circulation with more marked reductions in CO over time. Differences in venous compliance could also be a contributing factor. Reduced venous compliance in the low pressure range could affect the mobilization of peripheral venous blood to the central circulation in VVS (see Effects of venous compliance on LBNP tolerance, figure 14-15).

Net capillary fluid absorption was reduced by almost 40% in VVS during LBNP₃₀ (figure 12B). Fluid absorption from tissue to blood serves as a powerful mechanism to increase plasma volume during hypovolemic stress (27-30) and an effective fluid absorption is dependent on both a high hydrodynamic conductivity (i.e., CFC), and a reflex decline in capillary pressure due to α - and β -adrenergic adjustment of pre- and postcapillary resistance (25,26). The present finding of a similar calf CFC between VVS and controls makes capillary fluid permeability characteristics unlikely as an explanation of the differences in net fluid absorption. The reduced absorption is more likely to represent a dysfunctional regulation of the resistance vessels. This is consistent with several other studies that have indicated an impaired sympathetic response to both baroreflex (44,46,122,123) and non-baroreflex (131) functional testing in VVS.

Calf volume responses

Effects of venous capacitance and fluid filtration on LBNP tolerance

Increased lower limb blood pooling has been suggested to contribute to the symptoms of VVS due to greater reductions in venous return (72,79,80). However, calf venous capacitance response was comparable between VVS and controls during both VOP and single

step LBNP of 30 mmHg. These findings were confirmed during graded LBNP which also revealed a similar capacitance response (figure 13). Further, no correlation between the capacitance response and LBNP tolerance was found. Differences in venous pooling in other vascular segments cannot be excluded, e.g., splanchnic or pelvic region (75). Based on this it appears unlikely that excessive lower limb blood pooling is a major determinant in the pathophysiology of VVS.

Independent from the capacitance response, transcapillary fluid filtration is a significant contributor to lower limb volume load and thus important in the orthostatic reaction (10). Accordingly, it has been speculated that an increased capillary filtration during upright posture could affect stroke volume and thus contribute to a progressive fall in cardiac output in orthostatic VVS (66). However, we observed no differences in either net fluid filtration or CFC between VVS and controls during VOP or LBNP and it thus seems unlikely that an increased fluid filtration represents a crucial aspect in the vasovagal reaction.

Effects of venous compliance on LBNP tolerance

C_{calf} at high venous pressures was comparable in controls and VVS. However, C_{calf} was significantly reduced in VVS at low transmural pressures (Figure 14A-B). This is consistent with earlier findings of Freeman et al. (83), who reported, contrary to their expectations, a lower C_{calf} in patient with idiopathic orthostatic intolerance. They speculated that fluid shift from the vascular compartment to the interstitium during VOP could have affected their results. In the present findings, the impact of net fluid filtration was corrected for and C_{calf} was evaluated with the new VWM which is able to produce a valid characterization of the whole pressure-volume and pressure-compliance relation. Other factors, such as physical activity and sympathetic tone, could influence the measurements of C_{calf} (87,132). However, there were no differences in physical activity between the groups and although activation of the sympathetic system has been shown to lower unstressed venous volume, increased sympathetic activation does not appear to directly affect venous compliance (90,93).

There was a positive correlation between C_{calf} measured at the low pressure range (20 mmHg) and LBNP tolerance (figure 15), indicating that the functional elasticity of the veins at low transmural pressures rather than the amount of pooled blood are of importance regarding orthostatic stress. In contrast to the conventional view which states that a reduction in C_{calf} may be beneficial when it comes to orthostatic tolerance, (79-81) we suggest that a high compliance at low venous pressures may be advantageous due to the inherent possibility to mobilize substantial volumes of peripheral blood and concomitantly increase cardiac filling (12). In fact, this important first line of defense is initiated within seconds during acute hypovolemic stress (24,27,112,133). Considering this, we reason that the reduced C_{calf} at low venous pressures observed in VVS may adversely affect the compensatory mobilization of peripheral venous blood to the central circulation during hypovolemic stress and contribute to their hemodynamic instability. However, the extent and impact of mobilization of capacitance blood on cardiovascular control in VVS need to be further clarified.

An alternative explanation would be that the reduced C_{calf} seen in VVS women is an expression for adaptive changes in the dependent veins to counteract venous pooling. The present study was not designed to examine possible mechanisms of changes in C_{calf} but such adaptations require an effector stimulus. It is known that a chronic increase in venous pressure and volume can lead to structural remodeling of the venous vessel wall which, in turn, will alter the mechanical properties (134-136). Nevertheless, VVS and controls showed a similar lower limb blood pooling and we have no reason to believe that the otherwise healthy cohort of VVS women should suffer from a persistent increased venous pressure that predisposes for remodeling of the venous vessel wall (75). Thus, this explanation seems unlikely.

Effects of blood pooling time on LBNP tolerance

Conventionally, the main focus has been directed *on the amount* of the gravity-induced displacement of blood into the veins (5). Although blood pooling in the lower body is essential for the decrease in venous return to come about in the first place, our data, and others (75), indicate that increased blood pooling in lower limbs cannot explain the increased susceptibility to orthostatic intolerance in VVS.

Cardiovascular response to orthostatic stress has also been shown to be affected by the speed of the induced stimulus (27,77,78). Although the time to develop 50% of the capacitance response ($\text{pooling}_{\text{time}}$) was similar in controls and VVS, the groups presented with dichotomous correlation between $\text{pooling}_{\text{time}}$ and LBNP-induced cardiovascular responses as well as orthostatic tolerance. A recent study found that the time for lower limb blood pooling was much shorter in hemodynamic stable healthy women compared to otherwise healthy women experiencing vasovagal reactions during moderate levels of LBNP (77). In corroboration, we found that shorter $\text{pooling}_{\text{time}}$ correlated with higher LBNP-tolerance in healthy controls (figure 16A). In contrast, shorter $\text{pooling}_{\text{time}}$ correlated with lower LBNP-tolerance in VVS (figure 16B). The reliability of this unexpected finding was supported by the associations between $\text{pooling}_{\text{time}}$ and hemodynamic responses during $\text{LBNP}_{\text{stress}}$. In controls, shorter $\text{pooling}_{\text{time}}$ was associated with enhanced preservation of both SV and CO (figure 17A-C). In VVS, shorter $\text{pooling}_{\text{time}}$ was associated with a faster decline in SV (figure 17D) and similar trends were found for both CO and PP (figure 17E-F). Preservation of SV and CO are important factors for orthostatic tolerance and a steady decrease in CO, ultimately reaching a critical low limit has recently been suggested as a key factor for VVS (62,66).

The components associated with $\text{pooling}_{\text{time}}$ were different in controls compared to VVS (table 4). In controls, $\text{pooling}_{\text{time}}$ was associated with dynamic factors, i.e., baroreceptor induced changes in blood flow, vascular resistance and blood pooling during LBNP. Shorter $\text{pooling}_{\text{time}}$ correlated with increased vasoconstriction, indicating speed-dependent sympathetic activation (table 4). This is in agreement with recent findings from Kamiya et al. (78) who found greater MSNA responses during HUT with a rapid tilt compared to a slower. The mechanism for the speed-dependent MSNA activation is not clear but animal studies have suggested that the neural arc of the baroreflex has properties of a high-pass filter, i.e., faster changes in blood pressure are transmitted with higher gain (78,137). Further, Cui et al. (138) identified increased MSNA during rapid distension of the occluded venous circulation, and suggested a possible involvement of type III and IV afferent nerves in the vicinity of the veins. Thus, the

sympathetic response in healthy women seems to be dependent on the time by which the hypovolemic stimulus is instituted, where a faster initiation is related to a more marked sympathetic activation. Speed-dependent baroreflex-mediated sympathetic activation with greater compensatory responses in controls with rapid pooling_{time} may explain why these individuals showed a more efficient cardiovascular regulation during hypovolemia and higher LBNP-tolerance (figure 16A and 17A-C) (27,77).

In VVS on the other hand, factors that correlated with pooling_{time} were unrelated to baroreceptor activity and instead determined by static factors such as resting blood flow and venous compliance (table 4). Thus, it seems that sympathetic responses to LBNP-induced baroreceptor unloading are attenuated or at least divergent in women with VVS. Attenuated baroreflex function has previously been proposed to explain the incapacity of VVS patients to adequately increase sympathetic vasoconstriction in response to orthostatic stress (44,46), although the results are not conclusive (139). To further evaluate the sympathetic reflex arch and the role of the baroreceptor, sympathetic responses to cold pressor test (CPT) were measured. The hemodynamic responses to CPT are mediated through efferent sympathetic pathways of the baroreflex loop, while the afferent components are mediated via afferent pain and temperature fibres in the skin connecting to the central nervous system (140-142). Even though VVS responded with similar increases in MAP and HR during CPT, forearm vasoconstriction was attenuated (figure 18). This is in accordance with recent data from Jardine et al. (131), suggesting blunted efferent sympathetic control of the blood vessels. Thus, the present findings indicate an attenuation in the efferent part of the sympathetic baroreflex arc in VVS, further elucidating why pooling_{time} was determined by resting blood flow and not by hemodynamic changes induced by LBNP baroreceptor unloading.

Decreased baroreflex-mediated sympathetic control compared to healthy women may explain the overall lower LBNP-tolerance in VVS, but it cannot explain the lower LBNP-tolerance in women with shorter pooling_{time} within the VVS group (figure 16B and 17D-F). Interestingly, lower resting blood flow and thus higher vascular resistance were closely associated with longer pooling_{time} and as such

greater orthostatic tolerance in VVS. Although higher vascular resistance at rest (table 1) may reduce the vasoconstrictor reserve and orthostatic tolerance compared to healthy individuals (101) our finding suggest that high vascular resistance improves orthostatic tolerance *within* the VVS group. The mechanisms behind this novel findings are uncertain, but increased sympathetic tone at rest accompanied with higher arterial vasoconstriction may protect central blood volume during orthostatic stress in VVS by minimizing translocation of blood to peripheral parts of the body, e.g., the lower body and/or the splanchnic region. This may be particularly important when considering attenuated vasoconstriction during sympathetic stimulus and reduced ability to mobilize peripheral blood in VVS (table 2 and 4, figure 11-12). It is reasonable to suggest that a further understanding of the physiological mechanisms linking VVS women with longer pooling_{time} to increased LBNP tolerance will provide new insights in the pathophysiology of VVS.

Pathophysiological implications

The present data of an impaired increase in P-NE during graded LBNP in VVS as well as a reduced increase in TPR are in accordance with the concept of a decreased vasoconstrictor reserve (101,124,125). In addition, we suggest that the attenuated vasoconstrictor reserve is associated with a reduced capacity to mobilize an effective circulatory blood volume during orthostatic stress. Reduced venous compliance at the low pressure range may also contribute to the decreased mobilization of peripheral venous blood in VVS. Compensatory redistribution of blood and fluid towards the central circulation during hypovolemic stress is a crucial defense mechanism to preserve homeostasis (24,28,30,31). Due to its large tissue mass, skeletal muscle and skin represent an important reservoir for mobilization of venous capacitance blood and net fluid absorption. These mechanisms combined can increase the effective circulating blood volume well over 1 litre within the clinically relevant time frame for orthostatic VVS (28). Although it is currently debated whether decreased CO or vasodilation is the dominant hypotensive mechanism preceding VVS recent studies have suggested reduction in CO as the primary cause, rather than vasodilation (67). In agreement, the importance of increasing cardiac filling and CO have been stressed by several authors

investigating the clinical use of physical contra-maneuvers (PCM). Clinical trials have shown that PCM (leg crossing or hand grip and arm tensing) can, in many patients, induce significant increases in BP during impending vasovagal faint and avoid or delay loss of consciousness (1). The mechanisms underlying the effects of PCM have been attributed to an increase in CO (143,144). We propose that the present findings of progressively reduced mobilization of peripheral capacitance blood in VVS after several minutes of hypovolemia at LBNP levels equivalent to passive standing reduce venous return and contribute to earlier and more marked reductions in SV and CO (figure 10, figure 12A). In addition, net capillary fluid absorption is a slower but ongoing process to increase plasma volume during hypovolemic stress (30) and the almost halved fluid absorption in VVS may further seriously impede the ability to maintain CO (figure 12B). Collectively, these findings indicates that reductions in CO acts as a central hypotensive mechanism preceding VVS.

Interestingly, contrary to healthy women we found that VVS women with longer pooling_{time} demonstrated a higher LBNP tolerance and more effective cardiovascular responses during LBNP. These VVS women were characterized by a higher resting peripheral resistance and concomitantly lower peripheral blood flow (table 1). Our finding suggest that high vascular resistance improves orthostatic tolerance *within* the VVS group, although higher vascular resistance at rest may reduce the vasoconstrictor reserve compared to healthy individuals (101). These findings challenge the common view that individuals with VVS are a normal physiologic variant in one end of the Gauss curve, i.e., that healthy individuals and frequent fainters respond to orthostatic stimulus in the same way and only differ in the amount of hypovolemic stress required to provoke syncope (145,146). A further delineation of the physiology behind the well-defined differences could provide new insights in the pathophysiology and treatment of orthostatic VVS.

Methodological considerations and limitations

LBNP was used to create experimental orthostatic stress. There are some differences between LBNP and standing (or HUT which is used in clinical settings). Quiet standing or HUT, but not LBNP, is

associated with increased blood pooling in the splanchnic area (102) as well as gravity induced changes in transmural carotid pressure which may be of importance for unloading of the carotid baroreflexes (17). However, LBNP of 40 to 50 mmHg results in a comparable shift in central blood volume as HUT to 70° (102), and cardiovascular responses to LBNP, standing, and HUT seem to be both qualitatively and quantitatively similar (113,114). Further, calf volume increase during LBNP was measured as an indicator of orthostatic load, although it should be noted that blood pooling and fluid filtration occur in all of the body segments enclosed in the LBNP chamber. However, blood pooling in the lower limb, rather than the pelvic or abdominal region appears to be of greater importance in orthostatic stress during LBNP (79).

No women in the control group but 7 women in the VVS group used contraceptives. Still, all women were investigated during the first 10 days of the follicular phase, i.e., with low concentrations of estrogen. Modern oral contraceptives usually mimic the normal menstrual cycle and these women would also have low estrogen levels. Furthermore, no significant differences in cardiovascular responses during LBNP were found when normally menstruating women were compared to women using oral contraceptives (28), and calf venous compliance as well as capacitance seem to be unaffected by the use of contraceptives (28,86).

Previous studies point towards gender-specific differences in venous compliance and hemodynamic responses to LBNP, and women have lower tolerance of LBNP compared to men (28,70,91,147). Thus, the present findings cannot be directly transferred to men. However, young women have a higher prevalence of vasovagal syncope, emphasizing the importance of studying women (3).

Conclusions

- Net fluid filtration leads to underestimation of calf venous compliance evaluated with VOP. The effect of fluid filtration can be adjusted for by the developed correction model.
- The new venous wall model is able to adopt the curvilinear form of the venous pressure-volume curve and thus provide a valid characterization of venous compliance.
- Calf venous capacitance is similar in healthy women and women with VVS. Calf venous compliance is similar at high venous pressures, but reduced at low venous pressures in VVS and directly correlated to impaired LBNP-tolerance. Lower venous compliance could lead to reduced mobilization of capacitance blood during hypovolemic circulatory stress.
- Women with VVS displays attenuated vasoconstrictor response during circulatory stress caused by LBNP. Mobilization of peripheral capacitance blood to the central circulation is decreased and net capillary fluid absorption reduced compared to healthy women. This coincides with more marked reductions in CO. Reduced compensatory mechanisms to maintain CO could contribute to the pathogenesis of orthostatic VVS.
- In healthy women, rapid LBNP-induced lower limb blood pooling is associated with higher LBNP-tolerance and efficient cardiovascular responses, e.g., greater baroreflex-mediated vasoconstriction. This indicates a speed-dependent sympathetic activation. In women with VVS, rapid LBNP-induced lower limb blood pooling is associated with lower LBNP-tolerance as well as deficient cardiovascular responses. No speed-dependent sympathetic vasoconstriction is seen, indicating well-defined differences in cardiovascular regulation already in the initial responses to orthostatic stress.

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References

- (1) Task Force for the Diagnosis and Management of Syncope, European Society of Cardiology (ESC), European Heart Rhythm Association (EHRA), Heart Failure Association (HFA), Heart Rhythm Society (HRS), Moya A, et al. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J* 2009 Nov;30(21):2631-2671.
- (2) Colman N, Nahm K, Ganzeboom KS, Shen WK, Reitsma J, Linzer M, et al. Epidemiology of reflex syncope. *Clin Auton Res* 2004 Oct;14 Suppl 1:9-17.
- (3) Ganzeboom KS, Mairuhu G, Reitsma JB, Linzer M, Wieling W, van Dijk N. Lifetime cumulative incidence of syncope in the general population: a study of 549 Dutch subjects aged 35-60 years. *J Cardiovasc Electrophysiol* 2006 Nov;17(11):1172-1176.
- (4) Serletis A, Rose S, Sheldon AG, Sheldon RS. Vasovagal syncope in medical students and their first-degree relatives. *Eur Heart J* 2006 Aug;27(16):1965-1970.
- (5) Grubb BP. Neurocardiogenic syncope and related disorders of orthostatic intolerance. *Circulation* 2005 Jun 7;111(22):2997-3006.
- (6) Lewis T. A Lecture on vasovagal syncope and the carotid sinus mechanism. *Br Med J* 1932 May 14;1(3723):873-876.
- (7) van Dijk JG, Wieling W. Pathophysiological basis of syncope and neurological conditions that mimic syncope. *Prog Cardiovasc Dis* 2013 Jan-Feb;55(4):345-356.
- (8) Van Lieshout JJ, Wieling W, Karemaker JM, Secher NH. Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol* (1985) 2003 Mar;94(3):833-848.
- (9) Robertson D. The pathophysiology and diagnosis of orthostatic hypotension. *Clin Auton Res* 2008 Mar;18 Suppl 1:2-7.

- (10) Lundvall J, Bjerkhoel P, Quittenbaum S, Lindgren P. Rapid plasma volume decline upon quiet standing reflects large filtration capacity in dependent limbs. *Acta Physiol Scand* 1996 Oct;158(2):161-167.
- (11) Stewart JM. Mechanisms of sympathetic regulation in orthostatic intolerance. *J Appl Physiol* 2012 Nov;113(10):1659-1668.
- (12) Rowell L. *Human Cardiovascular Control*. New York: Oxford University Press; 1993.
- (13) Eckberg DL. High-Pressure and Low-Pressure Baroreflexes. In: Robertson DW, editor. *Primer on the Autonomic Nervous System* Burlington, MA, USA: Academic Press; 2004. p. 147-151.
- (14) Mifflin SW, Felder RB. Synaptic mechanisms regulating cardiovascular afferent inputs to solitary tract nucleus. *Am J Physiol* 1990 Sep;259(3 Pt 2):H653-61.
- (15) Mark AL, Mancia G. *Cardiopulmonary Baroreflexes in Humans*. Comprehensive Physiology: John Wiley & Sons, Inc.; 2011.
- (16) Victor RG, Mark AL. Interaction of cardiopulmonary and carotid baroreflex control of vascular resistance in humans. *J Clin Invest* 1985 Oct;76(4):1592-1598.
- (17) Smit AA, Halliwill JR, Low PA, Wieling W. Pathophysiological basis of orthostatic hypotension in autonomic failure. *J Physiol* 1999 Aug 15;519 Pt 1:1-10.
- (18) Cooke WH, Hoag JB, Crossman AA, Kuusela TA, Tahvanainen KUO, Eckberg DL. Human responses to upright tilt: a window on central autonomic integration. *J Physiol (Lond)* 1999;517(2):617-628.
- (19) Convertino VA. Neurohumoral mechanisms associated with orthostasis: reaffirmation of the significant contribution of the heart rate response. *Front Physiol* 2014 Jun 30;5:236.
- (20) Wieling W, de Lange FJ, Jardine DL. The heart cannot pump blood that it does not receive. *Front Physiol* 2014 Sep 18;5:360.

References

- (21) Weissler AM, Leonard JJ, Warren JV. Effects of posture and atropine on the cardiac output. *J Clin Invest* 1957 Dec;36(12):1656-1662.
- (22) Weissler AM, Warren JV, Estes EH,Jr, McIntosh HD, Leonard JJ. Vasodepressor syncope; factors influencing cardiac output. *Circulation* 1957 Jun;15(6):875-882.
- (23) van Lieshout JJ, Wieling W, Wesseling KH, Karemaker JM. Pitfalls in the assessment of cardiovascular reflexes in patients with sympathetic failure but intact vagal control. *Clin Sci (Lond)* 1989 May;76(5):523-528.
- (24) Rothe CF. Venous system: physiology of the capacitance vessels. In: Shepard J, editor. *Handbook of Physiology, section 2: The Cardiovascular System III, Peripheral Circulation and Organ Blood Flow* Bethesda, Maryland: American Physiological Society; 1984. p. 397-452.
- (25) Lundvall J, Hillman J. Fluid transfer from skeletal muscle to blood during hemorrhage. Importance of beta adrenergic vascular mechanisms. *Acta Physiol Scand* 1978 Apr;102(4):450-458.
- (26) Maspers M, Bjornberg J, Grande PO, Mellander S. Sympathetic alpha-adrenergic control of large-bore arterial vessels, arterioles and veins, and of capillary pressure and fluid exchange in whole-organ cat skeletal muscle. *Acta Physiol Scand* 1990 Apr;138(4):509-521.
- (27) Lanne T, Lundvall J. Mechanisms in man for rapid refill of the circulatory system in hypovolaemia. *Acta Physiol Scand* 1992 Nov;146(3):299-306.
- (28) Lindenberger M, Olsen H, Lanne T. Lower capacitance response and capillary fluid absorption in women to defend central blood volume in response to acute hypovolemic circulatory stress. *Am J Physiol Heart Circ Physiol* 2008 Aug;295(2):H867-73.
- (29) Lundvall J, Lanne T. Large capacity in man for effective plasma volume control in hypovolaemia via fluid transfer from tissue to blood. *Acta Physiol Scand* 1989 Dec;137(4):513-520.
- (30) Lundvall J, Bjerkhoe P, Edfeldt H, Ivarsson C, Lanne T. Dynamics of transcapillary fluid transfer and plasma volume during lower body negative pressure. *Acta Physiol Scand* 1993 Feb;147(2):163-172.

- (31) Lindenberger M, Lindstrom T, Lanne T. Decreased circulatory response to hypovolemic stress in young women with type 1 diabetes. *Diabetes Care* 2013 Dec;36(12):4076-4082.
- (32) Jacob G, Ertl AC, Shannon JR, Furlan R, Robertson RM, Robertson D. Effect of standing on neurohumoral responses and plasma volume in healthy subjects. *J Appl Physiol* (1985) 1998 Mar;84(3):914-921.
- (33) European Heart Rhythm Association (EHRA), Heart Failure Association (HFA), Heart Rhythm Society (HRS), European Society of Emergency Medicine (EuSEM), European Federation of Internal Medicine (EFIM), European Union Geriatric Medicine Society (EUGMS), et al. Guidelines for the diagnosis and management of syncope (version 2009): the Task Force for the Diagnosis and Management of Syncope of the European Society of Cardiology (ESC). *Eur Heart J* 2009 Nov;30(21):2631-2671.
- (34) Burke D, Sundlof G, Wallin G. Postural effects on muscle nerve sympathetic activity in man. *J Physiol* 1977 Nov;272(2):399-414.
- (35) Engel GL. Psychologic stress, vasodepressor (vasovagal) syncope, and sudden death. *Ann Intern Med* 1978 Sep;89(3):403-412.
- (36) Sharey-Schafer EP. Syncope. *Br Med J* 1956 Mar 3;1(4965):506-509.
- (37) Fenton AM, Hammill SC, Rea RF, Low PA, Shen WK. Vasovagal syncope. *Ann Intern Med* 2000 Nov 7;133(9):714-725.
- (38) Kosinski D, Grubb BP, Temesy-Armos P. Pathophysiological aspects of neurocardiogenic syncope: current concepts and new perspectives. *Pacing Clin Electrophysiol* 1995 Apr;18(4 Pt 1):716-724.
- (39) Mark AL. The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. *J Am Coll Cardiol* 1983 Jan;1(1):90-102.
- (40) Mosqueda-Garcia R. Role of the Autonomic Nervous System in Vasovagal Syncope. *Vasovagal Syncope* 2015 01:53.
- (41) Oberg B, Thoren P. Increased activity in left ventricular receptors during hemorrhage or occlusion of caval veins in the cat. A possible cause of the vaso-vagal reaction. *Acta Physiol Scand* 1972 Jun;85(2):164-173.

References

- (42) Goldstein DS, Spanarkel M, Pitterman A, Toltzis R, Gratz E, Epstein S, et al. Circulatory control mechanisms in vasodepressor syncope. *Am Heart J* 1982 Nov;104(5 Pt 1):1071-1075.
- (43) Kaufmann H. Neurally mediated syncope: pathogenesis, diagnosis, and treatment. *Neurology* 1995 Apr;45(4 Suppl 5):S12-8.
- (44) Mosqueda-Garcia R, Furlan R, Fernandez-Violante R, Desai T, Snell M, Jarai Z, et al. Sympathetic and baroreceptor reflex function in neurally mediated syncope evoked by tilt. *J Clin Invest* 1997 Jun 1;99(11):2736-2744.
- (45) Sra JS, Murthy V, Natale A, Jazayeri MR, Dhala A, Deshpande S, et al. Circulatory and catecholamine changes during head-up tilt testing in neurocardiogenic (vasovagal) syncope. *Am J Cardiol* 1994 Jan 1;73(1):33-37.
- (46) Bechir M, Binggeli C, Corti R, Chenevard R, Spieker L, Ruschitzka F, et al. Dysfunctional baroreflex regulation of sympathetic nerve activity in patients with vasovagal syncope. *Circulation* 2003 Apr 1;107(12):1620-1625.
- (47) Novak V, Honos G, Schondorf R. Is the heart 'empty' at syncope? *J Auton Nerv Syst* 1996 Aug 27;60(1-2):83-92.
- (48) Yamanouchi Y, Jaalouk S, Shehadeh AA, Jaeger F, Goren H, Fouad-Tarazi FM. Changes in left ventricular volume during head-up tilt in patients with vasovagal syncope: an echocardiographic study. *Am Heart J* 1996 Jan;131(1):73-80.
- (49) Scherrer U, Vissing S, Morgan BJ, Hanson P, Victor RG. Vasovagal syncope after infusion of a vasodilator in a heart-transplant recipient. *N Engl J Med* 1990 Mar 1;322(9):602-604.
- (50) Hainsworth R. Pathophysiology of syncope. *Clin Auton Res* 2004 Oct;14 Suppl 1:18-24.
- (51) Brignole M, Sutton R, Menozzi C, Garcia-Civera R, Moya A, Wieling W, et al. Early application of an implantable loop recorder allows effective specific therapy in patients with recurrent suspected neurally mediated syncope. *Eur Heart J* 2006 May;27(9):1085-1092.

- (52) Brignole M, Donato P, Tomaino M, Massa R, Iori M, Beiras X, et al. Benefit of pacemaker therapy in patients with presumed neurally mediated syncope and documented asystole is greater when tilt test is negative: an analysis from the third International Study on Syncope of Uncertain Etiology (ISSUE-3). *Circ Arrhythm Electrophysiol* 2014 Feb;7(1):10-16.
- (53) Raviele A, Giada F, Menozzi C, Speca G, Orazi S, Gasparini G, et al. A randomized, double-blind, placebo-controlled study of permanent cardiac pacing for the treatment of recurrent tilt-induced vasovagal syncope. The vasovagal syncope and pacing trial (SYNPACE). *Eur Heart J* 2004 Oct;25(19):1741-1748.
- (54) Barcroft H, Edholm OG. On the vasodilatation in human skeletal muscle during post-haemorrhagic fainting. *J Physiol* 1945 Oct 15;104(2):161-175.
- (55) Dietz NM, Joyner MJ, Shepherd JT. Vasovagal syncope and skeletal muscle vasodilatation: the continuing conundrum. *Pacing Clin Electrophysiol* 1997 Mar;20(3 Pt 2):775-780.
- (56) Epstein SE, Stampfer M, Beiser GD. Role of the capacitance and resistance vessels in vasovagal syncope. *Circulation* 1968 Apr;37(4):524-533.
- (57) Morillo CA, Eckberg DL, Ellenbogen KA, Beightol LA, Hoag JB, Tahvanainen KU, et al. Vagal and sympathetic mechanisms in patients with orthostatic vasovagal syncope. *Circulation* 1997 Oct 21;96(8):2509-2513.
- (58) Wallin BG, Sundlof G. Sympathetic outflow to muscles during vasovagal syncope. *J Auton Nerv Syst* 1982 Nov;6(3):287-291.
- (59) Jardine DL, Ikram H, Frampton CM, Frethey R, Bennett SI, Crozier IG. Autonomic control of vasovagal syncope. *Am J Physiol* 1998 Jun;274(6 Pt 2):H2110-5.
- (60) Vaddadi G, Esler MD, Dawood T, Lambert E. Persistence of muscle sympathetic nerve activity during vasovagal syncope. *Eur Heart J* 2010 Aug;31(16):2027-2033.
- (61) Cooke WH, Rickards CA, Ryan KL, Kuusela TA, Convertino VA. Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol* 2009 Oct 15;587(Pt 20):4987-4999.

References

- (62) Fu Q, Verheyden B, Wieling W, Levine BD. Cardiac output and sympathetic vasoconstrictor responses during upright tilt to presyncope in healthy humans. *J Physiol* 2012 Apr 15;590(Pt 8):1839-1848.
- (63) Fu Q, Levine BD. Pathophysiology of neurally mediated syncope: Role of cardiac output and total peripheral resistance. *Auton Neurosci* 2014 Sep;184:24-26.
- (64) Gisolf J, Westerhof BE, van Dijk N, Wesseling KH, Wieling W, Karemaker JM. Sublingual nitroglycerin used in routine tilt testing provokes a cardiac output-mediated vasovagal response. *J Am Coll Cardiol* 2004 Aug 4;44(3):588-593.
- (65) Murrell C, Cotter JD, George K, Shave R, Wilson L, Thomas K, et al. Influence of age on syncope following prolonged exercise: differential responses but similar orthostatic intolerance. *J Physiol* 2009 Dec 15;587(Pt 24):5959-5969.
- (66) Verheyden B, Liu J, van Dijk N, Westerhof BE, Reybrouck T, Aubert AE, et al. Steep fall in cardiac output is main determinant of hypotension during drug-free and nitroglycerine-induced orthostatic vasovagal syncope. *Heart Rhythm* 2008 Dec;5(12):1695-1701.
- (67) Wieling W, Jardine DL, de Lange FJ, Brignole M, Nielsen HB, Stewart J, et al. Cardiac output and vasodilation in the vasovagal response: An analysis of the classic papers. *Heart Rhythm* 2016 Mar;13(3):798-805.
- (68) Jardine DL, Melton IC, Crozier IG, English S, Bennett SI, Frampton CM, et al. Decrease in cardiac output and muscle sympathetic activity during vasovagal syncope. *Am J Physiol Heart Circ Physiol* 2002 May;282(5):H1804-9.
- (69) Levine BD, Lane LD, Buckey JC, Friedman DB, Blomqvist CG. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation* 1991 Sep;84(3):1016-1023.
- (70) Fu Q, Arbab-Zadeh A, Perhonen MA, Zhang R, Zuckerman JH, Levine BD. Hemodynamics of orthostatic intolerance: implications for gender differences. *Am J Physiol Heart Circ Physiol* 2004 Jan;286(1):H449-57.

- (71) Cote AT, Bredin SS, Phillips AA, Warburton DE. Predictors of orthostatic intolerance in healthy young women. *Clin Invest Med* 2012 Apr 1;35(2):E65-74.
- (72) Hargreaves AD, Muir AL. Lack of variation in venous tone potentiates vasovagal syncope. *Br Heart J* 1992 Jun;67(6):486-490.
- (73) Bellard E, Fortrat JO, Dupuis JM, Victor J, Leftheriotis G. Haemodynamic response to peripheral venous congestion in patients with unexplained recurrent syncope. *Clin Sci (Lond)* 2003 Sep;105(3):331-337.
- (74) Stewart JM, Weldon A. Contrasting neurovascular findings in chronic orthostatic intolerance and neurocardiogenic syncope. *Clin Sci (Lond)* 2003 Apr;104(4):329-340.
- (75) Stewart JM, McLeod KJ, Sanyal S, Herzberg G, Montgomery LD. Relation of postural vasovagal syncope to splanchnic hypervolemia in adolescents. *Circulation* 2004 Oct 26;110(17):2575-2581.
- (76) Olsen H, Verneris E, Lanne T. Cardiovascular response to acute hypovolemia in relation to age. Implications for orthostasis and hemorrhage. *Am J Physiol Heart Circ Physiol* 2000 Jan;278(1):H222-32.
- (77) Lindenberger M, Lanne T. Slower lower limb blood pooling in young women with orthostatic intolerance. *Exp Physiol* 2015 Jan;100(1):2-11.
- (78) Kamiya A, Kawada T, Shimizu S, Iwase S, Sugimachi M, Mano T. Slow head-up tilt causes lower activation of muscle sympathetic nerve activity: loading speed dependence of orthostatic sympathetic activation in humans. *Am J Physiol Heart Circ Physiol* 2009 Jul;297(1):H53-8.
- (79) Halliwill JR, Lawler LA, Eickhoff TJ, Joyner MJ, Mulvagh SL. Reflex responses to regional venous pooling during lower body negative pressure in humans. *J Appl Physiol* 1998 Feb;84(2):454-458.
- (80) Luft UC, Loeppky JA, Venters MD, Kobayashi Y. A Study of Factors Affecting Tolerance of Gravitational Stress Simulated by Lower Body Negative Pressure. Albuquerque, New Mexico: Lovelace Foundation; 1976. p. 2-60.

References

- (81) Tsutsui Y, Sagawa S, Yamauchi K, Endo Y, Yamazaki F, Shiraki K. Cardiovascular responses to lower body negative pressure in the elderly: role of reduced leg compliance. *Gerontology* 2002 May-Jun;48(3):133-139.
- (82) Hernandez JP, Franke WD. Age- and fitness-related differences in limb venous compliance do not affect tolerance to maximal lower body negative pressure in men and women. *J Appl Physiol* 2004 Sep;97(3):925-929.
- (83) Freeman R, Lirofonis V, Farquhar WB, Risk M. Limb venous compliance in patients with idiopathic orthostatic intolerance and postural tachycardia. *J Appl Physiol* 2002 Aug;93(2):636-644.
- (84) Whitney RJ. The measurement of volume changes in human limbs. *J Physiol* 1953 Jul;121(1):1-27.
- (85) Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* 2001 Dec;52(6):631-646.
- (86) Meendering JR, Torgrimson BN, Houghton BL, Halliwill JR, Minson CT. Effects of menstrual cycle and oral contraceptive use on calf venous compliance. *Am J Physiol Heart Circ Physiol* 2005 Jan;288(1):H103-10.
- (87) Monahan KD, Dinunno FA, Seals DR, Halliwill JR. Smaller age-associated reductions in leg venous compliance in endurance exercise-trained men. *Am J Physiol Heart Circ Physiol* 2001 Sep;281(3):H1267-73.
- (88) Monahan KD, Ray CA. Gender affects calf venous compliance at rest and during baroreceptor unloading in humans. *Am J Physiol Heart Circ Physiol* 2004 Mar;286(3):H895-901.
- (89) Young CN, Stillabower ME, DiSabatino A, Farquhar WB. Venous smooth muscle tone and responsiveness in older adults. *J Appl Physiol* 2006 Nov;101(5):1362-1367.
- (90) Halliwill JR, Minson CT, Joyner MJ. Measurement of limb venous compliance in humans: technical considerations and physiological findings. *J Appl Physiol* 1999 Oct;87(4):1555-1563.

- (91) Lindenberger M, Lanne T. Sex-related effects on venous compliance and capillary filtration in the lower limb. *Am J Physiol Regul Integr Comp Physiol* 2007 Feb;292(2):R852-9.
- (92) Gouloupoulou S, Deruisseau KC, Carhart R,Jr, Kanaley JA. Limb venous compliance responses to lower body negative pressure in humans with high blood pressure. *J Hum Hypertens* 2012 May;26(5):306-314.
- (93) Sielatycki JA, Shamimi-Noori S, Pfeiffer MP, Monahan KD. Adrenergic mechanisms do not contribute to age-related decreases in calf venous compliance. *J Appl Physiol* 2011 Jan;110(1):29-34.
- (94) Bartoletti A, Alboni P, Ammirati F, Brignole M, Del Rosso A, Foglia Manzillo G, et al. 'The Italian Protocol': a simplified head-up tilt testing potentiated with oral nitroglycerin to assess patients with unexplained syncope. *Europace* 2000 Oct;2(4):339-342.
- (95) Zachrisson H, Lindenberger M, Hallman D, Ekman M, Neider D, Lanne T. Diameter and compliance of the greater saphenous vein - effect of age and nitroglycerine. *Clin Physiol Funct Imaging* 2011 Jul;31(4):300-306.
- (96) Schnizer W, Klatt J, Baeker H, Rieckert H. Comparison of scintigraphic and plethysmographic measurements for determination of capillary filtration coefficient in human limbs (author's transl). *Basic Res Cardiol* 1978 Jan-Feb;73(1):77-84.
- (97) Pappenheimer JR, Soto-Rivera A. Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. *Am J Physiol* 1948 Mar 1;152(3):471-491.
- (98) Groothuis JT, van Vliet L, Kooijman M, Hopman MT. Venous cuff pressures from 30 mmHg to diastolic pressure are recommended to measure arterial inflow by plethysmography. *J Appl Physiol* 2003 Jul;95(1):342-347.
- (99) Cooke WH, Ryan KL, Convertino VA. Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol* (1985) 2004 Apr;96(4):1249-1261.
- (100) Olsen H, Lanne T. Reduced venous compliance in lower limbs of aging humans and its importance for capacitance function. *Am J Physiol* 1998 Sep;275(3 Pt 2):H878-86.

References

- (101) Fu Q, Witkowski S, Levine BD. Vasoconstrictor reserve and sympathetic neural control of orthostasis. *Circulation* 2004 Nov 2;110(18):2931-2937.
- (102) Taneja I, Moran C, Medow MS, Glover JL, Montgomery LD, Stewart JM. Differential effects of lower body negative pressure and upright tilt on splanchnic blood volume. *Am J Physiol Heart Circ Physiol* 2007 Mar;292(3):H1420-6.
- (103) Lightfoot JT, Tsintgiras KM. Quantification of tolerance to lower body negative pressure in a healthy population. *Med Sci Sports Exerc* 1995 May;27(5):697-706.
- (104) Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 1996 Mar;17(3):354-381.
- (105) Oberg B. The relationship between active constriction and passive recoil of the veins at various distending pressures. *Acta Physiol Scand* 1967 Oct-Nov;71(2):233-247.
- (106) Buckey JC, Lane LD, Plath G, Gaffney FA, Baisch F, Blomqvist CG. Effects of head-down tilt for 10 days on the compliance of the leg. *Acta Physiol Scand Suppl* 1992;604:53-59.
- (107) Melchior FM, Fortney SM. Orthostatic intolerance during a 13-day bed rest does not result from increased leg compliance. *J Appl Physiol* 1993 Jan;74(1):286-292.
- (108) Nicolaides AN, Cardiovascular Disease Educational and Research Trust, European Society of Vascular Surgery, The International Angiology Scientific Activity Congress Organization, International Union of Angiology, Union Internationale de Phlebologie at the Abbaye des Vaux de Cernay. Investigation of chronic venous insufficiency: A consensus statement (France, March 5-9, 1997). *Circulation* 2000 Nov 14;102(20):E126-63.
- (109) Pang CC. Autonomic control of the venous system in health and disease: effects of drugs. *Pharmacol Ther* 2001 May-Jun;90(2-3):179-230.

- (110) Sejrsen P, Henriksen O, Paaske WP, Nielsen SL. Duration of increase in vascular volume during venous stasis. *Acta Physiol Scand* 1981 Mar;111(3):293-298.
- (111) Lindenberger M, Lanne T. Decreased capillary filtration but maintained venous compliance in the lower limb of aging women. *Am J Physiol Heart Circ Physiol* 2007 Dec;293(6):H3568-74.
- (112) Lindenberger M, Olsen H, Lanne T. Impaired compensatory response to hypovolaemic circulatory stress in type 1 diabetes mellitus. *Diab Vasc Dis Res* 2011 Apr;8(2):136-142.
- (113) Blomqvist CG, Stone HL. Cardiovascular Adjustments to Gravitational Stress. *Comprehensive Physiology*: John Wiley & Sons, Inc.; 2011.
- (114) Gilbert CA, Stevens PM. Forearm vascular responses to lower body negative pressure and orthostasis. *J Appl Physiol* 1966 Jul;21(4):1265-1272.
- (115) Mosqueda-Garcia R, Furlan R, Tank J, Fernandez-Violante R. The elusive pathophysiology of neurally mediated syncope. *Circulation* 2000 Dec 5;102(23):2898-2906.
- (116) Ten Harkel AD, van Lieshout JJ, Wieling W. Effects of leg muscle pumping and tensing on orthostatic arterial pressure: a study in normal subjects and patients with autonomic failure. *Clin Sci (Lond)* 1994 Nov;87(5):553-558.
- (117) Convertino VA, Cooke WH, Holcomb JB. Arterial pulse pressure and its association with reduced stroke volume during progressive central hypovolemia. *J Trauma* 2006 Sep;61(3):629-634.
- (118) Xu TY, Staessen JA, Wei FF, Xu J, Li FH, Fan WX, et al. Blood flow pattern in the middle cerebral artery in relation to indices of arterial stiffness in the systemic circulation. *Am J Hypertens* 2012 Mar;25(3):319-324.
- (119) Goldstein DS, Benth O, Park MY, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. *Exp Physiol* 2011 Dec;96(12):1255-1261.

References

- (120) Moak JP, Goldstein DS, Eldadah BA, Saleem A, Holmes C, Pechnik S, et al. Supine low-frequency power of heart rate variability reflects baroreflex function, not cardiac sympathetic innervation. *Heart Rhythm* 2007 Dec;4(12):1523-1529.
- (121) Convertino VA, Rickards CA, Ryan KL. Autonomic mechanisms associated with heart rate and vasoconstrictor reserves. *Clin Auton Res* 2012 Jun;22(3):123-130.
- (122) Brown CM, Hainsworth R. Forearm vascular responses during orthostatic stress in control subjects and patients with posturally related syncope. *Clin Auton Res* 2000 Apr;10(2):57-61.
- (123) Thomson HL, Wright K, Frenneaux M. Baroreflex sensitivity in patients with vasovagal syncope. *Circulation* 1997 Jan 21;95(2):395-400.
- (124) Verheyden B, Ector H, Aubert AE, Reybrouck T. Tilt training increases the vasoconstrictor reserve in patients with neurally mediated syncope evoked by head-up tilt testing. *Eur Heart J* 2008 Jun;29(12):1523-1530.
- (125) Schondorf R, Wieling W. Vasoconstrictor reserve in neurally mediated syncope. *Clin Auton Res* 2000 Apr;10(2):53-55.
- (126) Vaddadi G, Guo L, Esler M, Socratous F, Schlaich M, Chopra R, et al. Recurrent postural vasovagal syncope: sympathetic nervous system phenotypes. *Circ Arrhythm Electrophysiol* 2011 Oct;4(5):711-718.
- (127) Goldstein DS, McCarty R, Polinsky RJ, Kopin IJ. Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension* 1983 Jul-Aug;5(4):552-559.
- (128) Esler MD, Hasking GJ, Willett IR, Leonard PW, Jennings GL. Noradrenaline release and sympathetic nervous system activity. *J Hypertens* 1985 Apr;3(2):117-129.
- (129) Meredith IT, Eisenhofer G, Lambert GW, Jennings GL, Thompson J, Esler MD. Plasma norepinephrine responses to head-up tilt are misleading in autonomic failure. *Hypertension* 1992 Jun;19(6 Pt 2):628-633.

- (130) Stewart JM, Lavin J, Weldon A. Orthostasis fails to produce active limb venoconstriction in adolescents. *J Appl Physiol* (1985) 2001 Oct;91(4):1723-1729.
- (131) Jardine DL, Krediet CT, Cortelli P, Frampton CM, Wieling W. Sympatho-vagal responses in patients with sleep and typical vasovagal syncope. *Clin Sci (Lond)* 2009 Sep 7;117(10):345-353.
- (132) Hernandez JP, Franke WD. Effects of a 6-mo endurance-training program on venous compliance and maximal lower body negative pressure in older men and women. *J Appl Physiol* 2005 Sep;99(3):1070-1077.
- (133) Lundvall J, Lanne T. Much larger transcapillary hydrodynamic conductivity in skeletal muscle and skin of man than previously believed. *Acta Physiol Scand* 1989 May;136(1):7-16.
- (134) Monos E, Lorant M, Feher E. Influence of long-term experimental orthostatic body position on innervation density in extremity vessels. *Am J Physiol Heart Circ Physiol* 2001 Oct;281(4):H1606-12.
- (135) Monos E, Lorant M, Dornyei G, Berczi V, Nadasy G. Long-term adaptation mechanisms in extremity veins supporting orthostatic tolerance. *News Physiol Sci* 2003 Oct;18:210-214.
- (136) Safar ME, London GM. Arterial and venous compliance in sustained essential hypertension. *Hypertension* 1987 Aug;10(2):133-139.
- (137) Tank J, Heusser K, Diedrich A, Brychta RJ, Luft FC, Jordan J. Yohimbine attenuates baroreflex-mediated bradycardia in humans. *Hypertension* 2007 Nov;50(5):899-903.
- (138) Cui J, McQuillan PM, Blaha C, Kunselman AR, Sinoway LI. Limb venous distension evokes sympathetic activation via stimulation of the limb afferents in humans. *Am J Physiol Heart Circ Physiol* 2012 Aug 15;303(4):H457-63.
- (139) Sneddon JF, Counihan PJ, Bashir Y, Haywood GA, Ward DE, Camm AJ. Assessment of autonomic function in patients with neurally mediated syncope: augmented cardiopulmonary baroreceptor responses to graded orthostatic stress. *J Am Coll Cardiol* 1993 Apr;21(5):1193-1198.

References

- (140) Fagius J, Karhuvaara S, Sundlof G. The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta Physiol Scand* 1989 Nov;137(3):325-334.
- (141) Victor RG, Leimbach WN, Jr, Seals DR, Wallin BG, Mark AL. Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension* 1987 May;9(5):429-436.
- (142) O'Mahony D, Bennett C, Green A, Sinclair AJ. Reduced baroreflex sensitivity in elderly humans is not due to efferent autonomic dysfunction. *Clin Sci (Lond)* 2000 Jan;98(1):103-110.
- (143) Krediet CT, de Bruin IG, Ganzeboom KS, Linzer M, van Lieshout JJ, Wieling W. Leg crossing, muscle tensing, squatting, and the crash position are effective against vasovagal reactions solely through increases in cardiac output. *J Appl Physiol* (1985) 2005 Nov;99(5):1697-1703.
- (144) van Dijk N, Quartieri F, Blanc JJ, Garcia-Civera R, Brignole M, Moya A, et al. Effectiveness of physical counterpressure maneuvers in preventing vasovagal syncope: the Physical Counterpressure Manoeuvres Trial (PC-Trial). *J Am Coll Cardiol* 2006 Oct 17;48(8):1652-1657.
- (145) Jardine DL. Vasovagal syncope: new physiologic insights. *Cardiol Clin* 2013 Feb;31(1):75-87.
- (146) Alboni P, Brignole M, Degli Uberti EC. Is vasovagal syncope a disease? *Europace* 2007 Feb;9(2):83-87.
- (147) Convertino VA. Gender differences in autonomic functions associated with blood pressure regulation. *Am J Physiol* 1998 Dec;275(6 Pt 2):R1909-20.

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