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The Impact of Urinary Uromodulin (Tamm-Horsfall Protein) on Renal Function and Haemodynamic Factors in a Community Sample with a High Prevalence of Hypertension

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2024
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Charles, Aaliah. (2024). The Impact of Urinary Uromodulin (Tamm-Horsfall Protein) on Renal Function and Haemodynamic Factors in a Community Sample with a High Prevalence of Hypertension [Master`s dissertation, University of the Witwatersrand, Johannesburg]. WIReDSpace. https://hdl.handle.net/10539/46875

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THE IMPACT OF URINARY UROMODULIN (TAMM-HORSFALL PROTEIN) ON RENAL FUNCTION AND HAEMODYNAMIC FACTORS IN A COMMUNITY SAMPLE WITH A HIGH PREVALENCE OF HYPERTENSION

Aaliah Charles

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in the fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 2024

Declaration

I, **Aaliah Charles**, declare that this dissertation is my own, unaided work unless otherwise stated. It is being submitted for the Degree Masters of Science in Medicine at the University of the Witwatersrand, Johannesburg. The work in this dissertation has not been submitted before for any degree or examination in this university or any other university.

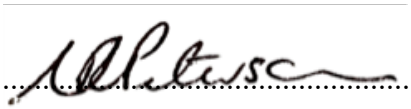
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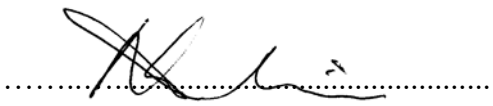
I certify that the studies contained in this dissertation have been approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg under the ethics approval numbers M02-04-72 and renewed as M07-04-69, M12-04-108, M17-04-01 and M22-03-93.



Vernice R. Peterson (Supervisor)

21-02-2024

Date



Angela J. Woodiwiss (Supervisor)

21-02-2024

Date

Dedication

I'd like to thank the Almighty, the source of all wisdom and guidance, for giving me the strength and inspiration to complete this dissertation.

I dedicate this dissertation:

To my late Grandmother, Mareldia Moosa, whose enduring support, motivation, and active role in my education will forever be etched in my heart. Your legacy of wisdom and encouragement will forever be my guiding light.

To my parents Aamila and Rafick, my pillars of strength, for their unwavering support through every twist and turn. Your love has been my motivation, and your strength has been my refuge. Thank you for always being present, believing in me and being the foundation upon which I built my aspirations.

To my siblings, Thaakir and Na'eelah, for their support and camaraderie throughout this process. Your encouragement has been a constant source of strength and I am grateful for the bond we share.

Conference Presentations

"Impact of Urinary Uromodulin Concentrations on Renal and Haemodynamic Parameters in a Community of African Ancestry with a High Prevalence of Hypertension."
Authors: Aaliah Charles, Manishka Reddy, Tanisha Naidoo, Monica Gomes, Angela J Woodiwiss, and Vernice R Peterson. Southern Africa Hypertension Society 2nd NextGen Virtual Spring School held on the 12th October 2023. (First Prize – Best Oral Presenter).

Abstract

Populations of African ancestry have a high prevalence of primary hypertension and its comorbidities. As they primarily exhibit a volume-dependent form of hypertension, the role of nephron components needs to be explored. Uromodulin is a potential biomarker for renal function and tubular reserve; however, its relationship with renal function, haemodynamic parameters, and hypertension in a population of African ancestry is unknown.

I therefore explored the relationship between urinary uromodulin (uUMOD) concentration and renal as well as hemodynamic parameters in an African community with a high prevalence of volume-dependent hypertension.

Haemodynamics (central pressures [SphygmoCor], echocardiographic aortic velocity and diameter in the outflow tract), uUMOD concentrations (ELISA assay), renal function (creatinine clearance from 24-hour urine [n = 370]) were determined in a community of African ancestry (n = 397).

No relationships between uUMOD concentrations and renal function, age, BMI, BP or hypertension were noted. However, uUMOD concentrations were higher in females than males, even after adjusting for confounders (P = 0.0007). An inverse relationship was observed between stroke volume (SV) and uUMOD (P = 0.0023). This inverse relationship was independent of confounders and present in hypertensives (P= 0.007) but not normotensives (P = 0.43). Hypertensives had a higher SV than normotensives (P = 0.047).

In a community sample with a high prevalence of volume-dependent primary hypertension, uUMOD was inversely related to SV, particularly in hypertensives. Although uUMOD is not a biomarker for renal function in this population, these data suggest the need to investigate mechanisms linking uUMOD to SV to assist in identifying novel pathways to better treat volume-dependent hypertension.

Acknowledgements

I would like to express my sincere gratitude to the following individuals for their invaluable contribution to the successful completion of this dissertation:

- The late Professor Gavin Norton, whose wisdom, guidance, and early encouragement significantly influenced my understanding of this project.
- Dr Vernice Peterson, my primary supervisor, and Professor Angela Woodiwiss, my co-supervisor, for their guidance, insightful feedback, and continuous support throughout the research process. Their commitment and expertise played a pivotal role in shaping this dissertation.
- Dr Monica Gomes, a senior technician in the School of Physiology, for her guidance and assistance with the ELISA.
- Dr Grace Tade, for her continuous support, encouragement, and advice throughout the process of my dissertation.
- All the individuals that assisted with the collection and retrieval of samples, your contribution is deeply appreciated.
- Dr Wessel (Wikus) du Toit, a postdoctoral fellow in the Cardiovascular Pathophysiology and Genomics Research Unit, for his thoughtful advice, comments, and feedback on my drafts.
- I extend my gratitude to NRF for funding this study and making it possible to pursue this research.

Table of Contents

Declaration i

Dedication ii

Conference Presentations iii

Abstract iv

Acknowledgements v

List of Figures ix

List of Tables x

Abbreviations xi

CHAPTER 1: Literature Review 1

1.1 Broad Overview 2

1.2 Characteristics of Hypertension in Individuals of African Ancestry 2

1.3 Role of the Kidney in Hypertension 3

1.3.1 Renal Control in Regulating BP 3

1.3.2 Abnormal Renal Control and BP Regulation 4

1.3.3 Biomarkers used to Assess Renal Function 6

1.4 UMOD 6

1.5 UMOD Function in Ion Transport 7

1.5.1 UMOD and NKCC2 7

1.5.2 UMOD and ROMK 10

1.5.3 UMOD and NCC 11

1.6 UMOD and Renal Determinants 11

1.6.1 Impact of UMOD on eGFR 11

1.6.2 Impact of UMOD on Fractional Excretion of Sodium ($FENa^+$) 12

1.7 UMOD and Haemodynamic Determinants 12

1.8 Urinary UMOD vs Serum UMOD as a Marker for Renal Function in Individuals of African Ancestry 13

1.9 Study Rationale 14

1.10 Objectives 14

CHAPTER 2: Methodology 16

2.1 Study Group 17

2.2 Demographic, Anthropometric and Clinical Data 17

2.3 Renal Function 17

2.4 Urinary UMOD Concentrations 18

2.5 Haemodynamic Assessment 20

2.5.1 BP Measurements 20

2.5.2 SphygmoCor Measurements 20

2.5.3 Echocardiograph Measurements 23

2.6 Data Analysis 26

CHAPTER 3: Results 27

3.1 Characteristics of Participants 28

3.2 Factors associated with uUMOD Concentrations (Bivariate Correlations) 28

3.3 Characteristics of Male verses Female Individuals 33

3.4 Sex Differences in uUMOD Concentrations (Multivariate Adjusted) 33

3.5 Characteristics of Hypertensive and Normotensive Individuals 33

3.6 Impact of Hypertension on uUMOD Concentrations (Multivariate Adjusted) 38

3.7 Relationship of uUMOD Concentrations and SV in Normotensive versus Hypertensive Individuals 38

CHAPTER 4: Discussion 43

4.1 Summary of Findings 44

4.2 Factors Contributing to Variations in uUMOD Concentrations 444

4.2.1 The Impact of Ethnicity on uUMOD Concentrations 44

4.2.2 Impact of Sex on uUMOD Concentrations 45

4.2.3 The Impact of Chronic Medication on uUMOD Concentrations 46

4.2.4 Impact of Salt Intake and Salt Sensitivity on uUMOD Concentrations 47

4.2.5 The Impact of Various Methods on uUMOD Concentrations 48

4.3 Cardiovascular Implications of uUMOD Concentrations....49
4.4 Renal Implications of uUMOD Concentrations....51
4.5 Potential Limitations....53
4.6 Future Studies....53
4.7 Clinical Relevance of Findings....54
4.8 Conclusion....54
Reference:....55
Website References:....71
Appendices **72**
Appendix 1: Human Ethics Approval....73
Appendix 2: Turnit-in Report (Plagiarism Report)....74

List of Figures

CHAPTER 1: Literature Review **1**

Figure 1.1: Mechanisms of Normal and Impaired Renal Pressure Natriuresis……..……..5

Figure 1.2: Nephron Structure and Key Ion Channels in DCT and TAL of the Loop of Henle..……....8

Figure 1.3: Potential Functions of UMOD (adapted from Takata & Isomoto, 2024…….....9

CHAPTER 2: Methodology **16**

Figure 2.1: Diagrammatic Representation of the Sandwich ELISA Process Used to Quantify uUMOD ..…….19

Figure 2.2: SphygmoCor System Interfaced with a Computer Running SphygmoCor, Version 9.0, Software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia)……. 21

Figure 2.3: SphygmoCor Validated Incorporated Function Conversion of Radial Waveforms to Central Aortic Waveforms……..…….....22

Figure 2.4: ACUSON SC2000 Diagnostic Ultrasound System (Siemens Medical Solutions, USA, Inc.) used to acquire Aortic Velocity and Diameter Measurements……..24

Figure 2.5: ACUSON SC2000 Echocardiographic Image Illustrating Aortic Velocity Waveform.…….....25

CHAPTER 3: Results **27**

Figure 3.1: Comparisons of Multivariate Adjusted uUMOD Concentrations in Males and Females of African Ancestry with a High Prevalence of Hypertension…….....…36

Figure 3.2: Comparisons of Multivariate Adjusted uUMOD Concentrations in Hypertensive and Normotensive Individuals in a Community sample of African Ancestry with a High Prevalence of Hypertension…….....400

List of Tables

CHAPTER 3: Results **27**

Table 3.1: General and Renal Characteristics of a Community Sample of African Ancestry with a High Prevalence of Hypertension……...29

Table 3.2: Haemodynamic Characteristics in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)……...30

Table 3.3: Bivariate Relationships Between General Characteristics and uUMOD Concentrations as well as Renal Characteristics and uUMOD Concentrations in a Community Sample of African Ancestry with a High Prevalence of Hypertension…….31

Table 3.4: Bivariate Relationships between Haemodynamic Parameters and uUMOD Concentrations in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)……...32

Table 3.5: Comparison of General and Renal Characteristics in Male and Female Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension…..34

Table 3.6: Comparison of Haemodynamic Characteristics in Male and Female Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension……..35

Table 3.7: General and Renal Characteristics of Normotensive and Hypertensive Individuals in a Community Sample of Africa Ancestry with a High Prevalence of Hypertension…....37

Table 3.8: Haemodynamic Characteristics of Hypertensive and Normotensive Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)……...39

Abbreviations

ACEI	Angiotensin converting enzyme inhibitor
ANGII	Angiotensin II
AT1	Angiotensin II type 1 receptor
BMI	Body mass index
BP	Blood pressure
CCB	Calcium channel blockers
CKD	Chronic kidney disease
Cl^-	Chloride
CO	Cardiac output
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCT1	Early part of the distal convoluted tubule
DM	Diabetes mellites
EDV	End diastolic volume
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
$FENa^+$	Fractional excretion of sodium
GFR	Glomerular filtration rate
GWAS	Genome-wide association studies
HbA1c	Percentage of glycated haemoglobin
HR	Heart rate
HRP	Horseradish peroxide
IHP	Interstitial hydrostatic pressure
K^+	Potassium
MAP	Mean arterial pressure
MI	Myocardial infarction
Na^+	Sodium
NaCl	Sodium chloride or salt
NCC	Sodium chloride cotransporter
NKCC2	Sodium potassium chloride cotransporter 2
NO	Nitric oxide

PD	Period
Q	Aortic flow
RAAS	Renin angiotensin aldosterone system
RAP	Right arterial pressure
RAS	Renin angiotensin system
ROMK	Renal outer medullary potassium channel
SAS	Statistical analysis software
SBP	Systolic blood pressure
SKIPOGH	Swiss Kidney Project on Genes and Hypertension
SOWETO	Southwest township
SPRINT	Systemic Blood Pressure Intervention Trail
sNa^+	Serum sodium
sUMOD	Serum uromodulin
SV	Stroke volume
SVR	Systemic vascular resistance
TAC	Total arterial compliance
TAL	Thick ascending limb of the loop of Henle
uNa^+	Urinary sodium
UMOD	Uromodulin
UMOD	Uromodulin gene
uUMOD	Urinary uromodulin
Zc	Characteristic impedance

CHAPTER 1: Literature Review

1.1 Broad Overview

Cardiovascular disease (CVD) is the world's leading cause of death, accounting for approximately 32% of all mortalities (WHO, 2019). A major risk factor for almost all acquired CVD is hypertension (hypertension) (Giles et al., 2009; Kjeldsen, 2018). Hypertension is characterised as the chronic and persistent elevation in arterial blood pressure (BP), commonly defined as BP readings of 140/90 mm Hg or higher (Giles et al., 2009; Kjeldsen, 2018; Wright et al., 2018). Hypertension is also known to affect numerous organs including the brain, heart, and kidneys (Giles et al., 2009; Kjeldsen, 2018; Wright et al., 2018). Over a billion adults worldwide are hypertensive, with developing countries accounting for three-quarters of cases due to low-middle income, limited health resources, poor BP management, and a lack of hypertension awareness (Arima et al., 2011; Ibrahim & Damasceno, 2012; WHO, 2022). South Africa, as a developing country, is heavily impacted by the burden of hypertension, with evidence of an increased prevalence of hypertension from 38.4% in 2012 to 48.6% in 2016 (Kandala et al., 2021). Interestingly, the highest prevalence of hypertension is reported in urban populations of African ancestry (Kandala et al., 2021). Apart from a higher prevalence of hypertension being observed in individuals of African ancestry, individuals of this ethnicity have also been observed to have a unique form of hypertension that is more susceptible to hypertension-related comorbidities, such as strokes and CVD (Fuchs, 2011; Musemwa & Gadegbeku, 2017; Nwankwo et al., 2013).

1.2 Characteristics of Hypertension in Individuals of African Ancestry

Predetermined genetic alterations in renal tubular sodium (Na^+) reabsorption, which enhance renal fluid retention, are thought to play a significant role in hypertension among individuals of African ancestry (Young et al., 2005). Malan et al., (2021) demonstrated a strong association between the age-related increase in systemic blood flow and the alterations of renal tubular function. This association between systemic blood flow and renal tubular function has been identified as a major determinant of increased BP in individuals of African ancestry (Malan et al., 2021). The impact of systemic blood flow in this cohort was also observed to have a far greater effect on BP than systemic vascular resistance (SVR)(Mmopi et al., 2021; Woodiwiss et al., 2020). Recent studies on individuals of African ancestry have further illustrated a unique renin-independent, volume-dependent form of sustained hypertension with a high stroke volume (SV) (Malan et al., 2021; Woodiwiss et al., 2020). Consequently, individuals of African ancestry have been observed to be more resistant to antihypertensive drugs,

specifically drugs targeting the renin-angiotensin-aldosterone system (RAAS) (Fuchs, 2011; Musemwa & Gadegbeku, 2017; Nwankwo et al., 2013). BP in this cohort is thus difficult to control and frequently necessitates multiple drug combinations, which include diuretics (Flack et al., 2010; Malan et al., 2021; Woodiwiss et al., 2020). Although renal mechanisms explain the marked increase in systemic blood flow, current antihypertensive medication, including diuretics, does not effectively sustain a decrease in systemic blood flow (Bochud et al., 2009; Malan et al., 2021). The lack of response to specific antihypertensive therapies alongside the unique form of hypertension observed in individuals of African ancestry thus raises the questions of whether the underlying cause of hypertension is being treated in this cohort and whether novel renal mechanisms should be considered.

1.3 Role of the Kidney in Hypertension

Obesity, advancing age, ethnicity, salt sensitivity, elevated salt and alcohol consumption, along with low potassium and calcium intake, have all been identified as major risk factors for developing hypertension and hypertension-related comorbidities (Carretero & Oparil, 2000; Oparil et al., 2018). With the kidney being a major regulator of BP and electrolyte homeostasis, renal mechanisms are thought to play a vital role in the genesis of sustained primary hypertension in individuals of African ancestry (Boder et al., 2021; Malan et al., 2021; Woodiwiss et al., 2020). According to a study by Guyton et al., (1972), the effect of intravascular volume is primarily responsible for a chronic steady-state BP. Vast physiological and experimental evidence demonstrates the significant involvement of renal control and renal perfusion pressure in maintaining arterial circulation and BP (Gu et al., 2009; Wadei & Textor, 2012).

1.3.1 Renal Control in Regulating BP

Traditionally, an elevation in BP increases the renal arterial pressure, initiating a cascade of events that lead to an augmentation in renal Na^+ excretion (Figure 1.1A) (Boder et al., 2021). This renal process, which directly regulates plasma Na^+ concentrations, is referred to as pressure natriuresis (Burke et al., 2014; Seriki, 2018). Although the glomerular filtration rate (GFR) and the filtered load of Na^+ are well autoregulated, alterations in renal perfusion pressure are thought to arise from adjustments in tubular Na^+ reabsorption (Burke et al., 2014). Increased renal perfusion pressure has thus been suggested to increase the medullary blood flow which subsequently increases the renal interstitial hydrostatic pressures (IHP) (Figure 1.1A)(Baek &

Kim, 2021). Additionally, an intrarenal mechanism whereby increased renal perfusion pressure reduces tubular reabsorption of Na^+ has been found to relate to renal autocoids like nitric oxide (NO), renal kinins, renal prostaglandins E^2, and angiotensin II (ANGII) (Figure 1.1A)(Baek & Kim, 2021; Granger & Alexander, 2000; Granger et al., 2002).With the renin-angiotensin system (RAS) being a strong modulator of pressure-natriuresis and thus long-term BP control, ANGII (a Na^+ retaining hormone and product of RAS) plays a vital role in regulating BP (Baek & Kim, 2021; Natarajan & Jose, 2008). In this regard, raised renal perfusion pressures may facilitate a decrease in Na^+ reabsorption through decreasing intrarenal ANGII (Figure 1.1A) (Baek & Kim, 2021). Apart from ANGII, prostaglandin E^2, kinin, and NO released in response to raised renal perfusion pressure, are also known to influence BP (Figure 1.1A)(Baek & Kim, 2021). Nitric Oxide plays a role in pressure natriuresis through 2 mechanisms: (1) directly inhibits Na^+ reabsorption and (2) indirectly increases medullary blood flow and renal IHP (Baek & Kim, 2021). Together, these mechanisms act simultaneously to decrease the tubular reabsorption of Na^+, subsequently decreasing the tubular reabsorption of water (Figure 1.1A)(Burke et al., 2014; Wadei & Textor, 2012). This in turn reduces the blood volume ultimately resulting in BP homeostasis (Figure 1.1A) (Burke et al., 2014; Wadei & Textor, 2012).

1.3.2 Abnormal Renal Control and BP Regulation

Impairment in pressure-natriuresis impacts BP by abnormally increasing the tubular reabsorption of Na^+, consequently increasing blood volume, SV, and cardiac output (CO) via a Frank-Starling shift (Campese & Park, 2006; Hall, 2016). Renal abnormalities that increase Na^+ reabsorption or kidney damage (nephron injury or loss) have thus been strongly associated with sustained hypertension (Campese & Park, 2006; Hall, 2016). Inappropriate activation of the RAS is one example of a renal abnormality that results in an abnormal retention of Na^+, and thus is implicated in sustained hypertension (Figure 1.1B) (Manrique et al., 2009). This is supported by a study that illustrated that the chronic infusion of ANGII directly into the kidney causes hypertension with impaired natriuresis, marked by a shift in the pressure-natriuresis relationship (Hall, 1986; Seriki, 2018). In this regard, the binding of ANGII to ANGII type 1 receptors (AT_1) in the kidneys induces the local activation of RAS (Figure 1.1B) (Navar et al., 1994; Seriki, 2018). This local activation further increases the production of ANGII in the lumen of renal tubules, resulting in autocrine and paracrine stimulation of renal transporters

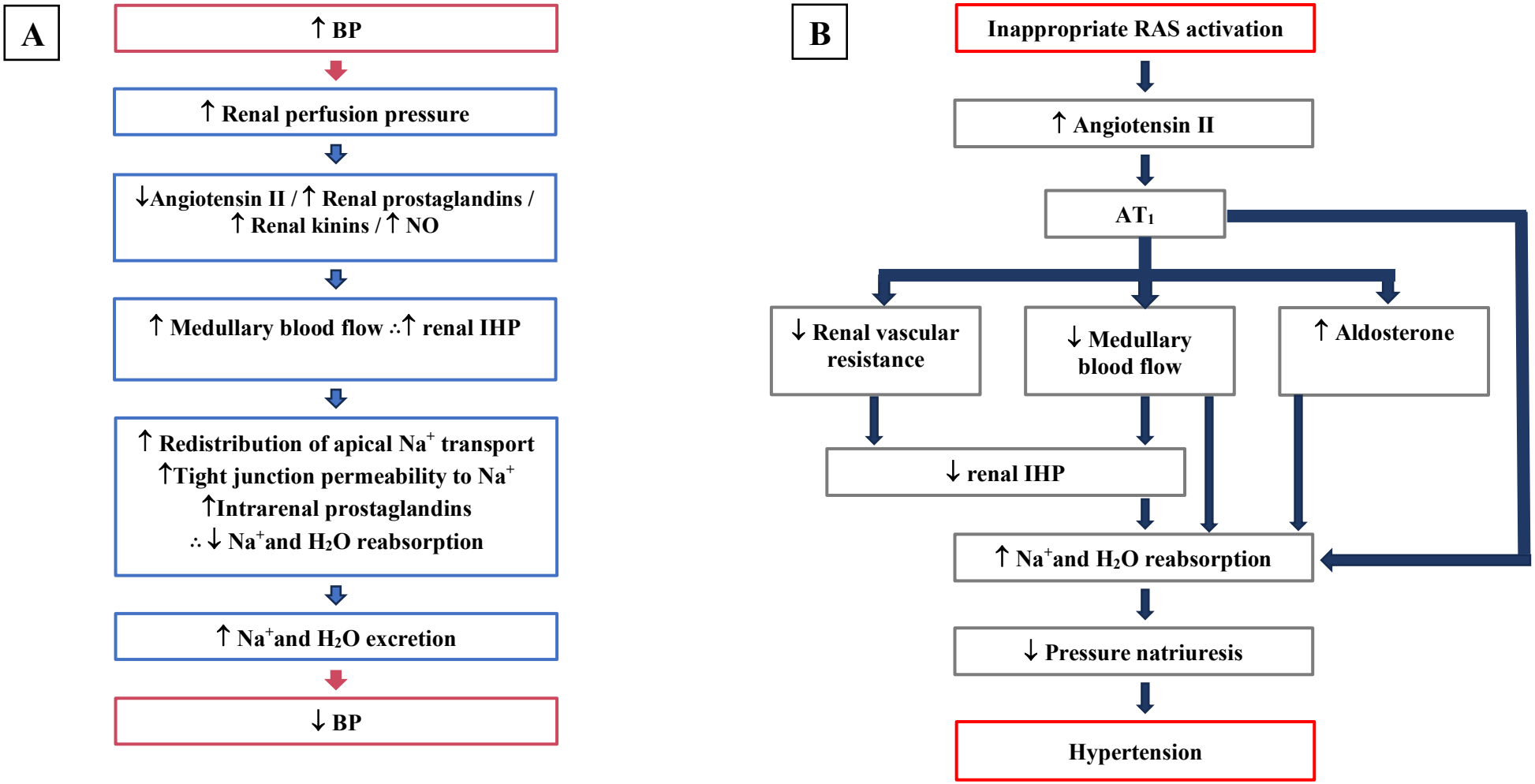


Figure 1.1: Mechanisms of Normal and Impaired Renal Pressure Natriuresis.

(A) Schematic representation of the mechanism underlying normal pressure natriuresis. (B) Schematic representation of the impaired renal pressure natriuresis mechanism leading to hypertension (Adapted from https://abdominalkey.com/role-of-the-kidneys-in-hypertension/#R24-39 and (Baek & Kim, 2021). The blue arrow illustrates the pathways activated by AT1 in response to angiotensin II. Abbreviations: BP, Blood Pressure; IHP, Interstitial Hydrostatic Pressure; Na^+, Sodium; RAAS, Renin-Angiotensin-Aldosterone System; AT1, angiotensin II type 1 receptor.

(Navar et al., 1994; Seriki, 2018). In this way, ANGII directly contributes to sustained hypertension through increasing Na^+ tubular reabsorption (Figure 1.1B)(Seriki, 2018).

Additionally, ANGII indirectly contributes to sustained hypertension in three ways: (1) through reducing renal vascular resistance, (2) by decreasing medullary blood flow and (3) by increasing the level of aldosterone release from the adrenal cortex (Figure 1.1B)(Seriki, 2018). The resulting reduction in renal vascular resistance and medullary blood flow leads to increased tubular Na^+ reabsorption through decreasing renal IHP (Figure 1.1B) (Seriki, 2018). Moreover, the ANGII-induced vasoconstriction of afferent arteries of the juxtamedullary nephrons diminishes the inner medullary blood flow which further enhances Na^+ reabsorption to increase medullary tonicity (Seriki, 2018). Given the renin-independent form of hypertension prevalent among individuals of African ancestry, there is a crucial need to investigate alternate renal-related mechanisms underlying hypertension. The unique form of hypertension in people of African ancestry thus necessitates the identification of novel renal biomarkers and the consideration of novel renal impairments that affect renal Na^+ handling as the cause of hypertension in this cohort (Campese & Park, 2006; Hall, 2016).

1.3.3 Biomarkers used to Assess Renal Function

With the most common form of hypertension in individuals of African origin thought to occur due to renal fluid retention or renal abnormalities, it is vital to understand the state of various components of the nephron. One way in which renal function is assessed is through investigating urinary biomarkers such as Na^+ excretion and creatinine clearance alongside calculating the estimated glomerular filtration rate (eGFR) (Devuyst & Bochud, 2015). Where well-established markers are available for glomerular filtration, tubular function markers are clinically less established (Devuyst & Bochud, 2015). Uromodulin (UMOD) also referred to as the Tamm-Horsfall protein, however, has piqued researchers' interest as a possible biomarker for renal function, tubular reserve, hypertension, and chronic kidney disease (CKD) (Devuyst & Bochud, 2015).

1.4 UMOD

UMOD is the most abundant protein excreted in urine (Devuyst & Bochud, 2015). This protein is primarily transcribed in the renal tubules of the thick ascending limb (TAL) of the loop of Henle (90%) and, to a lesser extent, in the early part of the distal convoluted tubule (DCT1)(10%)(Figure 1.2A) (Boder et al., 2021; Köttgen et al., 2009; Tokonami et al., 2018).

The TAL of the loop of Henle and the DCT1 have complex networks that work together to regulate ion homeostasis (Figure 1.2B and 1.2C) (Boder et al., 2021; Gonzalez-Vicente et al., 2019). The dysregulation of ion channels (including the sodium-potassium-chloride cotransporter [NKCC2] (Figure 1.2C), renal outer medullary potassium channel [ROMK] (Figure 1.2C) and sodium chloride cotransporter [NCC] (Figure 1.2B)) in the TAL and DCT1 is considered to be a major contributor to the pathogenesis of hypertension and renal abnormalities (Boder et al., 2021; Gonzalez-Vicente et al., 2019). Although the exact function of UMOD is unknown, UMOD has been linked to water and electrolyte balance, kidney innate immunity, protection against urinary tract infection, renal stone formation, and the regulation of ion channels on the apical membrane like NKCC2, ROMK, and NCC (Figure 1.3) (Devuyst & Bochud, 2015; Padmanabhan et al., 2010; Tokonami et al., 2018).

1.5 UMOD Function in Ion Transport

1.5.1 UMOD and NKCC2

Several studies, both human and animal-based, have linked NKCC2 to BP control and salt-sensitive hypertension (Glorioso et al., 2001; Haque et al., 2011; Jung et al., 2011). While NKCC2-dependent Na^+ and chloride (Cl^-) ion reabsorption are vital for the functioning of the TAL, Na^+, and Cl^- homeostasis, and BP control, the mechanism in which the NKCC2 ion channel is regulated remains unclear (Figure 1.2C) (Ares et al., 2011). It is, however, suggested that the amount of apical NKCC2 or the changes in affinity of NKCC2 for transported ions may play a role in its regulation (Ares et al., 2011). Only 3 molecular mechanisms are known to affect the amount of apical NKCC2: (1) protein-protein interaction, (2) protein trafficking, and (3) phosphorylation (Ares et al., 2011). UMOD has been suggested to play a crucial role in the apical transportation of NKCC2 with studies demonstrating a co-localisation of UMOD with NKCC2, indicating a possible protein-protein interaction (Boder et al., 2021). This interaction was further supported by a study on *UMOD* (uromodulin gene) knock-out mice which demonstrated a possible role of UMOD in the trafficking and phosphorylating of NKCC2 (Mary et al., 2022; Mutig et al., 2011). UMOD's role in NKCC2 trafficking was evident in this study, as the amount of apical NKCC2 ion channel was drastically reduced in *UMOD* knock-out mice, with 47% more intracellular NKCC2 located in subapical vesicles compared to wild-type mice (Mary et al., 2022; Mutig et al., 2011). From this, it is evident that UMOD plays a vital role in the transportation of subapical NKCC2. Additionally, UMODs role

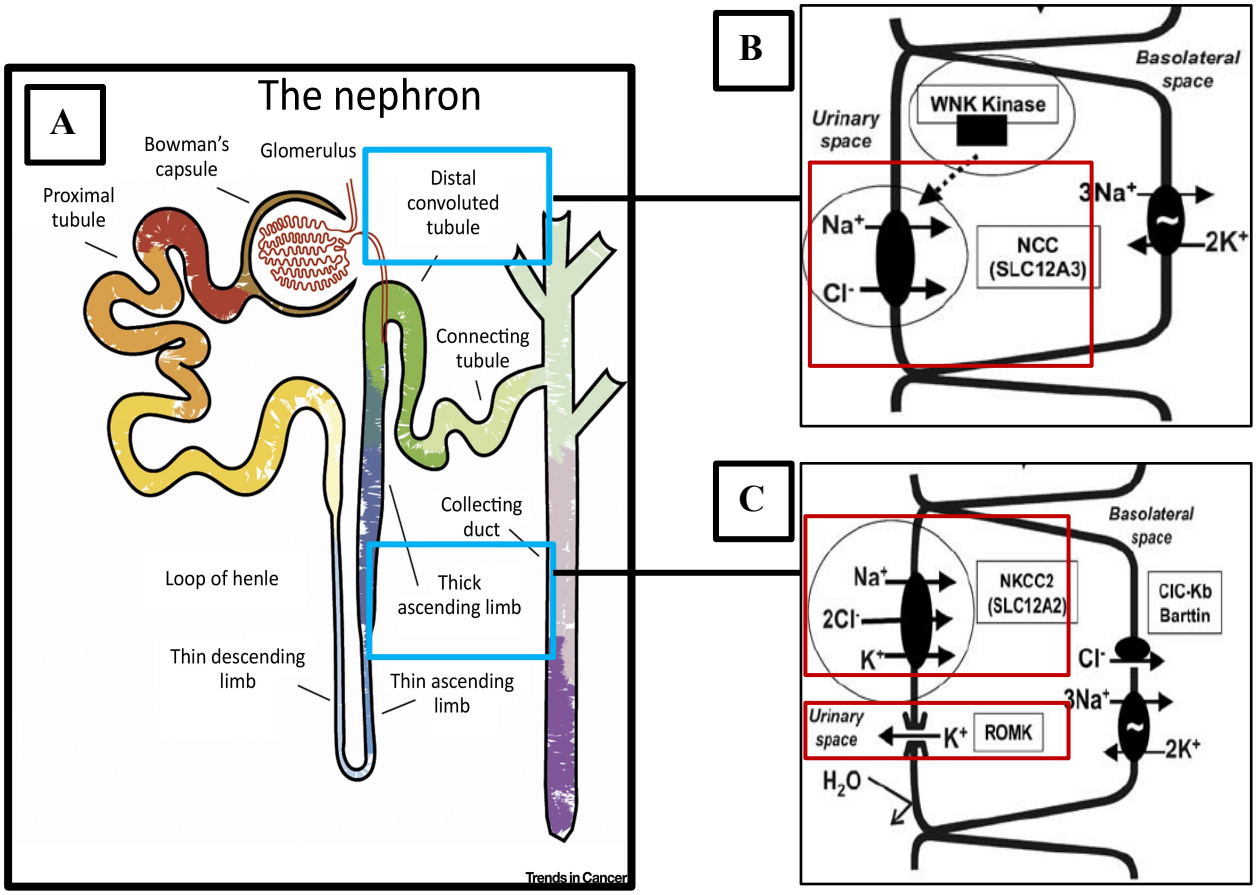


Figure 1.2: Nephron Structure and Key Ion Channels in DCT and TAL of the Loop of Henle.
(A) Diagram of a nephron, (B) enlarged section of the distal convoluted tubule illustrating the NCC ion channel and (C) enlarged section of the thick ascending limb of the loop of Henle illustrating the NKCC2 ion channel and ROMK. The blue boxes in (A) illustrate the part of the nephron that are enlarged in boxes B and C. The red boxes illustrate the ion channels that are regulated by UMOD and work together to ensure homeostasis. Abbreviations: NCC, sodium chloride cotransporter; NKCC2, sodium-potassium-chloride cotransporter; ROMK, renal outer medullary potassium channel.

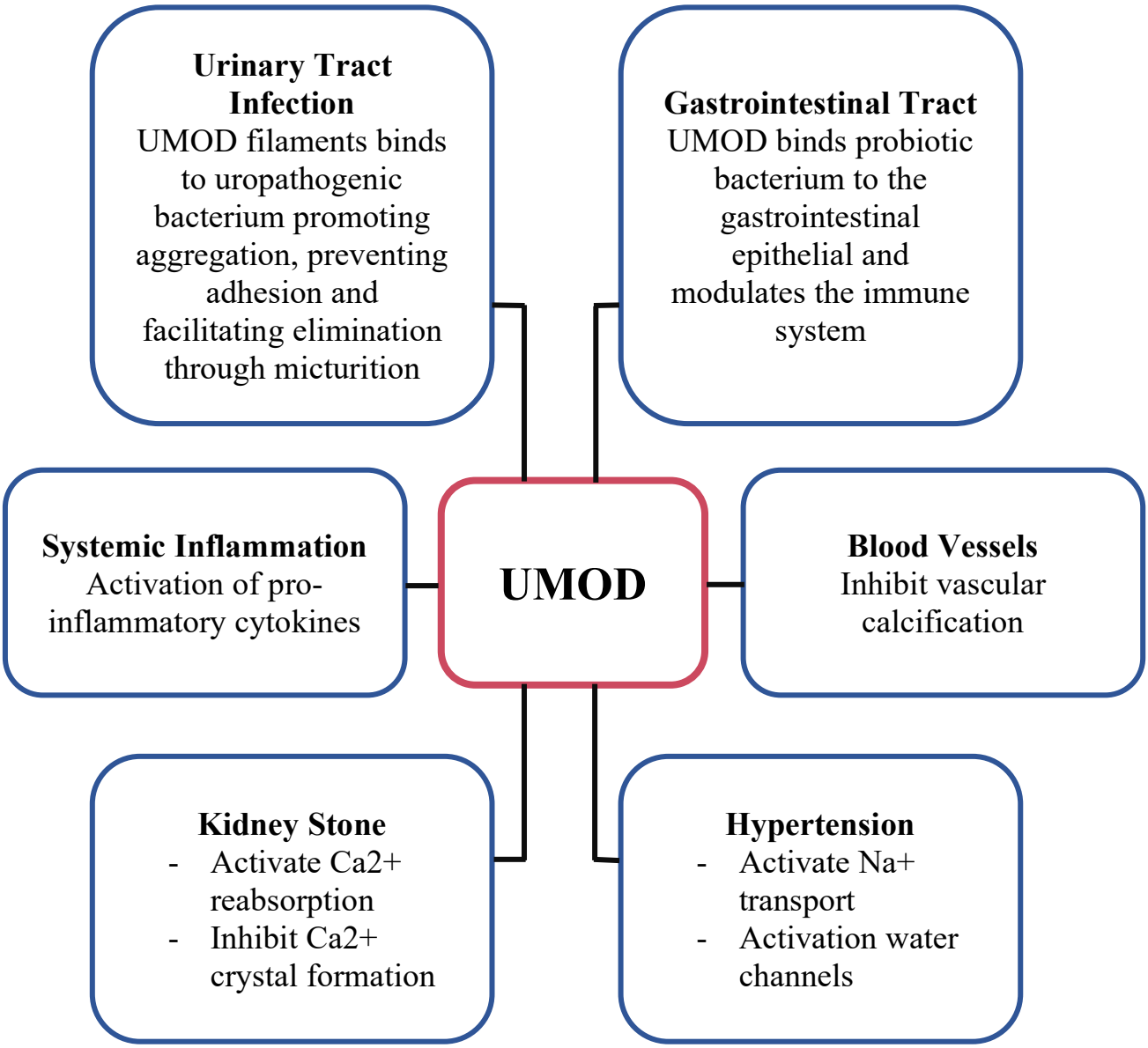


Figure 1.3: Potential Functions of UMOD (adapted from Takata & Isomoto, 2024).

Abbreviations: UMOD, uromodulin protein; Ca^{2+}, calcium.

in phosphorylation was demonstrated in the same study where 49% fewer NKCC2 ion channels were phosphorylated in the absence of UMOD compared to the wild-type mice (Mary et al., 2022; Mutig et al., 2011). Phosphorylation of NKCC2, in most studies on oocytes, has been associated with enhanced NKCC2-mediated Na^+ and Cl^- reabsorption (Ares et al., 2011). With 30% of Na^+ reabsorption occurring in the TAL, via the apical NKCC2 ion channels, it is evident that UMOD plays a vital indirect role in Na^+ reabsorption through not only affecting the amount of NKCC2 channels present on the apical membrane of the TAL, but also through the phosphorylation of these ion channels (Mary et al., 2022).

1.5.2 UMOD and ROMK

Similar to NKCC2, UMOD affects the transport of ROMK to the apical membrane of the TAL (Mary et al., 2022; Schiano et al., 2019). This is evidenced by a study on *UMOD* knock-out mice which exhibited an increase in the subapical vesicular accumulation of ROMK (Mary et al., 2022; Schiano et al., 2019). ROMK plays a crucial role in maintaining potassium (K^+) homeostasis in the TAL, sustaining the K^+ conductance required for the functioning of NKCC2 and creating a positive-lumen voltage required for the paracellular reabsorption of ions like calcium and magnesium (Boim et al., 1995; Renigunta et al., 2011; Schiano et al., 2019). Additionally, both human Bartter type 2 patients (individuals with a rare genetic disease characterised by salt wasting, hypokalaemia, metabolic acidosis, and hypercalciuria) with low or inactivating ROMK mutations and mice with tissue-specific and global ROMK knock-out illustrated reduced urinary uromodulin (uUMOD) levels coupled with increased kidney UMOD levels compared to wild types (Schiano et al., 2019). The pharmacological inhibition and genetic deletion of ROMK, which results in a significant reduction in uUMOD levels, suggests that ROMK directly regulates the processing and release of UMOD and supports the functional link between transport activity and UMOD relevant for BP control and the urinary concentrating ability (Schiano et al., 2019; Tokonami et al., 2018). While the precise mechanism linking ion channels and UMOD is unclear, with some studies illustrating the regulatory role of UMOD on ion channel transport, and other studies illustrating the regulatory role of the ion channels on uUMOD, a complex interplay between UMOD and ion channels is suggested.

1.5.3 UMOD and NCC

UMOD has also been observed to play a vital role in the regulation of NCC in the DCT1 (Tokonami et al., 2018). This is evident from a study done on *UMOD* knock-out mice which associated a decrease in UMOD with a decrease in the phosphorylation of DCT1 NCC (Tokonami et al., 2018). With the change in phosphorylation, the activity of NCC Na^+ uptake in the DCT1 was also observed to be reduced, and compensated for by increased activity of the downstream NCC (Tokonami et al., 2018). These findings were further evidenced with the chronic administration of furosemide (a loop diuretic that inhibits Na^+, Cl^- and water reabsorption from the proximal and distal tubules as well as the loop of Henle), where increased distal delivery of NaCl and calcium led to salt wasting and hypercalciuria only in *UMOD* knockout mice (Tokonami et al., 2018). The calcium loss was suggested to be a result of the shift in Na^+ and Cl^- reabsorption to the latter part of the DCT which is responsible for calcium uptake (Mary et al., 2022; Tokonami et al., 2018). With UMOD playing a vital role in the transportation of ion channels to the apical membrane thus indirectly influencing Na^+ reabsorption and BP, it is vital to understand its effect on the overall renal function determinants and haemodynamic parameters.

1.6 UMOD and Renal Determinants

1.6.1 Impact of UMOD on eGFR

Apart from UMOD's essential role in the regulation of ion channels, uUMOD has been found to be associated with height, birthweight, age, diabetes, eGFR, urine volume, and renal function and has been thought to play a vital role in the mechanisms leading to CKD (Takata & Isomoto, 2024). Studies on uUMOD have suggested two probable mechanisms in which uUMOD contributes to CKD: (1) through damaged renal tubules leading to reduced synthesis of UMOD and (2) UMOD affecting the amount of NKCC2, ROMK and NCC ion channels reaching the apical membrane, and its phosphorylation which in turn results in progressive kidney disease (Zhou et al., 2013). With both serum UMOD (sUMOD) and uUMOD being associated with eGFR and tubular reserve, UMOD has become a promising biomarker for renal function and CKD (Devuyst & Bochud, 2015; Takata & Isomoto, 2024). Numerous studies including the Swiss Kidney Project on Genes and Hypertension (SKIPOGH) have illustrated a positive association between uUMOD and eGFR, with lower uUMOD levels being linked to the rapid decline in eGFR and end-stage renal disease in CKD patients (Boder et al., 2021; Garimella et al., 2017; Mary et al., 2022; Steubl et al., 2020; Wang et al., 2021). Additionally, in a study

with a group of Hispanic individuals, higher uUMOD levels were associated with a 23% lower odds of experiencing more than a 30% decline in eGFR over a 10 year follow-up, thus indicating a protective role of uUMOD on renal function (Jotwani et al., 2020). In contrast to a positive correlation observed between UMOD and GFR, genetic studies have linked mutations in the promoter region of *UMOD* to eGFR, BP, and CKD incidents (Boder et al., 2021; Zhou et al., 2013). These studies have associated the G allele single nucleotide polymorphism (SNP) (rs13333226) with a lower risk of hypertension, reduced uUMOD levels, and higher eGFR, suggesting a potential protective role of this allele in regulating hypertension through renal excretion in Caucasians (Padmanabhan et al., 2010; Zhou et al., 2013).

1.6.2 Impact of UMOD on Fractional Excretion of Sodium ($FENa^+$)

$FENa^+$ is a marker for the amount of Na^+ excreted and provides insights into proximal tubule function (Seethapathy & Fenves, 2022). Additionally, $FENa^+$ plays a crucial role in distinguishing various causes of acute renal failure, aiding in the differentiation of tubular necrosis from prerenal uraemia (Seethapathy & Fenves, 2022). Studies have illustrated positive relationships between uUMOD and 24-hour excretion of Na^+, Cl^-, and K^+, thus suggesting a role of UMOD in influencing the tubular handling of electrolytes (Josipović, 2023). Given that $FENa^+$ reflects tubular function and uUMOD variation is linked to renal function, it is vital to investigate the relationship between uUMOD and $FENa^+$. Recent studies have illustrated a correlation between UMOD and Na^+ reabsorption in the proximal tubule, along with a correlation between uUMOD and $FENa^+$ in middle-aged pre-hypertensive individuals (Josipović, 2023). Although no difference was observed in uUMOD levels between hypertensive, pre-hypertensive, and normotensive individuals, variations in Na^+ excretion in response to renal UMOD level were observed based on the BP category (Josipović, 2023). Normotensives, however, exhibited the highest values of Na^+ excretion followed by the pre-hypertensive individuals, aligning with the proposed mechanism of higher renal UMOD levels inducing increased Na^+ reabsorption in TAL (Josipović, 2023). With UMOD levels being associated with BP, it is vital to explore the impact of UMOD on haemodynamic components.

1.7 UMOD and Haemodynamic Determinants

Both uUMOD and sUMOD levels have been associated with renal function, cardiovascular health, and mortality (Mary et al., 2022). Genome-wide association studies (GWAS) have highlighted the importance of mutations in the promoter region of *UMOD* and their role in

hypertension, CKD, and eGFR in individuals of European ancestry (Köttgen et al., 2009; Mary et al., 2022). The minor alleles of these SNPs have been associated with a decreased risk of hypertension, lower systolic blood pressure (SBP) and diastolic blood pressure (DBP), increased eGFR and reduced uUMOD levels (Giri et al., 2019; Mary et al., 2022; Padmanabhan et al., 2010; Ponte et al., 2021; You et al., 2021b). However, contradictory evidence exists regarding the role of haemodynamic parameters and their association with uUMOD. Where one study illustrated a positive relationship between mean arterial pressure (MAP) and uUMOD in elder hypertensives, another study demonstrated an inverse relationship between uUMOD and SBP (with higher uUMOD levels being associated with low SBP) and hypertension (Garimella et al., 2015; Trudu et al., 2013; Wang et al., 2021). Genetic and molecular studies further explored this link using transgenic mice models with *UMOD* risk variants (Trudu et al., 2013). These studies revealed that risk variants lead to the overexpression of UMOD-induced salt-sensitive hypertension and age-dependent renal lesions (Trudu et al., 2013). These findings were concurrent with those observed in elderly European individuals homozygous for the risk variants (Trudu et al., 2013). A genetic study reported similar findings in a Chinese cohort, which showed that high salt intake reduces uUMOD excretion (Du et al., 2021). Whereas numerous links have been found between UMOD and hypertension, renal function, and BP regulation, the majority of these studies were done on individuals of European, Chinese, and Hispanic origin. With individuals of African ancestry illustrating a unique form of hypertension, the question is whether the same UMOD associations and proposed mechanism of action are present in this population.

1.8 Urinary UMOD vs Serum UMOD as a Marker for Renal Function in Individuals of African Ancestry

Both uUMOD and sUMOD have established connections to renal regulation and hypertension. Given that UMOD is vastly more abundant in urine (roughly100-300 times higher concentrations than in sUMOD), actively regulates renal ion channels, and is synthesised in the TAL and DCT1, it is more appropriate to measure uUMOD levels compared to sUMOD levels when trying to understand UMODs role in hypertension, renal function, and tubular activity (Steubl et al., 2020). The synthesis of UMOD in the TAL and DCT1, coupled with its release in urine, thus makes uUMOD an ideal candidate for estimating nephron mass (Pivin et al., 2018). Notably, uUMOD levels have been associated with salt-sensitive hypertension and a potential detrimental elevation in BP, offering confirmation of its role and suggesting a

possible causal risk factor for hypertension in a population of African ancestry (Mary et al., 2022; You et al., 2021b). Individuals of African ancestry exhibit a unique form of renin-independent, volume and renal-dependant hypertension, which respond poorly to most current antihypertensive therapies, particularly inhibitors of the RAAS. Hence, it is vital to explore various mechanisms and biomarkers which could explain this distinct form of hypertension in this cohort, potentially leading to more effective and targeted treatment strategies. Given the strong associations of uUMOD with renal and haemodynamic parameters in individuals of European, Chinese and Hispanic ancestry, investigating whether uUMOD contributes to the unique for of hypertension in individuals of African ancestry and serves as a potential treatment target warrants further exploration. Therefore, the measurement of uUMOD will be invaluable for my study, providing insight into the role of UMOD in renal and haemodynamic parameters, particularly in a community sample with a high prevalence of hypertension. Urinary UMOD will thus be the focus of this dissertation.

1.9 Study Rationale

The rising prevalence of hypertension in developing areas such as South Africa is a major contributor to the rising cardiovascular epidemic. As noted in previous studies, individuals of African ancestry are more susceptible to a renin-independent volume-dependent form of hypertension. Although studies in individuals of African ancestry have shown that renal mechanisms of hypertension translate into increases in systemic blood flow, the mechanisms of this relationship need to be explored. As discussed above, uUMOD is a potential biomarker of renal function, particularly tubular function. However, the role of uUMOD in hypertension in people of African ancestry is currently unknown. Furthermore, whether a relationship exists between uUMOD, renal function and haemodynamic parameters in this population is unknown. Thus, in the present study, I aimed to investigate the relationship between uUMOD and both renal function (as assessed from $FENa^+$ and eGFR) and haemodynamic parameters in a large community of African ancestry.

1.10 Objectives

To achieve the aim, the objectives were as follows:

1. To investigate the uUMOD concentration in individuals of African ancestry using enzyme-linked immunosorbent assay kits.
2. To investigate the relationship between uUMOD concentrations and renal as well as

haemodynamic parameters in individuals of African ancestry.

3. To compare the relationship between uUMOD concentration and renal function as well as haemodynamic parameters in normotensive and hypertensive individuals of African ancestry.

CHAPTER 2: Methodology

2.1 Study Group

The current study was conducted according to the principles set out in the Helsinki declaration. Ethical approval (approval numbers: M02-04-72 and renewed as M07-04-69, M12-04-108, M17-04-01 and M22-03-93) was obtained from the University of the Witwatersrand Research on Human Subjects committee. The recruitment process involved the random selection of families (from the 2001 population census figures) from the Southwest Township (SOWETO) in Johannesburg, South Africa (Mmopi et al., 2021; Woodiwiss et al., 2020). Families were eligible for recruitment if they had Nguni and Sotho ancestry and if they had at least one or two siblings (above the age of 16), and one or both parents accessible for examination. No other recruitment inclusion/ exclusion criteria were employed. Written informed consent was obtained from all eligible participants. For this sub-study, participants were selected if they had full demographic, clinical and anthropometric measurements, high-quality velocity measurements in the outflow tract (further discussed under haemodynamic assessment), as well as 24-hour urine samples that met the previously described quality control criteria (Malan et al., 2021). This sub-study therefore consisted of 397 participants. However, data on renal function determinants ($FENa^+$ and eGFR) were only available for 390 of the participants.

2.2 Demographic, Anthropometric and Clinical Data

Participants demographic, anthropometric and clinical data (age, sex, ancestry, tobacco, and alcohol consumption, etc.) were collected using a questionnaire. The participants weight and height were measured using conventional approaches and were used to calculate their body mass index (BMI). Participants were considered overweight and obese if their BMI was ≥ 25 kg/m^2 and $\geq$ 30 kg/m^2 respectively. Blood glucose, and the percentage of glycated haemoglobin (HbA1c) were tested in an accredited diagnostic laboratory as previously described (Malan et al., 2021). The use of insulin, oral glucose lowering agents or an HbA1c value $>$ 6.5% were used as parameters to define diabetes mellitus (DM).

2.3 Renal Function

As previously mentioned by Bochud et al., (2009), renal function and urine Na^+ excretion were measured. Recognising that the initial urine void in the morning is the most concentrated sample of the day, which potentially could skew concentrations of specimens if collected twice, we took precautions (Miler & Šimundić, 2013). In this regard, the first urine collected before the commencement of the 24-hour collection period was discarded. The concentrations of Na^+

and creatinine in the urine were then determined. Creatinine concentrations were measured using the Advia Chemistry systems (Siemens, Medical Solutions, USA, Inc.) with calibration traceable to isotope dilution mass spectrometry. The quality of urine samples was determined by constructing regression relationships within gender-specific groups. These relationships were based on 24-hour urine creatinine to body weight and 24-hour urine volume relative to age. Based upon the 95% confidence intervals for each group, a 24-hour urine sample was considered acceptable if 24-hour urine creatinine (mmol) was > 3.5 and < 35 for males and > 3.5 and < 30 for females. Samples with urine volumes < 300 ml/day were also assumed to be incomplete urine collections. Estimated GFR was previously computed as creatinine clearance: $\frac{\text{Ucreat} \times \text{V}}{\text{Screat}}$, where Ucreat and Screat represent the urinary and serum concentrations of creatinine and V the urine volume in mls/minute. GFR was expressed per body surface area. $FeNa^+$ was determined as: $\frac{\text{clearance of } Na^+}{\text{clearance of creatinine}}$, where the clearance of Na^+ was computed as $\frac{uNa^+ \times V}{sNa^+}$,where uNa^+ and sNa^+ represents the urinary and serum concentrations of Na^+ and V the urine volume in mls/minute.

2.4 Urinary UMOD Concentrations

Pre-collected urine samples that met the above-mentioned conditions and were maintained in a -80°C freezer, were recovered and used to test for uUMOD concentrations using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (Cloud-clone corp., TX 77494, USA). The human UMOD ELISA kits made use of a sandwich ELISA approach whereby a UMOD specific capture antibody and a UMOD specific biotin-labelled detection antibody bound to UMOD at 2 antigenic sites (Figure 2.1). The micro-ELISA plate provided by the kit was pre-coated with capture antibodies. Samples were added to the microplate followed by the biotinylated detection antibody and Avidin-horseradish peroxide (HRP) conjugate. Microplates were consistently incubated (to allow for the binding of components) and washed (to remove the free components) throughout the process, adhering to the manufacturer's instructions. Only wells containing UMOD, HRP and the detection antibody appeared blue in colour. The enzyme-substrate reaction was terminated with the addition of the stop solution containing 3,3',5,5'-tetramethylbenzidine (TMB). The oxidation of TMB by HRP to TMB^{2+} causes the solution in the wells to change from blue to yellow (Guo et al., 2016). This colour change was proportionate to the amount of uUMOD-Avidin-Biotin-HRP complexes formed in the wells and was measured using a spectrophotometer. A Spectrophotometer (Multiskan

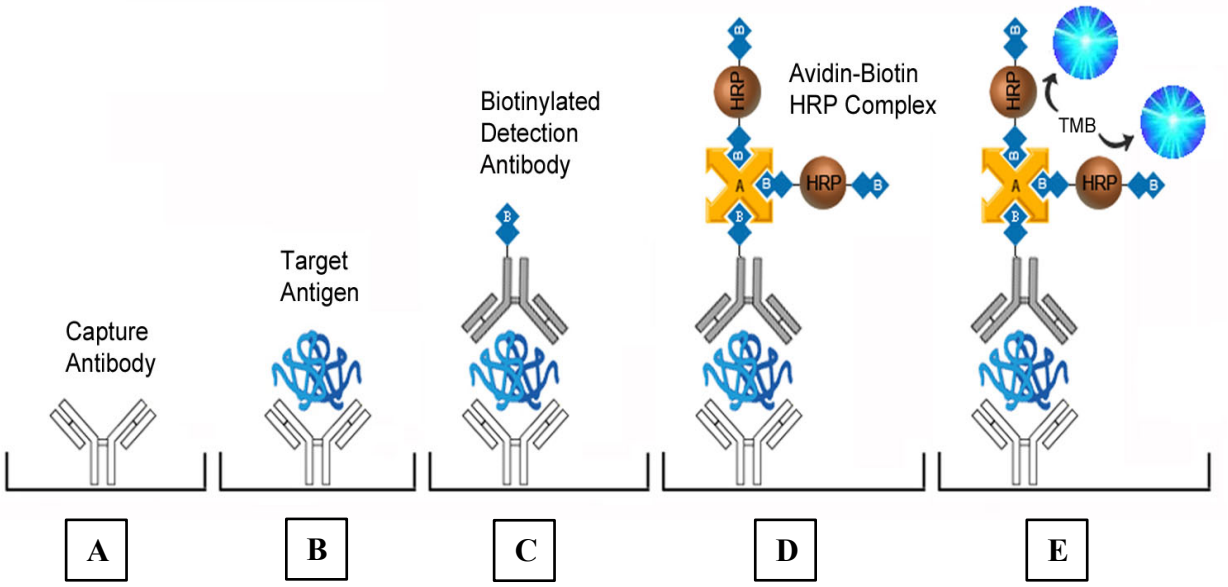


Figure 2.1: Diagrammatic Representation of the Sandwich ELISA Process Used to Quantify uUMOD (obtained from https://www.lsbio.com/elisakits/human-umod-uromodulin-sandwich-elisa-elisa-kit-ls-f57436/57436; accessed on 4 January 2024).

Panel (A) illustrates the UMOD specific capture antibody bound to a well. Panel (B) illustrates uUMOD bound to the capture antibody. Panel (C) illustrates the biding of the capture antibody-uUMOD-biotinylated detection antibody complex formed after the addition of the detection antibody. Panel (D) illustrates the binding of Avidin to the biotin-labelled capture antibody and biotin-labelled HRP to form the Avidin-Biotin-HRP complex. Panel (E) illustrates the oxidation of TMB found in the stop solution by HRP resulting in a colour change that is proportional to the amount of uUMOD- antibody complexes formed in the well. Abbreviations: HRP, Horseradish peroxidase; A, Avidin; B, Biotin; uUMOD, urinary uromodulin; TMB, 3,3',5,5'-tetramethylbenzidine.

Ascent, Lab system, model 354, Helsinki, Finland) set at a wavelength of 450nm ± 2nm was then used to measure the optical density which was directly proportionate to uUMOD concentrations. Urinary UMOD (uUMOD) concentrations were determined using the standard curve generated upon completion of the ELISA. The sandwich ELISAs were highly suited for the measuring of uUMOD as it allowed for high specificity and sensitivity in complex urine samples that were not purified. The human uromodulin ELISA had a lower detection limit of 15.6 pg /ml, intra-assay variability < 10% and inter-Assay variability < 12%.

2.5 Haemodynamic Assessment

2.5.1 BP Measurements

Using conventional mercury sphygmomanometer specifications, a professional nurse-technologist measured high quality office brachial BP after 5 minutes of repose, with the participant in a sitting posture (Woodiwiss et al., 2009). The office BP was calculated using the average of five BP readings taken at least 30 seconds apart. A mean office BP ≥ 140 mmHg systolic and ≥ 90mmHg diastolic or the use of antihypertensive medications were used to define hypertension in participants.

2.5.2 SphygmoCor Measurements

Non-invasive central arterial pressure measurements were collected from peripheral pulse wave analysis using a SphygmoCor device (Mmopi et al., 2021; Norton et al., 2012; Woodiwiss et al., 2020). Participants rested in supine position for 15 minutes before arterial waveforms at the radial pulse (on the dominant arm) were recorded. Arterial waveforms were recorded by applanation tonometry over an 8-second period using a high-fidelity SPC-301 micromanometer interfaced with a computer running version 9.0 SphygmoCor software (Figure 2.2) (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). Manual measurements of brachial DBP and SBP, taken immediately before recordings using an Omron professional digital BP monitor, were used to calibrate the pulse waves. Radial pressure waveforms were then converted into central aortic waveforms using SphygmoCor validated transfer function software (Figure 2.3). Recordings were discarded if the pulse wave signals were < 80 mV or the systolic or diastolic waveforms were > 5%. Characteristic impedance was determined in the time domain and was validated against invasive pressure measurements (Kelly & Fitchett, 1992; Segers et al., 2007). The foot (t0) of respective averaged

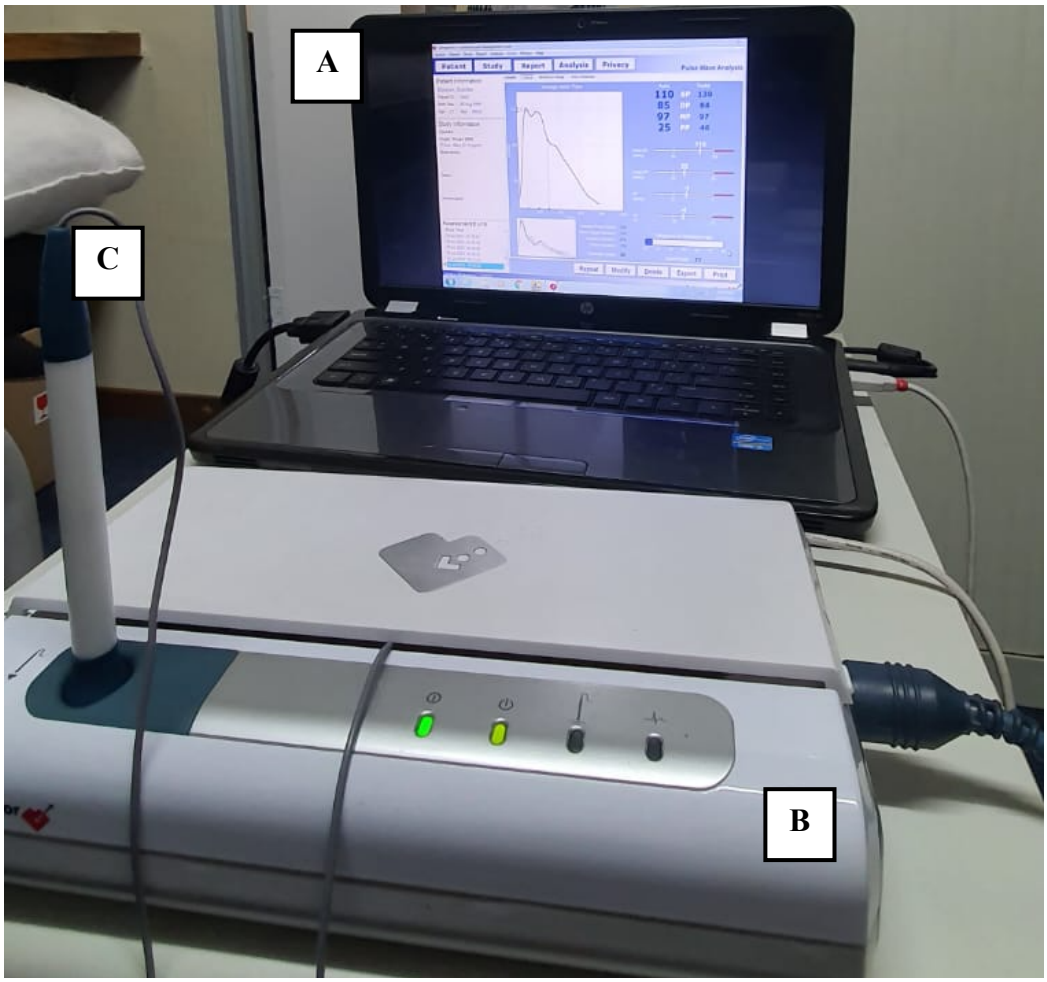


Figure 2.2: SphygmoCor System Interfaced with a Computer Running SphygmoCor, Version 9.0, Software (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia).

(A) Illustrates the computer interfaced with SphygmoCor software, (B) illustrates the SphygmoCor device and (C) illustrates the applanation tonometer.

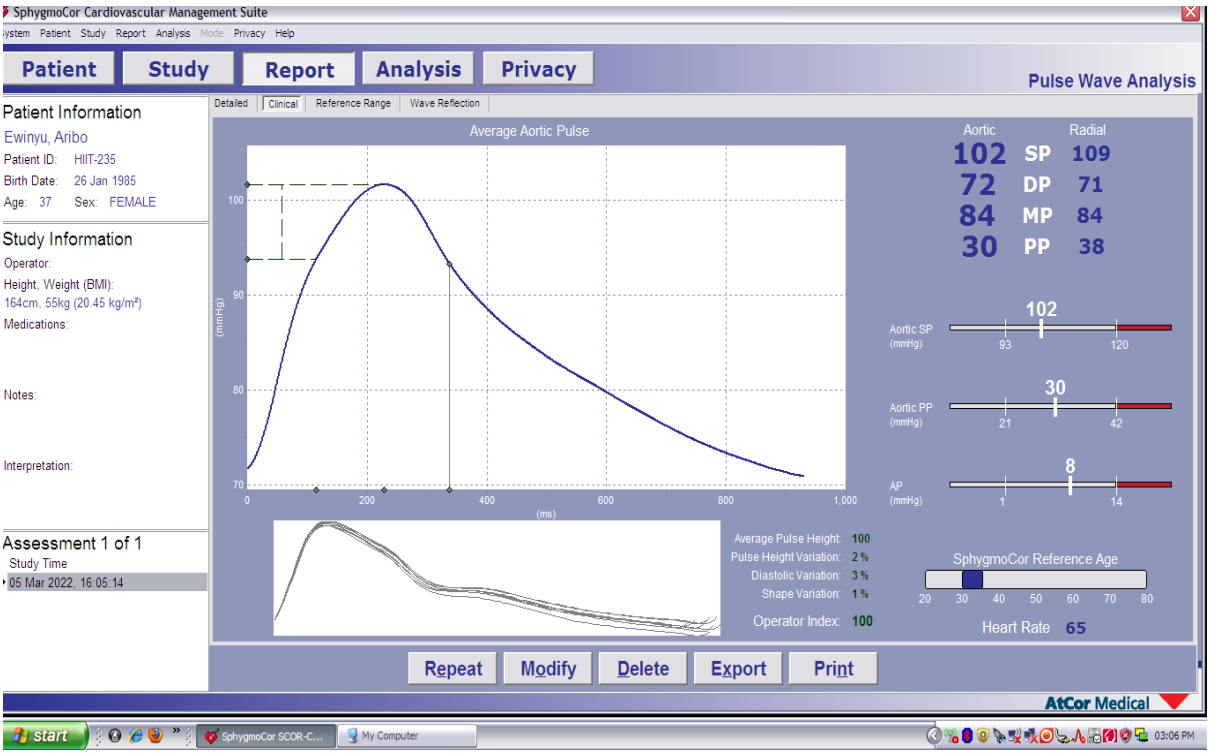


Figure 2.3: SphygmoCor Validated Incorporated Function Conversion of Radial Waveforms to Central Aortic Waveforms.

The y-axis illustrates pressures in mm Hg. The x-axis illustrates time in milliseconds (ms). Abbreviations: SP, systolic blood pressure; DP, diastolic blood pressure; MP, mean arterial pressure; PP, pulse pressure.

signal waveforms were aligned to paired volume flow waveforms and central arterial pressure waveforms. The point where flow reached 95% of its peak (tQ95) was identified, and the corresponding pressure change between t0 and tQ95 was determined. Zc was then computed as the ratio of change in pressure to change in flow between t0 and tQ95. To account for pressures generated in a pulsatile system, peak pressures were computed using the formula: flow (Q; measured using Echocardiography) × Zc (Woodiwiss et al., 2020).

MAPs (average arterial pressure for the peripheral waveform) were calculated using the formula: $\text{MAP} = \frac{(\text{TF}\sum i=\text{T0})\text{Pi}}{\text{n}}$, where TF is the end of the waveform, T0 is the start of the waveform, Pi is pressure points and n is the number of pressure points. Using the MAP and assuming the right arterial pressure (RAP) is equal to 0 mm Hg, SVR was computed using the formula $\text{SVR} = \frac{\text{MAP}-\text{RAP}}{\text{n}}$. Total arterial compliance (TAC) was calculated as $\text{TAC} = \frac{\text{SV}}{\text{aortic pulse pressure}}$ (Woodiwiss et al., 2020).

2.5.3 Echocardiographic Measurements

An ACUSON SC2000 Diagnostic ultrasound system (Siemens Medical Solutions, USA, Inc.) was used by experienced observers to acquire aortic velocity and diameter measurements in participants while in the left lateral decubitus position (Figure 2.4). High-quality velocity measurements in the outflow tract were obtained in the 5-chamber view (Woodiwiss et al., 2020). Smooth velocity waveforms with a dense leading outer edge and a clear maximum velocity were accepted as high-quality velocity measurements (Woodiwiss et al., 2020). Aortic diameter measurements, taken in the long axis parasternal view, were obtained just proximal to the aortic leaflets. Aortic flow waveforms were constructed using the largest diameter recorded in early systole (Figure 2.4A). Graphic software, DICOM viewer, was used to outline the densest/brightest portion of spectral image indicating velocity waveform (obtained from the ultrasound) while avoiding any overshot image (Figure 2.5).

Flow was calculated as flow (Q) = Velocity × aortic root cross-sectional area, where the aortic root cross-sectional area was calculated from the diameter assessment. SV was obtained using the formula SV = aortic velocity-time integral × aortic root cross-sectional area. Heart rate was determined using the average of length or period (PD) of a peripheral pressure waveforms



Figure 2.4: ACUSON SC2000 Diagnostic Ultrasound System (Siemens Medical Solutions, USA, Inc.) used to acquire Aortic Velocity and Diameter Measurements.

(A) Illustrates the how the aortic root in systole was measured at the tip of the aortic valve in the three-chamber parasternal long axis view. (B) Illustrates the echocardiogram lead. (C) Illustrates the standard linear array transducer used to obtain echocardiographic images.

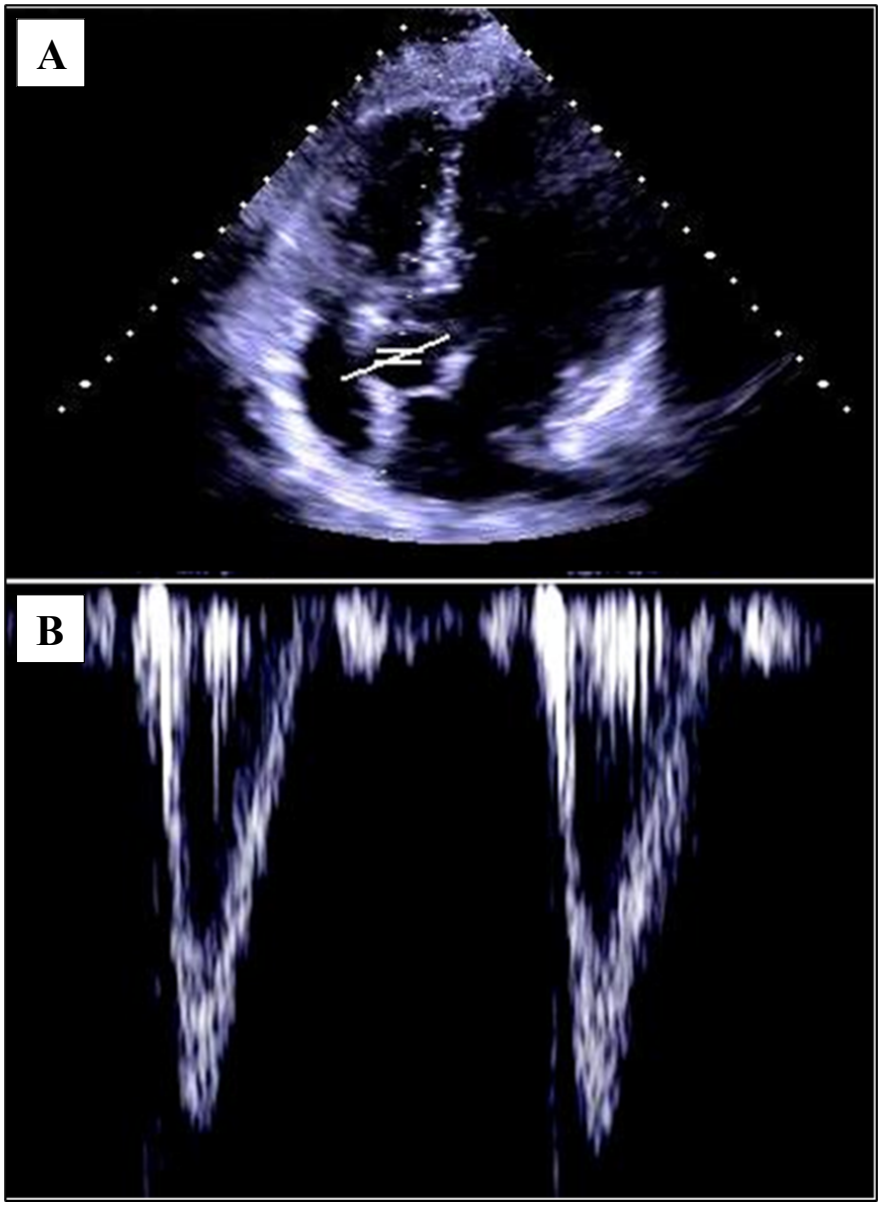


Figure 2.5: ACUSON SC2000 Echocardiographic Image Illustrating Aortic Velocity Waveforms. Aortic waveforms (Panel B) were obtained in the apical five chamber view (Panel A) of the heart. DICOM Viewer was used on images like this to outline the densest/brightest portion of spectral indicating velocity waveform while avoiding any overshot image.

that was captured over a period of 10 seconds using the formula: Heart rate (HR) = $\frac{1000}{\text{PD}\times 60}$. Cardiac output (CO) was then calculated as the product of SV and HR. The gold standard biplane Simpson approach, which quantifies left ventricular volume using a summation of disks approach and cross-sectional shape of the left ventricle, was used to quantify the left ventricle end diastolic volume (EDV) from the echocardiogram (Kim et al., 2022).

2.6 Data Analysis

SAS software, version 9.4 (SAS Institute Inc., Cary, NC), was used for database management and statistical analysis. The mean (SD) or median and interquartile range were used to express continuous variables. Percentages represented dichotomous variables. Multivariate linear or logistic regression analysis were used to assess relationships between uUMOD concentration, renal function, and haemodynamic parameters in normotensive vs hypertensive individuals. Further adjustments for non-independence of family members were made using non-linear regression analysis to determine probability values (mixed procedure as defined in the SAS package). Confounding variables such as age, sex, HR, MAP, regular alcohol intake, regular tobacco intake, BMI, DM and/or a HbA1c > 6.5%, antihypertensive treatment, and 24-hour Na^+ excretions were adjusted for in regression analysis (in assessments with renal function). A $P < 0.05$ was considered significant.

CHAPTER 3: Results

3.1 Characteristics of Participants

Table 3.1 outlines the general and renal characteristics in a community of African descent with a high prevalence of hypertension. In general, participants were middle-aged with over 50% of the individuals falling within the overweight or obese category. Of the 397 participants in this study, over 45% were hypertensive with approximately half of the hypertensive individuals receiving antihypertensive medication. Thiazide diuretic agents were the most commonly used antihypertensive medication (90%). A negligible percentage of hypertension treated individuals were receiving α-Blockers (< 1%) and only 14%, 27%, and 2% of hypertension treated individuals were receiving calcium channel blockers (CCB), angiotensin-converting enzyme inhibitor (ACEI) and central acting agents respectively. Renal assessment data was available for only 370 (93%) of the participants. Of the individuals with renal data available, approximately 6% had mild or moderate CKD (eGFR < 60 mL/min per 1.73 m^2) . None of the participants, however illustrated kidney damage with a severe decrease in eGFR or kidney failure (eGFR < 30 mL/min per 1.73 m^2).

Haemodynamic parameters of the respective 397 participants are summarised in Table 3.2. Apart from SV and EDV, which were observably higher than normal, the general haemodynamic characteristics were within normal ranges.

3.2 Factors associated with uUMOD Concentrations (Bivariate Correlations)

Table 3.3 shows the relationships between uUMOD concentrations and general characteristics as well as the relationships between uUMOD concentrations and renal function parameters in a community sample of African ancestry with a high prevalence of hypertension. The only general characteristic associated with uUMOD concentration was sex ($r = 0.177$; $P = 0.0004$). No significant relationships were observed between uUMOD concentrations and age, BMI, hypertension, and renal function parameters (eGFR and $FeNa^+$) in this community sample ($P \geq 0.05$).

Furthermore, no statistically significant ($P \geq 0.05$) correlations were found between uUMOD concentrations and haemodynamic parameters in this community sample, except for SV, which showed a significant inverse relationship ($r < 0$; $P = 0.045$) with uUMOD concentration (Table 3.4).

Table 3.1: General and Renal Characteristics of a Community Sample of African Ancestry with a High Prevalence of Hypertension

General characteristics	
n	397
Number of females (%)	64.5
Age (years)	46.7 ± 18.3
BMI (kg/m^2)	29.9 ± 7.5
Overweight/ obese (%)	26.2 /40.6
Diabetes mellitus (%)	12.8
Regular smoker (%)	14.9
Regular alcohol consumption (%)	19.9
Hypertensive individuals (%)	48.4
Hypertension treated individuals (%)	28.2
Individuals treated with α-Blockers (%)	0.5
Individuals treated with ACEI (%)	4.0
Individuals treated with CCB (%)	7.6
Individuals treated with thiazide diuretics (%)	25.4
Individuals treated with central acting agents (%)	1.3
Renal characteristics	
n	370
uCr concentration (mmol/L)	8.4± 5.6
Estimated GFR (mL/min per 1.73 m^2)	102 ± 31
$FENa^+$ (%)	0.8 ± 0.5
uUMOD concentration (pg/mL) (n = 397)	560.0 ± 369.4

Data is presented as mean ± standard deviation or proportion; Abbreviations: BMI, body mass index; ACEI, angiotensin converting enzyme inhibitor; CCB, calcium channel blocker; uCr, urine creatinine; GFR, glomerular filtration rate; $FENa^+$, fractional excretion of sodium; uUMOD, urinary uromodulin.

Table 3.2: Haemodynamic Characteristics in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)

Brachial pressures	
Systolic blood pressure (mm Hg)	130 ± 22
Diastolic blood pressure (mm Hg)	84 ± 11
Pulse pressure (mm Hg)	45.9 ± 16.5
Central pressures	
Systolic blood pressure (mm Hg)	121 ± 22
Diastolic blood pressure (mm Hg)	84 ± 11
Pulse pressure (mm Hg)	36.6 ± 15.4
Determinants of systemic flow	
Aortic flow (mL)	347 ± 173
Stroke volume (mL/beat)	83.7 ± 39.2
Heart rate (beats/min)	65.8 ± 11.6
Cardiac output (mL/min)	5.5 ± 2.6
Mean arterial pressure (mm Hg)	100 ± 15
End diastolic volume (mL)	114 ± 31
Vascular parameters	
SVR (mm Hg.min/L)	20.9 ± 9.2
TAC (mL/mm Hg)	2.5 ± 1.2
Zc (dynes.s /cm^5)	87.1 ± 43.4

Data is presented as mean ± standard deviation or proportion; SVR, systemic vascular resistance; TAC, total arterial compliance; Zc, characteristic impedance.

Table 3.3: Bivariate Relationships Between General Characteristics and uUMOD Concentrations as well as Renal Characteristics and uUMOD Concentrations in a Community Sample of African Ancestry with a High Prevalence of Hypertension

General characteristics (n = 397)	Pearson's r	P value
Sex (female)	**0.177**	**0.0004**
Age	0.079	0.114
BMI	0.034	0.495
Overweight/ obese	0.040	0.430
Diabetes Mellitus	-0.036	0.470
Regular smoker	0.055	0.269
Regular alcohol consumption	-0.004	0.942
Hypertensive individuals	0.028	0.584
Hypertension treated individuals	0.053	0.292
Renal characteristics (n = 370)		
Estimated GFR	0.038	0.465
$FENa^+$	0.067	0.195

Abbreviations: uUMOD, urinary uromodulin; BMI, body mass index; GFR, glomerular filtration rate; $FENa^+$, fractional excretion of sodium; significant correlations are shown in bold ($P \leq 0.05$)

Table 3.4: Bivariate Relationships between Haemodynamic Parameters and uUMOD concentrations in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)

Brachial pressures	Pearson's r	P value
Systolic blood pressure	-0.015	0.773
Diastolic blood pressure	-0.019	0.713
Pulse pressure	-0.049	0.327
Central pressures		
Systolic blood pressure	-0.005	0.915
Diastolic blood pressure	-0.022	0.665
Pulse pressure	-0.023	0.646
Determinants of systemic flow		
Aortic flow	- 0.026	0.605
Stroke volume	- **0.101**	**0.045**
Heart rate	0.036	0.477
Cardiac output	-0.082	0.101
Mean arterial pressure	-0.013	0.804
End diastolic volume	0.077	0.125
Vascular parameters		
SVR	0.073	0.147
TAC	- 0.074	0.142
Zc	- 0.011	0.828

Abbreviations: SVR, systemic vascular resistance; TAC, total arterial compliance; Zc, characteristic impedance; significant correlations are shown in bold ($P \leq 0.05$).

3.3 Characteristics of Male verses Female Individuals

Tables 3.5 compares the general and renal characteristics of males and females in a community sample of African Ancestry with a high prevalence of hypertension. In general, females had significantly higher BMIs than males, with over 50% of females falling within the obese category. Over 30% of the male participants were observed to partake in regular alcohol and tobacco consumption which was significantly higher than the female consumption rates which were less than 15% and 6% respectively. Although the proportion of hypertensives did not differ between males and females, over 70% of the treated hypertensive individuals were female. Additionally, females exhibited significantly higher uUMOD concentrations compared to male individuals ($P = 0.0007$). However, no differences were observed in other renal function parameters between the sexes (Table 3.5).

Furthermore, males exhibited a significantly higher aortic flow (approximately 58 mL difference in means), SV (approximately 12.5 mL/beat difference in means), EDV (approximately 11mL difference in mean) and TAC (approximately 0.50 mL/ mm Hg difference in mean) compared to females (Table 3.6). Females, however, illustrated a significantly higher HR (average of 6.1 beats/min difference) than male individuals (Table 3.6).

3.4 Sex Differences in uUMOD Concentrations (Multivariate Adjusted)

After adjusting for general characteristics (Figure 3.1A), general characteristics and BP (Figure 3.1B) and general characteristics, BP, and SV (Figure 3.1C), the difference in uUMOD concentration between males and females remained statistically significant ($P < 0.05$), with females having higher concentrations of uUMOD. Furthermore, after adjustments, the relationship between uUMOD concentrations and sex strengthened (initial $P = 0.0004$, after adjustments $P < 0.0001$, $P < 0.0001$, $P = 0.0002$ for general adjustments, general adjustments and BP, and general adjustments, BP, and SV respectively). However, no statistically significant ($P = 0.05$) bivariate correlations were found between uUMOD concentrations and SV in women ($r = -0.065$, $P = 0.299$) or men ($r = -0.090$, $P = 0.291$) when analysed separately.

3.5 Characteristics of Hypertensive and Normotensive Individuals

Table 3.7 compares the general and renal characteristics of hypertensive individuals to normotensive individuals in a community sample of African descent with a high prevalence of

Table 3.5: Comparison of General and Renal Characteristics in Male and Female Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension

General characteristics	Male	Female	P value
n	141	256	
Age (years)	45.6 ± 19.1	47.3 ± 17.9	0.380
BMI (kg/m^2)	**24.8 ± 5.4**	**31.2 ± 7.5**	**<0.0001**
Overweight/ obese (%)	**31.9 / 14.9**	**23.0 / 54.7**	**<0.0001**
Diabetes Mellitus (%)	12.1	13.3	0.727
Regular smoker (%)	**31.2**	**5.9**	**<0.0001**
Regular alcohol consumption (%)	**34.0**	**12.1**	**<0.0001**
Hypertensive individuals (%)	47.5	48.8	0.803
Treated individuals (%)	**14.9**	**35.5**	**<0.0001**
Renal characteristics			
n	133	237	
Estimated GFR (mL/min per 1.73 m^2)	102 ± 33	102 ± 30	0.917
$FENa^+$ (%)	0.8 ± 0.5	0.8 ± 0.6	0.456
uUMOD concentration (pg/mL)	**471.8 ± 399.9 (n = 141)**	**608.6 ± 342.7 (n = 256)**	**0.0007**

Data is presented as mean ± standard deviation or proportions; Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; $FENa^+$, fractional excretion of sodium; uUMOD, urinary uromodulin; significant differences are shown in bold ($P \leq 0.05$).

Table 3.6: Comparison of Haemodynamic Characteristics in Male and Female Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension

Brachial pressures	Male	Female	P value
n	141	256	
Systolic blood pressure (mm Hg)	132 ± 23	129 ± 21	0.200
Diastolic blood pressure (mm Hg)	84 ± 11	83 ± 12	0.224
Pulse pressure (mm Hg)	46.6 ± 19.3	45.5 ± 14.7	0.551
Central pressures			
Systolic blood pressure (mm Hg)	123 ± 23	121 ± 21	0.337
Diastolic blood pressure (mm Hg)	85 ± 11	84 ± 12	0.237
Pulse pressure (mm Hg)	37.1 ± 18.3	36.4 ± 13.6	0.671
Determinants of systemic flow			
Aortic flow (mL)	**385 ± 191**	**327 ± 159**	**0.002**
Stroke volume (mL/beat)	**91.7 ± 42.2**	**79.2 ± 36.8**	**0.002**
Heart rate (beats/min)	**61.9 ± 11.1**	**68.0 ± 11.4**	**<0.0001**
Cardiac output (L/min)	5.7 ± 2.8	5.3 ± 2.6	0.232
Mean arterial pressure (mm Hg)	101 ± 14	100 ± 15	0.419
End diastolic volume (mL)	**121 ± 32**	**110 ± 31**	**0.0008**
Vascular parameters			
SVR (mm Hg.min/L)	20.9 ± 9.8	20.9 ± 8.9	0.958
TAC (mL/ mm Hg)	**2.7 ± 1.3**	**2.3 ± 1.1**	**0.0026**
Zc (dynes.s/cm^5)	81.7 ± 44.4	90.0 ± 41.1	0.064

Data is presented as mean ± standard deviation or proportion; Abbreviations: SVR, systemic vascular resistance; TAC, total arterial compliance; Zc, characteristic impedance; significant differences are shown in bold ($P \leq 0.05$).

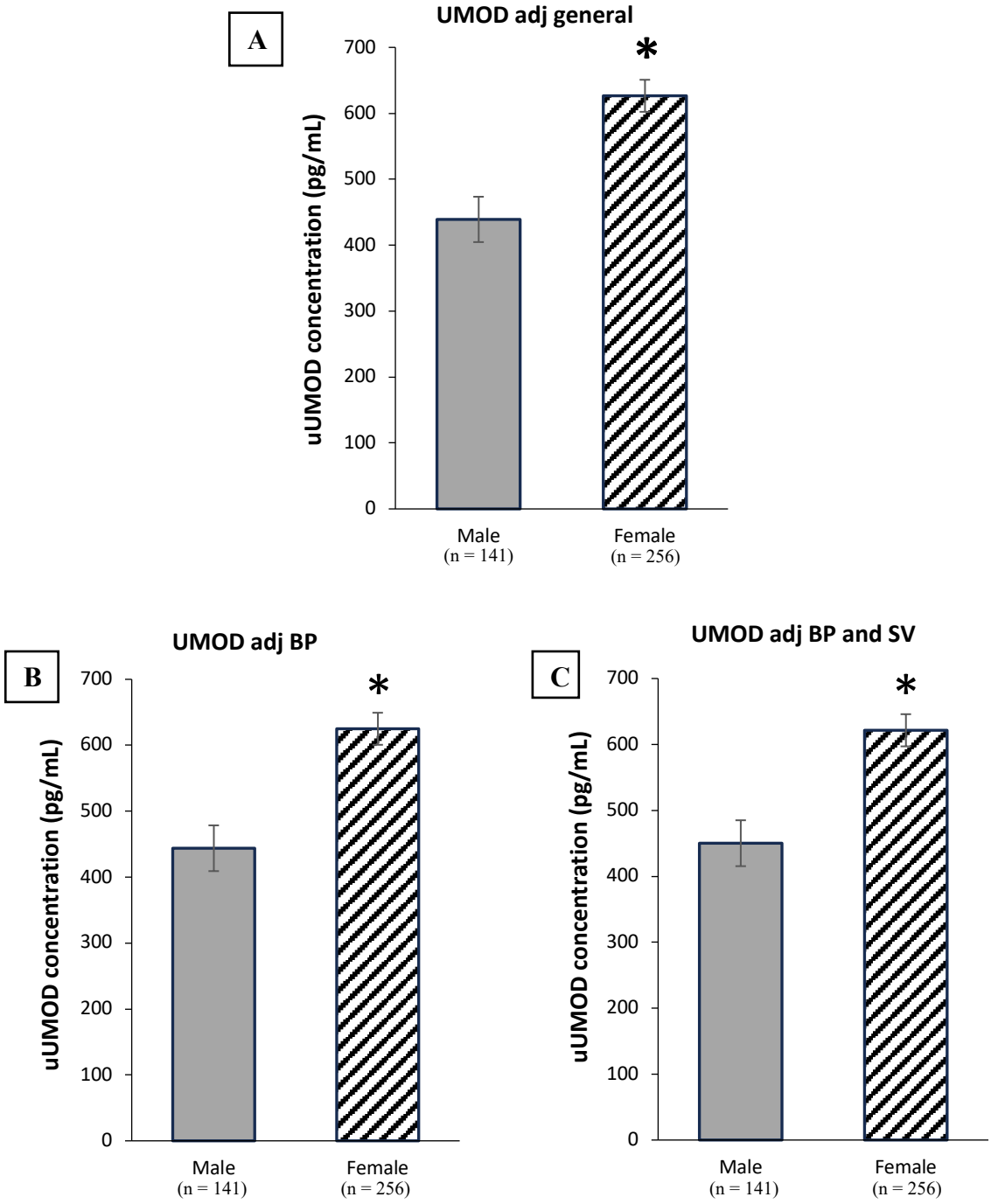


Figure 3.1: Comparisons of Multivariate Adjusted uUMOD Concentrations in Males and Females of African Ancestry with a High Prevalence of Hypertension.

Panel (A) refers to the comparison of uUMOD concentrations in male and female after adjusting for general characteristics such as age, BMI, DM, alcohol and tobacco consumption, treatment for hypertension and HR. Panel (B) refers to the comparison of uUMOD concentrations in male and female after adjusting for general characteristics and BP. Panel (C) refers to the comparison of uUMOD concentrations in male and female after adjusting for general characteristics, BP, and SV. * Indicates a $P \leq \mathbf{0.0005}$. The data is present as the mean with the respective bar's indication the standard error of the mean. Abbreviations: uUMOD, urinary uromodulin; adj, adjusted; BP, blood pressure; SV, stroke volume; HR, heart rate; DM, diabetes mellitus.

Table 3.7: General and Renal Characteristics of Normotensive and Hypertensive Individuals in a Community Sample of Africa Ancestry with a High Prevalence of Hypertension

General characteristics	Normotensive	Hypertensive	P value
n	205	192	
Sex (% female)	63.1	65.1	0.803
Age (years)	**36.8 ± 15.0**	**57.3 ± 15.5**	**<0.0001**
BMI (kg/m^2)	**27.0 ± 6.9**	**31.0 ± 7.6**	**<0.0001**
Overweight/ obese (%)	**27.3 / 30.2**	**25.0 / 51.6**	**<0.0001**
Diabetes Mellitus (%)	**4.9**	**21.3**	**<0.0001**
Regular smoker (%)	14.6	15.1	0.895
Regular alcohol consumption (%)	19.5	20.3	0.842
Renal characteristics			
n	190	180	
$FENa^+$ (%)	0.8 ± 0.5	0.8 ± 0.6	0.765
Estimated GFR (mL/min per 1.73 m^2)	**111 ± 30**	**92 ± 29**	**<0.0001**
uUMOD concentration (pg/mL)	555.2 ± 367.3 (n = 205)	570.5 ± 372.4 (n = 192)	0.584

Data is presented as mean ± standard deviation or proportion; Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; $FENa^+$, fractional excretion of sodium; Treated individuals, those receiving antihypertensive medication; uUMOD, urinary uromodulin; significant differences are shown in bold ($P \leq 0.05$).

hypertension. Hypertensive individuals were on average 21 years older than the normotensive individuals, with approximately 21 % more of the hypertensive individuals falling within the obese category. More hypertensive individuals had DM (16.4 % more) than normotensive individuals (Table 3.7). Furthermore, a higher eGFR (on average 19 ml/min per 1.73 m^2) was also observed in hypertensive individuals compared to normotensive individuals.

Apart from SVR, where no significant difference was observed between normotensive and hypertensive individuals ($P \geq 0.05$), hypertensive individuals illustrated significantly higher haemodynamic parameters when compared to normotensive individuals ($P \leq 0.05$) (Table 3.8).

3.6 Impact of Hypertension on uUMOD Concentrations (Multivariate Adjusted)

Figure 3.2 illustrates the uUMOD concentrations of hypertensive and normotensive individuals after adjustments for confounders, in a community sample of African descent with a high prevalence of hypertension. After adjusting for general characteristics (Figure 3.2A), general characteristics and SV (Figure 3.2B), general characteristics and $FENa^+$ (Figure 3.2C) and general characteristics and eGFR (Figure 3.2D), no significant differences were observed between the uUMOD concentrations in normotensive and hypertensive individuals ($P = 0.81$, $P = 0.988$, $P = 0.698$ and $P = 0.648$ respectively).

3.7 Relationship of uUMOD Concentrations and SV in Normotensive versus Hypertensive Individuals

Figure 3.3A illustrates the relationships between uUMOD concentrations and SV in hypertensive individuals and normotensive individuals. Figure 3.3B demonstrates the relationship between uUMOD concentration and SV after adjusting for general characteristics in hypertensive and normotensive individuals in a community sample of African ancestry with a high prevalence of hypertension. Importantly, a significant correlation was observed between uUMOD concentration and SV in hypertensive individuals both before ($P = 0.035$) and after adjustments for confounders. Succeeding adjustments the significant relationship observed between uUMOD concentration and SV strengthened in hypertensive individuals ($P = 0.007$). In contrast no significant relationship between uUMOD and SV was observed in normotensive individuals with the relationship diminishing further after adjustments ($P = 0.425$ before adjustments and $P = 0.948$ after adjustments). In women a clear impact of hypertension was

Table 3.8: Haemodynamic Characteristics of Hypertensive and Normotensive Individuals in a Community Sample of African Ancestry with a High Prevalence of Hypertension (n = 397)

Brachial pressures	Normotensive	Hypertensive	P value
n	205	192	
Systolic blood pressure (mm Hg)	**117 ± 11**	**144 ± 22**	**<0.0001**
Diastolic blood pressure (mm Hg)	**77 ± 7**	**90 ± 12**	**<0.0001**
Pulse pressure (mm Hg)	**38.8 ± 8.8**	**53.4 ± 19.2**	**<0.0001**
Central pressures			
Systolic blood pressure (mm Hg)	**108 ± 11**	**136 ± 21**	**<0.0001**
Diastolic blood pressure (mm Hg)	**78 ± 7**	**91 ± 12**	**<0.0001**
Pulse pressure (mm Hg)	**29.5 ± 8.0**	**44.2 ± 17.6**	**<0.0001**
Determinants of systemic flow			
Aortic flow (Q) (mL)	**309 ± 127**	**388 ± 204**	**<0.0001**
Stroke volume (mL/ beat)	**77.6 ± 35.6**	**90.2± 41.8**	**0.0015**
Heart rate (beats/min)	**64.5 ± 11.0**	**67.3 ± 12.1**	**0.019**
Cardiac output (L /min)	**4.9 ± 2.3**	**6.0 ± 2.9**	**<0.0001**
Mean arterial pressure (mm Hg)	**91 ± 8**	**110 ± 14**	**<0.0001**
End diastolic volume (mL)	**109± 30**	**118 ±31**	**0.0042**
Vascular parameters			
SVR (mm Hg.min/L)	20.6 ± 8.7	21.1 ± 9.8	0.623
TAC (mL / mm Hg)	**2.7 ± 1. 2**	**2.2 ± 1. 2**	**0.0002**
Zc (dynes.s/cm^5)	**83.0 ± 35.9**	**91.4 ± 48.2**	**0.050**

Data is presented as mean ± standard deviation or proportion; Abbreviations: SVR, systemic vascular resistance; TAC, total arterial compliance; Zc, characteristic impedance; significant differences are shown in bold ($P \leq 0.05$). .

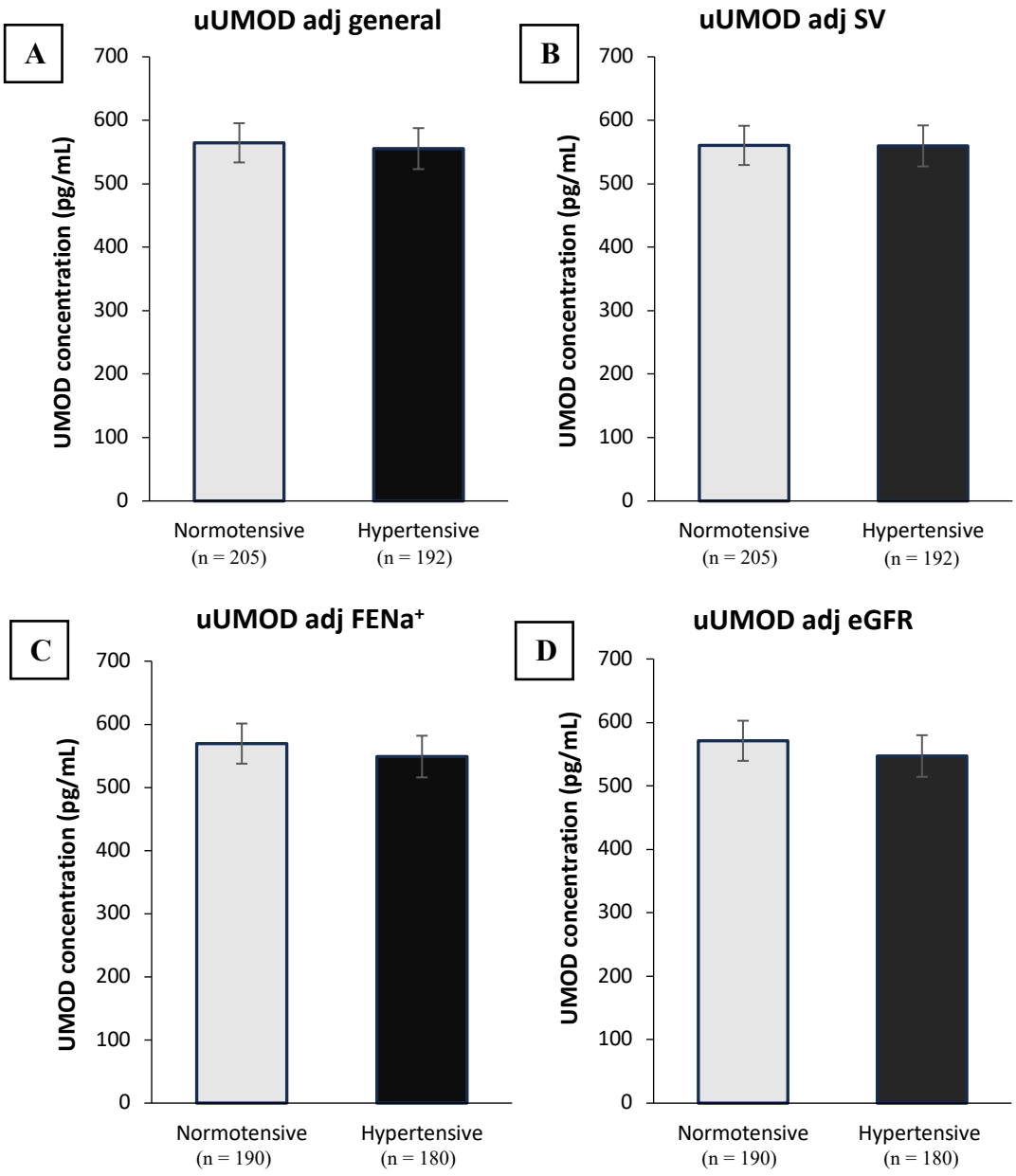


Figure 3.2: Comparisons of Multivariate Adjusted uUMOD Concentrations in Hypertensive and Normotensive Individuals in a Community sample of African Ancestry with a High Prevalence of Hypertension.

Panel (A) refers to the comparison of uUMOD concentration in hypertensive and normotensive individuals after adjusting for general characteristics such as age, sex, BMI, DM, alcohol and tobacco consumption, treatment for hypertension and HR. Panel (B) refers to the comparison of uUMOD concentration between hypertensive and normotensive individuals after adjusting for general characteristics and SV. Panel (C) refers to the comparison of uUMOD concentration between hypertensive and normotensive individuals after adjusting for general characteristics and $FENa^+$ and Panel (D) refers to the comparison of uUMOD concentration between hypertensive and normotensive individuals after adjusting for general characteristics and eGFR. Abbreviations: uUMOD, urinary uromodulin; SV, stroke volume; $FENa^+$ Fractional excretion of sodium; eGFR, estimated glomerular filtration rate; BMI, body mass index; DM, diabetes mellitus; HR, heart rate. * Indicates $P \leq 0.05$. Data presented as mean with bars indicating standard error of mean.

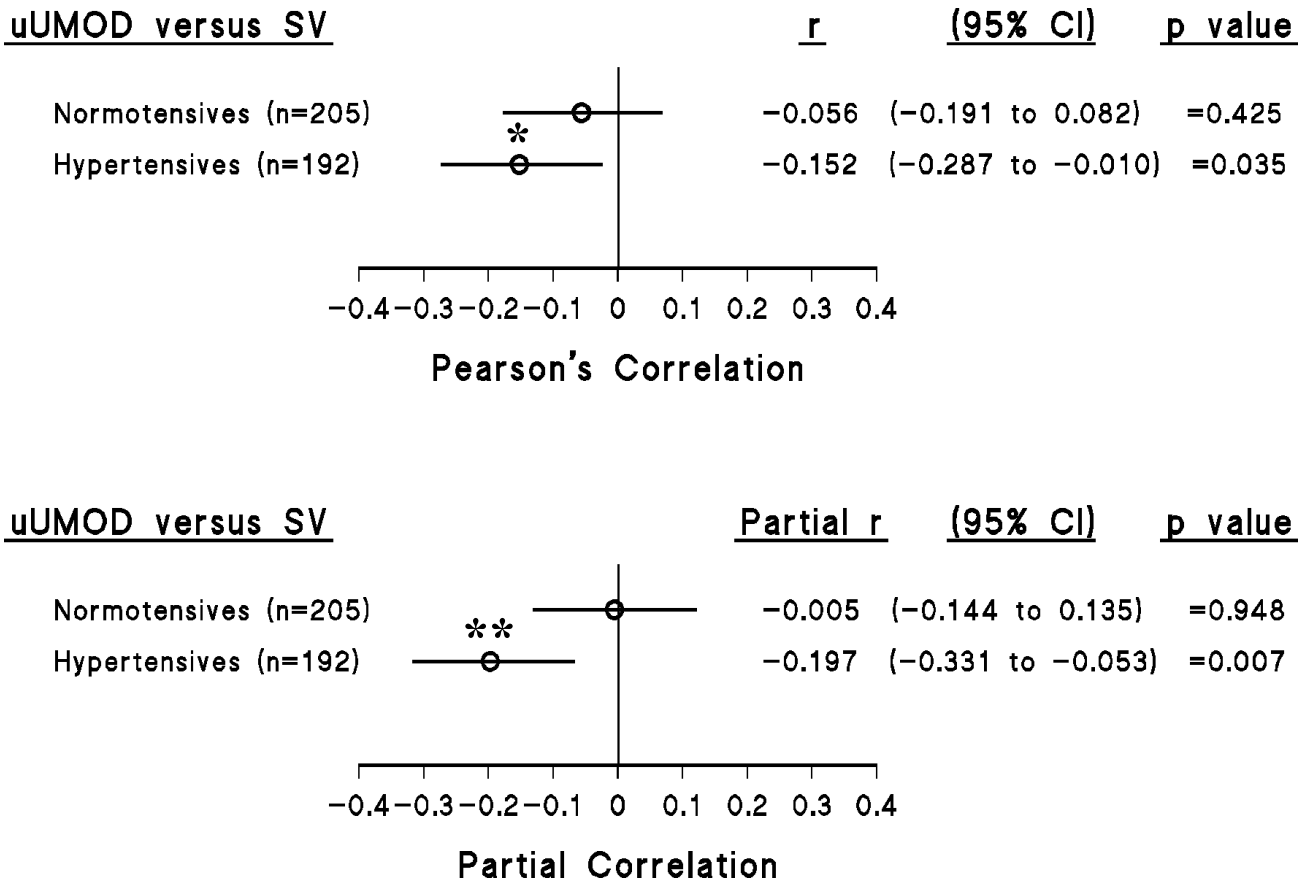


Figure 3.3: Correlations Between uUMOD Concentrations and SV in Hypertensive and Normotensive Individuals in Community Sample of African Ancestry with a High Prevalence of Hypertension.

Panel (A) refers to the bivariant analysis of uUMOD concentration (pg/mL) and SV in hypertensive and normotensive individuals. Panel (B) refers to the multivariant analysis of uUMOD concentration (pg/mL) and SV after adjusting for general characteristics including age, sex, BMI, DM, alcohol and tobacco consumption, treatment for hypertension and HR in hypertensive and normotensive individuals. Data point represents Pearson's r (partial r). Bars represents the range within the 95% CI. * $P \leq 0.05$ and ** indicates $P \leq 0.007$. Abbreviations: uUMOD, urinary uromodulin; SV, stroke volume; DM, diabetes mellitus; HR, heart rate; CI, confidence interval; BMI, body mass index.

noted, with uUMOD being independently and inversely associated with SV in hypertensives (n = 125, partial r = -0.206, P = 0.025), but not in normotensives (n = 131, partial r = 0.122, P = 0.174). However, in men, the inverse relationship between uUMOD and SV was not significant in either normotensives (n = 74, partial r = -0.186, P = 0.130) or hypertensives (n = 67, partial r = -0.102, P = 0.438).

CHAPTER 4: Discussion

4.1 Summary of Findings

In this study, I investigated the uUMOD concentrations in Nguni and Sotho individuals and its relationship with various general, renal and haemodynamic components. My findings illustrated that the average uUMOD concentrations were relatively low in this population. Notably, females exhibited higher uUMOD levels compared to males, even after controlling for multiple confounders. Following bivariate analysis, no significant associations were observed between uUMOD concentrations and renal determinants, age, BMI, BP, or hypertension. Although this study did not establish a link between uUMOD concentrations and renal function, an inverse relationship between uUMOD and SV was observed. The negative relationship between uUMOD and SV was predominantly observed in hypertensive individuals and in hypertensive women rather than normotensive individuals, with hypertensives exhibiting a greater SV than their normotensive counterparts.

4.2 Factors Contributing to Variations in uUMOD Concentrations

While the variability of uUMOD levels has been noted across diverse populations, the exact cause of these variations remains largely unknown (You et al., 2021b). Existing research has identified potential factors that influence uUMOD levels such as ethnicity, sex, medication, salt intake and salt sensitivity, and methods of measuring uUMOD levels (Du et al., 2021; Pruijm et al., 2016; Troyanov et al., 2016). The effect of these factors on uUMOD levels will be discussed in more detail below.

4.2.1 The Impact of Ethnicity on uUMOD Concentrations

Several studies have highlighted variability in uUMOD concentrations within racial groups and between different ethnicities (Köttgen et al., 2012; You et al., 2021b). While the exact reason for intraracial variability has not been fully studied to date, particular GWAS focussing on Caucasian populations have often linked uUMOD variability to variations in genetic factors (Köttgen et al., 2012; Olden et al., 2014; Troyanov et al., 2016). These variations include: (1) monogenic mutations leading to various kidney diseases, and (2) common variants near or in the promoter region of the *UMOD* gene, frequently associated with CKD and hypertension (Köttgen et al., 2012; Olden et al., 2014; Troyanov et al., 2016). To support the genetic findings, a meta-analysis study involving individuals both with and without acute kidney injury demonstrated variability in uUMOD levels among those of similar ethnicities (You et al., 2021b). Additionally, age has been identified as a factor influencing uUMOD levels, with

uUMOD levels fluctuating throughout different life stages, initially rising with age until maturity, and subsequently declining after the age of 60 years (Campese & Park, 2006). However, even in individuals of similar age, the meta-analysis revealed variability in uUMOD levels (You et al., 2021b).

Beyond intraracial variations in uUMOD levels, interracial differences have also been observed. Notably, Nqebelele et al., (2019), not only illustrated uUMOD variability between Black South Africans and Caucasians (with lower uUMOD concentrations observed in Black South Africans compared to Caucasians), but also provided the first characterisation of uUMOD concentrations in Black South Africans. While my study corroborated the lower uUMOD levels in Black South Africans compared to Caucasians, the uUMOD concentrations were notably lower than those reported by Nqebelele et al., (2019). It is crucial to note that Nqebelele et al., (2019) focused on individuals with CKD, while only 6% of my participants had CKD, potentially explaining the intraracial differences observed between our studies. Given that the majority of research on uUMOD levels has been done on individuals of European descent, further intraracial and interracial studies are warranted to understand the role of ethnicity on uUMOD levels.

4.2.2 Impact of Sex on uUMOD Concentrations

It is highly controversial as to whether uUMOD levels are directly or indirectly linked to sex. While evidence linking uUMOD concentrations to sex are contradictory in heterogenous populations, my study aligns with the findings of Troyanov et al., (2016). Research by this group revealed a significant association between the uUMOD levels and sex, indicating that females generally exhibit higher uUMOD concentrations (Pruijm et al., 2016; Troyanov et al., 2016). Similar to my study, a recent Cardiovascular Health Study found an association between uUMOD levels and sex in a population of 933 individuals of which only 15% were of African descent (Steubl et al., 2020). However, in contrast to my study, males were observed to have higher uUMOD concentrations (Steubl et al., 2020). It is unclear whether the differences observed between my population and the Cardiovascular Health Study are due to hormonal differences, which affect uUMOD levels differently in heterogeneous populations, or if the differences observed may result from ethnic, demographic and anthropometric influences. Due to different sexes being observed to have higher uUMOD concentrations in different populations, it is highly unlikely that the difference is due to levels of reproductive hormones

such as oestrogen, progesterone and testosterone. No studies, however, investigated the effects of reproductive hormones on uUMOD levels. In the Cardiovascular Health Study, limited data was available on males for a direct comparison of the sexes between their study verses my study (Steubl et al., 2020). In my population, however, females exhibited significantly higher BMIs with the majority of the females falling within the obese category. Despite this, no association between BMI and uUMOD concentrations were observed in my study, making it unlikely that BMI influences the difference in concentrations observed between the sexes in this population. It is not uncommon for BMI to be associated with uUMOD levels as prior studies have linked higher BMI with lower uUMOD levels (Troyanov et al., 2016), which further contradicts what I found. Even after adjusting for potential confounders like BMI, higher uUMOD levels persisted in females, further diminishing the likelihood of the sex difference being a result of BMI in my cohort.

Furthermore, despite a higher number of males engaging in regular alcohol and tobacco consumption, no significant link between these factors and uUMOD levels were observed in my study and other studies (Troyanov et al., 2016). Regardless of males and females having more or less similar percentages of hypertensive individuals, females were observed to be more adherent to antihypertensive medications compared to the males. No significant difference was observed between normotensives and hypertensives in uUMOD levels, however whether antihypertensive treatment explains the difference between the male and female concentrations cannot be entirely overlooked and requires further discussion.

4.2.3 The Impact of Chronic Medication on uUMOD Concentrations

To date, the direct effect of many chronic medications on uUMOD levels remain unclear. Studies have associated a decrease in uUMOD concentration with longstanding diabetes (> 15 years) (Pruijm et al., 2016; Torffvit et al., 1992; Vikulova et al., 2018). Subsequent investigations further illustrate that chronic diabetes medications, such as insulin and glucagon-like peptide 1 receptor agonist (which stimulates the body to produce more insulin), increase uUMOD concentrations (Pruijm et al., 2016; Torffvit et al., 1992; Vikulova et al., 2018). Given that only an eighth of my population had DM and lower levels of uUMOD were observed, it is highly unlikely that the overall diminished uUMOD levels observed in my study were due to longstanding diabetes or influenced by diabetes related medication. Similarly, recent research has found that certain medications, such as losartan and fenofibrate (which includes uricosuric

medication that increases the amount of uric acid excreted in cases of gout), decrease the amount of uUMOD excreted (Troyanov et al., 2016). However, none of my participants were on uricosuric medications thus I cannot attribute the concentrations observed in my population to this medicine usage.

Additionally, a study has demonstrated that the use of RAAS inhibiting medication has no effect on uUMOD levels, contrary to furosemide which has been noted to decrease uUMOD concentrations (Troyanov et al., 2016). This finding contradicts the observations in a study involving Sprague-Dawley rats, where an increase in the steady state UMOD mRNA levels and excretions were observed after the administration of furosemide (Ying & Sanders, 1998). These findings suggest a potential species-specific uUMOD excretion response to furosemide. Notably, studies have yet to explore the direct impact of hydrochlorothiazide medications, used by at least a quarter of my study population, on uUMOD levels. Despite a marginal increase being observed in the uUMOD levels in hypertensives compared to normotensive participants, this difference lacked statistical significance. It is thus improbable that chronic medication (primarily hydrochlorothiazide) used by my cohort significantly impacted uUMOD concentrations. Nonetheless, the impact of various types of chronic medication on uUMOD levels needs to be further investigated.

4.2.4 Impact of Salt Intake and Salt Sensitivity on uUMOD Concentrations

An intervention study conducted on hypertensive Caucasian individuals, revealed an increase in uUMOD levels with higher dietary salt consumption (Torffvit et al., 2004). This positive correlation between salt intake and uUMOD levels was not exclusive to Caucasians, as evidenced by a recent intervention study in a Chinese population, which also suggested a potential role for UMOD in the pathophysiological process of salt sensitivity in this population (Du et al., 2021). Notably, both of these intervention studies required a substantial salt intake of 200 mmol/day or more in order to elicit an increase in uUMOD concentrations (Du et al., 2021; Torffvit et al., 2004). However, a prior study by Scott et al., (2011) on individuals of African ancestry illustrated an average salt intake of 104 mmols/day, with only 42% of participant's salt intake falling above 105 mmol/day (Scott et al., 2011). While the salt intake in individuals of African ancestry were notably lower than the intervention studies, studies by Maseko et al., (2006) and Scott et al., (2011) have illustrated that the majority of individuals of African ancestry, 89% and 69% respectively, had a daily salt intake over 65 mmol/day,

thus surpassing the recommended daily allowance (RDA) (Maseko et al., 2006; Scott et al., 2011). In this regard, it unclear whether salt intake between 65 mmol/day – 200 mmol/day (with 60 mmol/ day being the RDA and 200 mmol/day being the definition of high salt intake) impacts on uUMOD concentrations particularly in individuals of African ancestry, and if so, what the implications of this relationship are in individuals of African descent (Castiglioni et al., 2017). While no investigations have specifically explored the impact of salt consumption on uUMOD levels in persons of African descent, studies indicate that 73% of hypertensive and 36% of normotensive individuals of African ancestry are salt sensitive (Jones & Rayner, 2021; Svetkey et al., 1996). Salt sensitivity has also been established by these studies as a characteristic feature of African hypertensives (Jones & Rayner, 2021; Svetkey et al., 1996). Nevertheless, the influence of salt sensitivity on uUMOD levels in individuals of African descent remains unexplored. More research is required to investigate the impact of salt sensitivity on uUMOD in African populations.

4.2.5 The Impact of Various Methods on uUMOD Concentrations

a) *Spot Urine vs 24-Hour Urine*

A study by Pruijum et al., (2015) illustrated similar uUMOD concentrations in morning spot urine and 24-hour urine in Caucasians. However, the variability of uUMOD levels throughout the day is yet to be determined. Apart from uUMOD's associations with salt sensitivity and certain medications, studies have linked uUMOD levels to protein intake, thyroid hormone, hydration status and antidiuretic hormone (Devuyst et al., 2017; Duława et al., 1998; Schmitt et al., 2004; Thielemans et al., 2023; Torffvit et al., 2004; Ying & Sanders, 1998). Given the apparent susceptibility of uUMOD to diverse environmental, dietary factors and hormonal changes, it is plausible that the levels of uUMOD fluctuate throughout the day. This dynamic nature of uUMOD could thus potentially influence spot uUMOD concentrations based on the time of the day the urine is collected. Consequently, variations in the time of the day at which spot urine samples are collected may influence the parity in uUMOD levels between spot urine and 24-hour urine. To my knowledge, no study has investigated and contrasted spot uUMOD concentrations throughout the day to 24 -hour uUMOD concentrations, therefore future studies are warranted.

b) *ELISA vs Meso Scale Discovery*

Various approaches are available for measuring uUMOD concentrations, with the majority of studies, as indicated by a meta-analysis, opting for ELISA approaches or Meso Scale Discovery (MSD) (You et al., 2021b). MSD is a multiplex assay which uses electrochemiluminescence to quantify one or more proteins and is recognised for its heightened sensitivity and broader detection range compared to ELISA (Sourial et al., 2009). Despite these advantages, limited information regarding how MSD compares with ELISA in detecting uUMOD concentrations is available. While both methods have successfully quantified uUMOD levels, no study has explored the potential variations in concentrations of the same uUMOD sample using either approach. Methodological dependencies may underlie variations in uUMOD concentrations. However further research is essential to elucidate these aspects.

4.3 Cardiovascular Implications of uUMOD Concentrations

Numerous studies have associated variations in uUMOD levels with various human diseases such as CKD, hypertension and CVD (Thielemans et al., 2023). It is, however, unclear whether uUMOD levels predict, CKD, hypertension or future CVD events in individuals of African descent. Although contentious, the Systolic Blood Pressure Intervention Trail (SPRINT) discovered that decreased uUMOD concentrations increased the risk of incident CVD (Garimella & Sarnak, 2016). In contrast to SPRINT, GWAS indicated that lower uUMOD levels (induced by common polymorphisms in the *UMOD* gene promoter area) reduced the risk of CVD, heart failure, and CKD mortality in Caucasians (Padmanabhan et al., 2010). A second investigation on Europeans confirmed the GWAS findings, demonstrating that greater uUMOD concentrations increased the risk of myocardial infarction (MI) via raised BPs (Jian et al., 2022). Dulawa et al., (1998), also found lower uUMOD levels in elderly normotensive individuals but increased uUMOD levels in elderly hypertensive individuals. While this study did not explain the observed phenomenon, Dulawa et al., (1998) rationalised that the decrease in uUMOD concentrations in normotensive individuals may be due to the natural physiological decrease in renal function with age. The higher uUMOD concentrations in hypertensives were suggested to result from renal adaptations to hypertension (Duława et al., 1998).

Although many studies favour the pathogenicity of higher uUMOD levels as opposed to low uUMOD concentrations in Caucasians, it remains questionable whether the opposite could apply to individuals of African ancestry ruling in favour of the SPRINT study. Lower uUMOD

levels have been associated with an 8-fold increased risk of cardiovascular deaths (Thielemans et al., 2023). Given the heterogeneity and variations in disorder susceptibility amongst different populations, low concentrations of uUMOD in my study population may be associated with pathogenicity. This is especially relevant considering the high prevalence and susceptibility of hypertension and hypertension-related comorbidities in this population. However, whether low uUMOD levels directly influence BP in my cohort needs to be further explored. It also remains questionable whether uUMOD levels directly impacts on hypertension in my population. Prior evidence linked higher uUMOD levels with higher SBP and DBP in Caucasians (Jian et al., 2022). Individuals with higher uUMOD levels exhibited a 69% and 87% higher risk of elevated SBP and DBP, respectively, contributing to an overall increased risk of MI (Jian et al., 2022). In contrast to the Jian et al., (2022) study, a prior study linked higher DBP to lower uUMOD excretion (Troyanov et al., 2016). However, I found no association between both brachial and central SBP and DBP with uUMOD concentrations in my cohort. Whether lower concentrations of uUMOD provide a protective function in individuals of African ancestry or affects BPs through alternative means, such as increased serum UMOD (sUMOD) concentrations or increased renal accumulation of UMOD, requires further investigation.

Additionally, there is an extensive debate about whether haemodynamic parameters are affected by or affect uUMOD concentrations in different populations. My study showed no association between BP, hypertension, vascular parameters and the majority of systemic flow determinants with uUMOD concentrations. However, a negative association was observed between SV and uUMOD in hypertensives. A study investigating the relationship between uUMOD and BP changes in response to salt found no association between BP and uUMOD levels with the change in salt intake (Bakhoum et al., 2021). In contrast, a study done during the same year found the complete opposite result substantiating the role of uUMOD in the salt sensitive component of BP regulation via the regulation of the NKCC2 (Ponte et al., 2021). Where my study favours the former results found by Bakhoum et al., (2021), it is uncertain whether uUMOD indeed does not affect the regulation of BP, as a negative association was found between SV and uUMOD excretion only in hypertensives. It is highly possible that uUMOD levels indirectly affects BP by influencing SV, where the low uUMOD excretion levels found in our population translates into the elevated SV observed in hypertensives. A previous study from our laboratory illustrated that age-related increases in BP are strongly related to CO and SV, and thus blood flow, in people of African ancestry (Woodiwiss et al., 2020). In addition to this, a subsequent study from our laboratory found SV to be strongly and

inversely associated with renal function determinants (both eGFR and $FENa^+$) (Malan et al., 2021). The absence of association between both BP and renal determinants with SVR indicates that renal mechanisms translate into a volume-dependent form of hypertension in this cohort. Despite this, my study revealed no association between uUMOD and renal functions or BP, thus suggesting that uUMOD levels do not directly influence BP through renal function but instead through flow i.e. SV. The mechanism underlying this phenomenon is unknown, therefore this study may be the first to demonstrate an association between low uUMOD levels and elevated SV in individuals of African ancestry. Furthermore, whether serum or localised renal UMOD concentrations affect BP and influence SV needs to be further investigated.

4.4 Renal Implications of uUMOD Concentrations

In addition to cardiovascular events, lower uUMOD levels have been associated with reduced risk of progressive renal disease (Gudbjartsson et al., 2010; Köttgen et al., 2010). Many studies, however, share opposing views indicating that lower levels, in fact, increase the risk of nephron damage and renal disease (Garimella & Sarnak, 2016; You et al., 2021b). Studies have also rationalised that higher levels of uUMOD concentrations are indicative of a higher percentage of functioning tubules and tubular reserves, thus limiting the progression of kidney disease (Garimella et al., 2015). Moreover, a recent study on mice illustrated that the loss of the *UMOD* gene caused a defect in anti-natriuresis with salt wasting and polyuria (Liu et al., 2018). The finding of the Liu et al., (2018) study was consistent with the effects observed in TAL of the Loop of Henle reabsorption dysfunction which results in reduced NKCC2 activity and ultimately affects Na^+, Cl^- and K^+ reabsorption. However, this study did not evaluate the level of UMOD remaining in the kidney and the UMOD reabsorbed into the serum. Consequently, it remains uncertain whether individuals in our population generally have low UMOD production, which impacts on NKCC2 activity. Additionally, it is unclear whether the low uUMOD concentration infers the accumulation of UMOD within the kidney, which would upregulate NKCC2 activity. This potential upregulation may lead to increased Na^+ and Cl^- reabsorption, indirectly causing hypertension through increased blood volume. In this regard, the population that I studied have previously been reported to have high-volume hypertension associated with high SV (Woodiwiss et al., 2020). High Na^+ and Cl^- ion reabsorption in the TAL is known to increase the amount of water reabsorbed. This could explain the volume-dependent form of hypertension observed in this cohort. However, further studies on NKCC2 activity, renal UMOD localisation and serum reabsorbed UMOD concentrations are required.

Over the last three decades, clinical and experimental kidney transplant studies (in which kidney donors with a family history of hypertension increased the BP of genetically normotensive recipients and vice versa) have associated renal function with primary hypertension (Curtis et al., 1983; Graham et al., 2014; Guidi et al., 1985, 1996). Several subsequent studies (both rat and human) have shown renal mechanisms to be a key predictor of primary hypertension (Curtis et al., 1983; Graham et al., 2014; Guidi et al., 1985, 1996). A recent study from our lab further supported the theory of renal mechanisms playing a fundamental role in hypertension, considering that individuals of African ancestry exhibit a renin-independent, volume-dependent form of hypertension (Malan et al., 2021; Woodiwiss et al., 2020). In this regard, we examined the effect of uUMOD levels on renal function, however, in this study we found no link between renal function (as assessed from $FENa^+$ and eGFR) and uUMOD levels. A recent study on black South Africans found a positive correlation between eGFR and uUMOD excretion (Nqebelele et al., 2019). Nqebelele et al., (2019) study population comprised of individuals with CKD (n = 71), their first degree relatives (n = 52) and controls (n = 58). As the majority of the participants had CKD or were close relatives of individuals with CKD, it is highly possible that the presence or absence of CKD or the cause of CKD if present impacts the relationship between uUMOD excretion and eGFR in Black South Africans. Both spot urine and 24-hour uUMOD concentrations were associated with eGFR in European CKD patients (Pruijm et al., 2016). However, only 6% of our population had mild to moderate CKD (eGFR < 60ml/min) thus further studies are required to compare the relationship between uUMOD and renal determinants in CKD patients with individuals without CKD. Numerous studies have indicated associations between renal determinates and uUMOD one of which was an American study on 5888 participants (Garimella et al., 2015; Thielemans et al., 2023). This American study associated higher uUMOD levels with eGFR decline, thus favouring an inverse association and suggesting that uUMOD may be a biomarker for kidney function (Garimella et al., 2015; Thielemans et al., 2023). Although normotensives had significantly higher eGFR than hypertensives (stage 1 CKD indicating possible kidney damage with normal kidney functions) in this study, no association between uUMOD and renal function was found in either group. The lack of association between uUMOD and renal function makes it unclear whether uUMOD can serve as a renal biomarker for kidney function in my study population. However, further studies need to be done on this population to confirm whether uUMOD can be a determinant of renal function in this cohort.

4.5 Potential Limitations

I faced several potential limitations in my study. Firstly, the extended duration and conditions of urine sample storage, and potential thaw-freeze cycles may have impacted uUMOD concentrations due to its sensitivity to storage conditions. However, efforts were made to maintain consistency in storage conditions (the samples used had not undergone many freeze-thaw cycles), and storing samples at -80 °C, an optimal temperature recommended by studies to prevent uUMOD degradation (Youhanna et al., 2014). Secondly, this was an association study, therefore my research cannot establish causality. Thus, future intervention studies are warranted to verify whether low uUMOD concentrations lead to elevated SV or vice versa. Thirdly, the participants of this study were exclusively individuals of African ancestry. Thus the results obtained in my study may not apply to individuals of other ethnicities. Future studies encompassing diverse ethnic groups are therefore necessary to ascertain the broader application of my results. Lastly, the majority of the participants in the study had normal renal function, making results less applicable to individuals with any form of renal dysfunction.

4.6 Future Studies

As previously mentioned, several GWAS have established a link between common variations in *UMOD* loci (which increases both sUMOD and uUMOD concentrations) and hypertension in Caucasians (Gudbjartsson et al., 2010; Köttgen et al., 2010; Padmanabhan et al., 2010; Ponte et al., 2021; You et al., 2021a). These studies alongside, transgenic mouse models, revealed that risk variants induce salt-sensitive hypertension and age-dependent renal lesion (Trudu et al., 2013). Despite these findings, the association between these *UMOD* variants and hypertension in individuals of Nguni or Sotho ancestry remains unexplored. With low uUMOD concentrations being associated with high SV and high SV being associated with renal-related mechanisms (as mentioned in the discussion), further investigating the possible indirect mechanisms that link renal-related influences to uUMOD levels in hypertensive individuals of African ancestry is needed. While our study does not show a direct association between uUMOD levels and hypertension, it serves as a foundation for future investigations. These could include exploring *UMOD* polymorphisms in our population, understanding their role in low uUMOD levels observed, examining serum and renal UMOD concentrations, evaluating the impact of uUMOD concentrations on CKD, studying hormone and medicinal effects on uUMOD levels, investigating uUMOD role in salt sensitivity in individuals of African ancestry

and investigating whether the uUMOD levels indirectly contribute to hypertension through elevated SV in individuals of African ancestry.

4.7 Clinical Relevance of Findings

These findings highlight the need to investigate the mechanisms linking uUMOD to haemodynamic parameters, specifically SV. Identifying the potential mechanistic link between uUMOD and SV may assist in identifying novel pathways to target for better treatment strategies in volume-dependent hypertension. Such future studies will be invaluable in improving BP control in volume-dependent hypertension.

4.8 Conclusion

In a community sample with a high prevalence of volume-dependent primary hypertension, we observed an inverse relationship between uUMOD and SV. This association was particularly notable in participants with hypertension. While this study does not support the use of uUMOD as a biomarker for renal function in individuals of African ancestry, due to the lack of association with renal determinants, future studies which investigate the possible indirect influence of uUMOD on renal function in hypertensive individuals need to be considered. Furthermore, pending the results of future intervention studies to establish the causal link between uUMOD and SV (a marker of increased blood volume), uUMOD may assist in establishing novel treatment strategies to better treat volume dependent-hypertension.

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Appendices

Appendix 1: Human Ethics Approval

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Professors GR Norton and AJ Woodiwiss

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220393

NAME: (Principal Investigator)	Professors GR Norton and AJ Woodiwiss
DEPARTMENT:	School of Physiology Cardiovascular Pathophysiology & Genomics Res Unit Medical School University
PROJECT TITLE:	*Gene candidates as determinants of blood pressure and intermediary phenotypes in the pathogenisis of hypertension in Black South Africans*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Renewal of M170401- expires on 2022/04/06
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Not applicable
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/04/04

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **March** and therefore reports and re-certification will be due in the month of **March** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

Appendix 2: Turnitin Report (Plagiarism Report)

Final uUMOD Dissertation Aaliah Charles.docx

ORIGINALITY REPORT

13%	1%	4%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Witwatersrand Student Paper This info used was from my Protocol submitted on 19/07/2022.	9%
2	Olena Maydan, Paul G. McDade, Yan Liu, Xue-Ru Wu, Douglas G. Matsell, Allison A. Eddy. "Uromodulin deficiency alters tubular injury and interstitial inflammation but not fibrosis in experimental obstructive nephropathy", Physiological Reports, 2018 Publication	1%
3	Joey P. Granger, Barbara T. Alexander, Mayte Llinas. "Mechanisms of pressure natriuresis", Current Hypertension Reports, 2002 Publication	1%
4	www.zora.uzh.ch Internet Source	<1%
5	Raïsa Thielemans, Reinhart Speeckaert, Charlotte Delrue, Sander De Bruyne, Matthijs Oyaert, Marijn M. Speeckaert. "Unveiling the Hidden Power of Uromodulin: A Promising Potential Biomarker for Kidney Diseases", Diagnostics, 2023	<1%

Publication

6	Alexandre Loktionov, Hester Vorster, Ian K. O'Neill, Theo Nell, Sheila A. Bingham, Shirley A. Runswick, John H. Cummings. "Apolipoprotein E and methylenetetrahydrofolate reductase genetic polymorphisms in relation to other risk factors for cardiovascular disease in UK Caucasians and Black South Africans", Atherosclerosis, 1999 Publication	<1 %
7	Nico Malan, Gavin R. Norton, Vernice R. Peterson, Suraj M. Yusuf et al. "Independent relationships between renal mechanisms and systemic flow, but not resistance to flow in primary hypertension in Africa", Journal of Hypertension, 2021 Publication	<1 %
8	research.library.mun.ca Internet Source	<1 %
9	Scolari, F., C. Izzi, and G. M. Ghiggeri. "Uromodulin: from monogenic to multifactorial diseases", Nephrology Dialysis Transplantation, 2014. Publication	<1 %