Associations between dietary electrolytes and pulse wave velocity

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ABSTRACT

Background- Pulse wave velocity (PWV) is a non-invasive measure of arterial stiffness, and a recognised predictor of cardiovascular morbidity and mortality. Given this, it is likely that investigating the determinants of PWV will improve our understanding of cardiovascular health. At present, there is disagreement regarding the relationship between dietary sodium and potassium intakes, and PWV. Hence, further research is needed in order to confirm whether dietary sodium and potassium are determinants of PWV.

Objective- The aim of this cross-sectional study was to investigate the associations between dietary sodium and potassium intake, and PWV in the general population.

Methods- This cross-sectional study used baseline data from Health And Bread Intervention Trial (HABIT). Spot urine samples were used to estimate dietary sodium and potassium intake. Weighed three-day diet records were analysed for self-reported dietary sodium and potassium intake. Brachial blood pressure, and carotid-femoral PWV were measured with the SphygmoCor 2000.

Results- Sixty-five HABIT participants were included in this study. Overall, 52.3% were males, with a mean±SD age of 34.5±18.3years, body mass index (BMI) of 24.9±4.5kg/m^2; BP of 126.5/74.8±17.7/11.2mmHg, and PWV of 7.2±1.6m/s. Mean sodium intakes as assessed by spot urine samples and diet records were above New Zealand's Upper Limit of 2300mg/day (urinary sodium: 3021±756mg/day; dietary sodium: 2784±1067mg/day). Mean potassium intakes, also assessed by spot urine samples and diet records, were below the Adequate Intake for New Zealand of 3800mg/day for males and 2800mg/day for females (male, urinary potassium: 2002±386.9mg/day; male, dietary potassium: 3500±1242.2mg/day; female, urinary potassium: 1902.6±428.5mg/day; female, dietary potassium: 2783.3±991.3mg/day). Dietary intakes of sodium, potassium, and sodium-to-potassium ratio as assessed by spot urine samples and diet records were not independently

associated with PWV. In multi-variate analysis age was positively associated with PWV (a 1-year increase in age was associated with a 0.05 m/s increase in PWV).

Conclusions- This small cross-sectional study found dietary intakes of sodium, potassium, and sodium-to-potassium were not independent predictors of PWV, suggesting the prediction of PWV is multi-factorial. Future adequately-powered studies should examine these relationships.

Key Words- pulse wave velocity, arterial stiffness, cardiovascular health, sodium, potassium, sodium-to-potassium

PREFACE

This research project was part of the Health And Bread Intervention Trials (HABIT) study. It was supervised by Dr Katherine Black and Dr Rachel Brown from the University of Otago's Department of Human Nutrition.

Under supervision, the candidate was responsible for the following:

- Laboratory analysis of urine samples
- Dietary analysis of weighed-diet records
- Compilation of results
- Interpretation of results
- Writing of the thesis

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LIST OF ABBREVIATIONS

Blood pressure	BP
Body mass index	BMI
Cardiovascular disease	CVD
Diastolic blood pressure	DBP
Double blinded	Db
Food frequency questionnaire	FFQ
Health And Bread Intervention Trial	HABIT
Marinobufagenin	MBG
Metallopeptidase	MMP
Not applicable	N/A
Placebo controlled	Pc
Potassium	K
Pulse wave velocity	PWV
Randomised control trial	Rct
Sample size	n
Single blinded	Sb
Sodium	Na
Systolic blood pressure	SBP
Twenty-four hours	24h

1 - INTRODUCTION

Pulse wave velocity (PWV) is a non-invasive measure of arterial stiffness (Laurent et al. 2006; van Bortel et al. 2012). Arterial stiffness describes the inability of arteries to expand and contract, which can contribute to an increase in systolic pressure (Wilkinson et al. 1998), and manifest as hypertension (>140/90 mmHg) (Zieman, Melenovsky and Kass 2005). Given the relationship between PWV and arterial stiffness, PWV is a recognised predictor of cardiovascular morbidity and mortality (Mancia et al. 2013).

Cardiovascular disease (CVD) is the leading cause of death in New Zealand (Ministry of Health 2015). The high number of CVD deaths highlights the importance of understanding the disease. As PWV is significantly associated with cardiovascular health outcomes, investigating the determinants of PWV could improve our understanding of CVD, and possibly identify prevention and treatment strategies. Age, hypertension and diabetes are known determinants of PWV (Zieman et al. 2005), however, little is known about the dietary determinants of PWV – for example whether dietary electrolytes determine PWV.

Sodium is important for maintaining extracellular volume, the active transport of molecules, and the depolarisation of cells (Robinson 2012), and is primarily found as sodium chloride in processed foods (e.g. bread, cheese, sauces), and as table or cooking salt (National Health and Medical Research and Ministry of Health 2006). New Zealand's Ministry of Health reports an Upper Limit of 2300mg per day (National Health and Medical Research and Ministry of Health 2006). However, the most recent research on a sample of New Zealand adults indicates males are consuming an average of 3865mg per day and females an average of 2934mg per day (McLean et al. 2015), which are both above the Upper Limit. Excessive consumption of sodium is associated with elevated blood pressure (Polonia et al. 2006) and the development of cardiovascular disease (Lennon-Edwards, Schellhardt, Ferreira, Farquharand and Edwards 2014). However, there is disagreement in the literature about

sodium's effect on PWV (i.e. arterial stiffness), with studies suggesting a positive relationship (e.g. He et al. 2009; Sonada, Takase, Dohi and Kimura 2012) and others no relationship (e.g. Dickinson and Keogh 2013; Gijsbers et al. 2015) (for further details see section: 2.3 dietary sodium and pulse wave velocity).

Potassium is important for the depolarisation and repolarisation of cells (Robinson 2012) and is found prominently in leafy green vegetables, root vegetables, tree fruit, and vine fruit (National Health and Medical Research and Ministry of Health 2006). New Zealand's Ministry of Health reports and Adequate Intake of 3800mg per day for men and 2800mg per day for women (National Health and Medical Research and Ministry of Health 2006). However, recent data indicates New Zealand's mean potassium intake was 3031mg per day for men and 2436mg per day for women (McLean et al. 2015), which are both below the Adequate Intake. It is thought potassium blunts sodium's effect on blood pressure (Whelton et al. 1997) and it is protective against CVD (Lennon-Edwards et al. 2014). However, like sodium, there is disagreement in the literature about potassium's relationship with PWV (i.e. arterial stiffness), with studies suggesting no relationship (e.g. Berry, Chowlenczyk and Sanders 2010; Garcia-Ortiz et al. 2012) and others suggesting an inverse relationship (e.g. Graham, McCance, Young and Mullan 2014; Lennon-Edwards et al. 2014) (for further details see section: 2.4 dietary potassium and pulse wave velocity).

Given the contradictions in the existing literature, there is need for further research regarding the relationship between dietary electrolytes and arterial stiffness. This research aims to address this discrepancy by undertaking a cross-sectional study to investigate the association between current sodium and potassium intake, and PWV, in the general population.

2 - LITERATURE REVIEW

2.1 - Pulse wave velocity

2.1.1 – Defining pulse wave velocity

The ejection of blood from the left ventricle into the aorta causes the propagation of pressure waves along the arterial tree. Compliant arteries absorb wave energy during systole and release it during diastole, which aids coronary perfusion. Some pressure waves are reflected back towards the heart and summate with forward-moving waves, producing pressure waveforms (Nichols 2011; Wilkinson et al. 1998).

PWV is the speed at which pressure waves travel. It is defined by the Moens-Korteweg equation – see below (Nichols 2011). This equation illustrates that PWV is inversely related to the elasticity of vessel walls (i.e. greater velocity corresponds to reduced elasticity). Therefore, PWV can be used to measure arterial stiffness (Laurent et al. 2006; van Bortel et al. 2012), and as a result, PWV is also recognised as a predictor of cardiac morbidity and mortality (Mancia et al. 2013) (for further details see section: 2.2 pulse wave velocity and health).

$$c_o=\sqrt{(Eh/2R\rho)}$$ [a]

2.1.2 - Measuring pulse wave velocity

PWV can be measured by using two pressure catheters placed at a known distance from each other, and calculated with the equation below (for further detail see section: 4.5.3 pulse wave velocity) (van Bortel et al. 2012).

$$PWV=d/t$$ [b]

Carotid-femoral PWV is the gold standard measure of PWV (Mancia et al. 2013). It has been used in landmark studies of arterial stiffness, including the Framingham Heart Study

[a] c_o represents PWV, E represents the vessel's modulus of elasticity, h represents the thickness of the vessel wall, R represents the radius of the vessel, and ρ represents the density of blood (Nichols 2011).

[b] d is the distance between the catheters, and t is the time it takes the pressure wave to go between the catheters (Van Bortel et al., 2012).

(Mitchell et al. 2004; Tanaka et al. 2009). However, over recent years, brachial-ankle PWV has emerged and is being used in clinical settings in Asia (Sugawara and Tanaka 2015). Its main advantage over carotid-femoral PWV is that the specialist equipment is easy to use. Carotid-femoral PWV requires a technician to manually find a pressure wave, where brachial-ankle PWV uses four automated blood pressure cuffs (Sugawara and Tanakacal 2015). Literature suggests that there is a significant correlation between PWV values recorded by both methods, and they predict stroke and coronary artery disease risk comparably (Sugawara et al. 2005; Tanaka et al. 2009).

In practical settings, measurement of the carotid-femoral PWV can be undertaken with specialist equipment. The most common are the SphygmoCor 2000, and Complior (Calabia et al. 2011). They use different methods to calculate the pulse wave distance (Rajzer et al. 2008), resulting in slightly different PWV readings. This highlights that uniform principles for the measurement of pulse wave distance should be established and used by all researchers. Furthermore, until the SphygmoCor 2000 and Complior show comparable results, they should use their own cut off values for determining arterial stiffness. The SphymoCor 2000 should use values above 10 m/s and the Complior should use values above 12m/s as an indication of arterial stiffness (Rajzer et al. 2008, van Bortel et al. 2012).

A limitation of using the SphygmoCor 2000 and Complior is that it is challenging to perform accurately due to the difficulty recording good pressure waves (Calabia et al. 2011). The Doppler ultrasound is another method that can be used to measure PWV and it overcomes the above limitation. The Doppler produces an anatomical image that may increase the accuracy of the measurement (Calabia et al. 2011). Despite this apparent benefit, the literature suggests that the Doppler ultrasound can produce similar PWV results to the Complior (Calabia et al. 2011) and the SphygmoCor 2000 (Jiang, Liu, McNeill and Chowienczyk 2008).

At present, it appears that the measurement method chosen depends on the equipment and expertise available. Hence, a variety of measurement tools are used in PWV and health literature.

2.2 - Pulse wave velocity and health

2.2.1 - Health outcomes associated with pulse wave velocity

Much of the literature agrees that PWV is positively associated with cardiovascular event risk (Blacher, Asmar, Djane, London and Safar 1999a; Boutouyrie, Tropeano, Asmar and Gautier 2002), cardiovascular mortality (Meaume, Benetos, Henry, Rudnichi and Safar 2001; Sutton-Tyrrell et al. 2005), and all-cause mortality (Blacher et al. 1999b; Laurent et al. 2001; Laurent et al. 2003; Mattace-Raso et al. 2006; Mitchell et al. 2010; Vlachopoulos, Aznaouridis and Stefanadis 2010; Willum-Hansen et al. 2006).

The literature exposed one conflicting result. PWV does not always predict all-cause mortality in older adults (>70 years of age) (Meaume et al. 2001; Sutton-Tyrrell et al. 2005). Sutton-Tyrrell et al. (2005) found the highest PWV quartile had a 70% higher risk of all-cause mortality than the lowest PWV quartile, where Meaume et al. (2001) found no association between PWV and all-cause mortality. The difference in results may be attributed to the marked difference in the number of older adults studied (n=141 (Meaume et al. 2001) versus n=2488 (Sutton-Tyrrell et al. 2005)) or the different methods used to measure carotid-femoral PWV (Complior (Meaume et al. 2001) versus Doppler ultrasound (Sutton-Tyrrell et al. 2005)). Even so, only having two studies in this area highlights the need for further exploration of the role PWV plays in the health of older adults.

PWV likely predicts cardiac morbidity and mortality, as it is related to arterial stiffness (Laurent et al. 2006; Mancia et al. 2013; van Bortel et al. 2012). Direct measurement of arterial stiffness is invasive, and thus is not feasible in humans. Instead, non-invasive measures of PWV, like those mentioned previously, are used to estimate arterial stiffness

(Blacher et al. 1999a). These measures have been widely used in research and validated in both patient and healthy populations (Nichols 2011).

2.2.2 - Arterial stiffness

Arterial stiffness describes the inability of arteries to expand and contract. It is made up of two modifiable aspects: distensibility and compliance. Distensibility is the amount the vessel's diameter changes in relation to its initial diameter. Compliance is the change in the vessel's diameter in response to blood pressure changes. Arterial stiffness is characterised by a decline in both distensibility and compliance (O'Rourke et al. 2002).

Collagen and elastin play a large role in arterial stiffness, as they determine the strength and elasticity of vessel walls. In compliant arteries, normal collagen and elastin are present, and their production and degradation is in equilibrium. Conversely, their production and degradation is not in equilibrium in non-compliant arteries. Instead, there is an overproduction of abnormal collagen and a breakdown of normal elastin (Najeeb and Ming-Hui 2010; Zieman et al. 2005).

2.2.3 - Factors impacting arterial stiffness

The literature suggests that arterial stiffness is somewhat genetically determined but is also influenced by hormones, glycaemic state, endothelial dysfunction and inflammation (Najeeb and Ming-Hui 2010).

Firstly, it is thought that angiotensin II and aldosterone play a role in the development of arterial stiffness. Dietary sodium and hyperglycaemic states have been shown to stimulate the renin-angiotensin-aldosterone axis. Increased levels of angiotensin II stimulate the production of collagen and the degradation of elastin, reduce nitric oxide synthesis and increase reactive oxygen species production. Whereas aldosterone stimulates smooth muscle hypertrophy, and fibrosis (Najeeb and Ming-Hui 2010; Zieman et al. 2005). Secondly, hyperglycaemic states can cause the production of abnormal collagen and elastin molecules

via glycation. The glucose cross-links make collagen stiffer and reduce the elasticity of elastin (Najeeb and Ming-Hui 2010; Zieman et al. 2005). Thirdly, researchers believe endothelial dysfunction plays a role in arterial stiffness. However, there is no clear consensus regarding the mechanism. Currently, the literature suggests that neither one causes the other but both play an important role in each other's development (i.e. a cyclic relationship) (Najeeb and Ming-Hui 2010; Zieman et al. 2005). Finally, inflammatory states cause the expression of catabolic metalloproteases (MMP). These molecules cause the production of weak collagen and frayed elastin, which are both found in non-compliant arterial walls (Najeeb and Ming-Hui 2010; Zieman et al. 2005).

2.2.4 - Mechanism linking arterial stiffness and cardiovascular health

In a healthy individual, vessel walls absorb pressure wave energy during systole and release energy during diastole, which optimises coronary perfusion (Wilkinson et al. 1998). In contrast, non-compliant arteries absorb less wave energy, resulting in the propagation of larger and faster waves. These large, fast waves are reflected, and arrive during systole, contributing to an increase in systolic pressure (Wilkinson et al. 1998). As a result, arterial stiffness manifests itself as hypertension (>140/90mmHg) and elevated pulse pressure (systolic blood pressure – diastolic blood pressure) (Zieman et al. 2005).

There are adverse consequences to chronic hypertension and elevated pulse pressure. Firstly, the heart must generate higher systolic pressures to produce the same stroke volume. This requires the heart to generate more energy per contraction. Hence, it can stimulate ventricular hypertrophy, reducing the efficiency of each cardiac ejection (Zieman et al. 2005). Additionally, when reflected pressure waves arrive in systole, coronary perfusion will start in systole. This causes a decline in coronary perfusion, which is further exacerbated by a cardiac event (Zieman et al. 2005). Finally, at the periphery, arterial stiffness and the associated

increase in pulse pressure causes turbulent blood flow. This increases the shear stress and pressure on vessel walls, which causes endothelial dysfunction (Zieman et al. 2005).

From the literature discussed above, we can conclude that PWV is associated with cardiovascular outcomes due to its relationship with arterial stiffness. Therefore, it is important to investigate the determinants of PWV (e.g. dietary determinants) to improve our understanding of cardiovascular disease.

2.3 - Dietary sodium and pulse wave velocity

2.3.1 – Proposed mechanism linking dietary sodium and pulse wave velocity

The mechanism linking sodium and PWV is unknown. Researchers speculate that sodium may promote fibrotic changes to arterial walls via aldosterone. This is supported by an observational study that suggests PWV is only elevated in high sodium, high aldosterone participants, and PWV is normal in high sodium, low aldosterone participants (Kotliar et al. 2014). Additionally, there is evidence to suggest that sodium may increase arterial stiffness via matrix MMP-9 , which is involved in the remodelling of collagen and elastin in the vessel wall (Todd et al., 2010). The literature also supports the role of marinobufagenin (MBG) in promoting arterial stiffness in response to high sodium intakes. MBG is produced in response to the ingestion of salt, and it inhibits Na/K ATPase to increase urinary sodium excretion. A consequence of this is the stimulation of vasoconstriction and oxidative stress, which may lead to arterial stiffness (Jablonski et al. 2013). At present, this is a relatively new area of research and hence it is unclear which mechanism(s) if any influence PWV.

2.3.2 - Association between dietary sodium and pulse wave velocity

Observational evidence supports a relationship between dietary sodium intake and PWV (Garcia-Ortiz et al. 2012; Polonia et al 2006; Sonoda et al. 2012). However, there is some debate as to whether the relationship between sodium and PWV is positive (Polonia et al. 2006; Sonoda et al. 2012) or J-shaped (Garcia-Ortiz et al. 2012). It is likely these different

conclusions are a result of different study methodologies. The two studies that found a positive relationship between sodium and PWV used urinary sodium excretion to measure of dietary sodium intake (Polonia et al. 2006; Sonoda et al. 2012). In contrast, the study that found a J-shaped relationship between sodium and PWV used a food frequency questionnaire (FFQ) to assess dietary sodium (Garcia-Ortiz et al. 2012). Dietary intake measures have different limitations and hence, this difference could explain the inconsistent findings For instance, FFQ is associated with a high rate of under and over reporting, which impacts the validity of results and thus it is the least accurate method of dietary intake assessment (McLean 2014) (for further details see section: 2.6 measuring dietary sodium and potassium intake).

2.3.3 - Effect of dietary sodium on pulse wave velocity

As with the observational studies, intervention trials have shown mixed results for a relationship between dietary sodium and PWV (for further detail see: table 1).

The majority of intervention studies have shown that sodium intake has no effect on PWV in pre-hypertensive or normotensive individuals (Dickinson et al. 2013; Dickinson et al. 2009; Gijsbers et al. 2015; Todd et al. 2012; Wang et al. 2014). Studies utilised an intervention with high sodium groups consuming 3450mg to 7079mg of sodium per day for one to six weeks. It is unlikely the null findings were due to study duration, as three of the five trials conducted an intervention for four weeks or more (Dickinson et al. 2013; Gijsbers et al. 2015; Todd et al. 2012), and changes in arterial stiffness can be seen as early as this (Todd et al. 2010). Furthermore, all studies saw a significant increase in sodium excretion (urinary sodium was 1050-3474mg/24-hours higher and sodium/creatinine was 9.6 units higher than low sodium groups), which suggests adherence to the intervention (Gijsbers et al. 2015; Todd et al. 2012; Wang et al. 2014).

In contrast to the above findings, several other research groups found sodium intake is positively associated with PWV (Avolio et al. 1986; He et al. 2009; Jablonski et al. 2013; Seals et al. 2001; Todd et al. 2010). Studies conducted interventions with high sodium groups consuming 3600 to 4600mg of sodium per day and found the high sodium groups had a 0.35-1.43m/s higher PWV than the low sodium groups. The reason why these research groups found a positive relationship, which contradicts other research remains unclear.

At present, there is not enough conclusive evidence to draw the exact relationship between dietary sodium and PWV, which is similar to the conclusions drawn from the dietary potassium and PWV literature.

Table 1: A description of intervention trials investigating the effect sodium has on pulse wave velocity [1]

First author, year	Intervention	Design	n	Population	Dose	Duration	Outcome measures	Results	Effect size
Dickinson, 2013	Low Na diet	Rct, sb Not pc	25	Overweight or obese, normotensive adults	Low Na diet (2340mg Na per day, from diet), versus high Na control diet (3510mg Na per day, from diet and supplement)	6 weeks low Na diet, versus 6 weeks control diet	Doppler, carotid-femoral PWV	• Urinary Na significantly lower on low Na diet than control diet • PWV was not significantly affected by low Na diet	• 1050mg/24h lower At week 6: • High Na 9.6±2.9m/s, versus low Na 10.7±3.5m/s
Dickinson, 2009	Low Na diet	Rct Not pc, db	29	Overweight or obese, normotensive adults	Low Na diet (1150mg Na per day, from diet), versus high Na control diet (3450mg Na per day, from diet)	2 weeks low Na diet, versus 2 weeks control diet	Doppler, carotid-femoral PWV	• Urinary Na significantly lower on low Na diet than control diet • PWV was not significantly affected by low Na diet	• 2121mg/24h lower At week 2: • High Na 10.5±3.1m/s, versus low Na 10.5±4.1m/s
Gijsber, 2015	Na supplement (tablet), and K supplement (tablet)	Rct, db, pc	36	Pre-hypertensive adults	Low Na control diet (2300mg K and 2400mg Na per day, from diet and placebo), versus Na supplement (5400mg Na per day from diet, and supplement), versus K supplement (5124mg K per day from diet and supplement)	4 weeks control diet, versus 4 weeks Na supplement, versus 4 weeks K supplement	SphygmoCor, carotid-femoral PWV	• Urinary Na significantly higher with supplement than control diet • Urinary K significantly higher with supplement than control diet • PWV not significantly affected by either intervention	• 2457mg/24h higher • 2457mg/24h higher At week 4: • High Na 13.1±2.9 m/s, versus low Na 13.1±3.0m/s

Todd, 2012	Na supplement (tomato juice)	Rct, sb, pc	25	Normotensive adults	Low Na control diet (1380mg Na per day, from diet and placebo), versus moderate Na supplement (3450mg Na per day, from supplement and diet), versus high Na supplement (4600mg Na per day, from supplement and diet)	4 weeks control diet, versus 4 weeks moderate Na supplement, versus 4 weeks high Na supplement	SphygmoCor, carotid-femoral PWV	• Urinary Na/creatinine significantly higher with moderate Na supplement than control diet • Urinary Na/creatinine significantly higher with high Na supplement than control diet • PWV was not significantly affected by either intervention	• 9.6 units higher • 17.1 units higher At week 4: • Low Na 6.6±0.1m/s, versus moderate Na 6.7±0.1m/s, versus high Na 7.0±1.2m/s
Wang, 2014	High Na diet (added salt to meals), and K supplement (tablet)	Crossover Not random or pc Db not reported	49	Normotensive, Chinese adults	Low Na control diet (1180mg Na per day, from diet), versus high Na diet (7079mg Na per day, from diet), versus high Na diet with K supplement (7079mg Na and 2340mg K per day, from supplement and diet)	1 week control diet, versus 1 week high Na diet, versus 1 week high Na diet with K supplement	Brachial-ankle PWV	• Urinary Na significantly higher with high Na diet than control diet • Urinary K significantly higher with K supplement than control diet • PWV was not significantly affected by either intervention	• 3474mg/24h higher • 1229mg/24h higher In 1 week: • Low Na reduced by 14.4±103.4cm/s, versus high Na reduced by 25.2±103cm/s

Avolio, 1986	Voluntary low Na diet	Not rct, db, pc	114	Normotensive, children and adults	Low Na diet, versus high Na control diet (mg Na not reported, as voluntary),	24.8 months low Na diet, versus 24.8 months control diet	Doppler, aortic to femoral PWV, femoral to tibial PWV, and brachial to radial PWV	• Urinary Na significantly lower on low Na diet than control diet • PWV significantly lower in all three locations on low Na diet than control diet	• 1564mg/24h lower • Not quantified
He, 2009	Low Na diet	Rct, db, pc	169	Hypertensive adults	Low Na diet (1955mg Na per day, from diet and placebo), versus high Na control diet (4025mg Na per day, from supplement and diet)	6 weeks low Na diet, versus 6 weeks control diet	Complior, carotid-femoral PWV	• Urinary Na significantly lower on low Na diet than control diet • PWV significantly lower on low Na diet than control diet • PWV significantly lower among blacks on low Na diet than control diet • PWV not affected by low Na diet among whites and Asians	• 1265mg/24h lower At week 6: • All: high Na 11.5± 2.3m/s, versus low Na 11.1±1.9m/s • Black: high Na 11.7±2.0m/s, versus low Na 11.2±1.8m/s • White: high Na 11.3±2.6 m/s, versus low Na 11.1±1.9m/s • Asian: high Na 11.3±2.2m/s, versus low Na 11.2±2.2m/s

Jablonski, 2013	Low Na diet	Rct, db, pc	11	Pre-hypertensive adults	Low Na diet (1500mg Na per day, from diet and placebo), versus high Na control diet (3600mg Na per day, from diet and supplement)	5 weeks low Na diet, versus 5 weeks control diet	Doppler, carotid-femoral PWV	• Urinary Na not reported • PWV significantly lower with low Na diet than control diet	• N/A At week 5: • Low Na 700±40cm/s, versus control diet 843+36cm/s
Seals, 2001	Low Na diet	Random Not crossover, db, pc	35	Post-menopausal, pre-hypertensive or hypertensive women	Low Na diet (2300mg Na per day, from diet), versus exercise (30min walking/day)	3 months low Na diet, versus 3 months exercise	Doppler, aortic to femoral PWV, and brachial to radial PWV	• Urinary Na significantly lower on low Na diet than on exercise • Aortic to femoral PWV significantly lower on low Na diet than on exercise • Brachial to radial PWV was not affected by low Na diet	• 874mg/24h lower In 3 months: • Low Na ~125cm/s lower, versus exercise ~25cm/s lower • Low Na ~50cm/s lower, versus exercise ~25cm/s higher
Todd, 2010	Na supplement (tomato juice)	Rct, sb, pc	35	Hypertensive adults	Low Na control diet (1380mg Na per day, from diet and placebo), versus moderate Na supplement (3450mg Na per day, from supplement with diet), versus high Na	4 weeks control diet, versus 4 weeks moderate Na supplement, versus 4 weeks high Na supplement	SphygmoCor, carotid-femoral PWV	• Urinary Na/creatinine significantly higher with moderate Na supplement than control diet • Urinary Na/creatinine significantly higher with high Na supplement	• 4.5 units higher • 8.2 units higher

					supplement (4600mg Na per day, from supplement with diet)			than control diet • PWV significantly higher with moderate Na supplement than low Na • PWV significantly higher with high Na supplement than low Na	At week 4: • Low Na 7.34±1.12m/s, versus moderate Na 7.63±0.95m/s, versus high Na 7.84±1.20m/s

[1] n = sample number, Na = sodium, K = potassium, rct = randomised crossover study, db = double blinded, pc = placebo controlled, sb = single blinded, PWV = pulse wave velocity, 24h = twenty-four hours, NA = not applicable

2.4 - Dietary potassium and pulse wave velocity

2.4.1 - Association between dietary potassium and pulse wave velocity

Few observational studies investigated the relationship between dietary potassium intake and PWV. Furthermore, there is little consensus regarding the relationship, with some findings supporting an inverse relationship between potassium and PWV (Dart and Qi 1995; Lennon-Edwards et al. 2014) and others suggesting there is no relationship (Garcia-Ortiz et al. 2012).

2.4.2 - Effect of dietary potassium on pulse wave velocity

Although few observational studies have been published, there are many intervention studies adding to the body of evidence regarding the relationship between dietary potassium and PWV. Intervention studies have reported inconsistent effects of high potassium diets on PWV (for further detail see: table 2). The majority of studies conclude that potassium supplementation has no effect on PWV (Berry et al. 2010; Blanch, Clifton, Petersen, Willoughby and Keogh 2014; Gijsbers et al. 2015; Wang et al. 2014); however, some report a reduction (Graham et al. 2014; He et al. 2010) and others an increase in PWV (Matthesen, Larsen, Vase, Lauridsen and Pedersen 2012).

There are some methodological differences that may explain the inconsistencies in results. The study that found an increase in PWV used the largest potassium supplement dose, of 3900mg per day in addition to the diet (Matthesen et al. 2012). Hence, this study may suggest large doses of potassium are detrimental to PWV. However, Matteson recruited the smallest sample size (n=21) (Matthesen et al. 2012). A power calculation was not given, but the study may have been underpowered and produced a result that we must interpret with caution. Furthermore, the studies that found no relationship between potassium and PWV, either supplemented participants' diets with high potassium foods (rather than supplements) (Berry et al. 2010; Blanch et al. 2014), or used a high sodium diet in their crossover design

(Gijsbers et al. 2015; Wang et al. 2014). All of which may have attenuated the effect potassium has on PWV. Adding to this, the studies that found a reduction in PWV both used a supplement dose of 2496mg of potassium (on top of usual intake) per day (Graham et al. 2014; He et al. 2010), and the trials that found no change added 780 to 2824mg of potassium per day to their participant's usual diet (Berry et al. 2010; Blanch et al. 2014; Gijsbers et al. 2015; Wang et al. 2014). This difference may imply that 2496mg of potassium per day is the optimal dose for the reduction of arterial stiffness. However, the difference in dose may only be accounting for some of the variation in results.

2.4.3 – Possible mechanism linking dietary potassium and pulse wave velocity

There is no clear mechanism explaining how potassium is linked to PWV and arterial stiffness. Currently, the literature supports two main theories. Firstly, high potassium levels may improve endothelial function by increasing nitric oxide synthesis, which causes vasodilation in response to shear stress (Blanch et al. 2014; He et al. 2010; Lennon-Edwards et al. 2014). However, Blanch, Clifton and Keogh (2015) did not find biomarker evidence to support this theory. Secondly, researchers suggest that potassium may reduce arterial stiffness via improvements in blood pressure (Blanch et al. 2015; Graham et al. 2014; Lennon-Edwards et al. 2014). However, it seems that this relationship has only been found in individuals with high cardiovascular disease risk (Blanch et al. 2015).

The lack of consensus regarding the mechanism linking dietary potassium and PWV supports the lack of consensus amongst observational and intervention studies that a link even exists. Therefore, no conclusions can be formed until further exploration in this area has been conducted

Table 2: A description of intervention trials investigating the effect potassium has on pulse wave velocity [1]

First author, year	Intervention	Design	n	Population	Dose	Duration	Outcome measures	Results	Effect size
Berry, 2010	K supplement (tablet) and high K diet	Rct, pc Db not reported	48	Pre-hypertensive adults	Low K control diet (585mg K per day from diet and placebo), versus moderate K diet (1365mg K per day from diet and placebo – 780mg more than control), versus high K diet (2145mg K per day from diet and placebo – 1560mg more than control), versus K supplement (585mg K per day from diet, and 1560mg K per day from supplement)	6 weeks control diet, versus 6 weeks moderate K diet, versus 6 weeks high K diet, versus 6 weeks K supplement	SphygmoCor, carotid-femoral PWV	• Urinary K significantly higher with moderate K diet than control diet • Urinary K significantly higher with high K diet than control diet • Urinary K significantly higher with K supplement than control diet • PWV was not significantly affected by either intervention	• 345mg/24h higher • 552mg/24h higher • 621mg/24h higher In 6 weeks: • Compared to baseline: moderate K diet 0.1m/s lower, versus high K diet 0.0m/s lower, versus K supplement 0.1m/s lower
Blanch, 2014	High K diet	Rct, db Not pc	35	Normotensive adults	Low K control diet (3110mg K per day, from diet), versus high K diet	6 days control diet, versus 6 days high K diet	SphygmoCor, carotid-femoral PWV	• Urinary K significantly higher with high K diet than control diet	• 1872mg/24h higher

					(5690mg K per day, from diet – 2580mg more than control)			• PWV was not significantly affected by high K diet	At day 6: • Control 6.0±0.9m/s, versus high K 6.1±0.9m/s
Gijsber, 2015	Na supplement (tablet), and K supplement (tablet)	Rct, db, pc	36	Pre-hypertensive adults	Low K control diet (2300mg K and 2400mg Na per day, from diet and placebo), versus Na supplement (5400mg Na per day, from diet and supplement), versus K supplement (2300mg K per day from diet, and 2824mg K per day from supplement)	4 weeks control diet, versus 4 weeks Na supplement, versus 4 weeks K supplement	SphygmoCor, carotid-femoral PWV	• Urinary Na significantly higher with supplement than control diet • Urinary K significantly higher with supplement than control diet • PWV not significantly affected by either intervention	• 2457mg/24h higher • 2457mg/24h higher At week 4: • Control 13.1±3.0, versus high K 12.7±2.6m/s
Wang, 2014	High Na diet (added salt to meals), and K supplement (tablet)	Crossover Not randomised, or pc Db not reported	49	Normotensive, Chinese adults	Low Na control diet (mg K not reported, and 1180mg Na per day, from diet), versus high Na diet (7079mg Na per day, from diet), versus K supplement with high Na	1 week control diet, versus 1 week high Na diet, versus 1 week K supplement with high Na diet	Brachial-ankle PWV	• Urinary Na significantly higher with high Na diet than control • Urinary K significantly higher with K supplement than control • PWV was not significantly affected by	• 3474mg/24h higher • 1229mg/24h higher In 1 week: • Control 14.4±103.4 cm/s

					diet (adds 2340mg K per day from supplement and 7079mg Na per day, from diet)			either intervention	lower, versus high K 28.2±104.8cm/s higher
Graham, 2014	K supplement (tablet)	Rct, db, pc	40	Adults with cardiovascular risk >10%	Low K control diet (mg K not reported, from diet and placebo), versus K supplement (adds 2496mg K per day)	6 weeks control diet, versus 6 weeks K supplement	SphygmoCor, carotid-femoral PWV	• Urinary K significantly higher with K supplement than control diet • PWV was significantly lower with K supplement than control diet	• 647mg/24h higher At week 6: • Control 8.3±0.1m/s, versus high K 7.9±0.2m/s
He, 2010	K supplement (tablet)	Rct, db, pc	42	Mild hypertensive adults	Low K control diet (mg K not reported, from diet and placebo), versus K chloride supplement (adds 2496mg K per day), versus K bicarbonate supplement (adds 2496mg K per day)	4 weeks control diet, versus 4 weeks K chloride supplement, versus 4 weeks K bicarbonate supplement	Complior, carotid-femoral PWV	• Urinary K significantly higher with K chloride supplement than control diet • Urinary K significantly higher with K bicarbonate supplement than control diet • PWV significantly lower with K chloride supplement,	• 1755mg/24h higher • 1872mg/24h higher At week 4: • Control 11.6±1.9m/s, versus K chloride

								than control diet • PWV significantly lower with K bicarbonate supplement, than control diet	10.8±1.7m/s, versus K bicarbonate 11.1±2.0m/s
Mattheson, 2012	K supplement (tablet)	Rct, pc Db not reported	21	Normotensive adults	Low K control diet (mg K not reported from diet and placebo), versus K supplement (adds 3900mg K per day)	28 days control diet, versus 28 days supplement	SphygmoCor, carotid-femoral PWV	• Urinary K was significantly higher with K supplement than control diet • PWV significantly higher with K supplement than control diet	• 390mg/sample higher At day 28: • Control 5.6±0.7m/s, versus high K 5.9±0.8m/s

[1] n = sample number, Na = sodium, K = potassium, rct = randomised crossover study, db = double blinded, pc = placebo controlled, sb = single blinded, PWV = pulse wave velocity, 24h = twenty-four hours

2.5 - Dietary sodium-to-potassium ratio and pulse wave velocity

There is evidence to suggest the dietary sodium-to-potassium ratio is a better predictor of cardiovascular disease outcomes (Castro and Raij 2013) and more strongly associated with blood pressure than the individual electrolytes (Tabara et al. 2015). In light of this research, there is the notion that the dietary sodium-to-potassium ratio may also have a stronger relationship with PWV.

The research groups that have investigated the association between the dietary sodium-to-potassium ratio and PWV have produced inconsistent results. Most of the evidence is observational, and does not support a relationship between the dietary sodium-to-potassium ratio and PWV (Lennon-Edwards et al. 2014; Redelinghuys et al. 2010; Tabara et al. 2015; Wang et al. 2014). However, two observational studies suggest the ratio is more strongly correlated with PWV than individual electrolytes (Garcia-Ortiz et al. 2012; Wei-Zhong, Ning-Ling and Hong-Yi 2012). Garcia-Ortiz et al. (2012) and Wei-Zhong et al. (2012) recruited hypertensive, diabetic participants, where the other studies recruited healthy samples. It is likely the response of a hypertensive, diabetic sample differs from a healthy sample. Therefore, sodium loading, with consistently low potassium intakes may only impact the arterial stiffness of populations at risk of cardiovascular disease. However, there is not enough consistent evidence to draw the exact relationship between the dietary sodium-to-potassium ratio and PWV.

2.6 - Measuring dietary sodium and potassium intake

2.6.1 - Urinary assessment

Twenty-four-hour urine sampling is used to calculate dietary potassium and sodium intake. It is calculated by multiplying total urine volume by urine-sodium concentration (Land et al. 2014). This method is the gold standard measure of dietary sodium (McLean 2014; Wen et al. 2015; Wielgosz et al. 2016) and potassium intake (Mizéhoun-Adissoda et al. 2016). This

is because the kidneys excrete approximately 90% of ingested sodium (Holbrook et al. 1984; McLean 2014; Wielgosz et al. 2016) and approximately 77% of ingested potassium (Holbrook et al. 1984; Mizéhoun-Adissoda et al. 2016). Unfortunately, 24-hour urine collection is burdensome and thus many participants return incomplete samples (Bentley 2006; McLean 2014; Wielgosz et al. 2016). No researcher has formulated an accurate method to overcome this limitation (Huang et al. 2014; McLean 2014; Wielgosz et al. 2016). Therefore, repeating samples, which is costly, is the only way to increase the accuracy of results (Bentley 2006; Huang et al. 2014).

Spot urine samples are less burdensome than 24-hour urine sampling. It only requires the collection of one urine sample in a day and hence it is very convenient and affordable. However, researchers are still looking at how well this predicts actual dietary sodium and potassium intake (McLean 2014). There are three traditional equations used to calculate estimated 24-hour sodium excretion from spot urine samples (Peng et al. 2016). From these studies, we can conclude that no matter the equation used to estimate 24-hour sodium, all three will correlate comparably with actual 24-hour sodium (Brown et al. r=0.505; Kawasaki et al. r=0.531; Tanaka et al. r=0.54) (Brown et al. 2013; Kawasaki, Itoh, Uezono and Sasaki 1993; Tanaka et al. 2002). The same is true for estimated 24-hour potassium (Kawasaki et al. r=0.443; Tanaka et al. r=0.56) (Kawasaki et al. 1993; Tanaka et al. 2002). However, a recent study concluded that all methods are still relatively inaccurate, and hence, more accurate methods are needed before population sodium intakes can be estimated from spot urine samples (Peng et al. 2016).

2.6.2 - Dietary assessment

Dietary assessment is a useful tool, as it not only quantifies dietary intake, but can be used to identify foods associated with high or low intakes (e.g. high sodium diets associated with high processed food intake (e.g. cheese, sauces)) (McLean 2014). Most of the literature

regarding dietary assessment of electrolyte intake focuses on measuring sodium, and the general consensus is that for sodium, dietary assessment is less accurate than 24-hour-urine sampling. This is likely because participants find it difficult to accurately measure salt added during cooking or at the table, participants misreport foods and portion sizes, and research assistants find it difficult to quantify sodium in home-cooked and processed foods as the sodium content can vary greatly (McLean 2014; Bentley 2006).

Out of the four dietary assessment methods, weighed diet records are considered the most accurate form of dietary assessment (Bentley 2006). A possible reason for this is diet records analyse current diet, as they are used prospectively, and they analyse usual intake, as multiple days are usually sampled (Bentley 2006). Huang et al. (2014) supports this, as they found diet records, in comparison to 24-hour sampling, only under-estimated sodium intake by 2%, where 24-hour diet recalls and FFQ under-estimated sodium intake by 8% and >10%, respectively. However, Huang et al. (2014) also found diet records were less accurate for measuring potassium and the sodium-to-potassium ratio than for measuring sodium. This is likely showing the limitations of using diet records. Some general limitations are than diet records require participants to be literate and numerate for accurate records, and highly motivated to overcome the high participant burden. Furthermore, many participants misreport or forget items and this often leads to incorrect estimation of dietary intake (Bentley 2006; McLean 2014).

In comparison to diet records, twenty-four-hour diet recalls are easy to administer, and usually have a high response rate, as participant burden is low (Bentley 2006). Unfortunately, 24-hour diet recall estimations of dietary sodium and potassium intake correlate weakly with 24-hour urine samples. Studies found correlation coefficients range from 0.16-0.46 for dietary sodium intake, and from 0.29-0.39 for dietary potassium intake (Mercado et al. 2015; Wen et al. 2015). An explanation for this is that 24-hour diet recalls are conducted by

interviewers, who can create bias (Bentley 2006). Additionally, 24-hour diet recalls do not account for day-to-day diet variation and hence, the results do not depict usual intake (Bentley 2006).

FFQ is a useful dietary assessment method for collecting data from large groups on usual intake, as they ask participants to answer questions about their diet over a long time period (McLean 2014). However, it is the least accurate method of dietary assessment. When measuring sodium and potassium intake, FFQ error is not only greater, but varies two times more than diet record error (Day, McKeown, Wong, Welch and Bingham 2001). One explanation for this is the prevalence of under and over reporting (McLean 2014). With this in mind, FFQ results must be interpreted with caution when reviewing literature.

2.7 - Summary

This literature review explored PWV and its possible association with dietary sodium and potassium intake. The first and second section defined PWV, a non-invasive measure of arterial stiffness, and highlighted that PWV is positively associated with cardiovascular morbidity and mortality. The third, fourth, and fifth section highlighted that there is disagreement regarding the relationship between dietary sodium, potassium, or sodium-to-potassium and PWV. Finally, the sixth section highlighted that all measures of dietary electrolyte intake have strengths and limitations that must be considered due to their impact on the validity of results.

3 - OBJECTIVE STATEMENT

The overall objective of this cross-sectional study was to investigate associations between sodium and potassium intake and PWV in the general population.

The specific aims were to:

1. Examine the association between sodium intake and PWV in the general population
2. Examine the association between potassium intake and PWV in the general population
3. Examine the association between the sodium-to-potassium ratio and PWV in the general population

4 - PARTICIPANTS AND METHODS

4.1 - Study design

This cross-sectional study was part of the Health And Bread Intervention Trials (HABIT) study, which was conducted in Dunedin, New Zealand, between January 2015 and December 2016. The aim of HABIT is to determine if changing the composition of bread, so it is lower in sodium, higher in nitrate (beetroot), or higher in L-arginine (nut) alters markers of cardiovascular disease in the general population. HABIT is a 12-week, randomised, controlled, parallel intervention trial with four arms – low sodium, beetroot, nut, or normal control bread. Participants were asked to continue with their usual diet, while substituting their usual bread for the study bread.

This cross-sectional study aimed to investigate the association between sodium and potassium intake, and PWV in the general population using the baseline data collected for the HABIT study.

4.2 - Ethics

The study received ethical approval from the University of Otago Human Ethics Committee (Health) in 2014 (H14_144). Prior to screening for eligibility, the participants were informed of the purpose and protocols of the study. If they felt they required more information, they were encouraged to discuss it further. Participants that chose to volunteer gave informed written consent to participate prior to any data collection.

4.3 - Participants

4.3.1 - Sample size

HABIT aimed to recruit 200 adult participants – 50 participants per intervention. This allowed HABIT to detect a clinically meaningful difference in systolic blood pressure (SBP) between any two groups, assuming a standard deviation of 0.8 or lower, a correlation between baseline and follow-up of r=0.85 or higher, with 80% power to detect an effect size using a

two-sided test at the 0.05 level, and allowing for a dropout rate of 10%. For the present study all participants recruited at the time of analysis were included in the study.

4.3.2 - Recruitment

HABIT participants were recruited via a database of previous study participants, community papers, local newspapers, flyers in shops, and via social media. Participants were recruited between January 2015 and December 2016.

4.3.3 - Eligibility

HABIT recruited participants from the general population, who were over 18 years of age, and reported they consumed four to six slices of bread per day.

Participants were excluded if they had a chronic disease (e.g. cancer, cardiovascular disease, diabetes), had food allergies, were on a energy-restricted diet, were under 18 years of age, were pregnant or lactating, consumed less than four slices of bread per day, or had an incomplete data set (i.e. missing a PWV measure, spot urine sample or diet record).

As of January 2016, sixty-five participants were eligible to participate in the study.

4.4 - Experimental protocol

Participants attended clinic visits at the University of Otago, Human Nutrition clinic. All visits occurred in the morning (0700-1000). This thesis utilized baseline visit one and two data (before any intervention was undertaken). Baseline visit one occurred two weeks prior to starting the intervention, and baseline visit two occurred the day before starting the intervention. At baseline, demographic data wer collected, anthropometric measures were taken, blood pressure was measured, carotid-femoral PWV was measured, and spot urine samples were collected. Participants were also given weighed-diet record equipment, and an accelerometer. They were taught how to complete the weighted diet record and use the accelerometer accurately. They returned their weighed-diet record and accelerometer at their next clinic visit.

4.5 - Outcome measures

4.5.1 - Anthropometry

Height was measured with a stadiometer, with the participant's head in the Frankfort plane and their heels against the wall. Weight and body composition was measured with a segmental body composition analyser (BC-418, Tanita, Illinois, United States of America). Height and weight were used to calculate body mass index (BMI=weight (kg) / height $(m)^2$).

4.5.2 - Blood pressure

The SphygmoCor 2000 (manufactured by AtCor Medical Pty Ltd, Sydney, Australia) was used to measure systolic and diastolic blood pressure. The equipment and software was calibrated annually, using the calibration kit provided by AtCor Medical Pty Ltd.

At all clinic visits, participants were asked to lie in the supine position, and rest for at least five minutes prior to the measurement. Firstly, the brachial cuff was positioned around the participant's upper arm, and was aligned with their brachial artery. The cuff was inflated, and systolic and diastolic blood pressure was measured automatically. Blood pressure was reported as the average of the three consecuative recordings at baseline.

4.5.3 - Pulse wave velocity

The SphygmoCor 2000 was also used to measure carotid-femoral PWV. As mentioned previously, the equipment and software was calibrated annually.

At all clinic visits, participants were asked to lie in the supine position, and rest for at least five minutes prior to the measurement. Firstly, the femoral cuff was positioned at the highest point on the participant's thigh. Secondly, a tape measure was used to measure the distance from the sternal notch to the femoral site (i.e. at the femoral cuff), and from the sternal notch to the carotid site (i.e. where the carotid pulse can be palpated). These two distances were entered into the SphygmoCor software, and the distance the pressure wave travelled along the aorta (d) was calculated automatically. Thirdly, the tonometer was held at

the carotid site to record carotid pressure waves, and the femoral cuff was used to record femoral pressure waves. The time difference between the beginning of the carotid pressure waves and the beginning of the femoral pressure waves was measured automatically (t). Finally, the SphygmoCor software calculated PWV with the equation below (AtCor Medical 2012). PWV was presented as the average PWV of the two consecutive recordings at baseline.

$$PWV = d/t$$

4.5.4 - Spot urine samples

At all clinic visits, participants were asked to complete a single-void morning urine sample. Their urine was transferred into a 20mL storage container. These were frozen at -20 degrees Celsius until later analysis.

From these samples, urinary sodium, potassium and creatinine excretion were measured. On the Cobas c 311 (manufactured by Roche, Rotkreuz, Switzerland), urinary sodium and potassium were measured using the ion selective electrode, and urinary creatinine was measured using the calorimetric assay. Urinary sodium, potassium and creatinine were used to estimate dietary sodium and potassium intake via the Tanaka method - see equation below (Tanaka et al. 2002; Hooft van Huysduynen et al. 2014; Toft et al. 2014).

Predicted 24-hour creatinine = (-2.04*age (year))+(14.89*weight (kg))+(16.14*height (cm)) – 2244.45

Estimated 24- hour sodium intake = $21.98*(X^{0.392})$
X = (spot urine sodium/spot urine creatinine)*predicted 24-hour creatinine

Estimated 24-hour potassium intake = $7.59*(Y^{0.431})$
Y = (spot urine potassium/spot urine creatinine)*predicted 24-hour creatinine

4.5.5 - Diet records

Prior to starting the intervention, HABIT participants were asked to complete a non-consecutive three-day weighed diet record, which included at least one weekend day. They were provided with electronic scales (Salter Housewares, HoMedics Group Ltd., Kent, United

Kingdom) a diet record book, and portion size photos. In addition to this, they were instructed on how to complete their weighed diet record by a nutritionist or dietitian. Participants were asked to record food and fluid intake at the time of consumption and return their diet record at the next clinic visit. At this visit, the records were checked, and any clarifications were requested.

Diet record data were entered into Kaiculator (2011). The listed foods were matched to the same or a similar food in the Kaiculator database (e.g. 'brown bread' matched with 'bread, wholemeal/wheatmeal'). Following this, the portion sizes were entered as weights, volumes or measure descriptors (e.g. two slices). Once all listed foods and portions were entered, Kaiculator (2011) calculated the nutrient composition of the diets using food composition data from 2014 Plant and Food Research. Dietary energy, carbohydrate, protein, fat, sodium, and potassium intake were reported as the average of the three reported days.

4.5.6 - Accelerometer

Prior to starting the intervention, HABIT participants were asked to wear an accelerometer (New-Lifestyles NL-1000, New-Lifestyles Inc., Lee's Summit, Missouri, USA) for seven consecutive days. This provided information on number of steps per day, distance travelled per day, and minutes of physical activity. The data were reported as the average of the seven days.

4.6 - Statistical analysis

Stata Statistical Software 14.1 (Statacorp LP, College Station Texas, USA) was used for all statistical analyses.

Descriptive, haemodynamic, and intake data was reported as mean and standard deviation. A two-sided t-test was performed to detect significant differences between males and females.

Univariate linear regression was used to determine the association between electrolyte intakes estimated from diet record and urine sample data (i.e. sodium, potassium, sodium-to-potassium ratio), and PWV. Multivariate linear regression was used to determine the adjusted relationship. To build the multivariate model, confounding factors were added individually, and were kept in the model if they contributed sufficiently (i.e. percentage of variance increased by more than one percent). The confounding factors included were: sex, age, BMI, and minutes of physical activity. Fat mass, steps per day, energy intake, fat intake, protein intake, and carbohydrate intake were not included in the multivariate model as they did not contribute sufficiently. In all models, regression coefficients, 95% confidence intervals and p-values were calculated for the electrolytes and confounding factors, and residuals of the models were assessed for homoscedasticity and normality.

5 - RESULTS

5.1 - Participant characteristics

5.1.1 - Descriptive data

Sixty-five HABIT participants were included in this cross-sectional study. Participant characteristics are reported in **Table 3, 4 and 5**. **Table 3** shows there were significant differences between males and females for a number of descriptors. Compared to males, females weighed significantly less, had significantly more body fat, and reported consuming significantly less energy, carbohydrate, protein and fat. There were no significant differences for BMI, steps and minutes of physical activity between the sexes.

Table 3: Characteristics of study participants (n=65) [1]

	Total	Male (n=34 (52.3%))	Female (n=31 (47.7%))	p-value [+]
Ethnicity (n (%))				
NZ European	42 (64.6%)	22 (64.7%)	20 (64.5%)	-
Maori/Pacific Island	2 (3.1%)	0 (0.0%)	2 (6.5%)	-
Other	20 (30.8%)	11 (32.4%)	9 (29.0%)	-
Age (years) [2]	34.5 ± 18.3	35.7 ± 18.2	33.0 ± 18.5	0.424
Weight (kg) [2]	73.6 ± 13.9	78.1 ± 12.7	68.5 ± 13.6	0.030 [+]
BMI (kg/m^2) [2]	24.9 ± 4.5	24.6 ± 3.1	25.2 ± 5.7	0.507
BMI (n (%))				
≤18.4 kg/m^2	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
18.5-24.9 kg/m^2	40 (61.5%)	19 (55.9%)	21 (67.7%)	-
25-29.9 kg/m^2	18 (27.7%)	13 (38.2%)	5 (16.1%)	-
≥30 kg/m^2	7 (10.8%)	2 (5.9%)	5 (16.1%)	-
Body fat (%) [2]	26.3 ± 10.5	19.3 ± 6.1	34.1 ± 8.7	<0.001 [+]
Steps per day [2]	9312.2 ± 4157.6	9612.3 ± 4759.2	8983.1 ± 3427.4	0.553
Physical activity (minutes/day) [2]	62.7 ± 24.4	61.1 ± 26.8	64.5 ± 21.8	0.662
Smokers (n (%))	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Energy intake (kJ/day) [2]	9152.9 ± 2915.3	10328.8 ± 2833.6	7863.3 ± 2452.1	<0.001 [+]
Carbohydrate intake (g/day) [2]	252.3 ± 83.2	285.6 ± 89.8	215.8 ± 57.2	<0.001 [+]
Protein intake (g/day) [2]	85.3 ± 34.8	100.3 ± 37.9	68.9 ± 21.8	<0.001 [+]
Fat intake (g/day) [2]	86.2 ± 32.6	96.7 ± 33.8	74.6 ± 27.3	0.012 [+]

[1] n=number of participants, BMI=body mass index
[2] mean ± standard deviation
[+] p-value <0.05 indicates statistically significant difference between males and females

5.1.2 - Haemodynamic data

As shown in **Table 4,** there were no significant differences between male and female haemodynamic data. The average blood pressure was not indicative of hypertension (<140/90mmHg (Zieman et al. 2005), and the average PWV did not suggest arterial stiffness (<10m/s (Rajzer et al. 2008).

Table 4: Haemodynamic data (n=65) [1] [2]

	Total	Male (n=34 (52.3%))	Female (n=31 (47.7%))	p-value [+]
SBP (mmHg)	126.5 ± 17.7	127.1 ± 17.2	125.8 ± 18.4	0.710
DBP (mmHg)	74.8 ± 11.2	51.3 ± 9.3	51.2 ± 14.0	0.991
PWV (m/s)	7.2 ± 1.6	7.5 ± 1.9	6.9 ± 1.2	0.243

[1] n=number of participants, SBP=systolic blood pressure, DBP=diastolic blood pressure, PWV=pulse wave velocity
[2] mean ± standard deviation
[+] p-value <0.05 indicates a statistically significant difference between males and females

5.1.3 - Urinary and dietary electrolyte intake data

Table 5 shows there were significant differences between male and female electrolyte data. Females had a significantly lower potassium intake as assessed by diet records than males. Furthermore, females had a significantly lower sodium intake as assessed by diet records and spot urine samples. Once adjusted for energy intake, these differences were no longer significant.

Table 5: Urinary and dietary electrolyte intake data (n=65) [1] [2]

	Total	Male (n=34 (52.3%))	Female (n=31 (47.7%))	p-value [+]
Urinary Na intake (mg/day) [3]	3021.3 ± 755.9	3204.2 ± 687.3	2820.6 ± 787.2	0.028 [+]
Dietary Na intake (mg/day) [4]	2783.7 ± 1067.4	3203.9 ± 1067.9	2322.8 ± 869.7	<0.001 [+]
Urinary K intake (mg/day) [3]	1954.7 ± 407.2	2002.2 ± 386.9	1902.6 ± 428.5	0.493
Dietary K intake (mg/day) [4]	3158.4 ± 1177.5	3500 ± 1242.2	2783.3 ± 991.3	0.008 [+]
Urinary Na:K ratio [3]	2.7 ± 0.7	1.6 ± 0.4	1.5 ± 0.4	0.147
Dietary Na:K ratio [4]	0.9 ± 0.4	1.0 ± 0.4	0.9 ± 0.4	0.267

[1] n=number of participants, Na=sodium, K=potassium
[2] mean ± standard deviation
[3] From spot urine samples (for further detail see: 4.5.4 spot urine samples)
[4] From 3-day weighed diet record (for further detail see: 4.5.5 diet records)
[+] p-value <0.05 indicates a statistically significant difference between males and females

5.2 - Adequacy of electrolyte intakes

5.2.1 - Sodium

Figure 1 and 2 present male and female sodium intakes in relation to New Zealand's Upper Limit (2,300mg per day for females and males (National Health and Medical Research and Ministry of Health 2006)). The figures show average sodium intakes, measured with diet records and spot urine samples, were above the Upper Limit.

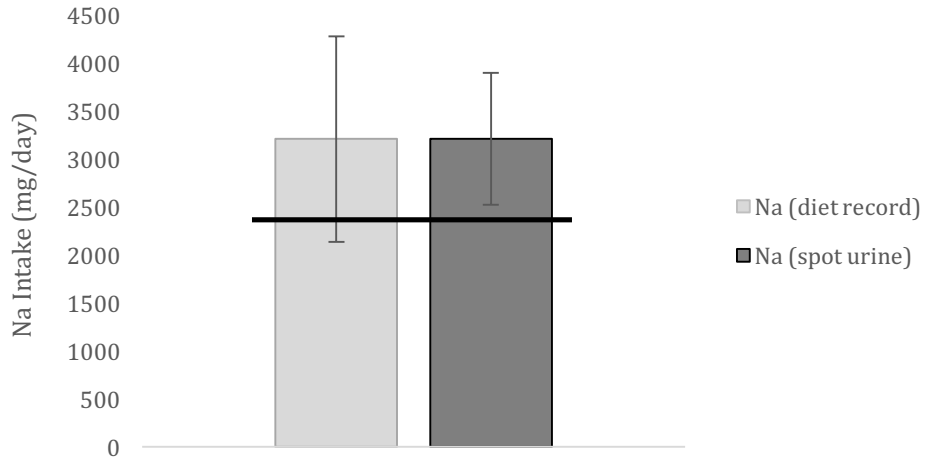

Figure 1: Male sodium intakes in relation to New Zealand's Upper Limit [1] [2]
[1] Na=sodium
[2] Black line–Ministry of Health advises an Upper Limit of 2,300mg of sodium per day (National Heatlh and Medical Research and Ministry of Health 2006)

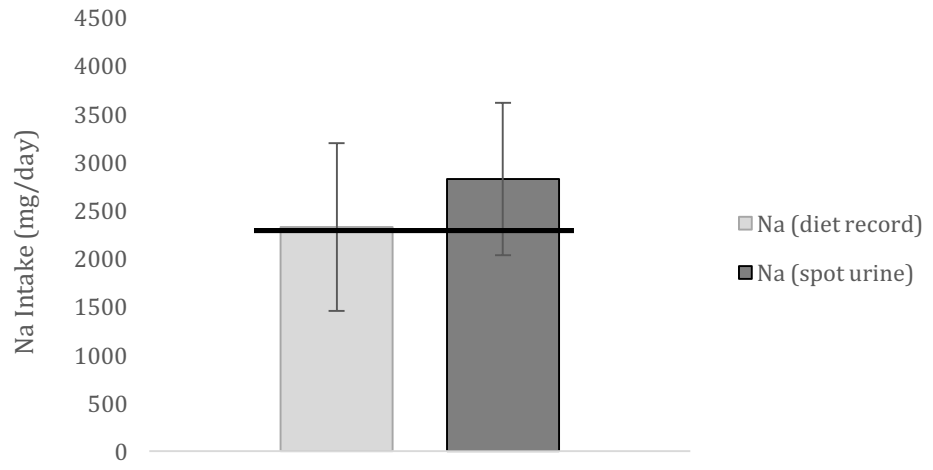

Figure 2: Female sodium intakes in relation to New Zealand's Upper Limit [1] [2]
[1] Na=sodium
[2] Black line–Ministry of Health advises an Upper Limit of 2,300mg of sodium per day (National Heatlh and Medical Research and Ministry of Health 2006)

5.2.2 - Potassium

Figure 3 and 4 present male and female potassium intakes in relation to New Zealand's Adequate Intake (2,800mg per day for females, 3,800mg per day for males (National Health and Medical Research and Ministry of Health 2006)). The figures show average potassium intakes as assessed by spot urine samples and diet records, were below the Adequate Intake.

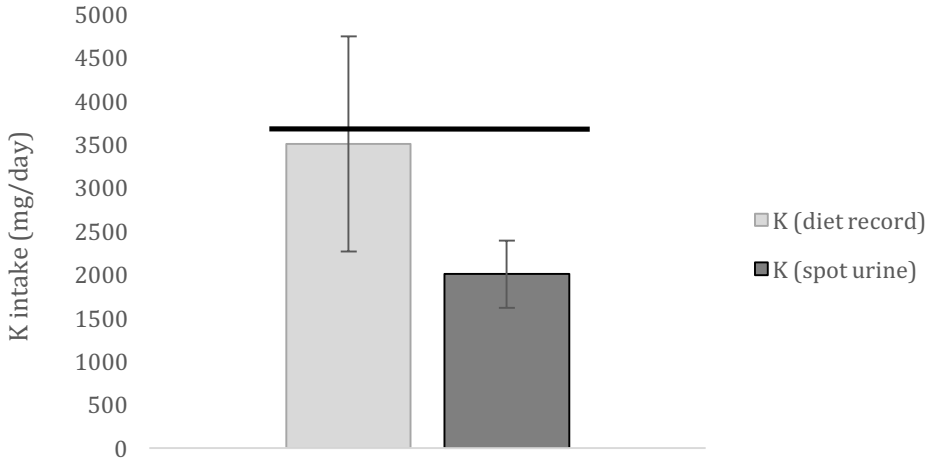

Figure 3: Male potassium intakes in relation to New Zealand's Adequate Intake [1] [2]
[1] K=potassium
[2] Black line-Ministry of Health recommends an Adequate Intake of potassium of 3,800mg per day for males (National Health and Medical Research and Ministry of Health 2006)

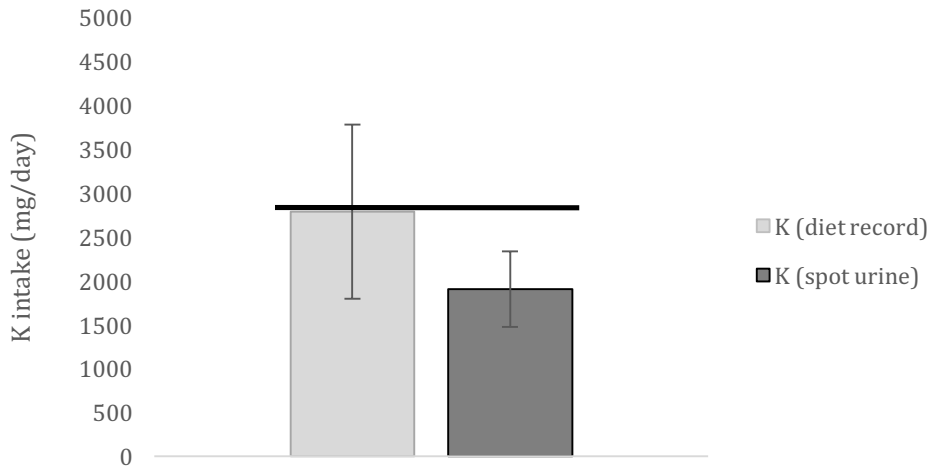

Figure 4: Female potassium intakes in relation to New Zealand's Adequate Intake [1] [2]
[1] K=potassium
[2] Black line-Ministry of Health recommends an Adequate Intake potassium of 2,800mg per day for females (National Health and Medical Research and Ministry of Health 2006)

5.3 - Association between dietary electrolytes, and PWV

Table 6 shows sodium, potassium, and the sodium-to-potassium ratio, measured with diet records, were not significantly associated with PWV.

Table 6: Predicting pulse wave velocity from dietary electrolyte intake, demographics, anthropometrics, and physical activity (n=65) [1]

Variables	Regression coefficient (95% CI)	p-value [+]
4.a Na Model		
Univariate regression		
Dietary Na intake (g) [2]	0.17 (-0.21, 0.55)	0.364
Age- and sex-adjusted regression		
Dietary Na intake (g) [2]	0.16 (-0.16, 0.49)	0.318
Sex	-0.28 (-0.97, 0.41)	0.424
Age (years)	0.06 (0.04, 0.07)	<0.001 [+]
Multivariate regression		
Dietary Na intake (g) [2]	0.15 (-0.17, 0.47)	0.344
Sex	-0.30 (-0.97, 0.38)	0.388
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.03 (-0.05, 0.11)	0.431
Physical activity (minutes)	-0.01 (-0.03, 0.00)	0.051
4.b K Model		
Univariate regression		
Dietary K intake (g) [2]	0.20 (-0.14, 0.54)	0.246
Age- and sex-adjusted regression		
Dietary K intake (g) [2]	0.09 (-0.20, 0.37)	0.539
Sex	-0.36 (-1.02, 0.30)	0.277
Age (years)	0.05 (0.04, 0.07)	<0.001 [+]
Multivariate regression		
Dietary K intake (g) [2]	0.11 (-0.17, 0.39)	0.430
Sex	-0.35 (-1.00, 0.30)	0.286
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.03 (-0.05, 0.11)	0.456
Physical activity (minutes)	-0.02 (-0.03, -0.001)	0.042 [+]
4.c Na:K Model		
Univariate regression		
Dietary Na:K ratio [2]	-0.17 (-1.23, 0.90)	0.757
Age- and sex-adjusted regression		
Dietary Na:K ratio [2]	0.11 (-0.74, 0.95)	0.802
Sex	-0.41 (-1.05, 0.22)	0.199
Age (years)	0.05 (0.04, 0.07)	<0.001 [+]
Multivariate regression		
Dietary Na:K ratio [2]	0.00 (-0.83, 0.83)	0.998
Sex	-0.43 (-1.056 0.20)	0.174
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.03 (-0.05, 0.11)	0.424
Physical activity (minutes)	-0.01 (-0.03, 0.0001)	0.052

[1] Na=sodium, BMI=body mass index, K=potassium
[2] From 3-day weighed diet record (for further detail see: 4.5.5 diet records)
[+] p-value <0.05 indicates a statistically significantly significant association with PWV

5.4 - Association between urinary electrolytes, and PWV

Table 7 shows the sodium-to-potassium-ratio, measured with spot urine samples, was not significantly associated with PWV. Individually, sodium and potassium, measured with spot urine samples, were significantly associated with PWV. However, the effect sizes were

small (a 23mg (1mmol) increase in urinary sodium was associated with a 0.02m/s increase in PWV; and a 39mg (1mmol) increase urinary potassium is associated with a 0.04m/s increase in PWV), and when adjusted for age, sex, BMI and minutes of physical activity the relationships were not significant.

Table 7: Predicting pulse wave velocity from urinary electrolyte intake, demographics, anthropometrics, and physical activity (n=65) [1]

Variables	Regression coefficient (95% CI)	p-value [+]
5.a Na Model		
Univariate regression		
Urinary Na intake (per 23mg) [2]	0.02 (0.01, 0.03)	0.002 [+]
Age- and sex-adjusted regression		
Urinary Na intake (per 23mg) [2]	0.01 (-0.004, 0.02)	0.221
Sex	-0.33 (-0.97, 0.32)	0.314
Age (years)	0.05 (0.03, 0.07)	<0.001 [+]
Multivariate regression		
Urinary Na intake (per 23mg) [2]	0.00 (-0.01, 0.02)	0.366
Sex	-0.35 (-0.99, 0.29)	0.281
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.02 (-0.06, 0.10)	0.580
Physical activity (minutes)	-0.01 (-0.03, 0.003)	0.054
5.b K Model		
Univariate regression		
Urinary K intake (per 39mg) [2]	0.04 (-0.001, 0.07)	0.058
Age- and sex-adjusted regression		
Urinary K intake (per 39mg) [2]	0.01 (-0.02, 0.04)	0.585
Sex	-0.41 (-1.04, -0.23)	0.205
Age (years)	0.05 (0.04, 0.07)	<0.001 [+]
Multivariate regression		
Urinary K intake (per 39mg) [2]	0.01 (-0.03, 0.04)	0.765
Sex	-0.41 (-1.05, 0.23)	0.202
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.02 (-0.07, 0.12)	0.625
Physical activity (minutes)	-0.01 (-0.03, -0.002)	0.048 [+]
5.c Na:K Model		
Univariate regression		
Urinary Na:K ratio [2]	0.38 (-0.22, 0.99)	0.209
Age- and sex-adjusted regression		
Urinary Na:K ratio [2]	0.15 (-0.33, 0.64)	0.533
Sex	-0.40 (-1.03, 0.24)	0.218
Age (years)	0.05 (0.04, 0.07)	<0.001 [+]
Multivariate regression		
Urinary Na:K ratio [2]	0.14 (-0.35, 0.63)	0.573
Sex	-0.41 (-1.03, 0.21)	0.192
Age (years)	0.04 (0.02, 0.06)	<0.001 [+]
BMI (kg/m^2)	0.04 (-0.04, 0.12)	0.367
Physical activity (minutes)	-0.01 (-0.03, 0.001)	0.063

[1] Na=sodium, BMI=body mass index, K=potassium

[2] From spot urine samples (for further detail see: 4.5.4 spot urine samples)

[+] p-value <0.05 indicates a statistically significantly significant association with PWV

5.5 - Association between confounding factors, and PWV

Table 6 and 7 show age was significantly and positively associated with PWV (i.e. a 1 year increase in age was associated with a 0.05m/s increase in PWV). Furthermore, these tables show minutes of physical activity was significantly associated with PWV in both potassium models (i.e. a 1-minute increase in physical activity was associated with a 0.01-0.02 decrease in PWV).

6 - DISCUSSION

The findings of this small study suggest that sodium, potassium, and the sodium-to-potassium ratio, measured with diet records and spot urine samples, do not independently predict PWV. Instead, the present study demonstrates age is the primary determinant of PWV.

6.1 - Sodium and pulse wave velocity

The present findings are in line with previous research by Dickinson and Keogh (2013), Dickinson et al. (2009), Gijsber et al. (2015), Todd et al. (2012), and Wang et al. (2014). They utilised interventions comparing low sodium groups consuming 1150-2400mg per day, with high sodium groups consuming 3450-7079mg per day, and found sodium has no effect on PWV. Dickinson and Keogh (2013), Dickinson et al. (2009), Todd et al. (2012), and Wang et al. (2014) recruited normotensive adults, which matches the present normotensive sample (mean blood pressure (BP): 127/75mmHg). The previous and present sample likely respond similarly to differing sodium intakes, as they are both normotensive, and this may explain why the present study supports this research.

In contrast, He et al. (2009), Jablonski et al. (2013), Seals et al. (2001), and Todd et al. (2010), conducted interventions with pre-hypertensive and hypertensive adults. They compared low sodium groups consuming 1380-2300mg per day, with high sodium groups consuming 3600-4600mg per day, and found high sodium groups have a 0.3-1.43m/s higher PWV, suggesting a positive relationship between sodium and PWV. This is supported by Polonia et al. (2006), and Sonada et al. (2012), who found a positive association in cross-sectional trials.

A possible reason why the present study did not find a positive association between PWV and sodium could be due to the methods used to quantify sodium intake. Sodium intake was measured with diet records (i.e. 595-6681mg per day) and spot urine samples (i.e. 1233-4655mg per day). Firstly, the diet record sodium range observed in this study shows extreme

low, and high intakes. This may have been caused by the limitations of using diet records. On its own, dietary assessment is not considered a reliable measure of sodium intake (University of Otago and Ministry of Health 2011), as sodium is difficult to quantify due to the numerous ways it is included in the diet (McLean 2014; Weilgosz et al. 2016). Most of the previous sodium and PWV literature used dietary assessment, with 24-hour urine urine samples - the gold standard measure of electrolyte intake (McLean 2014; Wen et al. 2015; Wielgosz et al. 2016). However, the present study used spot urine samples. Spot urine samples are known to be less reliable than 24-hour samples, as they may only represent intake over a few hours, may produce inaccurate data when used on small samples, and have only been validated in a few populations (McLean 2014). Given the limitations of these sodium intake measures, it is likely the present sodium intake data is unreliable, and hence may be why the present study supports a null finding.

Another reason for the discrepancy in results could be sample size. Previous observational studies recruited significantly more participants than the current study, with sample sizes ranging from 426-911 participants (Polonia et al. 2006; Sonada et al. 2012), compared to the present sample of 65. Although, Polonia et al. (2006), and Sonada et al. (2012) do not report a power calculation, it is likely their sample size provided enough power to detect a clinically meaningful difference in the PWV of groups with differing sodium intakes. The present study required 200 participants to detect a clinically meaningful difference, but only 65 participants were included, as that was the number recruited at the time of analysis. The present study was therefore likely to be underpowered, which may be why no relationship between PWV, and sodium, measured with diet records and spot urine samples was observed.

6.2 - Potassium and pulse wave velocity

The present findings support research by Berry et al. (2010), Blanch et al. (2014), Gijsber et al. (2015), and Wang et al. (2014), who showed potassium supplements, with doses ranging from 780-2824mg per day (on top of usual intake) have no effect on PWV. It is possible this study supports this research, as the interventions simulate a usual diet, like what was presently observed. Firstly, Berry et al. (2010) and Blanch et al. (2014) used high potassium foods, instead of supplements, and Gijsber et al. (2015) and Wang et al. (2014) used high sodium intakes in conjunction with high potassium diets. All of which may have attenuated potassium's effect and may explain why the present findings support this research.

However, several other research groups contradict the above finding. He et al. (2010) conducted an intervention study comparing a usual diet group with potassium supplementation groups, consuming an additional 2496mg of potassium per day (on top of usual intake), as potassium chloride or bicarbonate. They found the chloride group had a 0.8m/s, and the bicarbonate group had a 0.5m/s lower PWV. This finding was later supported by Graham et al. (2014), who used the same potassium dose, and found it reduced PWV by 0.4m/s, suggesting an inverse relationship between potassium and PWV. In line with this, Dart and Qi (1995), and Lennon-Edwards et al. (2014) found an inverse association using cross-sectional study designs.

A possible reason why the present study did not find an association between PWV and potassium, measured with spot urine samples and diet records, could be the present observation of usual intake, rather than supplemental intake. The present study observed usual potassium intakes, from diet records ranging between 948-6637mg per day, and from spot urine samples ranging between 1241-3004mg per day. In contrast, previous intervention trials supplemented usual intake with additional 780-2824mg of potassium per day. Given this, the

present levels of potassium observed may not have been high enough to find a significant inverse association.

Another reason for the discrepancy between previous and present results could be the methods used to quantify potassium intake. Previous observational studies used 24-hour urine sampling (Lennon-Edwards et al.; Dart and Qi 1995), again the gold-standard measure of dietary potassium intake (Mizéoun-Adissoda et al. 2016). As mentioned previously, the present study used spot urine samples and diet records. Spot urine samples are relatively inaccurate due to their limitations mentioned previously (Peng et al. 2016) (for further details see: 6.1 sodium and pulse wave velocity). Furthermore, dietary assessment is an accepted measure of potassium intake (University of Otago and Ministry of Health 2011), but, diet records are still associated with under and over reporting due to social bias, forgetfulness, and participant burden (Bentley 2006; McLean 2014). Given this, the present potassium intake data may be less reliable than previous research using 24-hour sampling, and may be why the present study does not support an association.

6.3 - Sodium-to-potassium ratio and pulse wave velocity

The present findings are in line with research by Lennon-Edwards et al. (2014), Redelinghey et al. (2010), and Tabara et al. (2015), who found no association between the sodium-to-potassium ratio and PWV in cross-sectional studies on healthy samples. This is further supported by Wang et al. (2014), who conducted an intervention comparing a high sodium group consuming 7079mg per day, with a high potassium group consuming the same amount of sodium, and 2340mg of supplemental potassium per day.

It is likely the present study supports research by Lennon-Edwards et al. (2014), Redelinghey et al. (2010), and Tabara et al. (2015), as they conducted cross-sectional studies and recruited a sample with similar characteristics to the present (i.e. present mean age of 35 years, compared to 41 years; present mean BP of 127/75mmHg, compared to 124/75mmHg;

present mean carotid femoral PWV of 7.2m/s, compared to 6.1 m/s). Where, research who found a positive association (Garcia-Ortiz et al. 2012; Wei Zhong, Ning-Ling and Hong-Yi 2012) conducted cross-sectional studies, recruiting samples with high cardiovascular risk (i.e. diabetic and hypertensive adults). Given this, it is possible the previous and present findings are explained by the different way hypertensive and normotensive samples respond to differing electrolyte intakes. However, at present, there is not enough evidence to support this conclusion, and thus future investigation is required.

6.4 - Age predicts PWV

The present study found age is a significant predictor of PWV, with PWV increasing by 0.05m/s each year. This finding supports the current body of evidence showing a positive association between age and PWV (Alghatrif et al. 2013; Cecelja and Chowienczyk 2009; Alecu et al. 2006). Benetos et al. (2002), and Asmas et al. (1995), for example, all report that on average, PWV increases by 0.1m/s per year. Similarly, Alecu et al. (2006) reports PWV increases gradually and continuously until 50 years of age.

6.5 - Sodium and potassium intake comparison

The present study found mean sodium intakes, measured with spot urine samples and diet records, are above the Upper Limit suggesting many New Zealanders are consuming too much sodium. Also, mean potassium intakes are below the Adequate Intake, indicating our sample's potassium intake may be less than adequate (National Health and Medical Research and Ministry of Health 2006). The Ministry of Health set sodium and potassium recommendations as there is evidence showing an increase in BP with a high sodium, or low potassium intake (Sacks et al. 2001; Stamleter et al. 1991; Whelton et al. 1997). As our sample's mean sodium and potassium intakes do not meet Ministry of Health criteria, our sample is at risk of increased BP.

High sodium, low potassium diets are typical in New Zealand (McLean 2013; McLean et al. 2015; University of Otago and Ministry of Health 2011). Most recently, McLean et al. (2015) showed a sample of New Zealand had a mean sodium intake of 3865mg per day for men and 2934mg per day for women, and a mean potassium intake of 3031mg per day for men, and 2436mg per day for women. These data supports the present findings, and highlights the need for strategies to reduce sodium and increase potassium intake.

6.6 - Strengths and limitations

The present study had a number of strengths. Firstly, the present study investigated the relationship between PWV and the sodium-to-potassium ratio. Few studies have investigated whether the ratio is a better predictor of PWV, and hence the present study addressed this gap. Secondly, this study used carotid-femoral PWV, which is the gold-standard measure of arterial stiffness (Mancia et al. 2013). The use of this method most likely increased the reliability of our data.

There are several limitations of this study that should be considered when interpreting the results. Firstly, this is a cross-sectional study. Therefore, these findings cannot be used to establish a longitudinal or causal relationship. Secondly, the present study was limited by its sample size, as it did not have enough power to detect a clinically meaningful difference between the PWV of groups with differing electrolyte intakes. Thirdly, spot urine samples were used instead of 24-hour urine samples. As mentioned above, the estimation equations have only been validated in three samples – an elderly Japanese, all female and a Danish population (Hooft van Huysduynen et al. 2014; Tanaka et al 2002; Toft et al. 2014). As these populations do not match the present sample, the present electrolyte data, measured with spot urine samples, is likely to be less reliable.

6.7 - Implications for future research

The present study identified areas that have implications for future research. Firstly, there are inconsistencies between present and previous findings, and this has identified the need for continued investigation of the relationship between dietary electrolytes and PWV. Intervention trials provide stronger evidence due to their ability to determine cause and effect. Therefore, it is advantageous for more intervention trials that are adequately powered, to be conducted in this area, so the exact relationship can be determined.

Secondly, the use of spot urine samples could have influenced the present findings, as the prediction equations have only been validated in three populations (Tanaka et al. 2002; Hoof van Huysduynen et al. 2014). Researchers should continue to validate the equations in a range of populations. This will ensure spot urine prediction equations can be matched to samples, and produce more reliable data. This is important because a validated urinary measure is needed to counter the high participant burden associated with 24-hour sampling.

Thirdly, the present study was limited by its sample size. It is important that future researchers recruit a sufficient number of participants to ensure their study has sufficient power. The main reason the present study had a small sample size was because only 65 participants had been recruited by the time of analysis. Therefore, future studies should ensure adequate time is allocated to recruitment before analysis is undertaken.

6.8 - Conclusion

The present study investigated the association between dietary electrolytes, and PWV. These findings suggest there is no association between sodium, potassium or the sodium-to-potassium ratio, and PWV. Given this, the present findings indicate dietary electrolytes do not predict arterial stiffness, and rather indict that prediction of PWV is multi-factorial. However, the present study was limited by its cross-sectional design, small sample size, dietary

assessment and urine collection method. Therefore, the exact relationship between dietary electrolytes and PWV cannot be determined until further research is conducted.

7 - APPLICATION TO PRACTICE

The present findings suggest sodium and potassium intake do not determine arterial stiffness independently. However, in light of previous research, the exact relationship between electrolyte intake and arterial stiffness remains unclear. As dietitians rely on new findings to guide their evidence-based practice, the present findings will add to the evidence base regarding micronutrients (i.e. sodium and potassium), and cardiovascular health (i.e. arterial stiffness). However, it will not change the way dietitians practice, as there is insufficient information to support a low sodium, high potassium diet for improvements in arterial stiffness. However, there is still plenty of research to support such a diet in terms of improving BP (Tabara et al. 2015), which dietitians should continue to promote one-on-one and in group settings.

The present study also supports previous literature (McLean 2013; McLean 2015; University of Otago and Ministry of Health 2011), as it found a sample of New Zealand's population had an average sodium intake above the Upper Limit (>2300mg per day (National Health and Medical Research and Ministry of Health 2006) and an average potassium intake below the Adequate Intake (<2800mg per day for women, and <3800mg per day for men (National Health and Medical Research and Ministry of Health 2006). This highlights the need for a population-based strategy to reduce sodium and increase potassium intake. This would require the skills of public health dietitians to develop food reformulation (e.g. salt reduction in bread), consumer education (e.g. label reading), and behavioural change strategies (e.g. reducing table salt use, increasing fruit and vegetable intake) (Lofthouse, Te Morenga and McLean 2016).

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