Investigating Syncope in Children and Adolescents

by

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Abstract

Syncope, or fainting, is a heterogeneous condition and hence difficult to diagnose. Treatments for syncope are vast and largely ineffective because individual variability impacts success. Research into the mechanisms and treatment of syncope has focused on adults, despite 15-25% of children experiencing episodes. Thus, we aimed to investigate syncope in children and adolescents and explore a non-pharmacological approach to its treatment.

We conducted cardiovascular autonomic function testing (Valsalva manoeuvre, cerebral reactivity to hypo- and hypercapnia, and orthostatic stress testing) to evaluate cardiovascular factors predisposing to syncope in 21 children. We also evaluated the efficacy of graded calf compression stockings for the treatment of syncope in 15 young adults.

We found that (1) autonomic function testing in children was appropriate, but current diagnostic criteria may need to be altered for paediatric populations; and (2) the utility of calf compression stockings to improve orthostatic tolerance is dependent on specific anthropometric variables.

Keywords: syncope; paediatric; autonomic function testing; orthostatic tolerance.

I would like to dedicate this thesis to the children and adolescents who graciously gave up their time to contribute to this area of work. It is my hope that professionals and patients will better understand syncope through this and future work.

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List of Acronyms or Glossary

ADH	Antidiuretic hormone
BBFV	Brachial blood flow velocity
BPV	Blood pressure variability
CO	Cardiac output
CO_2	Carbon dioxide
CMAP	Cerebral mean arterial pressure
CVR	Cerebral vascular resistance
DAP	Diastolic arterial pressure
ECG	Electrocardiogram
f_D	Doppler shift frequency
F_{opt}	Optimum frequency
F_r	Ultrasound frequency
F_0	Emitted frequency
FVR	Forearm vascular resistance
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency
LBNP	Lower body negative pressure
LQTS	Long QT Syndrome
MAP	Mean arterial pressure
MCA	Middle cerebral artery
NET	Norepinephrine reuptake transporter
NO	Nitric oxide
NTS	Nucleus tractus solitarius
O_2	Oxygen
OT	Orthostatic tolerance
P_aCO_2	Partial pressure of arterial carbon dioxide
$P_{ET}CO_2$	Partial pressure of end tidal carbon dioxide
POTS	Postural Orthostatic Tachycardia Syndrome
RRI	R-R Interval

SAP	Systolic arterial pressure
SSRI	Selective serotonin reuptake inhibitor
SV	Stroke volume
TPR	Total peripheral resistance
TRPV4	Transient receptor potential vanniloid 4
VASIS	Vasovagal Syncope International Study
VLF	Very low frequency
VVS	Vasovagal syncope

Project Objectives

Syncope, or fainting, is a heterogeneous condition that is difficult to diagnose because its underlying physiological mechanisms are unknown (1). Between 20-40% of the population is affected by syncope at some point in their lifetime, and many affected individuals suffer recurrent episodes (2). Presyncope, orthostatic dizziness prior to fainting, is commonly reported to physicians but its prevalence is unknown (3-6). Both syncope and presyncope affect the quality of life of those who suffer recurrent episodes, thus prompting investigation into their prevention. The autonomic nervous system controls many functions such as blood pressure and heart rate and impaired autonomic function is, therefore, implicated in the development of (pre)syncope (7). Treatments for (pre)syncope are vast and largely ineffective because individual variability impacts success (8). More importantly, research into the mechanisms, precipitating factors, and treatment of (pre)syncope has focused on adults, despite 15-25% of children and adolescents experiencing episodes (9-14). Therefore, we aim to:

1: Evaluate syncope, presyncope, and autonomic control in children and adolescents.

2: Treat syncope and presyncope in children and adolescents.

1. Background and Rationale

1.1. Syncope is prevalent and debilitating

Syncope, or fainting, is a heterogeneous condition whose underlying mechanisms remain elusive (15). It is defined as a transient loss of consciousness and postural tone due to global cerebral hypoperfusion, with a subsequent return to pre-existing neurological function (15-17). The association with cerebral hypoperfusion differentiates the condition from other disorders such as epilepsy, concussion and transient ischemic attacks that also include a loss of consciousness and postural tone (15, 18). Although accurate durations are rarely obtained, syncopal episodes are typically brief; however, there are cases in which the loss of consciousness occurs for several minutes, making the differential diagnosis between syncope and other causes of loss of consciousness difficult (18).

Syncope is particularly prevalent in the general population, accounting for 1-6% of emergency room visits, 1-3% of hospital admissions, and is a frequent reason for referral to cardiologists and neurologists (2, 18). However, these data may represent an underestimation of the prevalence because those who seek medical attention usually do so only when the event led to a fall, injury, or emotional scare (19, 20). Those in whom the episode is perceived to be unremarkable rarely seek medical attention (21, 22). Nevertheless, it is estimated that 6% of affected patients present with major injury such as fractures and motor vehicle accidents and 29% present with minor injury such as bruises (18). Presyncope, symptoms of impending loss of consciousness, is also commonly reported to physicians, but its prevalence is unknown, despite the fact that it severely impairs quality of life for those affected (3, 23).

There is a bimodal age distribution of episodes of both syncope and presyncope, with a high prevalence in children and adolescents (15-25% of children will experience one syncopal episode by the end of adolescence), a decline of incidence into middle age,

and another peak occurring in the elderly after age 70 (3, 9, 13, 14, 20, 24). This resurgence of syncope in the elderly may be partly explained by their large number of co-morbidities and use of drugs predisposing to syncope (25). Certainly, the age group represents a high percentage of hospital admissions due to syncope (25). The main difference between the two age groups is the mechanism underlying the event. In children and adolescents, syncope generally occurs secondary to an inappropriately triggered cardiovascular reflex response, with a benign prognosis. However, in the elderly the aetiology appears more diverse (possibly as a result of polypharmacy, and age-related deterioration of vascular health and autonomic impairment) (7, 25). It is important to note that approximately 1% of toddlers may have a form of vasovagal syncope (VVS) and exhibit symptoms even at this young age (18).

Interestingly, females, in all age groups, are more prone to syncope than males, although the reasons for this are unclear (18, 26). In all demographics affected, recurrence rates are high and particularly devastating (2, 21). Although the exact incidence of syncope and presyncope is unclear, it is known that they are highly prevalent and severely impair quality of life for those affected, particularly because patients live with the threat of reoccurrence (18, 23, 26, 27).

1.2. Classifications of syncope

There are numerous subtypes of syncope and this presents both a diagnostic and treatment challenge (22). Many of these forms share a common trigger: orthostatic or gravitational stress and as a result (pre)syncope is commonly initiated following prolonged motionless standing (22).

Orthostatic stress represents a considerable physiological challenge. Upon assuming an upright position approximately 700 mL of blood pools in the abdomen and lower extremities (28). This is due to gravitational fluid shifts causing a redistribution of hydrostatic pressures, with the lowest pressures in the head, and the highest pressures in the ankles (Figure 1.1) (29). Furthermore, fluid movement between the circulation and tissues occurs when there is an imbalance between hydrostatic and osmotic pressures; in an upright position due to the effect of gravity, hydrostatic pressure in the lower

extremities is greater than osmotic pressure (which remains relatively constant), leading to plasma filtration into the tissues (29). It is estimated that plasma filtration contributes to an increase in leg volume of 8.41±1.02 $mL.min^{-1}$ in the first ten minutes of tilt in healthy controls (30). Furthermore, if standing motionless, the absence of muscle pumping activity further contributes to plasma filtration because the normal compression of the lymphatic vessels or veins that occurs during locomotion or static postural sway, and which returns blood into the effective circulation is lost (29, 31). The net effect is a reduction in venous return to the heart leading to a lower stroke volume and thus reduced cardiac output (29).

The subsequent reduction in arterial pressure relieves the distension of arterial baroreceptors located in the carotid sinus, aortic arch and coronary arteries, with a reduced firing rate in the glossopharyngeal, and vagus nerves, respectively (28, 32, 33). The carotid sinus baroreceptors are particularly important during postural change because they detect the largest change in arterial pressure due to their location above the heart (Figure 1.1) (29, 34, 35). The afferent signal from the baroreceptors is relayed to the nucleus tractus solitarius (NTS) of the medulla (Figure 1.2) (28). During orthostatic stress, reduced afferent firing leads to decreased efferent parasympathetic activity coupled with increased sympathetic activity, and thus tachycardia and increased cardiac contractility (28). In addition, decreased inhibition of the C1 area in the medulla leads to increased sympathetic nerve activity to the vasculature causing vasoconstriction (Figure 1.2) (28). This baroreflex response is a key mechanism by which arterial pressure, and hence cerebral perfusion pressure is maintained during orthostatic stress, and (pre)syncope is prevented (28, 29).

With prolonged orthostatic stress other neurohumoral regulators of arterial blood pressure are activated including the renin-angiotensin aldosterone system, within 15 to 30 minutes, and antidiuretic hormone (ADH), within 30 to 45 minutes (35, 36). Renin is secreted in response to low mean arterial pressure (MAP) and a cascade of reactions leads to the formation of angiotensin II which causes vasoconstriction and thus higher blood pressure (35). Angiotensin II also increases aldosterone production which increases sodium reabsorption at the kidney, and in turn, plasma volume and then blood pressure are increased (35). Similarly, vasopressin or ADH is released in response to

low blood pressure, which increases plasma volume and mean arterial pressure (35). ADH can also directly act on blood vessels causing vasoconstriction (35).

Individuals with poor baroreflex responses to orthostatic stress are particularly prone to (pre)syncope (29). However, it is important to note that healthy subjects can still experience presyncope or even syncope during severe orthostatic stress; the distinguishing feature between patients with recurrent (pre)syncope and healthy controls is the amount of orthostatic stress required to induce the reaction (29).

Syncope and presyncope are typically classified as cardiovascular (reflex and cardiac), non-cardiovascular (neurologic and metabolic) or unexplained syncope (37). The focus of this thesis will be the classifications of cardiovascular causes of syncope. Many cardiovascular forms of syncope share a common mechanism, in which there is a failure to compensate for orthostatic stress, with decreased cardiac output, decreased cerebral blood flow and ultimately (pre)syncope. In general, there are four mechanisms that differentiate cardiovascular causes of syncope: (1) decreases in heart rate (HR) and blood pressure; (2) an inappropriate increase in HR; (3) decreases in blood pressure; and finally, (4) a cardiac abnormality.

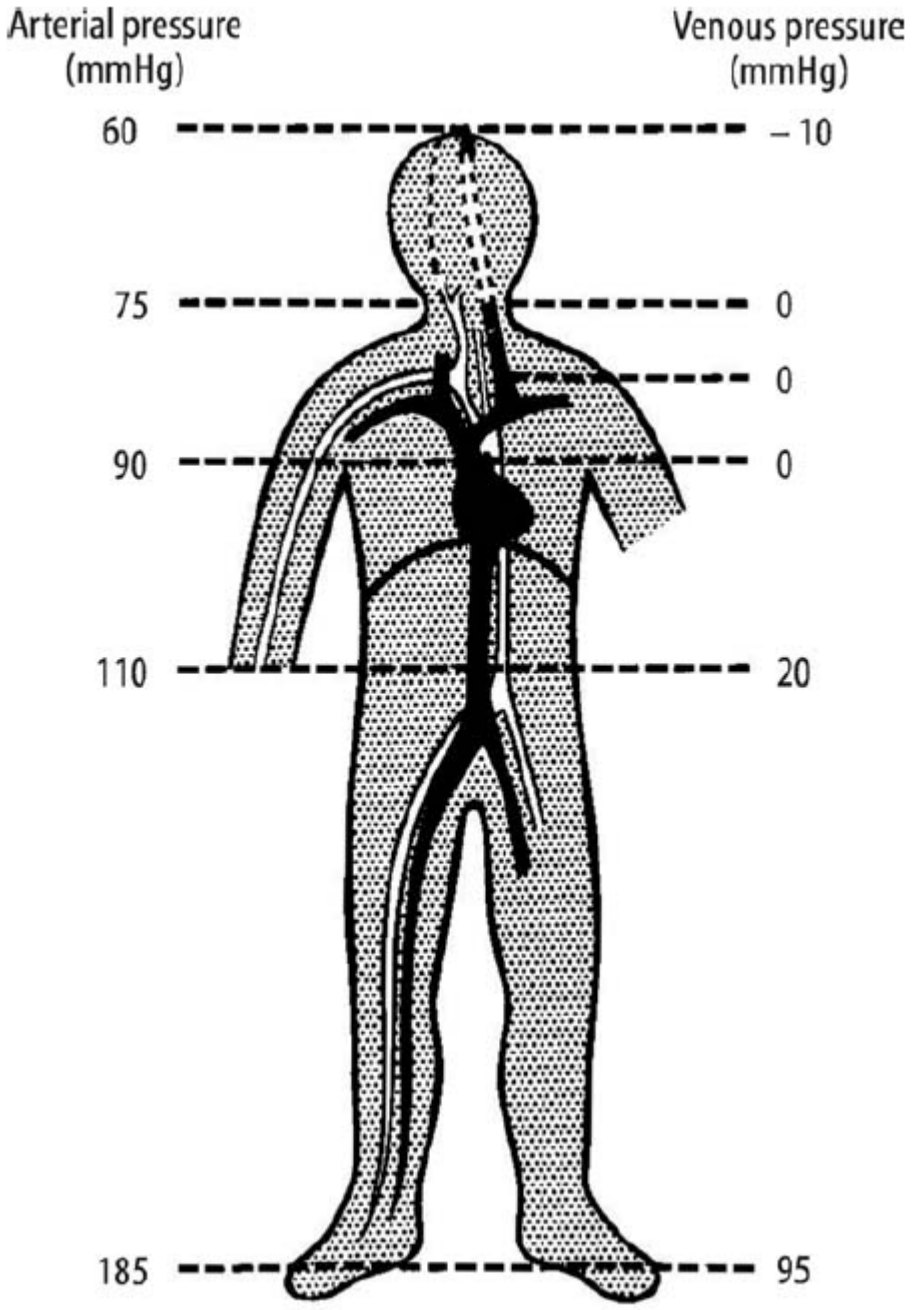

Figure 1.1 The effect of gravity on arterial and venous hydrostatic pressure in an upright, motionless human.

The highest pressures are in the ankles, leading to plasma filtration into the tissues. In the absence of muscle pumping and postural sway, oedema in the tissues contributes to diminished venous return, stroke volume (SV) and thus cardiac output (CO) (29). Prolonged orthostatic stress increases the physiological challenge and the baroreflex must compensate to maintain systemic blood pressure and cerebral perfusion.

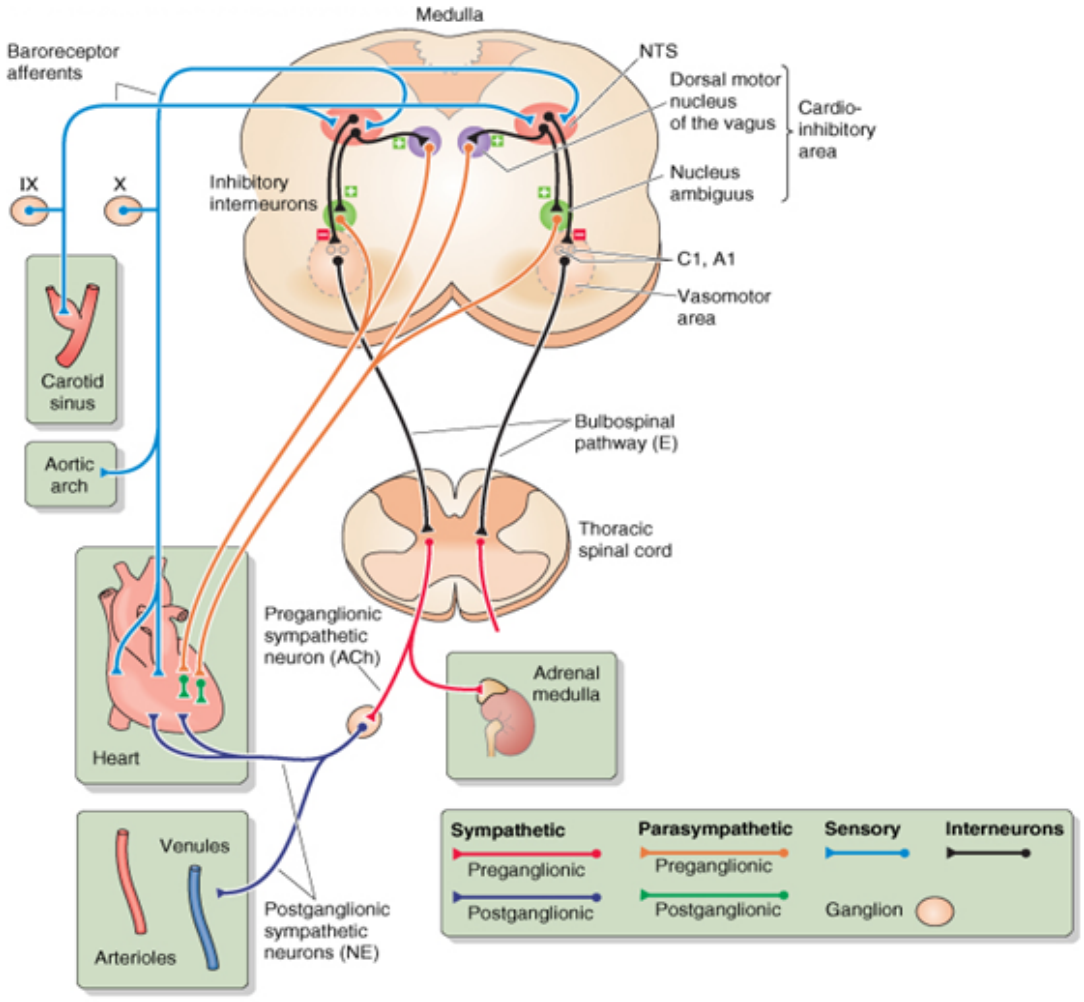

Figure 1.2. ***The afferent projections from the aortic and carotid baroreceptors to the cardiovascular centres in the medulla oblongata and their efferent projections to the heart and vasculature.***

During orthostatic stress, lower venous return and lower CO result in lowered arterial blood pressure (35). This is detected in the arterial walls by reduced stretch of the baroreceptor regions. There is a subsequent decrease in afferent firing from the carotid and aortic baroreceptors (and coronary baroreceptors, not shown) that is relayed to the nucleus tractus solitarius (NTS) (28). Excitatory interneurons project from the NTS to the dorsal motor nucleus of the vagus and the nucleus ambiguus; in turn, parasympathetic neurons project from these areas back to the heart (28). The reduced firing from the afferent nerves leads to decreased excitation of the parasympathetic branch, and thus tachycardia and increased cardiac contractility (28). Inhibitory interneurons also project from the NTS to the vasomotor area, and vasoconstriction results from decreased inhibition of the C1 area with a consequent increase in sympathetic nerve activity to the vasculature (28). The sensitivity of the baroreceptors is one important factor that influences the cardiovascular response mounted to a given orthostatic stress.

1.2.1. Vasovagal and carotid sinus syncope

Vasovagal syncope and carotid sinus syncope both involve a decrease in HR and blood pressure. VVS, also known as neurally mediated syncope and depicted in Figure 1.3, is thought to be the most common mechanism underlying orthostatic syncope in children and adolescents (2, 24). For an as yet unknown reason, in this form of syncope, the appropriate baroreflex-mediated vasoconstriction and tachycardia that occur during orthostatic stress is withdrawn, with an abrupt switch to vasodilatation and (often marked) bradycardia (29). This leads to cerebral hypoperfusion and loss of consciousness, or presyncope (29).

VVS is further subdivided into three types according to the relative contribution of the vasodilatation and bradycardia using the Vasovagal Syncope International Study (VASIS) classification (38):

- **Type 1, mixed response**: The blood pressure falls before HR, but HR declines at the time of syncope. HR does not fall to less than 40 bpm or falls to less than 40 bpm for less than 10 s, with or without asystole of less than 3 s (38).
- **Type 2A, cardioinhibition without asystole:** HR falls to less than 40 bpm for more than 10 s but asystole for more than 3 s does not occur. Blood pressure falls before HR falls (38).
- **Type 2B, cardioinhibition with asystole:** An asystole occurs for more than 3 s. The HR fall coincides with or precedes the blood pressure fall (38).
- **Type 3, vasodepressor:** HR does not fall more than 10% from its peak at the time of syncope (38).

Carotid sinus syncope has a similar mechanism but the onset of vasodilatation and bradycardia is triggered by external pressure on the carotid sinus (39). The typical scenario is a gentleman wearing a high collared shirt, which exerts pressure on the carotid sinus baroreceptors, located in the wall of the internal carotid artery (39). The body falsely interprets this deformation as arterial hypotension, which causes a decrease in the afferent firing rate of the glossopharyngeal nerve (Figure 1.2) (39). Consequently the cardiovascular centres within the medulla modulate efferent cardiovascular control with profound decreases in cardiac vagal activity leading to tachycardia, coupled with increased vascular sympathetic activity and subsequent vasoconstriction and

hypertension (35). Syncope can occur, upon quick removal of the stimulus, if the sudden switch to bradycardia and hypotension render cardiac output (CO) too low to maintain cerebral perfusion (39). In addition, the collar may also provide direct mechanical stimulation to the vagus nerve leading to bradycardia that has the potential to decrease cardiac output and in turn cerebral perfusion leading to syncope (39).

Vasovagal syncope is of particular interest in the young as it is common, and easily assessed with orthostatic stress testing (18).

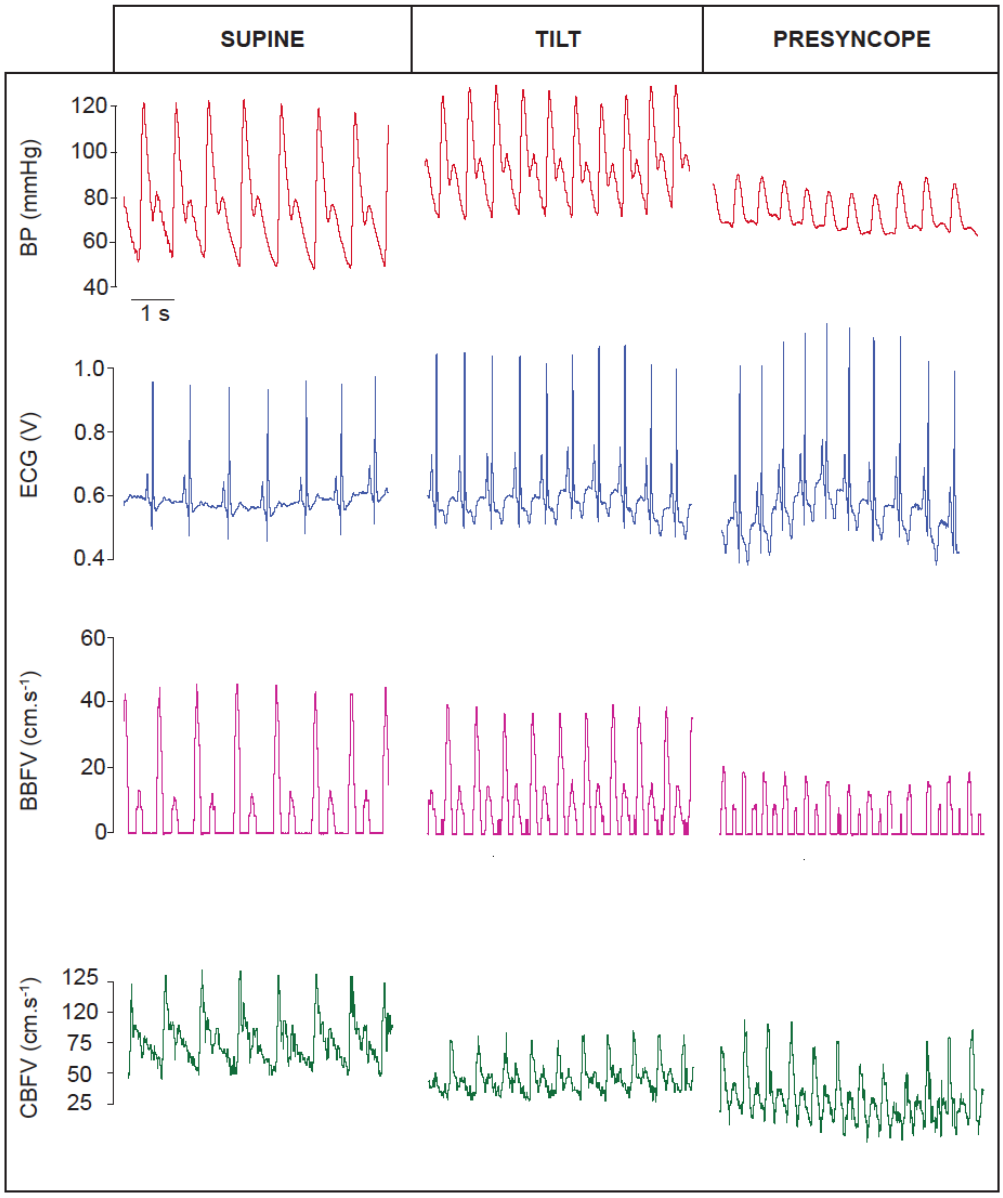

Figure 1.3. Example tracing showing cardiovascular responses to orthostatic stress in one paediatric subject.

Response of blood pressure (red), electrocardiogram (ECG, blue), and cerebral blood flow velocity (CBFV, green) are shown. Data are presented from a 16 year-old girl who experiences presyncope two to three times a week (usually when standing from a sitting position) and who exhibits VVS during the tilt test. Note the abrupt and profound fall in blood pressure at presyncope that is characteristic of VVS. There is a relative bradycardia at presyncope compared to her maximum HR (122 bpm during -20 mm Hg of lower body negative pressure (LBNP)).

1.2.2. Postural orthostatic tachycardia syndrome

An excessive increase in HR that compromises CO upon standing is termed Postural Orthostatic Tachycardia Syndrome (POTS) (12, 26, 27). Although increases in HR are usually associated with increases in CO, the lower venous return that results from assuming an upright position, accompanied by decreased ventricular filling during profound tachycardia leads to diminished CO during orthostasis. It is estimated that POTS is 5-10 times more common than orthostatic hypotension (Section **1.2.3**), typically occurring between the ages of 14-50 years (12, 26, 27). Five times more females are affected than males (12, 26, 27). To be diagnosed with POTS, a patient must have an increase in HR of ≥30 bpm, usually reaching ≥120 bpm within five minutes of standing and instability of blood pressure (18, 27). However, these criteria are based on data in adults, and may not apply well to children and adolescents in whom the normal HR response to orthostatic stress is greater (40). Further testing is required to determine the correct diagnostic criteria in a paediatric population (27).

POTS can also be subdivided into three types: neuropathic; hyperadrenergic; and the 'Grinch Syndrome' (12, 26, 27). The most common form of POTS is called neuropathic, or 'partial dysautonomic,' and is associated with a mild peripheral autonomic neuropathy (12, 27). During orthostatic stress, these patients are unable to vasoconstrict the peripheral vasculature to maintain blood pressure (12). As such, blood pooling in the lower extremities causes an excessive increase in HR and contractility to maintain cerebral perfusion (12). The body is able to compensate for orthostasis initially, but over time, tachycardia alone is insufficient to prevent syncope. Interestingly, partial dysautonomic POTS is commonly seen in adolescents aged around 14 years (12). It is thought that its onset follows a period of rapid growth and consequently a period of autonomic imbalance (12). The subsequent orthostatic intolerance is typically accompanied by severe headaches (12). The progression of this form of POTS worsens at age 16, but often disappears by the age of 19 (12, 40).

In hyperadrenergic POTS, patients experience orthostatic tachycardia as well as orthostatic hypertension due to elevated serum catecholamine levels with norepinephrine levels ≥600 ng/mL (12, 27). This disorder is thought to be due to a genetic point mutation that leads to dysfunction of the reuptake transporter protein that

clears norepinephrine from the intrasynaptic cleft (12). The high levels of norepinephrine spill over into serum, and the individual is thus in a hyperadrenergic state, reporting symptoms of sympathetic activation such as palpitations and anxiety (12, 27). As with all forms of POTS they also experience presyncope during orthostasis due to reduced ventricular filling time with lower CO and diminished cerebral perfusion (27).

Finally, POTS can occur in patients who are deconditioned, with poor exercise tolerance, or with prominent fatigue, which, in turn, contribute to a decreased heart size (27). Studies have shown that some POTS patients have a smaller left ventricular mass that is compounded by low blood volumes when compared to controls, and this leads to syncope because the heart is unable to maintain an adequate stroke volume (SV) to support cerebral perfusion during orthostasis (26). However, the role of hypovolemia has been questioned, with some studies suggesting that this is not a consistent feature of POTS (27). When blood pools in the extremities there is a consequent reduced blood volume, which further exacerbates the small SV (26). A compensatory baroreflex-mediated tachycardia is initiated to maintain CO, but this fails to adequately compensate for the impaired SV, and may even compound the problem by reducing left ventricular filling time (27). The small cardiac size in these patients has led to the condition being termed the “Grinch Syndrome” after the Grinch character from children’s books who has a heart ‘two sizes too small’ (26).

Given the relationship between some forms of POTS and adolescence, this form of syncope requires further investigation in this population (18).

1.2.3. Orthostatic hypotension and autonomic failure

Syncope secondary to a fall in blood pressure occurs in orthostatic hypotension and autonomic failure. Orthostatic hypotension is defined as a drop in systolic blood pressure of 20 mm Hg or diastolic blood pressure of 10 mm Hg after assumption of an upright position (41). It is common in patients with Parkinson’s disease and Multiple System Atrophy (MSA), as well as in other autonomic disorders (42). The resulting autonomic impairment leaves the individual incapable of mounting adequate baroreflex responses to counter the orthostatic stress (42). This is particularly devastating with lesions in the sympathetic nervous system (SNS), as occurs in MSA (preganglionic lesion) and

Parkinson's disease (postganglionic lesion) (42, 43). Thus, there is a steady decline in blood pressure until syncope occurs. However, these forms of syncope are extremely rare in paediatric populations.

1.2.4. Syncope secondary to cardiac abnormalities

Cardiac abnormalities can also lead to syncope, and are often life-threatening (44). Severe, non-sustained cardiac arrhythmias that compromise cardiac output will lead to syncope (44). Long QT syndrome (LQTS), a lengthening of ventricular repolarization or QT interval, is one such example (44). This prolongation of repolarisation increases the vulnerable period (the phase of cardiac repolarisation in which susceptibility to re-entry arrhythmia is greatest), with increased susceptibility to ventricular ectopy and ventricular tachycardia. In LQTS a characteristic polymorphic ventricular tachycardia (Torsades de Pointes) is commonly seen, and rapidly degenerates into ventricular fibrillation (44). This leads to profound and rapid decreases in CO, and subsequent reductions in cerebral blood flow with loss of consciousness (44). Sustained episodes are a leading cause of sudden cardiac death. Ultimately, a careful history must be taken when a patient presents with syncope (45). Although syncope is a risk factor for LQTS, patients with LQTS may also suffer from recurrent VVS and thus a medical history will help diagnose and treat those patients with an underlying cardiovascular cause (45, 46).

Structural heart disease such as valvular disease also negatively affects CO (8, 47). Aortic stenosis is one such example, where a narrowing of the aortic valve results in a limited CO (47). This becomes a problem when there is an increased requirement for oxygen (O_2) in the periphery in response to exercise, for example. If the heart cannot supply blood to meet the body's demands, a constant CO during increased activity can result in diminished cerebral blood flow and syncope (47).

Key features of cardiac (pre)syncope are a lack of prodromal symptoms, and episodes that occur unrelated to orthostatic stress.

1.2.5. Syncope due to cerebral vasoconstriction

Syncope can be triggered by cerebral vasoconstriction in the absence of systemic hypotension and bradycardia (48, 49). It is thought that impaired cerebrovascular

autoregulation results in inappropriate cerebral arteriolar constriction, which, in turn, reduces blood flow sufficiently to result in hypoxia and loss of consciousness, without systemic hypotension (50). This type of syncope has been misdiagnosed as psychogenic disorders in the absence of monitoring devices assessing the cerebral vasculature, such as transcranial Doppler ultrasound (51). This is because systemic hemodynamic variables remained constant despite patients complaining of orthostatic dizziness (51). There is limited evidence that children also suffer from this type of syncope (51). However, although reports of “cerebral syncope” and “paradoxic cerebral vasoconstriction” are commonly cited as evidence of this pathology, it should be noted that these observations are often taken in the absence of assessment of hyperventilation, which could precipitate cerebral vasospasm via hypocapnia (51).

1.2.6. Syncope in infants

It is important to consider syncope at a younger age as trends throughout the ageing process may help explain or uncover the triggers and mechanisms by which syncope occurs; again, a detailed medical history is pertinent in understanding this progression (52).

1.2.6.1. Reflex Anoxic seizures

In infants 6 months old to 2 years, reflex anoxic seizures or pallid infantile syncope can occur, typically triggered by a painful or frightening stimulus (53). This, in turn, leads to hyperstimulation of the vagus nerve and resultant bradycardia or cardiac arrest. Subsequently, cerebral ischaemia induces an anoxic seizure or attack (53).

1.2.6.2. Breath-holding spells

Breath-holding spells begin with a provocation such as an emotion or pain that leads to the child crying and whimpering (52). Eventually, the child becomes silent often with their mouth open in full expiration, and cyanosis or pallor may be present (52). A laboured inspiration resolves the spell, but the child loses consciousness (52).

The spells start within the first year of life up until the age of 2 years and occur in approximately 5% of children (54). By the age of six years, most children grow out of

these spells (54). Syncope has been shown to develop in late childhood and adolescent in about 17% of breath-holders following earlier breath-holding spells (54).

1.3. Data concerning the mechanisms of (pre)syncope in paediatric populations are lacking

Evidently, syncope is a complex condition that requires further investigation to uncover the underlying mechanism(s). VVS occurs most frequently in children and adolescents and appears to have a benign prognosis (37); however, further investigation is relevant for comparison with adult data. Similarly, POTS is seen in children and adolescents but it is unclear if adult diagnostic criteria are appropriate for this population. Therefore, the first aim of this thesis is to investigate and classify these specific types of syncope in children and adolescents. We hypothesize that adult diagnostic criteria will not apply to children and adolescents, because of altered cardiovascular reflex control in this population (10).

1.4. Pharmacological and non-pharmacological treatments for syncope are often ineffective

There is currently no gold standard treatment option for those suffering with syncope; however because syncope is multifactorial in origin, there are also numerous treatments options, each tailored to a specific underlying mechanism (55). The exact mechanism by which a faint occurs is often unknown, however, and the large variation in patients' experiences at presyncope and responses to medication render treatment challenging.

Typically, the first approach to treatment begins with a detailed history, physical exam, and an ECG (9, 56). Patients are educated on the underlying mechanism, if known, to help them prevent future episodes and encourage patient compliance with treatments (8, 57). Behavioural management is usually the first-line approach to treatment; if the patient is still unable to reduce the burden of their syncopal episodes, pharmacological treatment is initiated (57). Some patients will be considered for cardiac pacing (57). Although the importance of attention to the medical history and a basic physical examination is emphasized in identifying the cause of unexplained syncopal episodes, at

least one-third of patients remain undiagnosed (and therefore untreated) with this approach (24).

Behavioural management includes: salt loading; water drinking (to elicit the osmopressor response); tilt training; physical counter-manoeuvres; exercise training; and compression stockings (57). Salt loading and water drinking, through different mechanisms, have the common goal of increasing plasma volume to maintain blood pressure and prevent syncope (58-60). Recent research has uncovered a pressor response to water drinking within the hepatic circulation (61, 62). Osmoreceptors, thought to be transient receptor potential vanniloid 4 (TRPV4) channels, increase afferent firing in response to hypoosmolality in the liver and portal vasculature, which increases sympathetic activity and thus blood pressure through an unknown transduction mechanism (62). Tilt training is designed to teach an individual to recognize presyncopal symptoms to sit or lie down and thereby remove the orthostatic stimulus and abort a faint (55). This has a varied success rate, largely because not all individuals experience prodromal symptoms prior to an event. Physical counter-manoeuvres, exercise training and compression stockings assist with venous return (10, 58, 63, 64). Muscle pumps in the lower extremities aid veins in returning blood back to the heart instead of pooling in the tissues. Thus exercises such as squats, building muscle through exercise, or wearing a garment that applies external positive pressure to counteract the movement of fluid into the tissues are employed to maintain venous return and thus SV (58, 63, 64). Finally, cardiac pacing may be used to prevent the bradycardia of VVS (57). However, this approach is controversial because cardiac pacing cannot prevent the accompanying vasodilatation and hypotension, and accordingly it has limited success at preventing syncope (57, 65).

Pharmacological treatments include: beta blockers; alpha agonists; selective serotonin reuptake inhibitors (SSRI); and norepinephrine reuptake transporter (NET) inhibition (57). Beta-blockers have a negative inotropic effect on the heart, and β_1 antagonists block the action of norepinephrine preventing the increase in HR and contractility that occurs with sympathetic stimulation (66, 67). Historically they were used in VVS with the rationale that they would inhibit the Bezold-Jarisch reflex (pathological cardiac mechanoreceptor activation thought to precipitate the reflex withdrawal of sympathetic tone that occurs in VVS). However, their efficacy in the treatment of VVS is poor, and this reflex has since been shown not to be the trigger for VVS (1). Nevertheless, beta

blockers may be of use in the prevention of excessive tachycardia associated with POTS (27). Alpha agonists activate adrenergic receptors of the arteriolar and venous vasculature leading to increased vascular tone and blood pressure (68). Unfortunately, some of these drugs, for example midodrine, can result in supine hypertension associated with baroreflex failure and cardiovascular disease (68). SSRI diminish the actions of serotonin, which has been shown to cause vagally-mediated bradycardia, and decreased blood pressure (69, 70). There are a number of side effects with use of this drug, however, and it has shown limited success in the management of syncope in randomized controlled trials (71). Finally, NET inhibition may be of benefit in those with impaired HR responses to orthostatic stress, with some reports of efficacy (67). However, the long-term effects of NET inhibition are unclear (67).

1.5. Treatments are diverse and uniquely tailored to specific mechanisms

There is no gold standard treatment for syncope or presyncope because the ultimate trigger is often unknown (8). In children and adolescents, further evaluation to uncover an optimal treatment would be beneficial, with an emphasis on a non-pharmacological approach to prevent side effects from pharmacological options. Compression stockings are often cited as a non-pharmacological treatment option, with a benefit of being, at least theoretically, applicable to all forms of orthostatic syncope and presyncope. The rationale underlying their use is that they would reduce venous pooling and plasma filtration during orthostasis, and ameliorate the fall in venous return that occurs when upright. However, there is little research proving their efficacy (10). Therefore, the second aim of this thesis is to evaluate the efficacy of compression stockings as a potential universal treatment for orthostatic syncope and presyncope. We hypothesize that compression stockings are an effective non-pharmacological treatment option for the improvement of orthostatic tolerance (OT).

1.6. Project Aims

Aim 1: To evaluate syncope, presyncope, and autonomic control in children and adolescents

a. To determine the type and severity of cardiovascular autonomic dysfunction in children and adolescents with syncope and presyncope.
b. To evaluate the relative contribution of impaired cerebral autoregulation and/or hyperventilation to syncope or presyncope in children and adolescents.
c. To identify diagnostic criteria for children and adolescents with syncope and presyncope.

We hypothesize that the diagnostic criteria for the different forms of presyncope and syncope in children and adolescents will differ to existing adult data. There is limited research investigating autonomic control in children and adolescents so we determined whether autonomic function testing is appropriate in this population and enabled differentiation between different classifications of syncope.

Aim 2: To treat syncope and presyncope in children and adolescents with a non-pharmacological approach

a. To perform a pilot study in healthy young adults to test the efficacy of compression stockings for the treatment of orthostatic syncope and presyncope.
b. To perform a second pilot study (if stocking efficacy is proven in adults) evaluating the efficacy of compression stockings in children and adolescents.

We hypothesized that compression stockings will improve OT and thus provide a universal, non-pharmacological treatment for children, adolescents, and adults with orthostatic (pre)syncope. We performed this study in healthy adults first to determine their efficacy before moving into a patient population.

2. Methodology

2.1. Diagnostic Tests for Syncope of Cardiovascular Origin

2.1.1. The Valsalva manoeuvre assesses baroreflex control but may be unsuitable for children

Given the key role of the baroreflex in orthostatic cardiovascular control, many diagnostic tests for syncope incorporate a measure of baroreflex function. The Valsalva manoeuvre is one such test, which provides an assessment of the mechanical status of the circulation, the effectiveness of autonomic receptors, and efferent sympathetic and parasympathetic effectors (72). The manoeuvre is carried out by an individual forcefully expiring against a closed glottis, usually via a tube connected to a pressure gauge (73). The subject expires either at the end of a normal inspiration or after a maximal inspiration and is required to maintain a pressure in the range of 35-60 mm Hg for 10-40 seconds (73). The test is conducted in the supine position and HR and beat-to-beat blood pressure are continuously recorded throughout the different phases (73). The procedure is usually repeated three times at five-minute intervals (73). A typical response can be seen in Figure 2.1. The Valsalva ratio is the shortest R-R interval (RRI) during phase II or after phase II of the manoeuvre to the longest RRI in phase IV and is calculated as a measure of parasympathetic autonomic function (74). In this population, the Valsalva ratio of the 95th percentile is: 2.87 in males and 2.73 in women aged 10-29 years (75).

The main benefit to this assessment is its non-invasive nature, and minimal equipment required for testing. However, it is not possible to determine vascular resistance responses using this test, and not all individuals are capable of generating a strain of 40 mm Hg (76). Of relevance to the present study, children have particular difficulty in generating the required pressures, although there is evidence that they can produce a strain at 30 mm Hg and improve the magnitude of the strain with practice (72). Due to

the routine clinical use of the Valsalva manoeuvre, non-invasive nature and relative simplicity to conduct, we will evaluate its use for discriminating children with different types of syncope.

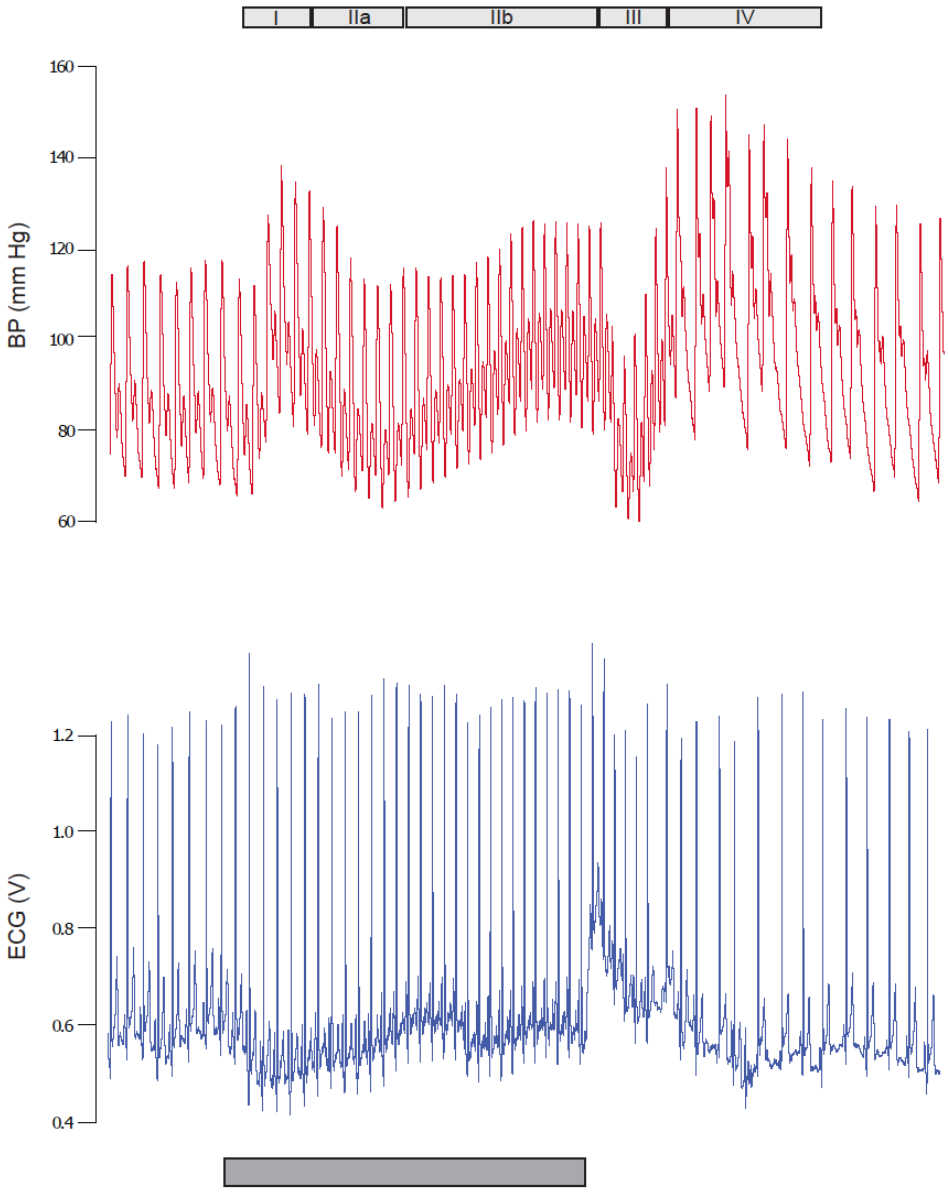

Figure 2.1 ***A beat-to-beat arterial blood pressure and ECG tracing highlighting the four phases of the Valsalva manoeuvre.***

The Valsalva strain (30 mm Hg for 20 s) is shown in the solid grey bar. The Valsalva response begins with an increase in arterial blood pressure due to increased intra-thoracic pressure at the onset of the strain and bradycardia (phase I) (77, 78). As the strain is maintained, venous return is diminished due to the pressure exerted onto the vessels and chambers of the heart. In turn, SV and CO decline leading to decreased arterial blood pressure and tachycardia (phase IIa) (77, 78). A baroreflex-mediated response increases peripheral vascular resistance and heart rate to maintain blood pressure (phase IIb) (77, 78). At the release of strain, arterial blood pressure returns to near baseline as intra-thoracic pressure decreases (phase III) (77, 78). This is followed by an overshoot in arterial blood pressure due to an increase in SV with concomitant decrease in peripheral vascular resistance and reflex bradycardia (phase IV) (77, 78).

2.1.2. Impaired cerebral autoregulation may contribute to syncopal events, and can be assessed non-invasively

Cerebral autoregulation maintains cerebral blood flow relatively constant over a range of cerebral perfusion pressures from 60 mm Hg to 150 mm Hg (Figure 2.2) (79, 80). Orthostatic stress is a challenge for autoregulation because when an individual is upright, gravitational fluid shifts will decrease cerebral perfusion pressure by approximately 20 mm Hg relative to the pressure at heart level (Figure 1.1) (29). For this reason, consciousness begins to be lost during orthostasis when systolic arterial pressure (SAP) approaches 80 mm Hg, corresponding to a cerebral perfusion pressure at the lower limit of the autoregulatory range (18, 29, 80, 81). Ultimately, the associated failure to maintain cerebral blood flow will lead to syncope.

Orthostatic stress is known to induce hyperventilation and the resulting hypocapnia causes peripheral vasodilatation and cerebral vasoconstriction (29, 79, 81). In the periphery, vasodilatation leads to reduced systemic blood pressure, exacerbating the orthostatic reduction in cerebral perfusion pressure (29). In the cerebral vasculature, vasoconstriction leads to increased cerebral vascular resistance (CVR), coupled with a decrease in cerebral perfusion pressure, leading to a decrease in cerebral blood flow (29, 79). Thus, hypocapnia-induced hypoperfusion of the cerebral vasculature could play a key role in orthostatic (pre)syncope (81).

Indeed, adults with syncope are reported to have increased cerebral and peripheral vascular reactivity to carbon dioxide (CO_2) compared to healthy controls (81). In this past study, both patients and controls exhibited a similar hyperventilatory response to orthostatic stress, with a similar decline in end-tidal CO_2 ($P_{ET}CO_2$) (81). However, there was a greater reduction in cerebral blood flow and greater vasodilatation in the forearm vasculature in the patients, and this would increase susceptibility to fainting (81). In addition, some adult patients have impaired autoregulatory control, and this could also contribute to their susceptibility to syncopal episodes (80).

2.1.2.1. Cerebral autoregulation

Data in adults highlight the importance of cerebral responses in the aetiology of syncope (29, 80, 81). However, responses in children are unknown. The analysis of cerebral

autoregulation in children, not commonly carried out in a clinical setting, will be an important addition to this study (81).

2.1.2.1.1. Static autoregulation

Static autoregulation refers to the assessment of cerebral autoregulation during steady-state, before and after blood pressure manipulation (82). Transcranial Doppler ultrasound is typically used to measure cerebral blood flow velocity (CBFV) (Section **2.2.4**). The autoregulatory curve describes the range over which CBFV is maintained despite changes in cerebral perfusion pressure (Figure 2.2) (79, 83). If cerebral perfusion pressure is outside of this range, autoregulation fails and there is a subsequent decrease or increase in CBFV.

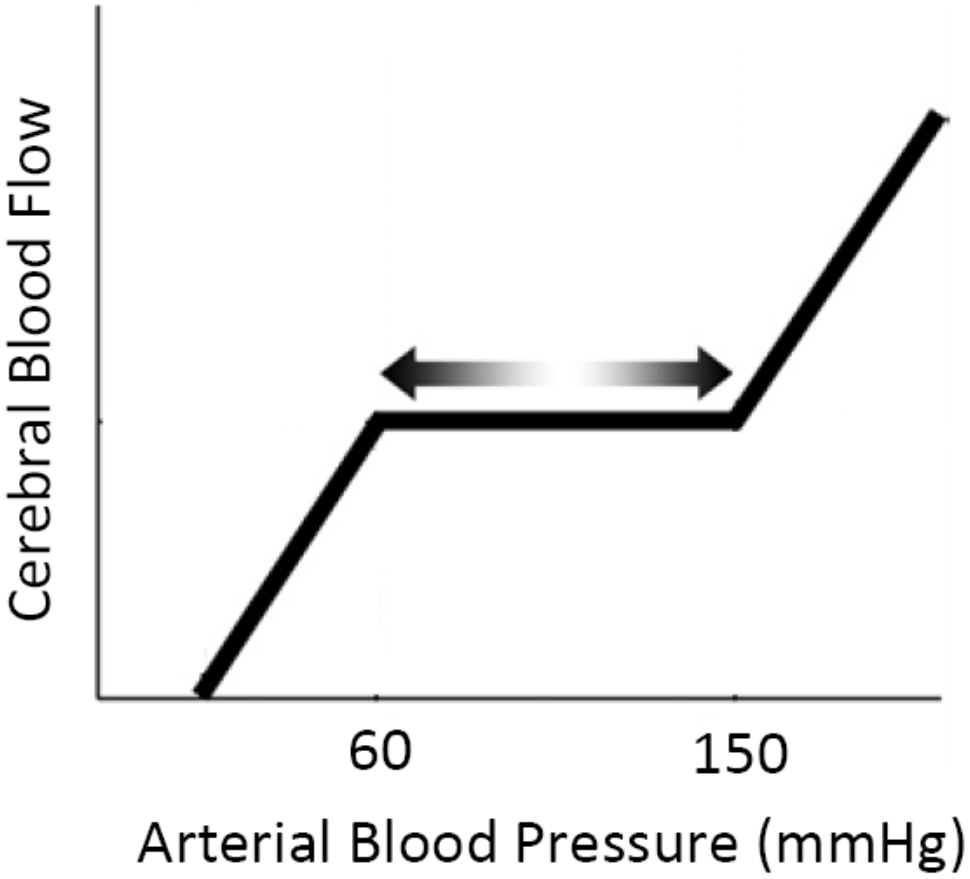

Figure 2.2. The cerebral autoregulatory range.

Cerebral autoregulation maintains cerebral blood flow despite fluctuations in systemic blood pressure within the range 60–150 mm Hg (79, 80, 84). An increase or decrease in systemic blood pressure instigates a reflex modulation of cerebrovascular resistance (CVR) typically via small cerebral arterioles (<100-250 μm in diameter) (84). According to Ohm's law, flow is kept constant if a decrease in pressure is accompanied by a decrease in resistance or alternatively if an increase in pressure is accompanied by an increase in resistance. The ability to autoregulate cerebral blood flow is influenced by CO, sympathetic nerve activity, and the arterial partial pressure of carbon dioxide (P_aCO_2) (79). One particularly potent modulator is P_aCO_2, and this is of particular relevance during prolonged orthostasis when P_aCO_2 declines markedly (79).

Static autoregulation can be determined from the correlation and gradient describing the relationship between cerebral mean arterial pressure (CMAP) and cerebral blood flow velocity (CBFV) over the autoregulatory range (between 60 mm Hg and 150 mm Hg) (80). A correlation and gradient of zero indicates intact autoregulation, with cerebral resistance responses buffering any change in CMAP such that CBFV remains constant (80). In adults with syncope there is a strong correlation between CMAP and CBFV, indicating impaired autoregulation that could contribute to episodes of cerebral hypoperfusion and syncope (80).

2.1.2.1.2. Dynamic autoregulation

Dynamic autoregulation refers to beat-to-beat control of CBFV in response to systemic pressure changes (83). Cross-spectral analyses of spontaneous oscillations in CMAP and CBFV are commonly used to evaluate dynamic autoregulation (80). Again, the use of transcranial Doppler ultrasound is essential for measures of dynamic autoregulation, which requires beat-to-beat CBFV recordings.

Cross-spectral analyses will be applied to CBFV and CMAP signals to retrieve the sum of their sinusoidal components (83). Specific sinusoids will be evaluated at low (LF, 0.075-0.15 Hz) and high (HF, 0.15-0.4 Hz) frequencies to measure the phase difference between CBFV and CMAP (83). This will allow quantification of the time delay between changes in CMAP and subsequent oscillations in CBFV (83, 85). A phase of zero indicates perfect autoregulation (80). However, in practice a positive phase is typically seen, and this has been shown to be due to the physiological delay in modifying CVR in smooth muscle (83). The gain of the CBFV-CMAP dynamic relationship can also be evaluated and represents the amplitude ratio between output, CBFV, and input, CMAP, at each frequency (83). Adequate autoregulation is associated with low gain so that with every increase or decrease in CMAP, there is only a small increase or decrease in CBFV. Finally, the coherence function provides information on the reliability of the phase and gain estimates at each frequency, as well as the strength of the relationship between CMAP and CBFV. Changes in CBFV are considered to be linearly related to changes in CMAP when coherence is greater than 0.5 (83, 85).

2.1.2.2. Cerebral reactivity to arterial carbon dioxide levels is easily assessed in pediatric patients

Cerebrovascular carbon dioxide (CO_2) reactivity refers to the response of CBFV to changes in P_aCO_2 as depicted in Figure 2.3 (86). It is easily evaluated clinically and noninvasively using respiratory manipulation to alter P_aCO_2, while measuring any induced changes in CBFV using transcranial Doppler ultrasound (87, 88). Hence, cerebral reactivity to P_aCO_2 is calculated as the percentage change in CBFV per mm Hg change in the partial pressure of end tidal CO_2 ($P_{ET}CO_2$) (87-89). It is usual to assume $P_{ET}CO_2$ is an accurate estimate of P_aCO_2, negating the need for invasive measures of arterial blood gases (87-92). While this is generally accepted to be valid, there are reports of situations in which $P_{ET}CO_2$ does not accurately represent P_aCO_2, and this should be considered when interpreting the results of these measures (93).

This additional test will provide insight into the possible role of hyperventilation-induced hypocapnia in syncope and presyncope in children and adolescents.

2.1.3. Tilt testing with lower body negative pressure is the "gold standard" for orthostatic stress testing

In the late 1980s tilt testing was reported as useful in diagnosing VVS (2). Since then it has been used to assess OT in patients with syncopal episodes of unknown cause in the absence of structural heart disease, as routine evaluation in POTS, as well as in healthy subjects to study the VVS reflex (2, 19, 27, 38, 55, 80, 94). Head-up tilt is commonly used in adults for the diagnosis of syncope but its use in paediatrics is debated (9). However, for children with syncope of unknown origin, tilt testing is reported to be both useful and necessary (9). Tilt testing has also been useful in distinguishing syncope from epilepsy and from falls in the elderly (18).

There are a number of head-up tilt protocols to determine an individual's OT that fit into three categories: passive head-upright tilt only; passive tilt accompanied by pharmacological provocation; and passive tilt with LBNP (2).

Typically, a passive tilt includes 20-60 minutes of orthostatic stress continued until the onset of hypotension, bradycardia and/or presyncope in patients (2, 94). However, the main drawback of this procedure is its inability to invoke symptomatic hypotension in all

individuals undergoing the test and subsequent lack of sensitivity (94). As a result, different methods were explored to heighten the orthostatic stress and improve sensitivity (94). Additional pharmacological provocation using sublingual nitrates or intravenous isoprenaline was introduced to increase the orthostatic challenge (4, 38, 94). However, these interventions typically increase sensitivity at the cost of unacceptable decreases in specificity (95). Other problems associated with these pharmacological approaches relate to their mode of delivery, often requiring an intravenous catheter, which is itself known to provoke a vasovagal reaction (94). This is mediated either through a psychological response, the so-called “blood phobia” or “needle faint,” or in response to endothelial injury and activation of an immune response that releases endogenous vasodilators such as nitric oxide (NO) (81). This is likely a key reason for the loss of specificity of these interventions. Furthermore, isoprenaline is given based on the rationale that it will stimulate ventricular receptors and trigger the Bezold-Jarish reflex, a phenomenon now known not to be the trigger for syncope (29, 94). Other problems with this approach are related to catecholamine-associated complications such as ventricular arrhythmia (13, 19, 94). In addition, sublingual nitrate causes systemic vasodilatation with a high positive response rate immediately after receiving the drug, again contributing to loss of specificity of the test (19, 38).

A final approach is to combine a passive tilt test with the application of LBNP, providing a stronger orthostatic stress without invasive procedures or drug side effects (94). Tilt testing with LBNP is able to obtain an endpoint in all subjects undergoing the test with a specificity of 92% and sensitivity of 85% (94-96). It is also highly reproducible (4, 94-96). For these reasons, it has been argued to be the “gold standard” for orthostatic stress testing, and as such was the method used in the present study.

Tilt testing is considered a safe technique with low risk to individuals undergoing the test; however, incidences of life-threatening ventricular arrhythmia occurring in individuals with structural heart disease have been reported when isoproterenol was administered during the protocol (97-99). No contraindications have been reported with the use of glycerol trinitrate (18).

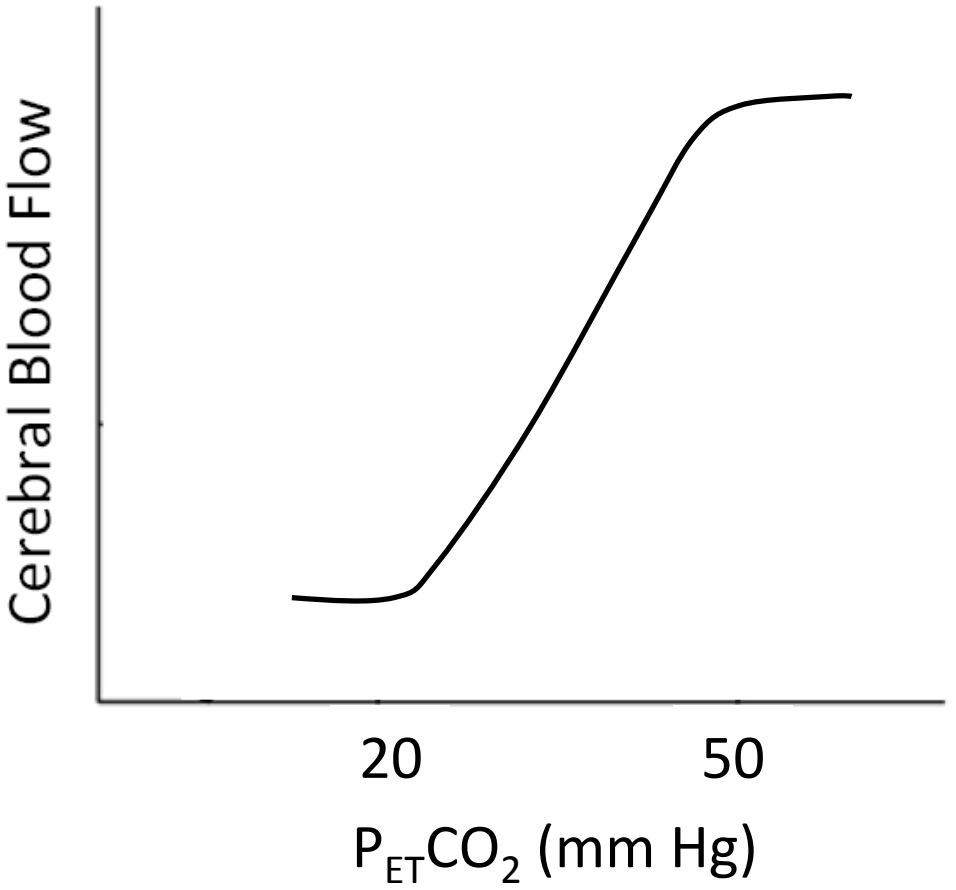

Figure 2.3 The influence of $P_{ET}CO_2$ on cerebral blood flow.

There is a linear response in cerebral blood flow across the range of 20 mm Hg to 50 mm Hg of $P_{ET}CO_2$ (86). The gradient describing the linear portion is an indication of cerebral reactivity to $P_{ET}CO_2$ with a steep gradient indicating greater reactivity to changes in $P_{ET}CO_2$. In patients with syncope, this gradient has been shown to be steeper than in controls and therefore one factor contributing to presyncope because low $P_{ET}CO_2$ in the cerebral vasculature causes vasoconstriction and a reduction in cerebral blood flow (81).

2.1.4. Spectral analysis: a non-invasive measure of cardiovascular neural regulation

Blood pressure and HR are tightly regulated on a beat-to-beat basis through a delicate interplay between two branches of the autonomic nervous system: the sympathetic and the parasympathetic nervous systems (100). Accordingly, blood pressure and HR continuously oscillate with each beat of the heart; these oscillations are driven, at least in part by fluctuations in autonomic activity (100). By examining their variability we can delineate the independent roles of the sympathetic and parasympathetic responses as well as cardiac baroreflex control (37, 100). Their variability can be described as a function of time, in the time domain, or with power spectral analyses in the frequency domain, as the sum of their oscillatory components defined by their frequency and amplitude (100). Spectral analysis, therefore, is a useful tool used to gain a clearer understanding of the role of the autonomic nervous system in the pathogenesis of (pre)syncope, providing a sensitive, non-invasive, quantitative approach that is suitable for use in children and adolescents (37, 100, 101).

Spectral analysis breaks down the blood pressure and HR waveforms, or fluctuating time series, into their frequency components by computing the power of each signal at predetermined frequency values (101). These predetermined frequency values reflect the relative contribution of the sympathetic and parasympathetic responses to these oscillations (37). There are three frequency regions of interest: very low frequency (VLF, 0.02-0.07 Hz); low frequency (LF, 0.075-0.15 Hz); and high frequency (HF, 0.15-0.4 Hz) (102, 103). We used autoregressive modeling of spectral analysis for blood pressure and HR signals. This technique is preferable because it ignores components of the signal that do not fit with the model, minimizing the confounding influence of occasional background noise (104).

2.1.4.1. Blood pressure variability

In the VLF range, blood pressure variability (BPV) is thought to represent the influence of catecholamines, angiotensin, heat stress and hypovolemia, driven by myogenic vascular responses to perturbations in blood pressure (102, 104). The LF oscillations

represent sympathetic drive to the resistance vessels, and the HF oscillations result from mechanical changes in intrathoracic pressure associated with respiration (102, 103, 105).

2.1.4.2. Heart rate variability

The VLF heart rate variability (HRV) represents many influences on the heart, such as thermoregulation, the renin-angiotensin system, and endothelial factors such as NO (103). LF HRV is due to both vagal and sympathetic cardiac control due to baroreflex mediated oscillations in vagal outflow driven by sympathetically induced LF BPV. HF HRV represents cardiac vagal control (102-104).

2.1.4.3. Baroreflex control

A bivariate autoregressive model was used to quantify the frequency-related squared coherence, phase shift, and transfer function gain between SAP and RRI in the LF range (106). The values for each of these were recorded where the coherence was greater than 0.5 (showing a statistically significant correlation between the two signals) and the central frequency at this point was noted. Coherence represents the relationship between blood pressure and RRI with zero indicating no relationship and 1 indicating perfect interdependence (106). The phase shift qualifies the baroreflex delay, and the transfer function gain provides a measure of the cardiac baroreflex sensitivity (107).

As with the Valsalva manoeuvre and cerebral reactivity to CO_2, if this non-invasive technique can be used to distinguish different types of syncope, then other more onerous testing procedures could be avoided, which would be particularly pertinent in paediatric populations.

2.2. Equipment

2.2.1. Continuous blood pressure monitoring using the Finometer™

The rapid onset of presyncope, and the dynamic cardiovascular measures proposed, requires beat-to-beat blood pressure monitoring for patient safety and integrity of the data. Beat-to-beat blood pressure can be measured invasively with an arterial catheter

(108, 109). However, because of the known effect of invasive procedures on OT and the young age of the volunteers, this technique may not be suitable for the present study (94). Recent advances have led to the development of non-invasive beat-to-beat blood finger pressure monitoring devices, the most notable of which is the FINger Arterial PRESsure, or Finapres™ (109). This device was superseded by the Finometer™, which uses the same technology. These devices employ the volume clamp method to measure finger arterial pressure (109). In this technique, an inflatable finger cuff with an infrared light source and detector on either side of the cuff is used to generate a beat-to-beat plethysmogram from variations in the amount of infrared light absorbed by the blood during systole and diastole. During measurements the diameter of the digital artery is kept constant (clamped), regardless of the changes in arterial pressure with each heartbeat. This is achieved by varying the pressure of the inflatable bladder, which is connected to a fast pressure servo controller system via the signal from the plethysmograph (110, 111). In this way the cuff pressure provides an indirect measure of intra-arterial pressure (112).

An additional height correction unit consists of a pressure transducer placed on the finger cuff, a liquid filled tube, and a compliant plastic bag, closed, at the other end (112). This measures height changes at the finger compared to the reference at heart level to avoid a hydrostatic error when blood pressure is measured if the hand is not positioned at the heart level (112).

In addition to measuring beat-to-beat arterial pressure, these devices incorporate a lead II ECG, and permit the calculation of HR, SV, CO and total peripheral resistance (TPR) using the Modelflow™ technique (112). This technique generates an aortic flow waveform from finger arterial pressure by simulating a non-linear three-element (including aortic characteristic impedance, arterial compliance, and peripheral vascular resistance) model of aortic input impedance (112). The computed aortic flow waveform per beat is integrated (from the area under the flow pulse in systole) to provide left ventricular SV and consequently CO (the product of SV and HR) (110). The Modelflow™ technique takes into account the elastic behaviour of the aorta, which varies nonlinearly with changes in distending pressure, by incorporating age, sex, height and weight into the algorithm (110). The Modelflow™ aortic age has been characterized in adults aged 20-88 year olds based on age-related changes in aortic compliance (113, 114); but this

technique has not been validated in children. Since the aortic stiffness index is more important in elderly populations, this technique has already been used in children (72, 115). TPR is calculated from the measured blood pressure divided by the derived CO, according to Ohm's Law (114).

Overall, this technique is an effective method to study rapid blood pressure changes during orthostasis and cardiovascular manoeuvres such as Valsalva straining, and has been extensively validated against measures of intra-arterial blood pressure as well as measures of CO by thermodilution and indirect Fick techniques (77, 114, 116-120). Two internal calibration methods are incorporated into the device that ensure its accuracy (112). Additional benefits are its non-invasive nature, which is crucial during tilt testing to ensure an accurate measure of OT is obtained, as well as the ability to obtain continuous beat-to-beat data. This is important for safety when inducing rapid blood pressure changes, as well as to ensure integrity of the data at presyncope. The inclusion of indirect measures of SV, CO, and TPR would also be expected to be of use when differentiating between types of syncope.

2.2.2. Arrhythmia detection using the electrocardiogram

The Dutch physiologist Willem Einthoven is credited with the development of the clinical ECG and although vector cardiography with three standard leads was introduced in 1912, it was not until approximately the 1930s that it became common use in a hospital setting (121, 122). The ECG is now a well-established method for monitoring HR and detecting arrhythmias (121, 123).

2.2.3. End tidal gases to evaluate a possible role for hyperventilation

The measurement of P_aCO_2 and P_aO_2 is difficult and invasive, requiring arterial catheterization, but monitoring $P_{ET}CO_2$ and $P_{ET}O_2$ is convenient, continuous and noninvasive (123). In addition, $P_{ET}CO_2$ and $P_{ET}O_2$ provide reliable estimates of arterial values (123). Therefore, we used $P_{ET}CO_2$ and $P_{ET}O_2$ to evaluate the potential contribution of hyperventilation-induced hypocapnia upon cerebral and peripheral vascular tone during (pre)syncope in children and adolescents (123).

$P_{ET}CO_2$ and $P_{ET}O_2$ was continuously recorded using an Oxigraf analyzer (124). The gas sample flows from a nasal cannula into the analyzer, where the concentration of O_2 in the sample is first analyzed using absorption spectroscopy (124). A laser diode emits a beam of light at 760 nm, the wavelength absorbed by O_2, which is focused onto a signal photo-detector (124). The laser is tuned thermally and electronically to an O_2 absorption line chosen for its absorption strength (124). Any O_2 present in the gas sample absorbs light from the laser and the amount of light registered on the detector is thus inversely proportional to the O_2 concentration (124, 125). The analysis occurs every 10 ms to obtain O_2 levels between 0 and 100% (125). Between measurements the laser is tuned to an O_2 non-absorption wave length (125). The gas sample is then directed to a sample chamber within a non-dispersive infrared sensor for measuring CO_2 (124). Again, infra-red light is directed through the chamber towards a detector, this time at a wavelength of 4289 µm, which is absorbed by CO_2 (125). There is an optical or wavelength filter in front of the detector that eliminates all light except the wavelength absorbed by CO_2 (124). The amount of light registered on the detector is inversely proportional to the gas concentration. This analysis also occurs every 10 ms over a range of 0 to 13% CO_2 (125).

2.2.4. Blood flow velocity in the middle cerebral and brachial arteries

Transcranial Doppler ultrasound is a noninvasive technique for determining beat-by-beat relative changes in CBFV (Figure 2.4) (126-129). It relies on the Doppler shift principle, whereby the frequency of an emitted wave differs from the frequency of the wave reflected back from a moving target, for example a red blood cell (130, 131). The difference in frequency is called the Doppler shift, and is dependent on the speed of the moving target (blood flow) and the direction of motion either toward or away from the receiver (the ultrasound probe) (130).

The difference between the emitted (f_0) and the reflected (f_r) ultrasound frequencies, the Doppler shift frequency (f_D) is determined as follows:

$$f_D = (f_r - f_0) = \frac{2f_0 v}{c} \cos \Theta$$

where v is the red blood cell velocity in $m \cdot s^{-1}$, cos Θ is the cosine of the angle between the direction of blood flow and the path of the ultrasound beam from the transducer, c is the velocity of sound in the medium, or average speed of ultrasound in soft tissue (1540 $m \cdot s^{-1}$), and 2 represents two Doppler shifts that occur (from the stationary receiver to the moving red blood cells, and from the moving red blood cells back to the stationary receiver) (126, 129). The angle between the ultrasonic beam and the artery is important to determine accurate velocity measurements: if the angle varies between 0-30˚ its cosine varies between 1-0.86 resulting in a maximum error that is less than 14% in the measurement of the peak flow velocity (127, 131). If the angle changed from 30˚ to 40˚ (cosine varying from 0.866-0.766) this would introduce an error of 10% between angles, but an error of 23% compared to an angle of 0˚. Conversely, at a higher angle of 50˚, if this was changed to 60˚, (cosine varying from 0.642-0.5) an error of 14% would occur between angles, but an error of 50% compared to an angle of 0˚. Thus, at higher angles the error in the measurement of peak flow velocity increases. In our experiments we maintain a fixed angle between phases of the orthostatic stress test, ideally of ≤30˚. We aim to determine the change in flow velocity from supine to tilt phases of the test, thus our angle of insonation can be greater than 30 if it is maintained throughout the phases.

With transcranial Doppler ultrasound a transducer is aimed at the blood flowing through the middle cerebral artery (MCA) and emits bursts of ultrasound at a frequency of 2 MHz (127). The MCA is appropriate for these measurements because anatomically the artery runs towards the probe, which is typically positioned on the temporal window (126). The emission frequency is determined by the following formula:

$$f_{opt} = \frac{90}{d}$$

Where f_{opt} is the optimum frequency in MHz and d is the distance in mm between the transducer and the structure being examined (130). Typically, the MCA in adults is found at depths ranging from 45-60 mm, therefore the optimum frequency is 2 MHz (130). We also used an 8 MHz ultrasound probe to determine peripheral vascular resistance responses from the brachial artery (Figure 2.5). The transducer emits electrical activity at 8 MHz because the brachial artery is located at depths 10-30 mm.

This technique provides reliable estimates of blood velocity. However, the parameter of interest is blood flow. If one assumes that the diameter of large conduit vessels, such as the MCA and brachial artery remains constant, with resistance responses occurring in smaller downstream vessels, then velocity would be proportional to flow. This assumption is valid during perturbations including orthostatic stress and hypo and hypercapnia (126, 129, 132).

Reproducibility data (unpublished) has been conducted in our laboratory with (1) a mean difference between two supine measurements on two different days of 0.28±-8.4 $cm.s^{-1}$ ±one standard deviation in the MCA (p=0.95) and (2) a mean difference between two supine measurements on two different days of -2.3±-5.1 $cm.s^{-1}$ ±one standard deviation in the brachial artery (p=0.43).

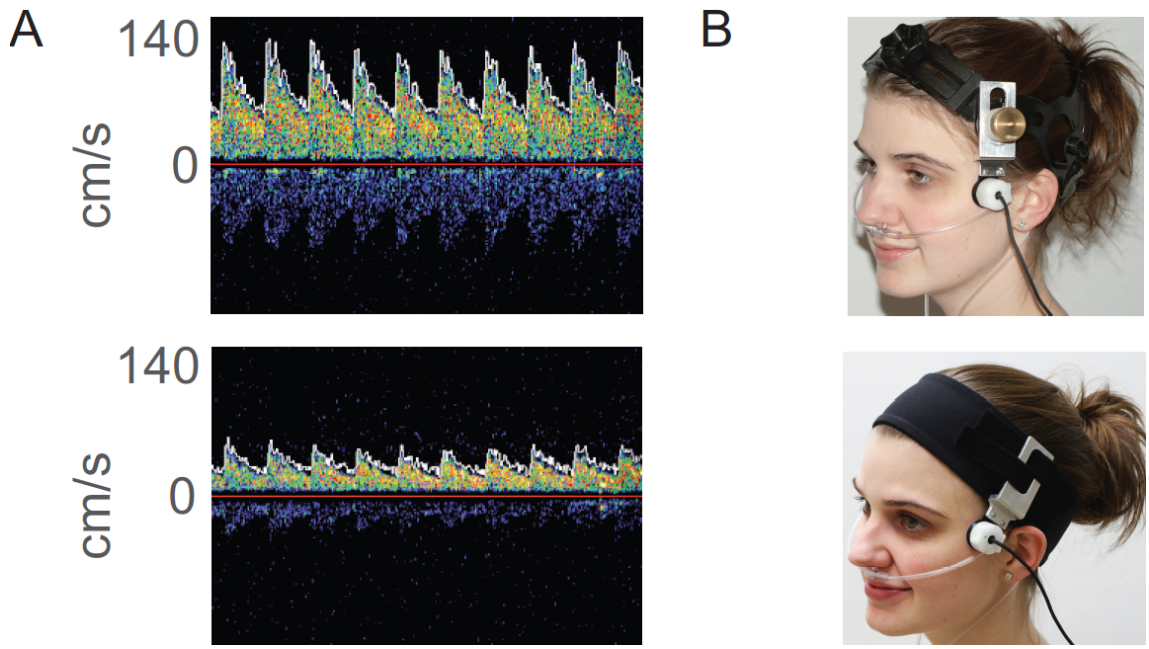

Figure 2.4 ***Middle cerebral artery blood flow velocity measurement.***

A. A 2MHz ultrasound probe is positioned on the transtemporal window overlying the MCA. An example showing MCA 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 MCA. The bottom panel shows an example from the posterior cerebral artery. Although the flow profile is similar to the MCA, 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) (133).

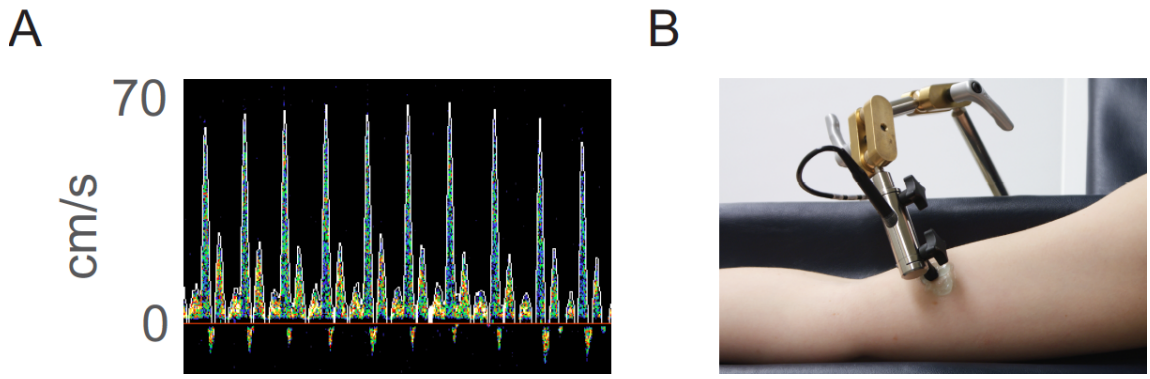

Figure 2.5 ***Brachial blood flow velocity measurement.***

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 (133).

3. Investigating syncope in children and adolescents

3.1. Background

Syncope is defined as a transient loss of consciousness and postural tone due to global cerebral hypoperfusion and is commonly reported in clinical paediatrics. It is estimated that 15-20% of all children and adolescents will experience at least one episode of presyncope (orthostatic dizziness prior to fainting) or syncope by the time they reach 18 years of age (14, 20). The prevalence is often underestimated because many do not report their symptoms to physicians if the episode is perceived as being unremarkable (21, 22). Research to date focuses on adults and therefore our understanding of the mechanisms underlying syncope in children and adolescents is limited.

The autonomic nervous system is responsible for the interplay between sympathetic and parasympathetic activity, which regulate blood pressure and heart rate during orthostasis via the baroreflex. Its dysfunction can be temporary or due to a diseased state in an individual and in both cases prompts further clinical evaluation (14). Autonomic function testing evaluates the autonomic nervous system to obtain an understanding of cardiovascular reflex control during orthostasis. It is thought that the autonomic nervous system plays a role in the pathogenesis of VVS, the most commonly diagnosed classification in children and adolescents, but the mechanism by which this occurs is unknown (134). Assessment of any autonomic deficits in children is hampered by uncertainty as to whether adult diagnostic criteria can be applied in younger populations.

The most appropriate autonomic function test(s) for children and adolescents with orthostatic (pre)syncope is unclear. The Valsalva manoeuvre is frequently used in adult clinical practice and represents a simple test if proven to be effective and attainable in children and adolescents. Tilt testing has been performed in adults to achieve diagnostic criteria for the various classifications of syncope and represents a repeatable, sensitive, and specific test (94, 96, 135). One benefit of this test is the ability to evaluate

presyncope by reproducing symptoms in patients and then comparing their responses, and the time taken to provoke presyncope, to those of controls. Finally, given the final common pathway of cerebral hypoperfusion in all forms of orthostatic (pre)syncope, it is pertinent to assess the relative contribution of impaired cerebral autoregulatory control, and cerebral reactivity to orthostatic hypocapnia, in children and adolescents with orthostatic presyncopal and syncopal episodes. We aimed to evaluate the severity and type of cardiovascular autonomic dysfunction in children with orthostatic (pre)syncope using these three tests. Ultimately, we aimed to ascertain whether children are capable of performing these tests in order to guide future diagnosis with tools that are accessible in a clinical setting. We hypothesize that adult diagnostic criteria will not be applicable in children and adolescents and will need modification to properly classify presyncopal and syncopal episodes in this population.

3.2. Methods

3.2.1. Subjects

A paediatric cardiologist referred children with orthostatic presyncope and/or syncope from the Heart Centre in BC Children's Hospital. Controls were recruited through poster advertisements. All children were aged 6-18 years old and provided written informed assent; where applicable their parent or guardian provided written informed consent (Appendix A). Ethical approval was obtained from the University of British Columbia Children and Women's Research Ethics Board and the Office of Research Ethics at Simon Fraser University. All experiments were conducted in accordance with the Declaration of Helsinki of the World Medical Association. We conducted a sample size calculation based on the assumption that we would have three groups: controls, patients with VVS, and patients with POTS. Based on previous blood pressure and HR data, we determined that 11 subjects in each group, a sample size of 33, are required to obtain a power of 0.80 or greater.

Prior to testing all subjects completed a detailed medical history; all patients had been seen by a paediatric cardiologist and were referred for testing for further investigation of their reflex-mediated syncope. Patients were asked to describe their (pre)syncopal

events, identify triggers of each episode, and report how often they occurred. In addition, any medications or interventions they were participating in were recorded. All controls were apparently healthy and free of cardiovascular and neurological disease.

3.2.2. Study Design

Subjects completed a Valsalva manoeuvre, assessments of cerebral reactivity to changes in $P_{ET}CO_2$, and orthostatic stress test consisting of head-upright tilting and LBNP. Testing was completed on the same day, in the morning. A report describing the response to orthostatic stress testing was created for every patient. This was given to their paediatric cardiologist for review at a follow up consultation.

3.2.3. Test Protocol

Subjects were asked to avoid caffeine on the morning of testing, have a light breakfast, and avoid strenuous exercise twelve hours prior to testing. Tests took place in a temperature-controlled laboratory in the morning. The onset of menarche in females was documented and testing did not take place during menstruation. The test protocol is depicted in Figure 3.1.

3.2.3.1. Monitoring Equipment

Throughout testing we continuously recorded non-invasive beat-to-beat finger arterial pressures (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). This device also calculates beat-to-beat CO, SV, and TPR, using the Modelflow technique (114, 117, 136). HR and rhythm were monitored using a lead II ECG (Finapres ECG Module, Finapres Medical Systems, Amsterdam, The Netherlands). We also monitored $P_{ET}O_2$ and $P_{ET}CO_2$ on a breath-by-breath basis (O_2Cap Oxygen Analyser, Oxigraph Inc, California, USA). Mean MCA CBFV was measured using a 2 MHz ultrasound probe located at the right temporal window and secured in position using a headband; similarly, brachial artery blood flow velocity (BBFV) was measured with an 8 MHz ultrasound probe held in place over the brachial artery by an adjustable clamp, with the arm supported at heart level (Doppler Box, Compumedics Germany GmbH, The DWL Doppler Company, Singen, Germany). Data acquisition was performed with a sampling

frequency of 1 KHz using an analog-to-digital converter (Powerlab 16/30, AD Instruments, Colorado Springs, CO).

3.2.3.2. Valsalva Manoeuvre

Subjects were asked to breathe normally for five minutes. They held a tube connected to a pressure gauge and generated a Valsalva strain of 30 mm Hg during a 20 s forced expiration with a closed glottis. Responses of blood pressure and HR were monitored continuously. Following this forced expiration, the subject breathed normally for another five minutes before repeating the strain. This was repeated for a third time and the strain closest to 30 mm Hg was used for analysis.

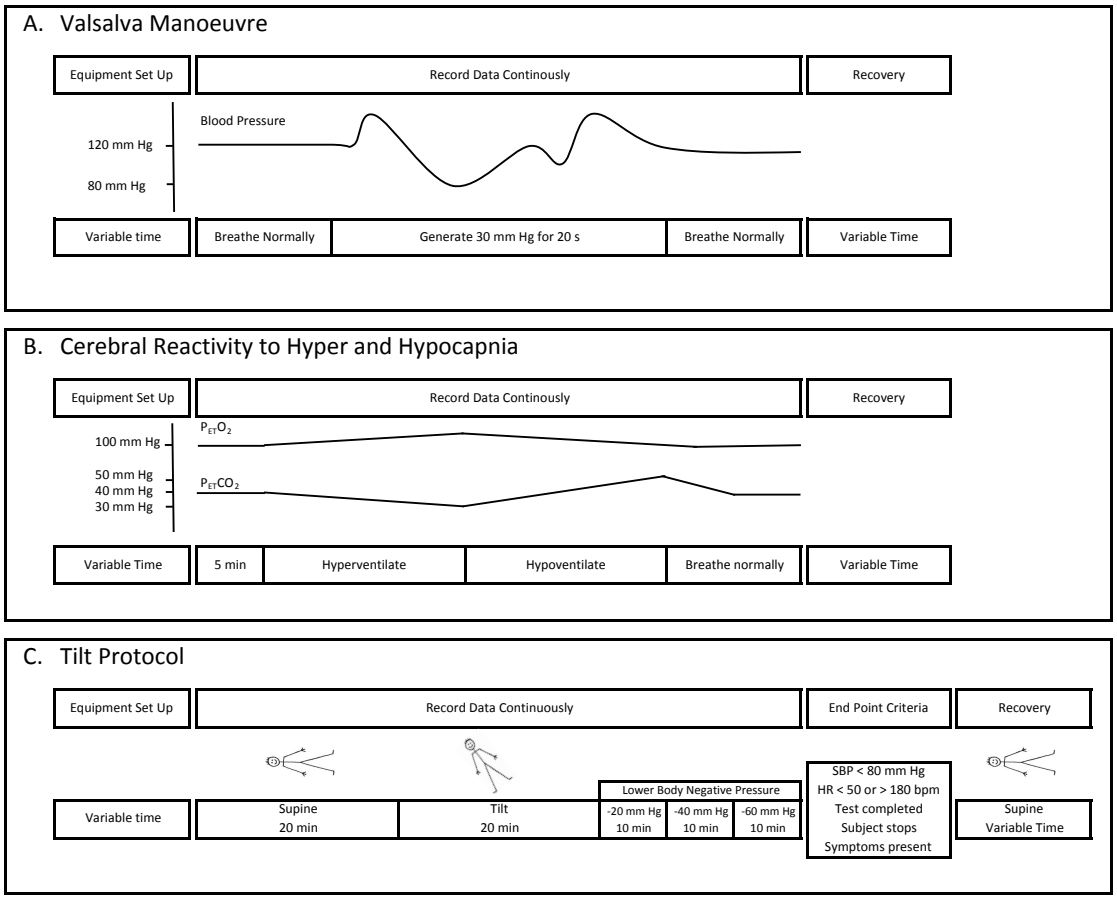

Figure 3.1 Schematic diagram showing the experimental protocol employed in Aim 1.

The protocol was completed in the order shown: **A.** Subjects carried out a Valsalva manoeuvre three times at a stain of 30 mm Hg for 20 s. **B.** Subjects then breathed through a tube before voluntarily hyperventilating and hypoventilating. **C.** Finally, a head-upright tilt test with LBNP was carried out until presyncope.

3.2.3.3. Cerebral Reactivity to Hypocapnia

Subjects breathed normally for five minutes through a 71 mL tube, following which they voluntarily hyperventilated to reduce $P_{ET}CO_2$ levels to a target level of 20 mm Hg. Breath-by-breath end tidal gases and beat-to-beat CBFV were measured continuously. Subjects breathed normally for four minutes and then hypoventilated with the addition of added dead space (227 mL) to increase $P_{ET}CO_2$ levels to a target level of 50 mm Hg. Cerebral reactivity was evaluated as the percentage change in CBFV in the MCA per mm Hg change in $P_{ET}CO_2$.

3.2.3.4. Head-up Tilt Test with Lower Body Negative Pressure

On each test day subjects completed an orthostatic stress test consisting of combined head-upright tilting and graded LBNP continued until presyncope (94, 96). After twenty minutes of supine rest, they were tilted to 60º, for twenty minutes. This was followed by incremental increases in LBNP at -20 mm Hg, -40 mm Hg and -60 mm Hg for ten minutes each. The test was terminated if either: their SAP fell below 80 mm Hg; their HR was less than 50 bpm or greater than 180 bpm; they experienced presyncopal symptoms such as light-headedness, nausea, perspiration and warmth; or the entire protocol was completed. At test termination, the tilt table was rapidly returned to the supine position. Orthostatic tolerance was taken as the time to presyncope in minutes, from the start of tilting until the test was terminated (133).

3.2.4. Data Analysis

Baseline blood pressure and HR was calculated from the five beats immediately prior to the Valsalva manoeuvre. The Valsalva ratio was calculated from the ratio of the longest R-R interval (RRI) during phase IV and the shortest R-R interval (RRI) during phase II of the Valsalva manoeuvre. According to convention, the response was considered to be abnormal if the ratio was greater than 2.87 in men and 2.73 in women or the MAP fell by more than 23.2 mm Hg (75, 77).

Cerebral reactivity to CO_2 was determined from values of CBFV and $P_{ET}CO_2$ extracted from the dataset for each breath during voluntary respiratory manipulation of $P_{ET}CO_2$. The reactivity was quantified as the slope or gradient of the CBFV-$P_{ET}CO_2$ relationship,

expressed as the percentage change in CBFV from baseline per mm Hg change in $P_{ET}CO_2$.

Each phase of the tilt test, supine, tilt, and LBNP, was broken down into 30 second averages, every two-minutes throughout the tilt test. At presyncope, the test endpoint, values were not averaged, rather the endpoint was chosen based on the blood pressure and SV values at termination. Note that because of variable times at which presyncope was initiated and the tests stopped, the number of subjects included for each data point decreased as the test progressed. Thus, cardiovascular responses are presented only for the first 30 minutes of orthostasis. MAP was calculated as diastolic arterial pressure (DAP) + 1/3 pulse pressure. The estimated forearm vascular resistance (FVR) was calculated as MAP divided by BBFV. CMAP was calculated from MAP at heart level, corrected for the measured height difference between the temporal window and heart when upright (80). CVR was estimated from CMAP/CBFV.

Subjects were asked to verify if their symptoms during the orthostatic stress test were similar to their spontaneous episodes of orthostatic (pre)syncope. Sub-types of syncope were evaluated according to the adult VASIS classification and POTS criteria. If adult diagnostic criteria did not fit the physiological responses seen we evaluated new diagnostic criteria for this population. OT (time to presyncope in minutes) was determined.

Static cerebral autoregulation was determined from the gradient and correlation coefficient describing the relationship between averaged CMAP and CBFV data obtained during orthostatic stress testing (whereby a steep gradient and strong correlation denotes impaired autoregulation) (93).

Dynamic cerebral autoregulation was determined from cross-spectral analysis of beat-to-beat CMAP and CBFV during tilt testing. CMAP and CBFV data were taken from each phase of the tilt test. The length of each signal was dependent on the continuous signal of CBFV and therefore differed between individuals, but all with a minimum of 550 beats. A bivariate autoregressive model was fitted to the time series to quantify the frequency related squared coherence, phase shift, and transfer function gain between CMAP and CBFV. Recordings were only used when the squared coherence was >0.5, indicating a

statistically significant correlation between the signals (137). The time delay between signals was calculated as: phase / ((1/frequency)/360).

Spectral analyses of HRV and BPV were conducted on time series of successive beats extracted from RRI, SAP, DAP, and MAP. Occasional ectopic beats were corrected by linear interpolation of adjacent normal beats. An autoregressive model was fitted to each time series and VLF, LF, and HF peaks were identified for each spectrum. Normalized units of LF and HF power were calculated by: (LF or HF Power/(Total Power – VLF Power)*100. Baroreflex sensitivity was determined from cross-spectral analyses of beat-to-beat SAP and RRI during tilt testing in the LF range. A bivariate autoregressive model was fitted to the time series to quantify the frequency related squared coherence, phase shift, and transfer function gain between blood pressure and RRI. Similarly, recordings were only used when the squared coherence was >0.5, indicating a statistically significant correlation between the signal (137). Similarly, the time delay between signals was calculated as: phase / ((1/frequency)/360).

3.2.5. Statistical Analysis

Statistical analyses were performed using SigmaPlot version 11 (Systat Software Inc, San Jose, CA). Data were tested for normality using the Kolmogorov and Smirnov assumption. Data are reported as means ± SEM for patients and controls respectively, unless otherwise indicated, with significance assumed when $p<0.05$. Comparisons between groups and over time were conducted using two-way ANOVA, with the Holm-Sidak post hoc test. Comparisons between patients and controls were conducted using t tests. Possible gender differences between groups were evaluated using Chi squared analyses.

3.3. Results

3.3.1. Subjects

Eleven patients (five male) and ten controls (five male) were recruited for this study. There were no significant differences in age or gender between patients and controls. Table 3.1 displays the subject demographics.

All patients had a history of (pre)syncopal episodes with at least one episode in the last year, but the majority of patients had experienced multiple episodes per week. Symptoms were typically felt either during a change in position from sitting to standing or shortly after stopping activity such as running or during sports. The symptoms included palpitations, dizziness, loss of consciousness, and warmth. All patients had a consultation with a paediatric cardiologist and were evaluated for cardiac arrhythmia or abnormality via ECG and echocardiography. Following these tests, the patients were diagnosed with “dysautonomia” and referred to take part in this study. Five patients were taking medications, such as Florinef, but only when symptoms were present. All patients had been advised to make dietary and lifestyle changes to help control symptoms, for example, increasing salt in their diet and/or tensing their legs before standing.

All controls were apparently healthy and not taking any medication. Six controls had never had a presyncopal or syncopal episode before. Four controls had experienced presyncope: three when changing position from lying or sitting to standing and one after having a blood test. None of the controls had lost consciousness before.

Table 3.1. Subject demographics.

PATIENTS					CONTROLS				
ID#	**Sex**	**Age (years)**	**Height (cm)**	**Weight (kg)**	**ID#**	**Sex**	**Age (years)**	**Height (cm)**	**Weight (kg)**
60458	M	12	157.48	39.46	06080	F	9	135.00	31.80
28891	F	13	170.20	59.1	39626	M	11	144.75	37.60
80951	M	13	180.34	61.23	05505	M	11	151.00	36.40
96973	F	14	157.48	62.6	45420	F	13	157.50	50.00
58362	M	14	175.26	59.77	56299	M	14	162.56	49.90
16194	M	14	160.02	45.36	12312	M	14	177.80	61.23
38196	M	15	180.30	65.90	90270	M	14	162.56	50.80
70437	F	16	160.02	50.80	33331	F	15	160.00	47.70
84981	F	16	165.10	49.90	51803	F	15	152.40	41.96
92069	F	17	165.10	54.50	77705	F	15	167.60	54.50
92403	F	18	154.94	52.16					
Mean±SEM		**14±0.56**	**166.02±2.79**	**54.62±2.41**	**Mean±SEM**		**13±0.66**	**157.12±2.87**	**46.19±2.87**

3.3.2. Cardiovascular responses to the Valsalva Manoeuvre.

Three patients and five controls successfully completed at least one Valsalva manoeuvre that was sustained for 20 seconds with a strain of 30 mm Hg. One control (ID# 77705) was eliminated from the analysis due to technical difficulties. All subjects produced a response with all four identifiable phases; however, the responses were not the same as seen in adults (138). All subjects reported that the manoeuvre was easier to carry out after practice and one male control subject was able to maintain 40 mm Hg pressure for 20 seconds, but only during one attempt, demonstrating that this higher stain was difficult in the majority of children. The attempt at 30 mm Hg was used for the analysis of this control subject.

Table 3.2 depicts the blood pressure and HR responses during the Valsalva manoeuvre in patients and controls. The blood pressure responses were variable whereas the HR responses were similar in patients and controls, but the differentiation between phases was clear. None of the patients or controls had a blood pressure fall during phase IIa that exceeded the limit of normal (23.3 mm Hg, based on data in young adults). All patients and controls had Valsalva ratios within the normal range for their age and gender (75).

Table 3.2. Blood pressure and HR responses to the Valsalva manoeuvre.

	PATIENTS	CONTROLS
Baseline SAP, mmHg	115.33±1.76	109.25±9.28
Baseline DAP, mmHg	72.00±3.21	67.50±5.04
Max SAP rise (phase I), mmHg	131.00±4.04	135.25±5.34
Max DAP rise (phase I), mmHg	83.67±0.67	84.00±1.96
SAP overshoot (phase IV), mmHg	130.33±11.57	131.5±14.60
DAP overshoot (phase IV), mmHg	76.67±1.67	75.5±7.51
MAP drop (phase IIa), mmHg	-8.6±5.8	10.3±5.4
Baseline HR, bpm	77.23±6.33	82.91±13.34
Max HR rise (IIb), bpm	10.72±14.10	14.81±4.29
Max HR decrease (phase IV), bpm	19.34±7.29	12.62±8.21
Valsalva ratio	1.42±0.33	1.27±0.12

3.3.3. Cerebral Reactivity to hypocapnia and hypercapnia

One patient and 3 controls carried out the CO_2 reactivity test. None of the subjects were able to increase their $P_{ET}CO_2$ to above 40 mm Hg during the hypoventilation phase. The patient became dizzy during the hyperventilation and this did not resolve very quickly even after resuming normal breathing. One control interrupted the hyperventilation stage of the test because she found breathing through the tube difficult and this brought on a coughing spell. This quickly resolved and she resumed the rest of this test.

The patient achieved the lowest $P_{ET}CO_2$ value (12 mm Hg) of all subjects who carried out this test, associated with a 34% decline in CBFV compared to baseline. She achieved a maximum $P_{ET}CO_2$ of 31 mm Hg during hypoventilation associated with a maximum 13% increase in CBFV from baseline. In contrast, the minimum $P_{ET}CO_2$ values were 13, 17 and 18 mm Hg for the three controls during hyperventilation with maximum decreases of -26, -46, and -41% in CBFV, respectively. During hypoventilation, the maximum $P_{ET}CO_2$ values were 36, 39 and 34 mm Hg with maximum increases of 50, 50 and 47% in CBFV compared to baseline. Data from one control is depicted in Figure 3.2A.

The correlation coefficient describing the relationship between the percent change in CBFV per mm Hg $P_{ET}CO_2$ was 0.53 in the patient and 0.26, 0.85, and 0.86 in the controls. The gradient in the patient was 2.43 %.mm Hg^{-1} compared to the average gradient in the controls of 3.23±1.10 %.mm Hg^{-1}. Figure 3.2B depicts the patient and control gradients compared to adult control data.

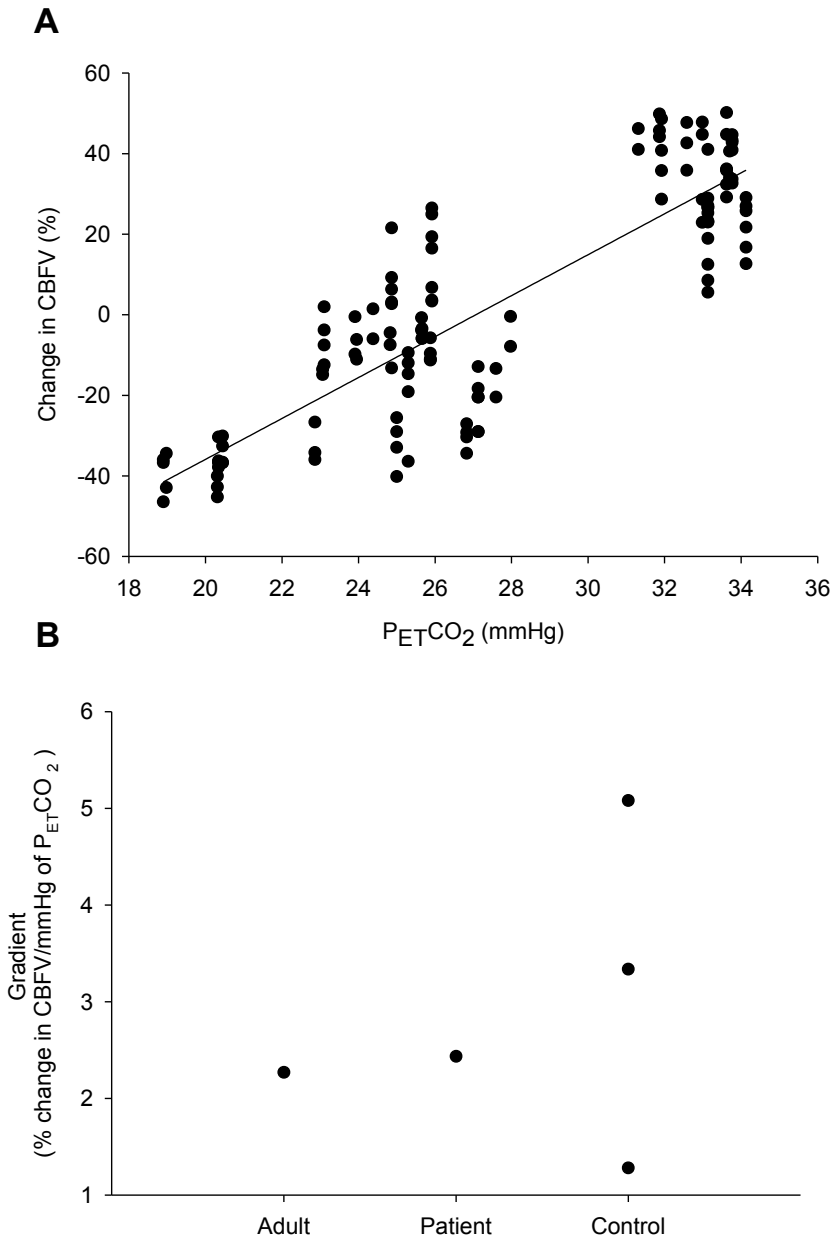

Figure 3.2 Cerebral reactivity to hypo- and hypercapnia.

A: The percent change of CBFV in the MCA per mmHg change in $P_{ET}CO_2$ in a 12 year-old boy. During hyperventilation he reached 13 mm Hg of CO_2 and during hypoventilation he reached 36 mm Hg of CO_2. The gradient of the response is drawn and is assumed to represent the linear portion of the relationship between CBFV and $P_{ET}CO_2$. **B**: The gradient values in the patient and three controls compared to adult control data.

3.3.4. Cardiovascular responses to orthostatic stress

3.3.4.1. Orthostatic Tolerance

Twenty-one subjects, 11 patients and ten controls, completed the tilt test. Nineteen subjects experienced presyncope with hypotension triggering termination of the tilt test, consistent with a vasovagal response. One patient asked to stop the test because the symptoms she was experiencing were too uncomfortable to continue into the LBNP phase and mimicked those she experiences in her activities of daily living. One control subject (ID# 77705) was eliminated from analysis due to technical difficulties. The time to presyncope was not significantly different between patients and controls (20.8±3.3 and 20.1±4.3 min, respectively; p=0.90) (Figure 3.3A). Kaplan-Meier plots also highlight the lack of significant difference in OT between patients and controls, Figure 3.3B.

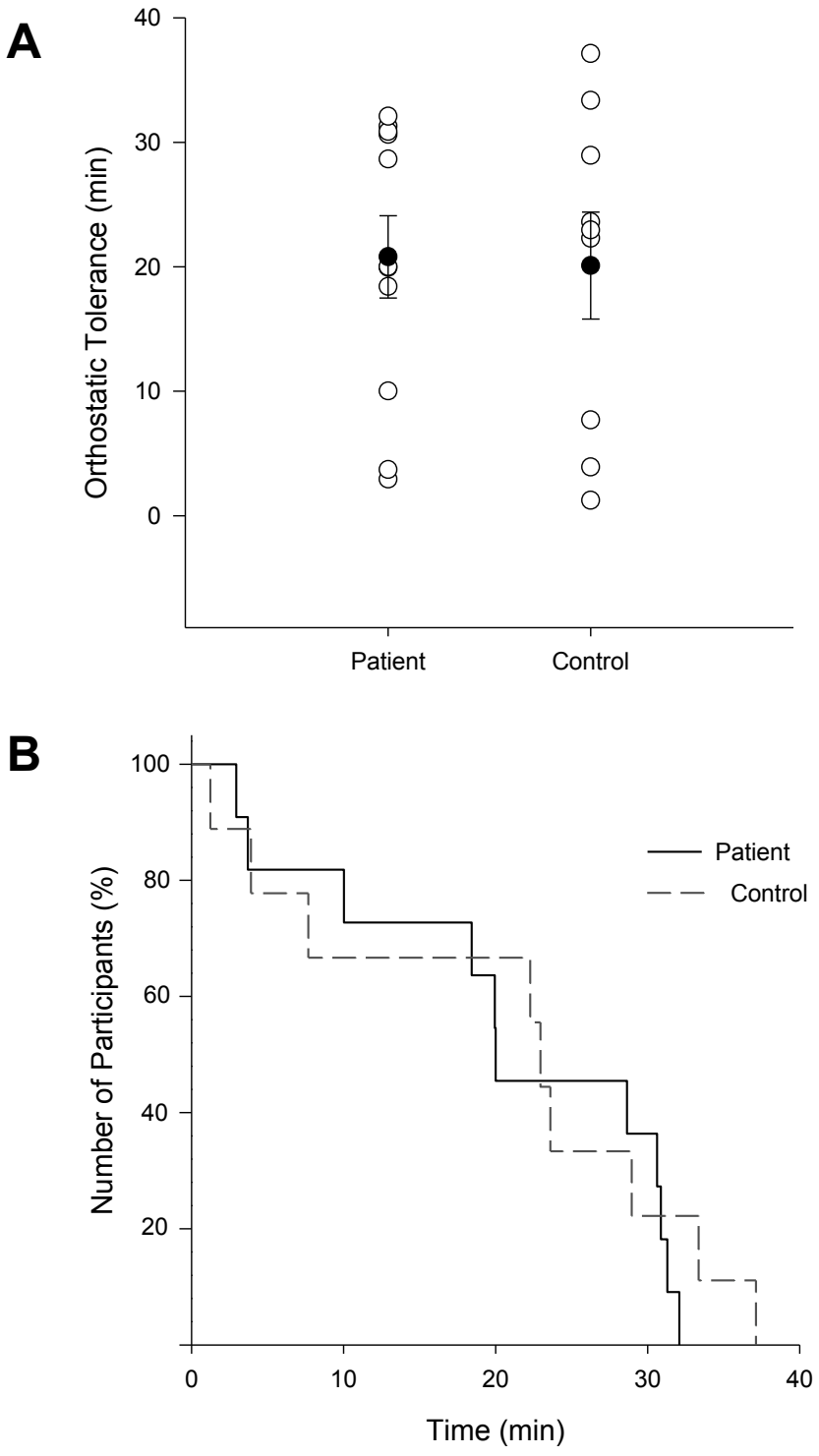

Figure 3.3 OT in patients and controls.

There was no significant difference in OT between patients and controls (A). Kaplan-Meier plots also showed similar times to presyncope in patients and controls (B). Filled circles denote mean data.

3.3.4.2. Blood pressure

There was no significant difference in resting blood pressure between patients (SAP: 119.5±4.4 mm Hg, DAP: 67.2±3.1 mm Hg) and controls (SAP: 108.2±4.4 mm Hg, DAP: 63.9±3.1 mm Hg). Systolic arterial pressure and DAP were also not different between tilt and LBNP **(**Figure 3.4**)**. At presyncope, both SAP and DAP were significantly lower than at any other phase during the test ($p \leq 0.001$). In patients, DAP during LBNP was significantly lower than supine but this was not evident in the controls ($p < 0.001$). There were no significant differences between patients and controls during orthostasis.

3.3.4.3. Stroke volume, heart rate and cardiac output

Resting SV was significantly higher in patients (42.5±1.1 mL) than controls (38.7±1.1 mL) when supine ($p = 0.006$). Stroke volume dropped significantly in each phase of the test compared to supine, including at presyncope, in both patients and controls ($p \leq 0.014$). However, the decrease in SV between tilt and LBNP phases was significant only in the patients (Figure 3.5A).

There were no significant differences between patients and controls in resting (66.6±4.1 bpm and 71.2±4.1 bpm, respectively) or maximum HR responses (108.1±3.9 bpm and 114.5±4.9 bpm, respectively). Heart rates were not different between groups at any stage of testing ($p > 0.05$) (Figure 3.5B). In both groups of subjects, HR were significantly higher at tilt, during LBNP and at presyncope when compared to baseline ($p \leq 0.001$). In two patients and one control, at five minutes of tilt, their HR had increased by more than 30 bpm when compared to supine levels (with increases of 42, 33 and 31 bpm, respectively).

Resting CO (4.0±0.2 L/min and 3.7±0.2 L/min) and CO at tilt, LBNP and presyncope were not significantly different between patients and controls ($p = 0.289$). CO at presyncope (2.6±0.2 L/min and 2.5±0.2 L/min) was significantly lower than the supine, tilt and LBNP phases ($p \leq 0.040$), Figure 3.5C.

3.3.4.4. Peripheral resistance

The angles of insonation for the brachial artery were 58.9±2.66 deg in patients and 56.8±1.83 deg in controls. Resting TPR (1902.8±157.4 dyne.s.cm^{-5} and 1909.7±157.4 dyne.s.cm^{-5}) and resting FVR (16.1±4.7 units and 13.7±4.7 units) were not significantly

different between patients and controls. TPR and FVR maximum responses (TPR: 2193.0±194.0 dyne.s.cm^{-5} and 2412.2±216.9 dyne.s.cm^{-5} FVR: 18.7±4.7 units and 33.2±5.6 units) were not significantly different between groups ($p>0.05$) (Figure 3.6 A and B**)**. However, the maximum percent change in FVR during tilt was significantly different between patients (29.6±15.1%) and controls (73.5±27.8%), $p<0.001$.

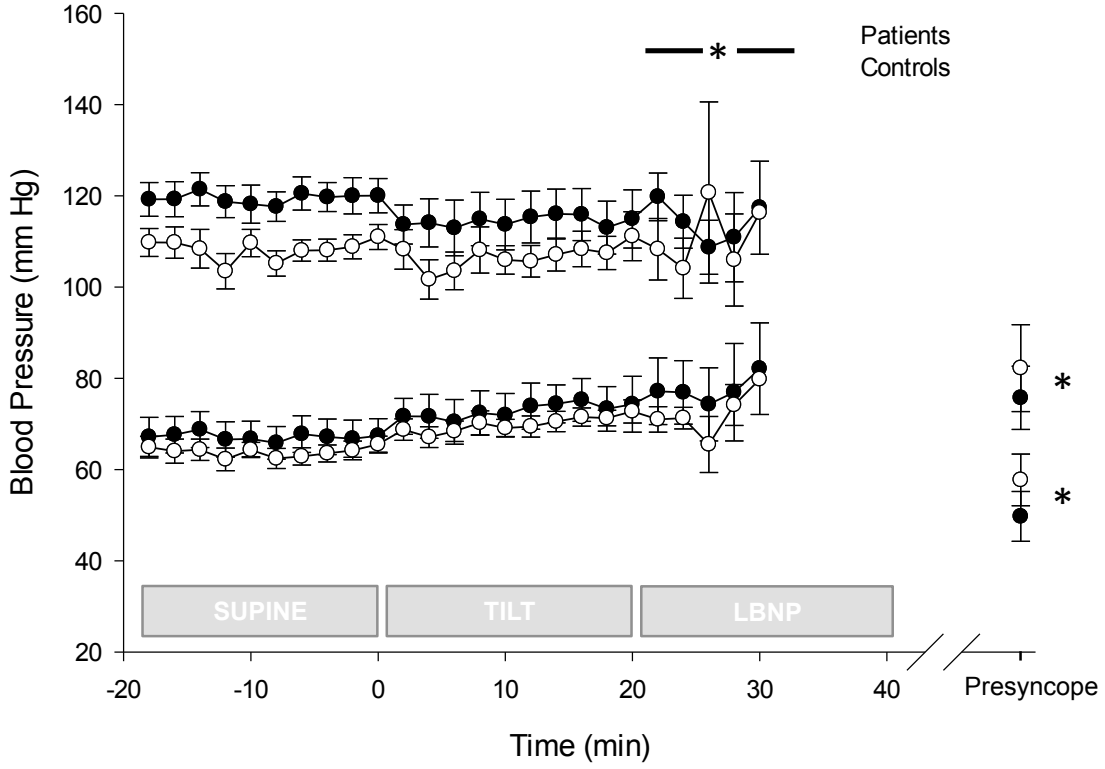

Figure 3.4 Blood pressure responses during orthostatic stress.

Systolic arterial pressures (upper) and DAP (lower) during the tilt test and at presyncope. There were no significant differences in blood pressures between patients and controls at any time point. Blood pressure during LBNP was significantly lower than supine in patients only. Values at presyncope were significantly reduced compared to supine in both patients and controls (*denotes $p<0.001$).

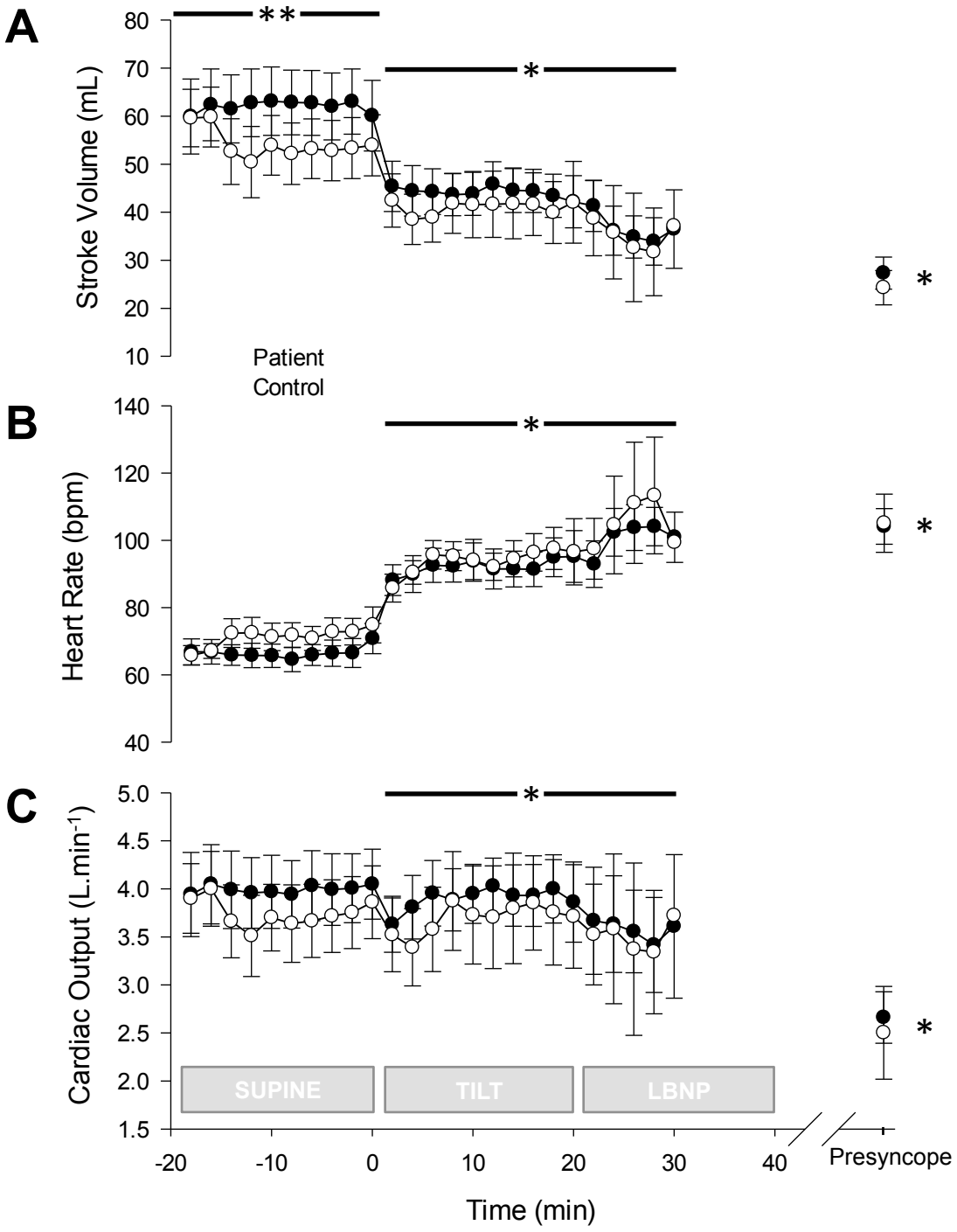

Figure 3.5 SV, HR, and CO responses to orthostatic stress.

There were no significant differences in HR, SV or CO between patients and controls except for during supine when patients had a significantly higher SV, denoted with ** (A). Significant differences from supine in all phases of the tilt test are indicated by the * ($p<0.05$).

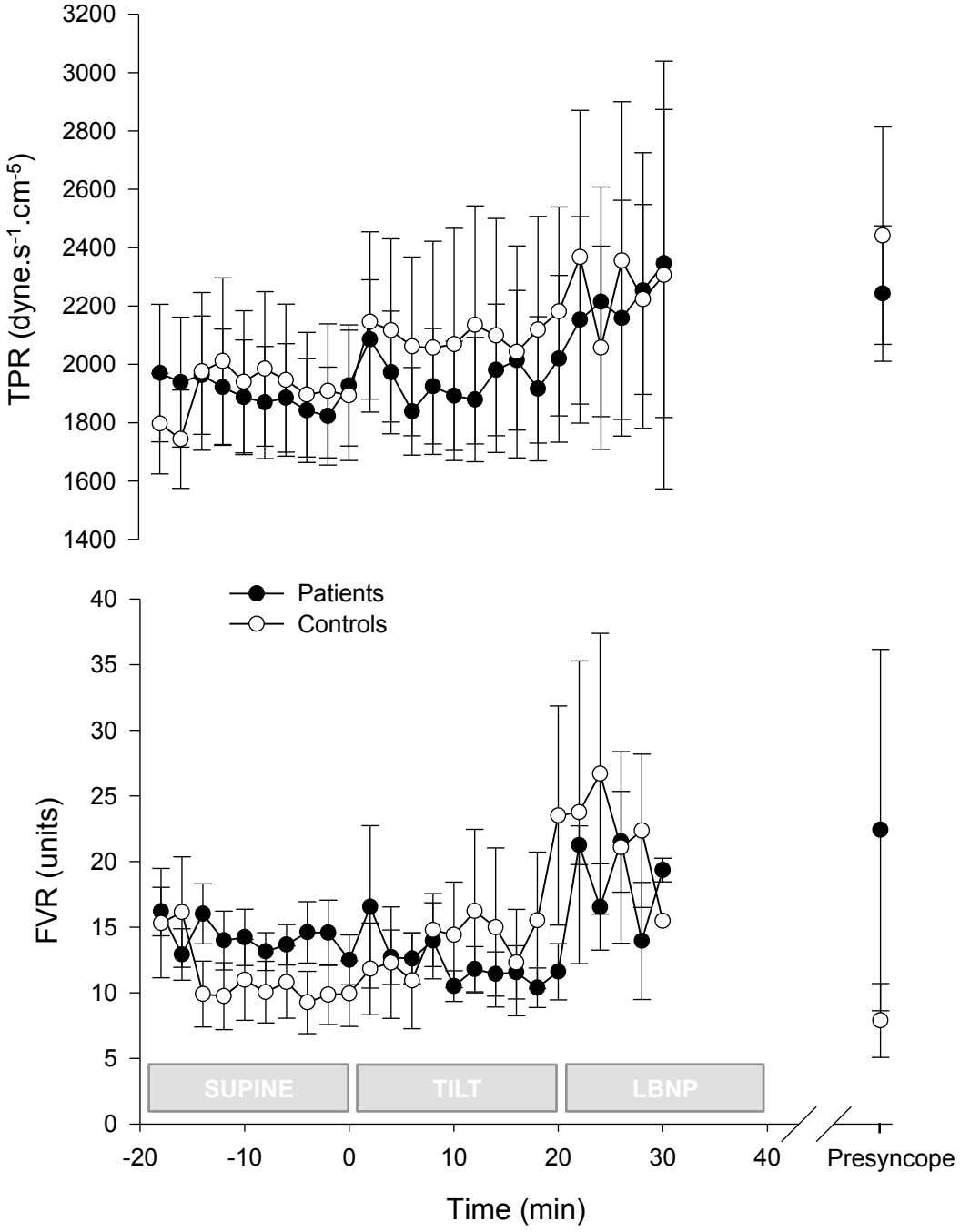

Figure 3.6 TPR and FVR responses to orthostatic stress.

There were no significant differences in TPR or FVR between patients and controls at any time point during the test.

3.3.4.5. Cerebral hemodynamics

Cerebral mean arterial pressure was significantly reduced during tilting compared to the supine phase in both patients and controls. CMAP was also reduced during LBNP compared to supine in the patients; this reduction did not achieve statistical significance in controls (p=0.06). Values at presyncope (35.7±3.3 mm Hg and 45.6±3.7 mm Hg) were significantly lower than any other phase in the tilt test (Figure 3.7A). Cerebral mean arterial pressure was not significantly different between patients and controls at any stage of testing.

In controls, CBFV was significantly lower at presyncope compared to supine, tilt and LBNP phases Figure 3.7B ($p< 0.001$). In patients, CBFV was significantly lower in all phases when compared to supine values of 66.1±2.6 $cm.s^{-1}$. In controls CBFV at tilt and LBNP were not significantly lower than supine values (64.1±2.6 $cm.s^{-1}$), and LBNP was not significantly lower than tilt. Values at presyncope were, however, significantly reduced compared to supine and all other phases (6.2±3.7 $cm.s^{-1}$ in patients and 10.2±4.7 $cm.s^{-1}$ in controls), $p<0.001$. There was no difference in the magnitude of reduction of CBFV at presyncope between patients and controls.

Cerebral vascular resistance was significantly higher in patients (1.4±0.1) than in controls (1.2±0.1) during the LBNP phase of the test. Cerebral vascular resistance was significantly lower at presyncope than any other phase of the test in both controls and patients ($p<0.001$). In patients, CVR was significantly lower during LBNP when compared to tilt ($p=0.011$) but this was not the case in controls. Figure 3.7C.

3.3.4.5.1. Static cerebral autoregulatory control

There was no significant difference in the correlation coefficient ($p=0.202$) and gradient ($p=0.637$ $cm.s^{-1}.mm\ Hg^{-1}$) describing the relationship between CBFV and CMAP in patients and controls **(**Figure 3.8**)**.

3.3.4.5.2. Dynamic cerebral autoregulatory control

During the supine phase, averages of 566±95.5 and 729.5±138.6 beats for the CMAP and CBFV signals, for patients and controls respectively, were used for analysis. Eight out of 11 patients and 6/10 controls exhibited coherence >0.5 between CMAP and CBFV and were therefore used in the analysis. The central frequency of the LF and HF ranges

was not significantly different between patients and controls. In the LF range, the phase was not different between patients (69.3±2.6 deg) and controls (66.3±18.0 deg) but the transfer function gain was significantly lower in patients (0.82±0.09 ms.mm Hg^{-1}) than in controls (1.56±0.04 ms.mm Hg^{-1}), p=0.008. In the HF range, only one control had coherence greater than 0.5. This control had a phase of 82.58 deg and a transfer function gain of 2.64 ms.mm Hg^{-1} with a time delay of 1.17 s.

During tilt, averages 690.7±264.1 and 706.3±163.4 beats for the CMAP and CBFV signals, for patients and controls respectively, were used for analysis. Three patients and three controls had coherence greater than 0.5 in the LF range and only two patients and two controls had coherence greater than 0.5 in the HF range, and these individuals were used in the analysis. The phase was positive in patients (37.93±4.7 deg) and controls (90.2±5.5 deg) and the transfer function gain was similar to supine values in both patients (0.7 ms.mm Hg^{-1}) and controls (0.91 ms.mm Hg^{-1}) in the LF range. The phase was also positive in the HF range for patients (80.4±15.4 deg) and controls (131.6±5.4 deg) with a lower gain when compared to supine (1.2±0.5 ms.mm Hg^{-1} and 1.6±0.1 ms.mm Hg^{-1}).

The time delay between CBFV and CMAP decreased from supine to tilt in patients (from 1.9±0.1 s to 0.9±1.0 s) but was unchanged in controls (from 2.9±0.3 s to 3.0±1.1 s) in the LF range. The controls had a significantly greater time delay than patients during supine (p=0.01). In the HF range, the time delay increased in both patients (from 0.5±0.2 s to 0.8±0.1 s) and controls (from 1.17, in one control, to 1.31±0.1 an average of 3 controls).

3.3.4.6. End-tidal gases

$P_{ET}CO_2$ was significantly lower in patients than in controls during both supine and tilt ($p \leq 0.05$, Figure 3.9A). In both patients and controls $P_{ET}CO_2$ decreased at presyncope when compared to supine and tilt values, indicating hyperventilation ($p \leq 0.008$). $P_{ET}O_2$ was higher in patients than controls during every phase of the test ($p < 0.049$, Figure 3.9B), but there was no difference in $P_{ET}O_2$ between test phases.

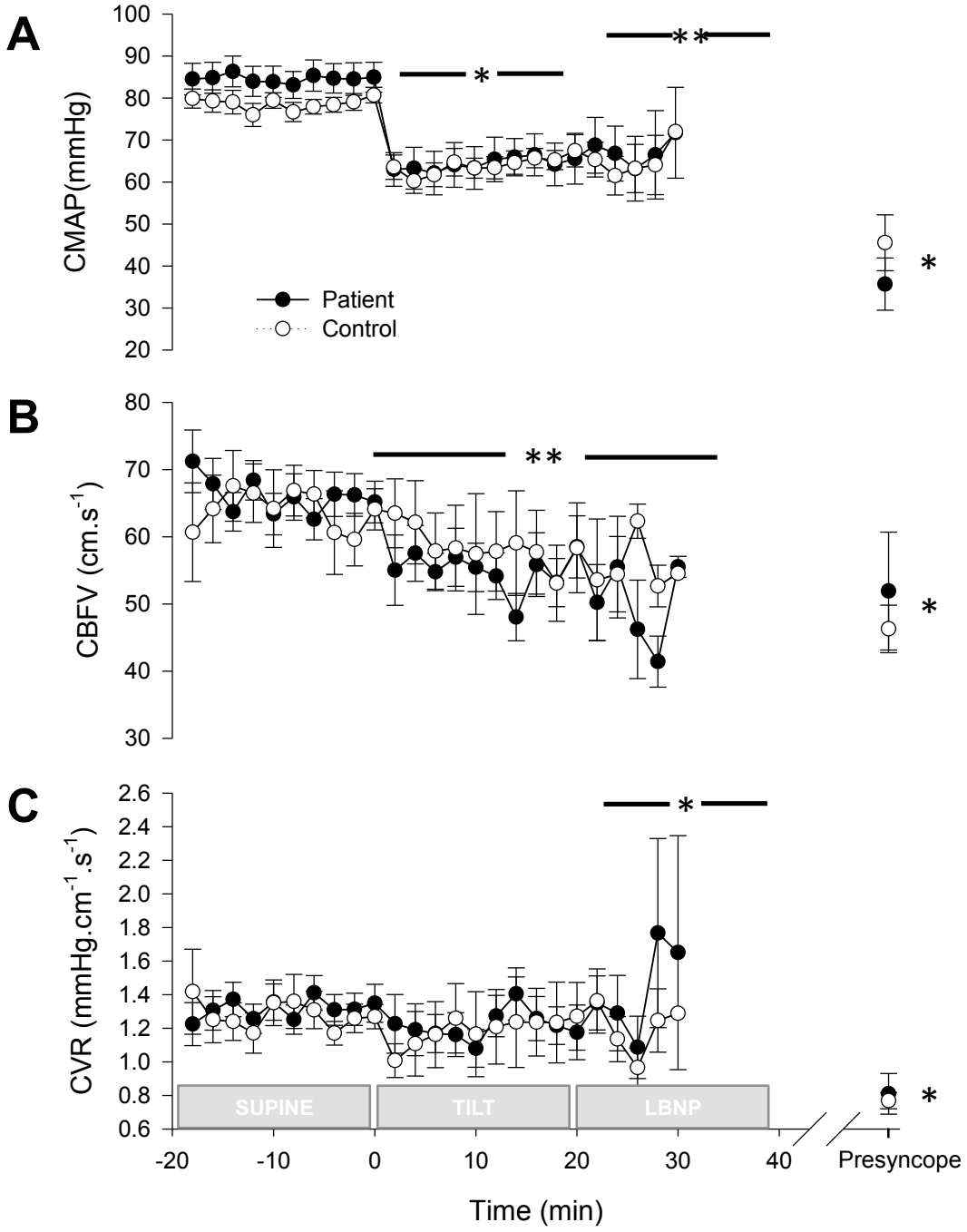

Figure 3.7 ***Cerebral mean arterial pressure, CBFV and CVR during orthostatic stress.***

Significant differences from supine in both patients and controls are denoted with * ($p<0.05$). Significant differences between tilt and LBNP when compared to supine in patients only are denoted with ** ($p<0.05$).

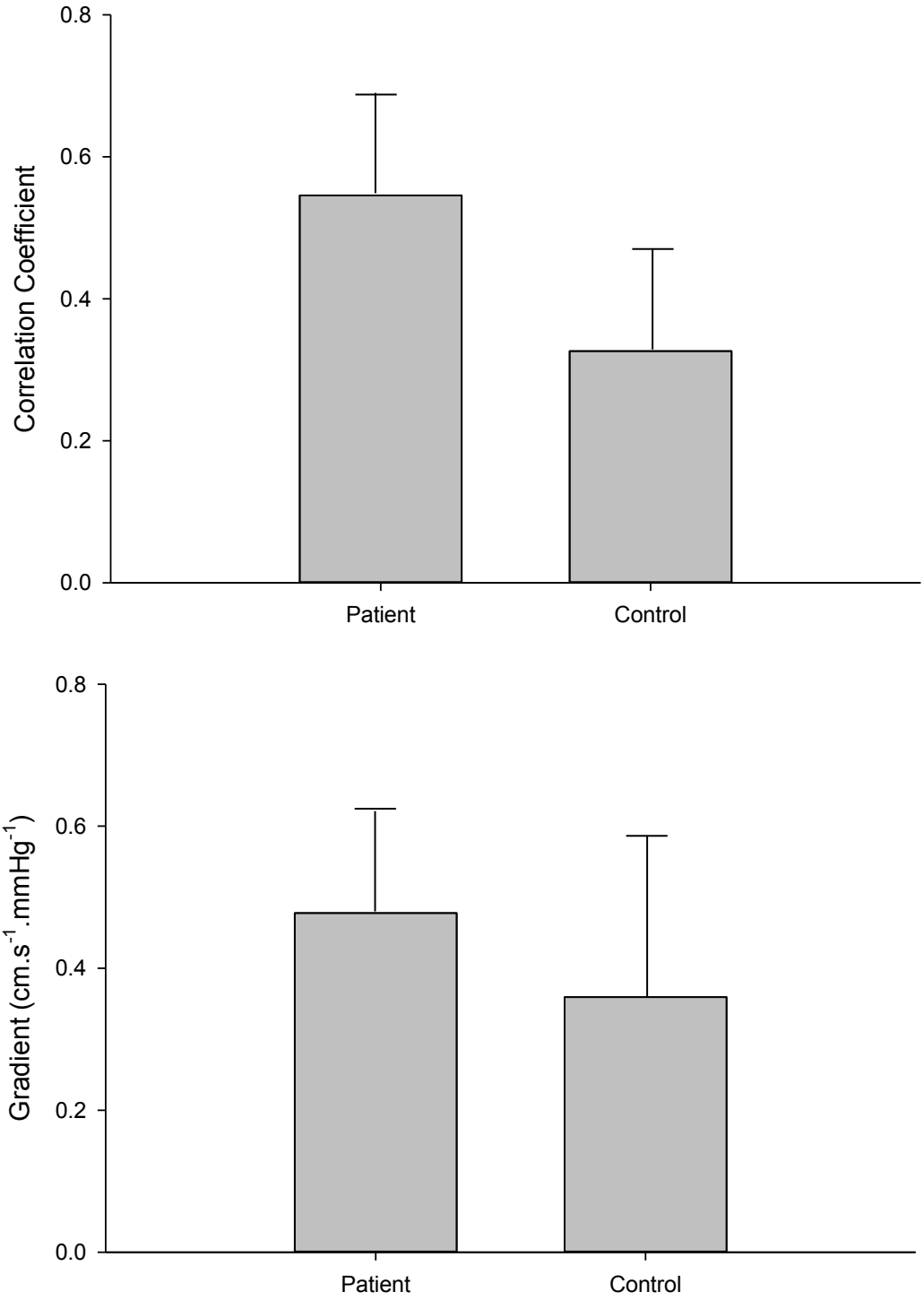

Figure 3.8 Static cerebral autoregulation.

There were no significant differences between patients and controls.

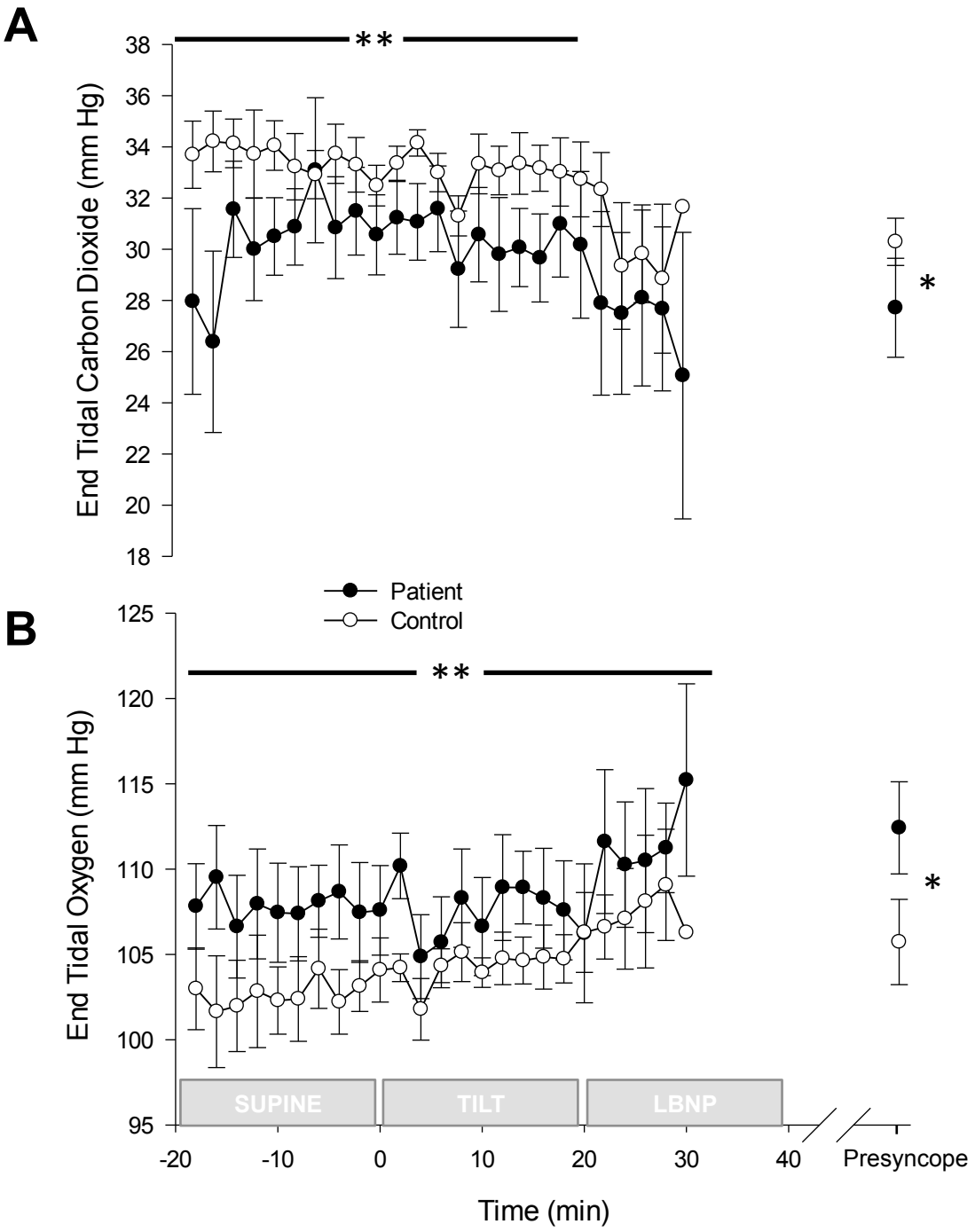

Figure 3.9 End tidal carbon dioxide and oxygen during orthostatic stress.

Significant differences from supine are denoted with * ($p<0.05$). Significant differences between patients and controls are denoted by ** ($p<0.05$).

3.3.5. Heart rate and blood pressure variability

3.3.5.1. Heart Rate Variability

The mean HR, LF and HF, and their corresponding powers, percent, and normalized values for each subject during supine and tilt are listed in Table 3.3.

HF central frequency was significantly lower in patients (0.2±0.01 Hz) than in controls (0.3±0.01 Hz) during the supine phase (p=0.02). From supine to tilt in both patients and controls, LF and HF power decreased. The LF:HF ratio increased in both patients and controls from supine to tilt. The percentage of power in the LF range was greater in patients (35.5±6.9%) than in controls (16.3±2.6%) during tilt (p=0.01).

3.3.5.2. Blood Pressure Variability

The mean SAP, LF and HF, and their corresponding powers, percent, and normalized values for each subject during supine and tilt are listed in Table 3.4. Similar responses were seen for analyses of DAP variability (data not shown).

The power of the LF oscillations in SAP was significantly lower in patients (1.8±0.3 mm Hg^2) than controls (3.6±0.8 mm Hg^2) during supine. At tilt, LF, HF and VLF % had decreased in patients whereas only the VLF % decreased in controls. During tilt, controls had significantly higher percentage of power in the VLF range (70.1±10.4%) and VLF power (37.6±7.4 mm Hg^2) when compared to patients (64.9±4.7% and 31.1±5.5 mm Hg^2, respectively), $p<0.03$.

There were no significant differences in the power of the oscillations in DAP between patients and controls during supine and tilt. At LBNP, patients' normalized HF power was significantly higher (16.4±6.6 nu) than in controls (5.7±1.6 nu). In patients and controls, HF %, VLF %, and normalized HF were decreased at tilt when compared to supine. HF power was also reduced in patients, but not in controls.

Table 3.3. Heart rate variability in patients and controls.

Significant differences between patients and controls during either supine or tilted phases are denoted with '*'.

	PATIENTS		CONTROLS	
	SUPINE	**TILT**	**SUPINE**	**TILT**
Mean HR, bpm	65.6±3.6	91.4±5.7	71.4±4.0	89.5±5.2
LF, Hz	0.11±0.01	0.13±0.02	0.12±0.01	0.12±0.001
LF Power				
ms^2	4713.8±2698.3	1226.7±363.4	1811.10±435.0	889.1±334.6
nu	42.2±4.81	74.9±6.8	40.8±4.8	52.5±9.1
%	26.4±3.5	35.5±6.9	25.4±3.0	16.3±2.6*
HF, Hz	0.25±0.01	0.30±0.04	0.28±0.01*	0.27±0.02
HF Power				
ms^2	4427.0±2480.5	181.7±95.2	1625.3±467.0	884.8±583.4
nu	29.0±3.9	15.3±4.9	0.7±0.2	1.0±0.4
%	20.7±3.6	5.2±1.5	20.7±3.7	9.4±3.3
LF:HF ratio	2.2±0.7	7.7±1.7	1.8±0.6	5.9±2.6
Total variance, ms^2	16072.5±8546.6	3312.7±1106.5	7563.0±1764.6	5496.4±1925.9
VLF Power				
ms^2	2558.9±920.4	1733.0±574.2	2355.3±442.3	2628.0±477.2
%	31.6±8.0	55.9±7.1	36.0±4.6	65.1±7.7

Table 3.4. Blood pressure variability in patients and control.

Significant differences between patients and controls during either supine or tilted phases are denoted with '*'.

	PATIENTS		CONTROLS	
	SUPINE	**TILT**	**SUPINE**	**TILT**
Mean SAP, mmHg	119.6±3.5	113.6±5.5	108.5±2.6	104.7±3.1
LF, Hz	0.12±0.01	0.11±0.01	0.10±0.1	0.15±0.05
LF Power				
ms^2	1.8±0.3	13.2±3.1	3.6±0.8*	8.9±2.8
nu	56.4±6.1	68.1±7.7	63.1±6.0	64.2±7.9
%	11.6±3.0	25.6±4.8	12.5±2.7	23.2±9.7
HF, Hz	0.27±0.02	0.26±0.01	0.25±0.03	0.26±0.04
HF Power				
ms^2	0.5±0.1	2.7±0.5	0.9±0.2	1.8±0.3
nu	16.4±4.2	24.7±6.9	15.8±2.4	23.4±6.7
%	2.9±0.9	7.1±1.9	2.7±0.6	4.2±0.7
LF:HF ratio	7.0±2.3	7.3±2.7	6.2±1.9	5.2±1.5
Total variance, ms^2	22.2±4.0	47.9±7.5	41.8±11.6	49.4±7.5
VLF Power				
ms^2	18.6±3.6	31.1±5.5	36.3±11.6	37.6±7.4
%	78.8±4.6	64.9±4.7	81.7±3.1	70.1±10.4

3.3.5.3. Baroreflex control

Eleven patients and five controls (in whom the supine coherence was greater than 0.5) were included in the supine analyses. In patients, the average phase was -25.6±6.6 deg and average transfer function gain was 27.5±9.1 ms.mm Hg^{-1} with a time delay between BP and HR of -0.8±0.23 s. In comparison, controls had an average phase of -32.4±15.0 deg, an average transfer function gain of 18.4±3.4 ms.mm Hg^{-1} with a time delay between BP and HR of -1.1±0.5 s.

Nine patients and six controls (in whom coherence was greater than 0.5) were included in the analyses of the tilt phase. Phase increased in both patients (-15.3±8.0 deg) and controls (-25.0±6.4 deg) from supine levels whereas the transfer function gain decreased (7.4±0.9 ms.mm Hg^{-1} and 7.6±1.6 ms.mm Hg^{-1} in patients and controls respectively). The time delay was -0.5±0.26 s in patients and -0.8±0.27 s in controls.

Five patients and two controls (in whom coherence was greater than 0.5) were analyzed during LBNP. Similarly, phase increased further between tilt and LBNP to -5.91±7.0 deg in patients but decreased from supine to tilt in the two controls (-40.9±8.4 deg). The transfer function gain decreased in both patients (5.8±0.7 ms.mm Hg^{-1}) and controls (4.9±0.9 ms.mm Hg^{-1}). The average time delay was -0.08±0.18 s in patients and -1.38±0.27 s in controls.

The LF central frequency was not significantly different between patients and controls at any phase of the test (p=0.6). There was no significant difference between the values for patients and controls at any point during the tilt test.

3.4. Discussion

We have demonstrated that autonomic function testing in children and adolescents is feasible and enables a greater understanding of the cardiovascular parameters that influence and contribute to orthostatic intolerance. Given that cardiovascular reflex control appears to differ in children and adolescents compared to previously published young adult normative data, further research is required to finalize diagnostic criteria in paediatric populations.

All patients had at least one episode of syncope or presyncope in the last year, but the majority had experienced multiple episodes per week. In addition, four of the ten controls had experienced presyncope, but never lost consciousness. This supports other studies that confirm the high prevalence of syncope and presyncope in children and adolescents (22).

We selected three tests to evaluate autonomic control in our subjects: the Valsalva manoeuvre; cerebral autoregulatory responses to perturbations in cerebral perfusion pressure and $P_{ET}CO_2$; and an orthostatic stress test continued until presyncope. Overall, all subjects (ranging in age from 9-18 years) were able to complete the tests, showing that they are applicable in this population. The equipment in our laboratory, although initially designed for adults, was well adapted where necessary and functioned as expected in our population of children and adolescents.

3.4.1. Valsalva manoeuvre

The arterial pressure changes during and after the Valsalva manoeuvre were similar to those described previously in a similar age group (139). During phase I, systolic and diastolic pressures increased and were accompanied by bradycardia. Blood pressure fell during phase IIa, but was maintained during phase IIb as HR increased. The responses in phase IIa were atypical because although the blood pressure decreased, it did not always decrease relative to baseline. In phase III, there was another fall in blood pressure and an increase in HR, and finally in phase IV there was an overshoot in blood pressure and accompanying bradycardia, as seen previously (74). Responses to strain were variable in patients and controls with some subjects showing greater increases in blood pressure and HR, while others exhibited minimal change. Overall, the magnitude and direction of the response was consistent with previous reports in paediatric populations (72). However, in other work the level of strain was correlated with the blood pressure and HR response; and therefore, reference values could not been ascertained due to the marked differences in the level of strain (72). In this study, although the response was variable between subjects, they were all able to maintain 30 mm Hg of strain during at least one attempt. As such, with a larger cohort, perhaps reference values can be obtained.

Our data show that the Valsalva manoeuvre is a non-invasive test that can be use in a clinical setting to obtain an understanding of cardiovascular autonomic control in paediatric patients. However, it does not discriminate between patients or controls or provide insight into the classification of syncope. The level of strain has been found to be proportional to the level of sympathetic nerve activity, so it is thought that with a higher strain, there will be a larger response (138). In children, it is not necessary to produce a larger strain, which may be prohibitively difficult if an estimate of autonomic control can be obtained from a strain at 30 mm Hg (72).

It has been suggested that the arterial pressure increase after the release of the Valsalva strain (phase IV) estimates the level of sympathetic activity and the arterial baroreceptor-sympathetic control mechanisms just preceding this elevation (138). This was found during measurements of sympathetic nerve activity, but in the presence of ganglionic blockade, there was no pressure increase (138). In our subjects both patients and controls had an increase in blood pressure from baseline of 15/4±11.7/1.8 mm Hg and 22/8±7.4/2.7mm Hg, respectively. Thus, it appears that the burst of efferent sympathetic activity during phase IV was detected in our subjects (139).

None of the subjects exhibited Valsalva ratios or blood pressure falls during the Valsalva that were abnormal compared to data collected in 10 to 29 year olds (75, 77). However, responses were atypical compared to adult reference data, and the fall in blood pressure during phase IIa, particularly in controls, was small. This may reflect the use of a slightly lower strain in this study, and a concomitant reduction in the magnitude of the responses seen (75). It is possible that had a larger strain been conducted some patients would have exhibited an abnormal response, and thus may be more helpful in differentiating between patients and controls. If so, new normative data for this modified procedure should be identified for this population to facilitate the identification of abnormalities in autonomic cardiovascular control using this technique.

Overall, there was no difference in the ability to produce the Valsalva strain at 30 mm Hg between patients and controls, supporting use of the Valsalva manoeuvre as a simple clinical evaluation of autonomic control in children. However, it may not be a useful test in diagnosing classifications of syncope; and therefore, more subjects are required in this study to find out first, whether the Valsalva ratio norms do in fact apply to patients

and controls aged 6 years to 18 years, and second, whether this should be included in a clinical setting in patients with “dysautonomia.”

3.4.2. Cerebral Reactivity to CO_2

One patient and three controls were able to tolerate cerebral reactivity testing, and perform the required respiratory manipulations. Only in the patient hyperventilation was associated with dizziness that prompted normal breathing more quickly than in the controls. All subjects had difficulty with hypoventilation in that $P_{ET}CO_2$ did not go above 40 mm Hg despite the addition of dead space to the breathing tube. While this limits our ability to determine cerebral reactivity to both hypo- and hypercapnia, in the present study the primary parameter of interest is the reactivity to hypocapnia, as this is implicated in the initiation of (pre)syncope, and these data were reliably obtained. Furthermore, given that the relationship between $P_{ET}CO_2$ and CBFV is linear over the targeted range (81, 86), our data can be extrapolated to apply to hypercapnia in addition to hypocapnia.

The correlation between $P_{ET}CO_2$ and CBFV was similar in controls and the patient, and was similar to that of adult controls (81). This is in opposition to adult data in which adults with syncope have a higher gradient describing the relationship between the percent change in CBFV in the MCA per change mmHg of CO_2 (81). In adults, this hypersensitivity to changes in $P_{ET}CO_2$, particularly when coupled with the known hyperventilation-induced hypocapnia observed during orthostatic stress testing in this and other studies (81, 140) would be expected to contribute to episodes of (pre)syncope (29). Hyperventilation decreases $P_{ET}CO_2$ and the resultant cerebral vasoconstriction leads to a reduction in CBFV. An individual is then more susceptible to presyncope through cerebral hypoperfusion in the face of hypotension. In this case report, the patient produced a range of $P_{ET}CO_2$ values from 12 mm Hg to 32 mm Hg suggesting that this was not representative of only the linear portion of the cerebral blood flow-CO_2 relationship depicted in Figure 2.3 (86). As such, the calculated gradient may be lower than expected because it also incorporates the response to values of $P_{ET}CO_2$ below 20 mmHg. Further studies are required to determine whether a similar relationship between the percent change in CBFV per mm Hg change in $P_{ET}CO_2$ exists and predisposition to (pre)syncope occurs in children as is documented in adults.

The controls produced a much higher gradient than in adult controls and this finding supports earlier work in young adults, where the cerebral reactivity to CO_2 was greater than in elderly adults (141). The present study underscores the need to measure cerebral reactivity to CO_2 in children and adolescents to identify the mechanisms that may have an important role in their (pre)syncopal episodes.

3.4.3. Responses to orthostatic stress

We have shown that tilt testing with LBNP is appropriate and successful in children and adolescents with the ability to attain the same endpoint, presyncope, in both patients and controls. We selected a test that has a clearly defined end-point in all subjects, and is highly reproducible, sensitive, and specific, at least in young adults (94, 96, 135). We are also confident that the test end point (presyncope), and therefore the OT, was reliably determined because the terminating blood pressures and HR (as well as the other cardiovascular variables) were similar between patients and controls. One female patient was too symptomatic to continue into the LBNP phase of the test despite her blood pressure and SV being well maintained. However, her symptoms mimicked those of her episodes in activities of daily living and as such, an appropriate end point in the test was reached. Vasovagal syncope was the most common mechanism by which presyncope occurred, consistent with other research (22).

The average OT was similar between the patients (20.8±3.3 min) and controls (20.1±4.3 min), but lower than previously reported averages in healthy men (35±1.4 min) and women (29±1.5 min) aged 20 to 35 years (133). This may suggest that cardiovascular reflex control is less efficient in children and adolescents when compared to adults, but the causes of this are not clear. Possible contributing factors could include the complex interplay between hormone regulation, growth spurts, and autonomic regulation during the transition from childhood to adolescence. In addition, in adult populations, patients with (pre)syncope have lower OT when compared to healthy controls (59, 81). However, this was not the case in the children and adolescents in this present study. This may be because the patients in this study had already sought medical attention and were given suggestions for lifestyle and dietary changes to prevent and reduce the frequency of their episodes. As such, when evaluated in this study, these interventions may have improved their responses to orthostatic stress, making them more similar to those of

controls. Conversely, some of the controls in this study could be considered to have abnormally poor OT. Indeed, two 15-year old male controls demonstrated OT of just over one and three minutes, clearly abnormal values based on young adult data. It may be that these controls naturally manage their orthostatic intolerance through behavioural means, and ultimately do not interpret episodes of presyncope as interfering with their activities of daily living. Certainly it is known that in young adults, a subset of controls with poor OT are protected from symptomatic episodes of orthostatic (pre)syncope through enhanced postural sway (31, 142). It is clear that further testing is required to resolve these discrepancies. The implications of these observations are that the tilt test could be considered as having low sensitivity and specificity for differentiating between paediatric patients and controls. However, the differences in cardiovascular responses (until presyncope) across this population of patients and controls suggest otherwise, and a study evaluating OT before and after intervention in both patients and controls could clarify the sensitivity and specificity of this protocol.

Consistent with a vasovagal response, systolic and diastolic arterial pressures were well maintained throughout testing, until the point of presyncope suggesting appropriate cardiovascular responses to orthostatic stress were mounted, at least initially. In all subjects the test was terminated using our endpoint criteria. In 19 subjects, the test was terminated because the subjects had a systolic arterial pressure below 80 mm Hg and in one subject it was terminated due to symptoms that were recognized as being similar to their previous spontaneous attacks, and not tolerable enough to continue the test into the LBNP phase.

A baroreflex-mediated tachycardia was observed in patients and controls. Three patients and one control exhibited slight bradycardia at presyncope compatible with the onset of cardioinhibitory syncope. In other subjects, prompt termination of the test likely terminated the bradycardia component of the reflex before it became severe. It is likely that had the test been continued until syncope occurred more individuals would have experienced profound bradycardia and potentially asystole. However, the additional diagnostic utility of provoking frank syncope is unclear, and we feel the additional patient burden of this more unpleasant approach prohibits its use in young children.

Stroke volume decreased with orthostatic stress, confirming that orthostatic stress leads to reduced venous return, secondary to venous pooling and plasma filtration. The HR increase in patients was 66.4±9.3%, which fairly closely matched the SV decrease of 51.3±6.4%. In controls, the HR increase of 60.7±10.7% again is close to the SV decrease of 49.3±10.2%. As such, CO was maintained in each test until presyncope when it decreased. Many POTS patients have smaller left ventricular masses, and consequently lower blood volumes when compared to controls (27). It is thought that the lower SV is inadequate in maintaining cerebral perfusion during orthostasis thus providing an explanation for their susceptibility to presyncope. Contrary to what is expected, patients in this study had higher SV than in controls. We speculate that this finding suggests a different mechanism underlying orthostatic tachycardia in children with POTS. In adults, the lower ventricular mass is thought to contribute to the onset of syncope; however, in children, perhaps autonomic dysfunction plays a greater role. Certainly there was evidence of abnormal cardiovascular autonomic responses to orthostatic stress in the patients in the present study, with small baroreflex mediated vascular resistance responses, but normal or enhanced cardiac baroreflex responses. As such, the diagnostic criteria for POTS in this population require further investigation.

It is expected that TPR and FVR will increase with tilt due to baroreflex-mediated increases in sympathetic drive to resistance vessels. In healthy adults, a maximum vascular resistance response of 100±12% is considered normal and smaller responses are indicative of impaired vascular resistance responses (143, 144). In the present study, the maximum FVR was significantly reduced in patients when compared to controls during tilt. Both patients and controls showed a lack of vasoconstriction in comparison to adult data and this impairment was greatest in the patient group, and thus may be implicated in their symptoms of (pre)syncope. There were no significant differences in HR responses between patients and controls, unlike in adult populations, underscoring the need for new HR criteria for children with syncope (115, 145). Furthermore, the disconnect between normal cardiac baroreflex responses and impaired vascular resistance responses in children with syncope provides mechanistic insight into the cause of their symptoms.

Cerebral mean arterial pressure decreased at tilt when compared to supine, due to the hydrostatic gradient imposed when the subject is upright, but CMAP was then

maintained at this new lower level in both patients and controls until presyncope. Cerebral blood flow velocity was maintained throughout the test (despite the drop in CMAP) indicating normal autoregulatory responses in controls, until presyncope when perfusion pressures were presumably closer to the lower limit of autoregulation and CBFV decreased. In patients, CBFV was significantly lower during all phases of the orthostatic stress when compared to supine values. This indicates impaired cerebral autoregulation in patients and is supported by the trend for a higher correlation and gradient describing the relationship between CMAP and CBFV when compared to controls. This warrants further investigation in a larger cohort, but it may be that, as in young adults with syncope, static cerebral autoregulation is impaired in children and adolescents with syncope, and this may be a contributing factor to their poor orthostatic tolerance.

The interpretation of dynamic autoregulatory responses is complicated by the varying sample size for analysis, based on the requirement for significant coherence between CMAP and CBFV to produce reliable estimates of autoregulatory function. In the evaluation of dynamic cerebral autoregulation, given that the relationship between these variables in the LF range is thought to be most influenced by alterations in autonomic (sympathetic) function (146), and HF coherence tends to be less, compounding the low sample size for these data, it seems prudent to focus on the more pertinent and robust LF autoregulatory data. Both patients and controls had a positive phase between spontaneous oscillations in CMAP and CBFV in the LF range. This is compatible with previous work, and is thought to represent the delay in smooth muscle responses in modifying CVR (83). At tilt, LF phase decreased in patients when compared to supine, suggesting impairment in cerebral autoregulation. In controls, there was an increase in phase, or an increase in the time delay between CMAP and CBFV, as expected during orthostasis. These findings are compatible with the reduction in CBFV that occurred during tilting in the patient group, in whom dynamic autoregulation was impaired. In controls, dynamic autoregulation was intact and CBFV did not decrease during the orthostatic stress, until presyncope when perfusion pressures below the lower limit of autoregulation were obtained. In contrast, both patients and controls tended to exhibit reductions in coherence during tilt, and patients exhibited lower gain during tilt than controls. These data are actually compatible with improvements in autoregulation and

seem at odds with the phase shifts and changes in absolute CBFV observed. This discrepancy likely reflects the low coherence values, and subsequent low sample sizes for these data. Certainly given the reduction in CBFV during orthostasis in the patient group only, in the face of similar perfusion pressures to controls, it seems likely that autoregulation is impaired in these patients, as suggested by the phase shift data.

$P_{ET}CO_2$ was lower at presyncope compared to the other phases of the tilt test indicating that both patients and controls were hyperventilating. In addition, $P_{ET}CO_2$ was decreased in patients throughout the test, again indicating hyperventilation in comparison to controls. This finding is consistent with data in adults, and is thought to contribute to orthostatic intolerance, particularly in those with increased cerebral reactivity to $P_{ET}CO_2$ (81). This is also consistent with the reduced FVR in these patients that predisposes to hypotension and subsequent cerebral hypoperfusion. This is compatible with the reduction in CBFV during orthostatic stress relative to the supine phase observed in the patients, but not the controls. The reason for patients hyperventilating more than controls is unclear; however, it may represent anxiety that is known to accompany POTS.

3.4.3.1. Diagnostic criteria

We found that blood pressure and HR responses at presyncope were different in this paediatric population when compared to young adults. The end point criterion for blood pressure during the tilt test was 80 mm Hg; however, in four patients, when SAP was 80 mm Hg, they were not symptomatic. As the goal of the orthostatic stress test was to reproduce episodes experienced in daily life, in these cases, we continued the orthostatic stress. The terminating SAP ranged from 41 mm Hg to 60 mm Hg in these patients, which is considerably lower than in adults (59, 147). This suggests that new criteria for the minimum blood pressure at presyncope may be necessary in this population.

Similar to previous studies, HR responses in this population were elevated compared to previously reported responses in young adults (115, 145). One 13-year old male subject met the adult diagnostic criteria for POTS as his HR increase was 42 bpm at five minutes of tilt; however, based on paediatric studies that have evaluated orthostatic HR and HR increment during tilt testing he would not be considered a paediatric POTS patient (145). This young male had symptoms during orthostatic stress, a HR increment

above 40 bpm, but his absolute HR was only 111 bpm during tilt and not greater than or equal to 130 bpm as previously suggested for diagnostic criteria in children (145). In addition, this patient's tilt test was stopped because of hypotension suggesting a vasovagal mechanism at presyncope. In comparison, a 13-year old female met the adult criteria for POTS with a HR increment of 33 bpm by five minutes of tilt. Despite her OT of 20 minutes, she was extremely symptomatic throughout the tilt phase with a maximum HR during tilt of 118 bpm and well-maintained blood pressure. Based on previous data this patient would also not meet the criteria for paediatric POTS. Thus, these examples highlight that HR cannot be the only parameter by which POTS can be diagnosed and also that there is considerable variation in other cardiovascular variables that must be considered when defining new diagnostic criteria.

Interestingly, CBFV was significantly reduced at each phase of the tilt test when compared to supine levels in patients, but not in controls. This is a new finding that could contribute to the classification of (pre)syncope in children and adolescents. A larger cohort of subjects will be required to define diagnostic criteria for this variable, but it may add valuable insight into the mechanism by which (pre)syncope occurs.

The VASIS classification helps identify the subtype of VVS in individuals undergoing an orthostatic stress test (148). In our tilt protocol, the end point is presyncope and therefore, to appropriately classify the type of faint, this must be taken into consideration. There was only one control with bradycardia (55 bpm) at test termination that was close to the classification of type 2A and 2B (defined in Section **1.2.1**), probably largely due to prompt termination of the test prior to the onset of syncope. As such, these may be irrelevant classifications in a test in which frank syncope is avoided.

3.4.4. Spectral Analysis

We have shown that spectral analysis is appropriate in children and adolescents and can identify the increase in sympathetic activity and decrease in parasympathetic activity during orthostatic stress as well as the sensitivity of the baroreflex revealed using cross-spectral analyses. It should be noted that patients with cardiac arrhythmias and thus high HRV are highlighted with the use of this analysis (through increases in total power) and represent as a potential drawback to this method of evaluating autonomic control.

3.4.4.1. Heart rate variability

At rest, patients tended to have higher cardiac vagal control when compared to control subjects, compatible with their lower resting HR when compared to controls. The decrease in LF and HF power during tilt and increase in LF:HF ratio, demonstrates a withdrawal of cardiac vagal control and dominance of cardiac sympathetic activity, consistent with a baroreflex response (106, 149).

3.4.4.2. Blood pressure variability

At rest, LF power was significantly lower in patients than in controls. At tilt, LF and HF central frequency decreased in patients but not in controls. This suggests reduced sympathetic drive to resistance vessels in patients at supine and during tilt. This is consistent with the observed decreased vasoconstriction in the periphery in the patient group. In POTS patients there is often increased cardiac sympathetic activity and decreased sympathetic activity to the periphery indicative of a selective impairment in the baroreflex responses to orthostasis (27). Our data are compatible with these findings.

At tilt, normalized HF power was significantly greater in patients than controls. This is compatible with the greater hyperventilatory response to orthostatic stress in the patient group.

3.4.4.3. Frequency domain assessments of cardiac baroreflex function

We have shown that the cardiac baroreflex sensitivity (transfer function gain) decreased with tilt in both patients and controls, consistent with other work examining responses to orthostasis (150). However, in patients the time delay (phase) between oscillations in blood pressure and HR was reduced in comparison to controls, and in some cases, not compatible with baroreflex function (106). Furthermore, the increase in coherence at tilt was significantly higher in patients than in controls suggesting that during orthostasis HR becomes more dependent on increases and/or decreases in blood pressure. This suggests that in patients, the impaired baroreflex control of the peripheral vasculature is somewhat compensated by enhanced cardiac baroreflex function and thus they exhibit brisk HR responses to orthostatic stress. This has important implications for the management of orthostatic tachycardia in children with presyncope. If it is indeed a

compensatory response, treatment of the tachycardia *per se* may in fact worsen their OT and worsen their symptoms. Importantly, this technique is able to capture differences between paediatric patients and controls suggesting this is an effective tool in evaluating cardiac autonomic control.

3.5. Limitations

The main limitation of this pilot study is that we were constrained by a small sample size, and as such, the study was underpowered. We have shown that these simple non-invasive measures of autonomic function are applicable to children and adolescents, and yield useful information concerning cardiovascular autonomic control and orthostatic intolerance. Extension of this study to a larger population seems warranted to further evaluate the potential diagnostic utility of these tools.

There were some technical difficulties with the procedures. The children we tested had difficulty initiating hypercapnia when breathing through a mouthpiece, even with the additional of extra dead space, and in fact tended to hyperventilate. Testing using a face-mask may ameliorate this effect to more accurately evaluate the response to hypercapnia or the use of end tidal forcing may be appropriate. Similarly, the subjects only produced a Valsalva strain of 30 mm Hg when in adults a strain of 40 mm Hg is recommended. This may reduce the magnitude of the responses expected, and as such, new diagnostic criteria may need to be developed (72).

Frequency domain analyses of baroreflex function, HR and blood pressure variability and dynamic cerebral autoregulation are dependent on lengthy and continuous data acquisition. In some cases this was difficult due to subject movement or technical difficulties and this limits the applicability of these analyses to paediatric (and adult) populations. However, we maintain that their noninvasive nature and the breadth of information contained within these analysis techniques renders them suitable candidates for continued evaluation in this population. Furthermore, because any technical difficulties and/or subject movement tends to occur in the upright portion of the orthostatic stress test, the use of these analyses on data collected while supine is not likely to be adversely affected.

The equipment in the laboratory is designed for adults and this had to be modified to cater for the children's small stature. We made a new LBNP waist board with extra neoprene designed to fit around slim children and still provide a sealed chamber for the LBNP phase of the test. We used headbands to keep the cerebral Doppler probe in place for the whole test as opposed to the plastic headset (depicted in Figure 2.4B). This was easy to put on and very effective at keeping the probe stable for the duration of the test. Overall, the adjustments to our equipment worked well and we were able to obtain data as we would in adults.

It is important to note that in children and adolescents the Modelflow aortic age has not been validated. However, in this and other studies, the Finometer has been used in younger populations indicating that this technique is applicable (72, 115).

3.6. Conclusions

We have shown that these simple non-invasive measures of autonomic function are applicable to children and adolescents, and yield useful information concerning cardiovascular autonomic control and orthostatic intolerance. Although we have identified some limitations to the utility of these tests in paediatric populations, we believe that the breadth of information available, coupled with the non-invasive nature of these techniques, renders them strong candidates to aid the diagnosis and subclassification of disorders of autonomic function that contribute to syncopal episodes in children and adolescents. We propose that adult diagnostic criteria may need to be reviewed for children and adolescents with syncope. In addition, the evaluation of peripheral vascular resistance responses and impaired static and dynamic cerebral autoregulation, which have not previously been performed in children to our knowledge, may add further insight into the mechanisms associated with presyncope, and should be considered when defining new diagnostic criteria. As such, we advocate that further testing be undertaken to increase the power of this study and identify the focus of future diagnostic criteria in a clinical setting. In the future this may enable targeted therapeutic approaches for patients with syncope in whom a better understanding of the contribution of cardiovascular autonomic impairment to their symptoms has been achieved.

4. Are Compression Stockings an Effective Treatment for Orthostatic Presyncope?

4.1. Background

Syncope is associated with reduced cerebral blood flow, often attributed to sudden onset hypotension and bradycardia: the “vasovagal” response (16). Many syncopal events are triggered by orthostatic stress, likely due to concomitant venous pooling and enhanced capillary filtration when upright (29). This reduces venous return and, if not adequately compensated, leads to profound reductions in blood pressure and cerebral blood flow (29).

The prevalence of syncope and presyncope is high and both have a marked negative impact on quality of life, with many individuals reporting injury secondary to an associated fall or accident during the event; recurrent episodes are particularly debilitating (3, 18, 151-155).

The treatment of orthostatic syncope can be particularly challenging. Usually the initial approach is patient counselling (152, 155) incorporating avoidance of known triggers, encouraging adequate hydration (often with salt supplementation) (156, 157), and physical countermanoeuvres (59). While these strategies aid in the management of occasional syncope, they are not usually sufficient for the treatment of frequent or severe episodes (155). Additional treatment strategies include cardiac pacemakers for syncope with cardioinhibition, and pharmacologic therapy, although their utility and efficacy has been questioned (158, 159).

The use of compression hosiery is commonly recommended for those affected by recurrent orthostatic intolerance, based on the rationale that external counter-pressure of the lower limbs or abdomen will reduce venous pooling and capillary filtration, thereby increasing venous return and preventing or delaying the onset of syncope (160-162).

Graduated compression garments are thought to be most effective for the treatment of orthostatic syncope, because the movement of body fluid when upright redistributes hydrostatic pressures throughout the body, with the highest pressures found at the ankles (163). Thus, garments designed to apply greater counter-pressures at the extremities might be expected to be more efficacious. However, despite the common recommendation for patients with orthostatic intolerance to utilise compression stockings (152, 159), there is little research proving their efficacy.

Short term improvements in orthostatic blood pressures with garments applying counter-pressure to the whole leg and/or abdominal segments have been reported (30, 164-167), although compression of the thighs may actually promote venous pooling when sitting, due to a reversal of the pressure gradient (168), with an expected deleterious effect on OT. Generally, garments that compress the abdomen show greater promise for the prevention of orthostatic intolerance (165-167). However, these are reported to be uncomfortable, difficult to put on and remove, and are associated with poor patient compliance (169, 170).

We aimed to evaluate whether graded calf compression stockings increase OT using a randomised, placebo-controlled, double-blind design. We evaluated calf-high compression stockings so that, if effective, there would be higher compliance and garment comfort for the target patient population (170). We hypothesized that graded calf compression stockings would improve OT during a progressive orthostatic stress test consisting of combined head-upright tilting and LBNP (171, 172).

4.2. Methods

4.2.1. Subjects

Fifteen young adults (six females; aged 25.5±1.3 years) were recruited for this study, and provided written informed consent. Ethical approval was obtained from the Simon Fraser University Research Ethics Board and experiments were conducted in accordance with the Declaration of Helsinki. Prior to testing subjects completed a brief medical history; all volunteers were healthy and free of cardiovascular and neurological

disease. None of the volunteers was taking any medication, except for three females who were using oral contraceptives.

4.2.2. Study Design

Each subject completed testing on three separate days wearing each of three different types of stocking: calf-length graded compression stocking (Knee-high Graded Support Therapy Socks, Sigvaris Inc, Peachtree City, USA); standard calf-length socks not designed to provide compression, but visually similar to the compression stocking (calf placebo); and ankle-length socks that did not compress the calf (ankle placebo). Testing was conducted in a randomised double-blind fashion, at the same time of day (in the morning). Female subjects were tested in the same phase of their menstrual cycle, achieved by testing on either consecutive days, or at monthly intervals. Subjects were asked to refrain from strenuous exercise twelve hours prior to each test, eat a light breakfast, and avoid caffeine on the morning of each test.

Prior to testing, anthropometric measures were taken. Circumference and skinfold thickness of the calf were determined using a standard tape measure and skinfold callipers (Slim Guide®, Creative Health Products, Plymouth, USA) at the widest level of the calf. Measures were taken in triplicate on the right leg, and the average used for analysis. Calf cross-sectional area (cm^2) was estimated from the circumference (cm), assuming circularity (173):

$$Calf\ cross\ sectional\ area = \frac{calf\ circumference^2}{4\pi}$$

Measures of circumference and skinfold thickness (cm) were used to calculate subcutaneous adipose tissue cross-sectional area (cm^2):

$$Adipose\ \ tissue\ cross\ sectional\ area = \frac{calf\ circumference \bullet skinfold\ thickness}{2}$$

Muscle cross-sectional area (cm^2), also assumed to be circular, was estimated by the difference between cross-sectional area of the whole limb and adipose tissue with an assumed cross-section of bone with its constituent marrow (6 cm) (173):

$$Muscle\,cross\,sectional\,area = \frac{calf\ circumference^2}{4\pi} - \frac{calf\ circumference \bullet skinfold\ thickness}{2} - 6$$

To account for leg shape, height, and foot size, calf circumference was expressed as ratios relative to the subject's height and shoe size.

4.2.3. Test Protocol

As discussed in section **3.2.3**, the same restrictions applied to all subjects in this study.

4.2.3.1. Monitoring Equipment

The equipment used for these experiments is as discussed in section **3.2.3.1**.

4.2.3.2. Head-up tilt test with lower body negative pressure

The protocol is as described in section **3.2.3.4** and is depicted in Figure 4.1.

4.2.3.3. Measure of calf compression

Stocking compression data were obtained at three sites (at the level of the malleoli [ankle], the widest point of the calf [mid-calf], and one inch below the top of the stocking [knee]). Compression measures were not conducted for the ankle placebo stocking, which terminated below the malleoli. A custom-made rig was used to measure compressive pressure, consisting of a load cell (Futek Advanced Sensor Technologies, Inc, Irvine, CA, USA, model LLB350) mounted between two semi-cylindrical plastic parts (Figure 4.2). The stocking was stretched around the rig and force measured by the load cell via a data acquisition board (National Instruments USB 6259). Custom software was used to process the acquired data (LabVIEW 2009, National Instruments).

The relationship between force (F) and pressure (P) exerted by the stocking was derived by integrating the component of pressure along the axis normal to the load cell (y-axis, Figure 4.2) on the interval [0, π]:

$$F = \int_0^{\pi} P \bullet \sin\theta \bullet h \bullet r \quad d\theta$$

where *h* and *r* are the height and radius of the two semi-cylindrical plastic parts and θ is

the angle represented in Figure 4.2. The pressure exerted by the stocking was computed from the measured force as follows:

$$P = \frac{F}{2 \bullet h \bullet r}$$

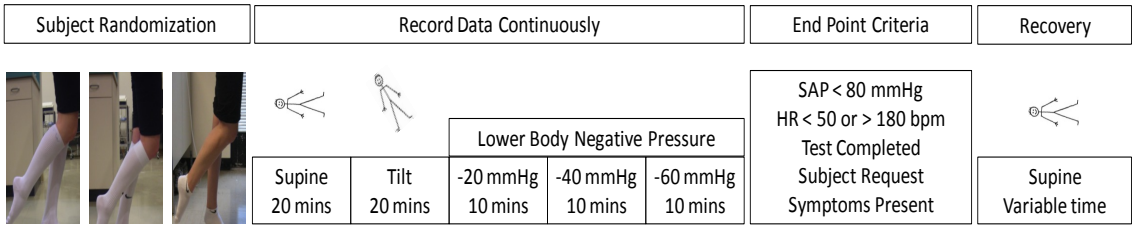

Figure 4.1 Schematic diagram showing the experimental protocol to be employed in Aim 2.

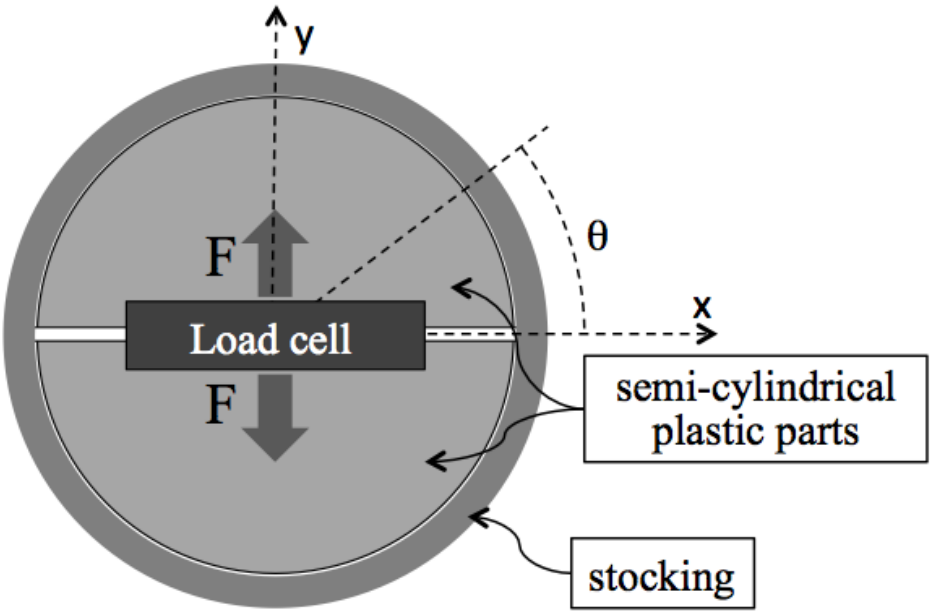

Figure 4.2. ***Schematic representation of a stocking stretched around the custom-made rig.***

Compression was derived from the measured force (F).

4.2.4. Data Analysis

MAP was calculated as DAP + 1/3 pulse pressure. FVR was calculated as MAP divided by BBFV. CMAP was calculated from MAP at heart level, corrected for the measured height difference between the temporal window and heart when upright (80). CVR was taken as CMAP/CBFV. Data are presented as 30-second averages, every two minutes throughout testing. Data at presyncope represent the final value for each variable prior to the return to the supine position. Note that because of variable times at which presyncope was initiated and the tests stopped, the number of subjects included for each data point decreased as the test progressed. Thus, cardiovascular responses are presented only for the first 30 minutes of orthostasis.

4.2.5. Statistical Analyses

Statistical analyses were performed using SigmaPlot version 11 (Systat Software Inc, San Jose, CA) and JMP (Statistical Analysis Systems, Cary, North Carolina.) Data were tested for normality using the Kolmogorov and Smirnov assumption and parametric or non-parametric testing used accordingly. Data are reported as means ± SEM. Significance was assumed where $p<0.05$. Comparisons between groups and over time were conducted using repeated measures ANOVA, with the Tukey or Bonferroni post hoc test. Differences in OT between conditions were determined using a randomised complete block design ANOVA. We also used a two factor blocked analysis of variance to analyze the OT data, where stocking condition and order of intervention were the explanatory variables (factors) and the subject was the block. Correlations between variables were determined using Pearson Product Moment Analyses or Spearman Rank Order tests for parametric and non-parametric data respectively. Multiple regression analyses were used to develop a predictive model for the expected change in OT from selected anthropometric characteristics.

4.3. Results

4.3.1. Orthostatic tolerance

All subjects experienced presyncope with hypotension, which triggered termination of each test, consistent with a vasovagal response. The time to presyncope was not significantly different between the three conditions (calf placebo 29.9±1.8, ankle placebo 27.6±2.4, and compression stocking 26.0±2.0 min; data for each experimental condition will be presented in the text in this order throughout), Figure 4.3A. Kaplan-Meier plots also indicated no differences in OT on the three test days (Figure 4.3B). Therefore, we combined data from the two placebo conditions. The OT remained similar between the placebo and compression stocking conditions (Figure 4.3C). There was no significant effect of the order in which the interventions were received, and no significant interaction between the stocking condition and order in which the stockings were applied.

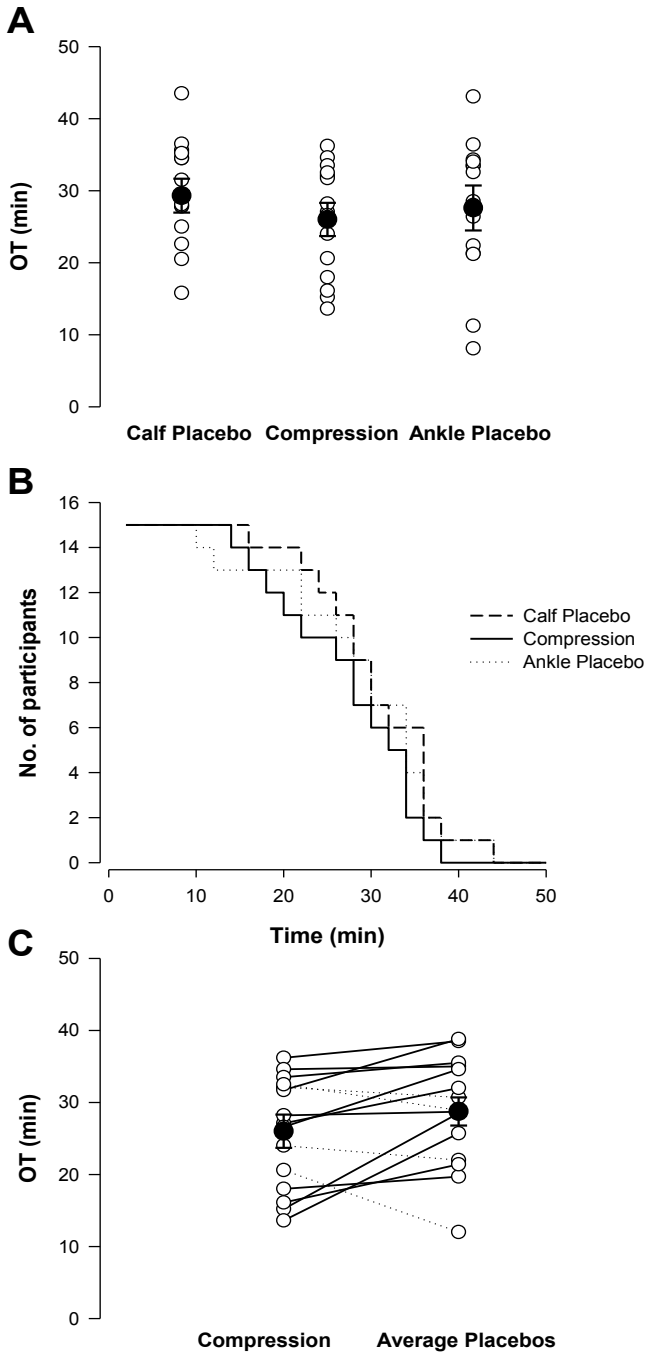

Figure 4.3. OT in the three test conditions.

There were no significant differences in OT between conditions (A). Kaplan-Meier plots also revealed similar times to presyncope in all test conditions (B). When the two placebo conditions were combined, OT remained similar in placebo compared to compression stocking conditions (C). Solid lines denote those in whom OT improved with compression stockings compared to placebo conditions, and dashed lines denote those in whom OT was worse with compression stockings. Filled circles denote mean data.

4.3.2. Cardiovascular responses

4.3.2.1. Blood pressure

Resting blood pressures were similar in all three conditions (114.9±4.1/61.7±3.0 mm Hg, 116.4±3.3/63.8±2.6 mm Hg and 117.6±2.5/66.8±2.3 mm Hg). There were no significant differences in systolic or diastolic arterial pressures between conditions at any stage of testing (Figure 4.4). Blood pressure falls at presyncope were similar for all conditions (72.2±2.6/52.8±2.2 mm Hg, 66.8±3.7/48.3±1.9 mm Hg and 71.8±1.5/51.3±2.4 mm Hg).

4.3.2.2. Stroke volume, heart rate, and cardiac output

Supine SV were similar in all three conditions (82.6±4.2, 83.9±3.9 and 83.1±3.2 mL, Figure 4.5). Values at presyncope, during tilt and LBNP, were significantly reduced compared to supine ($p<0.001$).

Resting HR (61.2±2.7, 61.7±1.9 and 62.2±2.3 bpm), as well as the maximum HR responses to the orthostatic stress (115.9±5.5, 110.3±4.9 and 113.9±6.0 bpm), were similar in all conditions. Maximum responses, and values at presyncope and during LBNP, were significantly greater than supine in all conditions ($p<0.01$).

Resting CO were similar in all conditions (5.0±0.3, 5.2±0.3 and 5.2±0.3 L). Values at presyncope were significantly reduced compared to supine, tilt and LBNP ($p<0.01$).

There were no significant differences in SV, HR or CO between conditions at any stage of testing.

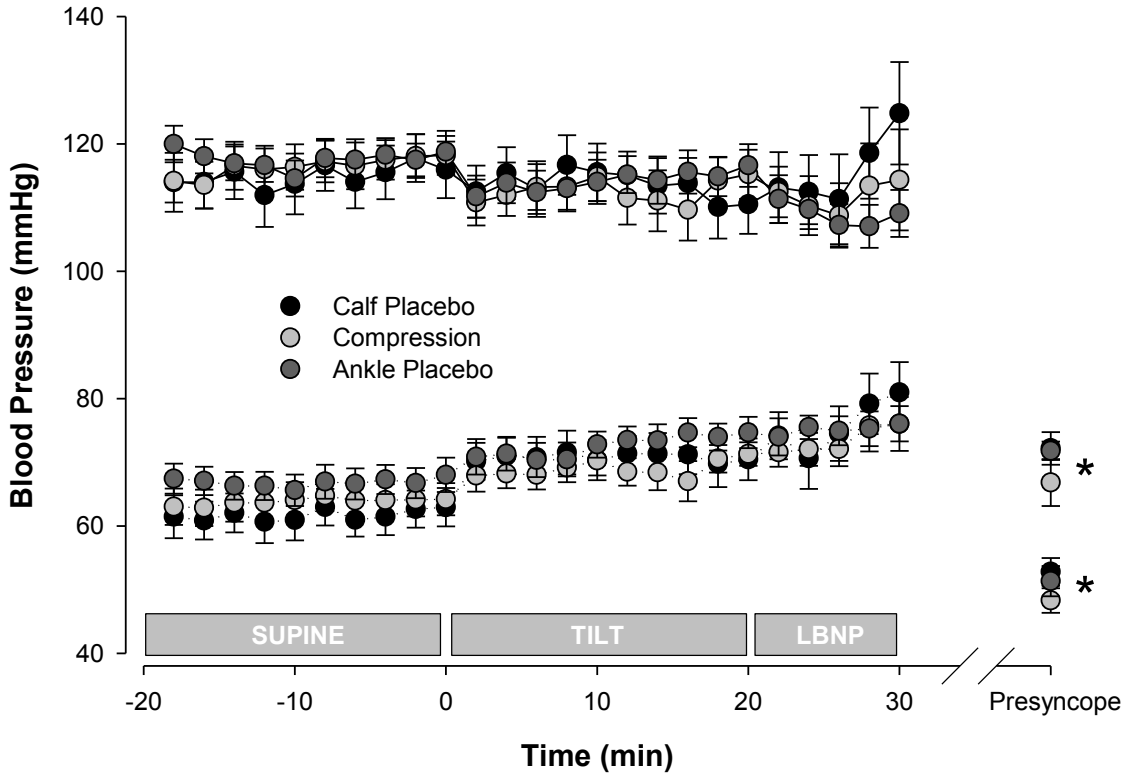

Figure 4.4. Blood pressure responses in the three test conditions.

Solid lines, systolic arterial pressures; dotted lines, diastolic arterial pressures. There were no significant differences in systolic or diastolic arterial pressures between test conditions at any time point. Values at presyncope were significantly reduced compared to supine in all conditions (* denotes $p<0.01$).

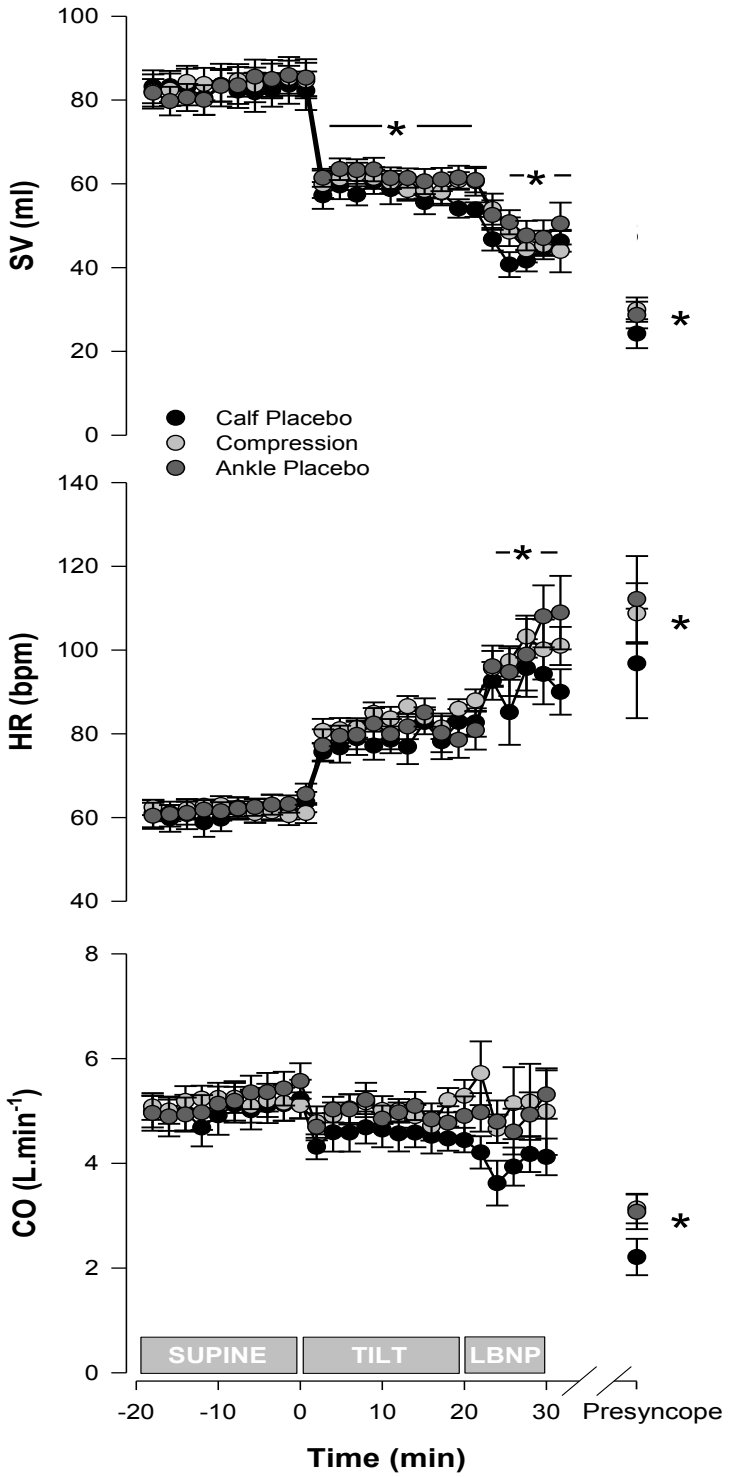

Figure 4.5. HR, SV and CO responses in the three test conditions.

There were no significant differences in HR, SV or CO between test conditions at any time point. Significant differences from supine in all conditions are indicated by the * ($p<0.05$).

4.3.2.3. Peripheral resistance responses

Resting TPR (1527±121, 1422±70 and 1373±85 dyne.s.cm^{-5}) and FVR (11.7±1.7, 10.5±1.6 and 13.6±2.1 units) were similar in all three conditions. There was a significant increase ($p<0.05$ compared to supine) in both TPR (maximum response 2492±419, 1983±200 and 1632±88 dyne.s.cm^{-5}) and FVR (maximum response 30.1±4.9, 17.9±2.3 and 45.1±13.4 units) during orthostatic stress in each condition. The magnitudes of these responses were similar for each test condition.

There were no significant differences in TPR or FVR between conditions at any stage of testing.

4.3.2.4. Cerebral haemodynamics

CMAP was significantly reduced in all conditions during orthostatic stress compared to supine (Figure 4.6). In each condition, there was a further significant reduction in CMAP at presyncope compared to supine. Values at presyncope were similar in each condition. There were no significant differences in CMAP between conditions at any stage of testing.

CBFV was similar at rest in all conditions (54.4±3.5, 58.9±3.3 and 64.6±3.8 cm.s^{-1}), Figure 4.6. CBFV was maintained, until presyncope, at levels not significantly different from supine for each condition. At presyncope, CBFV decreased compared to supine values, to 37.2±3.8, 41.0±4.0 and 44.0±5.2 cm.s^{-1} for each test respectively. The magnitude of the reduction in CBFV was similar for each condition (-19.8±4.2, -22.2±4.2 and -14.1±5.8 cm.s^{-1}).

CVR was not significantly different between conditions at any stage of testing and did not change significantly within each test compared to supine. Both the correlation coefficient and the gradient describing the relationship between CMAP and CBFV were similar for all conditions (Figure 4.7).

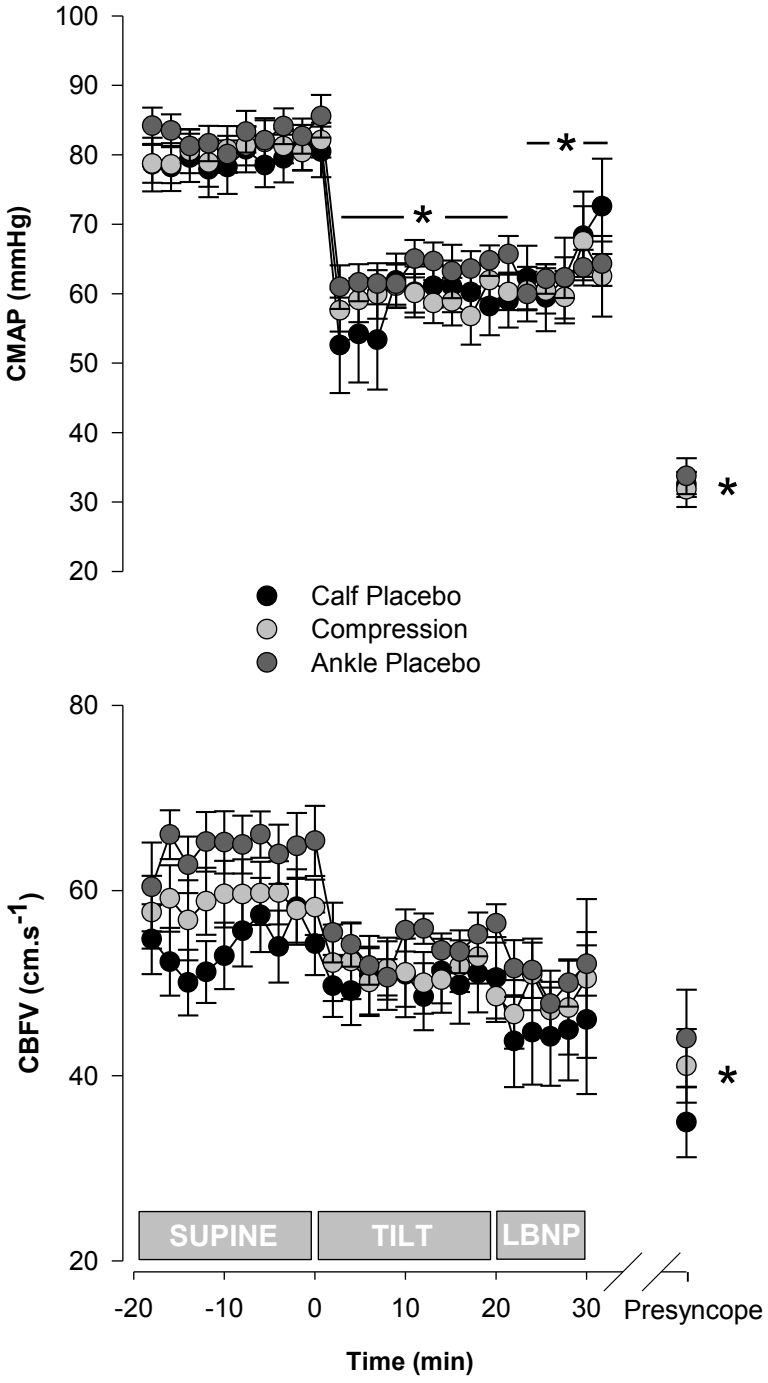

Figure 4.6. CMAP and CBFV in the three test conditions.

There were no significant differences in CMAP or CBFV between conditions at any time point. Significant differences from supine in all conditions are indicated by the * ($p<0.05$).

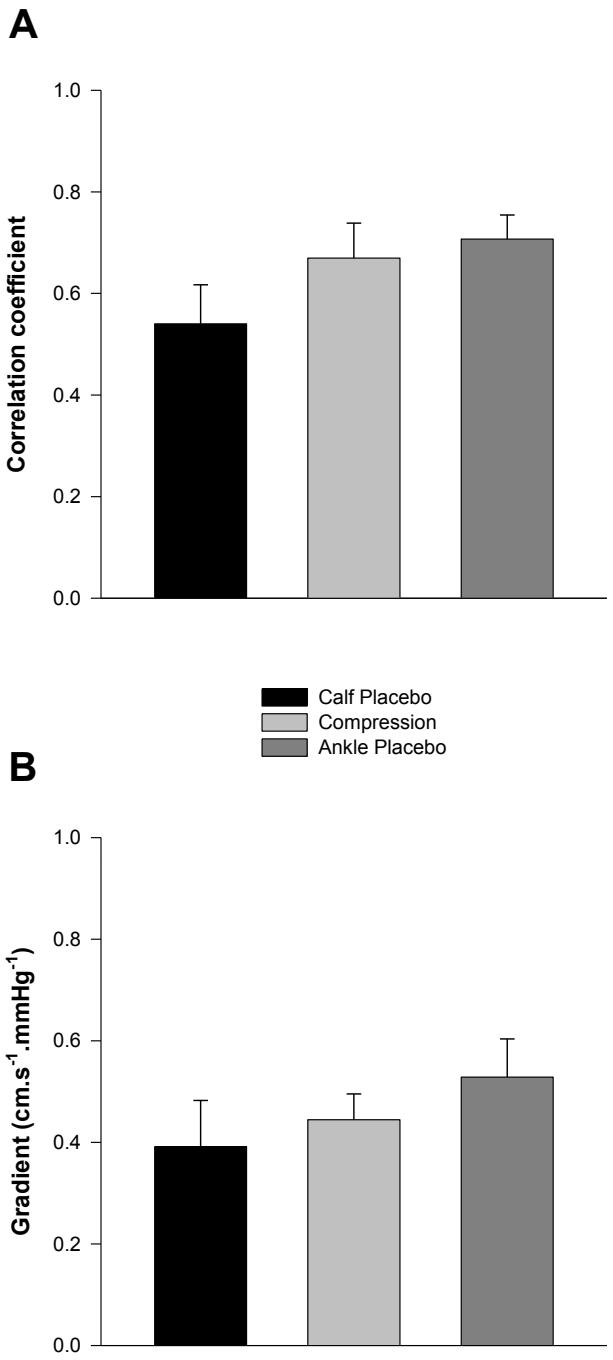

Figure 4.7. Cerebral autoregulatory responses in the three test conditions.

There were no significant differences in either the correlation coefficient (A) or the gradient (B) describing the efficiency of cerebral autoregulation between the three conditions.

4.3.2.5. End-tidal gases

There were no significant differences in $P_{ET}CO_2$ or $P_{ET}O_2$ between conditions at any stage of testing. The $P_{ET}CO_2$ decreased, and $P_{ET}O_2$ increased, at presyncope compared to supine in each condition ($p<0.001$), suggesting hyperventilation relative to baseline values. The absolute values were not significantly different at presyncope between conditions. The magnitude of the reduction in $P_{ET}CO_2$ from supine to presyncope was also similar in all conditions (-7.4±1.2, -6.6±1.0 and -7.0±1.1 mm Hg).

4.3.3. Relationships between orthostatic tolerance and anthropometric variables

Although the mean OT was not different between conditions, we noted considerable variability between individual responses; with some showing greater OT with compression stockings, and some showing reduced OT (Figure 4.3C). To examine whether this might be related to anthropometric variables, we qualified the OT while wearing the compression stocking relative to the mean of the two placebo conditions. The change in OT was positively correlated to the height: calf circumference ratio and negatively correlated to the calf circumference: shoe size ratio (Figure 4.8A & B). The efficacy of the compression stocking was predicted by a model based on the calf circumference and shoe size (Figure 4.8C) as follows:

$$Change\ in\ OT\ (min) = 26.266 - [1.398 \bullet calf\ \ circumference\ (cm)] + [2.538 \bullet shoe\ size]$$

There was no significant relationship between the change in OT and the calf circumference measurements when expressed as muscle or adipose cross-sectional areas.

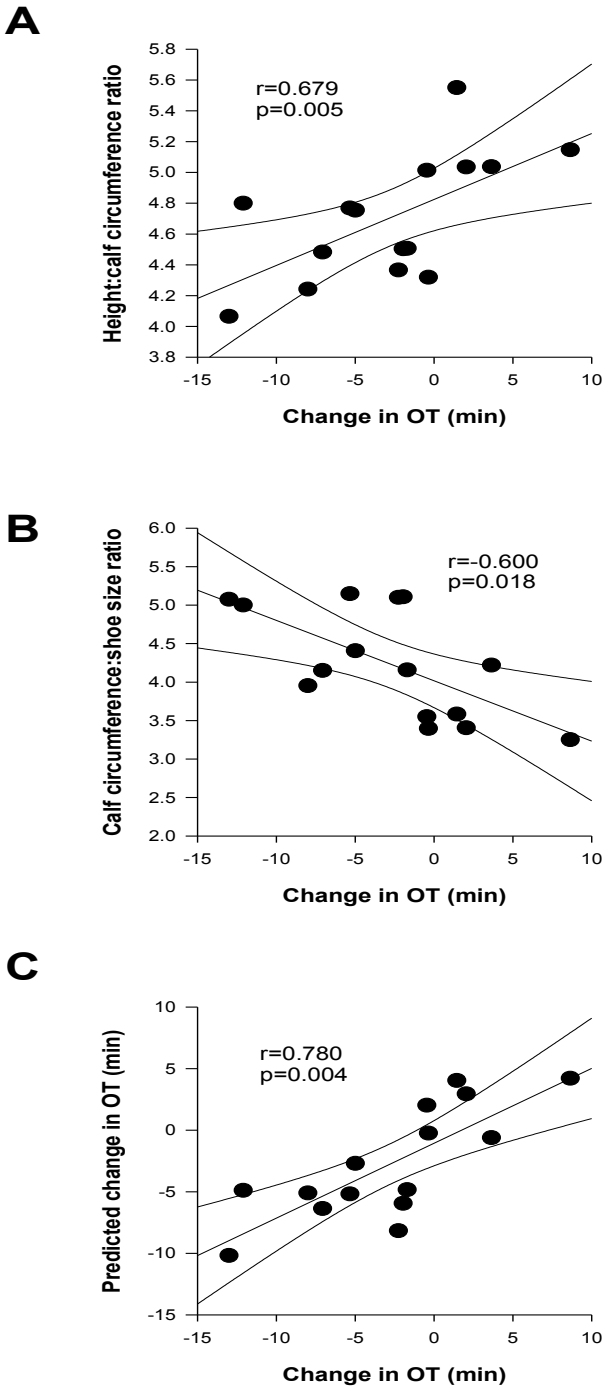

Figure 4.8. Relationship between the change in OT with compression stockings and anthropometric variables.

There was a significant positive correlation between the change in OT and the height: calf circumference ratio (A). There was a significant negative correlation between the change in OT and the calf circumference: shoe size ratio (B). The efficacy of the compression stockings could be predicted from the subject's shoe size and calf circumference (C).

4.3.4. Calf compression measurements

Compression data could not be collected for the ankle placebo stocking. The calf placebo stocking applied minimal compression at low distending circumferences (Figure 4.9A), but a tight band at the knee resulted in high compression levels at this point with larger distending circumferences. The compression stocking applied graded compression at all distending circumferences, with the highest levels at the ankle, and lowest levels at the knee (Figure 4.9B). The measured calf circumference at the mid-calf in our volunteers was 37.1±0.8cm (range 32.3-41.5cm), similar to previous reports (174, 175). Typical values for leg circumferences at the ankle and knee are 22.7±0.1cm and 39.9±0.4cm (174, 175). When the two stockings were compared at these physiological distending circumferences for each region of interest (Figure 4.9C) it was seen that the compression stocking applied higher pressures at the ankle and mid-calf, but lower pressures at the knee compared to the calf placebo stocking.

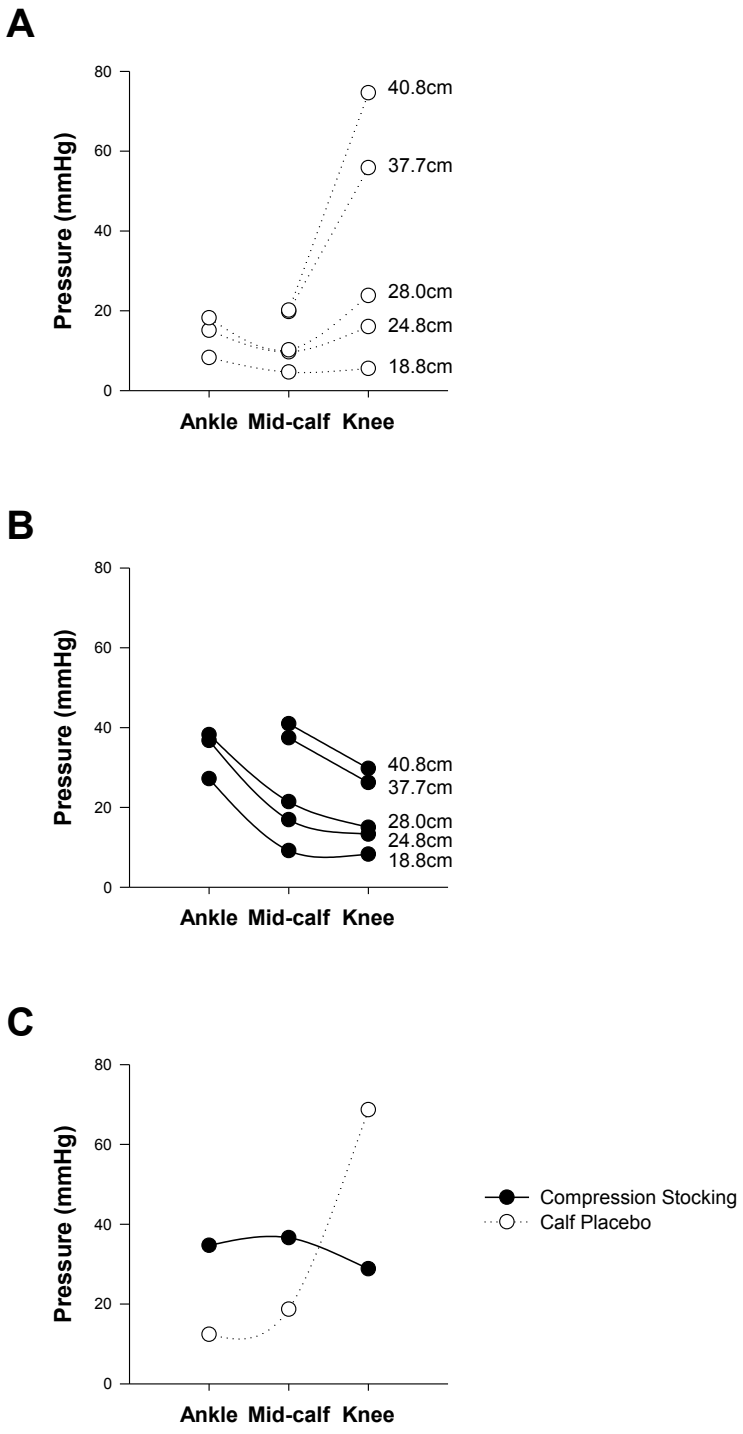

Figure 4.9. Compression levels for the compression and calf placebo stockings.

Compression pressures applied over a range of distending circumferences can be seen at each region of interest for the calf placebo (A) and compression stockings (B). The compression pressures applied by the two stockings were compared at physiological distending circumferences for each region of interest (C).

4.4. Discussion

We have demonstrated that graded calf compression stockings had no significant effect on OT in a randomised, double-blind, placebo-controlled study. Despite the lack of improvement in OT with compression stockings in the group as a whole, some individuals exhibited modest improvements in OT with compression stockings whereas others did not. From our anthropometric analysis we found that the calf circumference was a key determinant of the efficacy of the compression stocking. This has implications for their clinical use for the treatment of orthostatic intolerance, and underscores the need for individualised therapy when considering compression stockings as a treatment paradigm.

We selected a test that has a clearly defined end-point in all subjects, and is highly reproducible, sensitive and specific (94, 96, 135). As such, we are confident that had there been a significant effect of the compression stocking on OT we would have been able to detect it. We are also confident that the test end point (presyncope), and therefore the OT, was reliably determined because the terminating blood pressures and HR (as well as the other cardiovascular variables) were similar in all conditions. Furthermore, the investigator responsible for terminating the test was blinded to the test condition, to ensure this could not have influenced the result.

We tested a compression stocking reported to generate 20 mm Hg compression at the ankle, graduated to 15 mm Hg at the knee (www.sigvarisusa.com). At the mean leg circumferences of our group we measured compression of 35 mm Hg at the ankle, graduated to 29 mm Hg at the knee for this stocking, higher than quoted by the manufacturer. We found that the calf placebo may not have been a true placebo because although it applied minimal compression at the ankle and mid-calf (<15 mm Hg) it did apply high compression just under the knee. However, we also included the ankle placebo, which could not have applied calf compression. Given that the responses were similar in all conditions, we are confident in our assertion of the lack of efficacy of the compression stocking tested. Finally, although this study was randomised, we also examined the possibility that there could be an effect or interaction between the order in which the stockings were tested and the orthostatic tolerance. This was not the case.

Our findings are compatible with earlier observations that compression of the calf has minimal effect on OT (168-170). However, some subjects had modest improvements in OT from calf compression stockings, and this could be predicted from simple anthropometric variables. The question as to why the anthropometric data influence the efficacy of the stockings remains. It could be that the compression stockings were over-stretched in those with large calf circumferences relative to their shoe size, applying higher pressures than intended. If sufficient to impede venous return, this could exacerbate venous pooling and so reduce OT. In contrast, in those with a smaller calf circumference relative to their shoe size, the compression may be just sufficient to enhance venous return, and delay the onset of syncope. Further studies are required to examine these possibilities. The fact that the efficacy of the compression stockings was related to the calf circumference, and not the proportion of muscle, suggests that this effect is not mediated via alterations in the mechanics of the skeletal muscle pump with compression stockings. However, we acknowledge that with tilt testing the skeletal muscle pumps are largely inactivated, so their potential role in the efficacy of compression stockings during active standing is unclear.

4.4.1. Cardiovascular responses

Despite testing in healthy adults, six subjects exhibited poor OT during each test, compared to previously published “normal” values (94). We and others occasionally observe poor OT in apparently healthy controls (142, 176), and this false positive response during tilt testing appears to reflect impaired reflex control of the circulation that is compensated by greater activation of the skeletal muscle pump during active standing (31, 142). The fact that our control population included some individuals with poor OT does not negate our finding that compression stockings were ineffective at improving OT. In fact, it strengthens this argument, because the potential ceiling effect of testing only individuals with high OT is lessened. Furthermore, sub-analyses revealed that the influence of compression stockings was not related to the baseline OT.

Cardiovascular responses to the test were similar in all conditions at all time points. These observations underscore both the repeatability of the test, and the lack of efficacy of the compression stockings.

As expected in healthy controls, systolic and diastolic arterial pressures were maintained throughout testing, until the point of presyncope, reflecting appropriate arterial baroreflex responses to the gravitational fluid shifts imposed. At presyncope there was a sudden fall in arterial pressures, consistent with the onset of a vasovagal response (16, 167). In all subjects each test was terminated with a systolic pressure below 80 mm Hg, associated with symptoms of presyncope.

We observed baroreflex-mediated tachycardia that increased in a stepwise fashion at the beginning of each test phase (167). The magnitude of this response was similar for all conditions. We did not observe significant bradycardia at presyncope in all subjects, likely due to either prompt termination of the test (prior to the bradycardia that typically accompanies a vasovagal response), or reflecting that this cardioinhibitory component of the reflex is not always present (177).

SV also decreased in a stepwise fashion during the orthostatic stress, decreasing by approximately 67% in all conditions at presyncope. This is compatible with reduced venous return when upright, secondary to venous pooling and plasma filtration (167).

CO was maintained throughout each test, until presyncope, when it decreased precipitously. The maintenance of CO prior to presyncope likely reflects the intact baroreflex response in these healthy control volunteers, whereby reductions in SV were accompanied by compensatory increases in HR. Indeed, the increase in HR in all conditions was approximately 73%, closely matching the fall in SV.

We observed baroreflex-mediated increases in FVR and TPR during orthostatic stress in each condition. This response was smaller in magnitude than has previously been observed in healthy control volunteers, presumably reflecting that some volunteers in this study had poor OT, and impaired vascular responses (143, 144, 167).

In each condition, due to the hydrostatic gradient imposed when upright, CMAP decreased similarly with the initial postural change, but was then maintained until presyncope. Despite the fall in CMAP, CBFV was maintained throughout each test indicating intact autoregulatory responses, until presyncope when the perfusion pressures were below the lower limit of autoregulation (178). Indeed, when we quantified autoregulation from the correlation coefficient and gradient describing the relationship

between CMAP and CBFV (whereby a steep gradient and high correlation coefficient indicate impaired autoregulation) (80) we confirmed similar autoregulatory control in each test condition. Accordingly, CVR responses were also similar between conditions. Again, this is compatible with minimal haemodynamic effect of the compression stockings. We also determined $P_{ET}CO_2$ and $P_{ET}O_2$ throughout testing, because of their known effect on CBFV (81). Although $P_{ET}CO_2$ decreased and $P_{ET}O_2$ increased at presyncope, compatible with the modest hyperventilation that is known to accompany presyncopal episodes (81), the magnitude of these changes was similar for each test, confirming a similar challenge to cerebral autoregulation on each occasion.

Thus, the use of graded calf compression stockings did not influence cardiovascular responses during an orthostatic stress continued to presyncope.

4.5. Limitations

We evaluated the efficacy of graded calf compression stockings on OT, and accordingly our results may not extend to other compression garments. The existing literature suggests that compression garments extending to the thigh and abdomen may be more effective at preventing orthostatic intolerance (168-170), but are associated with poor patient satisfaction and compliance (171, 173). Future studies may wish to examine the optimum compromise between efficacy, comfort, and patient compliance.

Although subjects were not informed which stocking they were wearing on each test day, nor were they told the anticipated outcome of the test, it is possible that the study was not truly double-blinded. The ankle placebo is visually distinct from the calf placebo and compression stocking, and subjects may have been aware of different sensations of compression or tightness of the stockings. However, when questioned after completion of all three conditions, volunteers could not consistently identify the compression stocking.

Thirdly, we chose to conduct testing in healthy volunteers, and it is not known whether the results would extend similarly to patient populations. However, we expect this would be the case, because a number of our control volunteers actually had poor OT, similar to that of patients with syncopal episodes. Furthermore, other non-pharmacological

approaches to prevent or delay syncope apply equally well to both patients and controls (60, 179).

Finally, it may be that the application of compression stockings prior to rising in the morning would have a greater effect, due to the "water jacket effect", whereby oedema accumulating during the day restricts further venous pooling (180). However, it has been shown that 20 minutes of supine rest is sufficient to normalise any prior venous pooling/capillary filtration effect (30), at least in control subjects, so we consider this unlikely.

4.6. Compression stockings in other populations

We aimed to evaluate a potential non-pharmacological approach for the treatment of orthostatic presyncope and syncope in young adults and, subsequently, in children and adolescents. Given the lack of efficacy demonstrated in young adults, this study was not extended into a paediatric patient population. The patient burden for affected children to report to the laboratory for testing until presyncope on multiple occasions was felt to be unwarranted, particularly in the light of the lack of significant improvement in orthostatic tolerance in young adults with compression stockings. However, it may be that some children could experience benefit from compression stockings, if the relationship between their efficacy and leg anthropometric variables seen in young adults also extended to paediatric populations.

One patient population that may be hypothesised to experience benefit from compression stockings might be individuals with spinal cord injury (SCI). Up to 74% of SCI patients are susceptible to episodes of presyncope and syncope, specifically orthostatic hypotension, due to a combination of factors including injury to descending spinal sympathetic pathways (and thus the inability to mount an appropriate vascular resistance response to orthostasis), and the absence of skeletal muscle pumping activity to counteract venous pooling in the paralysed lower limbs (181). These episodes are devastating, and impede rehabilitation, negatively impacting quality of life for those affected. However, SCI patients often experience lower limb muscle atrophy secondary to paralysis, and as such, their slender calves may provide better promise for a positive

outcome of the stockings. The potential drawback of compression stocking use in this population relates to the loss of functional independence in those who may require assistance with putting on the stockings. As a consequence, patient compliance with stocking therapy is likely to be adversely affected. However, if efficacious this intervention has the potential to greatly improve quality of life for individuals living with SCI, and as such, the ability to use this non-pharmacological option in this population should be evaluated in the future.

4.7. Conclusions

These data question the use of calf compression stockings for orthostatic intolerance and highlight the need for individualised therapy accounting for anthropometric variables when considering treatment with compression stockings.

5. Final Discussion

This project has given further insight into the complexities of syncope and presyncope in children and adolescents. We have highlighted not only the applicability of autonomic testing in a paediatric population, but we have also uncovered important additions for existing diagnostic criteria for (pre)syncope, such as measures of static and dynamic cerebral autoregulation. Furthermore, we have evaluated the utility of compression stockings for the prevention of orthostatic intolerance in young adults. This work highlights the need for further research in this area to define and apply new diagnostic criteria for children with syncope, as well as to develop and evaluate more appropriate treatment options for paediatric patients.

We **determined the type and severity of cardiovascular autonomic dysfunction in children and adolescents with syncope and presyncope**. This project highlighted that (pre)syncope is prevalent among children and adolescents, with four of the controls reporting presyncope on one occasion before the tilt test. This further supports that (pre)syncope is under-reported and could affect a larger number of individuals than is presently known. In the patient group, based on their medical history it was clear that the severity and frequent recurrence of their syncopal episodes was having a severe impact on their quality of life.

We **evaluated the relative contribution of impaired cerebral autoregulation and hyperventilation to syncope or presyncope in children and adolescents** during orthostatic stress. Both static and dynamic autoregulation appeared to be impaired in patients with syncope; however, a larger cohort is required to evaluate the statistical significance of this apparent impairment. This novel observation in children and adolescents supports previous work in adult syncope patients who also exhibit impaired autoregulation, which, in turn, makes them more susceptible to postural syncope (80). Hyperventilation was present in patients during orthostatic stress, particularly in the early phases of tilt, and this could contribute to syncopal episodes, particularly if the cerebral

circulation exhibited enhanced sensitivity to hypocapnia. There was a disconnect between cardiac and peripheral baroreflex function in children with syncope that would be expected to predispose them to hypotension and tachycardia during orthostasis. These findings support previous work in adult syncope patients, and should be considered in future work in identifying the susceptibility to (pre)syncope in children (22, 182).

We aimed to **identify diagnostic criteria for children and adolescents with syncope and presyncope**. Our sample size was too small to identify new diagnostic criteria for the use in a clinical setting; however, it supports existing work that there are differences in cardiovascular responses to orthostatic stress between adults and children (115, 145). Further testing in a larger cohort of children with dysautonomia, as well as in healthy controls, is necessary to define new criteria using this experimental protocol.

The second part of this project was **to treat syncope and presyncope in children and adolescents with a non-pharmacological approach.** We hypothesized that compression stockings would improve OT and thus be a universal, non-pharmacological treatment for children, adolescents, and adults suffering from disorders of orthostatic intolerance. This work is of particular interest because of the common practice of prescribing compression stockings for orthostatic intolerance, despite little evidence of their efficacy. We **performed a pilot study in healthy adults to test the efficacy of compression stockings for the treatment of orthostatic syncope and presyncope** before moving into a paediatric population. It was found that compression stockings were not effective in every individual tested. A multiple regression analysis, using anthropometric variables, produced an equation for use when prescribing compression stockings in a clinical setting. This may provide a tool for physicians to use to enable them to predict the effect of compression stockings on OT, on an individual basis, based on individual patient shoe size and calf circumference. As such, the potential use of compression stockings as a non-pharmacological treatment can be assessed before their use. Importantly, if OT is predicted to diminish with compression stockings, their use can be avoided.

Finally, we aimed **to perform a second pilot study (if stocking efficacy was proven in adults) evaluating the efficacy of compression stockings in children and**

adolescents with syncope. As the efficacy of compression stockings was controversial and not proven in all adults, this experiment was not conducted. The associated patient burden with multiple tilt tests and little promise of efficacy (based on our study in young adults) was one factor in deciding not to perform this study.

Overall, this project highlighted the complexity of presyncope and syncope in children and adolescents. There are many future directions for this body of work to gain a better understanding of the mechanisms by which episodes occur, which will then guide diagnosis, treatment and ultimately improvements in the quality of life for patients affected by these devastating episodes.

6. Future Directions

6.1. Syncope in children and adolescents

It is imperative that these studies be extended to a larger cohort to ensure statistical confidence in our data. We aim to include additional studies in both patients and controls that continue to be age and gender matched to accurately define normal and abnormal responses to orthostatic stress in this population. There are many other factors that relate to blood pressure regulation and thus affect orthostatic stress. We have ethical approval to analyze blood samples for levels of plasma adrenaline, noradrenaline, dopamine and serotonin at the end of the supine period and at five minutes after tilt. However, the use of an intravenous catheter may decrease the sensitivity of the test due to the invasive component of this test. It is well known that the use of needles during orthostatic testing decreases OT when compared to a non-invasive protocol (94). However, the analysis of plasma catecholamines may provide additional insight into the neurohumoral control of the circulation, and the benefit of this information may outweigh the associated risks of diminishing OT. All future subjects will be given the option to take part in this protocol and its analysis will enable a deeper understanding of the roles of other molecules impacting cardiovascular responses to orthostasis.

Often syncope in adolescents is associated with a recent growth spurt (12). Consequently, it may be useful to evaluate hormone levels associated with puberty to evaluate the effects, if any, on autonomic control. It is well known that oestrogen and progesterone have direct effects on the vasculature and therefore, these actions could potentially alter OT and the cardiovascular responses during orthostatic stress. (183, 184). This would complement the plasma catecholamine data and could be retrieved at the same time point without any further additions to the protocol or burden to the subject.

6.2. Compression stockings as a treatment for presyncope

A pilot study in an SCI patient population should be conducted to test the efficacy of compression stockings. Based on the evidence that thin calves are associated with the greatest benefit from the stockings, this population may be particularly well positioned to experience an improvement in their OT with compression hosiery due to the lower limb atrophy that accompanies SCI. If their efficacy is proven in this population, this would improve quality of life and also rehabilitation experiences for those living with SCI, especially in SCI patients with higher lesions who have greater autonomic dysfunction.

Finally, a commonly discussed recommendation for children and adolescents with dysautonomia is an increase in dietary salt intake in the hope it will improve OT. Physiologically, an increase in salt intake leads to an increase in plasma volume. In turn, a higher plasma volume improves OT through improved maintenance of blood pressure. Salt supplementation can be achieved in the diet alone or by the prescription of salt tablets. Previous work has evaluated the efficacy of 6 g slow-release sodium chloride tablets for improving OT in adult patients and has demonstrated considerable benefit (59). Unlike the compression stocking data, this body of work strongly suggests that salt supplementation could also be successful in preventing presyncopal and syncopal episodes in paediatric populations. As such, evaluation of salt supplementation for the treatment of orthostatic tolerance in paediatric patients would be of interest. Subjects would perform a tilt test prior to this intervention and after three months of salt supplementation. If OT was improved, this would guide further treatment for other patients suffering from orthostatic intolerance. Importantly, blood pressure would be closely monitored during this intervention. It is well known that a precursor for hypertension is a high salt diet and therefore, before salt supplementation becomes part of routine prescription in children and adolescents, it would have to be shown that supine blood pressure is not elevated with the use of 6 g tablets (59).

7. Publications from this thesis

7.1. Manuscripts

C.L. Protheroe, H.J.C. Ravensbergen, J.A. Inskip, and V.E. Claydon (2012). Tilt testing with combined lower body negative pressure: a “gold standard” for the determination of orthostatic tolerance. Journal Of Visualized Experiments. In press.

C.L. Protheroe, A. Dikareva, C. Menon, and V.E. Claydon (2011). *Are compression stockings an effective treatment for orthostatic presyncope?* PLoS One, 6(12): e28193.

7.2. Abstracts

CL Protheroe, A Dikareva, VE Claydon (2010) The effect of compression stockings on orthostatic tolerance. Proceedings of the Disability Health Research Network Annual Research Conference, Meeting Proceedings. Vancouver, Canada. *This presentation won the prize for the best poster by an undergraduate student.*

CL Protheroe, A Dikareva, VE Claydon (2010) Compression stockings: a controversial treatment for orthostatic intolerance. Proceedings of the Biomedical Physiology and Kinesiology Research Day, Meeting Proceedings. *This presentation won the prize for Best Undergraduate Student Poster Presentation.*

CL Protheroe, A Dikareva, VE Claydon (2010) Are compression stockings really an effective treatment for orthostatic intolerance? *Clinical Autonomic Research,* 20: 312.

CL Protheroe, C Albaro, JM Roller, K Gibbs, S Sanatani, VE Claydon (2011) Investigating syncope in children and adolescents: preliminary observations. Clinical Autonomic Research, 21: 280.

CL Protheroe, C Albaro, JM Roller, K Gibbs, S Sanatani, VE Claydon (2011) Investigating syncope in children and adolescents: preliminary observations. Autonomic Neuroscience: basic and clinical, 163: 104.

CL Protheroe, C Albaro, JM Roller, K Gibbs, S Sanatani, VE Claydon (2011) Investigating syncope in children and adolescents: preliminary observations. Proceedings of the Biomedical Physiology and Kinesiology Research Day, Meeting Proceedings.

CL Protheroe, A Dikareva, VE Claydon (2011) Are compression stockings really an effective treatment for orthostatic intolerance? *Canadian Journal of Cardiology,* 27: S179.

CL Protheroe, A De Souza, K Gibbs, VE Claydon, S Sanatani. (2012) Running: How is it taught and evaluated in British Columbian Schools. Paediatrics & Child Health. 17: 26A.

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Appendices

Appendix A.

Example Patient Consent Form

Study Title: Cardiovascular Autonomic Control in Children and Adolescents Consent Form

Principal Site Investigator:

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NOTE: The pronouns “you” and “yours” should be read as referring to the participant rather than the parent/legal guardian who is signing the consent form for the participant.

INVITATION TO PARTICIPATE

You are invited to participate in a research study designed to gather medical information about the circulatory system (how your heart pumps and your nerves work to control your blood pressure) in young people ages 6 to 18. You have been invited to participate because you have had episodes of fainting (syncope) or what is known as pre-syncope that is the term used to describe the feelings of dizziness, or unwellness a person experiences just before they faint.

The purpose of this form is to provide you with the information you require to make an informed decision about whether or not to participate in this research study.

YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

Please take time to read the following information carefully and to discuss it with your family, friends, and family doctor before you decide. You will have an opportunity to ask the study doctors questions you may have about the study.

WHO IS CONDUCTING THE STUDY?

Dr. Shubhayan Sanatani BSc, MD, FRCPC Director of Cardiac Pacing and Electrophysiology at British Columbia Children’s Heart Centre and Dr. Victoria Claydon, PhD., who is an Assistant Professor with the School of Biomedical Physiology and Kinesiology, Simon Fraser University along with Dr. Cecilia Albaro MD, Cardiology Fellow at British Columbia Children’s Heart Centre, and Dr. Gabriella Horvath MD, FRCPC, CCMG, who is an Assistant Professor with the Faculty of Medicine, University of British Columbia, have designed this study together. Dr. Claydon and one of Drs. Albaro, Horvath or Sanatani will supervise the study procedures

WHAT IS THE PURPOSE OF THE STUDY?

The primary purpose of this investigation is to determine the normal control mechanisms of the heart and circulation in healthy adolescents, and how these control systems may be affected in adolescents with autonomic impairment. The control of the blood pressure, heart beat and circulation is normally regulated by nerves (called autonomic nerves) that adjust these variables according to your activity. These nerves bring about appropriate changes that allow you to regulate your heart beat, blood pressure and circulation properly, for example when you stand up, or start exercising.

We have a good understanding of how these nerves work in adults, but we do not know whether they work in exactly the same way in children or adolescents.

In some children and adolescents these nerves may not be working properly, and this can cause abnormal heart rate or blood pressure responses to occur when standing or during exercise, predisposing to dizzy spells, tiredness, or fainting episodes (pre-syncope).

In this study we would like to test the cardiovascular autonomic control in healthy adolescents, so we can find out how they normally function. We would also like to test these control systems in children who are experiencing dizziness or fainting when standing or exercising.

WHO CAN PARTICIPATE IN THE STUDY?

You are eligible to take part in this study if you are English speaking; male or female aged 6-18 years inclusive.

You must have been examined by a paediatric cardiologist and been told that you have “reflex mediated pre-syncope”, this means that your episodes of dizziness or fainting are not because you have something wrong with your heart, but from some type of reflex that occurs in your nervous system.

You must be able to tolerate the “tilt test” to participate in this study.

WHO SHOULD NOT PARTICIPATE IN THE STUDY?

If you are younger than 6 or older than 18 you cannot participate.

You should not participate if you do not want to.

If you have had episodes of pre-syncope or syncope but have not been examined by a paediatric cardiologist you cannot take part in this study.

If you have been diagnosed with any cardiac arrhythmias (irregular heart rhythm), cardiac disease or cardiovascular disease (diabetes or high blood pressure) you cannot participate in this study.

If you cannot understand enough English to follow instructions during the testing you cannot participate.

If you have had a “tilt test” preformed before and could not tolerate the test you cannot participate in this study.

You cannot participate if you are female and may be pregnant.

WHAT DOES THE STUDY INVOLVE?

Before you start the test one of the study team will ask you some questions about your medical history, and your general health.

You will participate in two breathing exercises. The first evaluates the effects of changes in carbon dioxide and oxygen levels upon the control of blood flow to the brain. For this test we will ask you to breathe quickly, then slowly, and then back to normal again. The second exercise determines the response of heart rate and blood pressure while you blow into a tube for twenty seconds.

OPTIONAL BLOOD WORK: You can volunteer to have two blood samples drawn during the testing. This is optional, meaning you can say no to the blood work but still take part in the rest of the study

You will be asked to undergo a “tilt test”. This test measures your blood pressure control, and your susceptibility to dizzy spells.

For the test procedure, you will be asked to lie down on a bed while we attach monitoring equipment to your body. This will include:

An electrocardiogram (ECG). This is a monitor that will measure your heart beat (how fast and how regularly your heart is beating). We will attach six adhesive electrodes (stickers) to the skin of your chest, one on each of your wrists and one on each of your ankles, and connect them to the ECG machine. An alcohol swab will be used to clean the skin prior to electrode placement.

The stickers will be removed after we print the ECG recording from the machine. Removing the stickers can be uncomfortable, like removing a bandaid.

If you agree to blood work, a sterile needle will be inserted into a vein in your left forearm. Around the outside of the needle is a tiny flexible plastic tube called a cannula. The needle will place the cannula into a vein just under the skin of your arm, and the needle will be removed, leaving the cannula in place. This will allow blood testing while the other testing is taking place, but you will only need to have one needle poke. You will feel the poke as the needle goes in, (feels like a pin prick) but the cannula should feel comfortable once it is in place. This tube will stay in place for the rest of the test. The cannula will be taped securely to keep it from moving during the testing. You can keep your arm still during the testing to prevent the cannula being moved about.

A second ECG monitor with only three adhesive electrodes, will be attached to your chest, to assess your heart rate and rhythm during the rest of the tests.

A blood pressure monitor. A small Velcro cuff will be placed around your right middle finger that will pulse gently against the small arteries along the side of the finger, and records your blood pressure with every heart beat. This measurement is non-invasive and painless.

We will measure your breathing rate and the gases in the air you are breathing out with a small nasal cannula (a flexible tube). This small plastic tube will be placed under your nose, on your top lip, and will sample your breathing. You will be able to breathe and talk normally while wearing this device.

We will measure the blood flow to your brain in an artery called the middle cerebral artery. We will do this using ultrasound (imaging device). We will place a little ultrasound gel on your temple and will position an ultrasound probe overlying the gel. The probe will be held in place with a head band. This means the investigators will not need to touch you to hold the probe. You can move your head freely when wearing the ultrasound probe. You will not be able to feel the ultrasound.

We will also measure blood flow in your right arm, in the brachial artery, also with ultrasound. Your arm will be placed on a support with a probe positioned over a little ultrasound gel near the elbow. We will ask you to keep your arm still during the test.

We will place a strap over your knees and a box over your legs that seals against your waist (a bit like a canoe skirt). The strap is to help you stand in a relaxed position without fidgeting your legs too much. The box is placed over your legs so that we can apply lower body negative pressure to your legs later on in the test without disturbing the monitoring.

Once the monitors are in place we will make recordings from them for 20 minutes while you lie on your back and rest. After ten minutes, we will begin the first breathing exercise. We will measure any changes in blood flow to the brain during alterations in carbon dioxide levels in the body that occur when you change your breathing rate. First we will ask you to breathe quickly (hyperventilate) to decrease the carbon dioxide levels. Then we will ask you to breathe slowly through a tube to increase carbon dioxide levels. You will then be asked to breathe normally until the carbon dioxide levels return to normal. Most people do not find this unpleasant, as they control the rate at which they are breathing, so they are able to breathe at levels that are comfortable for them. You will not be able to feel the changes in the carbon dioxide levels. Some people feel dizzy when they breathe very fast. If this happens you can simply breathe a bit slower, and any dizzy feelings will go away.

You will then rest for another ten minutes. Next we will ask you to breath out forcefully (blow hard) into a closed tube attached to a pressure gauge. You can watch the pressure gauge and try to keep the pointer at 40 mm Hg for 20 seconds by adjusting how hard you blow. We will monitor your heart rate and blood pressure while you do this.

You will then be asked to lie back and rest for a further 20 minutes while we continue to make recordings. After 20 minutes, if you agree to have blood work done for the study, we will take a 10 mL blood sample (about 2 teaspoons) from the cannula in your arm. We do this to measure the

presence of molecules (called noradrenaline, adrenaline, serotonin and dopamine) in the blood that can be increased in some people who are prone to fainting or dizzy spells. We will then tilt the table into an upright position (at 60 degrees) so you are leaning backwards slightly. We will make recordings from the monitors for a further 20 minutes. We will ask you not to move your legs much during the test. After 5 minutes of standing, we will take another 10mL blood sample, to see if the levels of these molecules changed when you were standing. Next, After 20 minutes of standing, we will apply lower body negative pressure to the box over your legs. This will feel a little bit draughty, and may be a little noisy, but is not painful or unpleasant. The effect mimics prolonged standing. We will apply the lower body negative pressure at three different levels for 10 minutes each (-20 mm Hg, -40 mm Hg and -60 mm Hg).

The test will be stopped immediately if:

You complete the whole procedure (20 minutes lying down, 20 minutes standing, and 30 minutes of lower body negative pressure).

You experience symptoms of dizziness or light-headedness and/or your blood pressure or heart rate begin to decrease.

You request the test to stop.

You will then be returned to the lying down position. If you experienced dizziness at the end of the test, lying down will quickly resolve this. The monitors and cannula will be removed and any residue from the ultrasound gel will be removed. It is common to feel a bit hot and sweaty at the end of the test. There are showers near to the lab, and we can provide clean towels etc. for you to freshen up after the test if you wish. The testing will take approximately 2 1/2 hours of your time.

WHAT ARE THE POSSIBLE HARMS AND SIDE EFFECTS OF PARTICIPATING?

The study will take place in a controlled laboratory environment and most subjects do not find the assessments unpleasant. Every effort will be made to ensure your safety, privacy and comfort. Testing will be performed by a cardiovascular physiologist, with a medical doctor present. This will usually be one of the doctors in the Paediatric Cardiology departments at BC Children's Hospital. The following are discomforts or risks that may be associated with your procedures.

Placing the needle in the vein to draw blood may be painful. On rare occasions bruising may develop at the site of the blood draw. This may last a few days, but will eventually completely go away. There is also a less than 0.1% risk of infection at the puncture site. You may experience dizziness or feel faint when having the blood drawn or just afterwards.

During the tilt table test and breathing exercises you may experience some dizziness or lightheadedness associated with reduced blood pressure and/or heart rates. Rarely, subjects have been known to faint briefly. Actual fainting is unusual and is always very short in duration with rapid return to consciousness.

These assessments will take time to perform and you will be asked to keep still for about 1 hour during the assessments. You may find that you become uncomfortable or bored during the course of these investigations. Every effort will be made to maintain your comfort throughout the study. You will be provided with pillows, blankets etc as appropriate to ensure your comfort.

Preparing the skin for electrode placement may cause minor irritation or redness. It is possible that you will experience an allergic reaction to the electrode gel or adhesive.

You may find the procedure long and boring.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

There are no direct benefits to you from taking part in the study. It is hoped that the results of this study will ultimately aid in the treatment and management of autonomic impairment in children and adolescents, and so improve quality of life for those affected. It is possible that we may learn

new information about you from this test that you or your cardiologist is currently not aware of. If this happens we will provide the information to you and your parents so that you may discuss it with your doctor.

WHAT ARE THE ALTERNATIVES TO THE STUDY TREATMENT?

This is not a treatment study. You are being asked to share your health care information.

WHAT IF NEW INFORMATION BECOMES AVAILABLE THAT MAY AFFECT MY DECISION TO PARTICIPATE?

If information is gained during the course of the study which might affect your health care you will be notified by writing and during a visit to the British Columbia Children's Heart Centre.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You are free to choose not to participate and if you do become a subject you are free to withdraw from this study at any time during its course. If you choose not to participate or if you withdraw it will not harm your relationship with your own doctors nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

The study doctors may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

AFTER THE STUDY IS FINISHED

You will be notified of the study conclusions when the study is complete and all the data is reviewed. You will be responsible to keep your contact information current with Dr. Sanatani and his research staff so that they can contact you with the study findings.

WHAT WILL THE STUDY COST ME?

You will be given a $30.00 movie pass for your participation in the study. If there are any costs incurred for your participation in the study you will be reimbursed for your expenses if you provide the original receipts for any costs incurred.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected; this means that all your personal information will be kept private. The information collected in the study will be given a study code instead of your name and health care number. If there is reason to use your personal information in any way this would only be done if you allow it. Research records and medical records with your name and personal information may be inspected in the presence of Dr. Sanatani or his representative, by the UBC Research Ethics Boards and Simon Fraser University Research Ethics Board for the purpose of monitoring the research. However, these individuals are required to keep all information confidential. No records that identify you by name or initials will be allowed to leave the investigators' research office.

Your personal information will not be revealed, if any information is presented or written for other health care professionals

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, you can contact Dr. Shubhayan Sanatani at 604-875-3619 or his study coordinator at 604-875-2345 extension 7955.

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services by e-mail at RSIL@ors.ubc.ca or by phone 604-822-8598. *(Toll Free number 1-877-822-8598).* You could also contact Dr. Hal Weinberg, Director of the Office of Research Ethics at Simon Fraser University.

OPTIONAL BLOODWORK

I understand that having the blood testing for this study is optional and it is ok for me to participate in all the other study procedures even if I don't want to have my blood tested.

I AM willing to have a needle and cannula placed in my arm to draw blood for testing.

☐

I am NOT willing to have a needle and cannula placed in my arm to draw blood for testing.

☐

SUBJECT CONSENT TO PARTICIPATE

I have read and understand the subject information and consent form.

I have had sufficient time to consider the information provided and to ask for advice if necessary.

I have had the opportunity to ask questions and have had satisfactory responses to my questions.

I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.

I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.

I understand that I am not waiving any of my legal rights as a result of signing this consent form.

I understand that there is no guarantee that this study will provide any benefits to me.

I have read this form and I freely consent to participate in this study.

I have been told that I will receive a dated and signed copy of this form.

The parent(s)/legal guardians and the Investigator are satisfied that the information contained in this consent form was explained to the child to the extent that the child is able to understand it, that all questions have been answered, and that the child assents to participating in the research.

SIGNATURES

Printed name of subject _____

Signature_____Date_____

Printed name of subject's parent/legal guardian _____

Signature_____Date_____

Printed name of principal investigator/ _____

Designated representative

Signature_____Date_____

ASSENT FOR SUBJECTS FROM 14 – 18 YEARS OF AGE

(Legally incompetent to give consent)

I have had the opportunity to read this consent form, to ask questions about my participation in this research, and to discuss my participation with my parents/guardians. All my questions have been answered. I understand that I may withdraw from this research at any time, and that this will not interfere with my access to other health care. I have received a signed and dated copy of this consent form. I assent to participate in this study**.**

Printed name of subject _____

Signature _____ Date _____