

**Pediatric syncope diagnosis and management:
Validation of continuous blood pressure monitoring
and alternatives to 24-hour urine sodium sampling**

**by
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B.H.K., University of Windsor, 2017

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biomedical Physiology and Kinesiology
Faculty of Science

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SIMON FRASER UNIVERSITY
Fall 2019

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Ethics Statement



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Abstract

Syncope, or fainting, is common and has a devastating impact on quality of life. Diagnosis and management of syncope is challenging. In pediatric populations the current diagnostic gold standard, a tilt test, cannot be safely or properly performed, because the necessary non-invasive beat-to-beat blood pressure monitoring is not validated for children. In addition, low sodium intake is common in syncope patients, and salt supplementation is recommended. However, standard assessments of sodium from urine collections are difficult and unpleasant. This thesis demonstrated that: (i) continuous non-invasive finger blood pressure monitoring provides a novel, comfortable, and accurate approach for use in children compared to an intra-arterial catheter, (ii) Quantab test strips provide a valid alternative to flame photometry for the determination of 24-hour urine sodium levels, and corrected spot sample averages offer an acceptable and convenient alternative to 24-hour urine sampling. These advancements in diagnostic tools for syncope will enhance quality of life for affected individuals.

Keywords: Syncope; pediatric; blood pressure; finger plethysmography; urine sodium

This book is dedicated to my Mom for always believing that I could touch the stars and to my Brother for always trying to move them.

Most notably to Patrick, for being my nice boulder.

Acknowledgements

I would first and foremost like to acknowledge all of my participants who dedicated their time to make these projects possible. I would also like to thank Finapres Medical Systems for allowing us to use their equipment. From the children at BC Children's Hospital, to everyone who consented to allow me to analyze their pee, for your time and dedication, thank you.

I would also like to acknowledge my lab mates and friends who have taught me so much about what it is to be a graduate student and for all the help and laughs throughout my time here at Simon Fraser University. To Matthew Dorton, Brooke Hockin, Matthew Lloyd, and Vera-Ellen Lucci, as well as all of the other students, particularly Rebekah, who have found a home in the Cardiovascular Physiology Lab, thank you.

I would also like to acknowledge my supervisory committee members, Dr. Dawn Mackey and Dr. Tara Sedlak for all of their guidance and support throughout this process. Being able to learn from your insight and feedback has been such a valued experience, thank you.

Finally, and most importantly, I would like to thank and acknowledge Dr. Victoria Claydon, without whom I would not have been able to accomplish this work. Vic, I want to thank you for your expertise, direction, napkins, and support – it has made my time here unforgettable. You took me under your wing without having met me, and I cannot say how lucky I am you took that chance. Having you as a mentor these last couple years has taught me more than I ever thought possible in such a short time, and I want to thank you for always being there not just for me, but for all of us when we needed you. Vic, thank you.

Table of Contents

Approval	ii
Ethics Statement	iii
Abstract	iv
Dedication	v
Acknowledgements	vi
Table of Contents	vii
List of Tables	x
List of Figures.....	xi
List of Acronyms.....	xiii
Chapter 1. Background and Rationale	1
1.1 What is syncope?	1
1.2 Syncope has a devastating effect on quality of life	1
1.2.1 Syncope represents a large healthcare burden	1
1.2.2 Syncope effects all aspects of quality of life.....	2
1.3 General mechanisms of syncope	4
1.3.1 The role of orthostasis and baroreflex control	4
1.3.2 Classifications of syncope	6
1.3.2.1 Cardiac syncope	7
1.3.2.2 Neurally mediated syncope.....	8
Vasovagal syncope	9
Carotid sinus hypersensitivity	11
1.3.2.3 Neurological causes of syncope	12
Orthostatic hypotension.....	12
Postural orthostatic tachycardia syndrome	13
1.4 Syncope diagnosis and management is difficult	14
1.4.1 Diagnosis.....	14
1.4.1.1 Common diagnostic components.....	15
1.4.1.2 Tilt testing is the gold standard in the evaluation of autonomic orthostatic impairments	17
1.4.2 Management	18
1.4.2.1 Non-pharmacological management	18
1.4.2.2 Medical management.....	20
1.5 Project aims.....	21
Chapter 2. General Methodology	23
2.1 Continuous blood pressure monitoring.....	23
2.1.1 The need for continuous blood pressure monitoring.....	23
2.1.2 Finger plethysmography as an alternative to intra-arterial blood pressure ...	24
2.2 Urine sodium sampling and analysis techniques	25
2.2.1 The need for urine sodium sampling.....	25
2.2.2 24-hour urine sodium sampling.....	26

2.2.3	Flame photometry	26
2.2.4	Spot samples.....	27
2.3	Measurement instrument validation	27
Chapter 3. Validation of Finger Blood Pressure Monitoring in Children		31
3.1	Background	31
3.1.1	Attempts to validate finger plethysmography in children.....	31
3.1.2	New approaches to validate finger plethysmography and volume clamping in children	32
3.2	Methods.....	33
3.2.1	Ethical approval.....	33
3.2.2	Participants.....	33
3.2.3	Study design.....	33
3.2.3.1	Intra-arterial blood pressure measurement.....	33
3.2.3.2	Finger plethysmography blood pressure measurement.....	34
3.2.4	Protocol	34
3.2.5	Data analysis.....	35
3.2.5.1	Numerical comparisons.....	35
3.2.5.2	Morphological comparisons	36
3.3	Results	37
3.3.1	Numerical results.....	38
3.3.2	Morphological results	45
3.4	Discussion	50
3.4.1	Finger plethysmography with waveform correction provides a reasonable alternative to an intra-arterial catheter for measurement of beat-to-beat blood pressure in children	50
3.4.2	Waveform reconstruction improves numerical accuracy but impairs waveform morphology in children.....	51
3.5	Limitations	52
3.6	Conclusions.....	54
Chapter 4. Validation of a Novel Technique for Determining Urine Sodium Concentration		55
4.1	Background	55
4.1.1	Urine sodium levels in syncope patients are low	55
4.1.2	Using spot samples as an estimate of 24-hour urine sodium	55
4.2	Methods.....	57
4.2.1	Ethical approval.....	57
4.2.2	Participants.....	57
4.2.3	Study design.....	57
4.2.4	Protocol	58
4.2.5	Data analysis.....	60
4.3	Results	61
4.3.1	Creation of a conversion equation between Quantab units and millimoles... 61	
4.3.2	Accuracy of Quantab chloride test strips compared to flame photometry..... 63	

4.3.3	Protocol one	66
4.3.4	Protocol two.....	70
4.3.5	Model validation for use with time-irrelevant spot urine samples	79
4.3.5.1	Removal of the effect of time	79
4.3.5.2	Creation of a time-independent regression equation	82
4.3.5.3	Single use spot samples compared to flame photometry analyzed 24-hour urine collection	83
4.4	Discussion	87
4.4.1	Quantab chloride test strips in comparison to flame photometry	87
4.4.2	Utilizing spot samples compared to a 24-hour urine sample	88
4.4.3	Considerations for at-home testing	90
4.5	Limitations	91
4.6	Conclusions	93
Chapter 5. Final Discussion		95
5.1	Validation of finger blood pressure in children	95
5.2	Urine sodium analysis	96
5.3	Conclusions	97
5.4	Future directions.....	98
Chapter 6. Publications from this Thesis.....		99
6.1	Manuscripts	99
6.2	Abstracts	99
References		100
Appendix A Consent Forms		111
Appendix B Instruction Sheets		126
Appendix C At-Home Conversion Chart		129

List of Tables

Table 1	Participant indication for intra-arterial blood pressure monitoring and pre-existing medical conditions	38
Table 2	Summary statistics for blood pressure values from the intra-arterial catheter, FinAP, and reBAP.....	39
Table 3	Individual blood pressure values from the intra-arterial catheter, FinAP, and reBAP	40
Table 4	Absolute mean differences for the blood pressure value from the FinAP and reBAP compared to the intra-arterial catheter	41
Table 5	Cumulative percentages and corresponding grade according to British Hypertension Society (BHS) guidelines (106)	43
Table 6	Demographic data for comparisons between Quantab chloride test strips and flame photometry	63
Table 7	Demographic data for comparisons between multiple morning spot samples and flame photometry.....	67
Table 8	Demographic data for comparisons between multiple morning or evening spot samples and flame photometry.....	70
Table 9	Combinations of AM and PM spot sample averages explored to compare to flame photometry	71
Table 10	Regression-corrected spot sample averages	72
Table 11	PAP average and flame photometry initial and prospective values.....	78
Table 12	Descriptive statistics for the normalized spot sample averages	81
Table 13	Statistical analysis for various spot sampling averages.....	83
Table 14	Comparison of single morning and evening spot samples	84

List of Figures

Figure 1	Changes in arterial and venous blood pressure gradients in an upright motionless human (3)	5
Figure 2	Classification of the types of syncope and their mechanisms	7
Figure 3	Simple vasovagal faint during baseline, head-up tilt, and presyncope showing blood pressure (BP) and heart rate (HR).....	11
Figure 4	Age of onset for patients with vasovagal syncope (VVS) or POTS	14
Figure 5	Finger plethysmography with volume clamping mechanism	24
Figure 6	TOST equivalency testing outcomes	29
Figure 7	Bland-Altman analyses comparing reBAP to the intra-arterial catheter...	42
Figure 8	Equivalency plots for index and middle finger reconstructed brachial pressure compared to the intra-arterial catheter.....	45
Figure 9	Individual traces showing the 10 beats selected for morphology analysis and the smoothed average waveform	47
Figure 10	Group average waveform morphology for intra-arterial catheter, FinAP, and reBAP measurements.....	48
Figure 11	Average intra-arterial catheter trace compared to group difference plots for FinAP and reBAP	49
Figure 12	Urine sodium sampling protocol diagram	58
Figure 13	Protocol outline of data processing for comparing chloride test strips and spot samples to flame photometry analyzed 24-hour urine samples.....	61
Figure 14	Equations for conversion of Quantab units (a.u.) to standard units (mmol)	62
Figure 15	Correlation between Quantab chloride test strip 24-hour urine sodium values compared to flame photometry.....	64
Figure 16	Bland-Altman analyses comparing the bias and limits of agreement between Quantab 24-hour values and flame photometry.....	65
Figure 17	Equivalency between flame photometry and Quantab 24-hour urine samples	66
Figure 18	Box plots showing the data for 5-day, 4-day, and 3-day, 2-day, and 1-day Quantab spot sample averages.....	68
Figure 19	Comparison of flame photometry values to 3-day spot sample averages	69
Figure 20	Box plots showing the spread of the original and regression corrected spot samples compared to flame photometry.....	72
Figure 21	Correlations between spot sample averages (both in mmol and regression corrected) compared to flame photometry	74
Figure 22	Bland-Altman comparing the corrected spot sample averages to flame photometry.....	76
Figure 23	Equivalency plot showing the mean difference for the corrected spot samples compared to flame photometry.....	77

Figure 24	Bland-Altman plot comparing the PAP-prospective corrected values to flame photometry	79
Figure 25	Residual plot for the regression of raw spot sample values compared to the time the spot sample was taken	80
Figure 26	Raw and normalized spot samples compared to flame photometry	81
Figure 27	Correlation, residuals, and Bland-Altman plots for the time-normalized model	82
Figure 28	Correlations between single spot samples (both raw and regression corrected) compared to flame photometry	85
Figure 29	Bland-Altman plots of single use spot samples compared to flame photometry	86

List of Acronyms

AAMI	American Association for the Advancement of Medical Instrumentation
ANF	Autonomic failure
ANOVA	Analysis of variance
BHS	British Hypertension Society
COSMIN	Consensus-based standards for the selection of health measurement instruments
DAP	Diastolic arterial pressure
ECG	Electrocardiogram
FinAP	Finger arterial pressure
MAP	Mean arterial pressure
POTS	Postural orthostatic tachycardia syndrome
reBAP	Reconstructed brachial arterial pressure
ROC	Receiver operator characteristic
SAP	Systolic arterial pressure
SD	Standard deviation
SE	Standard error
SFU	Simon Fraser University
TLOC	Transient loss of consciousness
TOST	Two one-sided tests

Chapter 1.

Background and Rationale

1.1 What is syncope?

Syncope is defined as a transient loss of consciousness (TLOC) that occurs due to hypoperfusion of the brain and is characterized by a rapid onset, short duration, loss of postural tone, with complete and spontaneous recovery (1,2). There are many disorders that present with TLOC, but not all are classified as syncope due to their inability to meet the other requirements of the definition. There is a need to differentiate between syncope and other disorders (for example epilepsy, cataplexy, psychogenic pseudosyncope, and metabolic disorders) for the purpose of obtaining a defined diagnosis and appropriate management (2). TLOC is divided into two main categories: traumatic and non-traumatic forms (1). Traumatic forms of TLOC include events due to external causes, such as a concussion. Non-traumatic causes of TLOC are the most common and include epileptic seizures, psychogenic pseudosyncope, other rare miscellaneous causes, as well as the more frequent causes of syncope (1). In many cases, syncope is not associated with any particular pathophysiology, but rather is a result of varying levels in the amount of stress an individual is able to tolerate before a reaction (the same reaction across both healthy and patient populations) that culminates in loss of consciousness is evoked (3).

1.2 Syncope has a devastating effect on quality of life

1.2.1 Syncope represents a large healthcare burden

While syncope is common, it is hard to identify its true prevalence rate, as it is often under-reported in the general public (4). Current statistics indicate 15% of children will experience at least one episode of syncope during their lifetime (5), but the actual prevalence rate is thought to be much higher as many patients do not seek medical attention, particularly if there are few episodes and they do not sustain injury with coincident loss of consciousness (6). It is important that individuals with syncope are identified and properly diagnosed as those with cardiac syncope have a 33% increase in mortality and are at risk of sudden cardiac death (7,8).

Syncope accounts for 2% of all emergency department admissions in the United States, resulting in a considerable economic burden. The economic cost of syncope in the United States is approximately \$3.8 billion per year or \$8700 per syncope admission (1,9,10), with undiagnosed syncope (individuals who present with symptoms of syncope, but do not received a definitive diagnosis) costing \$1.5 billion or \$6700 per admission (10). This cost does not include individuals who present to the emergency department with syncope, and who receive a diagnosis, other than syncope, on presentation. Part of the reason for the large cost associated with syncope diagnosis is due to the overwhelming number of tests that can be necessary in order to confirm a diagnosis (10). This is further complicated by the presence of several different subtypes of syncope, many with overlapping heterogeneous symptoms. Even after completing the diagnostic process and seeing an average of eight different healthcare providers (11), up to 20% of patients remain undiagnosed (12,13). Furthermore, even within those who receive a diagnosis, many are misdiagnosed as having an anxiety or panic disorder due to lack of awareness of the various syncope disorders (14), or they may be given an actual syncope diagnosis, but are discharged with no further evaluation or follow-up (15).

1.2.2 Syncope effects all aspects of quality of life

Not only is syncope burdensome to the healthcare system, but it has a devastating impact on many aspects of a patient's life, and can continue to occur throughout the lifespan, with many subtypes first presenting at a young age (16,17). The quality of life for these individuals is similarly impaired to patients with other chronic diseases such as chronic fatigue syndrome (18), chronic obstructive pulmonary disorder (14,18), rheumatoid arthritis (10), and congestive heart failure (14). In pediatric populations, children with syncope have a quality of life impairment similar to other children with renal disease, asthma, and structural heart disease, and have a significantly worse quality of life than children living with diabetes mellitus (19). Quality of life impairments in children with syncope include a perception of low physical health, poor mental health, and increased fear, depression, and anxiety, as well as impairments to activities of daily living (19). The patient's perception of their health is an important consideration – the psychosocial and physical functioning for syncope patients is profoundly dissimilar, indicating a disconnect between the patient's perception of their physical limitations and the actual impairment to their physical function due to syncope (15). Issues with sleep

quality, particularly in postural orthostatic tachycardia syndrome (POTS) patients, are also a problem as they result in daytime sleepiness and fatigue (18). Furthermore, symptoms for POTS patients may be so severe that they limit everyday activities such as bathing, housework, and even eating (20). POTS patients also have a reduced health-related quality of life (21) (the impact of a condition on different areas of a patient's life in terms of their physical, psychological, or social functioning), commonly seen in other disorders associated with recurrent syncope (22,23).

Recurrent syncope is typically defined as more than one episode in the last year, with at least one episode in the last six months and significantly impairs quality of life across all indices, as well as interfering with every day activities (24). In fact, 76% of individuals with syncope feel their disorder interferes with their regular activities (15). Many individuals feel they are unable to drive (64% report an impairment with driving) and work (15,25). Quality of life is especially impaired in working age individuals as it is associated with a loss of employment – 39% of working individuals with syncope have reported a loss of employment due to syncope (15). In addition, a study found that one quarter of POTS patients are classified as disabled and unable to work due to their syncope disorder (18,26). For individuals who are able to work, syncope has been shown to cause injuries in the workplace, as well as in everyday life (13). Up to 30% of syncope patients will experience injury or physical trauma secondary to the loss of consciousness (13,27), with about 5% of individuals experiencing significant injury that leads to further impairments in quality of life (12). Even individuals with only a single episode of syncope have a 1.4-fold increased risk of occupational accident, and those with recurrent syncope have a further 1.4-fold increased risk over those with only one episode of syncope (28). Individuals who have been hospitalized due to syncope have twice the risk of employment termination during the two-years following discharge (28).

Syncope interferes with the lives of patients through everyday activities, with the impact taking its toll both physically and mentally. Grief over the loss of physical ability, even if the loss is perceived rather than functional, has been shown to lead to depression in individuals with syncope (29). Despite episodes being intermittent, it is the constant fear and anxiety surrounding the potential for another episode that results in the perceived loss of physical function and in the continuous impairment of quality of life (1). Indeed, 73% of patients with syncope report feelings of anxiety and depression (15), as well as impairments in close relationships, and an increased suicide risk (11,15). Individuals with

syncope also report/experience an increased prevalence of anxiety and depression, elevated somatization, and obsessive compulsive disorder (15,21). For those with recurrent syncope, psychosocial impairment impacts a large portion of their daily activities (1). While the quality of life for individuals with syncope is greatly impaired, it has been shown to marginally improve after a diagnosis has been received (10); however, obtaining a diagnosis is a long, tedious process with very few effective management options.

1.3 General mechanisms of syncope

Mechanistically, there are several different subtypes of syncope, classified according to their underlying mechanism, although the ultimate common feature is cerebral hypoperfusion (2,3). There are thought to be four main mechanisms underlying syncope, with the first being through insufficient pumping action of the heart – typically associated with cardiac arrhythmias and/or structural heart disease (2). The second mechanism is through insufficient effective circulating blood volumes, typically due to hypovolemia (2). The third mechanism relates to low blood pressure from reduced vascular tone, which results in pooling in the legs and abdomen, leading to orthostatic hypotension (2). Finally, syncope can be the result of a counterproductive neural circulatory response, which results in neurally mediated syncope, the most common subtype of syncope (2).

1.3.1 The role of orthostasis and baroreflex control

During a syncopal episode, cerebral perfusion is reduced to about half of its resting value (3,30). Syncope most commonly occurs in the upright position, and this is largely because of the impact of gravitational stress on arterial pressure gradients throughout the body. One key effect of gravitational, or orthostatic, stress when upright is a profound decreased in arterial pressure at the level of the brain, as can be seen in **Figure 1**.

Accordingly, the upright cerebral arterial pressure is about 30 mmHg lower than the brachial arterial pressure (the standard location for arterial blood pressure measurements) due to the hydrostatic effects of gravity (3). Below the level of the heart the pressure will increase in proportion to the distance from the heart, leading to the pooling of blood in the legs and increased filtration of plasma out of the capillaries and into the surrounding tissues (3). The reduction in arterial pressure above the heart is sensed

by arterial baroreceptors, in particular those in the carotid arteries of the neck. Activation of the baroreflex results in associated increases in vessel constriction, as well as increases in heart rate (tachycardia). These responses combine to compensate for the hydrostatic pressure gradient and fluid loss to the lower extremities and maintain arterial pressure. However, with a sufficiently severe orthostatic stress, this compensation can fail, resulting in a plummeting blood pressure, often accompanied by bradycardia, or a slowing of the heart rate (3). This compensatory failure can occur gradually as the compensation slowly becomes insufficient, or it can be an abrupt switch to vasodilation and bradycardia from vasoconstriction and tachycardia.

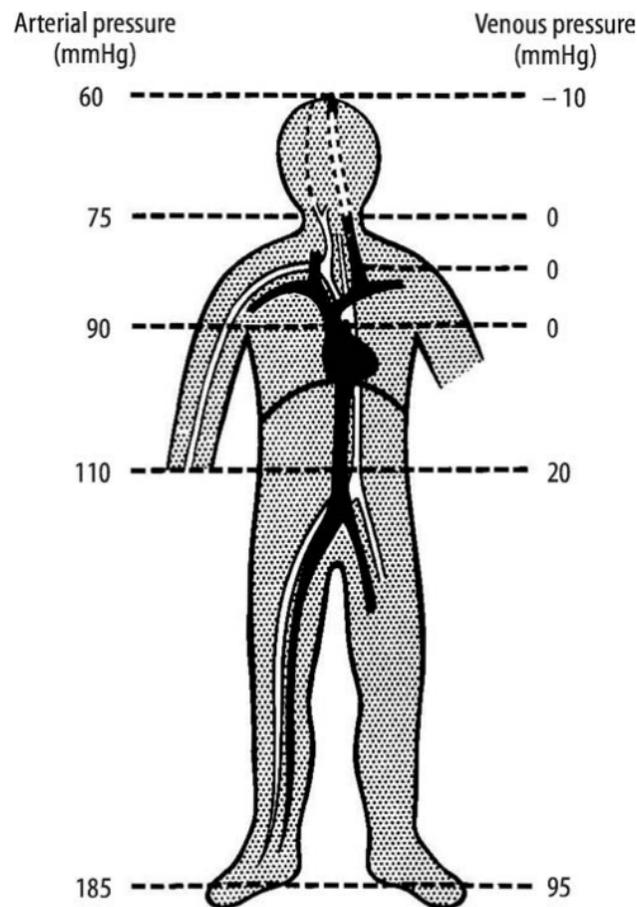


Figure 1 Changes in arterial and venous blood pressure gradients in an upright motionless human (3)

Arguably the most important control mechanism for the prevention of syncope is the response of the arterial baroreceptors, which are mediated by stretch receptors present within the vessel walls (30). In response to increases in arterial pressure, the baroreceptors – stretch receptors in the arterial walls of the carotid arteries and the aorta

– detect the increase in transmural pressure and increase afferent firing, which is relayed to the medulla, the primary centre for the integration of these signals. As a result, efferent sympathetic and parasympathetic nerve traffic to the heart, blood vessels, and kidneys is modified (31). The efferent limb of the baroreflex regulates peripheral vascular resistance and capacitance by regulating vessel tone, through modulation of vasoconstriction and vasodilation. There is also efferent baroreflex regulation of heart rate as both the sympathetic and parasympathetic pathways innervate the heart, enabling the baroreflex to increase or decrease heart rate, depending on the pressure sensed by the stretch receptors. The net response to an increase in arterial pressure is a reduction in arteriolar tone, bradycardia, and decreased force of cardiac contraction, with a reduction, and thus restoration, of arterial blood pressure back down to normal levels; this response is thought to be largely due to a sudden inhibition of sympathetic activity (3). The reduction in vascular tone occurs predominantly in skeletal muscle, of which there is a great mass, and thus potential, to generate huge changes in blood pressure (3).

Increased baroreflex sensitivity and sympathetic inhibition can be observed immediately prior to a syncopal event (3). The mechanism behind this increased sensitivity is thought to be mediated via the actions of vasopressin (3,32). Vasopressin is released in response to hypotensive stressors, where it acts peripherally to cause vasoconstriction of the blood vessels – thus it helps the body to maintain blood pressure upon orthostasis. However, vasopressin also causes increases in the sensitivity of the baroreflex (3), which results in central sympathetic inhibition and consequent vasodilation, culminating in syncope.

1.3.2 Classifications of syncope

There are several different classifications of syncope (**Figure 2**) based on the specific mechanisms and effects that culminate in the failure to maintain global cerebral hypoperfusion. These are broadly categorized into three main types, including cardiac syncope, neurally mediated syncope (reflex syncope), and neurological causes of syncope (divided into autonomic nervous system failure and POTS).

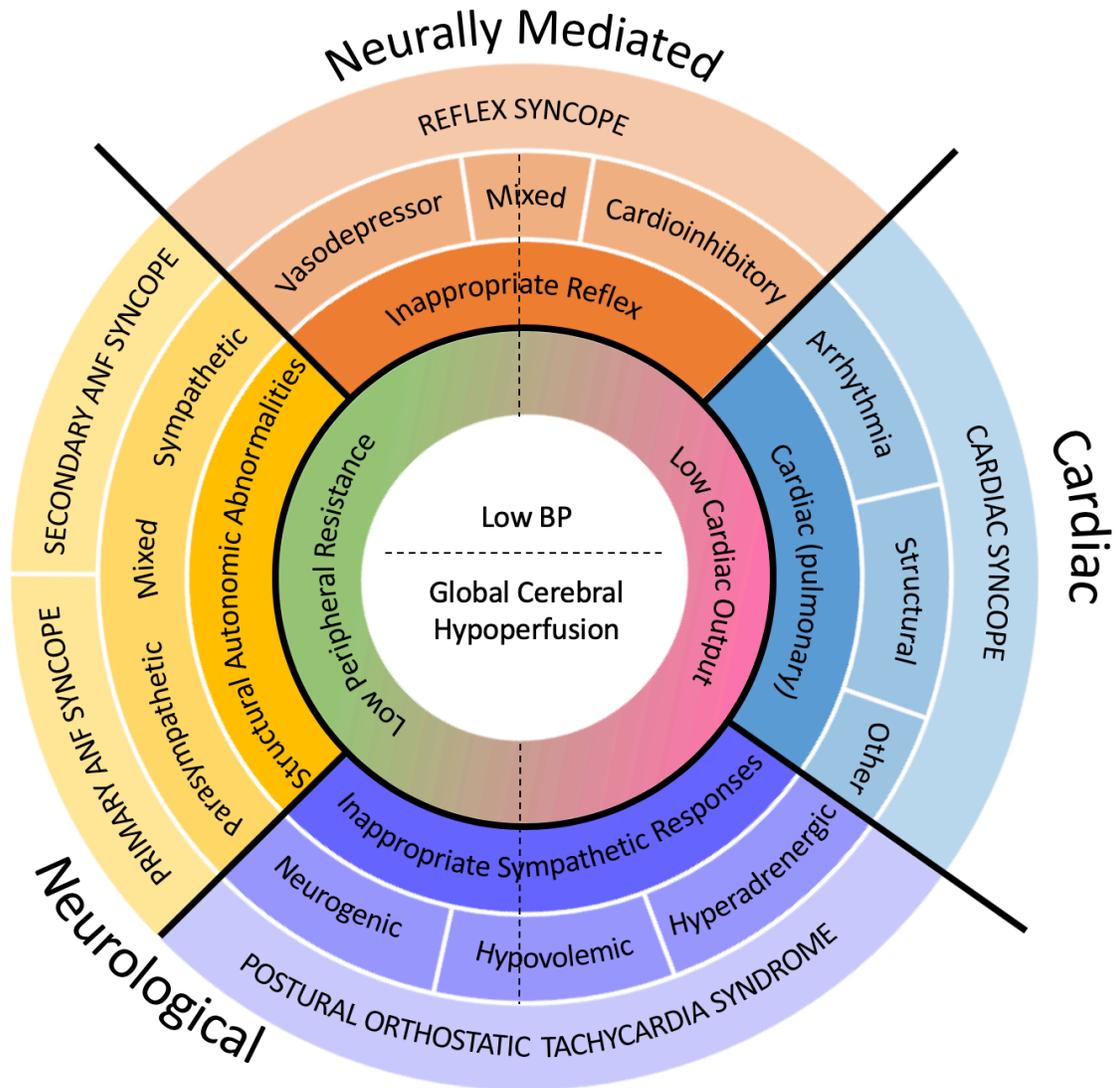


Figure 2 Classification of the types of syncope and their mechanisms

This figure outlines the different classifications and subtypes of syncope. Neurally mediated syncope (also known as reflex syncope) is the most commonly occurring type of syncope and results from an inappropriate reflex with three possible mechanisms of action (vasodepressor, cardioinhibitory, and a mix of the two). There are two main subtypes of neurological syncope, postural orthostatic tachycardia syndrome and autonomic failure (ANF). Primary ANF occurs due to diseases of the autonomic nervous system, whereas secondary ANF occurs due to diseases which damage the peripheral nerves. ANF can occur due to abnormalities in the parasympathetic or sympathetic pathways, or a combination of the two. Cardiac causes of syncope result from low cardiac output usually occurring from either an arrhythmia or other structural abnormality of the heart. Modified from (1).

1.3.2.1 Cardiac syncope

The majority of cardiac causes of syncope are characterized as either structural or arrhythmic (1,33), as can be seen in **Figure 2**. Structural cardiac syncope stems from structural heart disease, while arrhythmic can be classified as either bradyarrhythmias or

tachyarrhythmias. Both of these yield low cardiac output, which is the primary mechanism resulting in cerebral hypoperfusion and ultimately syncope (33). Different disorders that may present with cardiac syncope include congenital long QT syndrome, ventricular tachycardia and bradyarrhythmias (29). Bradyarrhythmias are usually caused by sick sinus syndrome or complete heart block, both issues that are commonly seen in pediatric populations who have had cardiac surgery (29). Right ventricular outflow obstruction, which presents as primary pulmonary hypertension, also results in low cardiac output and cardiac syncope. Finally, causes of left ventricular outflow obstruction can result in cardiac syncope, as a limited cardiac output is present in these patients. These patients often present with heart murmurs and may have evidence of heart failure upon physical examination; physical activity among these patients should be restricted until they can be fully evaluated to minimize the risk of syncope (29).

Those with cardiac syncope have an increased mortality rate, even compared to those with other classifications of syncope; however, it is thought that the increased mortality is due to the nature of the underlying diseases and not necessarily the syncope *per se* (1,7,8). For this reason, it is important that a detailed family history be taken, paying particular attention to a history of sudden cardiac death and with cardiological follow-up (1,29).

1.3.2.2 *Neurally mediated syncope*

Neurally mediated syncope, also known as reflex syncope, is by far the most common and frequently occurring type (29,33). Neurally mediated syncope is usually classified based on the efferent or descending pathway involved, but may also be classified based on the trigger if it is specific to certain situations (referred to as situational syncope) (1). Situational syncope is a specific type of neurally mediated syncope that occurs when the event is triggered by an activity that activates the Valsalva maneuver including urinating, defecating or coughing (33). When performed clinically, the Valsalva maneuver is a breathing technique that induces a challenge to blood pressure regulation that is both reliable, reproducible, and provides information on the integrity of both parasympathetic and sympathetic function during beat-to-beat blood pressure and heart rate recordings (34). This maneuver requires an individual to maintain an increased intrathoracic pressure by performing a respiratory strain against a closed glottis – this impedes venous return, causing an abrupt initial decrease in arterial blood pressure that

must be compensated by the baroreflex. When performed incidentally during activities of daily living in susceptible individuals this maneuver can provoke syncope, particularly in weight lifters and trumpet players who often use this technique to create an increase in intrathoracic pressure with a consequent hypotensive reaction (9).

Neurally mediated syncope is often referred to as reflex syncope, as it reflects an inappropriate reflex response that initiates the path toward syncope. This type of syncope differs from others, particularly those classified as autonomic failure, in that, after an initially normal response, the autonomic nervous system then initiates an action that is inappropriate. In contrast, autonomic failure stems from reflex failure – the inability to deliver a response of sufficient magnitude to prevent syncope (2). There are three main pathways through which neurally mediated syncope occurs as shown in **Figure 2**, these are vagally-mediated bradycardia (cardioinhibitory), sympathetic withdrawal-mediated vasodilation (vasodepressor), or a mixture of the two (1,2). The dominance of these pathways during a syncopal event can define the type of faint that occurs. The two main types of neurally mediated syncope, vasovagal and carotid sinus hypersensitivity, are described below.

Vasovagal syncope

Vasovagal syncope (a type of neurally mediated syncope), is the most frequently occurring type of syncope, and is particularly common in pediatric and young adult populations, as seen below in **Figure 4** (6). Symptoms that present prior to a vasovagal event often include dizziness, light-headedness, visual changes (including either blackout or tunnel vision), and muffled hearing (29). Other classic symptoms or signs include nausea, warmth, pallor, sweating, and neck pain (29,30). Syncope occurs as a result of the body's inability to maintain global cerebral perfusion secondary to a reduced circulating blood volume or profound hypotension (30). Nausea may occur secondary to splanchnic vasodilation, which contributes to fluid loss from venous pooling and lowering the effective circulating volume, further reducing cerebral perfusion (35).

Several factors can precipitate vasovagal events including prolonged maintenance of an upright posture, emotional stress (including the sight of blood), pain, and venous puncture (3,9,33). Other common factors that predispose to syncope are those that promote vasodilation (e.g. vasodilatory medications, heat stress, alcohol ingestion, or vigorous exercise) or dehydration (including vomiting, diuretic medications, alcohol

ingestion) (33). Historically, the switch from appropriate vasoconstriction and tachycardia to inappropriate vasodilation and bradycardia (the vasovagal reaction) was thought to be explained by a phenomenon known as the Bezold-Jarisch reflex (33). This reflex was thought to occur when decreased venous return resulted in inadequate ventricular filling with vigorous cardiac contraction (33), pathologically stimulating left ventricular mechanoreceptors that then trigger a reflex bradycardia and hypotension secondary to sympathetic withdrawal and vagal activation (30). However, this mechanism has since been discredited because: (i) there are few ventricular afferent pathways that are excited by a near-empty vigorous heart contraction (36); (ii) this reflex has only been observed from chemical injections and not mechanical stimuli (the trigger for a faint); (iii) the vasodilation component of a faint has been shown to occur in individuals with denervated transplanted hearts (37); (iv) and studies using echocardiography indicate syncope may not be associated with near-empty ventricles (3,36). The precise mechanism underlying the vasovagal response remains elusive.

As mentioned, there are two predominant pathways for neurally mediated syncope, cardioinhibitory and vasodepressor (seen in **Figure 2**). If vasovagal syncope occurs with bradycardia (and possible asystole), it is considered cardioinhibitory, whereas if hypotension predominates, then it is considered vasodepressor (1). Mechanistically, vasovagal syncope is mainly characterized by vasodilation because baroreflex-mediated peripheral vascular constriction is the primary method for maintaining blood pressure during orthostatic stress (3,38).

There are four main stages of vasovagal syncope as shown in the tracing of a vasovagal faint seen in **Figure 3** (39). During the first stage, full compensation, the cardiac sensitivity of the baroreceptor reflex is reduced and heart rate and diastolic arterial pressure (DAP) increase (39). These increases are associated with large increases in peripheral vascular resistance (increased sympathetic outflow) and decreases in heart rate variability (decreased cardiac vagal tone) (3,39). The next stage is tachycardia, where the heart rate continues to increase, but with little further change in vascular resistance (39). Following this is the instability stage, characterized by oscillations in both blood pressure and heart rate, some of which may be of great magnitude, occurring approximately every 10 seconds (3,39). During this stage, cardiac vagal activity, as well as baroreflex sensitivity, remain low and heart rate reduces to below that of the previous

stage (39). Finally, during the fourth stage, presyncope and recovery, both heart rate and blood pressure drop rapidly.

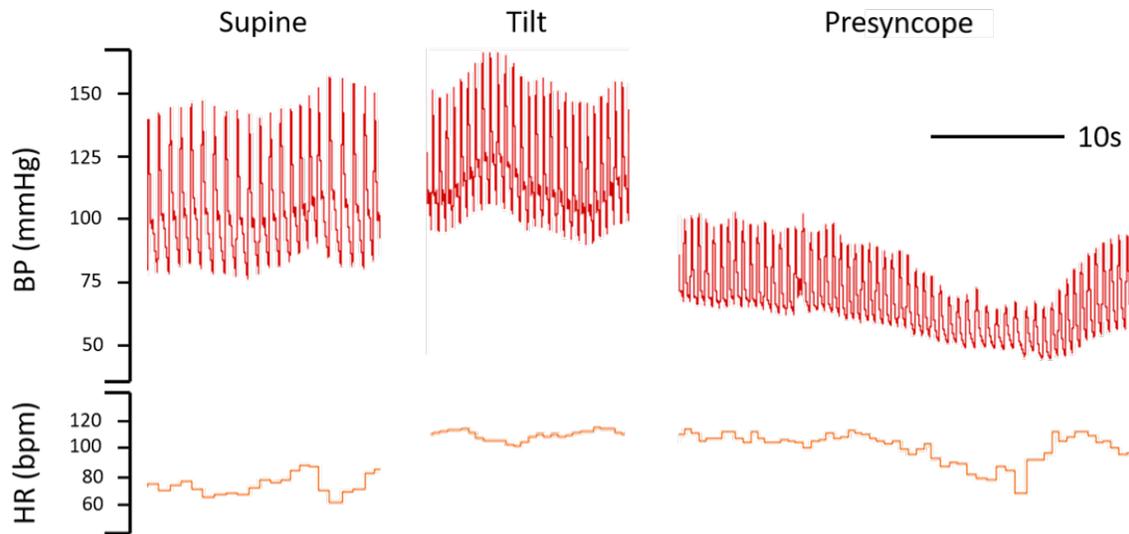


Figure 3 Simple vasovagal faint during baseline, head-up tilt, and presyncope showing blood pressure (BP) and heart rate (HR)

The drop in blood pressure at presyncope usually precedes the decrease in heart rate, with significant bradycardia occurring in only about 14% of cases (39). This stage is also marked by increases in vagal activity and baroreceptor sensitivity (39). While these four stages may be variable in terms of timing of initiation and length of phase, the occurrence and order of each phase is consistent in the prodrome for syncope.

Carotid sinus hypersensitivity

Carotid sinus hypersensitivity is another form of neurally mediated syncope that is typically seen only in older adults and commonly provoked by sudden movements of the head and neck (9,33), cervical compression of the spine, or use of a tight neck tie, through activation of a pathologically hypersensitive carotid sinus baroreflex response (33) with resultant bradycardia and hypotension. Carotid sinus hypersensitivity is thought to occur predominantly in individuals with atherosclerotic disease in the arteries containing the baroreceptors (the carotid or coronary vessels). In these individuals, predominantly males, the usual reduction in baroreceptor function with aging (40) instead becomes hypersensitive with pathologically large responses to baroreceptor stimulation that can result in syncope (9).

1.3.2.3 Neurological causes of syncope

Neurological causes of syncope include both primary and secondary autonomic failure, as well as orthostatic hypotension and POTS. Primary autonomic failure occurs with autonomic nervous system diseases such as Parkinson's disease, multiple system atrophy, or Huntington's disease, which impair central coordination of baroreflex responses with an associated inability to vasoconstrict in response to a baroreflex-mediated stimulus (33). Secondary autonomic failure also occurs in the face of impaired vasoconstriction, but due to diseases that damage the peripheral nerves, such as diabetes or spinal cord injury (33). Both types of autonomic failure can occur through either parasympathetic, sympathetic or even mixed mechanisms and both are commonly associated with orthostatic hypotension (low upright blood pressure). Accordingly, orthostatic hypotension is the most common presentation of syncope in patients with autonomic failure. POTS occurs secondary to abnormal autonomic nervous responses and is considered a neurological cause of syncope, although it is not an autonomic failure – quite the opposite – in that orthostatic cardiac responses in patients with POTS are generally excessive.

Orthostatic hypotension

Orthostatic hypotension is usually defined as a drop in systolic arterial pressure (SAP) of 20 mmHg or a drop in DAP of 10 mmHg within three minutes of adopting an upright position (9,30). However, more recently, recognition of a profound initial orthostatic hypotension upon the transition to an upright posture has gained prominence because of its association with falls, frailty, and cardiovascular mortality (41). Initial orthostatic hypotension is, therefore, defined as a drop in SAP greater than 40 mmHg during the first 30 seconds of assuming an orthostatic position (33). Progressive orthostatic hypotension refers to a more progressive fall of blood pressure that meets the diagnostic criteria between three minutes to a half hour after standing (33). Unlike other reflex mediated forms of syncope, orthostatic hypotension presents without bradycardia. Instead, the blood pressure gradually declines due to gravitational fluid shifts that are not compensated by the autonomic nervous system. Orthostatic hypotension is much less common in younger individuals (1) and the falls and dizziness occurring with this type of syncope often result in functional impairment or injury, which frequently require hospitalization (33). As with other forms of orthostatic syncope, symptoms associated with orthostatic hypotension are rapidly resolved with the assumption of a supine posture (30).

There are several factors known to exacerbate orthostatic hypotension including elevated environment temperature, hot food temperature, meals that are high in carbohydrates, exercise, and vasodilator medications (33). Individuals with orthostatic hypotension often experience postprandial hypotension, reduced blood pressure following a meal (33). Postprandial hypotension occurs with a drop of at least 20 mmHg in SAP or a SAP lower than 90 mmHg seen within two hours following a meal (33). This drop in blood pressure is due to sympathetic dysfunction causing inadequate vasoconstriction and an insufficient increase in heart rate, resulting in reduced cerebral perfusion and ultimately syncope (33).

Postural orthostatic tachycardia syndrome

POTS is another type of neurological syncope (**Figure 2**) (1). Individuals with POTS develop tachycardia upon maintaining an upright posture, with heart rate increases greater than 30 beats per minute, or a heart rate of over 120 beats per minute, within 10 minutes of the initiation of orthostatic stress (9,30). POTS, much like vasovagal syncope, is predominantly present in the young (**Figure 4**) and is much more common in women (approximately 80% of cases) than men (1,9).

Figure 4 shows the age of onset of symptoms for patients with vasovagal syncope and POTS. As can be clearly seen, these disorders typically first present in the young, with a typical onset between the ages of 10 and 15 years. Common symptoms of POTS include lightheadedness, dizziness, fatigue, and near syncope; however, hypotension and loss of consciousness are rare (9,30). The primary cause of symptoms is due to reduced effective circulating blood volume, largely due to excessive venous pooling in the legs. This evokes excessive cardiac compensation to try to prevent hypotension. In the face of impaired venous return, and reduced cardiac filling time secondary to the excessive tachycardia, cardiac output falls dramatically, and cerebral perfusion is reduced. Unfortunately, there is still much unknown about POTS including the underlying pathophysiology, although it is commonly acquired following an infectious disease, possibly due to the inflammatory response (42) with some reports suggesting POTS reflects a form of autoimmune autonomic ganglionopathy.

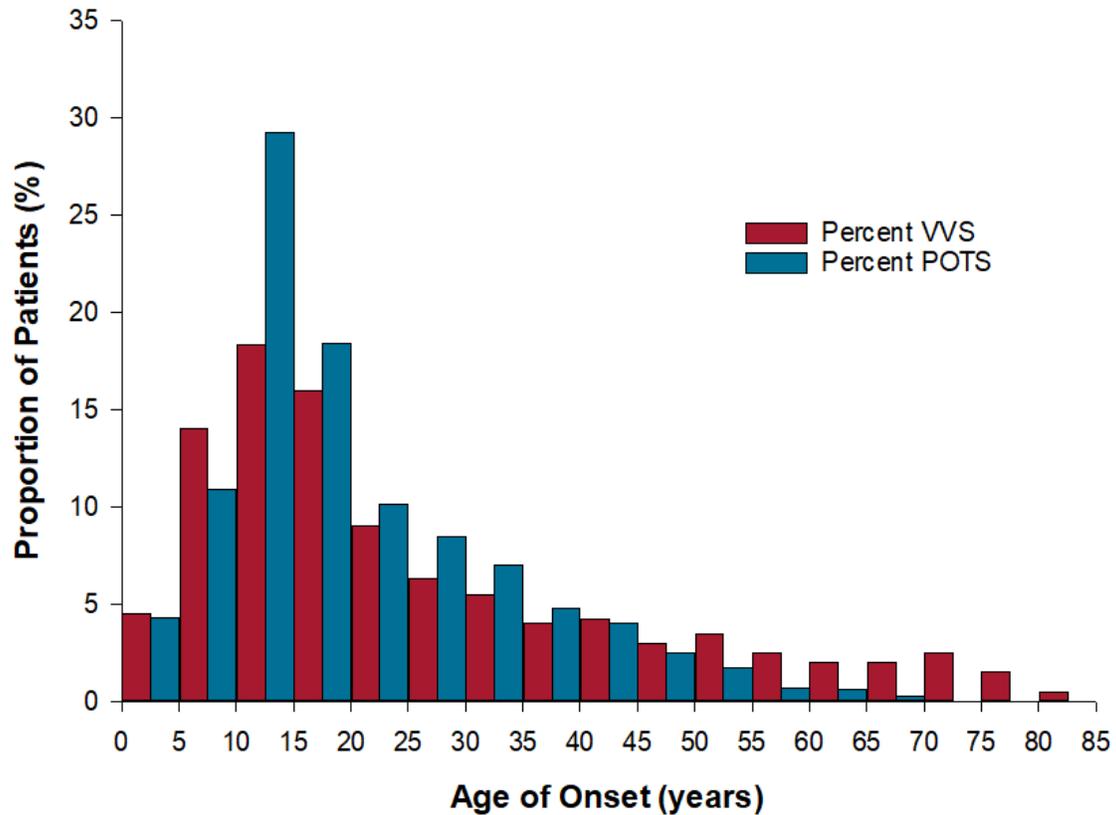


Figure 4 Age of onset for patients with vasovagal syncope (VVS) or POTS
Adapted from (16,17,43).

1.4 Syncope diagnosis and management is difficult

1.4.1 Diagnosis

A syncope diagnosis is a lengthy, difficult process involving numerous tests, visits with multiple physicians, and may still end in an inconclusive result. Individuals with possible syncope disorders are often passed from physician to physician, all with varying specialties (cardiologists, neurologists, pediatricians, psychiatrists, emergency room physicians), and all of whom have their own customs and practices on how to deal with syncope (2). Many physicians spend a large amount of time ruling out other possible causes of syncope, including epilepsy and arrhythmia, and when no underlying cause is found, simply release the patient (29). Part of the reason a syncope diagnosis is so challenging to achieve is due to the sporadic nature of the condition, meaning that physicians are rarely able to observe the physiological mechanisms that occur during a spontaneous syncopal episode.

There is minimal consensus as to which tests should be included in exploring a syncope disorder, partly because the many types of syncope present in very heterogeneous ways, but with similar symptoms. On average, six different diagnostic tests are conducted per patient, these commonly include both a neurologic and cardiac consultation, a 12-lead electrocardiogram (ECG), an echocardiogram, and Holter monitoring (44), but the problem of not receiving a definitive diagnosis still remains and continues to burden the community and individual (6,9). Furthermore, a lack of a definitive diagnosis means it is harder for these individuals to get proper management, an already challenging feat for those living with syncope. One item those in the field agree upon is that a detailed history is the first step required in achieving a syncope diagnosis (1,9,29). A standard physical clinical exam should also be conducted (including both neurological and cardiac examination to exclude underlying diseases), in addition to an ECG, and autonomic function testing (if indicated by the patient's personal history) (9,29).

1.4.1.1 Common diagnostic components

There are several aspects to a syncope diagnosis, but the patient history is agreed upon as the most important tool in identifying and classifying syncope disorders (33,38,45). There are many facets of a patient's history that need to be considered, and this is one reason why a syncope diagnosis is such a long and tedious process. It is argued that each episode of syncope should be detailed and characterized, including components such as the presence of prodromes (early symptoms), conditions surrounding each event and the possible precipitating factors, the position of the patient during each syncopal episode, if there was a state of confusion following the event, and any other signs or details that may aid in discerning a diagnosis (29,33). It is important to note if there was a state of confusion, as syncope often presents with fatigue, but is not associated with confusion, indicating a different underlying disease at play (29). Outlining every episode can be difficult as those with recurrent syncope may find their events blend together and those who have only had one episode may not have been paying attention to the circumstantial details (9). Geographical location should also be noted as infections or diseases that can cause syncope may not be found in the current region; furthermore, a tendency towards anxiety or panic attacks should be recorded as this may point to an underlying psychiatric disorder (9). A neurological history is important to ascertain if there are any family members with a history of epilepsy, seizures, migraines, neurovascular disease, or sleep disorders (29), as well as a cardiac family history, as patients with cardiac syncope tend

to have worse outcomes and increased mortality (8,29). A patient history is imperative, and while it may help distinguish between several autonomic disorders, an inconclusive result can still occur and require further testing.

The physical examination includes a variety of standard tests ranging from blood tests to questionnaires. Multiple blood pressure measurements should be taken, in both arms (to be detect the presence, if any, of subclavian steal), including measurements in a supine position, and within three minutes of obtaining an orthostatic position (33). Exercise testing may also be of use as those who have syncope during exercise tend to experience worse outcomes than those who do not (45). Blood testing to evaluate hematocrit, blood glucose, and the presence of anemia should also be performed and can help rule out other underlying diseases (6). Patients with neurally mediated syncope have a higher prevalence of anemia and this is important because iron deficiency can cause production of the vasodilator nitric oxide, which may affect an individual's ability to vasoconstrict, resulting in, or predisposing to, syncope (46). In addition, splanchnic vasodilation is thought to contribute to the reduction of cerebral perfusion through blood pooling, and also occurs to a greater extent in those with anemia (35). Further testing may include a 24-hour urine sample, as patients with syncope tend to have low urine sodium output (47). Finally, symptom questionnaires should be included as part of the physical examination. One such questionnaire is the Calgary score which has high sensitivity (89%) and specificity (91%) in identifying patients with vasovagal syncope compared to those with syncope secondary to another cause (48), and can be quite useful in providing physicians with another diagnostic tool to aid in confirming a syncope diagnosis.

Cardiological testing is often recommended for patients with syncope because of the association with abnormal regulation of blood pressure and heart rate. An ECG is recommended when attempting to confirm a syncope diagnosis (38) and is particularly useful in identifying heart rhythm irregularities that may be indicative of cardiac syncope, a type of syncope that, as mentioned, tends to have increased mortality rates (8,29,38). If an individual's personal history points to neurally mediated syncope, and their cardiac exam, including auscultation and an ECG, presents with no abnormalities, it is usually enough to rule out cardiac causes of syncope (29). If an atypical ECG is found, an echocardiogram may be performed to identify any structural cardiac abnormalities that might underlie abnormal electrical activity (29). A neurological evaluation is also often incorporated in the evaluation of syncope, including examination of the cranial nerves, a

funduscopy exam, a test of the vestibular system, and, if indicated by the patient history, an electroencephalogram (29).

1.4.1.2 Tilt testing is the gold standard in the evaluation of autonomic orthostatic impairments

Finally, tilt testing may be performed, as tilt testing with combined lower body negative pressure is considered the gold standard in autonomic function testing (49). Tilt testing is considered the cornerstone of autonomic testing, and is often used when a patient's history may point to neurally mediated syncope, the most common type, but is inconclusive (9,29,38). Tilt testing can be used to identify not only neurally mediated syncope, but also orthostatic hypotension, POTS and pure autonomic failure (9). Tilt testing occurs using a tilt table, a large table whose angle can be manipulated either manually or automatically. The protocol for a passive tilt test requires an individual to lie on the tilt table and it is then slowly tilted upright to about 60 degrees for 20-60 minutes. The current gold standard uses lower body negative pressure, which is achieved through a chamber placed over the lower legs to facilitate blood pooling and provokes syncope in almost all participants, unlike passive testing in the absence of additional provocation (49).

In order to be able to safely and properly perform a tilt test, a measure of beat-to-beat blood pressure must be used to allow for constant hemodynamic and cardiovascular monitoring. In adults this can be achieved non-invasively through finger plethysmography, and this is important because invasive measurements can cause psychic stimulation from anxiety and discomfort, which in turn interfere with the autonomic orthostatic reflex responses that are under scrutiny (50). However, non-invasive beat-to-beat blood pressure monitoring is not currently available for children, and the only way to obtain these measurements is through the placement of an intra-arterial catheter. This painful and unpleasant procedure is not only challenging in children, but renders the results of autonomic function testing meaningless, and leaves the search for a syncope diagnosis elusive. **Accordingly, it is imperative that non-invasive beat-to-beat blood pressure measurements are validated for children to enable the safe and accurate use of autonomic function testing in this population.** This is particularly pertinent given the high incidence of first occurrence of syncope in childhood and adolescence

1.4.2 Management

Management for syncope disorders, no matter the classification, is challenging as there is no universal therapy. For almost all syncope disorders, initial management is attempted through various lifestyle changes. These lifestyle changes include dietary control measures, in particular an increase in water and dietary sodium intake (which tends to expand plasma volume and so improve orthostatic blood pressure control). They also include increased exercise (which also expands plasma volume, increases the efficacy of skeletal muscle pumps, and enhances baroreflex function), as well as other physical approaches that may help counteract fluid shifts (such as postural counter maneuvers to bolster venous return through leg crossing or muscle tensing) (3,29,30). In creating a treatment plan, a psychological referral may be incorporated as early mental health referral is key in this population, especially if the patient's history points to psychological causes as the root of the syncope (29). Individuals are also educated on how to identify factors that trigger their syncopal events and given advice on avoidance of common triggers (33).

1.4.2.1 Non-pharmacological management

Diet alteration is one of the most important treatment options for individuals with syncope. It has been shown that improving hydration can alleviate some symptoms, in addition to improving orthostatic tolerance (3,51). Drinking excess water, also referred to as superhydration (38), in quantities of two-three litres per day is suggested to help increase plasma volume and increase the effective circulating blood volume upon standing (3,29,33). There is evidence that for patients with autonomic failure (30), POTS (52), and vasovagal syncope (53), bolus water drinking before a known trigger activity, such as taking a warm shower, is efficacious for increasing blood pressure and improves orthostatic tolerance (30,54). In this case, 500 mL of water should be rapidly consumed (over a two minute period) about 10 minutes prior to engaging in the activity (30).

In many cases, orthostatic symptoms are worse post-prandially, because of associated vasodilation and hypotension occurring after food ingestion (30). Patients are advised to plan triggering activities prior to mealtimes, and to follow a diet that avoids large carbohydrate-based meals that are particularly likely to provoke post-prandial hypotension. Similarly, the avoidance of alcohol and its associated vasodilatory effects is recommended (30).

Exercise training is also recommended for patients with syncope, with the rationale that it expands plasma volume, improves baroreflex function, and enhances the efficacy of physical counter maneuvers (54,55). Aerobic exercise is recommended to help reduce the recurrence of vasovagal syncope (54); swimming is also recommended – the hydrostatic effect of water can help oppose blood pooling during exercise (30). Home orthostatic (standing) training (standing against a wall for 30 minutes a couple of times a day) has been suggested, as most individuals are not able to stay in the hospital long enough to complete tilt training, but has limited efficacy, possibly due to low compliance (54,56). Other physical maneuvers such as contracting the abdomen, squatting, or obtaining a supine position, are recommended if the patient has sufficient warning of an impending episode to attempt preventative physical counter maneuvers (3,38). Lastly, compression garments have been suggested for individuals with syncope, but full compression garments that cover the abdomen can be difficult to put on (57), and have poor patient compliance, and calf compression garments have been shown to have limited efficacy (54,58).

One mainstay of syncope management is an increase in dietary salt intake. Salt loading involves ingesting a high salt diet (typically about 10 grams of salt per day) with the rationale that it will increase plasma volume, improve orthostatic tolerance, and ameliorate orthostatic symptoms (47). This is because salt supplementation increases renal water retention and so expands plasma volume. The plasma volume expansion will cause dilution of hematocrit, triggering erythropoiesis and thus expanding blood volume. While salt supplementation has been proven to be effective in the management of syncope, there are some challenges with this approach. Firstly, it is difficult for patients to achieve the desired intake of salt through dietary measures alone and still follow a healthy diet. Often their sodium intake is not increased sufficiently, and so the approach is not efficacious. Secondly, there is no way for individuals to measure the amount of sodium they are ingesting, unless they complete a 24-hour urine sample and send it to a hospital for analysis – an unpleasant and time-consuming task, particularly for children. Thirdly, salt supplementation, while shown to be effective in adults (59,60), has not been studied in pediatric populations and the effects of increased salt at such a young age are unknown. Finally, salt loading has been found to be effective only in syncope populations who already have low urine sodium values (60), which, again, would need to be measured through a 24-hour urine sodium sample. **Simple, practical approaches to determine**

urine sodium levels, ideally avoiding specialist analysis techniques and the need for a 24-hour urine collection, would be a great asset to identify those patients that are candidates for salt supplementation, and to assess compliance with dietary or pharmacological salt supplementation.

1.4.2.2 Medical management

If an individual has more severe syncope and lifestyle management options are not sufficient to improve symptoms, then alternative treatment options, such as cardiac pacing or pharmacological options, may be explored. While cardiac pacing is of obvious benefit to patients with significant cardiac arrhythmia, its use in those with neurological or reflex syncope is questionable. Cardiac pacing is generally reserved for patients with severe cardioinhibitory syncope associated with asystole or profound bradycardia, or older individuals who may experience general functional benefit from pacing (33,54). However, even in those with asystolic syncope, cardiac pacing has not been shown to prevent or delay the onset of syncope, largely because cardiac pacing will not improve blood pressure when venous return is low (3,61). As such, the efficacy of pacing for orthostatic syncope, particularly when the risk to benefit ratio is considered, is not typically advised.

Pharmacological options are reserved for extreme cases, as they are not usually sufficient unless combined with other lifestyle management options, and are often accompanied by intolerable side effects (30). The most common medications for syncope are fludrocortisone, midodrine, and slow release sodium chloride (29,33,62). Beta-blockers have been suggested for use with vasovagal syncope, but in fact blunt baroreflex mediated orthostatic heart rate responses, and in a randomized, double-blind, placebo controlled clinical trial, no evidence was found for beta-blockers to prevent recurrent syncope in young patients (62). In some instances, beta-blockers are recommended for patients with POTS to blunt their orthostatic tachycardia, but because this response is often compensatory in nature, blocking the tachycardia simply provokes orthostatic hypotension or vasovagal responses (63,64). Fludrocortisone is a corticosteroid that is exploited in syncope patients for its mineralocorticoid actions on the distal kidney tubules where it improves sodium reabsorption and thus increases blood pressure and blood volume, similar to the lifestyle management recommendation of increasing dietary salt (29,33,38). However, fludrocortisone also has some undesirable glucocorticoid actions, and can provoke supine hypertension, edema, and heart failure in susceptible individuals,

so is generally less desirable than salt supplementation. Midodrine is an alpha agonist and thus acts as a vasoconstrictor, promoting increases in peripheral resistance, but is not commonly used due to the frequent dosing required and its potential to cause supine hypertension, meaning dosing cannot occur in the afternoon or evening, further increasing the challenge of midodrine treatment (54). Thus, while some patients with severe and recurrent episodes may be helped with the addition of pharmacological management, the options are limited and have poor efficacy.

One option that seems to be effective is the use of pharmacological salt supplementation achieved through oral enteric coated, slow-release sodium chloride. The benefit of this approach is that the desired high salt load (10 g per day) can be achieved, which is difficult to do through lifestyle changes while maintaining a healthy diet. For this approach, the patient's diet does not need to be manipulated, and the side effects of nausea and gastrointestinal discomfort that can be associated with high oral salt intake are avoided with enteric coated slow release formulation. Accordingly, patient compliance with this approach is high. This management option is associated with a marked increase in orthostatic tolerance and a decrease in symptoms (47,51,60). However, while the efficacy of salt supplementation has been robustly demonstrated in adults, it has not been explored in children. *The lack of safety and efficacy data on the use of salt supplementation in children is in part due to two challenges in pediatric syncope management: (i) without validated beat-to-beat non-invasive blood pressure measurements it is not possible to properly and safely assess autonomic function or perform tilt testing – the gold standard for the determination of orthostatic tolerance, (ii) identification of pediatric patients with low urine sodium who would be expected to benefit from salt supplementation, or assessment of compliance with dietary or pharmacological approaches to increase salt intake, is difficult given the need for 24-hour urine collections and specialist testing equipment.*

1.5 Project aims

This thesis investigates the validation of alternatives to current diagnostic and management techniques for pediatric populations with syncope. The specific aims of this thesis are:

1. to evaluate the validity of a non-invasive finger plethysmography and volume clamping blood pressure monitoring device for pediatric populations as an alternative to the current gold standard of intra-arterial blood pressure monitoring.
2. to evaluate the validity of convenient at-home measurement of urine sodium through:
 - i. examining whether Quantab chloride test strips can be used to accurately measure urine sodium compared to the gold standard of flame photometry.
 - ii. examining whether the use of multiple spot urine samples provides a reasonable alternative to measure urine sodium concentrations compared to the gold standard of a 24-hour urine sample.

Chapter 2. General Methodology

2.1 Continuous blood pressure monitoring

2.1.1 The need for continuous blood pressure monitoring

The accurate determination of arterial blood pressure is a fundamental component of cardiovascular assessment. Continuous beat-to-beat blood pressure monitoring is often warranted for in-hospital intra-operative and hemodynamic monitoring of critical care patients, as well as for out-patient evaluation of cardiovascular reflex control. There is a need for beat-to-beat blood pressure monitoring in both clinical and research applications. Intra-arterial blood pressure monitoring is the gold standard for continuous blood pressure measurement and is comprised of a catheter attached to a pressure transducer that is inserted into an artery (usually the radial artery) to detect pressure changes (65,66). However, associated complications with this method include hematomas, ischemic necrosis, and infection, (67–69). Furthermore, this invasive procedure is not always available or desirable, particularly in children, for whom alternative methods to record continuous non-invasive arterial blood pressure would be of benefit.

In children and individuals with syncope, the gold standard for syncope testing and autonomic evaluation is through a tilt test with combined lower body negative pressure (49). In order for this test to be safely and properly performed, a method of beat-to-beat blood pressure must be available because the changes in blood pressure that are associated with syncope occur very rapidly, over just a few heart beats, and so are missed with intermittent monitoring. Using an intra-arterial catheter during a tilt test is problematic because this invasive method interferes with the compensatory reflexes that become engaged in order to help a person remain in an orthostatic position, leading to false positive responses (50). This creates a dilemma, a tilt test cannot be safely conducted without a method of beat-to-beat blood pressure monitoring; however, it cannot be properly conducted using an intra-arterial catheter, which is currently the only validated means to measure beat-to-beat blood pressure in children. There is a non-invasive beat-to-beat blood pressure monitoring device validated for use in adults, which uses finger

plethysmography to determine blood pressure (70,71) and allows for tilt-testing to be achieved properly and safely in this population.

2.1.2 Finger plethysmography as an alternative to intra-arterial blood pressure

Infrared finger plethysmography combined with volume clamping is the most common technique for non-invasive beat-to-beat blood pressure monitoring, and is based on the principle of Peñáz (72). This technique (**Figure 5**) utilizes an inflatable Velcro finger cuff (with an infrared light source and detector on either side of the cuff) to generate a beat-to-beat plethysmogram from variations in the amount of infrared light absorbed by the blood during systole and diastole (72). During measurements, the diameter of the artery is kept constant regardless of changes in arterial pressure with each heartbeat by varying the pressure of an inflatable bladder within the cuff using a rapid servo-controller system (73). In this way, cuff pressure provides an indirect measure of intra-arterial pressure within the digital arteries, allowing blood pressure to be continuously monitored (72,74,75).

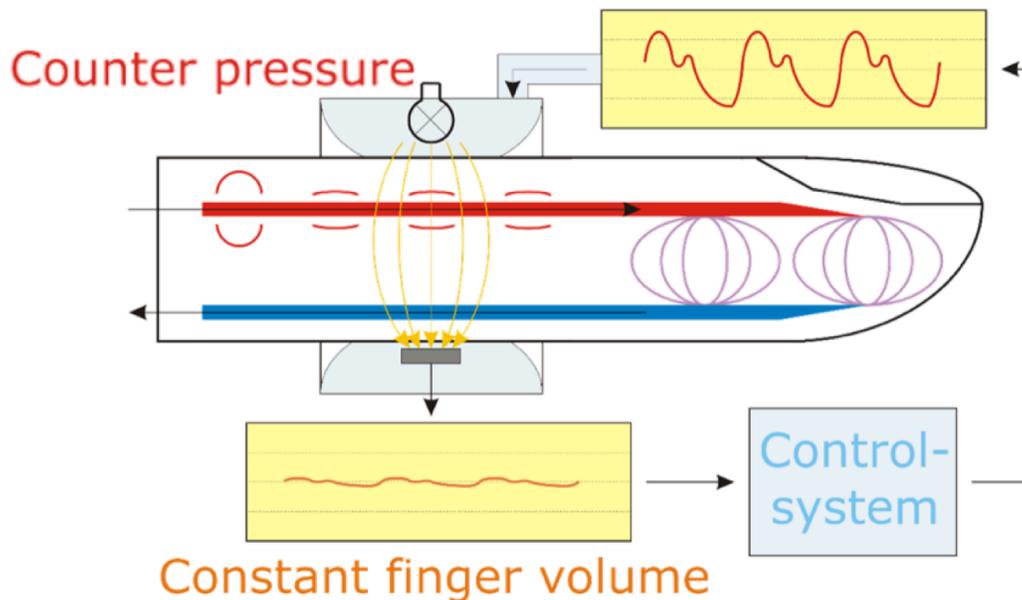


Figure 5 Finger plethysmography with volume clamping mechanism

The infrared light emitting diode detects the diameter of the blood vessels and the inflatable bladder maintains a constant diameter of the blood vessels. The counter pressure required to maintain this pressure is used as a proxy for the finger blood pressure and fed into a control system that converts the values into brachial arterial pressure. Adapted from ProfBondi, CC BY SA 3.0, 2012. Licensed as CC BY SA.

Many finger plethysmography devices also incorporate waveform filtering, height correction (to adjust for hydrostatic effects on finger blood pressure if the hand is not placed at heart level), internal calibration (through the Physiocal™ algorithm, which helps to reduce measurement drift over time by recalibrating the setpoint (76)), and calibration to brachial arterial pressure using the “return-to-flow” technique (74,77,78). This technique is required to correct for the phenomenon of pulse wave amplification, whereby systolic pressure is higher and diastolic pressures is lower as the pulse wave travels further down the arterial tree. This necessitates correction of the finger pressure values to those that would be expected at the brachial artery.

The use of a further reconstruction algorithm is often employed to estimate brachial arterial pressures from the recorded finger arterial pressure to improve accuracy (70,73,77,79). This algorithm takes into account an individual’s height, weight, age, sex, and estimated arterial compliance, as well as the return-to-flow brachial calibration (to estimate the compliance of the arteries and calibrate brachial pressures relative to the recorded finger arterial pressures) (70).

The accuracy of this technology has been confirmed by numerous studies in both healthy adults and adults with cardiovascular pathology (70,71,79–82). This has enabled finger plethysmography devices to be used as an alternative to intra-arterial catheters for both short-term and long-term monitoring, as well as for ambulatory use, in both clinical and research settings (70,71,79–82). However, due to improper cuff sizing, and concerns about the applicability of the assumptions used to estimate arterial compliance in children, use of finger plethysmography to determine beat-to-beat non-invasive arterial blood pressure has not been validated in children.

2.2 Urine sodium sampling and analysis techniques

2.2.1 The need for urine sodium sampling

Individuals with syncope have low urine sodium and benefit from salt supplementation. Determining an individual’s 24-hour urine sodium concentration can be used to identify those who may improve their orthostatic tolerance and symptoms with increases in dietary salt. This is because patients with low urine sodium concentrations benefit from salt supplementation, but those who are already on a high salt diet do not

receive the same advantages and do not need to be placed on such a management plan (60). A 24-hour urine sodium sample can also be used to determine compliance to salt supplementation, as many patients are unaware of the increases in sodium they are actually achieving when trying to salt load through dietary means alone. Urine sodium sampling can be used to determine a baseline concentration prior to salt loading, and afterwards as a comparative measure once salt supplementation has been undertaken.

Obtaining a 24-hour urine sodium sample can be challenging, particularly in pediatric populations. Validating an alternative at-home measure that is more convenient and would not need to be sent to a specialist laboratory for analysis would allow for easier determination of individuals who may benefit from salt supplementation, as well as enable patients to monitor their own sodium levels during treatment.

2.2.2 24-hour urine sodium sampling

The gold standard in determining urine sodium concentration is a 24-hour urine sample, giving a measure of a person's sodium concentration (83,84). This technique requires the individual to collect all of their urine in a container, typically beginning with the second morning void of the first day through until, and including, their first morning void of the following morning. The urine collection must be refrigerated until the sample is analyzed. Since sodium levels tend to fluctuate drastically, getting a measure over 24-hours is considered an accurate representation of an individual's overall salt levels (85–87). However, the collection of a 24-hour urine sample is difficult and inconvenient, and can be particularly challenging for pediatric populations (83,88–90). Accordingly, we aimed to investigate alternative ways to determine an individual's urine sodium level.

2.2.3 Flame photometry

The analysis of 24-hour urine sodium concentrations is typically conducted using flame photometry (85). Flame photometry makes use of a low temperature flame which activates the ions within a given metal (91). To measure urine sodium concentration, a urine solution is aspirated into the machine via an aspirator and atomizer using compressed air (91). The aspirated solution is then heated to incandescence in the flame, passed through photocells, and ultimately measured using a galvanometer (91). The

galvanometer gives a value based on the amount of light detected, and is proportional to the concentration of sodium present in the solution (91).

2.2.4 Spot samples

Spot samples require an individual to urinate in a cup and place a test strip inside the cup to determine the sodium concentration for that given sample. Unlike 24-hour urine samples, spot samples do not require the individual to keep any urine and the sample may be discarded as soon as the test strip has been interpreted. While much simpler, spot samples only give an idea of the sodium levels from the last few hours and are not considered to be as accurate as a 24-hour sample when determining overall sodium levels, although some argue they could be used as an alternative under circumstances where specific concentrations are not required (83,87,89). It has been suggested that use of averages from multiple spot samples can increase the accuracy of spot sample urine estimation of 24-hour urine sodium (83,87,90,92,93).

In order to complete a spot sample, a test strip is used to determine the sodium concentration in the urine; however, sodium test strips are not readily available. For this reason, chloride test strips have been investigated to determine urine sodium concentrations since dietary salt (sodium chloride) contains both sodium and chloride ions in a one-to-one ratio (92,94). This study will utilize Quantab chloride test strips (HACH, Loveland, Colorado) of high concentration (300-6000 ppm or 13-260 mmol). The typical range for a 24-hour urine sodium spans from 40-220 mmol, while a spot sample concentration typically spans from the low end of this range to 20 mmol (95), so the Quantab chloride strips are able to cover the full range of expected values. Should spot samples prove reasonably accurate compared to a 24-hour urine sample, they would provide a convenient, low-cost alternative that would be more comfortable for patients.

2.3 Measurement instrument validation

In order to validate measures of non-invasive blood pressure monitoring and multiple spot samples using chloride test strips to their respective gold standards, the type of validity being determined must be defined. In general, the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) initiative defines validity as:

“the degree to which an instrument measures the construct(s) it purports to measure.” (96)

There are various types of validity, including content validity (is the content an accurate representation of what is being measured), construct validity (are the scores consistent with the hypothesis), and criterion validity (are the scores an adequate reflection compared to a gold standard) (96,97). In this thesis, the pertinent consideration is criterion validity. Criterion validity deals with the agreement between the observed measurements and the true measurements (or the gold standard) (97). When comparing to a clinical gold standard, the implicit hypothesis is that the observed measurements are in good agreement with the true measurements; however, what is considered ‘good agreement’ and seen as sufficiently valid, is not always a clear-cut picture. In a clinical environment there are other factors such as cost benefit trade-offs, feasibility, or patient comfort, which need to be taken into consideration along with the consequences of false positive and false negative classifications when deciding if a new instrument is sufficient (97).

Criterion validity is outlined to consist of the following steps: (i) identify a suitable criterion and method of measurement, (ii) identify an appropriate sample in which the instrument will be used, (iii) define the required level of agreement prior to undertaking the experiment, (iv) obtain scores for the instrument and the gold standard independently from each other, and (v) determine the strength of the relationship between the two (97). In both aims of this thesis, a suitable method of measurement was identified (non-invasive finger plethysmography and multiple spot samples with chloride test strips), fulfilling step one. The next step, selecting the sample populations, was chosen based on the ability to cause minimal harm and discomfort to individuals. For aim one, pediatric patients in an intensive care unit who already had intra-arterial monitoring in place were chosen, with the knowledge that this population would likely perform worse than an out-patient population (arguably the target population), but in keeping with ethical constraints that would preclude the insertion of an arterial catheter in healthy children. The sample population for aim two was also chosen outside the target population, as asking syncope patients, particularly those in the pediatric population, to perform multiple spot samples and a 24-hour urine sample, when the measurements could be performed in a healthy control population, was seen as causing unnecessary discomfort. However, there is no reason to suspect that the validity of the approach would be different in children than in

adults. The levels of agreement were chosen based on clinically relevant criteria for both measurement instruments and the scores were obtained in an independent manner. For step five, determining the strength of the relationship, there are a few ways validity can be assessed. This thesis will primarily use Bland-Altman plots to determine agreement; however, equivalency testing will also be used in comparison to the gold standard for aim two.

Bland-Altman plots are used to assess validity by creating estimates of the mean bias and limits of agreement, that is 95% confidence intervals. These plots take the average between two measurements on the x-axis and compare it against the difference between the two measurements on the y-axis (98). This simple plot can easily detect if there is a systematic bias occurring in either measurement including over- or underestimation, truncation, or increasing error with increasing values of the mean, which cannot be detected with a simple correlation or t-test.

Equivalency testing is not as commonly used as other tests of agreement, but is an important consideration for criterion validity. The two one-sided tests (TOST) are a type of equivalency testing which investigates if two measurement instruments are equivalent with one another (99). While other tests of agreement, such as a t-test, examine the data to determine if they are different, they fail to conclude if the measurements are equivalent – just because two measurements are not different, does not necessarily mean they are equivalent (99). The main outcomes from a TOST test are shown in **Figure 6** below.

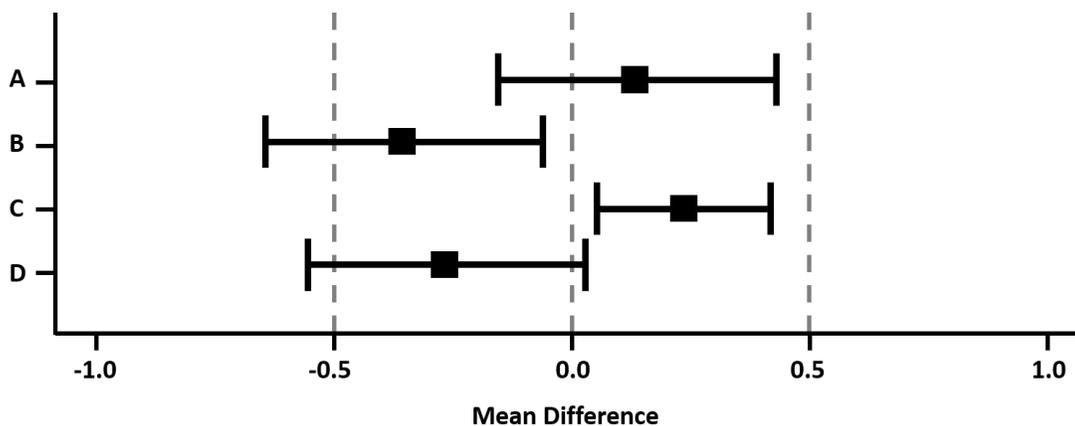


Figure 6 TOST equivalency testing outcomes

There are four main outcomes of a TOST test. This figure represents the outcomes of two measurements that are: (A) statistically equivalent and not different, (B) not equivalent and statistically different, (C) statistically equivalent and statistically different, (D) not equivalent and not different. Adapted from (99).

When a standard t-test is performed and the confidence intervals cross zero, the results of the test indicate that the two measurements are not statistically different. This is shown in outcomes (A) and (D) in **Figure 6**. Two measurements are deemed statistically equivalent by the TOST equivalency test if their confidence intervals do not cross the effect size boundaries. In **Figure 6**, these boundaries are set at -0.5 and 0.5, meaning that in outcomes (B) and (D), which cross this boundary, the measurement instruments are not equivalent. As can be seen from this figure, two measurements can be statistically different, yet still equivalent (C), but may also be statistically not different and not equivalent (D). The disparity in outcomes highlights the need for equivalency testing to also be performed when determining criterion validity.

Chapter 3.

Validation of Finger Blood Pressure Monitoring in Children

A version of this chapter was published as Heeney, N., Habib, F., Brar, G., Krahn, G., Sanatani, S., & Claydon, VE. Validation of finger blood pressure monitoring in children. *Blood Pressure Monitoring*. 24(3): 137-145, 2019.

3.1 Background

3.1.1 Attempts to validate finger plethysmography in children

Continuous beat-to-beat blood pressure monitoring is often required for both clinical measurements and research applications. Intra-arterial blood pressure monitoring is the gold standard for continuous blood pressure measurement (65,66); however, this approach is associated with complications, and invasive techniques interfere with reflex responses during orthostasis (50,67). A non-invasive alternative to this method has been validated in adults through the use of volume clamping and finger plethysmography, but the accuracy of such a device in children is unknown.

The use of finger plethysmography is limited in children, largely due to the lack of availability of pediatric finger cuffs – improper cuff sizing results in increased bias within the measurements. Application of adult cuffs on the wrists of neonates to record continuous non-invasive blood pressure has been described in comparison to intra-arterial recordings (78–80,100,101). However, the pressures were often over- or under-estimated with the plethysmography technique, presumably due to improper cuff fit (80,102). While this particular application of the non-invasive device shows promise in neonates, it is not possible for long duration recordings because of associated venous pooling in the hand (80). Furthermore, the extension of this approach to older children would not be possible once their wrist size exceeds the largest adult finger cuff dimension.

There is little evidence of the validity of finger plethysmography for arterial blood pressure monitoring in children. There are some reports comparing finger plethysmography cuffs with intra-arterial measurements in pediatric populations (73,102,103), but their validity exceeded current guidelines for blood pressure monitoring

devices (104) and their utility has been questioned (73), possibly due to improper cuff fit (80,102).

3.1.2 New approaches to validate finger plethysmography and volume clamping in children

New prototype pediatric finger plethysmography cuffs with improved fit for small fingers have now been developed, but their accuracy, reproducibility, mean differences or limits of agreement compared to the reference standard (intra-arterial monitoring) are not known. Furthermore, although the reconstruction of brachial arterial pressure from finger arterial pressure improves accuracy of finger blood pressure measurements in adults (70), it is unclear how the reconstruction algorithms perform in children. Children and younger individuals tend to have more compliant blood vessels, and this is not accounted for in the current algorithm which uses estimates of compliance that were generated using adult data. The accuracy of this algorithm, which is necessary in order to correct the digital artery values due to pulse wave amplification, needs to be validated before this device can be used in pediatric populations. It is also unclear how the waveform morphology compares between finger plethysmography and intra-arterial pressure monitoring in children. It may be that the arterial pressure waveform at the digital site is less faithfully captured in children, or that it is subject to distortion when the reconstruction algorithms are applied. This is important because the arterial pressure waveform is often used to aid in the determination of disease and state of care (105).

We aim to compare the accuracy, mean differences and limits of agreements, and waveform morphology between beat-to-beat finger arterial blood pressure monitoring based on finger plethysmography and volume clamping and intra-arterial blood pressure measurements, the current gold standard, in children. We will use established guidelines from the American Association for the Advancement of Medical Instrumentation (AAMI) (104), as well as the British Hypertension Society (BHS) (106) to evaluate the suitability and determine the criterion validity of finger plethysmography for continuous non-invasive arterial blood pressure measurement in children. We hypothesize that finger blood pressure measurements will be within the gold standard AAMI guidelines and provide a suitable non-invasive surrogate for beat-to-beat blood pressure monitoring in pediatric populations.

3.2 Methods

3.2.1 Ethical approval

This study was approved by both the Department of Research Ethics at Simon Fraser University and the University of British Columbia Children's and Women's Health Centre of British Columbia Research Ethics Board, and was conducted in accordance with the Declaration of Helsinki (2013) (107).

3.2.2 Participants

Prior to testing, participants provided written informed assent where applicable, and their parents or guardians provided written informed consent (**Appendix A**). A brief medical review was conducted to ensure compatibility with our inclusion and exclusion criteria. Participants were recruited by our collaborators from the pediatric intensive care unit at the British Columbia Children's Hospital and were included if they were: aged 3-13 years old; considered by their primary physician to be stable enough for the additional monitoring; already in need of an intra-arterial catheter for blood pressure measurement for their ongoing clinical care. Participants were excluded if they were known to have Methicillin-resistant Staphylococcus aureus, had burns affecting their upper extremities, needed extracorporeal life support, or had disorders affecting perfusion to the fingers.

3.2.3 Study design

3.2.3.1 Intra-arterial blood pressure measurement

All participants were receiving care in the pediatric intensive care unit and were already receiving continuous beat-to-beat intra-arterial blood pressure recording for their ongoing clinical care. Intra-arterial blood pressures were measured using either a Jelco catheter (22 or 24 gauge) for the radial intra-arterial lines (n=17) or a Cook catheter 3 French (5 cm) for the femoral intra-arterial lines (n=1). The catheter was connected to a Truwave pressure transducer (PSMK0695, Edwards Lifesciences, Irvine, USA) linked to an IntelliVue MX800 (Philips, Amsterdam, The Netherlands). Once the intra-arterial line had been inserted, patients received a continuous infusion of 0.9% NaCl with heparin at 2 ml/hr. Transducers were zeroed every 12 hours and leveled with the patient's mid-

thorax. The intra-arterial line was connected to an analog channel on the non-invasive finger plethysmography device.

3.2.3.2 Finger plethysmography blood pressure measurement

Continuous non-invasive finger blood pressure recordings were conducted using the Finapres NOVA™ (Finapres Medical Systems [FMS], Amsterdam, The Netherlands) and two prototype pediatric finger cuffs designed using Finapres™ technology. These prototype cuffs incorporate a miniaturised design to fit child-sized fingers, aiming to reduce the bias seen with larger fitting cuffs. Participant sex, age, height and weight were entered into the Finapres NOVA™ in order to provide an individualised calibration for each participant (70,108). Finger cuff measurements were calibrated to brachial blood pressure measurements, determined using sphygmomanometry with appropriate pediatric-sized cuffs, prior to data collection, using the built-in calibration mode (70). The participant's hand was kept approximately at heart level throughout testing, and a height correction unit was used to account for small changes in vertical height between the finger cuff and the heart (70). Continuous internal calibration was achieved using the Physiocal™ technique described earlier (70,76). These internal calibrations occur periodically throughout the test (every 10 beats initially and every 70 beats after a few minutes of “warm up”) (71,76). During the Physiocal™, finger blood pressure readings are interrupted for two heart beats, and accordingly, these calibrations were manually removed for the purposes of data analysis (71,76).

3.2.4 Protocol

The intra-arterial blood pressure waveform was acquired from the intra-arterial catheter as a time-synchronised analog input (sampled at 100 Hz) to the Finapres NOVA™ device. Beat-to-beat blood pressure waveforms were acquired from both blood pressure monitors (intra-arterial and the non-invasive device) simultaneously. The finger blood pressure was recorded (sampled at 200 Hz) as both the raw finger arterial pressure (FinAP) and the reconstructed brachial pressure (reBAP) (70) to evaluate the impact of waveform reconstruction on the values obtained.

For long term use (greater than 2 hours), finger plethysmography is recommended to employ “finger switching” as longer duration recordings can be associated with some discomfort and diminishing accuracy (65,109–111). Data were recorded for six minutes

per recording and recordings were made from both the middle and index fingers to evaluate the impact of changing fingers on the recordings obtained. Recordings were made when patients were medically stable and cardiovascular parameters were in steady state, typically the patients were not conscious at the time of recording. Where necessary for ongoing care, short rest periods were permitted between recordings.

3.2.5 Data analysis

3.2.5.1 Numerical comparisons

Group data are reported as mean \pm standard error (SE) unless otherwise stated and significance was assumed where $p < 0.05$. Data were analyzed using R (R Core Team, 2015), Labchart 8 (AD Instruments, Colorado Springs, CO, USA) and SigmaPlot Version 14 (Systat Software, San Jose, CA).

Data analyses were conducted on the waveforms recorded during the final minute of each data collection period for each patient, on each finger. FinAP and reBAP files were down sampled to 100Hz because of the lower sampling rate of the intra-arterial line, to enable valid comparisons between the two devices. Using R, values of systolic blood pressure (SAP) and diastolic blood pressure (DAP) were extracted for each beat across all three measurements (intra-arterial, FinAP and reBAP). Mean arterial pressure (MAP) was calculated as $DAP + 1/3[SAP - DAP]$. Comparisons between mean values were analyzed using a one factor ANOVA with a Holm-Sidak post-hoc comparison to determine the pairwise differences.

The SAP, DAP, and MAP values of all methods were averaged for each participant and used to determine the correlation between the FinAP and reBAP to the intra-arterial pressure. These values were also used to determine the bias or mean difference between the two devices (reBAP and FinAP with intra-arterial pressure), their limits of agreement ($1.96 \times$ standard deviation [SD]) and the creation of Bland-Altman plots. These values were compared to the guidelines outlined by the AAMI, whereby the bias must not exceed 5mmHg and the SD of the mean difference between the two devices must be ≤ 8 mmHg (104).

For numerical comparisons of beat-to-beat differences from the intra-arterial catheter, we focused primarily on the relationships with reBAP because these values have

been shown previously to be more closely associated with the gold standard intra-arterial catheter values – waveform reconstruction improves the accuracy of the values obtained (77,103,108). Beat-to-beat SAP, DAP and MAP values were expressed as the difference between reBAP and the intra-arterial catheter for every beat, for each participant, and the cumulative percentage of differences for values less than or equal to 5 mmHg, 10 mmHg and 15 mmHg were determined. These values were compared to the guidelines outlined by the BHS (106). This approach reflects the overall consistency of the device when monitoring blood pressure. Finally, equivalency testing using TOST testing was conducted to determine if the blood pressure values were within the 20% clinically recommended guidelines (99).

To evaluate any potential influence of age and weight, correlations between the differences in intra-arterial pressure and reBAP SAP, DAP, and MAP were conducted for both participant age and weight using the Spearman correlation coefficient.

3.2.5.2 Morphological comparisons

The waveform morphology of the intra-arterial, FinAP and reBAP waveforms were evaluated for morphological agreement between the two measures recorded from the finger and the intra-arterial pressure, and whether the reBAP algorithm impacts the waveform shape. We performed these analyses on 10 successive beats taken from the final one-minute of simultaneous data collection for the trials using the middle finger for cuff application. We used the middle finger for these analyses because this is the finger recommended by the manufacturer for optimal device use (111). Each heartbeat considered was evaluated from 50 ms before the systolic peak until 30 ms before the systolic peak of the following beat. These beats were aligned such that time was considered in units of heartbeat to allow for easier comparison of waveforms across different heart rates. Similarly, the blood pressure waveforms were expressed as the percentage of the systolic value to ensure morphological comparisons were considered independent of any vertical deviation of the blood pressure traces. The waveforms for each device were plotted together to aid visual determination of any morphological differences. These data were then analyzed in collaboration with Dr. David Campbell who created a smoothing and functional regression modelling approach in R (112). The functional regression model created uses the intra-arterial blood pressure waveform as the baseline model, as it is considered the gold standard. The model is defined as:

$$Y_{ijk}(t) = \mu(t) + \beta_j(t) + \alpha_k(t) + \gamma_{jk}(t) + \varepsilon_{ijk}(t) \quad (1)$$

where $Y_{ijk}(t)$ is a function for the curve for replicate i from measurement j on person k at heartbeat time (t) . $\mu(t)$ represents the average curve for the intra-arterial blood pressure such that $\mu(t)+\alpha_k(t)$ is the baseline for a typical intra-arterial blood pressure curve and the individual intra-arterial blood pressure behaviour. In a circumstance where no deviation from the baseline intra-arterial blood pressure morphology function occurs compared to either the FinAP or reBAP morphology, $\beta_j=0$, where β_j is the term for changing measurement type. $\varepsilon_{ijk}(t)$ is a model residual and an interaction term, $\gamma_{jk}(t)$ was incorporated into the model to evaluate the potential for interaction between the individual patient-specific deviations for each measurement type. A constraint was added to the model such that the sum of all the patient specific effects, $\alpha_k(t)$, was equal to zero, ensuring that the $\alpha_k(t)$ term is interpretable as the deviation from the mean behaviour for a specific participant k . Using this approach, where $\beta_j=0$ the waveform morphology of the two devices was considered identical.

A second model was also considered to determine if there were any effects of age or weight on the morphology of the FinAP and reBAP waveform or their deviation from the intra-arterial blood pressure morphology.

$$Y_{ijk}(t) = \mu(t) + \beta_j(t) + \alpha_k\beta_{age}(t) + W_k\beta_{weight} + \gamma_{jk}(t) + \varepsilon_{ijk}(t) \quad (2)$$

The average deviations for reBAP and FinAP over each heartbeat were also compared to the intra-arterial data to determine if the average waveform for the different measurement approaches were similar. Waveform comparisons only took into account the shape of the waveform, as each beat was normalized relative to the peak waveform (SAP) to remove any influence of vertical deviation on the analysis.

3.3 Results

Data were obtained from 18 participants (12 males) aged 7.5 ± 0.7 years (range 3-13 years) and weighing 25 ± 3 kg (range 12-57 kg). The indication for the intra-arterial catheter and any significant underlying medical conditions are listed in **Table 1**. Data from 5 participants (4 males) were excluded from analyses due to unsatisfactory recordings (inability to record data due to lack of plethysmogram). The 5 patients who were excluded

had the following conditions: septic shock (n=2); necrotizing fasciitis (n=1 [femoral intra-arterial catheter]); perimembranous ventricular septal defect repair (n=1); and hemispherectomy (n=1). This meant data from 13 participants (8 males) were used for final analyses, all with comparisons to radial intra-arterial monitoring.

Table 1 Participant indication for intra-arterial blood pressure monitoring and pre-existing medical conditions

Participant characteristics	n (%)
Reason for intra-arterial blood pressure monitoring	
Blood pressure monitoring (postoperative or hypertensive emergency)	15 (83)
Septic shock	2 (13)
Diabetic ketoacidosis	1 (5)
Concurrent medical conditions	
Hypertensive emergency	1 (5)
Surgery	11 (61)
Septic shock	2 (11)
Diabetic ketoacidosis	1 (5)
Other	3 (17)

Indications for surgery included spinal fusion (n=1), cardiac surgery (n=8), renal transplant (n=1), and hemispherectomy (n=1). Other concurrent medical conditions included epilepsy (n=1), severe necrotizing fasciitis (n=1), and cyanotic congestive heart disease (n=1).

The mean duration of data collection was 20±3 mins. For technical reasons, in one participant, recordings were made only on the index finger.

3.3.1 Numerical results

Summary statistics showing the mean SAP, DAP and MAP values obtained from the intra-arterial catheter, FinAP, and reBAP are provided in **Table 2**; individual data are provided in **Table 3**. The middle finger SAP was significantly lower for the FinAP than both the reBAP (p=0.039) and intra-arterial pressure (p=0.029). There were no significant differences in any other values obtained from the finger recordings compared to the intra-arterial recordings.

Table 2 Summary statistics for blood pressure values from the intra-arterial catheter, FinAP, and reBAP

		Index (n=13)	Middle (n=12)
SAP (mmHg)	Intra-arterial	102.0±5.1	99.8±4.0*
	reBAP	100.2±6.0	98.0±4.3*
	FinAP	95.2±7.1	83.0±4.6
DAP (mmHg)	Intra-arterial	54.6±4.2	50.9±3.6
	reBAP	57.5±5.5	51.2±3.5
	FinAP	54.8±5.2	47.1±3.1
MAP (mmHg)	Intra-arterial	70.4±4.4	67.2±3.6
	reBAP	71.7±5.4	66.8±3.5
	FinAP	68.2±5.7	59.1±3.5

Data (mean ± standard error) are shown with finger blood pressure monitoring applied to both index and middle fingers. There were no significant differences in the reBAP values obtained from the finger compared to the intra-arterial recordings. The FinAP SAP were significantly lower than both intra-arterial and reBAP measurements for the middle finger. *significant difference from FinAP (p<0.05)

Table 3 Individual blood pressure values from the intra-arterial catheter, FinAP, and reBAP

		Index (mmHg)			Middle (mmHg)		
		SAP	DAP	MAP	SAP	DAP	MAP
Patient 1	<i>Intra-arterial</i>	116.8±2.6	62.8±3.2	80.8±2.6	118.3±3.0	61.6±2.5	80.5±2.4
	<i>reBAP</i>	130.2±6.5	64.8±4.0	86.6±4.4	114.1±4.6	53.9±3.5	74.0±3.7
	<i>FinAP</i>	142.9±6.3	80.4±3.2	101.3±3.9	94.7±3.0	49.9±2.8	64.9±2.8
Patient 2	<i>Intra-arterial</i>	140.4±3.4	88.0±4.1	105.5±3.7	N/A	N/A	N/A
	<i>reBAP</i>	146.1±4.7	110.4±4.1	122.3±4.2	N/A	N/A	N/A
	<i>FinAP</i>	145.4±3.7	97.7±3.6	113.6±3.5	N/A	N/A	N/A
Patient 3	<i>Intra-arterial</i>	86.7±3.4	46.2±2.3	59.7±2.6	91.6±3.3	49.3±4.0	63.4±3.3
	<i>reBAP</i>	87.5±2.9	48.4±2.3	61.4±2.4	100.4±3.3	58.5±2.6	72.5±2.6
	<i>FinAP</i>	80.0±2.0	47.4±1.9	58.3±1.8	85.3±2.8	49.5±1.9	61.4±2.0
Patient 4	<i>Intra-arterial</i>	92.4±5.3	33.5±2.2	53.1±2.9	91.6±7.4	31.4±3.0	51.4±4.0
	<i>reBAP</i>	90.5±6.0	34.7±3.2	53.3±3.6	94.6±9.8	32.5±2.5	53.2±4.5
	<i>FinAP</i>	74.7±4.6	29.9±2.6	44.8±2.7	49.7±5.0	26.8±2.1	34.4±2.9
Patient 5	<i>Intra-arterial</i>	93.8±1.4	52.4±1.8	66.2±1.4	106.2±4.0	50.1±2.5	68.8±2.3
	<i>reBAP</i>	100.6±1.7	57.2±1.5	71.7±1.5	92.1±2.2	53.1±3.4	66.1±2.9
	<i>FinAP</i>	102.3±1.7	47.4±1.1	65.7±1.2	89.7±2.4	42.8±2.4	58.4±2.3
Patient 6	<i>Intra-arterial</i>	95.5±3.2	64.1±2.2	74.6±2.5	113.8±3.4	70.3±3.3	84.8±3.2
	<i>reBAP</i>	86.7±4.2	56.8±2.9	66.7±3.3	106.4±4.7	69.0±3.4	81.5±3.8
	<i>FinAP</i>	83.2±3.6	58.0±2.3	66.4±2.7	100.8±4.4	62.3±2.9	75.1±3.3
Patient 7	<i>Intra-arterial</i>	119.3±2.4	60.7±1.8	80.2±1.9	101.2±5.0	52.3±2.6	68.6±3.3
	<i>reBAP</i>	97.8±2.0	67.2±1.9	77.4±1.9	107.1±3.8	54.1±2.6	71.8±2.9
	<i>FinAP</i>	100.8±1.7	62.6±1.7	75.3±1.6	87.8±3.2	55.6±2.2	66.4±2.5
Patient 8	<i>Intra-arterial</i>	87.2±4.6	50.2±3.1	62.5±3.2	85.5±4.9	48.9±4.1	61.1±3.9
	<i>reBAP</i>	85.3±3.6	51.9±3.7	63.0±3.4	89.7±4.2	54.6±3.3	66.3±3.2
	<i>FinAP</i>	59.4±3.6	35.2±3.0	43.3±3.1	75.5±4.0	46.6±2.7	56.2±2.9
Patient 9	<i>Intra-arterial</i>	131.3±3.9	76.3±2.7	94.6±3.0	128.0±5.5	74.5±3.0	92.4±3.6
	<i>reBAP</i>	133.6±5.3	73.8±3.3	93.8±3.8	132.0±5.8	69.0±3.2	90.0±3.8
	<i>FinAP</i>	112.4±3.9	65.1±2.8	80.9±3.0	101.0±3.8	61.9±2.7	74.9±2.9
Patient 10	<i>Intra-arterial</i>	90.1±2.8	44.5±1.6	59.7±1.9	89.3±3.1	43.6±1.6	58.8±2.0
	<i>reBAP</i>	88.4±3.6	52.9±2.6	64.8±2.9	85.8±3.3	52.1±2.2	63.3±2.5
	<i>FinAP</i>	93.7±3.5	55.5±2.2	68.3±2.6	79.6±3.5	39.3±1.9	52.8±2.3
Patient 11	<i>Intra-arterial</i>	92.1±1.6	44.2±3.2	60.2±2.4	94.6±1.8	45.7±1.8	62.0±1.6
	<i>reBAP</i>	84.3±2.9	38.6±1.7	53.9±2.0	91.7±2.3	44.4±1.3	60.2±1.6
	<i>FinAP</i>	82.0±3.0	34.6±1.4	50.4±1.7	98.2±2.2	55.3±1.1	69.6±1.3
Patient 12	<i>Intra-arterial</i>	90.9±5.3	39.5±5.2	56.6±4.8	90.1±2.7	38.5±5.9	55.7±4.1
	<i>reBAP</i>	87.3±3.6	35.4±4.7	52.7±3.9	83.5±2.0	38.6±3.5	53.6±2.7
	<i>FinAP</i>	74.2±2.8	47.9±2.7	56.7±2.6	64.3±1.8	38.8±1.3	47.3±1.3
Patient 13	<i>Intra-arterial</i>	89.9±2.8	47.9±2.1	61.9±2.2	87.3±2.5	44.3±2.7	58.6±2.5
	<i>reBAP</i>	84.0±3.9	55.5±3.3	65±3.4	78.3±3.7	33.9±3.2	48.7±3.2
	<i>FinAP</i>	86.9±4.1	50.1±2.7	62.4±3.0	69.9±3.0	36.4±2.7	47.6±2.7

Data (mean ± standard error) are shown with finger blood pressure monitoring applied to both index and middle fingers.

In all cases (SAP, DAP, MAP), the reBAP values were strongly and significantly correlated with those from the intra-arterial catheter (mean $r=0.873$; range 0.753-0.940;

all $p < 0.002$). FinAP and intra-arterial catheter values were also significantly correlated (mean $r = 0.815$; range 0.753-0.888; all $p < 0.002$). Mean differences and standard deviations were determined for comparisons between intra-arterial and both reBAP and FinAP pressures (**Table 4**).

Table 4 Absolute mean differences for the blood pressure value from the FinAP and reBAP compared to the intra-arterial catheter

	reBAP		FinAP	
	Index (n=13)	Middle (n=12)	Index (n=13)	Middle (n=12)
SAP (mmHg)	1.9±8.5	1.8±6.9	6.8±14.6	16.8±11.7
DAP (mmHg)	2.9±7.7	0.3±6.1	0.1±9.7	3.7±6.4
MAP (mmHg)	1.3±6.4	0.4±5.3	2.2±10.5	8.1±7.2

Data (mean differences ± standard deviation of the differences) are shown with finger blood pressure monitoring applied to both the index and middle fingers. According to AAMI guidelines, values should fall within 5 ± 8 mmHg (104).

Given the poorer performance of the raw FinAP in terms of both the raw blood pressure values (**Table 2**) and the mean bias analyses (**Table 4**), we considered further numerical analyses only on reBAP data.

Bland-Altman analyses were conducted on the reBAP data to evaluate the bias, SD, limits of agreement, and visualise the agreement between the values obtained using the intra-arterial catheter and the reBAP values (**Figure 7**). In all cases, the bias (mean difference) was within 3mmHg. The SD of the bias was ≤ 8.5 mmHg for the index finger and < 7 mmHg for the middle finger for each parameter of SAP, DAP and MAP. When considering the mean bias and upper and lower limits of agreement the middle finger appeared to perform slightly better than the index finger across all parameters.

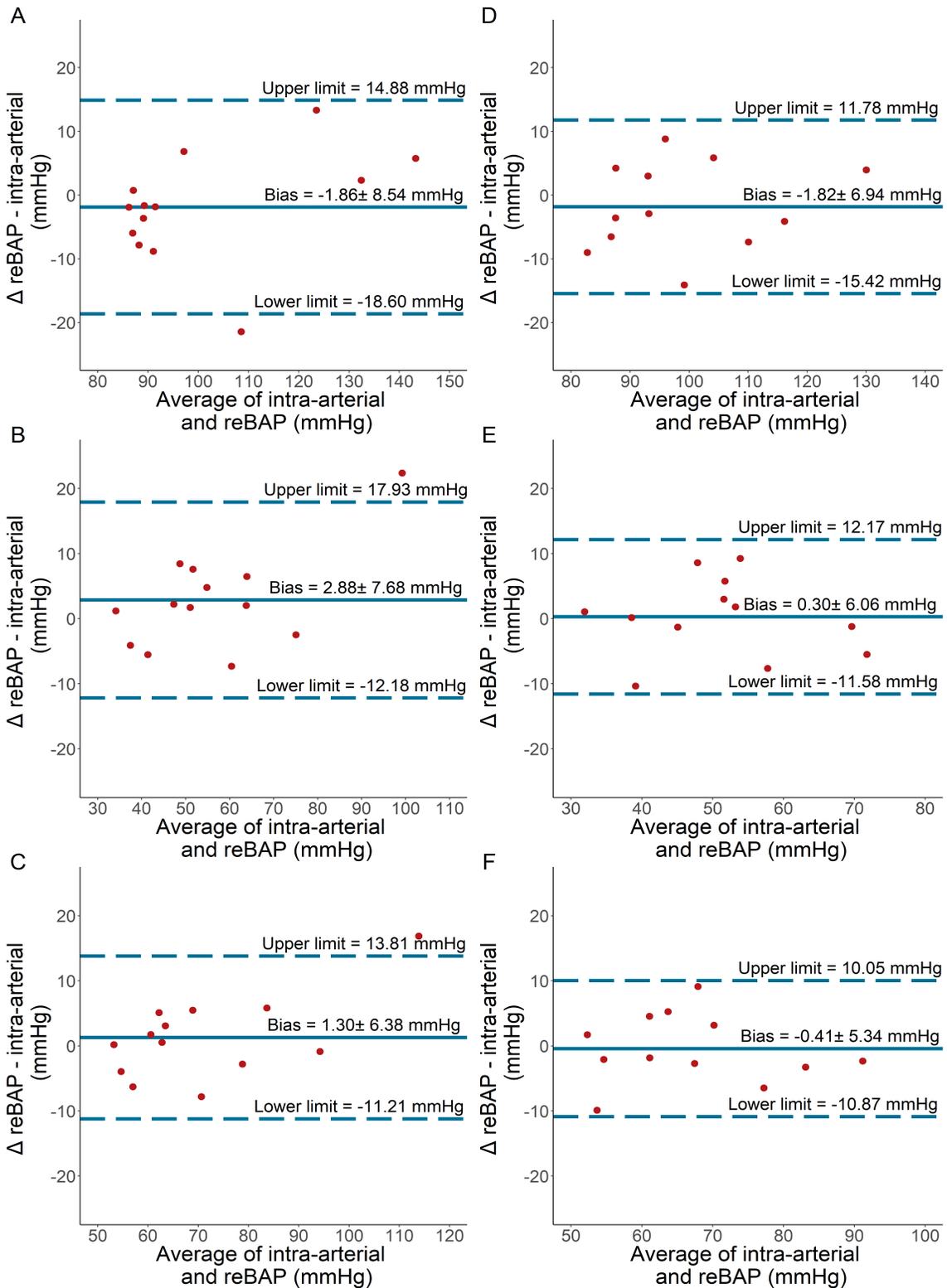


Figure 7 Bland-Altman analyses comparing reBAP to the intra-arterial catheter
 Data are shown for SAP, DAP and MAP comparisons for the index finger (A-C respectively) and the middle finger (D-F respectively). The solid line indicates the bias, reflected as the mean difference \pm SD. The dashed lines show the upper and lower limits of agreement respectively (mean difference \pm (1.96 x SD)).

These analyses can be compared to the AAMI guidelines, which require the mean difference to be $\leq 5\text{mmHg}$ and SD of the differences to be $\leq 8\text{mmHg}$ (104). Accordingly, in all cases for both reBAP middle and index finger recordings, the absolute mean differences met the AAMI criteria. Furthermore, the SD of the differences was also clearly within the AAMI criteria for all comparisons, with the exception of the SD of the SAP for the index finger, which just exceeded the 8mmHg criterion (8.5mmHg).

Table 5 outlines the cumulative percentage of heart beats where the absolute differences in blood pressure recorded from the reBAP compared to the intra-arterial catheter were $\leq 5\text{mmHg}$, $\leq 10\text{mmHg}$ and $\leq 15\text{mmHg}$. Cumulative percentages were reported over 1253 beats for the index finger and 1190 beats for the middle finger. The grade obtained for each criterion, as well as the cumulative grade, are provided according to the BHS guidelines (grades A and B meet the standard) (106).

Table 5 Cumulative percentages and corresponding grade according to British Hypertension Society (BHS) guidelines (106)

	Cumulative Percentage ($\leq 5\text{mmHg}$)	Cumulative Percentage ($\leq 10\text{mmHg}$)	Cumulative Percentage ($\leq 15\text{mmHg}$)	Overall Grade
Criteria	A \geq 60 B \geq 50 C \geq 40	A \geq 85 B \geq 75 C \geq 65	A \geq 95 B \geq 90 C \geq 85	
Index				
SAP	48.2 [C]	80.3 [B]	88.9 [C]	C
DAP	53.4 [B]	90.8 [A]	93.5 [B]	B
MAP	61.1 [A]	92.7 [A]	95.7 [A]	A
Middle				
SAP	41.9 [C]	85.6 [A]	95.7 [A]	C
DAP	56.7 [B]	91.1 [A]	99.4 [A]	B
MAP	68.5 [A]	94.0 [A]	99.9 [A]	A

Data are shown for reBAP monitoring applied to both index and middle fingers. Values reflect the cumulative percentage of heart beats in which the difference in the reBAP waveform compared to the intra-arterial catheter were $\leq 5\text{mmHg}$, $\leq 10\text{mmHg}$, and $\leq 15\text{mmHg}$ respectively. The percentage criteria for each letter grade according to the BHS guidelines are provided (106). A grade of D would be awarded where no other criteria are met. The grades attained for each parameter are indicated in parentheses. The overall grade is provided – all three percentages must meet criteria for a specific grade to be awarded.

Based on these criteria, both reBAP DAP and MAP values, with both index and middle finger application, meet the BHS standards. SAP values do not meet the standard (required to have at least 50% of difference readings $\leq 5\text{mmHg}$; 75% $\leq 10\text{mmHg}$ and 90% $\leq 15\text{mmHg}$), failing to meet these criteria by approximately 2% for the index finger and 8% for the middle finger.

We considered whether the accuracy of the reBAP readings relative to the intra-arterial values was related to the participant's weight or age. There were no significant correlations between the difference values for SAP, DAP or MAP and either age or sex for both index (SAP correlation with age: $r=-0.40$, $p=0.17$; or weight: $r=-0.47$, $p=0.10$) or middle fingers (SAP correlation with age: $r=-0.23$, $p=0.46$; or weight: $r=0.06$, $p=0.85$).

We also evaluated whether the reBAP values were equivalent to the intra-arterial catheter values using equivalency testing or TOST testing. We selected equivalency bands at 15% of the average gold standard intra-arterial catheter measurements, as differences less than 20% are generally considered clinically meaningless. It was found that all blood pressure parameters (index: SAP 15.3 mmHg, DAP 8.2 mmHg, MAP 10.6 mmHg; middle: SAP 15 mmHg, DAP 7.6 mmHg, MAP 10.1 mmHg) were equivalent to within 15% of the gold standard ($p<0.01$) (**Figure 8**).

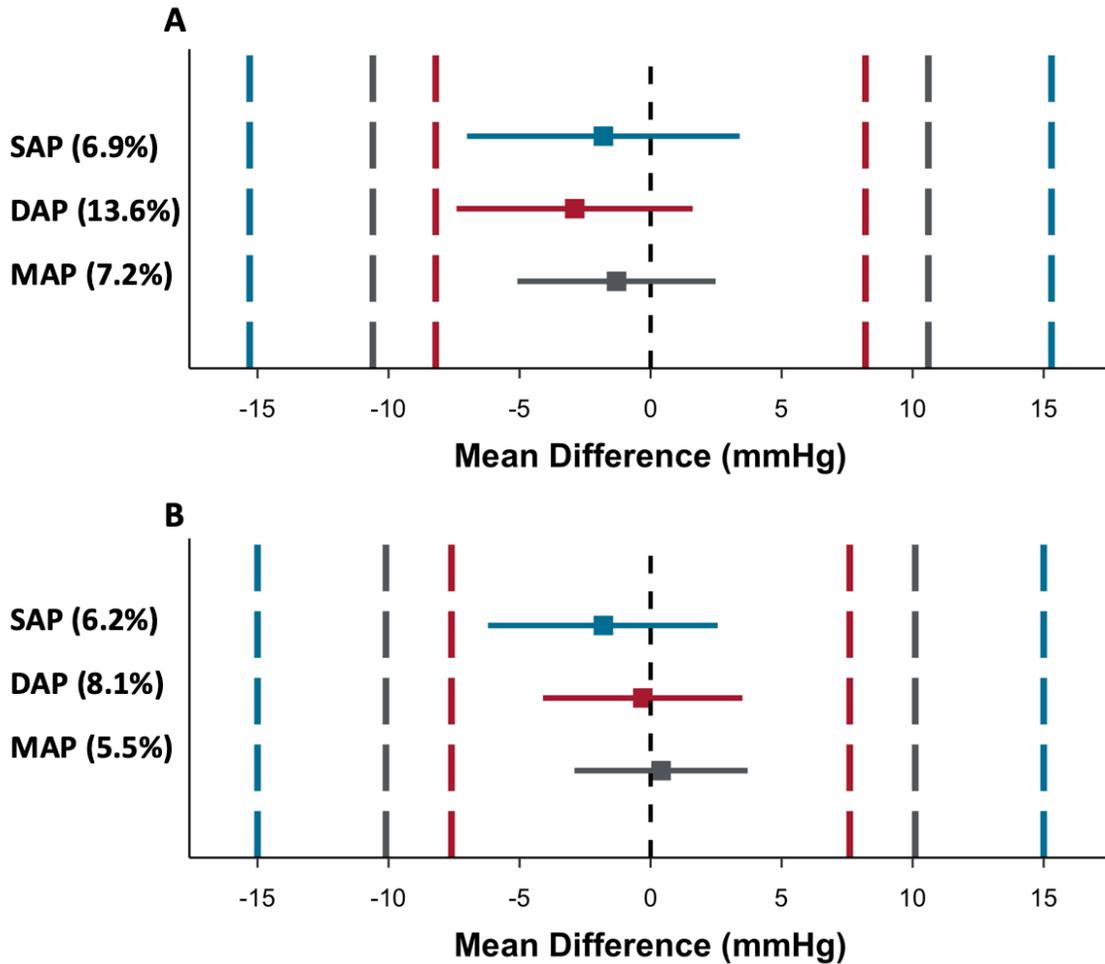


Figure 8 Equivalency plots for index and middle finger reconstructed brachial pressure compared to the intra-arterial catheter

A) Shows the equivalency plot for the index finger between the reconstructed brachial pressure (reBAP) and the intra-arterial catheter. B) Shows the equivalency plot for the middle finger between the same two measurement techniques. The mean differences, confidence intervals, and 15% equivalency bounds for all three blood pressure parameters (SAP systolic arterial pressure – blue, DAP diastolic arterial pressure – red; MAP mean arterial pressure – grey) are shown for each plot. The percentages to which these two measurement techniques are equivalent for each blood pressure parameter are listed on the left side of the plot.

3.3.2 Morphological results

We performed morphological analyses on both FinAP and reBAP waveforms, to evaluate whether the improved numerical accuracy of the reBAP data came with a compromise in the waveform morphology. We considered two models for our morphological comparisons, one which accounted for age and weight (**Equation 2**) and one which did not (**Equation 1**). There was no significant evidence for a change in the accuracy of the waveform or the deviations from the intra-arterial pressure based on

participant's age or weight. Accordingly, the simpler model (**Equation 1**) was used for the remaining analyses.

Figure 9 shows the individual tracings for the 10 middle finger beats selected for morphological analysis for the 12 participants for their intra-arterial catheter, FinAP and reBAP values. A smoothed regression line or "typical" waveform morphology for each participant is shown. As can be seen, the Finapres NOVA waveforms are extremely consistent within an individual over consecutive heart beats, and the smoothing regression equation is able to preserve the original average waveform shape of an individual.

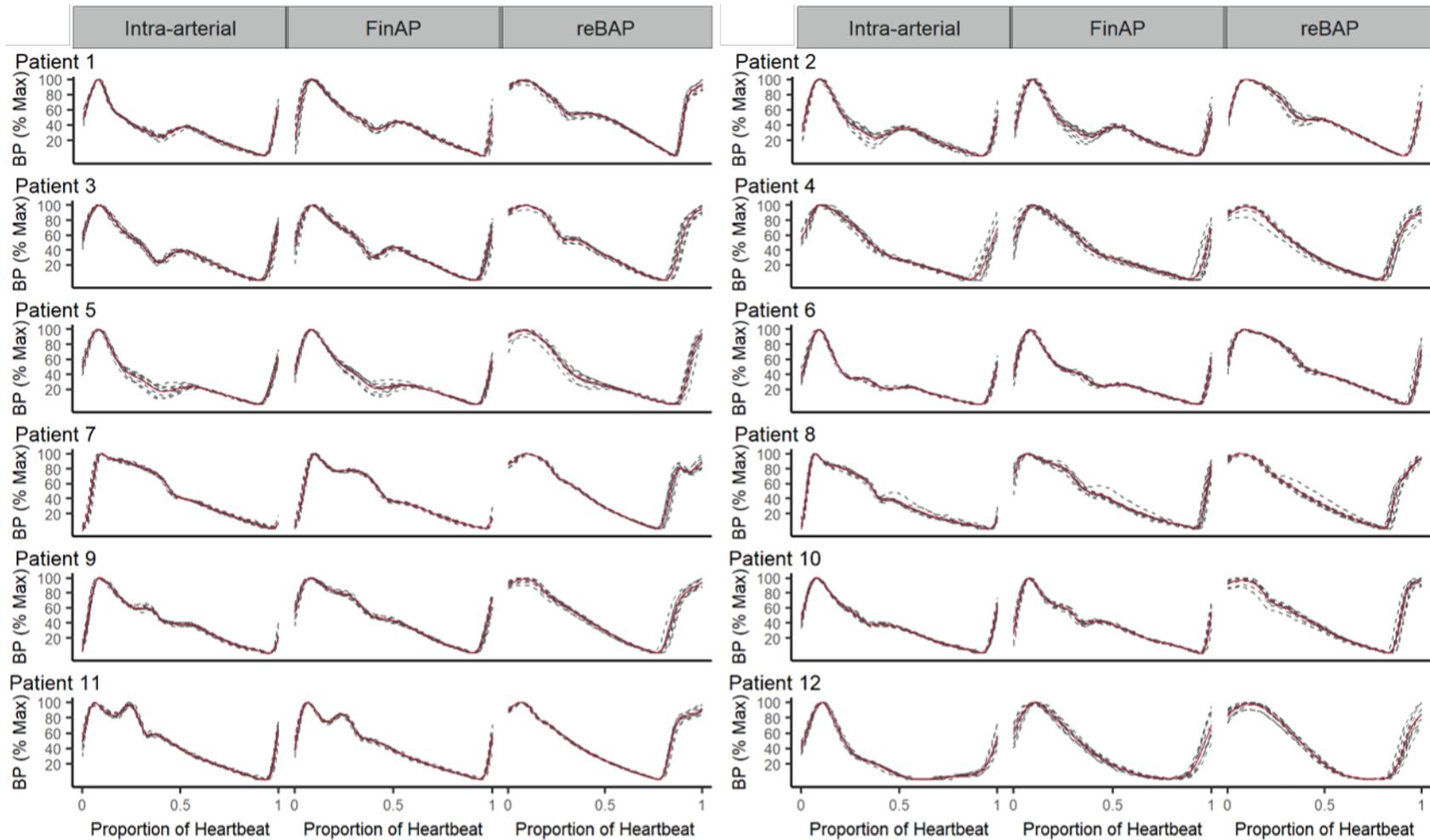


Figure 9 Individual traces showing the 10 beats selected for morphology analysis and the smoothed average waveform. Each beat is displayed in units of proportion of heartbeat to allow for easier comparison of waveforms across different heart rates; in addition, the y-axis blood pressure values are normalized relative to the peak SAP. These graphs show the morphology of 10 beats obtained simultaneously for all three measurement types for each participant with the smoothed waveform or the ‘typical’ waveform overlaid (red).

The group average intra-arterial catheter trace is considered the “gold standard” to which all other waveforms are compared. **Figure 10** shows the average group trace for the intra-arterial catheter, the FinAP, and the reBAP waveforms. As can be seen, the FinAP trace (blue line) waveform shape more closely resembles the intra-arterial catheter waveform (red line) than the reBAP trace (grey line).

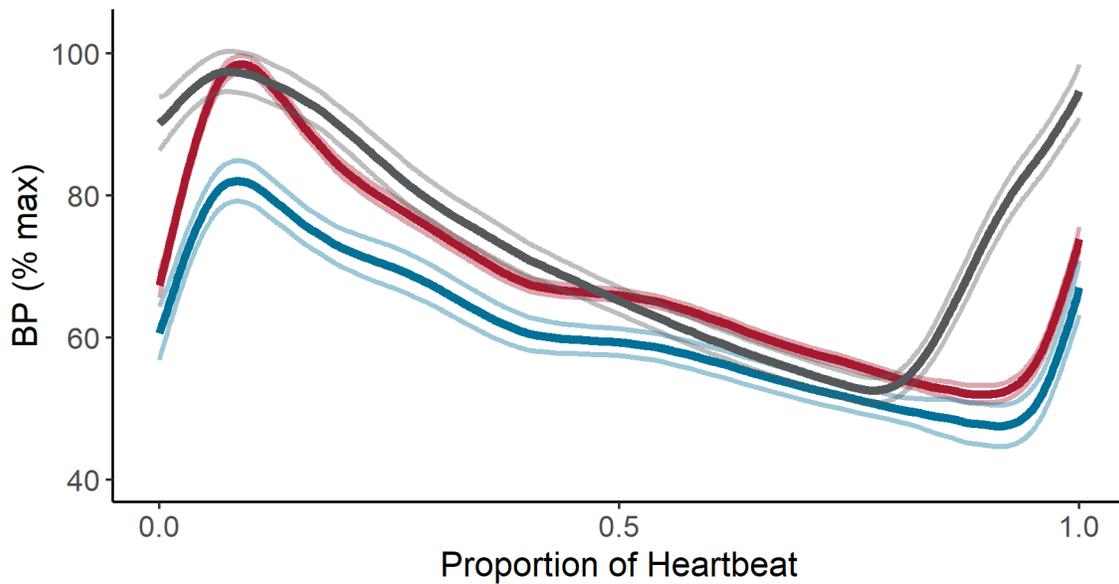


Figure 10 Group average waveform morphology for intra-arterial catheter, FinAP, and reBAP measurements

This figure shows the average group waveforms for the intra-arterial catheter (red line), FinAP (blue line) and reBAP (grey line) measurements along with their standard errors. A much larger deviation from the baseline waveform (red) can be seen in the reBAP waveform compared to the FinAP.

Figure 11 shows group averaged data for the differences between both FinAP and reBAP waveforms compared to the intra-arterial waveform. Patient specific effects account for waveform deviations specific to a patient’s heartbeat. There were significant deviations in reBAP waveforms compared to the intra-arterial baseline waveform, $\beta_j(t)$ in **Equation 1**.

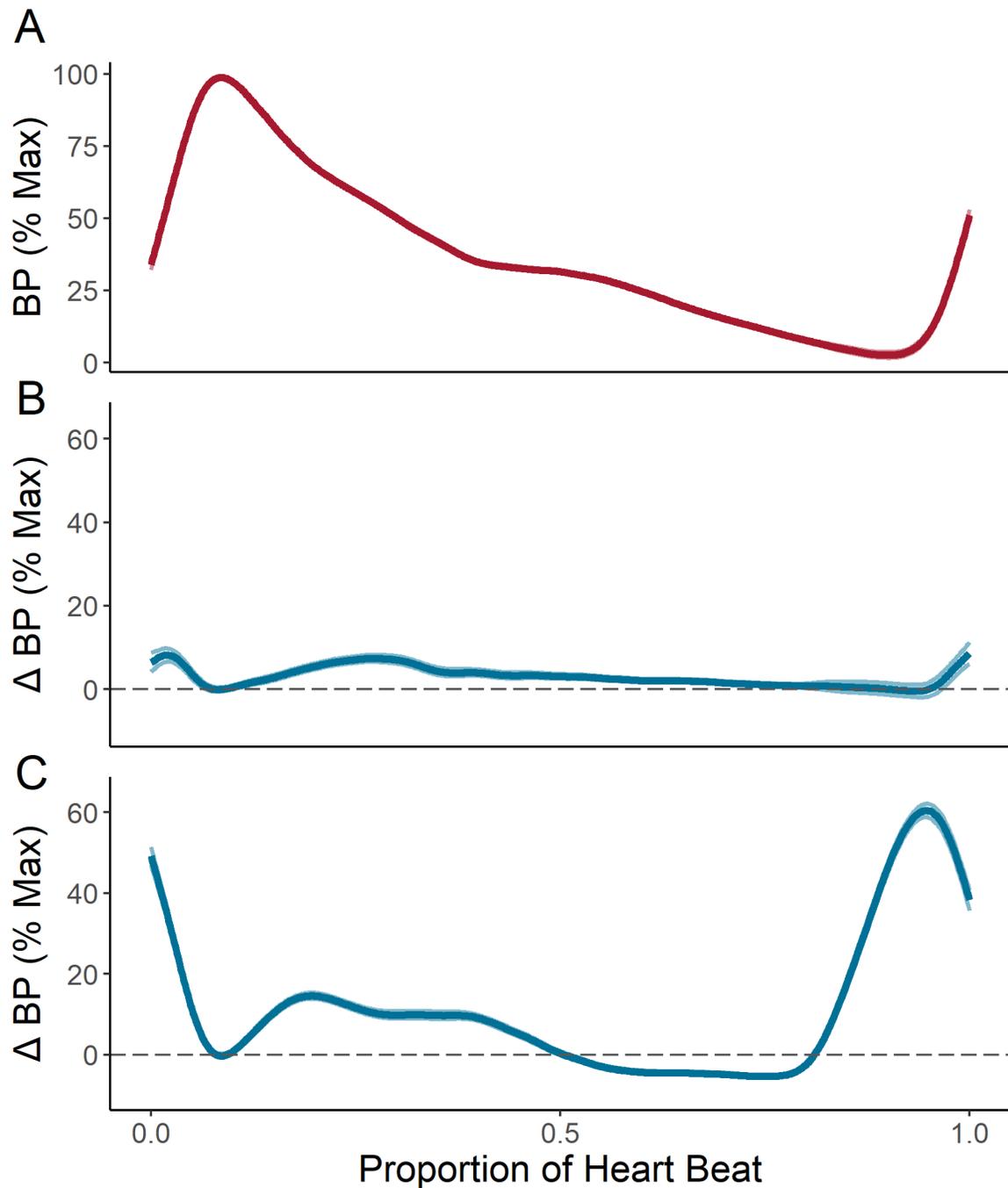


Figure 11 Average intra-arterial catheter trace compared to group difference plots for FinAP and reBAP

A) Shows the average baseline blood pressure waveform for the intra-arterial catheter group average with a normalized y-axis, B) shows the difference plot with standard errors for the group average FinAP waveform, and C) shows the difference plot with standard errors for the group average reBAP waveform. Regions where (B,C) differ from zero (the dashed horizontal line) indicate a significant difference between the waveform and the intra-arterial catheter.

This waveform discrepancy was particularly pronounced during the end of diastole and immediately prior to systole. The FinAP waveform morphology was not substantially different from the intra-arterial baseline throughout the beat. Overall, FinAP tended to outperform reBAP in terms of waveform morphology.

3.4 Discussion

3.4.1 Finger plethysmography with waveform correction provides a reasonable alternative to an intra-arterial catheter for measurement of beat-to-beat blood pressure in children

Excellent numeric agreement has been demonstrated between the non-invasive assessment of arterial blood pressure using finger plethysmography and data obtained using the gold standard of intra-arterial blood pressure monitoring in children. These agreements are particularly strong when the device is used on the middle finger and when brachial reconstruction algorithms are applied. While the FinAP raw values were found to be significantly different from the intra-arterial catheter values, the reBAP values were well within guidelines, indicating the need to use this reconstruction algorithm in order to obtain data that accurately reflect the arterial blood pressure at the level of the brachial artery. Using this approach, minimal bias (within 2 mmHg) was observed, as seen in **Table 4**, between the two measures (reBAP and intra-arterial catheterization) and AAMI (104) criteria were met. Performance for the index condition was slightly poorer, and although the bias (<3 mmHg) was within AAMI criteria (≤ 5 mmHg) (104), the SD of the differences for SAP just exceeded AAMI guidelines with a value of 8.5 mmHg rather than the recommended SD of 8 mmHg.

BHS (106) guidelines require the absolute differences between a novel device and a gold standard to achieve a grade of A or B for the SAP and DAP parameters. This study satisfied almost all conditions (including MAP parameters), except for SAP which did not quite achieve the B grade for all criteria. The cumulative SAP percentages were ~2% and ~8% away from meeting criteria for the index and middle fingers respectively. It is possible that this reflects use of the device in a pediatric intensive care population. The use of finger plethysmography has been previously shown to be limited by poor signal quality in cardiac surgery patients in whom peripheral perfusion is impaired (73,113).

In previous studies of finger plethysmography in both patient populations and in younger individuals, there was a tendency to overestimate SAP using plethysmography (74,77,114). This was not the case in the present study, at least once waveform reconstruction algorithms were considered, and may reflect better cuff fit with the novel miniaturised cuffs utilised.

3.4.2 Waveform reconstruction improves numerical accuracy but impairs waveform morphology in children

Due to pulse wave amplification, the pressure wave observed at the digital arteries is greater in amplitude than at the brachial artery. The reconstruction algorithm converts the FinAP into a brachial pressure (reBAP) based on average blood vessel characteristics for a given population (70,77). We found that use of the reBAP algorithm was beneficial and necessary to enable numeric comparisons within AAMI guidelines (104); however, the reconstruction algorithm applied was the standard algorithm, which applies demographic-adjusted corrections based on adult data (70). This algorithm does not incorporate further specifications for demographic-specific patient characteristics in those below the age of 18 years. In addition, the reBAP algorithm has previously been shown to be less accurate in individuals weighing less than 20 kg, and this was the case in 6 of the 13 participants in the study (111). The poor performance of waveform reconstruction algorithms in children has been noted previously (73).

Furthermore, several studies have commented on the reduced capability for these algorithms to perform in patient populations (74,113,115). Reduced performance in patient populations may be due to interventions or medications that alter blood vessel compliance, finger perfusion, or other variables incorporated in the algorithm (74,116). Use of the standard algorithm in children may also underestimate vascular compliance, which is known to be greater in children (102,113).

Despite these caveats, we found surprisingly good numeric agreement between the reBAP and intra-arterial blood pressure data, particularly when applied to the middle finger, suggesting that the algorithm performs at least reasonably well in pediatric patients. We evaluated whether there was an impact of participant age or weight on the accuracy of the numeric comparisons and waveform morphology and found no evidence that this was the case. This raises the question as to whether much of the early concerns were

related to improper cuff fit in pediatric populations, rather than poor waveform reconstruction. However, while our data were in good numeric agreement with the intra-arterial measurements, we recognise that the development of pediatric-specific algorithms may further enhance the utility of waveform correction using the reBAP algorithm (73,103).

Although the numeric agreement between reBAP and intra-arterial blood pressure measurements was good, the waveform morphology differed significantly for the reconstructed brachial waveform. Use of the adult reBAP algorithm may contribute to the relatively poor agreement between the waveform morphology from the intra-arterial line and the reBAP waveform. The waveform comparisons took into account differences from the intra-arterial line waveform shape, independent of any vertical deviation. Given that the reBAP algorithm corrects the vertical deviation, it is reasonable to find the numerical values for the reBAP to be within guidelines; however, the reBAP waveform was worse in terms of overall morphological comparisons. As noted previously, use of pediatric-specific reconstruction algorithms may be required to faithfully describe the waveform morphology from the finger plethysmogram. At the present time, while the numerical extraction of middle finger SAP, DAP, and MAP is accurate with waveform reconstruction, clinical inference based on reBAP waveform morphology obtained from finger plethysmography in children should be avoided. However, it should also be noted that the FinAP waveform was very closely related to the intra-arterial catheter waveform. Morphology-based insight may be acceptable with the use of the FinAP waveform, with the caveat that the absolute blood pressure values are subject to inaccuracy.

In some children, despite several attempts, we were unable to obtain usable data. In each of these cases the children were either hemodynamically unstable with low SAP or there was evidence of peripheral vasoconstriction and poor finger perfusion. This suggests that finger blood pressure monitoring should not be considered in these cases, something alluded to in previous studies that indicate poorer performance of these devices in patient populations (74,113,115).

3.5 Limitations

Although we showed good agreement between finger and arterial blood pressure monitoring in a pediatric intensive care unit population, use of finger plethysmography did not quite achieve all of the criteria outlined in the AAMI (104) and BHS (106) guidelines.

We do not consider this a critical limitation of the device – our data were within a few mmHg of criteria, and based on previous reports, the accuracy would be considerably better in an outpatient population (113,115) – arguably the target population for this technology. We also used prototype finger cuffs that incorporated standard technology but were not fabricated to final manufacturing standards – manufacturer grade cuffs would be expected to exhibit superior performance. Furthermore, as discussed above, we made use of the adult algorithm in a pediatric population whose blood vessels are expected to be more compliant and whose weight is likely to be less 20 kg. Another consideration is that our sample size was somewhat limited with an obvious potential to impact statistical power. We obtained data suitable for analysis in 13 (index finger) and 12 (middle finger) participants, with 25 recording periods compared. Blood pressure validation studies are recommended to include 35 pediatric participants (104). We recognize that this is a limitation in our study; however, we were limited by the use of an intra-arterial catheter as the reference standard, which would be undesirable for a healthy pediatric or outpatient population. Moreover, our sample size is in keeping with other similar studies that incorporated between 7-35 (average 15 ± 4) participants (73,78–80,100,102,103) during their validation of such devices.

In theory it would be of benefit to conduct these comparisons in healthy children or pediatric outpatient populations, in whom finger perfusion would be expected to be optimal, but as noted above, use of an intra-arterial catheter purely for research purposes is not recommended for healthy pediatric or outpatient populations. Instead we opted to test children in whom hemodynamic monitoring using an intra-arterial catheter was required for their ongoing clinical care, with the associated risk that their compromised hemodynamic status would impair device accuracy. Despite these concerns, the device performed well.

Furthermore, we did not assess the accuracy of blood pressure measurements during induced rapid or large blood pressure perturbations in this pediatric population, as manipulation of blood pressure in this manner in the intensive care unit would not have been clinically acceptable. Further investigation using tests that provoke large hemodynamic changes, such as a Valsalva maneuver or a tilt test, should be conducted in healthy children to determine the ability of the device to deal with such changes. While we could not induce blood pressure perturbations in this study, we were able to demonstrate that the device was able to consistently record accurate blood pressure in

patients in a pediatric intensive care unit, many of whom were hemodynamically unstable and who exhibited large natural variations in blood pressure. Of note, in adults the same technology has been shown to faithfully track both large and rapidly induced blood pressure alterations (117,118).

3.6 Conclusions

These data demonstrate the high accuracy and precision of continuous non-invasive blood pressure monitoring from the middle finger in a pediatric patient population. The device met all numeric criteria for agreement outlined by the AAMI (104) standards for SAP, DAP and MAP, once reconstruction algorithms were applied. Agreement with BHS (106) standards was met for DAP and MAP, and was very close to the required standard for SAP. Finger plethysmography using the middle finger provides a suitable alternative for beat-to-beat blood pressure monitoring in children in both clinical and research settings, although some caution should be applied when considering the accuracy of systolic blood pressure. Recordings from the middle finger were marginally superior to the index finger. Finger plethysmography was not suitable for use in children who were hemodynamically unstable or with impaired perfusion to the fingers, due to difficulty obtaining a reliable plethysmogram. Future development of manufacturer-grade cuffs, and pediatric-specific algorithms may help improve the morphological agreement of the reBAP waveform to the intra-arterial pressure waveform. Finger plethysmography offers a reliable and more comfortable non-invasive option for children who require beat-to-beat blood pressure monitoring.

Chapter 4.

Validation of a Novel Technique for Determining Urine Sodium Concentration

4.1 Background

4.1.1 Urine sodium levels in syncope patients are low

The quality of life of syncope patients is poor and reaching a confirmed diagnosis is a long, tedious process (13,19,119). One common diagnostic tool is the analysis of a 24-hour urine sample in order to determine urine sodium concentrations. This can provide important information for clinicians, since individuals with syncope tend to have low levels of urine sodium (47,60,120), and this can predispose to syncope because it is associated with low plasma volumes. In addition, adults with syncope and low urine sodium tend to have a greater response to treatment with salt supplementation (60). Enhanced ability to identify those individuals most likely to benefit from salt loading, and those who are unlikely to receive any benefit from it, would better inform management practices.

Individuals with syncope disorders have lower blood and plasma volumes, and this is thought to contribute to the underlying pathophysiology (47,51). As discussed, there is a decrease in cerebral perfusion with prolonged standing, in part due to blood pooling in the legs, with a subsequent reduction in the effective circulating volume (2). In adults with low urine sodium, salt supplementation improves orthostatic tolerance through increases in renal sodium and water retention, which in turn increase plasma volume (47,51). The increased plasma volume causes hemodilution and triggers erythropoiesis, resulting in an increase in blood volume, and thus improves orthostatic tolerance (121). Accurately determining urinary sodium excretion is, therefore, a useful tool to identify individuals with syncope and low sodium excretion who might benefit from salt supplementation, and to track compliance with salt supplementation approaches.

4.1.2 Using spot samples as an estimate of 24-hour urine sodium

Since 24-hour urine sodium sampling is inconvenient for many individuals, particularly children, validation of alternative methods for sampling that avoid the need for a 24-hour urine collection would be an asset. Previous research demonstrated that the

accuracy of a single spot urine sample compared to a 24-hour urine sample was poor (88–90). These researchers suggested that multiple spot samples may provide a more accurate alternative, although this has yet to be evaluated (88–90). There is also a lack of consensus on the best time of day to take a spot sample, as the first morning void would be the most convenient, but is also most likely to underestimate the true urine sodium concentration. Accordingly, one aim of this study is to investigate how many spot samples are necessary to provide a reliable estimate of 24-hour urine sodium, and whether morning or evening samples provide a more reliable estimate.

Currently, urine sodium samples are analyzed by flame photometry, which typically means they need to be sent away to a specialist laboratory for analysis. At-home testing would be a beneficial addition for patients with syncope disorders, to allow them to conveniently identify their suitability for salt supplementation, and to monitor their sodium levels to check compliance with dietary salt interventions. This is particularly pertinent given that it is recommended that individuals with syncope disorders increase their sodium and fluid intake (30,122), but few have a solid understanding of how to achieve this and maintain a healthy diet, and most struggle to hit the target salt recommendation (10 g per day). Chloride test strips have been suggested as an alternative to flame photometry since they provide convenient, inexpensive, and immediate results to the user; because sodium and chloride are present in the urine in an equal ratio, chloride can be used as a proxy for sodium (92,94,123). This approach has been used previously to determine sodium concentrations in individuals who are at risk of salt and water depletion (94).

We will evaluate use of Quantab chloride test strips to determine urine sodium. We aim to test whether chloride test strips accurately measure 24-hour urine sodium compared to the gold standard measurement for urine sodium, flame photometry. However, the current requirement for 24-hour urine sodium estimation can be tedious and uncomfortable for many individuals, particularly children. We will also determine whether use of chloride test strips applied to multiple spot samples will provide a more comfortable at-home alternative to 24-hour urine sampling with flame photometry.

4.2 Methods

4.2.1 Ethical approval

This study was approved by the Department of Research Ethics at Simon Fraser University and was conducted in accordance with the Declaration of Helsinki (2013) (107).

4.2.2 Participants

Participants aged 18 years and over were recruited through flyers posted around SFU Burnaby Campus and on the Graduate Student Society participation blog. Written informed consent (**Appendix A**) was obtained prior to the collection of any information or data from the participant. Participants were asked to refrain from participating in the study if they had a current urinary tract infection, and females were asked to complete the test on days they were not menstruating. If they were entered in the study while they were menstruating, we asked that they wait to begin collection until menstruation was complete.

4.2.3 Study design

The first aim of this study is designed to compare estimates of urine sodium derived from Quantab chloride test strips (high concentration: 300-6000 mg/L) to the gold standard, flame photometry (85), to determine their accuracy in analyzing urine sodium concentrations. Individuals were given a three-litre urine sampling jug and asked to complete a 24-hour urine sample, which was then analyzed using both flame photometry and the Quantab chloride test strips. The second aim of this study was to determine if multiple spot samples can be used as a reasonable alternative to a 24-hour urine sample.

Quantab chloride test strips detect chloride ions in increases of about 8 mmol/L; however, given that we expect to see increases of over 100 mmol/day in individuals who would be salt loading, the sensitivity of the measurement appears to be well within the range of our difference to detect. We determined that a difference to detect within 15% of the gold standard would be clinically meaningful, as differences less than 20% are generally deemed clinically meaningless (99).

Using a 15% difference from the gold standard, we will require approximately 20 individuals in order to be powered to detect a difference, assuming a conservative standard deviation of 25 mmol/day for individuals completing the 24-hour urine sample.

4.2.4 Protocol

Once volunteers provided their written informed consent, a brief demographic background was taken and an instruction sheet (**Appendix B**) given to them. This component comprised of two testing phases.

Protocol One: The first group of participants collected spot samples from their first morning void on five consecutive days (seen in the top row of **Figure 12**), and analyzed them at home using the Quantab chloride test strips, following which they discarded the urine – they collected a 24-hour urine sample at the midpoint.

Protocol Two: The second group of participants collected both AM (first morning void) and PM (void within an hour of their evening meal) spot samples on three consecutive days (seen in the bottom row of **Figure 12**), and analyzed them at home using the Quantab chloride test strips before discarding the sample – they collected a 24-hour urine sample at the midpoint. The spot samples were then compared with the 24-hour sample to determine whether they provide a valid alternative to 24-hour sampling.

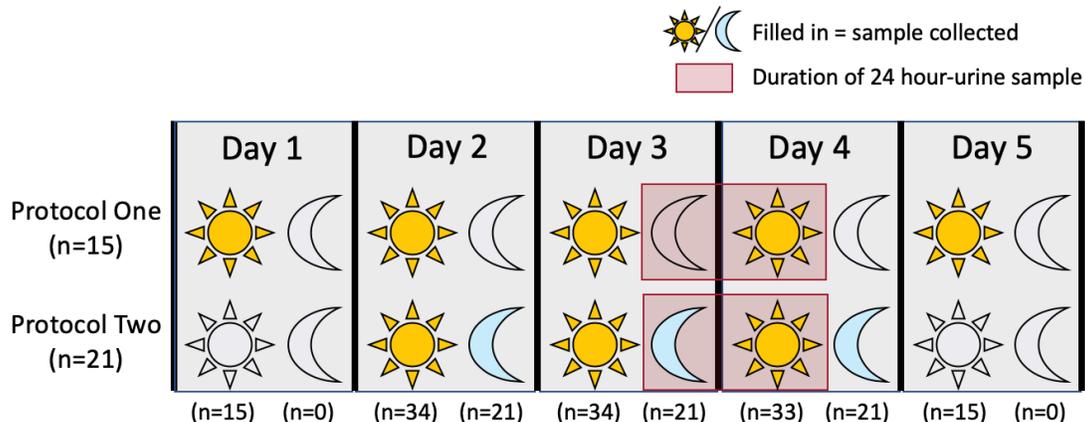


Figure 12 Urine sodium sampling protocol diagram

The suns represent the morning spot samples, the moons represent the evening spot samples; icons which are presented as coloured/filled in, indicate the sample was collected for that particular protocol. The total number of usable samples collected for each particular time point are listed in brackets below each column in the figure.

All participants were instructed to record the date of the first spot sample, urinate their first morning void in a container, and place the Quantab chloride test strip in the container so that the solution did not go above the yellow line on the strip. They were then told to wait five-seven minutes to allow the yellow colour indicator to turn black (**Appendix B**), before removing the strip. They then took a photograph of the strip before placing it in a storage bag and recording the Quantab value on the instruction sheet. Participants then discarded the urine in the toilet and rinsed the container out for the next sample (or discarded the container and used a new one each time). This process was repeated for consecutive samples and days, varying depending on the protocol. The test strips originally come in sealed bottles; however, in order to give participants the strips to use at home, the chloride test strips were placed in vacuum sealed packages prior to being handed to the participant. As noted above, in protocol one spot samples were completed only in the morning using the first morning void for five consecutive days. In protocol two spot samples were completed both in the morning and the evening for three consecutive days.

For protocol one, following the spot sample on the middle day, participants were instructed to collect all of their urine for a 24-hour period starting with the second time they urinated that day. The urine was collected in a three-litre container given to the participant by the laboratory and labelled with their ID number, but no identifying factors. Volunteers were told to write the date and time of the start of their 24-hour urine sample on the instruction sheet and to keep the sample in the fridge throughout the day. The fourth day first morning void was used as the fourth spot sample; however, instead of discarding this urine, participants were instructed to pour this sample into the 24-hour urine container, as it is the last void that marks the end of the 24-hour sample. At this point, participants were told they could bring the 24-hour sample up to the laboratory to be stored in our facilities, or they could keep it in the fridge and return it along with the strips and photos the following day, after they had completed the fifth morning spot sample.

For protocol two, the instructions were identical, except they were instructed to collect their urine starting with the second void of the middle day and to add both the middle day evening sample and following day first morning void to the 24-hour urine sample.

4.2.5 Data analysis

All files and information were saved according to a random and unique code number from which volunteers cannot readily be identified. Prior to analysis of the spot samples, an equation was created using known standards from the Quantab chloride test strip bottles to enable accurate interpretation of values not specified directly on the label (predicting values for 6.7 rather than using either the 6.6 or 6.8 specified values). This equation was used to convert the Quantab units (arbitrary units [a.u.]) to the standard urine sodium concentration expression of millimoles for the entire range of values rather than the given 8 mmol increases. In order to create this equation a line was fitted to the Quantab points; however, the best single fitted line had a rather complex equation, and we were concerned this might limit accessibility for clinical use. Accordingly, various alternative equations were created based on different properties (exponential, logarithmic, varying number of parameters). It was found that utilizing two equations, one for higher concentrations and one for lower concentrations, maintained the accuracy of the conversion across the entire range of values, as both the simpler equations tended to deviate from the specified points at either higher or lower test strip values.

After conversion of the test strip values, the 24-hour urine samples were analyzed using both the gold standard flame photometry and Quantab chloride test strips. These values were then correlated, and Bland-Altman plots produced to determine the bias, SD, and limits of agreement of the two measures. Equivalency testing using the TOST test was performed between the two measurement approaches. Once the chloride test strips had been validated, the mean spot sample values using protocol one were compared through Bland-Altman plots and equivalency testing to determine if they provided a reasonable alternative to 24-hour urine sodium samples, as well as to determine the minimum number of spot samples required for reasonable accuracy. Protocol two was then used to determine the sampling average that best correlated with 24-hour urine sampling. Additional secondary outcomes included correlations with single spot samples and participant demographic characteristics. An outline of the data processing can be seen in Figure 13. Data were analyzed using R (R Core Team, 2015), and SigmaPlot Version 14 (Systat Software, San Jose, CA). Statistical significance was assumed where $p < 0.05$. Data are presented as mean \pm SE unless otherwise stated.



Figure 13 Protocol outline of data processing for comparing chloride test strips and spot samples to flame photometry analyzed 24-hour urine samples

The order of analysis for this study began with the creation of a conversion equation, followed by comparison of the Quantab chloride test strips to the gold standard of flame photometry using the 24-hour urine samples. Afterwards, Protocol One was used to determine the number of spot samples required in order to be used as an alternative to 24-hour urine sampling. Protocol Two was then used to determine which sampling average best correlated with 24-hour urine sampling. A correction factor was created based on the best sampling averages and finally the use of a single spot sample in relation to 24-hour urine sampling was investigated.

4.3 Results

4.3.1 Creation of a conversion equation between Quantab units and millimoles

Quantab chloride concentrations (a.u.) can be converted to the standard measurement unit of millimoles through the fitting of an equation to the pre-determined Quantab values. Two equations were identified in order to compromise simplicity of the equation while ensuring as close a fit as possible to the original values; these equations along with the pre-determined Quantab values are shown in **Figure 14**.

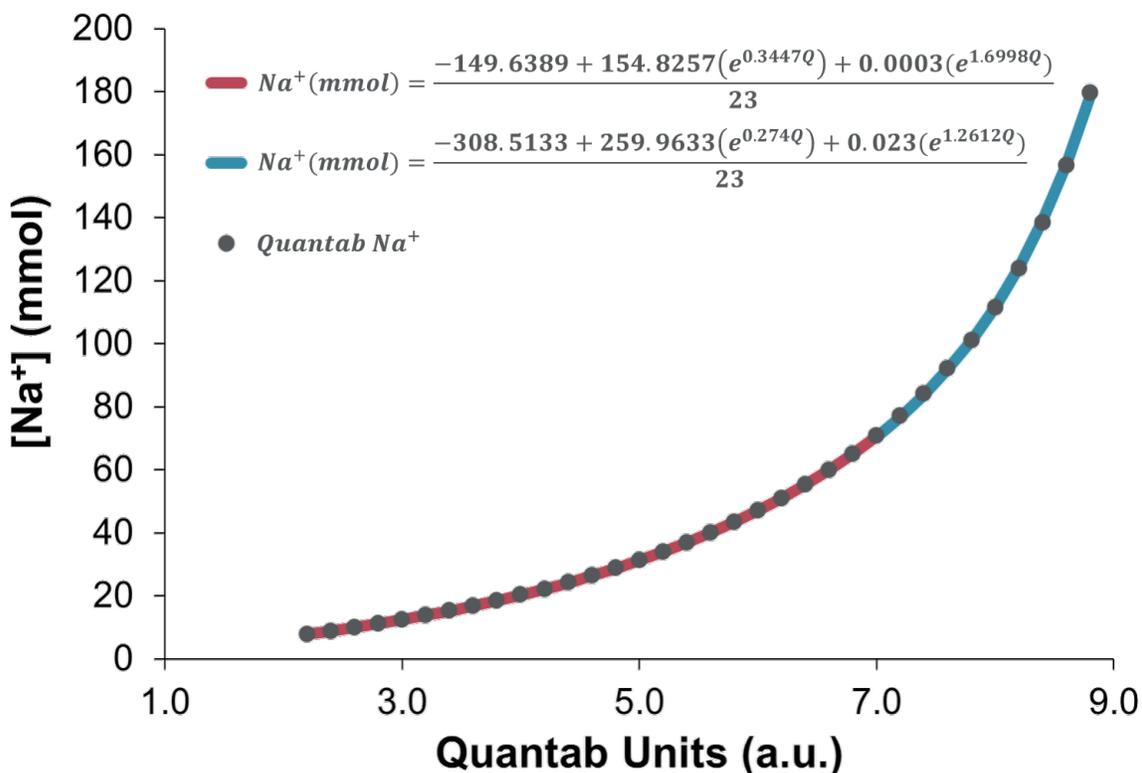


Figure 14 Equations for conversion of Quantab units (a.u.) to standard units (mmol)

The grey points specified on the figure represent the pre-determined values in arbitrary Quantab units. Two simpler equations were fitted to the curve of these points to allow for conversion between the Quantab units (a.u.) to millimoles (mmol), the standard concentration for urine sodium. The red equation is used for points below seven Quantab units (a.u.) and the blue equation is used for Quantab values of seven or more (a.u.).

For optimal accuracy, concentrations below seven Quantab units are converted using **Equation 3**, where Q is equal to the Quantab chloride measurement (a.u.):

$$Na^+(mmol) = \frac{-149.6389 + 154.8257(e^{0.3447Q}) + 0.0003(e^{1.6998Q})}{23} \quad (3)$$

and concentrations at or above seven Quantab units are converted using **Equation 4**, where Q is again equal to the Quantab chloride measurement (a.u.):

$$Na^+(mmol) = \frac{-308.5133 + 259.9633(e^{0.274Q}) + 0.023(e^{1.2612Q})}{23} \quad (4)$$

These two equations allow for conversion of the values obtained from the Quantab chloride test strips into millimoles for comparisons with the gold standard in urine sodium analysis, flame photometry.

4.3.2 Accuracy of Quantab chloride test strips compared to flame photometry

Data were obtained from 34 participants (17 males) aged 23.9 ± 0.7 years (range 19-35 years) who completed either protocol one or protocol two. Demographic data for these participants are listed in **Table 6**.

Table 6 Demographic data for comparisons between Quantab chloride test strips and flame photometry

Demographics	Mean \pm SE
Age (years)	23.9 \pm 0.7
Height (cm)	170.2 \pm 1.7
Weight (kg)	68.8 \pm 2.5
Final volume (L)	1.69 \pm 0.14
Q24 (mmol/day)	114.8 \pm 7.7
Q24-alt (mmol/day)	127.5 \pm 8.8
Flame photometry (mmol/day)	119.3 \pm 8.0

Final volume is the average volume of the 24-hour urine sample; Q24 is the average of the undiluted Quantab chloride test strips; Q24-alt is the diluted average of the Quantab chloride test strips.

Flame photometry 24-hour urine sodium concentration was not significantly correlated with age, height, or weight.

Since the Quantab conversion equation is on an exponential or polynomial scale, participants who had a 24-hour Quantab reading of more than 7 a.u. had their samples diluted by 50% and re-dipped to obtain a more accurate measurement (which was then multiplied by two). This grouping of Quantab 24-hour values is referred to as Q24-alt, whereas the original Quantab measurements without any dilutions applied are referred to as Q24. In general there was a strong and highly significant correlation between both Quantab 24-hour urine sodium estimates and the gold standard obtained by flame photometry; the correlation with flame photometry measurements was slightly stronger with Q24-alt ($r=0.959$, $p<0.0001$) than with Q24 ($r=0.902$, $p<0.0001$) (**Figure 15**).

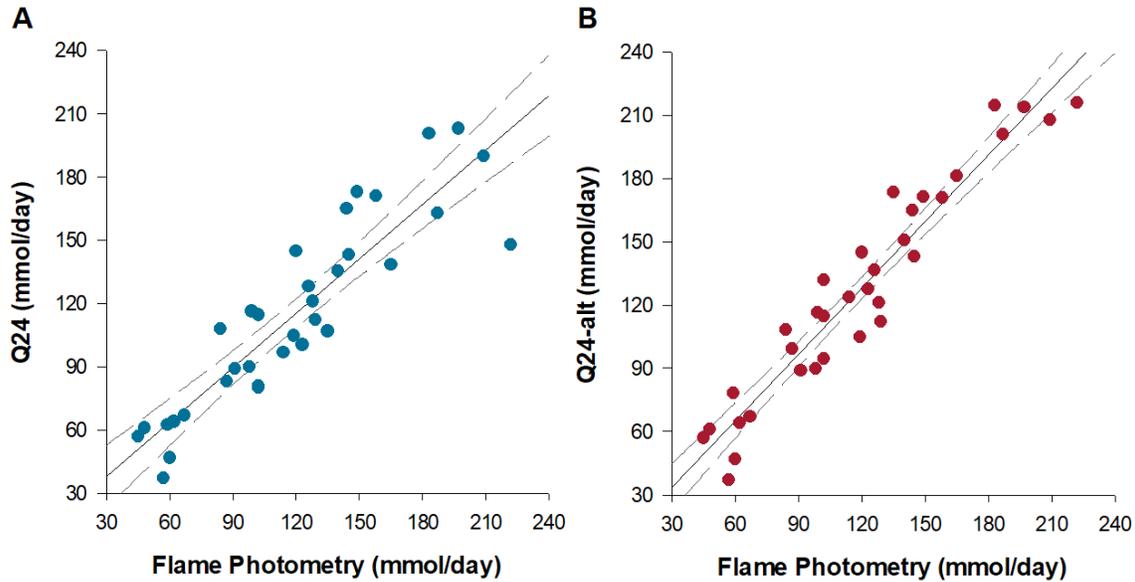


Figure 15 Correlation between Quantab chloride test strip 24-hour urine sodium values compared to flame photometry

A) Shows the correlation between the undiluted Quantab chloride test strip analyzed 24-hour samples (Q24) compared to the gold standard of flame photometry ($p < 0.0001$). B) Shows the correlation between the diluted Quantab chloride analyzed 24-hour urine sodium samples (Q24-alt) compared to flame photometry ($p < 0.0001$).

Furthermore, Bland-Altman analyses showed minimal bias between both measurements of Quantab 24-hour values and the flame photometry values. The Q24 values produced a bias of -4.51 ± 20.4 mmol/day (**Figure 16a**), whereas the Q24-alt values produced a bias of 8.16 ± 14.6 mmol/day (**Figure 16b**) when compared to flame photometry. An outlying data point can be observed in the undiluted Q24 Bland-Altman plot. The large disparity in this point may stem from the high concentration found, as the 24-hour urine sample analyzed through flame photometry placed this value at 222 mmol/day, above the upper average bound of 220 mmol/day. If this data point were to be removed, it should be noted that the bias for the undiluted samples becomes -2.4 ± 16.5 mmol/day with limits of agreement at -34.8 mmol/day and 30.0 mmol/day. Despite the slightly larger bias seen in the Q24-alt comparison, it should be noted that smaller limits of agreement were observed in this condition compared to both sets of undiluted sample values, indicating its increased accuracy.

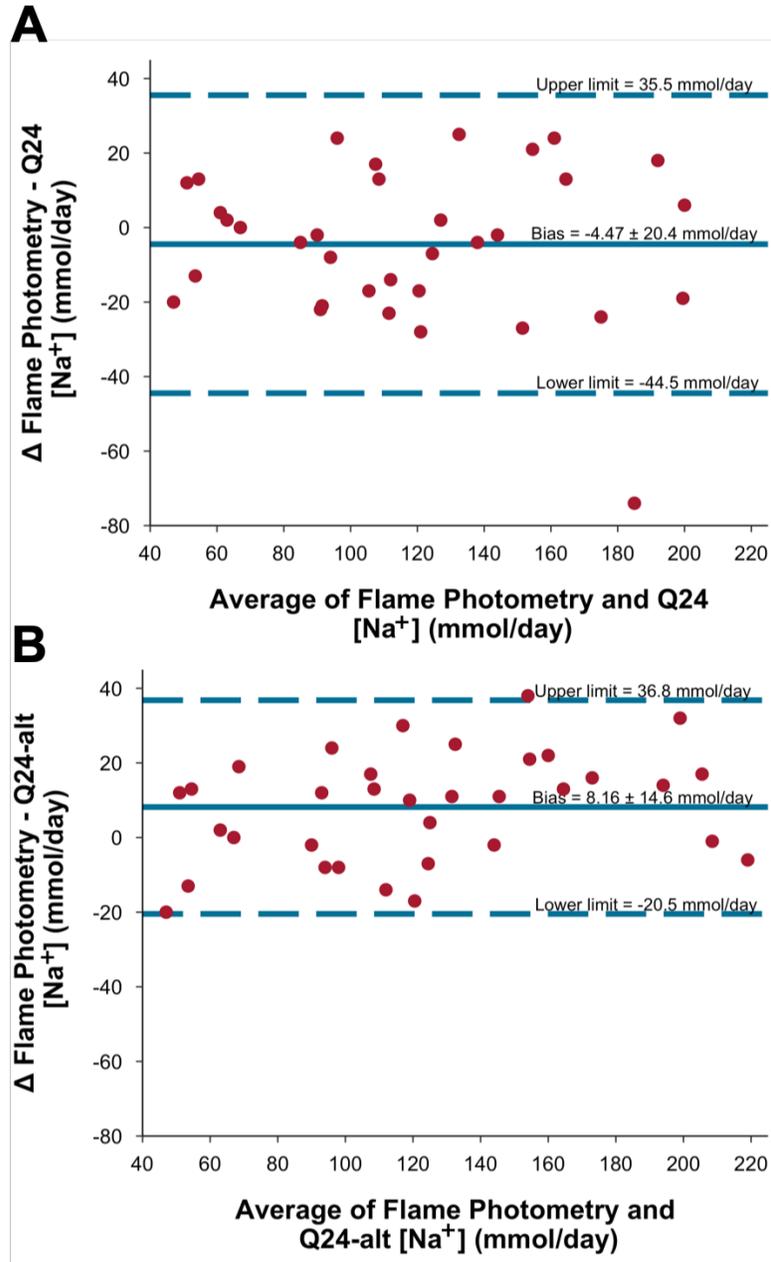


Figure 16 Bland-Altman analyses comparing the bias and limits of agreement between Quantab 24-hour values and flame photometry

A) Shows the bias and limits of agreement between the gold standard flame photometry and the original 24-hour Quantab values (Q24). B) Shows the bias and limits of agreement between flame photometry and the re-dipped Quantab values (Q24-alt).

The Quantab 24-hour urine sodium values were also compared to flame photometry using equivalency testing through a TOST test. We selected equivalency bands at 15% of the average gold standard flame photometry measurements (18 mmol/day) as a reasonable clinically meaningful difference to determine if the two

measurements were equivalent. It was found that the Q24-alt values, while significantly different from zero ($p=0.0026$), were also statistically equivalent ($p=0.0002$). Further analysis showed that the Q24-alt metric was equivalent to within 10% of the gold standard (12 mmol/day raw score) ($p=0.05$). The equivalency plot is shown below with both the 10% and 15% equivalence bounds (**Figure 17**). It should also be noted that the Q24 values were found to be statistically equivalent within 10% of the flame photometry measurements ($p=0.02$), as well as statistically not different ($p=0.25$). However, the lack of statistical difference may reflect the larger variance present within the sample that was corrected for by re-dipping samples at the higher end of the Quantab exponential range.

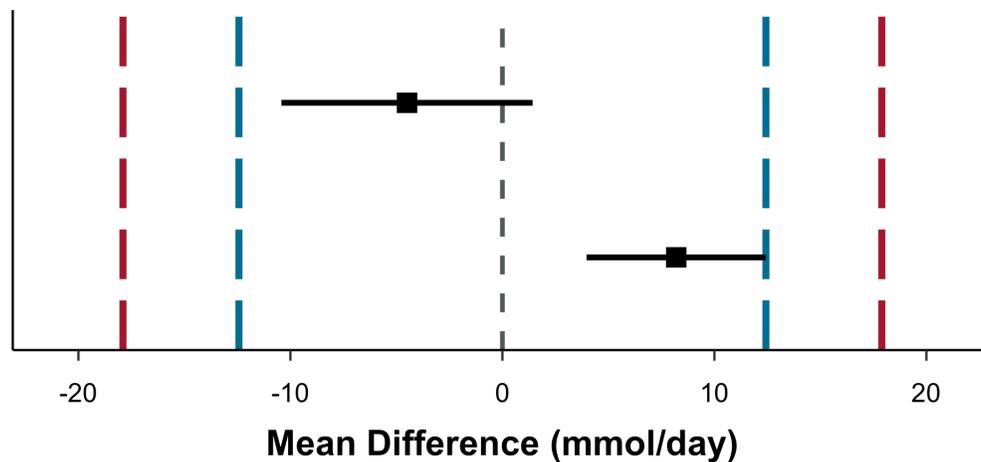


Figure 17 Equivalency between flame photometry and Quantab 24-hour urine samples

The red dashed lines indicate 15% equivalency with the flame photometry values, while the blue dashed lines indicate 10% equivalency with these gold standard values. As can be seen, the mean difference for both Q24 values (top) and the Q24-alt (bottom) lie within both bounds indicating equivalency to within at least 10% of the gold standard. The Q24-alt values do not cross zero, indicating a significant difference between the two measurement methods, but with statistical equivalence.

Collectively these data show that use of Quantab test strips to determine 24-hour urine sodium produces results that are equivalent to the gold standard of flame photometry, with minimal bias.

4.3.3 Protocol one

Having established the accuracy of the Quantab strips to determine urine sodium concentration, the primary purpose of protocol one was to determine whether the 24-hour

urine sodium concentration could be reasonably approximated with a series of spot samples. Participant characteristics and mean values for estimates of sodium over up to five days of morning spot samples are found in **Table 7**.

Table 7 Demographic data for comparisons between multiple morning spot samples and flame photometry

Method (n=15)	Mean±SE
Age (years)	23.6±1.1
Height (cm)	171.8±2.4
Weight (kg)	72.8±4.2
Flame photometry (mmol/day)	112.5±12.8
Q24 (mmol/day)	106.1±10.4
Q24-alt (mmol/day)	118.8±12.7
5-day average (mmol)	47.5±4.9*
4-day average (mmol)	46.1±5.1*
3-day average (mmol)	45.9±5.1*
2-day average (mmol)	48.2±5.8*
1-day average (mmol)	44.9±5.7*

*significant difference from flame photometry (p<0.001)

Flame photometry 24-hour urine sodium concentration was not significantly correlated with age, height, or weight for protocol one.

There were no differences determined between 5-day vs 4-day vs 3-day vs 2-day vs 1-day averages as determined by a repeated measures ANOVA (p=0.708). The prior suggestion to use multiple spot samples as a proxy for 24-hour urine sodium recommended the use of more than two spot samples (89). No significant difference was found between 5-day (the highest number of spot samples collected) and 3-day (the minimum number of spot samples recommended) spot sample averages (p=0.326). The spread of the data between all five sampling averages can be seen in the box plots in **Figure 18**. Since there were no significant differences and similar variance between the different sampling averages, the decision was made for protocol two to only require participants to collect spot samples over three consecutive days, as there was negligible additional benefit from additional samples, and considerable added participant burden.

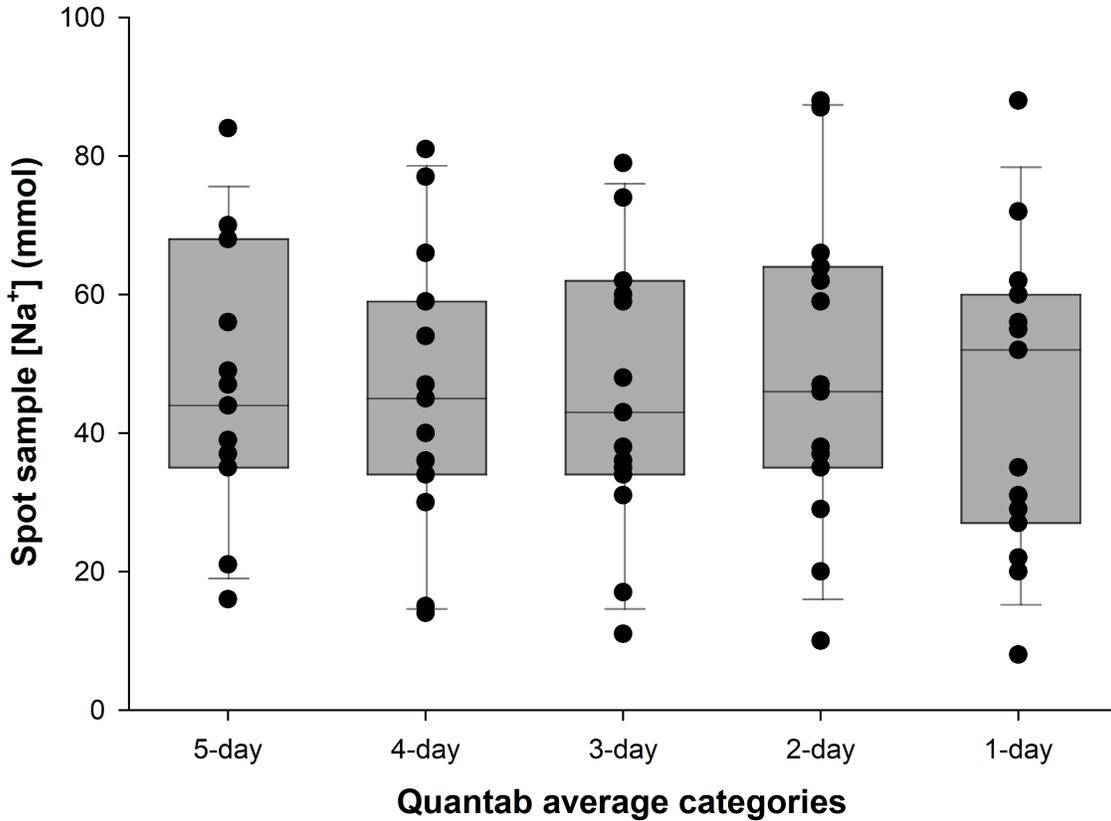


Figure 18 Box plots showing the data for 5-day, 4-day, and 3-day, 2-day, and 1-day Quantab spot sample averages

Box plots are shown for sampling averages from Protocol One showing 5-day through to 1-day morning sampling averages. A repeated measures ANOVA found no differences between any of the sampling averages ($p=0.708$).

The 3-day average spot samples were moderately correlated with flame photometry ($r=0.469$, $p=0.078$); however, there was a large and significant difference between the absolute values of the spot samples, regardless of the spot sample approach, when compared with flame photometry. When examining the box plots and Bland-Altman graphs for these two methods (**Figure 19**), the difference in values and the large systematic bias gives a clear indication that a correction factor needs to be applied to the spot samples. This is not surprising as spot samples usually contain a lower concentration of sodium compared to 24-hour urine samples, particularly spot samples that come from first morning voids, as is the case in our study.

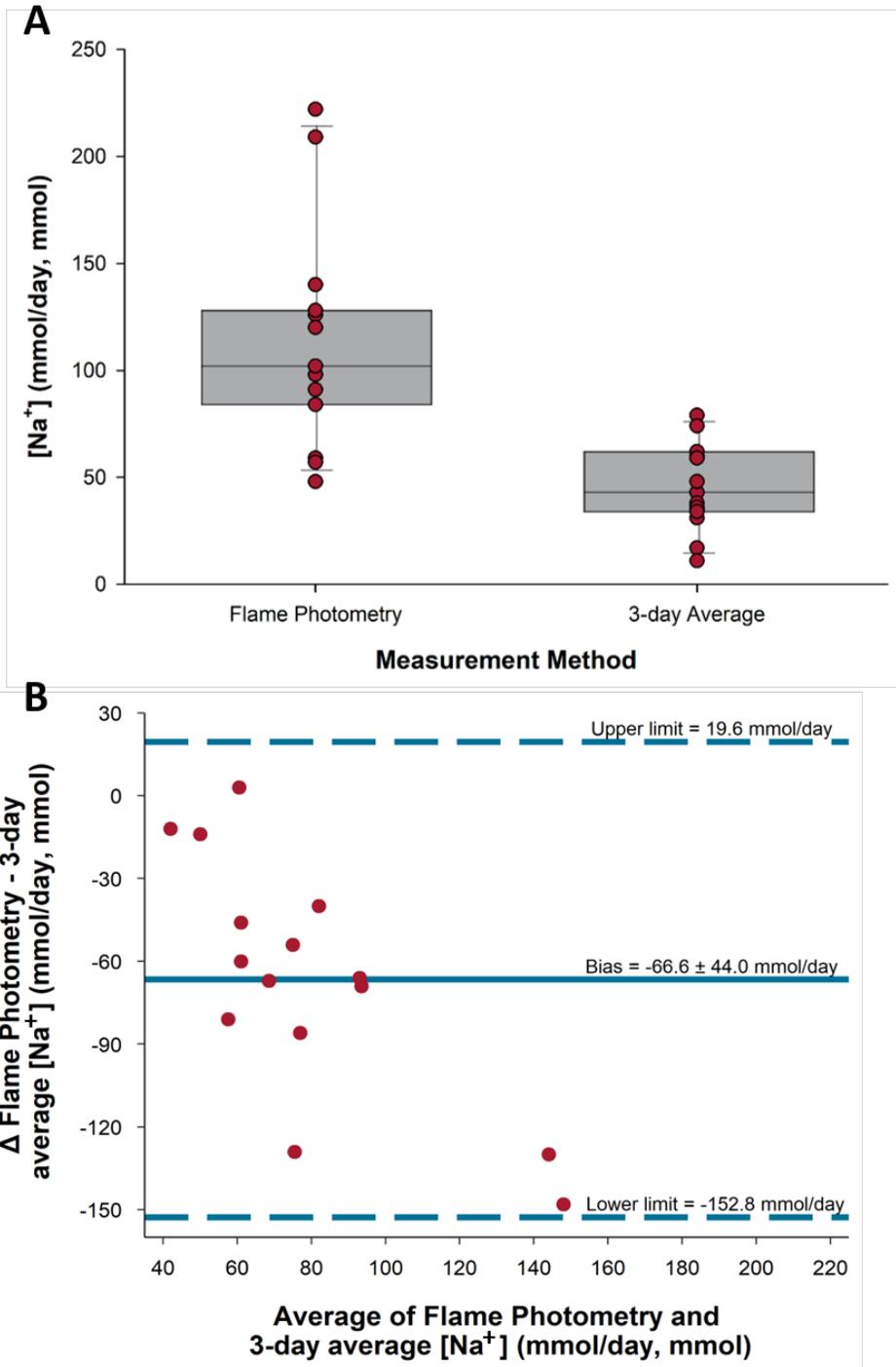


Figure 19 Comparison of flame photometry values to 3-day spot sample averages

A) Shows the spread of the data through box plots between flame photometry and 3-day spot sample averages, B) shows the Bland-Altman analyses for this comparison.

4.3.4 Protocol two

The demographic data for participants who completed protocol two can be found in **Table 8**. One individual had a technical error during day three of their morning spot samples, their data is included in the evening (PM) samples, but could not be used for comparisons that required a day three morning sample due to the missing data.

Table 8 Demographic data for comparisons between multiple morning or evening spot samples and flame photometry

Method (n=21)	Mean±SE
Age (years)	24.2±0.8
Height (cm)	168.8±2.1
Weight (kg)	66.5±2.6
SAP (mmHg)	120±1.8
DAP (mmHg)	74±1.8
MAP (mmHg)	89±1.6
Flame photometry (mmol/day)	124.6±9.4
Q24 (mmol/day)	122.8±10.0
Q24-alt (mmol/day)	134.2±11.0
3AM (mmol) <i>n</i> =20	47.2±5.5*
3PM (mmol)	57.0±5.1*
24 P/A (mmol) <i>n</i> =20	50.3±4.8*
6-all (mmol) <i>n</i> =20	51.9±5.3*
PAP (mmol) <i>n</i> =20	48.4±4.6*
APA (mmol) <i>n</i> =20	51.4±5.3*
PAPA (mmol) <i>n</i> =20	53.6±5.2*
APAP (mmol) <i>n</i> =20	50.5±5.2*

Abbreviations: SAP systolic arterial pressure; DAP diastolic arterial pressure; MAP mean arterial pressure; Q24 undiluted Quantab chloride test strip values; Q24-alt diluted Quantab chloride test strip values; 3AM three morning spot samples; 3PM three evening spot samples; 24 P/A two spot sample average; 6-all average of all six spot samples; PAP evening, morning, evening spot sample; APA morning, evening, morning spot sample; PAPA two evening and two morning spot samples; APAP two morning and two evening spot samples. Explanations for the different sampling averages (3AM, 3PM, 24 P/A, 6-all, PAP, APA, PAPA, APAP) are found in Table 9. *Significant difference from flame photometry ($p < 0.001$)

Flame photometry 24-hour urine sodium concentration was not significantly correlated with age, height, weight, or blood pressure (SAP, DAP, or MAP) for protocol two.

Protocol two was used to determine a correction factor for the spot samples in order to make them comparable to the gold standard flame photometry measurements. Additionally, protocol two also included evening spot samples, and so various

combinations of AM and PM samples were explored – these combinations are listed below in **Table 9**.

Table 9 Combinations of AM and PM spot sample averages explored to compare to flame photometry

Average	Composition
3AM	Average of all 3 AM spot samples
3PM	Average of all 3 PM spot samples
24 P/A	Average of the middle day PM and fourth day AM spot samples
6-all	Average of all 3 AM and all 3 PM spot samples
PAP	Average of the middle and fourth day PM and the fourth day AM spot samples
APA	Average of the middle and fourth day AM and the middle day PM spot samples
PAPA	Average of the second and middle day PM and the middle and fourth day AM spot samples
APAP	Average of the middle and fourth day AM and the middle and fourth day PM spot samples

Flame photometry measurements were significantly correlated with almost all protocol two spot sample averages ($p < 0.02$), except for 3AM ($p = 0.11$). Accordingly, regression equations based on the strongest three correlations were created as a simple method of correcting spot samples. The strongest three correlations were: PAP ($r = 0.603$, $p = 0.005$), APAP ($r = 0.555$, $p = 0.01$), and 3PM ($r = 0.546$, $p = 0.01$). However, because the next strongest correlation, APA ($r = 0.543$, $p = 0.01$), was negligibly weaker than the third strongest correlation, it was also included in the next phase of analysis for the sake of completeness. Moreover, given the only modest improvement in the correlation with the addition of a fourth spot sample in the APAP condition (and yet considerable further participant burden involved in the collection of additional samples), we decided not to pursue further analyses with the APAP condition. The three regression equations considered (PAP, 3PM, APA – **Equations 5-7** respectively) are listed below, where Q is the average of the spot samples in mmol.

$$\text{PAP: } 24 \text{ - hour urine sodium sample} = 1.301Q + 61.435 \quad (5)$$

$$\text{3PM: } 24 \text{ - hour urine sodium sample} = 0.998Q + 67.709 \quad (6)$$

$$\text{APA: } 24 \text{ - hour urine sodium sample} = 1.015Q + 72.213 \quad (7)$$

The means \pm SE for the corrected averages are listed in **Table 10**. None of the corrected averages were found to be significantly different compared to flame photometry ($p > 0.96$).

Table 10 Regression-corrected spot sample averages

Method (n=21)	Mean±SE
Flame photometry (mmol/day)	124.6±9.4
PAP (mmol/day) n=20	124.3±5.9
3PM (mmol/day)	125.0±5.1
APA (mmol) n=20	124.5±5.3

We can also see, through the use of box plots, the comparison of the spread of data between the corrected spot samples and flame photometry (shown below in **Figure 20**) is similar, as the correction factor is able to preserve the original distribution. This figure also demonstrates how the distribution of these samples mimics the gold standard flame photometry values.

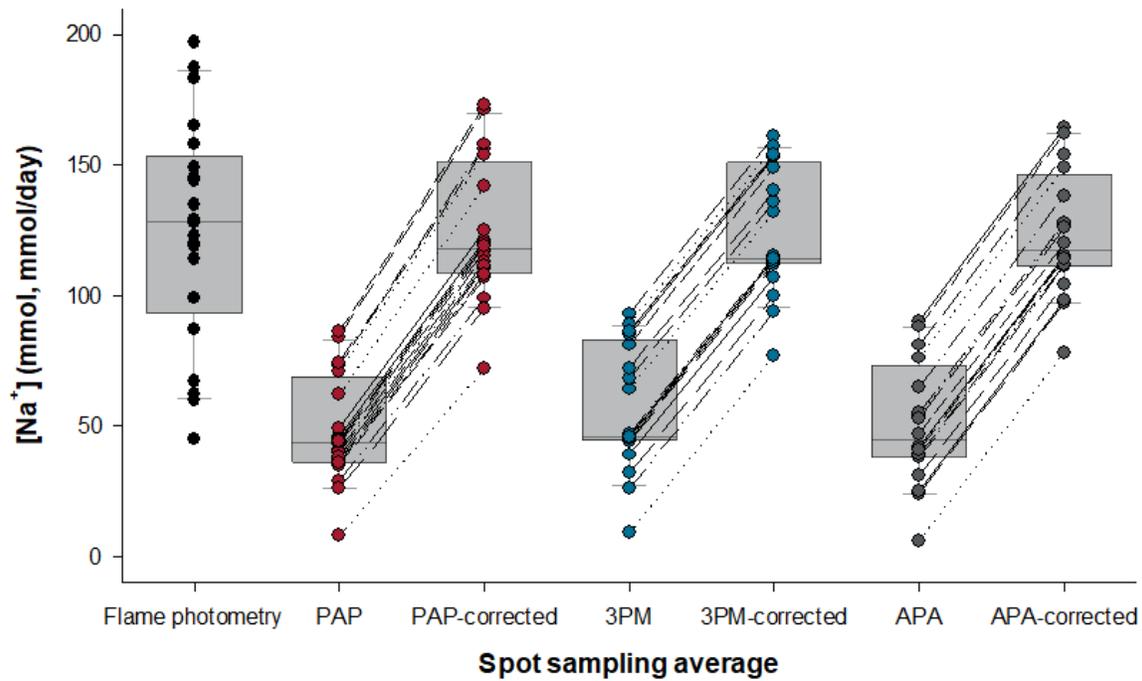


Figure 20 Box plots showing the spread of the original and regression corrected spot samples compared to flame photometry

Box plots showing the un-corrected sampling average from the top correlations compared to flame photometry and the new regression-corrected values from these sample spot sampling averages. This figure shows that the distribution of the spot samples after being corrected is able to preserve the original shape compared to flame photometry. All of these regression-corrected sampling averages were significantly correlated with flame photometry ($p < 0.01$).

After applying the regression equations, the PAP average was determined to have the strongest correlation with the gold standard ($r = 0.605$, $p = 0.005$). The correlations for the 3PM ($r = 0.546$, $p = 0.01$) and the APA ($r = 0.539$, $p = 0.01$) averages were also statistically significant; however, were outperformed by the PAP average. The correlations between

the three averages with flame photometry before and after the regression equations (**Equations 5-7**) are applied can be seen in **Figure 21**.

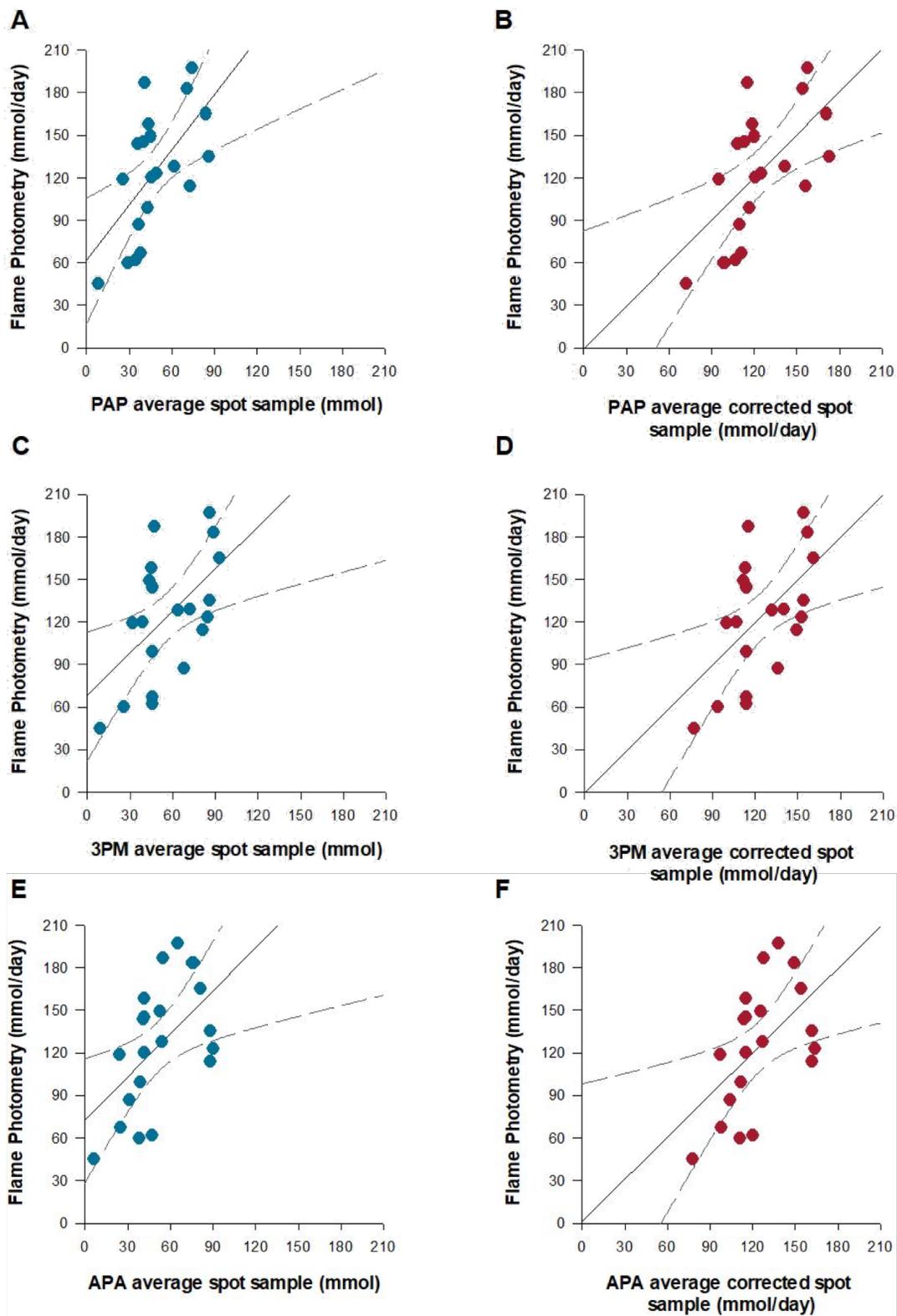


Figure 21 Correlations between spot sample averages (both in mmol and regression corrected) compared to flame photometry
 A-B) Show the PAP averages, C-D) show the 3PM averages and E-F) show the APA averages compared to flame photometry before and after Equations 5-7, respectively, are applied.

As can be seen, the corrected spot sample measurements are better correlated with flame photometry, rectifying the vertical deviation present. Further analysis was done using Bland-Altman plots (**Figure 22**) showing, once again, the PAP corrected averages produce the most accurate results. The PAP average produced a bias of -0.05 ± 35.0 mmol/day with random scatter around zero. Of note, the lower and upper limits of agreement (-68.7 and 68.8 mmol/day respectively) represent values below the 100-150 mmol/day increases expected to be seen in individuals who are salt loading for their syncope management.

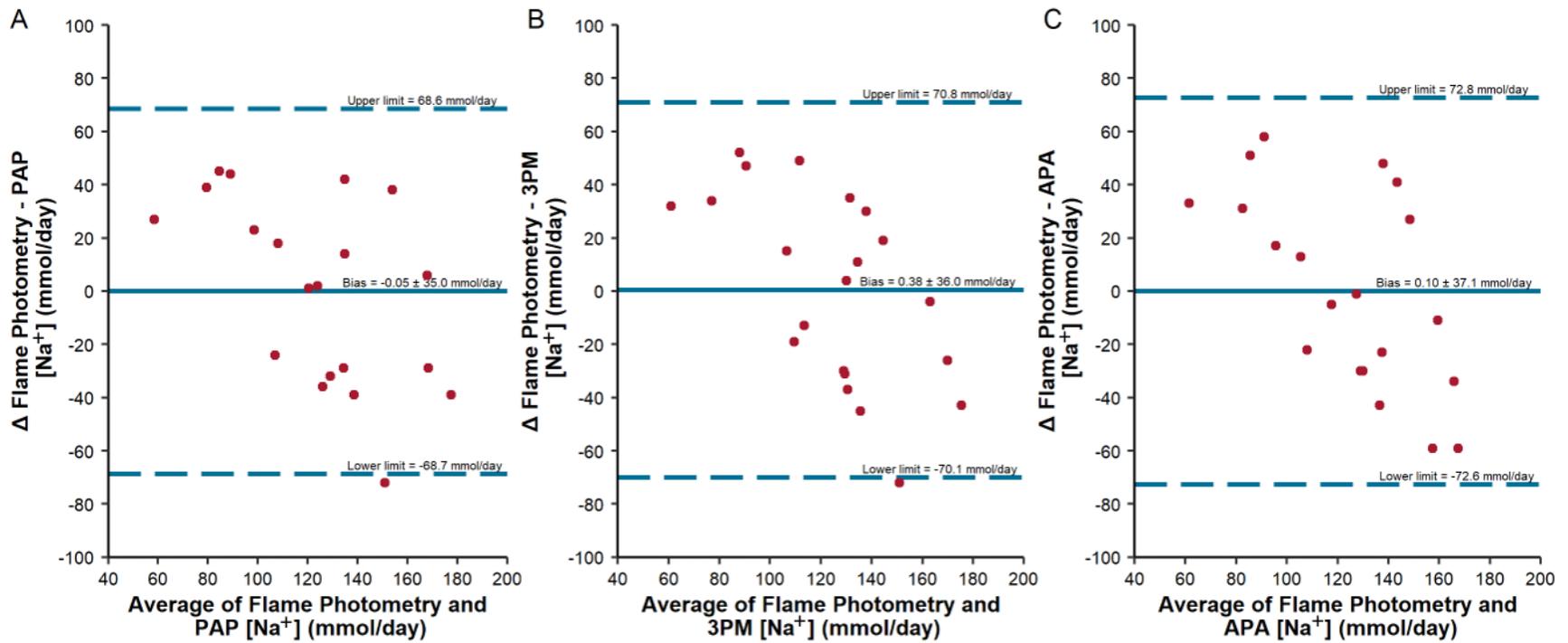


Figure 22 Bland-Altman comparing the corrected spot sample averages to flame photometry

A-C) show the corrected spot samples between PAP, 3PM, and APA averages compared to flame photometry. As can be seen, (A) or the PAP corrected average displays the smallest vertical bias with minimal indication of a systematic bias that appears to be present in (B-C).

Equivalency testing was also performed between the corrected samples and flame photometry to help determine the criterion validity of the two measurements. The equivalency bands were again selected at 15% of the gold standard (19 mmol/day). The PAP corrected averages were found to be significantly equivalent within the 15% bounds ($p=0.01$) as well as within 10.5% of the gold standard. The 3PM and APA averages were also within the 15% bounds ($p=0.01$ and $p=0.02$ respectively), with 3PM within 11.2% and APA within 11.6% of the gold standard. The equivalency plot is shown below in **Figure 23** with equivalency bands at both 15% and 10% indicated. The PAP corrected average marginally outperformed the other sampling options.

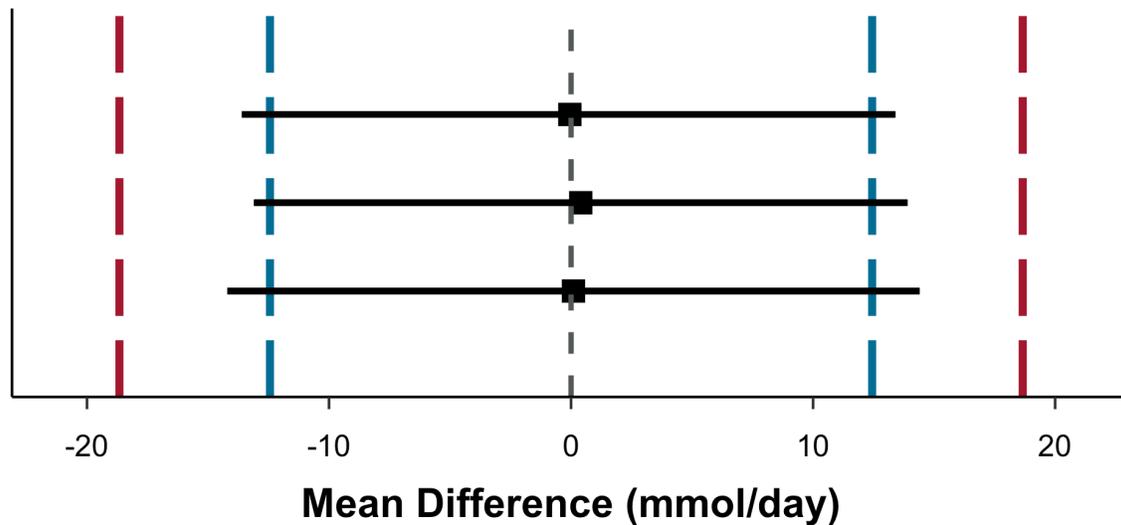


Figure 23 Equivalency plot showing the mean difference for the corrected spot samples compared to flame photometry

Equivalency plot with bands at 15% (red dashed lines) and 10% (blue dashed lines) showing the mean difference for the PAP (top), 3PM (middle), and APA (bottom) corrected spot sample averages compared to the gold standard – flame photometry.

The results of this study have consistently demonstrated the ability for PAP corrected spot sample averages to provide a reasonable alternative to 24-hour urine samples for the determination of urine sodium levels. However, regression equations are traditionally created and then further tested by application to prospective data. The application to prospective data using this sample was explored, despite the smaller sample sizes it created, with a regression line being derived from the first 10 participants and applied prospectively to the second set of 10 participants, to further explore the validity of this approach. The results for the initial participants (initial) and the second set of participants (prospective) are shown in **Table 11**.

Table 11 **PAP average and flame photometry initial and prospective values**

Method (n=20)	Mean±SE
PAP-initial (mmol)	56.9±6.8
PAP-prospective (mmol)	39.8±5.0
PAP-initial corrected (mmol/day)	124.8±7.3
PAP-prospective corrected (mmol/day)	106.3±5.4
Flame photometry-initial (mmol/day)	124.6±10.6
Flame photometry-prospective (mmol/day)	124.1±17.2

Mean ± standard error data for prospective analysis from protocol two.

The regression equation created from the PAP-initial data set is found in **Equation 8** below and was used to create the PAP-prospective corrected values.

$$24 - \text{hour urine sodium sample} = 1.069Q + 63.763 \quad (8)$$

After this equation was applied, the PAP-prospective corrected data were compared to the flame photometry-prospective data using the same statistical approach described above. It was found that the two prospective data sets were significantly correlated ($r=0.752$, $p=0.01$) and there were no statistical differences between the two measurement methods ($p=0.223$). A bias was observed in the Bland-Altman plot (**Figure 24**) for these two measurements, with both vertical deviation as well as a hint of systematic bias. However, it should be noted that with such a small sample size for the creation of the regression equation and the application to prospective data, observing a large variance and possible bias is not surprising.

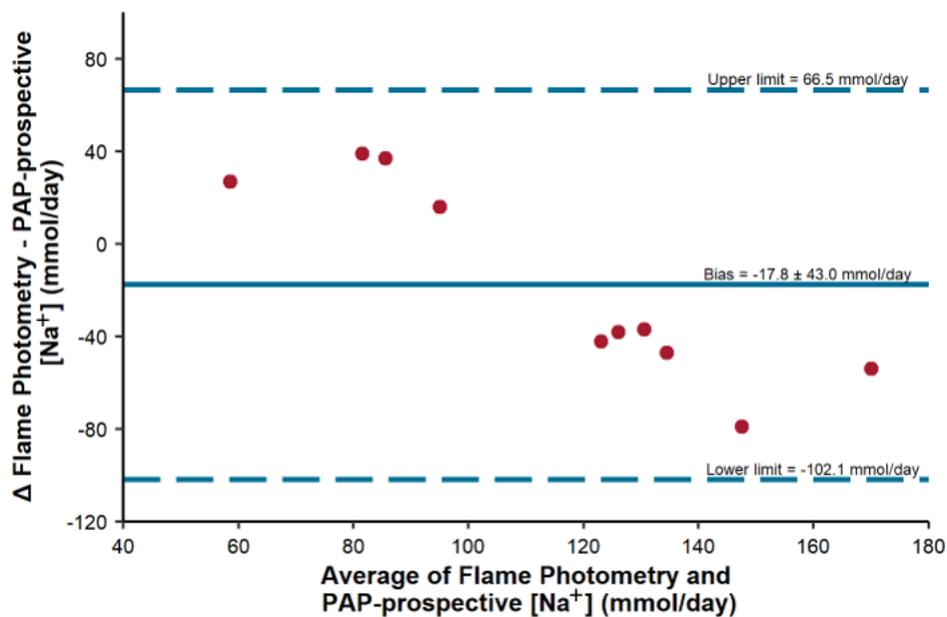


Figure 24 Bland-Altman plot comparing the PAP-prospective corrected values to flame photometry

Bland-Altman plot comparing the prospective values that have been regression corrected according to the PAP sampling average compared to flame photometry.

Furthermore, the PAP-prospective corrected data was found to be equivalent to the flame photometry-prospective values to within 35% of the gold standard ($p=0.05$), despite the large variance and small sample size. Based on the ability for this equation to perform prospectively with so many limiting factors, it was decided that the regression equation based on the full protocol two sample size (**Equation 5**) should be used.

4.3.5 Model validation for use with time-irrelevant spot urine samples

4.3.5.1 Removal of the effect of time

It is recognized that it may not always be convenient for individuals to provide evening spot samples, or samples collected over consecutive days, and so a stronger model, made using normalized spot sample values, was investigated in order to create an equation that could be used, regardless of the sample collection time. To do this, all spot samples for all participants, irrespective of time collected, were regressed against the flame photometry values. The residuals from this regression equation (**Equation 9**) were added to the intercept in order to create normalized spot sample values that were devoid of the timing effects of the initial raw spot samples.

$$24\text{ - hour urine sodium sample} = 11.54Q + 50.30 \quad (9)$$

The residual plot (**Figure 25**) for this model shows the large random distribution in the residuals, with no indication of bias. It should be noted that there are only two x-axis values as the x-variable is a categorical variable with time of day (morning or evening) as possible values.

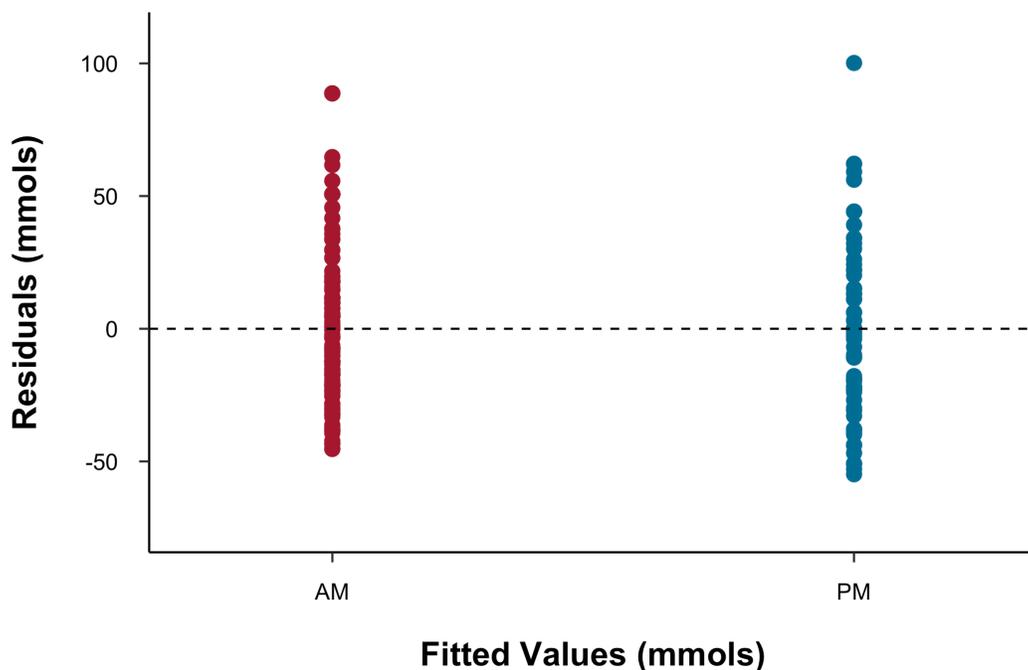


Figure 25 Residual plot for the regression of raw spot sample values compared to the time the spot sample was taken

This figure demonstrates the distribution of the residuals for the model which compares the raw spot sample values in millimoles to the time categorical variable (divided into morning [red] and evening [blue] samples).

The raw spot samples are also plotted against the gold standard, flame photometry, in **Figure 26A**. As can be seen, the morning samples tended to be lower in urine sodium concentration in comparison to the evening samples ($p=0.02$). This is corrected in **Figure 26B**, which correlates the normalized spot samples with flame photometry. We can use this model to determine the coefficient of variance of the typical error by taking the standard deviation of the residuals (30.37 mmol) and dividing it by the mean of the normalized spot samples (50.15 mmol), resulting in coefficient of variance of 0.61.

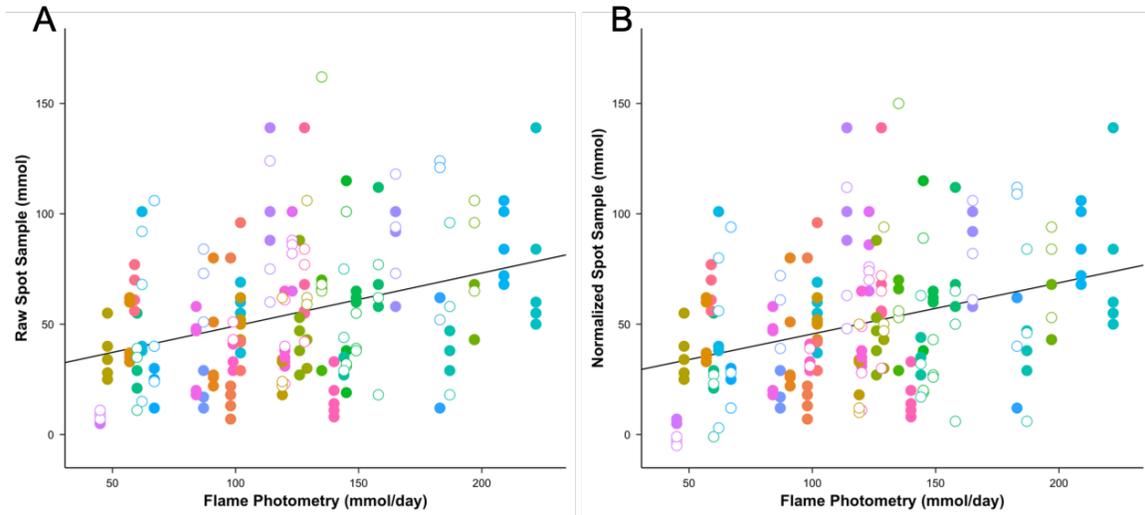


Figure 26 Raw and normalized spot samples compared to flame photometry
 A) Shows the raw spot samples, colour coded for each participant, including both morning (filled in circles) and evening (open circles) samples and B) shows the normalized spot sample values, colour coded by each participant, including both morning (filled in circles) and evening (open circles), compared to flame photometry.

Using the normalized spot sample values, we can establish another method to determine the number of spot samples required to get a suitably accurate 24-hour urine sodium surrogate. A table was created including the mean average of one spot sample through to the mean average of six spot samples (as this was the largest number of spot samples a participant completed), as well as their SD and SE (**Table 12**).

Table 12 Descriptive statistics for the normalized spot sample averages

	Average of one (mmol)	Average of two (mmol)	Average of three (mmol)	Average of four (mmol)	Average of five (mmol)	Average of six (mmol)
<i>n</i>	34	34	34	34	34	21
<i>Mean</i>	49	50	50	50	51	50
<i>SD</i>	28	25	23	23	21	22
<i>SE</i>	4.8	4.3	4.0	3.9	3.6	4.8

The standard deviation (SD) and standard error (SE) can be seen to level off at an average of three spot samples. No significant differences were found between any of the groups.

It was found that the mean of the normalized averages, the SD, and the SE tends to level off at three spot samples (SE of 4.0 compared to SE of 4.3 for an average of two spot samples). Here the values begin to plateau with further samples added, indicating that only three spot samples are required, providing confirmation of the findings from protocol one of this study.

4.3.5.2 Creation of a time-independent regression equation

The entire range of spot samples for each participant was used to calculate their normalized average spot sample concentration, resulting in one average spot sample value per participant. These normalized averages were regressed against the flame photometry values in order to create a regression equation that can be used to correct spot samples to represent 24-hour sample values. **Figure 27A** shows the regression of the normalized averages for each participant compared to flame photometry and **Figure 27B** shows the residual versus the fitted values for this model. This model resulted in **Equation 10**, which can be used to convert spot samples into 24-hour urine sodium values.

$$24\text{-hour urine sodium sample} = 1.172Q + 60.438 \quad (10)$$

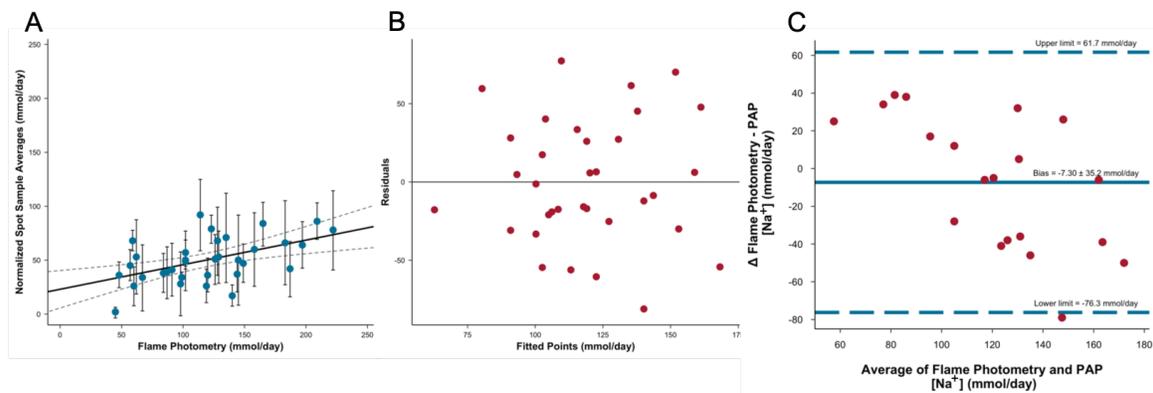


Figure 27 Correlation, residuals, and Bland-Altman plots for the time-normalized model

A) Shows the correlation between the normalized average spot samples, and their standard deviation, compared to flame photometry. B) Shows the residual plot for Equation 10 comparing the residuals against the fitted values. A random distribution with no bias can be seen from this plot. C) Shows a Bland-Altman plot looking at the corrected evening, morning, evening (PAP) spot sampling average compared to flame photometry.

The correlations, Bland-Altman biases and limits of agreement (the PAP Bland-Altman plot can be seen in **Figure 27C**), and TOST equivalency values can be found in **Table 13**. As can be seen, this equation corrects spot samples to reflect 24-hour urine sodium with less dependence on the time of day at which the samples were collected (including single spot samples which will be discussed in the next section).

Table 13 Statistical analysis for various spot sampling averages

	3AM	3PM	PAP	APA
<i>n</i>	34	21	21	21
<i>Correlation with flame photometry</i>	r=0.40, p=0.019	r=0.55, p=0.01	r=0.6, p=0.005	r=0.54, p=0.013
<i>Bland-Altman (mmol/day)</i>	Bias: -3.88±44.1 Limits: -90.4, 82.6	Bias: 2.48±36.1 Limits: -68.3, 73.2	Bias: -7.30±35.2 Limits: -76.3, 61.7	Bias: -3.55±37.1 Limits: -76.3, 69.2
<i>TOST equivalency</i>	14.2%	13.1%	16.8%	14.5%

Correlations, Bland-Altman biases and limits of agreement, and equivalency testing percentages in mmol/day for various sampling averages made up of different time combinations. As can be seen, the time-normalized model is able to adequately deal with the effects of time, producing similar results regardless of the sampling approach utilized.

Here we use combinations of three spot samples because our earlier analyses showed that three samples provided a small improvement in the limits of agreement between the corrected values and the gold standard. Of note, incorporating an evening sample appears to further minimize the limits of agreement, providing a more accurate surrogate, likely due to the physiological nature of not being in a fasted state at the time of sample collection.

4.3.5.3 Single use spot samples compared to flame photometry analyzed 24-hour urine collection

It was found that three spot samples consisting of two evening and one morning sample provides the most accurate alternative to the gold standard of flame photometry; however, it is expected that not everyone will have the ability to complete all three samples. Accordingly, we also investigated the accuracy of a single spot sample in comparison to flame photometry. The morning spot sample was selected as the morning sample following the middle day, as this was the morning spot sample included in the 24-hour urine collection and in the PAP spot sampling average. The evening spot sample was selected as the evening sample from the middle day, as this is the evening sample included in the 24-hour urine collection and the first evening sample in the PAP spot sampling average. These data are shown in **Table 14**, including the single spot sample corrected averages once the time-normalized equation has been applied

Table 14 Comparison of single morning and evening spot samples

Method (n=33)	Mean±SE
Flame photometry (mmol/day)	119.0±8.3
Flame photometry (mmol/day) <i>n=21</i>	124.6±9.4
AM spot sample (mmol)	49.9±5.1*
PM spot sample (mmol) <i>n=21</i>	65.2±8.2*
AM corrected spot sample (mmol/day)	118.9±5.96
PM corrected spot sample (mmol/day) <i>n=21</i>	136.9±9.60

*denotes significant different from flame photometry, $p < 0.05$

The uncorrected single morning spot sample was significantly correlated with flame photometry ($r=0.475$, $p=0.005$), as was a single uncorrected evening spot sample ($r=0.497$, $p=0.02$). After applying the correction equation (**Equation 10**), the morning and evening samples were still significantly correlated ($r=0.47$, $p=0.005$ and $r=0.50$, $p=0.02$ respectively); these correlations can be seen below in **Figure 28**.

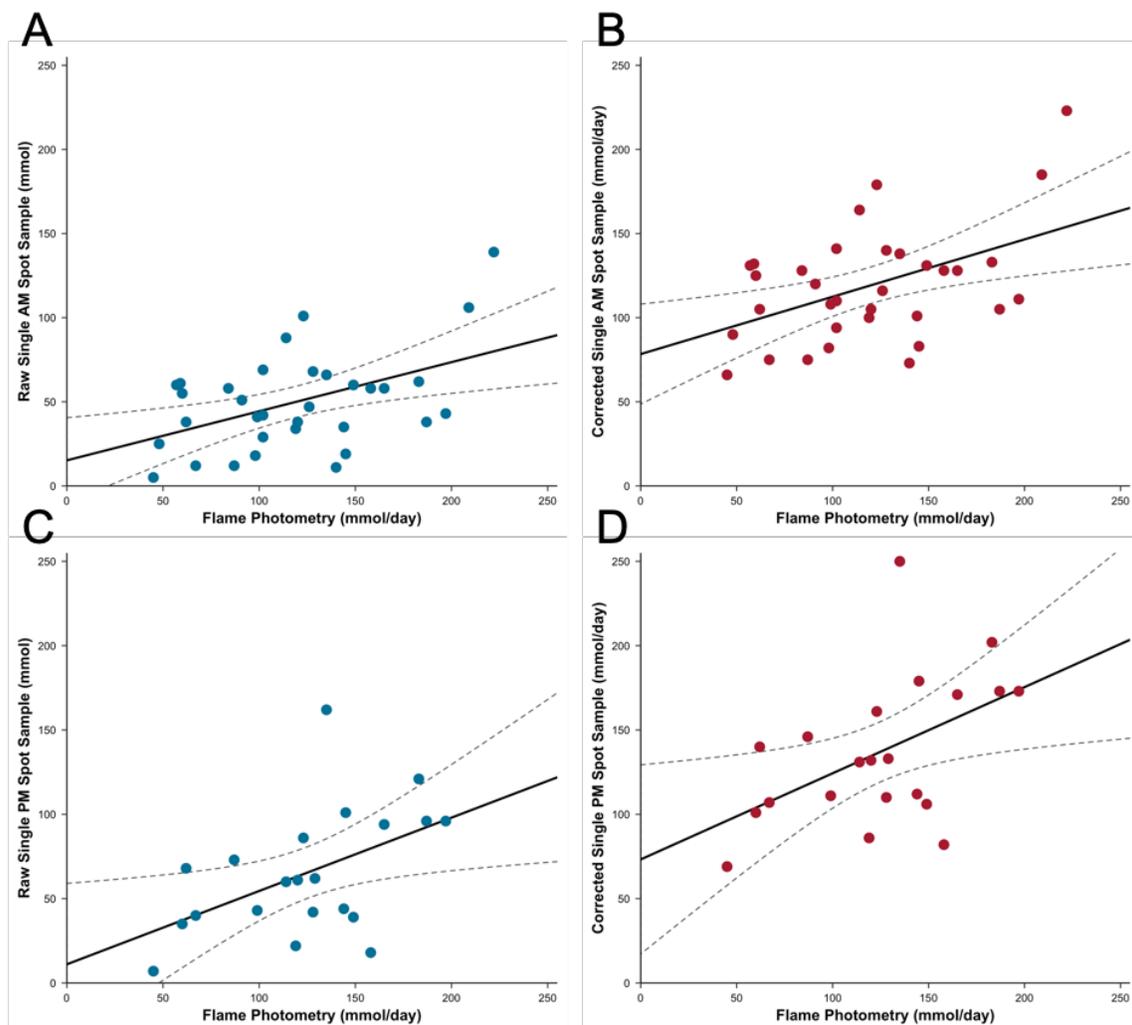


Figure 28 Correlations between single spot samples (both raw and regression corrected) compared to flame photometry

A) Shows the correlation between a raw single morning spot sample compared to flame photometry. B) Shows the same correlation except with corrected (Equation 10) single morning spot sample values. C) Shows the correlation between a raw single evening spot sample against the gold standard flame photometry. D) Shows the same single evening spot sample values except after they have been regression corrected (Equation 10).

Bland-Altman plots were produced for all four comparisons; however, a systematic bias (over-estimation at lower concentrations and under-estimation at higher concentrations) occurred in the uncorrected samples. Further analysis was considered only on the single spot sample corrected values. The Bland-Altman plots for these corrected samples are shown in **Figure 29**. The bias for the morning spot sample was -0.06 ± 43.5 mmol/day whereas the bias for the evening spot sample compared to flame photometry was 12.3 ± 43.5 mmol/day.

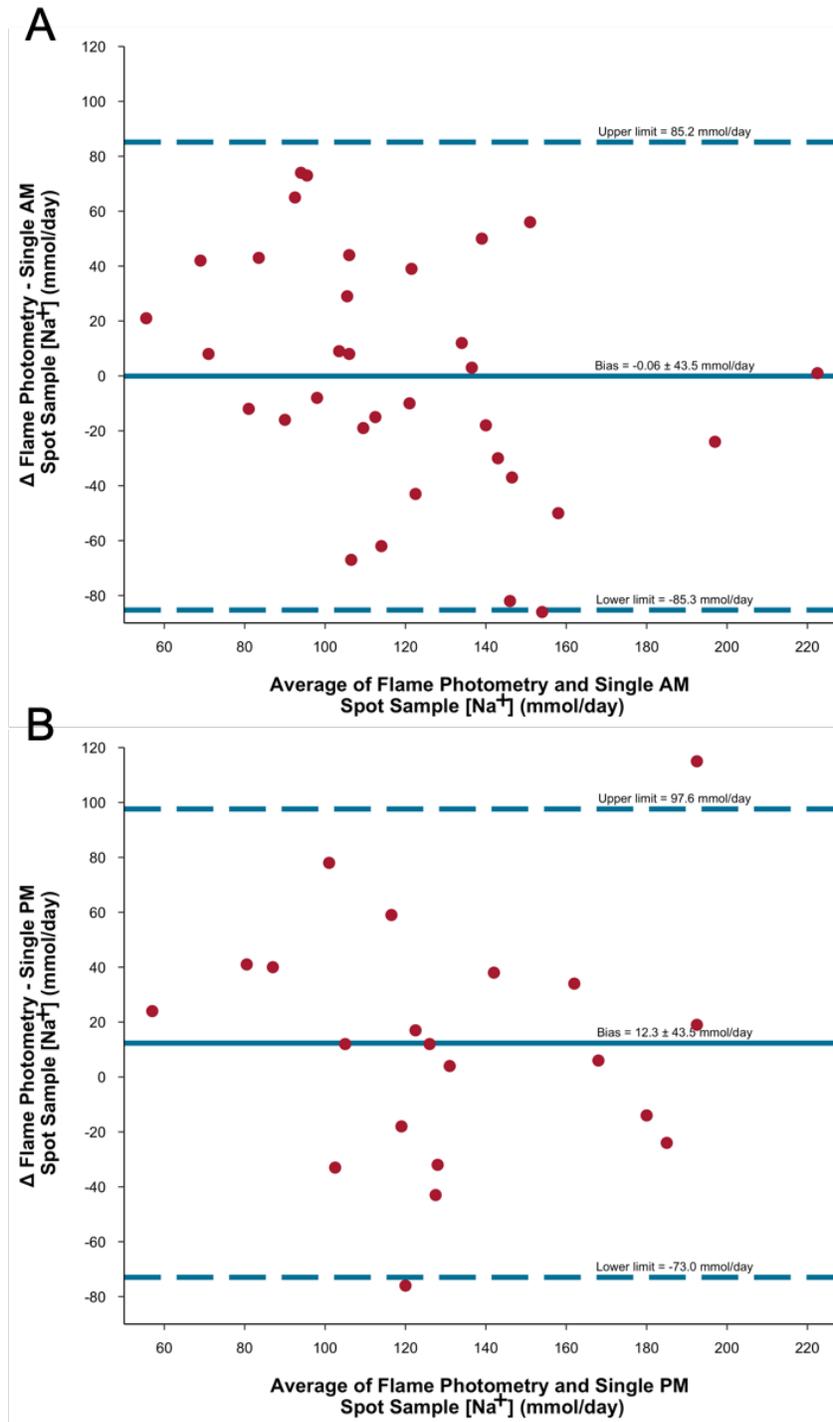


Figure 29 Bland-Altman plots of single use spot samples compared to flame photometry

A) Shows the bias and limits of agreement between a corrected (Equation 10) single morning sample and the gold standard. B) Shows the bias and limits of agreement between a corrected (Equation 10) single evening sample and flame photometry.

Equivalency testing was also conducted on the single use spot samples compared to flame photometry. It was found that a single morning spot sample is equivalent to within 11% of the gold standard, whereas a single evening spot sample is equivalent to within 23% of the gold standard. However, it is possible that the larger variance in the evening sample stems from a lower sample size, as the evening spot samples produce a Bland-Altman plot indicating no systematic bias and were more strongly correlated with the gold standard.

4.4 Discussion

4.4.1 Quantab chloride test strips in comparison to flame photometry

Quantab chloride test strips proved to be an accurate alternative to the gold standard in urine sodium analysis, flame photometry. This is an important finding in the syncope community as it allows individuals with syncope to be able to measure their urine sodium levels at home, without having to send the samples away to a laboratory. Individuals with syncope are encouraged to increase their salt intake as a way to manage their symptoms; however, many patients are unaware of how much sodium is required to be effective, and struggle to increase their sodium levels sufficiently while maintaining a healthy diet. Salt supplementation of approximately six grams on top of normal dietary intake, about a 100 mmol/day increase in urine sodium, is recommended for the treatment of symptoms of syncope (47,51,59,60). The accuracy of the Quantab assessments compared to the gold standard of flame photometry is clearly sufficient to allow patients to track changes in urine sodium of this magnitude from a 24-hour sample conducted at home.

It should be noted that, because of the exponential relationship between the Quantab chloride measurements and the sodium concentration in mmol, we found a modest increase in the accuracy of Quantab estimates of 24-hour urine sodium compared to flame photometry when any Quantab values at or above seven Quantab units were diluted by 50% and re-tested. This approach strengthened the correlation between the Quantab assessment and flame photometry, with a smaller SD and limits of agreement. However, this improvement in accuracy was small, and the standard Quantab approach was highly correlated with the gold standard ($r=0.902$, $p<0.0001$) with small bias and limits of agreement and high equivalency. Accordingly, we suggest that both the standard and

corrected Quantab measurement approaches can be used with confidence to conveniently determine 24-hour urine sodium concentration at home.

Currently, without a way to conveniently test sodium levels on a frequent basis, most patients are unaware as to whether they are getting the required amount of sodium, particularly since this is so hard to do while eating a healthy diet. Being able to use chloride test strips at home can help these individuals better understand how they are proceeding with their management options.

4.4.2 Utilizing spot samples compared to a 24-hour urine sample

Having patients conduct a 24-hour urine sample every time they need to determine their sodium levels is not only tedious, but quite challenging, especially in pediatric populations. This study has shown that multiple spot samples may be used as an alternative to 24-hour urine sampling. Protocol one and the normalized spot sample table (**Table 12**) investigated the number of spot samples needed to obtain an accurate representation of a 24-hour urine sodium sample. For protocol one, there were no differences found between using five, four, or three spot samples, and so three spot samples were chosen, as this is the most convenient option for patients and is in accordance with previous reports recommending three spot samples (89,124). Furthermore, the time-normalized spot sample averages found the SD and SE in the average variance plateaued at three spot samples, concluding that an average of three spot samples should be used as a surrogate to 24-hour urine samples.

Protocol two made use of averages created from three spot samples including both morning and evening samples. Various combinations of these samples were then created and correlated with flame photometry. Three averages emerged as the strongest correlates with the gold standard: PAP, 3PM, and APA. Since spot samples are generally lower in concentration than a 24-hour urine sample, a correction factor needs to be applied in order to convert the spot samples to 24-hour sample concentrations in mmol/day. Multiple correction factors were created based on regression data and combined with their spot sample average to determine which correction provided the most accurate results.

The regression equations, Bland-Altman plots, and equivalency testing all indicate that the PAP average, created from the middle evening sample and following day morning

and evening samples, consistently resulted in a more accurate alternative to 24-hour urine sodium sampling than the other spot sampling approaches considered. The correlation between the PAP average and the gold standard was the strongest out of these spot sample approaches, in addition to having minimal systematic bias (likely due to small sample sizes and fewer individuals with lower levels of urine sodium) and being the most equivalent. Should individuals be able to collect an evening sample followed by a morning and another evening sample, the PAP regression equation (**Equation 5**) gave the strongest method of identifying those with low sodium intake who are most likely to benefit from salt supplementation, and determining compliance with the expected 100 mmol/day increases for individuals with syncope undergoing sodium supplementation.

Protocol Two was also used to determine if the equation could be applied prospectively. Regression equations are generally created from a data set and prospectively applied to further values, which are then correlated with a gold standard in the case of determining criterion validity. Due to the smaller sample size of this study, when separating the data and creating a regression equation from the initial set, the application of the equation will likely produce large variances and less accurate results. While this approach is not suggested for such a small sample (the data in each group would be halved), it was completed during this study as an additional test to determine if the correction equation could be applied prospectively, even with such limiting factors. It was found that the regression equation created from only the first 10 participants and prospectively applied to the second set of 10 participants enabled the prospective data to still be significantly correlated with the gold standard, with no detected differences ($p=0.223$). The Bland-Altman did appear to have a trend towards a systematic bias; however, with such a small sample size, it is hard to accurately evaluate. Furthermore, the regression equation is likely to be less accurate when applied to a smaller sample. Nevertheless, the equivalency plot indicated that the prospective data was still accurate to within 35% of the gold standard. Considering the good performance of this smaller sample size regression equation, and the minimal differences between the two equations (**Equation 5** and **Equation 8**), it was decided that the original equation, **Equation 5**, would be recommended for use over that of the prospective equation (**Equation 8**), if individuals are able to complete the required PAP sampling approach. This equation was created from a larger sample size and, therefore, more likely to be representative of a larger population and thus more accurate.

While this study recommends the use of a three-spot sample average to obtain the most accurate results compared to a 24-hour urine sample, obtaining spot samples over multiple days may not always be possible. Because of this, we investigated the accuracy of a different model which removes the effect of the time of day the spot samples were taken (**Equation 10**). This equation was created from normalized spot samples and was shown to be suitable for conversion of various combinations of spot samples, including single spot samples, from both morning and evening samples. While we still recommend the use of an evening, morning, evening (PAP) spot sample average, where possible, this equation makes any combination of spot samples feasible to be used to determine the 24-hour urine sodium value. Corrected single spot samples (both morning and evening) were significantly correlated with flame photometry, although this correlation was weaker than the recommended three sample approach. The Bland-Altman values for the single spot sample approach (**Figure 29**), indicate no evidence of a systematic bias when using this regression model. It should be noted that using a single spot sample results in much larger variance, as shown by the larger limits of agreement seen in the single spot sample Bland-Altman plots (**Figure 29**). In addition, equivalency testing indicated a single morning spot sample to be more equivalent (within 11%) than the single evening spot sample with the gold standard; however, the morning spot sample had a weaker correlation, and larger limits of agreement. It is likely that the stronger equivalency of the morning spot sample reflects the increased sample size for this measure, resulting in reduced variance and smaller equivalency bounds. If multi-day spot testing is not feasible, it is possible that a single spot sample may be used as an alternative to 24-hour sampling; however, a single evening sample will likely give a more accurate estimate compared to a morning sample. It is our recommendation that the time-normalized equation (**Equation 10**) be the recommended approach, particularly if the PAP spot sampling average was not obtained.

4.4.3 Considerations for at-home testing

In regards to at-home testing, because of the exponential relationship between the Quantab chloride concentration (a.u.) and the converted sodium concentration (mmol), the 24-hour urine samples were diluted in the laboratory if the Quantab chloride test strip value exceeded seven Quantab units. Distilled water was used for these dilutions so as not to introduce any contaminants into the samples. As shown in the comparison of Quantab chloride test strips to flame photometry, the re-dipping of the Quantab strips after

dilution of highly concentrated samples helped to modestly increase the accuracy of the measurement method. In this study, we did not ask participants to dilute their samples when evaluating spot samples at home, and so the agreement between the spot samples and the 24-hour urine samples investigated are that of the original undiluted sample results. Despite not diluting the samples, the results indicate that there is still good agreement between the two methods; however, should individuals feel confident and have the ability to dilute any samples with initial concentrations over seven Quantab units, they should proceed to do so in order to further increase the accuracy of the results.

Another consideration is that not everyone may have access to distilled water for dilution of concentrated samples when they are completing at-home testing. Using tap water to dilute samples would be a feasible option, but tap water may have added chloride ions. This has the potential to skew the results, given that the Quantab test strips actually measure chloride ions, which we are using as a proxy for sodium concentration. It should be noted that local chlorine-treated tap water does not contain a high enough chloride ion concentration to even register on the Quantab chloride test strip, and so would not affect the urine concentration, rendering tap water usable for at-home dilutions if this approach were adopted. An at-home conversion chart has been created for distribution and can be found in **Appendix C**.

4.5 Limitations

There are several possible limitations to this study. The primary limitation is that the technique evaluated, use of chloride test strips, measures urine chloride ion concentration, and is not a direct measure of sodium. This may reduce their accuracy in capturing the sodium content; however, sodium and chloride are found in a one-to-one ratio in dietary salt (the substance being measured) and chloride test strips have been shown to be a suitable proxy for measuring sodium concentrations (92,94,123). In our study, the use of the test strips to evaluate 24-hour urine sodium compared extremely highly with the gold standard of flame photometry, with minimal bias, high equivalency, similar means, and a strong correlation. Accordingly, we conclude that the use of chloride test strips as a proxy for urine sodium is valid.

Secondly, the conversion between the chloride measurements in Quantab units (a.u.) and the sodium concentration in mmol is an exponential or polynomial shaped curve,

meaning that as sodium concentrations are higher, accuracy is reduced. This was accounted for when evaluating the 24-hour urine collection by diluting all samples by 50% that were in excess of seven Quantab units, to place the concentration on the shallower part of the curve (and then multiplying the result by two). This approach produced a modest increase in the precision, and reduction in the variance. However, given that this improvement was small compared to the undiluted samples, and the high performance of the undiluted samples as a proxy measure for urine sodium, we conclude that dilution is beneficial, but not necessary, for at-home determination of urine sodium concentration using chloride test strips.

Furthermore, we placed the chloride test strips in vacuum sealed packages for ease of participant use, but this causes them to be removed from their storage bottles for a period of time prior to being used, possibly reducing the accuracy of the test strips themselves. Prior to undertaking this study, we placed test strips in a series of environmental conditions (sunlight, a regular plastic bag, heat, vacuum sealed) and compared their results to those from strips kept in the correct storage container for a series of known concentrations of sodium chloride. The strips exposed to the various environmental conditions were found to be just as accurate as those properly stored, regardless of the environmental condition applied. Accordingly, we do not believe that the use of vacuum sealed strips affected the accuracy of the strips themselves.

In a study such as this, where the onus for the majority of data collection is on the participant, it is extremely difficult to ensure that the protocol is completely standardized, and more importantly, that the protocol is even followed. This likely resulted in much larger variance in the data, meaning the relationships between the measurement methods tested may in fact be an underestimate. The inability to enforce a standardized protocol was viewed as a positive aspect of the study, as it more closely mimics the real-life conditions in which the strips would be used.

We tested a healthy control population to validate this technique, and as such would expect their 24-hour urine sodium samples to be higher than those generally found in a syncope population, particularly a pediatric population (as children are generally advised to eat a low salt diet). As can be seen in the results section, individuals with higher urine sodium concentrations tend to have more variance. Being able to validate this test in the targeted patient population, while ideal, was seen as causing unnecessary

discomfort to an already burdened population, and so healthy controls were utilized, possibly increasing the variance in the results. However, some of our participants did in fact have very low sodium concentrations, enabling validation over the full physiological range of urine sodium concentrations, with high equivalency to the gold standard. The fact that some of the participants had high urinary sodium levels is actually an asset, as they are closer to the values one would expect in children and adults with syncope who are successfully salt loading.

Sample size is often a factor in studies where there is large variance. The large variance and smaller sample size observed in protocol two compared to those of the full group data used in the comparison of chloride test strips to flame photometry, may have accounted for the larger variance seen during the equivalency testing. With increased sample sizes there is a reduction in variance, resulting in smaller equivalence bounds compared to the gold standard. Despite the smaller sample size, equivalence was still observed within reasonable boundaries and both the use of Quantab chloride test strips and the use of spot samples were found to be reasonable alternatives to 24-hour urine sodium samples analyzed through flame photometry.

4.6 Conclusions

This study looked at the criterion validity between Quantab chloride test strips and the gold standard in 24-hour urine sodium analysis, flame photometry. It was found that the test strips provided an accurate alternative to the gold standard. The use of multiple spot samples provided a more comfortable alternative to 24-hour urine sampling and should consist of three spot samples, preferably made up of consecutive evening, morning, and another evening spot samples, averaged and corrected by a regression equation (**Equation 10**). If this is not feasible, an alternate number of spot samples or even a single spot sample can be used; however, results are reduced in accuracy compared to the use of multiple spot samples. The validation of chloride test strips and multiple spot samples as an alternative to 24-hour urine sodium sampling analyzed by flame photometry provides a comfortable, at-home option for individuals with syncope to help manage their disorder, providing estimates of their approximate 24-hour urine sodium levels to guide dietary management. While this would be beneficial for individuals to conveniently estimate 24-hour urine sodium concentrations, and the impact of dietary sodium modifications, we acknowledge that the measurement of urine sodium using this

approach is less accurate than the gold standard of 24-hour urine collections analyzed using flame photometry. In clinical decision-making where a precise measurement of urine sodium is required, the gold standard approach should be used.

Chapter 5.

Final Discussion

The quality of life for individuals with syncope is extremely poor (19,23) and it is important to uncover ways that might reduce the burden of their disorder for these patients, especially those in the pediatric population in whom syncope is particularly common (16,17). A first step to improving quality of life lies in improving diagnosis. This thesis has validated different diagnostic tools for use in children with syncope compared to their respective gold standards to aid the diagnosis and management of these disorders, particularly in pediatric populations. The favourable results will improve the quality of life for these individuals.

5.1 Validation of finger blood pressure in children

Intra-arterial catheters are currently considered the gold standard in continuous blood pressure monitoring in children (65,66); however, this approach is unsuitable for use during a tilt test, a standard diagnostic tool for the evaluation of patients with syncope, because it interferes with the reflexes that help maintain orthostatic cardiovascular control (50). This has made it difficult to safely and properly diagnose children with syncope disorders, highlighting the need for an alternative non-invasive method of continuous blood pressure monitoring. Finger plethysmography with volume clamping has been validated for non-invasive beat-to-beat blood pressure monitoring in adults (70,71,82), but prior to this thesis, had not been validated for use in children.

The first experimental chapter of this thesis validated the use of finger plethysmography with pediatric cuffs for use in children, allowing the gold standard in autonomic testing, a tilt test, to be utilized in future investigations regarding the diagnosis of pediatric syncope. This will enhance the diagnosis of syncope and other autonomic disorders in children. This is of particular importance as it has been shown that individuals tend to improve once a diagnosis has been confirmed – whether this is through psychological effects, efficient management strategies, or an alternate reason is unknown (10). This thesis found that finger plethysmography in children is most accurate when used on the middle finger and that the values obtained were well within the guidelines outlined by AAMI criteria (104).

While the numerical agreement between these two measurements was good, the morphology of the corrected waveform appears not to have been accurately transcribed. The shape of the waveform is often used in clinical settings to infer information about a patient's overall hemodynamic status. However, the morphology of the finger plethysmography waveform after applying the reconstructed algorithm appears unsatisfactory and is therefore not recommended for use in interpretation of state of care. This does not seem to be a fault in the device itself, as the raw values recorded from the finger are able to faithfully transcribe the intra-arterial waveform compared to the gold standard. Rather, it is the algorithm that is required in order to correct the vertical deviation of the values to within AAMI guidelines that causes the distortion. It is possible the inaccuracy of the waveform correction is due to the much higher vascular compliance in children, which may not be adequately accounted for in the current algorithm. This algorithm takes age, sex, height, and weight into account, but relies on assumptions of aortic compliance based on individuals aged 18 years and older and weighing more than 20 kg, which is of course not the case in pediatric patients. This study highlights that, while blood pressure values obtained using finger plethysmography in children are accurate, for adequate waveform transformation novel pediatric algorithms are necessary.

Having a method of continuous non-invasive blood pressure monitoring is useful not only in clinical, but also in research settings. As discussed, it can be used clinically in order to diagnose syncope or other autonomic disorders outside the scope of syncope, in addition to being used in out-patient populations. Children who require continuous hemodynamic monitoring, but do not require an intra-arterial catheter, now have an alternative to the invasive procedure. Furthermore, the use of tilt testing in the research setting allows for studies on diagnosis and management techniques for pediatric syncope populations to be pursued in the hopes of finding more efficient methods of alleviating symptoms for these individuals.

5.2 Urine sodium analysis

The accurate determination of 24-hour urine sodium provides useful information regarding the sodium intake of a patient. In the context of syncope, it can be used to detect if an individual has increased their salt levels enough to receive the benefits of salt loading compared to baseline, or even if salt supplementation might be a useful management option in the first place. However, many individuals find the task of a 24-hour urine

collection uncomfortable and difficult to achieve, particularly in pediatric populations. This thesis validated an alternative to this uncomfortable test, through the use of Quantab chloride test strips applied to multiple (three) spot urine samples. Chloride test strips combined with spot samples results in a quick, convenient, inexpensive and simple way to identify low urine sodium – a common characteristic of individuals with syncope disorders. This method can now be used to determine suitability for, and compliance with, salt supplementation, one of the few management options available to syncope patients.

In addition to the ability to now use chloride test strips in lieu of flame photometry, this study was able to validate the use of corrected spot samples as an alternative to a 24-hour urine sodium sample. Taking the average of an evening, a morning, and another evening spot sample, all completed consecutively, and applying a regression equation, provides a simplistic solution for individuals or even physicians to monitor approximate urine sodium levels. This method can be used for at-home monitoring, clinical, or research settings. Having a baseline measure of urine sodium is often a quite useful metric, but is frequently not collected due to the inconvenience of having to complete a 24-hour urine sample. The ability to use chloride test strips instead of flame photometry, in addition to removing the need to complete a 24-hour urine sample, opens a large avenue for urine sodium to be a more accessible metric that can be used in the diagnosis and management of those burdened with syncope disorders. In addition, it is possible that this assessment would also be of interest in other domains of medicine, for example in the evaluation of renal dysfunction or hypertension.

5.3 Conclusions

This thesis has validated a continuous non-invasive blood pressure monitoring device, utilizing techniques of finger plethysmography and volume clamping, as an alternative to an intra-arterial catheter for pediatric populations. Utilizing a pediatric sized finger cuff, the numeric agreement between the two measurement methods was within international guidelines. However, the algorithm used to correct the raw finger values distorts the initial waveform, so the morphology of the waveform should not be used to make inferences as to state of disease, until a pediatric-specific algorithm is created. This thesis was also able to validate the use of Quantab chloride test strips as an alternative to the gold standard in urine sodium analysis, flame photometry. Finally, this thesis showed that the use of corrected spot samples, in lieu of a 24-hour urine sample, can provide a

convenient way for individuals with syncope to be able to measure and monitor their sodium levels.

5.4 Future directions

The validation of non-invasive continuous blood pressure monitoring in pediatric populations will allow for easier diagnosis in those with syncope, by providing a way to safely measure continuous beat-to-beat non-invasive blood pressure during a tilt test or other autonomic function assessments. Future studies should be conducted to develop enhanced algorithms to minimize the morphological disruption of the waveform once the waveform correction algorithm is applied and to investigate the accuracy of the device during rapid and large blood pressure changes.

This thesis was able to show excellent agreement between Quantab chloride test strips and flame photometry for the determination of urine sodium concentration; however, despite the good agreement between spot samples and flame photometry, and between multiple spot samples and 24-hour urine collections, future research should extend these analyses to patient populations and larger sample sizes.

This work should be extended to the study of more efficient management options for individuals with syncope. While studies have been conducted in adults demonstrating the efficacy of salt supplementation on orthostatic tolerance (47,59), this management option has not been validated in children. With the validation of a non-invasive beat-to-beat blood pressure monitoring approach for use in children, a clinical trial should be conducted to determine the effect of salt supplementation on orthostatic tolerance (susceptibility to syncope, determined as the time to presyncope during a tilt test) in a pediatric population. This is important because the mechanisms of syncope differ in children and in adults, and it is currently unknown how children might respond to salt supplementation, or whether there are any long-term impacts of administering high doses of salt to children.

Chapter 6. Publications from this Thesis

6.1 Manuscripts

Coupal, KE., Heeney, ND., Hockin, BCD., Ronsley, R., Armstrong, K., Sanatani, S., & Claydon, VE. Pubertal hormonal changes and the autonomic nervous system: potential role in paediatric orthostatic intolerance. *Frontiers in Neuroscience*. 13:1197. Original review article.

Heeney, N., Habib, F., Brar, G., Krahn, G., Sanatani, S., & Claydon, VE. Validation of finger blood pressure monitoring in children. *Blood Pressure Monitoring*. 24(3): 137-145, 2019. Original research article.

6.2 Abstracts

Heeney, N., Hockin, BCD., Sanatani, S., Armstrong, K., & Claydon, VE. Can urine sodium concentration be determined through chloride test strips? *Clin Auton Res*. 29: 479-546, 2019. [Podium presentation]

Heeney, N., Hockin, BCD., & Claydon, VE. Are Quantab® chloride test sticks accurate in determining urine sodium concentration? *BPK Research Day*, SFU, BC. 2019. [Poster presentation]

Heeney, N., Habib, F., Brar, G., Krahn, G., Sanatani, S., & Claydon, VE. Validation of finger blood pressure monitoring in children. *Clin Auton Res*. 28: 453-505, 2018. Abstract. [Poster presentation]

Heeney, N., Habib, F., Brar, G., Krahn, G., Sanatani, S., & Claydon, VE. Validation of finger blood pressure monitoring in children. *BPK Research Day*, SFU, BC. 2018. [Poster presentation]

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Appendix A Consent Forms



Participant Information and Consent Form

The accuracy of finger blood pressure monitoring in children

Principal Investigator:	Dr Shubhayan Sanatani, MD Head, Division of Cardiology Associate Professor, University of British Columbia Children's Heart Centre 1F9 British Columbia Children's Hospital, Vancouver, BC
Co-Investigator(s):	Dr Victoria Claydon, PhD Associate Professor Department of Biomedical Physiology and Kinesiology Simon Fraser University Burnaby, BC Dr Fajish Habib, MD Clinical Fellow Paediatric Intensive Care Unit British Columbia Children's Hospital, Vancouver, BC Dr Gordon Krahn, MD Research Coordinator Division of Critical Care British Columbia Children's Hospital, Vancouver, BC

If you are a parent or legal guardian of a child who may take part in this study, permission from you and the assent (agreement) of your child may be required. When we say “you” or “your” in this consent form, we mean you and/or your child; “we” means the doctors and other staff.

1. Invitation

You are being invited to participate in a research study designed to compare how measurements of blood pressure from a new technique using a finger cuff compare to standard measurements from a device inserted into an artery with a needle.

2. Your participation is voluntary

Your participation is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled or are presently receiving.

You should be aware that there is a difference for both you and your doctor between being a patient and being a research participant. As a patient all medical procedures and treatments are carried out for your benefit only according to standard accepted practice. As a research participant you and your doctor also must take into account the requirements for the research study. These may include procedures and treatments that are not part of standard practice or are not yet proven. This consent form describes the diagnostic and treatment procedures that are being carried out for research purposes. Please review the consent document carefully when deciding whether or not you wish to be part of the research and sign this consent only if you accept being a research participant.

If you wish to participate in this study, you will be asked to sign this form.

Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

3. Who is conducting this study?

You are invited to take part in investigations conducted under the supervision of Dr Victoria Claydon who is an Associate Professor with the School of Biomedical Physiology and Kinesiology, Simon Fraser University, Dr Shubhayan Sanatani who is Head, Division of Cardiology at the British Columbia Children’s Hospital, Dr Fajish Habib who is a Medical Fellow in the Paediatric Intensive Care Unit at the British Columbia Children’s Hospital and Dr Gordon Krahn who is a Research Coordinator at the British Columbia Children’s Hospital.

4. Background

The overall goal of this investigation is to determine whether finger blood pressure monitoring can provide accurate measurements of continuous, beat-to-beat blood pressure in children when compared to an intra-arterial catheter.

An intra-arterial catheter is a small thin plastic tube that is inserted using a needle into an artery (a blood vessel that carries oxygen-rich blood) when continuous (with every

heart beat) blood pressure monitoring is needed. It enables close monitoring of blood pressure for long periods and allows doctors to respond quickly if there are changes in the patient's blood pressure. For this reason, doctors normally recommend blood pressure to be monitored via intra-arterial catheterization in young children or in infants during their recovery from cardiac surgeries or interventions.

Finger blood pressure monitoring provides non-invasive (meaning no needles are involved to place the monitor) beat-to-beat blood pressure readings using a small Velcro cuff wrapped around a finger. This device has been extensively tested in adults, and some studies have been conducted in newborn babies that have compared the blood pressure readings obtained when the finger cuff is placed on the wrist to intra-arterial catheters. However, it is not known how well blood pressure readings from finger blood pressure measurements compare to arterial catheter measurements *in children*. It may be that the finger blood pressures are less accurate in young children because their arteries are more stretchy.

Therefore, we wish to look at whether finger blood pressure monitoring in children is a viable alternative to measuring blood pressure invasively with an arterial catheter. This is important because if the readings are reliable, in some situations doctors could measure blood pressure with finger cuffs and avoid using needles to place arterial catheters; this might be less stressful and more comfortable for children.

5. What is the purpose of the study?

This study will determine whether finger blood pressure monitoring in children is a viable alternative to measuring blood pressure invasively with an arterial catheter.

6. Who can participate in this study?

You may be able to participate in this study if:

- You are aged 1-13 years old.
- Your primary care physician feels you are stable enough to undergo the additional finger blood pressure monitoring
- You will already need an intra-arterial catheter for blood pressure measurements for your ongoing clinical care.

7. Who should not participate in this study?

You will not be eligible to participate in this study if:

- You have a particular kind of infection, known as MRSA.
- You have burns *affecting the upper extremities*
- You need extracorporeal life support
- You have a disorder affecting perfusion to the fingers

8. What does the study involve?

You will be asked to undergo blood pressure monitoring using both finger cuffs and an intra-

arterial catheter at the same time. Your doctor will have already decided that intra-arterial catheter blood pressure measurements are needed for your clinical care. The only addition in this study will be the use of the finger blood pressure cuffs and a heart beat monitor (electrocardiogram).

The intra-arterial catheter will be placed by your medical doctor according to standard procedures. This would still be done even if you decide not to take part in the study, because your medical team have determined that this is necessary for your care.

A small Velcro cuff will be fitted around your middle and index fingers. When it is making measurements, it will gently squeeze the fingers to record blood pressure with each heartbeat. No needles are used to fit the device and it is painless. We will also record your heart rate using three stickers placed on the chest (one near each shoulder and one on the left hip).

Once the monitors are in place, the test will take 30 minutes. Blood pressure and heart rate recordings will be made from the finger cuff and electrocardiogram for four six-minute periods, with a two-minute break in between each recording phase. Sometimes recordings will be made from the middle finger, and sometimes from the index finger.

9. What are my responsibilities?

You do not need to make any special preparations for the procedure. A medical doctor will place the intra-arterial catheter that is needed for your ongoing care. This would still be done even if you decide not to take part in the study, because your medical team have determined that this is necessary for your care.

10. What are the possible harms and discomforts?

The study will take place in a controlled clinical environment and most people do not find finger blood pressure or electrocardiogram assessments unpleasant. Every effort will be made to ensure your safety, privacy and comfort. The following are discomforts or risks that may be associated with the study.

- These assessments will take 30 minutes to perform, and you will be asked to keep quite still during the assessments. You may find that you become a little 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. You do not need to be awake for the measurements, and if you are sleepy the study team will allow you to sleep during the measurements.
- Preparing the skin for the electrocardiogram stickers may cause minor irritation or redness. It is possible that you will experience an allergic reaction to the electrode gel or adhesive.
- You may be aware of the finger cuff squeezing your fingers. Some people find the sensation unusual, but it is not painful.

- The risks and side-effects of the standard or usual treatment of having blood pressure monitored with an intra-arterial catheter will be explained to you as part of your standard care.

11. What are the potential benefits of participating?

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 development of a new non-invasive blood pressure monitoring technique that might be less stressful and more comfortable for use in children.

12. What are the alternatives to the study treatment?

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

13. What if new information becomes available that may affect my decision to participate?

If you choose to enter this study and at a later date a more effective treatment becomes available, it will be discussed with you. You will also be advised of any new information that becomes available that may affect your willingness to remain in this study.

14. What happens if I decide to withdraw my consent to participate?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn, for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know.

15. Can I be asked to leave the study?

If you are not able to follow the requirements of the study or for any other reason, the study doctor may withdraw you from the study and will arrange for your care to continue. On receiving new information about the treatment, your research doctor might consider it to be in your best interests to withdraw you from the study without your consent if they judge that it would be better for your health. If you are asked to leave the study, the reasons for this will be explained to you and you will have the opportunity to ask questions about this decision.

16. How will my taking part in this study be kept confidential?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the C&W Research Ethics Board or Simon Fraser University Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information

or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. You also have the legal right of access to the information about you and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

17. What happens if something goes wrong?

By signing this form, you do not give up any of your or the participant's legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

18. What will the study cost me?

All research-related medical care and treatment and any related tests that you will receive during your participation in this study will be provided at no cost to you. You will not incur any personal expenses as a result of participation. You will not be paid for participating in this study.

19. 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, or if you experience any adverse effects, you can contact Dr Claydon (E-mail: [REDACTED] or Dr Sanatani [REDACTED]

Research results can be obtained upon request from Dr Claydon.

20. Who do I contact if I have any questions or concerns about my rights as a participant?

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at [REDACTED], or Dr. Jeff Toward, Director of the Office of Research Ethics at Simon Fraser University

21. After the study is finished

After the study is completed you may obtain a copy of the research results upon request from Dr Claydon

Participant Consent

My signature on this consent form means:

- I have read and understood the information in this consent form.
- I have had enough time to think about the information provided.
- I have been able to ask for advice if needed.
- I have been able 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 purposes.
- I understand that my participation in this study is voluntary.
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change 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.

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

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Printed Name of Participant	Signature	Date
_____	_____	_____
Printed Name of Parent/Guardian	Signature	Date
_____	_____	_____
Printed Name of Investigator/Designate	Signature	Date
_____	_____	_____
Printed Name of Translator	Signature	Date



The accuracy of finger blood pressure monitoring in children

Participant Information and Assent Form Assent Form for Children Ages 7-13 years old

- Principal Investigator:** Dr Shubhayan Sanatani, MD
Head, Division of Cardiology
Associate Professor, University of British Columbia
Children's Heart Centre 1F9
British Columbia Children's Hospital, Vancouver, BC
- Co-Investigator(s):** Dr Victoria Claydon, PhD
Associate Professor
Department of Biomedical Physiology and Kinesiology
Simon Fraser University
Burnaby, BC
- Dr Fajjish Habib, MD
Clinical Fellow
Paediatric Intensive Care Unit
British Columbia Children's Hospital, Vancouver, BC
- Dr Gordon Krahn, MD
Research Coordinator
Division of Critical Care
British Columbia Children's Hospital, Vancouver, BC

INVITATION TO PARTICIPATE

I am being invited to take part in this research study designed to compare how measurements of blood pressure from a new technique using a finger cuff compare to the standard way of measuring blood pressure which involves inserting a needle into me.

DATE: 11Feb2016
Version 1

Page 1 of 4



Research studies help doctors learn more about diseases and how to help children who have health problems. They can also allow doctors to learn more about how to better treat patients like me.

It is up to me if I want to be in this study. No one will make me be part of the study. Even if I agree now to be part of the study, I can change my mind later. No one will be upset with me if I choose not to be part of this study.

I will read this form so that I will understand the study and if I wish to participate I will be asked to write my name on this form. My parents/legal guardians will be asked to write their name on a different form.

WHO IS DOING THE STUDY?

Dr. Shubhayan Sanatani from BC Children's Hospital along with the investigators listed at the top of this form are doing this study. Dr. Claydon [REDACTED] telephone: [REDACTED] or Dr. Sanatani [REDACTED] will answer any questions I may have about the study. I can also contact the study coordinator Dr. Krahn [REDACTED] if I have any problems.

WHY ARE WE DOING THIS STUDY?

This study is being carried out to determine whether measuring blood pressure in children using a finger cuff is better than the standard way of measuring blood pressure in children which involves inserting a needle into me.

WHAT WILL HAPPEN IN THIS STUDY?

If I agree to be part of this study I will be asked to undergo blood pressure monitoring using both finger cuffs and the device that is inserted into my artery with a needle at the same time. My doctor will have already decided that the device inserted into my artery with a needle to measure blood pressure is needed for my care. The only addition in this study will be the use of the finger blood pressure cuffs and a heartbeat monitor (electrocardiogram).

The device inserted into my artery with a needle will be placed by my medical doctor according to standard procedures. This would still be done even if I decide not to take part in the study, because my medical team have determined that this is necessary for my care.

A small Velcro cuff will be fitted around my middle and index fingers. When it is making measurements, it will gently squeeze the fingers to record blood pressure with each heartbeat. No needles are used to fit the device and it is painless. My heart rate will be recorded using three stickers placed on my chest (one near each shoulder and one on the left hip).

Once the monitors are in place, the test will take 30 minutes. Blood pressure and heart rate recordings will be made from the finger cuff and electrocardiogram for four six-minute periods, with a two-minute break in between each recording phase. Sometimes recordings will be made from the middle finger, and sometimes from the index finger.



WHAT ARE MY RESPONSIBILITIES?

I do not need to make any special preparations for the procedure. A medical doctor will place the device into my artery with a needle that is needed for my ongoing care. This would still be done even if I decide not to take part in the study, because my medical team have determined that this is necessary for my care.

CAN ANYTHING BAD HAPPEN TO ME?

The study will take place in the hospital and most people do not find finger blood pressure or electrocardiogram assessments unpleasant. Every effort will be made to ensure my safety, privacy and comfort. The following are discomforts or risks that may be associated with the study.

- These assessments will take 30 minutes to perform, and I will be asked to keep quite still during the assessments. I may find that I will be bored during the course of these investigations. Every effort will be made to maintain my comfort throughout the study. I will be provided with pillows, blankets etc as appropriate to ensure my comfort. I do not need to be awake for the measurements, and if I am sleepy the study team will allow me to sleep during the measurements.
- Preparing the skin for the electrocardiogram stickers may cause minor irritation or redness. It is possible that I will experience an allergic reaction to the electrode gel or adhesive.
- I may be aware of the finger cuff squeezing my fingers. Some people find the sensation unusual, but it is not painful.
- The risks and side-effects of the standard or usual treatment of having blood pressure monitored with the device which is inserted into my artery with the needle will be explained to me as part of my standard care.

CAN ANYTHING GOOD HAPPEN TO ME?

There are no direct benefits to me from taking part in the study. It is hoped that the results of this study will ultimately aid in the development of a new non-invasive blood pressure monitoring technique that might be less stressful and more comfortable for use in children.

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

I may withdraw from this study at any time without giving reasons. If I wish to leave the study, I may call Dr. Claydon or contact Dr. Sanatani or the study coordinator or I can also send Dr. Sanatani a letter (at the address listed at the top of this form), asking that I be removed from the study but this is not required.



CAN I BE ASKED TO LEAVE THE STUDY?

If I am not able to follow the requirements of the study or for any other reason, the study doctor may withdraw me from the study and will arrange for my care to continue. On receiving new information about the treatment, my research doctor might consider it to be in my best interests to withdraw me from the study without my assent if they judge that it would be better for my health. If I am asked to leave the study, the reasons for this will be explained to me and I will have the opportunity to ask questions about this decision.

WHO WILL KNOW I AM IN THE STUDY?

Only my doctors and the people involved in the study will know I am in it. When the study is finished, the doctors will write a report about what is learned. This report will not say my name or that I was in the study. My parents/legal guardians and I do not have to tell anyone I am in the study if we don't want to.

DO MY PARENTS/LEGAL GUARDIANS KNOW ABOUT THIS?

My parents/legal guardians know about this study, and they said that I can be in it. They will have to write their name on a form like this to let me be in the study. I will talk this over with them before I decide. I do not have to be in the study even if my parents/legal guardians want me to be.

DO I HAVE TO BE IN THIS STUDY?

I do not have to be in this study. No one will be upset with me if I don't want to do this. If I don't want to be in this study, I just have to tell the research staff. I can say yes now and change my mind later. It is up to me.

It will not make any difference to my health care if I decide to say no to the study.

WHEN DO I DECIDE?

I have as much time as I want to decide to be part of the study. I have also been asked to discuss my decision with my parents/legal guardians before I make a decision.

If I **do not** want to be part of this study I will tell the study doctor or the research coordinator. No one will be upset with me if I don't take part in the study.

If I **do** want to be part of this study I will print my name and write my name and date on the lines below.

If I agree to participate by signing this form, I will receive a signed copy of this form for my own records.

Name _____

Signature

Date

DATE: 11Feb2016
Version 1

Page 4 of 4



Are Quantab Chloride sodium test sticks accurate in determining urine sodium concentration?

CONSENT FORM

Principal Investigator: Dr. Victoria Claydon, Associate Professor
Department of Biomedical Physiology and Kinesiology
Simon Fraser University
Vancouver, BC

INVITATION TO PARTICIPATE

In some people who are prone to fainting, their blood pressure is very low and this can be associated with low levels of salt in the body. Measures of body salt content might provide useful information in the care of these individuals. The usual way to measure body salt content is to ask a person to collect all their urine for a 24-hour period, so the salt levels in their urine (which reflects the levels in the body) can be measured. However, for some people the urine collection is inconvenient, particularly in children, and this is a problem because children are very susceptible to faints. We are testing a new method to measure the salt content of the body using test strips that can be done from a single sample of urine in the morning. You are invited to participate in a research study designed to figure out whether this alternative method of analyzing urine for sodium concentration is accurate. The purpose of this form is to provide you with information to help you 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.

Please take time to read the following information carefully and to discuss it with anyone you wish. You will have an opportunity to ask the study team any questions you may have about the study.

WHO IS CONDUCTING THE STUDY?

Dr Victoria Claydon, PhD., who is an Associate Professor with the School of Biomedical Physiology and Kinesiology, Simon Fraser University, along with Natalie Heeney and Brooke Hockin (both graduate students in Dr. Victoria Claydon's Lab), have designed this study together.

HAS THIS STUDY RECEIVED FUNDING?

This research is funded by the Heart and Stroke Foundation of Canada.

WHO CAN I CONTACT IF I HAVE CONCERNS ABOUT THE STUDY?

If you have concerns about your rights as a research participant and/or your experiences while participating in this study, please contact Dr. Jeffrey Toward, Director, Simon Fraser University Research Ethics

WHAT IS THE PURPOSE OF THE STUDY?

The primary purpose of this study is to test whether spot sample test strips can be used instead of 24-hour urine collections to accurately measure body sodium levels.

WHO CAN PARTICIPATE IN THE STUDY?

You are eligible to take part in this study if you are at least 18 years old and able to understand instructions in English. Women should not complete the study urine collections on days when they have, or expect, their menstrual period.

WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You should not participate if you do not want to.

If you have a current urinary tract infection, you are asked to wait until it has been successfully treated before volunteering for this study.

If you cannot understand enough English to follow instructions and communicate with the testers you cannot participate.

WHAT DOES THE STUDY INVOLVE/WHAT ARE THE PROCEDURES?

For this study you will be asked to complete six urine spot samples, one each morning and evening for three days. You will collect your first morning pee in a container and place one of the test strips provided by the research team in the sample. Once the strip has recorded the concentration, you may dispose of the sample down the toilet and either rinse the cup for the following day or discard the cup and use a different one the next day. The exception to this is on the 3rd day, as this first morning void will need to be collected. Each day you will take a photo of the test strip once it has changed colour, and place the used strip in a container provided. We are asking you to take photos of the test strips in case you have difficulty reading the colour change, you lose a strip, or the colour change fades over time. Once you have shown the pictures to the researcher, you can delete them from your device or camera.

You will also be asked to provide a 24-hour urine sample that you will collect on day two. You will be given a large jug in which you will collect all of your pee on day two. You will then bring this sample to the lab for analysis. The 24-hour urine collection needs to be refrigerated throughout the day. If you are a member of the SFU community, you may store the jug in a fridge in the research lab for convenience. There are washrooms near the lab and you can come and go to add to your sample collection whenever you need to. Alternatively, you can collect the entire sample on a weekend day from the comfort of your home. When you complete the spot sample on the 3rd morning, you will be asked to add this to the 24-hour sample, or bring it to the lab so we can add it for you.

Once the spot samples and 24-hour sample have been completed, we ask you bring all six spot sticks, the photos you took, and the 24-hour sample back to the lab. You are free to return the 24-hour sample back to the lab as soon as you like once it is collected. Upon returning to the lab we will measure your blood pressure in case this influences the measures we are taking.

WHAT ARE THE POSSIBLE HARMS AND SIDE EFFECTS OF PARTICIPATING?

The study is designed with every effort made to ensure the safety, privacy and comfort of participants. Some volunteers may feel uncomfortable or embarrassed with collecting and storing their urine for a day. If you feel this way, you are free to stop the study at any point.

WHAT ARE THE BENEFITS OF PARTICIPATING?

There are no direct benefits to the volunteers; however, it is hoped that through this study a method that can help aid in the diagnosis of fainting will be validated. This may result in earlier diagnosis for affected

patients, as well as potentially provide a cost-effective and quick method for 24-hour urine sodium estimations.

WHAT IF YOU LEARN NEW INFORMATION ABOUT MY HEALTH DURING THE STUDY?

Measures of urine sodium content are not an indication of your health. We do not expect to learn any information about your health during this study. If you like we can tell you the value of your blood pressure measurement, but only your doctor can tell you whether this is a healthy blood pressure for you. This study will only provide information on the accuracy of methods for testing sodium levels.

WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT?

You are free to choose not to participate, and if you do volunteer, you are free to withdraw from this study at any time. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

WHAT WILL THE STUDY COST ME?

There are no costs to you for taking part in this study. Free parking will be provided at Simon Fraser University, where you will need to come to collect and return the study materials. As a thank you for taking part, you will receive a \$20 honorarium for your time.

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 unique and random code instead of your name. Research records with your name and personal information may be inspected by the 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 will be allowed to leave the investigators' research office. We will keep these records for ten years, after which time they will be destroyed.

We plan to make the results of the study public so that doctors and people with fainting spells can learn from the study. This might be in written reports aimed at doctors and scientists, or in patient information leaflets aimed at people who faint and their families. These documents would also be available online. We will also present the results verbally, for example at science conferences or patient education meetings. In all cases, we will make sure that no one could tell who took part in the study (we would not include any names or other information about participants). If you would like to learn about the results of the study when it is finished, please let one of the study team know.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions or desire further information either before or during participation, you can contact Dr. Victoria Claydon [REDACTED]

PARTICIPANT INFORMED CONSENT

I, _____, (print full name), have received a copy of the consent form. I consent to participation in this study by signing this consent form. I have read and understand all the preceding information describing the study, and all by questions have been answered to my satisfaction. I voluntarily consent to participate in this study.

Signature _____

Witness _____

Date _____

Date _____

Appendix B Instruction Sheets

URINE SODIUM EXPERIMENT INSTRUCTIONS

ID# _____

Day 1A: _____ SPOT SAMPLE

write date and time here

- 1) Pee your **FIRST MORNING VOID (FMV)** into a container
- 2) Place **Day 1A** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



- 5) Discard urine and wash cup for tonight

Quantab reading: _____



Day 1P: _____ SPOT SAMPLE

write date and time here

- 1) Pee your **EVENING SAMPLE (PM)** into a container
- 2) Place **Day 1P** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



- 5) Discard urine and wash cup for tomorrow

Quantab reading: _____



Day 2A: _____ SPOT SAMPLE

write date and time here

- 1) Pee your **FIRST MORNING VOID (FMV)** into a container
- 2) Place **Day 2A** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



- 5) Discard urine and wash cup for tonight

Quantab reading: _____



Day 2: _____ 24-HOUR SAMPLE

write date and time here

- 1) **Discard** your FMV for Day 2
- 2) **All other urine today will need to be collected in the 3L container**



- 3) You may pee in a separate container and **pour it** into the 3L container
- 4) Once there is urine in the container, it must be **kept refrigerated**
- 5) **Day 2's PM sample and Day 3's FMV** must also be collected in the 3L container



URINE SODIUM EXPERIMENT INSTRUCTIONS

ID# _____

Day 2P: _____ SPOT SAMPLE
write date and time here



- 1) Pee your **EVENING SAMPLE (PM)** into a container
- 2) Place **Day 2P** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



5) Pour urine from PM into 3L container



6) Wash cup for tomorrow



Quantab reading: _____

Day 3A: _____ SPOT SAMPLE
write date and time here



- 1) Pee your **FIRST MORNING VOID (FMV)** into a container
- 2) Place **Day 3A** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



5) Pour urine from FMV into 3L container



6) Wash cup for tonight



Quantab reading: _____

Day 3P: _____ SPOT SAMPLE
write date and time here



- 1) Pee your **EVENING SAMPLE (PM)** into a container
- 2) Place **Day 3P** test strip into container
- 3) Wait for thread to change from yellow to black (*refer to Quantab Instructions*)
- 4) When done, take photo of test strip and place strip in bag labelled "Used"



5) Discard urine and wash cup or throw it out



OR



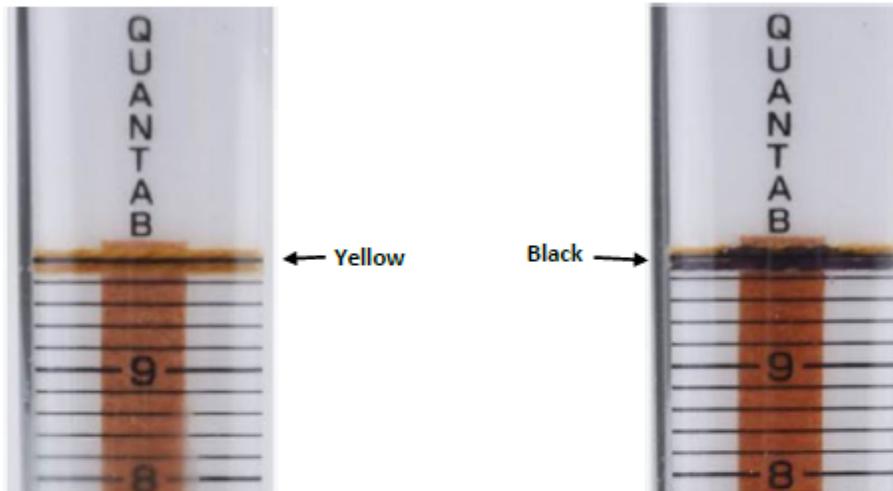
Quantab reading: _____

URINE SODIUM EXPERIMENT INSTRUCTIONS

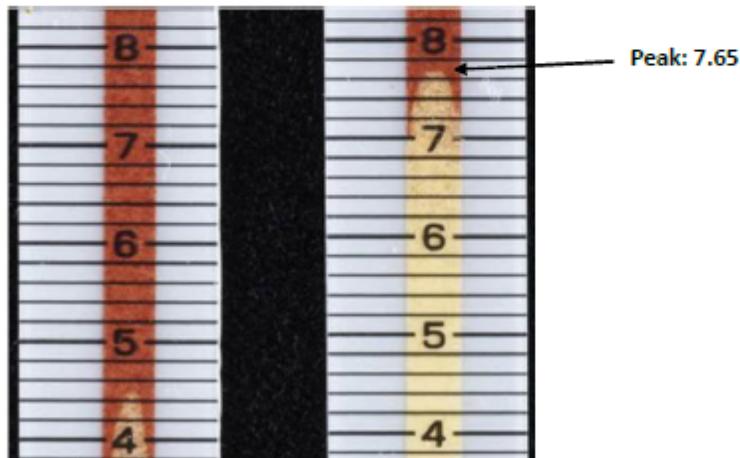
ID# _____

Quantab Instructions

- Open bag and remove Quantab stick
- Make sure the strip number is the correct number for the test day
- Place stick in urine, but do NOT let solution go above the yellow line
- Leave stick in urine until yellow line turns black



- Once black, remove Quantab stick and read number where white peak occurs



- Record number in the "Quantab reading: _____" space for that day
- Take a photograph of the test strip
- Place the test strip in the bag labelled "used"

Appendix C

At-Home Conversion Chart

At-Home Quantab Conversion Chart

After letting the yellow thread completely turn black, use the conversion chart below to obtain a urine sodium estimate. **If you are completing a three-sample average, use the average of the three spot samples as your Quantab value.** Alternatively, for a more precise estimate use the equations below.

Quantab value (a.u.)	24-hour urine sodium value (mmol/day)	Quantab value (a.u.)	24-hour urine sodium value (mmol/day)	Quantab value (a.u.)	24-hour urine sodium value (mmol/day)	Quantab value (a.u.)	24-hour urine sodium value (mmol/day)
0.1	61	2.6	72	5.1	99	7.6	168
0.2	61	2.7	73	5.2	100	7.7	173
0.3	62	2.8	74	5.3	102	7.8	179
0.4	62	2.9	74	5.4	104	7.9	185
0.5	62	3	75	5.5	106	8	192
0.6	63	3.1	76	5.6	107	8.1	199
0.7	63	3.2	77	5.7	109	8.2	206
0.8	63	3.3	77	5.8	111	8.3	215
0.9	64	3.4	78	5.9	113	8.4	224
1	64	3.5	79	6	116	8.5	234
1.1	64	3.6	80	6.1	118	8.6	245
1.2	65	3.7	81	6.2	120	8.7	257
1.3	65	3.8	82	6.3	123	8.8	270
1.4	66	3.9	83	6.4	125	8.9	284
1.5	66	4	84	6.5	128	9	300
1.6	67	4.1	85	6.6	131	9.1	318
1.7	67	4.2	86	6.7	134	9.2	338
1.8	67	4.3	88	6.8	137	9.3	360
1.9	68	4.4	89	6.9	140	9.4	384
2	69	4.5	90	7	143	9.5	411
2.1	69	4.6	91	7.1	146	9.6	441
2.2	70	4.7	93	7.2	150	9.7	475
2.3	70	4.8	94	7.3	154	9.8	512
2.4	71	4.9	96	7.4	159	9.9	554
2.5	71	5	97	7.5	163	10	602

$$\text{If Quantab value (Q) is below 7: } Na^+ (\text{mmol}) = \frac{-149.6389 + 154.8257(e^{0.3447Q}) + 0.0003(e^{1.6998Q})}{23}$$

$$\text{If Quantab value is 7 or above: } Na^+ (\text{mmol}) = \frac{-308.5133 + 259.9633(e^{0.274Q}) + 0.023(e^{1.2612Q})}{23}$$

Using the Na⁺ value above, use the equation below to calculate your **24-hour urine sodium value**:

$$24 - \text{hour urine sodium sample} = 1.172Q + 60.438$$