Video Article

Tilt Testing with Combined Lower Body Negative Pressure: a "Gold Standard" for Measuring Orthostatic Tolerance

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Abstract

Orthostatic tolerance (OT) refers to the ability to maintain cardiovascular stability when upright, against the hydrostatic effects of gravity, and hence to maintain cerebral perfusion and prevent syncope (fainting). Various techniques are available to assess OT and the effects of gravitational stress upon the circulation, typically by reproducing a presyncopal event (near-fainting episode) in a controlled laboratory environment. The time and/or degree of stress required to provoke this response provides the measure of OT. Any technique used to determine OT should: enable distinction between patients with orthostatic intolerance (of various causes) and asymptomatic control subjects; be highly reproducible, enabling evaluation of therapeutic interventions; avoid invasive procedures, which are known to impair OT[1].

In the late 1980s head-upright tilt testing was first utilized for diagnosing syncope[2]. Since then it has been used to assess OT in patients with syncope of unknown cause, as well as in healthy subjects to study postural cardiovascular reflexes[2-6]. Tilting protocols comprise three categories: passive tilt; passive tilt accompanied by pharmacological provocation; and passive tilt with combined lower body negative pressure (LBNP). However, the effects of tilt testing (and other orthostatic stress testing modalities) are often poorly reproducible, with low sensitivity and specificity to diagnose orthostatic intolerance[7].

Typically, a passive tilt includes 20-60 min of orthostatic stress continued until the onset of presyncope in patients[2-6]. However, the main drawback of this procedure is its inability to invoke presyncope in all individuals undergoing the test, and corresponding low sensitivity[8,9]. Thus, different methods were explored to increase the orthostatic stress and improve sensitivity.

Pharmacological provocation has been used to increase the orthostatic challenge, for example using isoprenaline[4,7,10,11] or sublingual nitrate[12,13]. However, the main drawback of these approaches are increases in sensitivity at the cost of unacceptable decreases in specificity[10,14], with a high positive response rate immediately after administration[15]. Furthermore, invasive procedures associated with some pharmacological provocations greatly increase the false positive rate[1].

Another approach is to combine passive tilt testing with LBNP, providing a stronger orthostatic stress without invasive procedures or drug side-effects, using the technique pioneered by Professor Roger Hainsworth in the 1990s[16-18]. This approach provokes presyncope in almost all subjects (allowing for symptom recognition in patients with syncope), while discriminating between patients with syncope and healthy controls, with a specificity of 92%, sensitivity of 85%, and repeatability of 1.1±0.6 min[16,17]. This allows not only diagnosis and pathophysiological assessment[19-22], but also the evaluation of treatments for orthostatic intolerance due to its high repeatability[23-30]. For these reasons, we argue this should be the "gold standard" for orthostatic stress testing, and accordingly this will be the method described in this paper.

Video Link

The video component of this article can be found at https://www.jove.com/video/4315/

Protocol

Throughout testing, continuous beat-to-beat blood pressure and electrocardiogram (ECG) monitoring is paramount. This ensures subject safety, and prompt termination of the test with the onset of presyncope. Beat-to-beat blood pressure recordings can be obtained through arterial catheterization, or finger plethysmography[31-33]. The latter is used in this protocol because it is non-invasive and can assess the onset of presyncope with the same accuracy as catherization[31,34], without the detrimental impact of invasive monitoring on OT[1]. Using the Modelflow technique changes in stroke volume, cardiac output, and total peripheral resistance can be derived from the finger arterial pressure waveform[35,36]. Additional noninvasive measures that may aid the haemodynamic evaluation can also be conducted, and will be described here. Continuous end tidal oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) monitoring using a nasal cannula allows the evaluation of any contribution of hyperventilation to the subject's symptoms. Finally, monitoring both brachial and cerebral blood flow velocities using Doppler ultrasound can be undertaken to allow the determination of peripheral and cerebral vascular responses to orthostasis. In addition, measurements of venous

pooling and capillary filtration could also be obtained using impedance plethysmography[20]. Ultimately, this protocol allows assessment of postural cardiovascular reflex control in a controlled and reproducible setting.

1. Equipment

1. The manually adjustable tilt table is capable of moving from -15 to 60 ° in 10 ° increments (**Figure 1**). It includes an adjustable right-arm rest with an adjustable holder for a brachial ultrasound probe (**Figure 1D**), an adjustable footplate for the subject to stand on, and a seat belt to secure the subject's legs (**Figure 1E**).
2. An LBNP chamber with an attached pressure gauge fits in a wooden groove filled with neoprene onto the bottom half of the tilt table and is secured in place by four adjustable straps (**Figure 1**). A clear Plexiglas material can be employed for the chamber if desired, to enable visualization of the subjects' legs, and thus visual monitoring of any skeletal muscle pumping activity.
3. A wooden waist board is attached to the top of the chamber with a neoprene surround at the level of the subject's iliac crest to provide an air-tight seal with the chamber (**Figure 1B**). The level of the iliac crest is chosen because it avoids the application of LBNP to the abdomen, which can be uncomfortable, but ensures a standardized stimulus for individuals of different statures.
4. Perform continuous beat-to-beat data acquisition with a sampling frequency of 1 KHz using an analog-to-digital converter. Visualize all data simultaneously in real time throughout testing using LabChart (Powerlab 16/30, AD Instruments, Colorado Springs, CO).
5. Conduct testing in a temperature-controlled room (20-22 °C) to avoid the known effect of heat stress upon OT[37]. Tests are ideally conducted in the mornings because of the effect of diurnal rhythms on baroreflex control[38]. In some cases, a familiarization session is advised to minimize the influence of a "stress response" on the procedure.
6. Instruct subjects to have only a light breakfast, to minimize possible confounders due to post-prandial hypotension, and to avoid caffeine and strenuous exercise on the morning of testing. Subjects should fast for 2 hr prior to testing. They should also abstain from drinking alcohol for 24 hr prior to testing, to eliminate the diuretic effect of alcohol and consequent reductions in plasma volume, which are known to reduce OT[39].
7. **Figure 2** outlines the protocol. Stand the subject on the table foot plate and move them into a supine position (**Figure 1**). Once supine, align the iliac crest with the center of the table. This allows for ease of tilting, and ensures standardized positioning of the LBNP chamber. Adjust the footplate accordingly. A foot plate support is preferred because tables with saddle supports are associated with increased false positive responses, probably due to excess compression of leg and pelvic veins[6].
8. Loosley position a strap just above the knee to promote passive standing and provide postural support. Instruct subjects not to move their legs during testing. Position the subject's right arm on the arm rest, adjusted so that it is comfortably supported at heart level. Attach monitoring equipment.
9. Conduct beat-to-beat blood pressure monitoring using the Finometer, according to the manufacturer's instructions[40]. Chose the finger cuff that fits appropriately onto the middle phalanx of the subject's right middle finger. Use a brachial cuff to internally calibrate the Finometer prior to data collection. Enter the subject's sex, age, height and weight into the Finometer to enable appropriate assumptions for the Modelflow algorithms[35,36].
10. Continuous ECG monitoring is important for the accurate determination of heart rate responses, and prompt identification of any cardiac arrhythmia, should they occur. Apply ECG electrodes in a modified lead II configuration, ensuring that the electrode sites do not interfere with positioning of the neoprene waist board.
11. Determine peripheral vascular responses using Doppler ultrasound of the brachial artery of the right arm supported at heart level (to avoid the influence of hydrostatic pressure changes on blood pressure and velocity in the arm). Palpate the artery until the pulse is located. Apply ultrasound gel to this area and position an 8 MHz ultrasound probe so that a brachial artery velocity waveform is obtained (**Figures 3A,B**).
12. Once the signal is identified, optimize the depth and gain, and tighten the adjustable holder to keep the angle of insonation constant for the duration of the test (**Figure 3B**). This is important because, according to the Doppler shift equation, changes in velocity will be proportional to changes in flow if the angle of insonation and arterial diameter are constant[19,21].
13. Determine cerebral vascular responses similarly (**Figures 4A,B**). Secure the cerebral ultrasound probe (2 MHz) in place using a plastic headset or fabric headbands (**Figure 4B**) to ensure faithful signal detection and a constant angle of insonation throughout testing.
14. Determine blood velocity continuously from the middle cerebral artery. This vessel is chosen because of its convenience of identification, large contribution to global cerebral perfusion, and constant diameter during orthostatic stress[41], ensuring changes in velocity are proportional to changes in flow. Apply ultrasound gel to the subject's temple and locate the vessel (**Figure 4A**). Once identified, optimize the depth and gain settings.
15. Attach the nasal cannula. Nasal sampling is preferred because it permits the subject to talk freely during testing (unlike with use of a mouthpiece). This is important to enable self-report and recognition of symptoms, while avoiding invasive blood sampling that may adversely impact OT. However, during speech, or when the subject is breathing through their mouth, accuracy of these readings may be affected. Encourage subjects to breathe through their nose.
16. Place the LBNP chamber on the table and secure with the straps. Select a waist board that fits snugly so that an airtight seal with the neoprene can be achieved (**Figure 1B**). The wooden component of the waist board should not be touching the subject. Secure the waist board to the chamber. Connect the chamber to a negative pressure source, *via* a variable resistor (**Figure 1C**).

2. Data Collection

1. Record data for 20 min in the supine position. Shorter rest periods are associated with greater falls in blood pressure when upright, presumably because the reabsorption of any fluid that has collected in the dependent limbs prior to lying down is not yet complete[20].
2. At the end of the supine phase maneuver the table to a tilt angle of 60 °. This angle is preferred because it affords nearly 90% of the maximal vertical displacement, while allowing the subject to remain relaxed and supported against the tilt table (minimizing the confounding effect of skeletal muscle pumping activity with higher tilt angles). Less steep angles may increase the false negative rate; although the physiological effects of tilting are similar for angles ≥60 °, higher tilt angles are associated with a reduction in specificity[6].
3. Complete the transition to upright within thirty seconds. The transition time is not known to affect OT and it is more pleasant for the subject if tilting is not excessively rapid. However, very slow tilt maneuvers may be associated with lower activation of muscle sympathetic nerve activity and should be avoided[42].

4. Continuously monitor the subject's cardiovascular parameters as well as their subjective experience. The test should be stopped immediately, and the subject returned to a supine position, if any of the following end-point criteria are met: blood pressure is 80 mmHg or below; heart rate is lower than 50 beats per minute (bpm) or higher than 170 bpm; and the subject experiences symptoms such as dizziness, warmth, and requests to stop; or the protocol is completed.
5. Unless there is a specific desire to initiate syncope, terminate the test rapidly at the onset of presyncopal signs and symptoms, avoiding frank syncope.
6. After 20 min of tilting apply LBNP, while still tilted, at -20 mmHg for a further 10 min. It is important to inform the subject of the impending onset of LBNP to prevent a startle response to the sensation and sound of the LBNP.
7. After 10 min, increase the LBNP to -40 mmHg for another 10 min.
8. After 10 min, increase the LBNP to -60 mmHg and continue for another 10 min. At the end of this phase, turn off the LBNP and return the subject to the supine position.
9. It is theoretically possible to increase the vacuum source to achieve -80 mmHg LBNP if a presyncopal end-point is not reached at the end of this phase. However, in practice this high level of LBNP is uncomfortable for the subject, and presyncope usually occurs at much lower levels of orthostatic stress, even in healthy controls. The largest OT we have recorded was 50 min (the end of -60 mmHg) and this was in a Peruvian high altitude resident with Chronic Mountain Sickness and an extremely high blood volume[43].
10. OT is defined as the time to presyncope in minutes from the onset of the head-up tilting phase.
11. To ensure the rapid termination of symptoms and signs of presyncope, and so to minimize the likelihood of syncope or asystole, a rapid return to supine is desired (ideally ~1 sec). For this reason, a manual tilt table may be preferred to an autonomic one, which may not have the capability for such rapid transitions. Returning the table to a slightly head-down position (-15 °) may promote faster resolution of the presyncopal event. Once all variables have returned to the supine levels and any symptoms are resolved, remove the monitoring equipment.
12. After removing the LBNP chamber, remove the strap over the subject's legs and lower the footplate to its original position. Ensure the subject is positioned with their feet on the plate.
13. Before returning the table to an upright position, instruct the subject to tense their leg muscles throughout the transition to avoid any symptoms from reoccurring as the table is tilted to facilitate them getting off[44,45]. Ask the subject to sit after stepping off the table to ensure they are symptom-free before leaving the laboratory.

Representative Results

Using this protocol, all subjects experience presyncope, and the definition of normal or abnormal responses is made largely based upon the time it takes to induce this reaction. OT is defined as the time to presyncope in minutes from the onset of upright tilting. Typical values for OT in healthy volunteers according to age and gender can be seen in **Table 1.** Patients with orthostatic intolerance exhibit presyncope earlier in the test, with 85% ending the test within the -20 mmHg phase compared to 23% of controls[17]. The threshold of normal is taken as the 20% incidence of syncope[17].

At termination of the test the haemodynamic responses fall into essentially three categories: vasovagal syncope; postural tachycardia syndrome (POTS); or autonomic failure[46]. The vasovagal response is characterized as sudden onset hypotension and bradycardia, although the relative contributions of each component can vary greatly, and have been characterized according to the VASIS classification[46,47]. A representative response in an adult control is depicted in **Figure 5.** At tilt, the baroreflex compensates for blood pooling in the lower extremities through vasoconstriction, reflected in the reduced brachial blood flow and increased diastolic pressure, and tachycardia. This response is maintained until presyncope, at which point there is a sudden switch to vasodilatation and bradycardia. In this case the subject experienced a vasovagal response at presyncope, associated with symptoms of dizziness and warmth. This is the most common response to tilting in healthy controls.

In patients with autonomic failure there is an absence of adaptation of blood pressure to the upright position, with a slow and progressive decline in blood pressure, and small or absent heart rate response to orthostatic stress. Patients will develop symptoms once a critical level of blood pressure is reached (typically at or below a systolic pressure of 80 mmHg[46], although many patients with autonomic failure tolerate much lower blood pressures than this) representing the lower limit for cerebral autoregulation. This response generally occurs only in older patients with syncope, who often present with other associated disorders[46].

POTS is characterized by a heart rate rise of 30 bpm within 10 min of head-upright tilting, or with an upright heart in excess of 120 bpm[48,49], associated with symptoms of presyncope. Blood pressure is usually well maintained. This disorder generally affects young females (<40 years old), and typically presents with presyncope, and only occasionally with syncope[48]. Heart rate responses to orthostatic stress are brisk in children and adolescents, so alternative diagnostic criteria for POTS are recommended in this population[50].

Regardless of the haemodynamic response, there is a drop in cerebral blood flow velocity at presyncope[51]. There may be impairments in cerebral autoregulation in patients with vasovagal syncope[22] and POTS[52].

In those with intact baroreflex control of vascular resistance there will be a progressive reduction in brachial blood flow velocity during the orthostatic stress, associated with increased forearm vascular resistance. In healthy controls a maximum vascular resistance response of +100±12% is considered normal[21]. Smaller responses are indicative of impaired vascular resistance responses[19,21], with 60% of patients with presyncope and poor OT having maximal vascular resistance responses below +80%, and 83% of controls having responses that exceed this value[19]. Patients with POTS often have particularly small peripheral vascular resistance responses[21]. There is a significant positive correlation between the maximum vascular resistance response and OT[19].Typical heart rate responses in healthy controls at the end of the head up tilt phase and peak heart rates during the orthostatic stress are 71-82 bpm and 98-133 bpm respectively[16,21].

Some degree of hyperventilation is common during orthostatic stress, with associated reductions in $P_{ET}CO_2$[53]. This tends to produce a hypocapnic cerebral vasoconstriction and peripheral vasodilation, and so contributes to the decreases in blood pressure and cerebral blood flow at presyncope. Some individuals are excessively sensitive to alterations in $P_{ET}CO_2$ and this may be a factor in predisposing them to syncopal events[53].

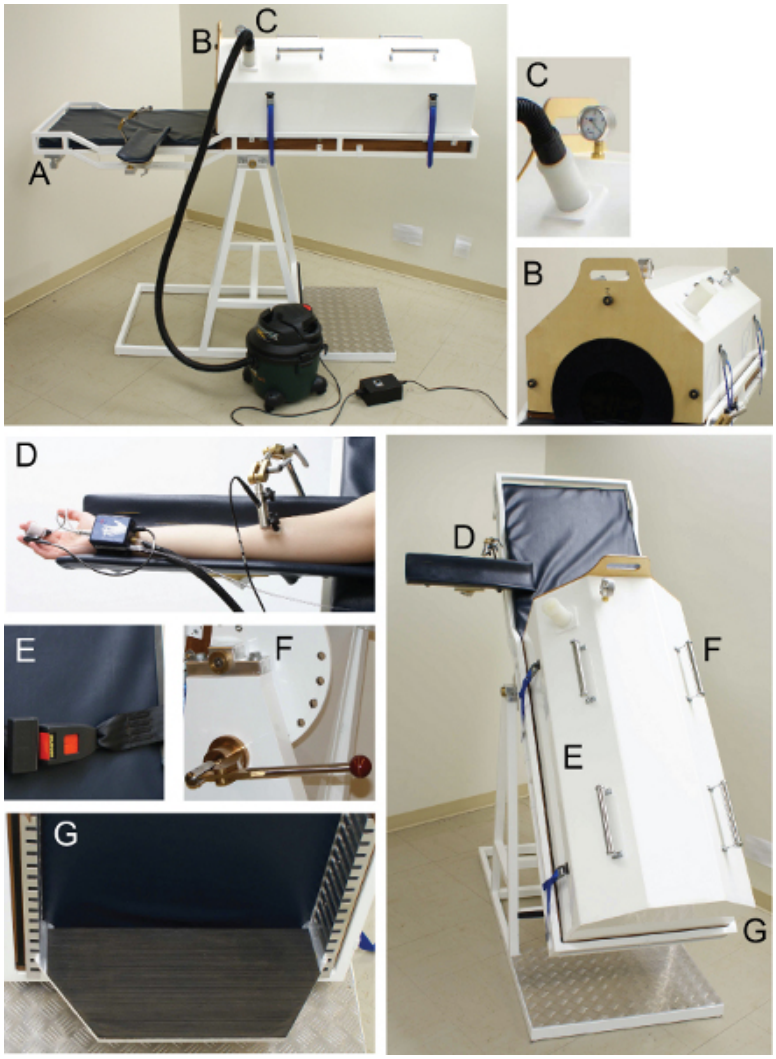

Figure 1. The manual, adjustable head-up tilt table and LBNP chamber. The manual design allows for rapid transitions between supine and tilt, ensuring the prompt recovery of presyncopal symptoms at test termination. **A.** The handle used to move the table into different positions. **B.** A pressure gauge monitors the level of LBNP during the protocol. **C.** A wooden waist board with neoprene is attached to the top of the chamber to ensure the chamber is air-tight. **D.** The adjustable arm rest with attached clamp for the brachial ultrasound probe. **E.** A seatbelt over the subject's legs minimizes skeletal muscle pumping activity during orthostatic stress. **F.** The lever used to adjust the table from -15 to 60 °. **G.** The footplate can be adjusted for the subject's height once their iliac crest is positioned in the center of the table.

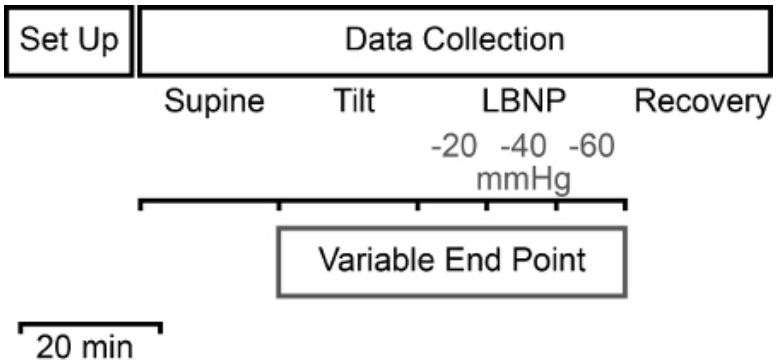

Figure 2. Head-up tilt protocol. There are three different phases in the protocol: supine for 20 min; tilt for 20 min; and LBNP for 10 min each at -20 mmHg, -40 mmHg, and -60 mmHg.

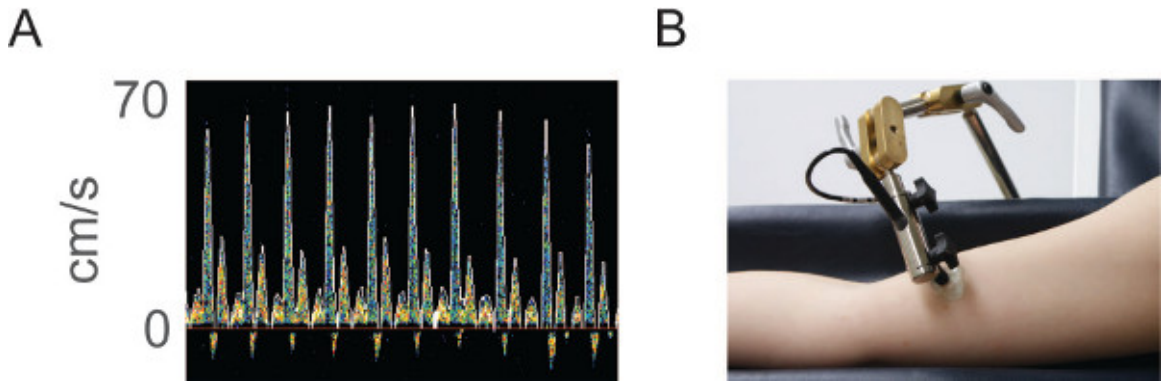

Figure 3. Brachial blood flow velocity measurement. A. The brachial ultrasound probe is positioned overlying the brachial artery to enable measurements of forearm blood flow velocity, and the calculation of vascular resistance responses. **B.** Once in place, the probe is secured using an adjustable clamp to ensure the angle of insonation does not change throughout the test.

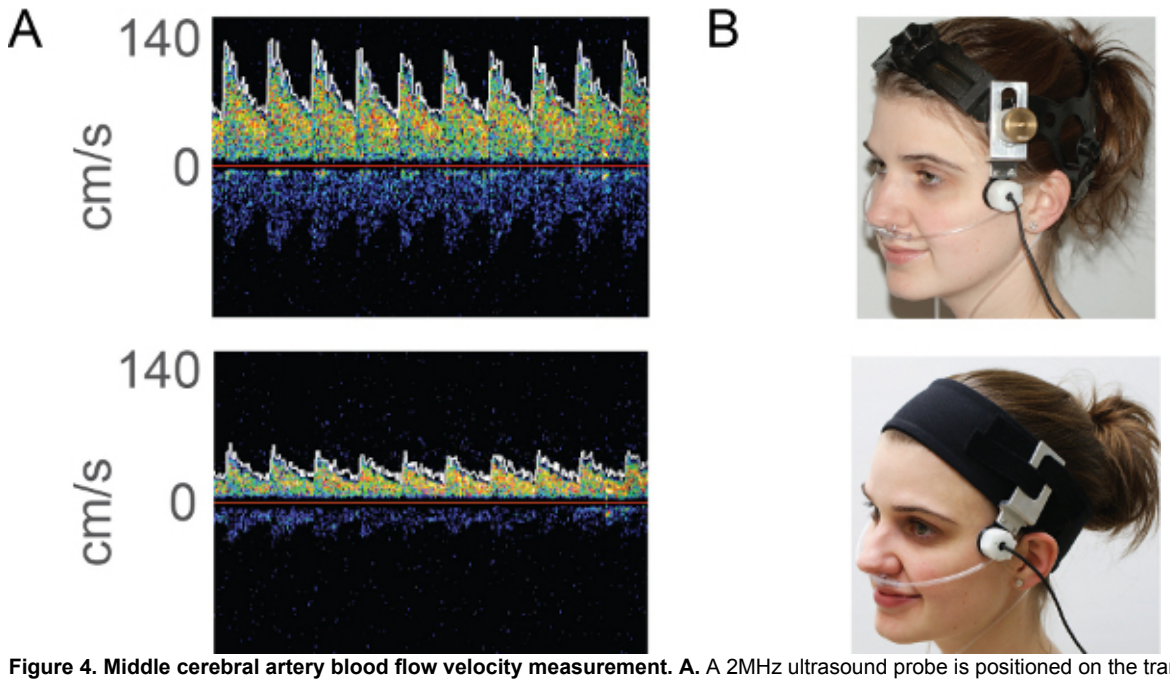

Figure 4. Middle cerebral artery blood flow velocity measurement. A. A 2MHz ultrasound probe is positioned on the transtemporal window overlying the middle cerebral artery. An example showing middle cerebral artery blood flow velocity is shown in the top panel. The birfurcation point, with the anterior cerebral artery waveform just visible as a negative deflection, enables confident identification of the middle cerebral artery. The bottom panel shows an example from the posterior cerebral artery. Although the flow profile is similar to the middle cerebral artery, the mean velocity is lower and insonation depth greater, enabling ease of discrimination between the two vessels. **B.** Once in place the probe is secured in position using either a plastic headset (upper) or fabric headbands (lower).

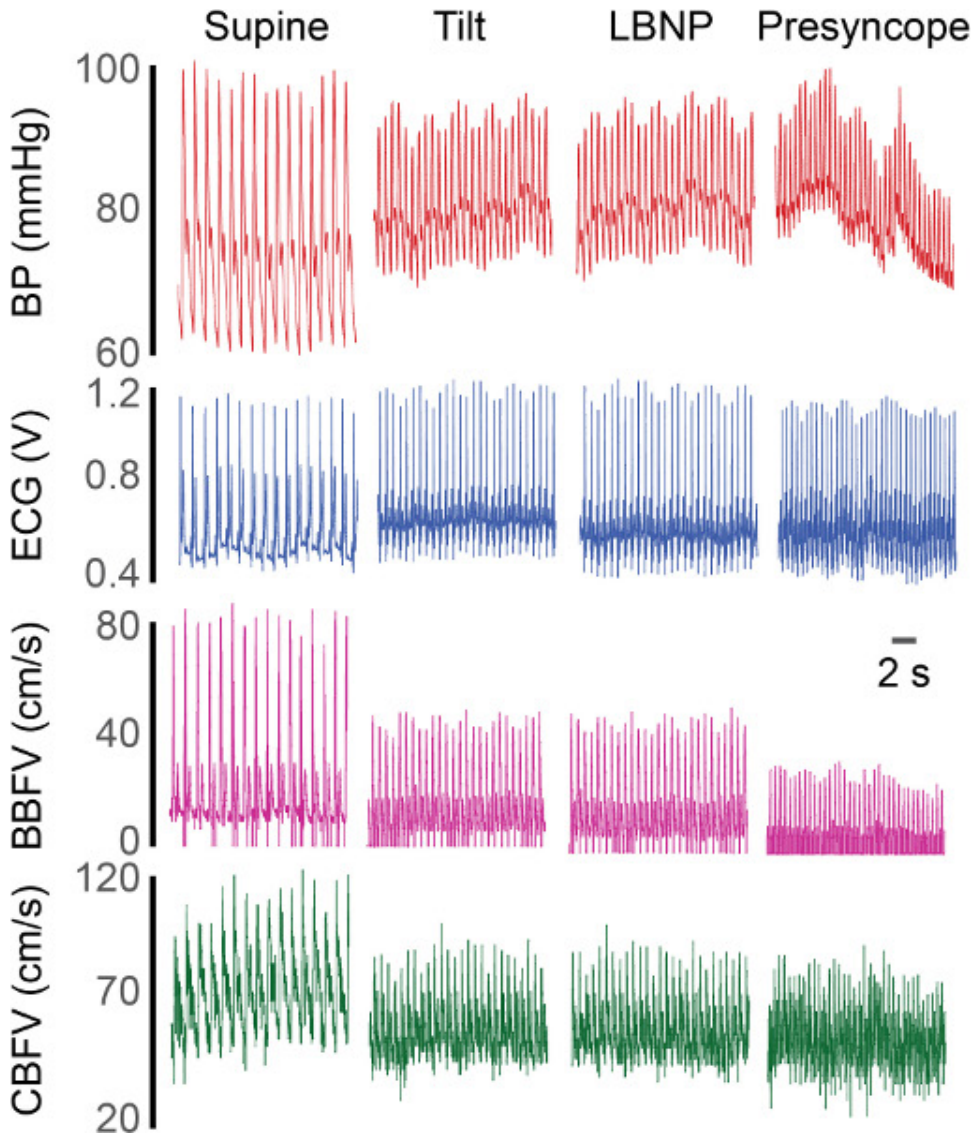

Figure 5. Example tracing from an adult control volunteer. Beat-to-beat blood pressure (BP), electrocardiogram (ECG), brachial (BBFV) and cerebral (CBFV) blood flow velocity are shown. During the orthostatic stress a progressive baroreflex mediated tachycardia and vasoconstriction can be seen. At presyncope, the baroreflex begins to fail and there is a drop in blood pressure and cerebral blood flow velocity.

	Orthostatic Tolerance (min)					
	Males			**Females**		
Age (years)	**20-35**	**36-50**	**>50**	**20-35**	**36-50**	**>50**
Mean	35±1.4*	35±1.7	35±1.7	29±1.5	31±1.8	37±2.3
20% incidence	30	34	32	24	28	30
50% incidence	34	37	36	29	32	38

Table 1. Predicted times to presyncope in healthy volunteers according to age and gender. Values are given for the mean orthostatic tolerance ± standard error, as well as the times at which 20% and 50% of individuals experienced presyncope. * denotes significant difference ($p<0.05$) between males and females within the age group. There was a significant correlation between age and onset of presyncope in females ($r=0.63$; $p=0.004$), but not males. Combined data (n=63) from El-Bedawi and Hainsworth (1994), and Claydon, unpublished observations.

Discussion

This technique is highly reproducible, has the ability to discriminate normal and abnormal responses with high sensitivity and specificity, and can provoke presyncope in all subjects, allowing for symptom recognition in patients with recurrent syncope. In a clinical setting, different types of

syncope can be distinguished, allowing tailored treatment and management approaches. The impact of interventions can readily be assessed. With additional cardiovascular monitoring, reflex responses can also be evaluated.

In order to differentiate certain sub-types of syncope in patient populations, the analysis of plasma catecholamine responses during orthostatic stress may be useful. However, the invasive nature of this procedure may interfere with the accurate determination of OT[1].

This protocol can easily be modified to account for specific clinical or research questions, or the needs of specific populations. The angle of the table can be altered in situations when 60 ° may be difficult to sustain, for example when evaluating orthostatic intolerance in spinal cord injured populations. In such cases, tilting to 30-35 ° may be more appropriate and tolerable[54-56]. If desired, the protocol may be shortened in specific populations by stopping after completion of LBNP at -20 mmHg - individuals who reach the end of this phase essentially have a normal OT (**Table 1**). This may be applicable if the goal is to determine whether the response is abnormal or not, but not necessarily to define the precise OT. This may be particularly pertinent in paediatric populations.

Often with an automatic table, the transition back to supine is slow and this can increase the likelihood of profound bradycardia or asystole, and brief loss of consciousness, occurring coincident with a vasovagal response[57]. As such, the benefit of the manual table is that the pathophysiological and symptomatic responses associated with presyncope are quickly recovered.

An experienced physiologist, technician or nurse must be present during testing. There is a lack of agreement as to whether a medical doctor should also be present or available (on call) in case of asystole or other complications[3,6,58]. The laboratory should have access to atropine should asystole occur, as well as a defibrillator. Serious adverse events are rare and no deaths have been reported[58]. Brief periods of profound bradycardia or asystole may accompany vasovagal responses[4,24,46], but they typically recover spontaneously with the return to a supine posture. Seizure-like activity may accompany syncope[59], but can be avoided with termination of the test at presyncope. There is one report of cardiopulmonary resuscitation being required in the case of tilt-induced coronary vasospasm in a patient with known coronary vasospastic angina[60].

Disclosures

No conflicts of interest declared.

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