

Drug dosing in the elderly:

The impact of age and tubular function

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Te Whare Wānanga o Otāgo

NEW ZEALAND

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Abstract

Renal drug elimination is a function of the combined processes of glomerular filtration, tubular secretion and reabsorption. Traditionally the dosing of renally excreted drugs has been modified according to estimates of glomerular filtration rates (eGFR) using equations based on serum creatinine^(1, 2). The assumptions inherent in this process are that all components of kidney function decline at the same rate and in direct relationship with eGFR⁽³⁻⁶⁾ and that eGFR is a reasonable estimate of measured GFR (mGFR). However, many drugs are eliminated by tubular anionic and cationic transport where a decline in eGFR may not necessarily reflect parallel changes in tubular function. This study investigates the relationship between GFR and renal tubular function with reference to drug handling using an established drug “cocktail” method⁽⁷⁻⁹⁾. Measured GFR (mGFR; Cr⁵¹ EDTA clearance) is compared to tubular anionic transport (urate clearance), tubular reabsorption (fluconazole clearance) and cationic transport (S-pindolol clearance). In addition, the influence of comorbid pathology (primarily hypertension) over and above that of aging alone is investigated by recruiting subjects with a range of ages, coexistent disease and renal function and comparing four groups. The groups investigated were “young” controls with normal serum creatinine, two groups above the age of 65 years with normal creatinine both with and without mild comorbid pathology known to affect renal function such as hypertension, diabetes and heart disease and those with established chronic kidney disease. To complete the study mGFR is compared to several of the commonly utilised estimating formulae for GFR (eGFR).

The major findings demonstrate a moderate positive correlation between mGFR and proximal tubular anion transport and reabsorption ($R^2=0.41$ and 0.44 , $p<0.05$). In contrast, cationic secretion correlated poorly with mGFR ($R^2=0.11$, $p<0.05$). In the study population with normal renal function the estimating equations significantly

underestimated measured GFR (-26-38 mL/min/1.73m²) with the Chronic Kidney Disease Epidemiology (CKD-EPI) formula the most accurate overall. The tubular processes investigated displayed declining clearances with age and GFR. However, in the elderly with normal creatinine, mild pathology unexpectedly increased clearances relative to those without disease.

In clinical practice the elderly, both with and without comorbid disease, form a significant proportion of any caseload where adjustments of drug dosing for renal function are extremely important to avoid significant side effects. However given that drug-dosing schedules utilise eGFR values as the basis for modifying drug dosing, these results suggest that a global recommendation of dose reduction according to eGFR alone should be treated with some caution. This is due to the significant underestimation in GFR by the estimating equations in those without chronic kidney disease (CKD) and the lack of parallel decline of tubular function to GFR. This is most significant where cationic tubular handling of the drug is the major component of elimination where reliance on eGFR may lead to under-dosing of these medications.

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Statement

This study was conducted under the approval of the Northern Y Regional Ethics Committee of the Ministry of Health, New Zealand in January 2010 (Protocol number NTY/10/12/103) and in accordance with the Declaration of Helsinki. It was registered with the Australian and New Zealand Clinical Trials Registry number: ACTRN 12611000035921

Written and informed consent was given by each subject before specific assessments and protocol began.

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Abbreviations

ABW	Actual Body Weight
ACE	Angiotensin Converting Enzyme
AGE	Advanced Glycosylation End Products
ARB	Angiotensin Receptor Blocker
ATN	Acute Tubular Necrosis
AUC	Area Under the Curve
BNF	British National Formulary
BSA	Body Surface Area 1.73 m ²
BLSA	Baltimore Longitudinal Study of Aging
CKD	Chronic Kidney Disease
CCB	Calcium Channel Blocker
CHF	Congestive Heart Failure
CG	Cockcroft Gault
CKD-EPI	Chronic Kidney Disease Epidemiology
CKD-MBD	Chronic Kidney Disease Mineral Bone Disorder
Cr ⁻⁵¹ EDTA	Chromium ⁻⁵¹ EthyleneDiamineTetraacetic Acid
CrCl	Creatinine Clearance
DTPA	DiethyleneTriaminePenta Acetate
eGFR	Estimated GFR
ESRF/ESRD	End Stage Renal Failure / Disease
FDA	Food and Drug Administration

GBM	Glomerular Basement Membrane
GFR	Glomerular Filtration Rate
K/DOQI	Kidney Disease Outcomes Quality Initiative
kDa	Kilo Daltons
KIM-1	Kidney Injury Molecule -1
h	Hour
HCT	Haematocrit
HIV	Human Immunodeficiency Virus
HTN	Hypertension
IDMS	Isotope Dilution Mass Spectrometry
Kg	Kilogram
L	Litre
LBW	Lean Body Weight
m	Metre
MDRD	Modified Diet in Renal Disease
mGFR	Measured Glomerular Filtration Rate
min	Minute
mL	Millilitre
Na/H	Sodium Hydrogen channel
Na/K/ATPase	Sodium Potassium Adenotriphosphatase
NONMEM	Non Linear Mixed Effects Modelling
NGAL	Neutrophil Gelatinase Associated Lipocalin
NKCC	Sodium Potassium 2 Chloride channel
NSAID	Non-Steroidal Anti-Inflammatory Drugs

OAT	Organic Anion Transporter
OCT	Organic Cation Transporter
RAS	Renin Angiotensin System
RBF	Renal Blood Flow
RPF	Renal Plasma Flow
SDHB	Southern District Health Board
Tc ⁻⁹⁹	Technetium ⁻⁹⁹
UA	Uric Acid
URAT	Uric Acid Anion Transporter
UV/P	Urinary concentration x Volume / Plasma concentration
WHO	World Health Organisation
μmol/L	Micromoles per litre

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Chapter 1

Literature Review

1.1 Introduction

Renal drug elimination is a product of the processes of glomerular filtration, tubular secretion and reabsorption. Conventional measures of renal function such as glomerular filtration rate or creatinine clearance may not detect alterations in the integrity of the tubular function and hence do not necessarily detect altered renal clearance of a drug. Recommendations in drug formularies⁽¹⁰⁾ for modification of drug dosing in the setting of kidney impairment are made based on calculated creatinine clearance derived from the Cockcroft Gault formula⁽¹¹⁾. Current clinical practice is to determine renal function by estimating glomerular filtration rate (eGFR) and then adjusting the dose based on eGFR and the fraction of the drug excreted unchanged. The assumptions inherent to this practice are first that a decline in GFR equally reflects a similar decline in tubular function and second that eGFR accurately reflects measured GFR (mGFR). The eGFR formulae all use age as a variable, thus automatically assuming that the elderly have a degree of renal impairment, which categorizes them into categories of chronic kidney disease. In the healthy elderly this assumption may not be valid.

This thesis explores the concept whether any decline in glomerular filtration rate reflects parallel changes in tubular function and the ramifications of this when considering modification of drug dosing in clinical practice. This is important as many drugs are in fact handled by tubular secretion and reabsorption rather than predominantly filtration. In addition, the study investigates the influence of pathological changes from co-morbid disease on any changes in kidney function occurring with advancing age - are these alterations due to the normal physiology

of aging, or the addition of pathological processes? Or do healthy elderly without pathology maintain normal kidney function?

Prior to investigating these issues it is necessary to review the measurement of renal function and current concepts of how renal drug handling alters with age and disease.

1.2 Chronic kidney disease

Chronic kidney disease (CKD) is defined according to the level of kidney function ($\text{eGFR} < 60 \text{ mL/min/1.73m}^2$ hereafter mL/min) and the presence of markers of kidney damage irrespective of the specific diagnosis, present over at least three months duration. This internationally recognized definition from the Kidney Disease Outcomes Quality Initiatives 2002 (K/DOQI)⁽¹²⁾ has gained widespread acceptance incorporating the concept that CKD reflects both structural and functional changes, using significant proteinuria and scarring as markers of disease, in addition to a decline in the standard estimation of the glomerular filtration capacity (eGFR).

Thus, there are two independent criteria for CKD:

- Kidney damage for ≥ 3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifested by either: pathological abnormalities (markers of kidney damage) such as abnormalities in the composition of the blood or urine; or abnormalities in imaging tests.

OR

- $\text{eGFR} < 60 \text{ mL/min}$ for ≥ 3 months, with or without structural kidney damage.

Staging of individuals (see Table 1) with CKD⁽¹²⁾ is defined on the level of kidney function primarily using eGFR, which is a derived determination of GFR using the

Modification of Diet in Renal Disease 4 variable (MDRD) formula⁽¹³⁾; which will be discussed in section 1.5.1. Stage 1 is further defined on the basis of albuminuria/proteinuria or other evidence of structural kidney disease. The CKD classification specifically defines albuminuria as an indication of both tubular and glomerular dysfunction although acknowledges there will be a small group with significant tubular proteinuria may be missed by screening for albuminuria alone.

Table 1: K/DOQI definition of CKD staging ⁽¹²⁾

Stage	Description	mL/min/1.73m²
1	Kidney damage with normal or ↑ GFR	≥90
2	Mild ↓ GFR	60-89
3	Moderate ↓ GFR	3a: 45-59 3b: 30-44
4	Severe ↓ GFR	15-29
5	ESRD	<15 (or dialysis)

ESRD: End Stage Renal Disease

An anomaly of this staging system is the disparity between outcomes of those with reduced eGFR alone (with age as major determinant of this) and those with evidence of kidney damage. For example those with stage 1 kidney disease (i.e. GFR > 90 mL/min and albuminuria or structural disease) have a worse prognosis than those with stage 2 disease where the eGFR is between 60 and 90 mL/min without proteinuria⁽¹⁴⁾. It has been found that those with a GFR less than 45 mL/min have significantly higher mortality and morbidity over the age of 65 years⁽¹⁵⁾ than those with an eGFR greater than 45 mL/min. Modifications to this basic categorization system to include proteinuria at all stages and to subdivide stage 3 into 3a: eGFR 45-59 mL/min and 3b: 30-44 mL/min to reflect the findings above have been mooted but not universally accepted. For most patients a GFR greater than 45-60 mL/min is entirely adequate for normal function, irrespective of

age, and is reflected in the absence of any sequelae of CKD such as anaemia or mineral bone disorder. A significant decline from a previous estimate may be a better indicator of kidney disease for the individual. The diagnosis of CKD therefore requires assessment of glomerular filtration – generally by using the plasma creatinine and extrapolating the creatinine clearance from estimating equations, and also urinalysis for quantification of albuminuria above 0.3g/day (the equivalent of 1+ proteinuria on dipstick).

Routine reporting of values of eGFR from laboratories can lead to labelling many elderly patients as having chronic kidney “failure” or disease, despite having no other evidence of kidney damage or loss of function. The use of age appropriate staging/reporting has been proposed but not taken up internationally due to inadequate discussion.

With successful aging there is an increasing proportion of adults with chronic disease such as diabetes, obesity, hypertension and heart disease that contributes to the escalation in incidence and prevalence of CKD both in Australasia and worldwide. A systematic review of 26 studies in predominantly Western cultures demonstrated the prevalence of CKD to be 25-35% of those over 65⁽¹⁶⁾. In Australia, and by inference New Zealand, the AusDiab study⁽¹⁷⁾ identified that 16% of Australians have CKD – although this was reduced to 11% if the Chronic Kidney Disease Epidemiology (CKD-EPI) formula^(18, 19) was used to estimate GFR instead of MDRD. In Australia and New Zealand, medical laboratories are now moving to standardise the reporting of eGFR using this CKD-EPI formula rather than the MDRD eGFR to reflect its greater accuracy in those without CKD – this will be discussed in section 1.5.1. A degree of accuracy in estimating GFR in the elderly is of increasing clinical importance given the increasing burden of CKD in this group likened to a “grey epidemic” of renal disease. In the elderly there is some uncertainty whether any decline in GFR is an actual disease process as opposed to a physiological decline.

1.3 Aging and the kidney

Conventional teaching states that kidney function declines with age from the fourth decade onwards. However the evidence for this is largely from population studies performed in the 1940-1950's^(20, 21) and experimental studies in primarily elderly men^(22, 23). Despite this many assumptions in clinical practice are based on this age related dogma as the effect of aging-alone versus pathological decline in kidney function on drug handling, metabolism and excretion have not been evaluated in any depth. These assumptions may be problematic when extrapolated to guide drug dosing.

It is not surprising that it is difficult to distinguish the effect of aging (physiology) from that related to co-existent disease (pathology). Pure aging or senescence of the kidney has been described as a “progressive involution with loss of nephron mass and decline of cellular function”⁽⁴⁾. The kidney is composed of complex, inter-relating glomerular, tubular and vascular components with linked and difficult to separate functions. It is not clear how normal aging alters these components of kidney function, nor is it clear that any changes, if they occur, are uniform and affecting each component equally.

Rodriguez-Puyol⁽²⁴⁾ suggests that “renal dysfunction of the elderly is due to the accumulation of damage induced by minimal, clinically undetected renal disease, and is not the consequence of the aging process itself”. The fact that almost one third of the participants in the sentinel Baltimore Longitudinal Study of Aging⁽²⁰⁾ did not demonstrate a declining GFR, suggests that renal dysfunction in the elderly is not simply just due to biological senescence. Rather, it more likely due to subtle and poorly understood glomerular and vascular changes, combined with superimposed injury from processes such as hypertension, atherosclerosis, diabetes, smoking, diet and obesity – all factors known to accelerate aging changes in other parts of the cardiovascular system and beyond⁽²⁵⁾.

There is a complex association with vascular disease, hypertension, arteriosclerosis and atherosclerosis affecting kidney function. Contributing to hypertension in aging is a loss of vascular compliance and endothelial dysfunction. The at-risk elderly have reduced functional reserve and an impaired ability to autoregulate glomerular pressure and renal blood flow (RBF) over a range of blood pressures, making the kidney vulnerable to additional insults such as acute illness, surgery (fluid losses and hypotension) and drugs (diuretics, NSAIDs and iodinated radio-contrast). Clinical evidence demonstrates a significant correlation between modest hypertension and the rate of decline of creatinine clearance in patients⁽²⁶⁾ which is not evident in normotensive individuals.

In addition, the Bronx Longitudinal Study⁽²⁷⁾ showed that, even in the very elderly, control of blood pressure can improve renal function. This short “longitudinal study” of three years duration in the “very old” (aged 75-85 years) found in many subjects, plasma creatinine concentrations and proteinuria improved with close attention to blood pressure control, even in those with significant CKD. Intriguingly, although there was little change in systolic blood pressure demonstrated there was a statistically significant fall in diastolic pressure in those who maintained their renal function which persisted at 6 years follow-up. This suggests, even in the very elderly, that renal function improves (and is preserved) with therapeutic control of hypertension, and provides further support that decline of renal function with aging is not predominantly due to senescence, but to loss of renal function from concurrent disease.

Further to the progressive glomerular and vascular changes described above, with increasing age many kidney specific pathological processes affecting large and small vessels such as amyloidosis, some forms of glomerulonephritis (such as membranous nephropathy), diabetes and arteriosclerosis are more prevalent. These processes also contribute to the incidence of CKD and end stage renal disease (ESRD) in the elderly.

Therefore the contribution of physiological changes and concurrent pathological disease is linked and additive in the development of CKD in the elderly. Thus, there is likely to be an intricate, integrated relationship between external influences and the genetic make-up of the individual that contribute to any decline in renal function.

1.3.1 Glomerular Structure and Function

The decline in GFR with aging is often attributed to progressive glomerulosclerosis i.e. loss of functioning glomeruli in conjunction with reduced RBF. The remaining glomeruli adapt with hyperfiltration and hypertrophy as haemodynamic compensation to maintain GFR⁽²⁸⁾. These adaptive changes can, unfortunately, lead progressively to haemodynamically mediated glomerular injury and subsequent glomerulosclerosis. Brickers' Intact nephron theory⁽²⁹⁾ (which will be discussed in section 1.4) suggests that any loss of the functioning glomeruli implies a parallel decline of tubular function (i.e. damage to one part of the nephron leads to the entire loss of that nephron). However there are complex interactions within and between the pathways involved in primary glomerular disease, subsequent tubular injury and secondary glomerular damage that have not been well studied⁽³⁰⁾.

Post mortem studies demonstrate that kidney volume, length and mass decline approximately 10% per decade after the age of 40 - from 400g in 4th decade to 300g by the 9th decade⁽³¹⁾. Grossly, the kidney appears granular and pitted secondary to cortical loss and arterial disease. Nyengaard⁽³²⁾ reports the reduction in kidney weight after the age of 50 is reflective of cortical glomerulosclerosis. The remaining glomeruli appear to undergo compensatory hypertrophy accompanied by mesangial expansion, arteriolar hyalinization and interstitial fibrosis. The degree of fibrosis is highly variable and increases from 5% in young adulthood to 10-30% in healthy 70 year olds^(33, 34). However, these earlier studies were uncontrolled for

related pathology that impact upon renal damage, in particular hypertension and arteriosclerosis. When these pathological comorbidities are excluded there is no evidence of significant loss of renal mass prior to the age of 75 years^(31, 35).

1.3.2 Tubular structure and function

The tubules also demonstrate structural and functional changes with aging. The greatest accumulation of cellular injury with time occurs in the cortex and outer medulla leading to tubular atrophy. The basic biochemistry of the tubular cell alters with age with a “reduction of mitochondria and Na/K/ATPase channels leading to decreased sodium handling and decreased oxygen consumption in older animal studies”⁽⁴⁾.

Despite the physiological alterations, there is meagre evidence of true decline in proximal tubular function attributable solely to aging⁽³⁶⁾. Nevertheless there are several clinical occurrences to suggest altered tubular function occurs with declining age including impaired electrolyte handling with prominent hyponatraemia. Water handling in the distal tubule is also impaired with both reduced concentration and diluting ability secondary to blunted responses to arginine vasopressin (anti-diuretic hormone)^(25, 37). Clinically this can manifest as nocturia and delayed response to disturbances in body water volume.

There appears to be some evidence of impaired salt and water handling involving the distal nephron with aging but there is a paucity of research evaluating any alteration to the proximal tubular function and its relationship to GFR – with most of our knowledge arising from early observational or animal studies. Dontas⁽³⁾ evaluated tubular function in the elderly, both with and without renal infection (probably now more accurately described as reflux nephropathy) in the early 1970’s, and compared the findings with young adults. Proximal tubular secretory function was evaluated with para-amino hippuric acid (PAH), distal tubular

function with water handling, medullary function via the achieved osmolar gradient, and glomerular filtration with inulin and creatinine clearance. There was a parallel reduction between inulin clearance and tubular function, with distal function declining greater than proximal tubular function. Similarly, Lindeman⁽³⁶⁾ reported that PAH proximal tubular secretion declines with age at a rate nearly parallel to inulin clearance. Earlier seminal work by Davies and Shock in 1949⁽²²⁾ demonstrated that tubular excretory capacity of iodinated contrast declines in a consistent ratio to GFR. This would suggest there is a parallel loss of glomerular and some aspects of tubular function, when injury occurs to any aspect of that nephron.

Again, these earlier studies did not correct for comorbid hypertension and atherosclerotic vascular disease, making it difficult to evaluate the impact of pure aging over pathological decline.

1.3.3 Renovascular structure and function

Subtle changes in renal dynamics and structure occur in aging. Biopsy studies⁽³²⁾ show that the outer layers of the cortex have significant obliterative microvasculopathy (matching the pattern of glomerulosclerosis) than other areas. This is the area where most other cellular damage occurs. Functional changes accompany the structural damage. As GFR declines with age there appears to be a parallel reduction in RBF^(20, 32, 38) with similar changes seen in patients undergoing unilateral nephrectomy as kidney donors^(32, 39, 40). As the pool of nephrons diminish with progressive insult or disease it appears that RBF is affected more significantly than GFR. This is likely due to afferent arteriolar dilation and efferent arteriolar constriction occurring in an attempt to maintain filtration pressure - at the expense of higher capillary flow rate and intraglomerular pressure i.e. glomerular hypertension with cortical shunting of blood flow. At a critical point, occurring around a GFR of 30-40% of normal (GFR<90mL/min/1.73 m²), the hypertension

within the glomerulus leads to tuft injury and sclerosis. Resultant scarring leads to further nephron loss. Modification of this process by reducing systemic blood pressure, afferent arteriolar dilation, and efferent constriction by medications (such as angiotensin converting enzyme inhibitors - ACEi) and diet can lessen trans-glomerular pressures and preserve nephrons⁽⁴¹⁾.

Renal blood flow (RBF) cannot be easily measured directly and is calculated from the equation: $RPF/1-HCT$ ⁽⁴²⁾ where RPF is renal plasma flow and HCT is the haematocrit. RPF is estimated by measuring PAH clearance which has a known extraction ratio of 92%. PAH clearance has been shown to decline with aging from 649 mL/min/1.73m² in fourth decade to 289 mL/min/1.73m² in the ninth decade, reflecting reduced RPF⁽²²⁾ and does not appear to be due simply to the loss of renal mass or reduced cardiac output^(40, 43). Altered arteriolar resistances arise following loss of vascular mass leading to glomerular tuft collapse and sclerosis. This implies that post-glomerular renovascular resistance (and hence microcirculation resistance) is elevated⁽²⁵⁾. This finding was shown in normotensive subjects but is accelerated in those with cardiovascular disease such as hypertension and congestive heart failure. It appears that the decline in renal plasma flow is, in least part, secondary to increased post-glomerular resistance from narrowed and obsolescent vessels⁽⁴⁰⁾.

It is generally accepted that the inability for vessels to dilate in response to stimuli such as acetylcholine and amino acids^(25, 37) is an important process occurring in the vessels of elderly individuals. This indeed may be the primary cause of the age related reduction in RBF. Protein loading normally causes hyperperfusion - resulting in an elevation in GFR⁽⁴¹⁾, but this response is diminished in patients with CKD, the elderly, and those with cardiovascular disease. This may imply a state of relative hyperfiltration and signifies loss of renal reserve due to comparative vascular vasoconstriction in these "disease" states i.e. a lack of ability to increase filtration as this is already working near maximally⁽³⁷⁾. The associated vasoconstriction with increased renovascular resistance could account for the age-

induced reduction in renal blood flow. It is possible that the afferent arterioles, under resting conditions, are in a relatively greater state of vasodilation compared to efferent arterioles. This produces an elevated perfusion pressure, hyperperfusion and hyperfiltration in the surviving glomeruli and would explain both the increased filtration fraction and maintenance of near normal creatinine function despite the pathological structural changes in aging⁽⁴⁴⁾.

Consequently, renal aging seems to be associated with two broad classes of kidney injury that are interactive and co-dependant. Firstly, glomerulosclerosis with mildly reduced GFR and adaptive hyperfiltration occurs predominantly in the cortex. Secondly, renovasculopathic alterations occur, in association with hypertension, resulting in potentially a more significant impact on kidney injury. Conflicting evidence of the primary inciting event likely reflects the complexity of the physiological/pathological mechanisms – as well as the structures involved. Senescence and pathology have been linked aetiologically via repetitive oxidative stress and free radical injury. This contributes to age-related telomeric shortening within the chromosomes and deregulated mitochondrial function and enhanced apoptosis⁽⁴⁵⁾. The histological changes of aging include glomerulosclerosis, tubular atrophy, interstitial fibrosis and arterial intimal fibrosis primarily in the cortex. Vascular adaption preserves GFR in the surviving nephrons but this promotes a vicious cycle of on-going injury and sclerosis⁽⁴⁵⁾. Diminished vasodilatory response likely contributes to the reduced functional reserve in the kidney (against a background state of vasoconstriction) to cope with additional stressors; predisposing the elderly kidney to acute (kidney) injury. The degree of vascular injury in glomeruli has been reported to be proportional to the degree of systemic atherosclerosis^(24, 31). Therefore a somewhat circular argument occurs where arteriolar hyalinization and fibrosis known to be accelerated by hypertension and other kidney disease, in turn, triggers both sclerosis of the glomerulus and interstitial fibrosis. Interstitial fibrosis can also be induced by the actions of cytokines such as tumour growth factor β (TGF- β) induced by proteinuria which

may have potential future therapeutic relevance. One inference would be that glomerulosclerosis is indicative of subclinical renal injury from comorbid conditions and not simply a consequence of aging.

1.4 Intact nephron theory and drug dosing in the elderly

In 1960, Neil Bricker propagated the intact nephron theory or hypothesis in discussing concepts of the pathophysiology of chronic kidney disease, then referred to as chronic Bright's disease^(29, 46, 47). This hypothesis describes that each nephron is either a fully functional unit or does not function at all and is lost. Surviving nephrons can increase their functional capacity by undergoing hypertrophy to retain a remarkably uniform relationship between glomerular and tubular function. This is independent of the underlying disease process but related to number of surviving nephrons. Bricker emphasized that "as number of functioning nephrons decrease, each remaining nephron must perform a greater fraction of the total renal excretion". However, as further nephrons are destroyed in progressive renal disease the kidney's capacity to accommodate to stressors diminishes and renal insufficiency develops. In contrast to this concept of a "uniform relationship" between glomerular filtration and tubular function there are differences noted in clinical practice between those substances primarily handled by filtration (such as creatinine) and those by tubular reabsorption (e.g. sodium, potassium and phosphate). This is evidenced by creatinine rising as GFR declines but sodium, potassium and phosphate concentrations maintained until much later in established kidney disease⁽⁴⁸⁾.

Despite these concerns, numerous studies⁽³⁾ have used this assumption of a constant relationship between glomerular filtration and tubular function to adjust drug dosing on the basis of changes in GFR, despite the fact that many drugs are primarily secreted or reabsorbed by the tubules e.g. frusemide and beta-lactam antibiotics. McLachlan⁽⁸⁾ states "for any decrease in filtration there is an equivalent

reduction in tubular secretion". Lindeman⁽⁵⁾ asserts "most decisions on drug dosage can be based on this information alone (i.e. eGFR or CrCl) as other tubular functions of the kidney decrease at rates paralleling the decrease in glomerular function rate". On the basis of this assumption Lindeman declares in a subsequent paper: "the most important clinical renal function to monitor with aging is the GFR (creatinine clearance)"⁽⁶⁾ and this sentiment is re-iterated in current literature and guidelines despite the paucity of evidence supporting this premise.

As previously discussed, tubular function with declining GFR has not been studied extensively. Nonetheless it appears that proximal and distal function decline with GFR with aging. However, there is limited evidence to substantiate the intact nephron hypothesis i.e. that all processes in the tubule decline at the same rate as GFR. Proximal tubular activity appears to decline at a similar rate to GFR⁽²²⁾, and to a lesser extent than distal function. One small study demonstrates a reduction in distal tubular water handling in the elderly compared to the young⁽³⁾. Most studies looking at tubular function tend to be undertaken in young, and often only male, subjects, although arguably the research may be more relevant in the elderly demographic. An accurate determination of tubular function in the aging kidney is of particular relevance with regard to drug dosing where polypharmacy and significant side effects are common in clinical medicine.

The concept of an inexorable decline of renal function with aging has arisen from a number of older studies⁽²¹⁻²³⁾. However the accuracy of this data is limited by significant methodological concerns including:

- 1) failure to correlate measurements of creatinine (and creatinine clearance calculations) with alteration in muscle mass as the source of creatinine generation. Generally with aging the percentage of body fat to muscle mass increases⁽⁴⁹⁾ although the female correction factor does take this into consideration.

- 2) inherent inaccuracies of 24 hour urine collections e.g. significant patient burden limiting reproducibility, and incomplete collections⁽⁵⁰⁾.

- 3) cross-sectional or very short (12-36 month) longitudinal study designs.
- 4) determinants of kidney function that have not controlled for comorbid conditions that influence kidney function such as hypertension, cardiovascular disease and atherosclerotic vascular disease^(22, 23).
- 5) cohort or period effect i.e. today's elderly are on average healthier and living longer than those that were part of those earlier longitudinal studies⁽⁴³⁾.

Direct measurement of GFR in routine clinical practice is virtually impossible and not applied outside of research studies. Therefore surrogate determinants based on serum creatinine and calculated creatinine clearance are used to estimate GFR. The formulae include age as a primary variable – based on the assumption that renal function declines with age alone (i.e. as a physiological change). However, recent studies^(20, 43) have shown that in healthy elderly, without hypertension, cardiac disease or malnutrition, that this so-called inevitable decline in kidney function does not always occur. In the sentinel Baltimore Longitudinal Study of Aging^(20, 26) (BLSA), the measured creatinine clearance (by 24 hour urine collection) declined from 140 mL/min at 25-34 years to 97 mL/min at age 75-84 whilst the mean serum creatinine remained stable at 72-74 $\mu\text{mol/L}$ from ages 25 to 75 years. This also demonstrates the creatinine blind phenomena where GFR declines before any rise in serum creatinine occurs due to increased tubular secretion. Despite this a later study using many of the subjects from the BLSA analysis showed that 22% of this group did not actually have any decline in GFR⁽²⁰⁾. To confound this finding within this group of normal subjects a small percentage had diabetes without proteinuria which may have clouded the findings due to periods of hyperfiltration. Similarly Fliser⁽⁵¹⁾ found, that in a group of healthy elderly although there was a decline in calculated creatinine clearance using the Cockcroft-Gault equation, that it remained within the normal range i.e. > 90 mL/min – and this was confirmed by more accurate determinants of GFR using isotope

clearance studies. This has raised the possibility that the age-related decline in GFR is not inevitable but as a result of superimposed pathology.

Other aspects of renal tubular function have not been well studied or routinely measured creating inevitably incomplete knowledge of drug handling in the elderly. Many medications are organic acids and bases that employ renal tubular secretion and/or absorption as significant mechanisms of metabolism and excretion. Consequently estimates of kidney function determined by GFR alone may not accurately reflect these processes. There is also a paucity of information with respect to the accuracy of calculated GFR and how this correlates with tubular function. Given that Fliser⁽⁵¹⁾ reported no major differences in tubular renal clearances between young and healthy older subjects, modification of drug dosing solely on the basis of impaired estimation of filtration function (determined by a formula where age is an independent variable) may lead to dosing errors for important renally excreted drugs such as antibiotics and chemotherapy. For these reasons the global recommendation of dose reduction according to age (or eGFR) is contentious, questionable and should be treated with some caution.

1.5 Measurement of renal function

Measurement of renal function gives an indication of the state of the kidney physiology at a point in time. Cumulative results for the same patient are also used to ascertain the baseline and previous responses to acute illness and recovery. This provides guidance to the clinician regarding appropriate adjustments to drug dosing, duration of therapy and other treatment options. It also provides diagnostic information for primary renal diseases and other conditions with primary and secondary renal dysfunction e.g. diabetes, sepsis and hydration status.

The term renal function is usually synonymous with a measurement of glomerular filtration rate (GFR) either directly or indirectly. This has arisen from the intact nephron hypothesis⁽⁴⁶⁾ as discussed prior with eGFR often the only

measure of kidney function recorded as “all other changes in renal function tend to parallel changes in the GFR”⁽⁶⁾.

1.5.1 Glomerular Filtration Rate (GFR)

GFR is the volume of fluid filtered from the glomerular capillaries into the Bowman’s capsule per unit time. The normal range is 100-130 mL/min – and is similar in both men and women. Most older cross sectional studies have found GFR to progressively decline by around 1 mL/min/year – with an accelerated decline after the 7th decade ^(21, 22). Initially serum creatinine fails to rise as compensatory tubular secretion occurs to give a creatinine blind period despite falling GFR. Clinically, GFR cannot be directly measured but is instead calculated using clearance studies of a marker (endogenous or exogenous) that is present in a steady state concentration in the blood, is freely filtered and neither reabsorbed or secreted. This allows plasma and urinary concentrations to be used as a measure of clearance per unit time (urine concentration x urine flow / plasma concentration: UV/P)⁽⁵²⁾.

Inulin (a 5.2 kDa polymer of fructose) clearance was the original accepted gold standard technique for GFR assessment. It is freely filtered and neither secreted or reabsorbed by the tubules. However, due to the requirement for intravenous infusion to achieve steady state, urinary catheterization with multiple timed collections and the requirement for chemical assay for measurement, it remains largely a research tool and is not in routine clinical use. Like inulin, the polymer sinistrin is not metabolized in human blood and passes the kidneys unchanged. Despite it being highly soluble in water and easier to handle than inulin it remains difficult to use clinically.

These issues led to the introduction of single dose radiolabelled agents such as ethylenediamine tetra acetic acid (EDTA) Cr^{51} or diethylene triaminepenta acetic acid (DPTA) Tc^{99} (that are solely cleared by glomerular filtration) into clinical use.

Cr⁵¹ EDTA clearance has been found to be as accurate as inulin clearance⁽⁵³⁾. GFR is determined from the area under the plasma clearance curve (area under the curve - AUC) of the isotope and requires multiple blood samples to be taken over a period of several hours with no need for urine collection. Although simpler than inulin infusion, this multiple sampling technique is very labour intensive and was subsequently simplified by restricting the blood sampling to the second of the two exponential components of clearance⁽⁵⁴⁾ and is referred to as the slope-intercept method. It is considered that, among the various methods for measuring Cr⁵¹EDTA plasma clearance, the slope-intercept method provides the best compromise between accuracy and reliability on the one hand and simplicity on the other. Because data on only the slow exponential phase is used, the total AUC is underestimated, and consequently the isotopic GFR overestimates the true value of GFR. For patients with low GFR this error is small and may be negligible but for patients with normal GFR the use of the slope-intercept method can overestimate the true GFR by 10-20%. The method uses between two and four plasma samples taken over 6 or 24 hours post injection depending on presumed renal function. As with any modification to an extensive method, this simplification introduced systematic errors in the values of GFR hence various methods of correction have been derived and incorporated into computer based programmes to allow interpretation and comparison to reference range. The method described by Chantler in 1972⁽⁵⁴⁾ is used widely and includes correction of the measured GFR to a nominal BSA figure for the "standard man" (BSA 1.73 m²) by the method of DuBois and DuBois⁽⁵⁵⁾ to allow standardisation across populations.

The normal range for GFR was originally defined in seminal papers by Davis and Shock⁽²²⁾. This was redefined for Cr⁵¹ EDTA plasma clearance by Granerus and Aurell⁽⁵⁶⁾ for adults between 20 and 75 years - with the mean population GFR in young adults of 105 mL/min/1.73 m². The GFR was noted to fall slowly up to the age of 50 years and then more rapidly thereafter. This compares to Rowe's original study⁽²¹⁾ where the creatinine clearance fell from 140 mL/min/1.73 m² at the age of 30

to 97 mL/min/1.73 m² at the age of 80. Granerus goes on to demonstrate the decline of isotopic GFR with age with the following simple equations⁽⁵⁶⁾ (95% CI +/-25 mL/min):

For age (a = years) between 20 and 50: $GFR = 116 - 0.35a$

For age between 50 and 75: $GFR = 148 - a$

However these age adjustments has not been widely taken up by the medical community and laboratories due to inadequate discussion.

Creatinine is derived from the breakdown of skeletal muscle creatine phosphate in a relative steady state. Creatinine UV/P clearance and formulae based on this are commonly used as an estimate of GFR. With normal kidney function creatinine is predominantly filtered by the glomerulus with minimal tubular secretion. With progressive kidney impairment the proximal tubular secretion increases to contribute 10-20% of creatinine excretion and thus creatinine clearance becomes a less reliable marker/measure of GFR overestimating GFR by 10-20%. Variations in dietary intake of animal protein and creatine supplements also alter creatinine production and impact on the accuracy of creatinine UV/P as a measure of GFR.

In conditions where there is proportionally less muscle mass such as in the elderly or in cachexic states, inaccuracies relating plasma creatinine to creatinine clearance occur and as such, formulae tend to underestimate GFR – with the converse in obesity. Serum creatinine follows a non-linear inverse relationship to GFR because of tubular secretion, variation in muscle mass and non-renal elimination via degradation by intestinal bacteria. Determination of creatinine clearance is made using the UV/P method involving an accurate 24 hour collection of urine and single serum measurement. Fundamental difficulties with accurate and complete urine collection invariably negate this method being used with any ease or precision in the clinical setting.

To avoid the difficulties with prolonged urine collections, a number of formulae using plasma creatinine have been derived as alternative methods of estimating of

GFR. Of these, three are in common clinical use: the Cockcroft-Gault equation⁽¹¹⁾, the 4-variable MDRD equation⁽⁵⁷⁾ and the CKD-EPI⁽¹⁹⁾ formula. These equations estimate GFR from the measured plasma creatinine by mathematically adjusting for factors that influence the production of creatinine from muscle mass such as age, gender, weight and race.

The Cockcroft-Gault formula, described in 1976⁽¹¹⁾, was derived from the relationship between age and 24-hour creatinine excretion in 249 hospitalized men in the 1960's. This formula uses creatinine, age, and mass with an adjustment for gender. The Cockcroft-Gault formula is known to underestimate actual GFR in those with normal kidney function⁽⁵⁸⁾. The original formula uses ideal or lean body weight (LBW) although it is well known to underestimate creatinine clearance in the obese and overestimate in the underweight⁽⁵⁹⁾. Modification for body surface area (BSA) to adjust for creatinine generation from muscle has been shown in some studies to improve accuracy dependant on body size⁽⁶⁰⁾. This was a significant advancement over creatinine alone even though in practice many clinicians tend to utilize actual body weight (ABW) in the equation for ease of use. In 1998 the US Food and Drug Administration (FDA) published *The Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function*⁽⁶¹⁾ which recommends that pharmaceutical companies use the Cockcroft-Gault formula to estimate kidney function, and incorporating it in the design of pharmacokinetics studies and the development of drug dosing guidelines. The rationale for the use of the Cockcroft-Gault equation is that it was the most commonly used method for assessment of kidney function in clinical practice at the time. Hence, the bulk of our knowledge of the impact of eGFR on drug dosing has been derived using the Cockcroft-Gault formula. A further assumption made with this equation is that females have up to 15% lower creatinine clearance than their male counterparts despite the same creatinine and weight. This adjustment was calculated using the averages of body and muscle mass (from which to generate creatinine) from that era, but was not

based on any evidence that the average females measured GFR was actually 15% less than males.

The MDRD₄ formula⁽¹³⁾ was derived from a population of 1628 patients with moderate kidney disease whose kidney function had been measured by renal ¹²⁵Iothalamate clearance. The original formula was based on six variables but the four item variation using creatinine, age, race and gender is comparable and easier to use. This does not require clinical characteristics to calculate, enabling automatic laboratory reporting. As it was derived from a population with moderate kidney disease (GFR 25 – 60 mL/min) it is most accurate at this level of dysfunction and has a poorer precision in those with GFR > 60 mL/min and will underestimate actual GFR. Routine reporting was introduced in many parts of the world by laboratories to improve decision making around drug dosing and monitoring of patients with kidney disease. However there is no adjustment for body mass and hence relative to Cockcroft-Gault, MDRD underestimates GFR for obese people and overestimates it in underweight individuals. Interestingly, back adjusting of MDRD by dividing by 1.73 (to de-standardize body surface area) improves the accuracy of the estimation of GFR^(62, 63). A French group showed the MDRD equation to be more accurate than Cockcroft-Gault at lower GFR, but the reverse occurring when GFR > 60 mL/min⁽⁶⁴⁾.

In an effort to improve precision of estimating GFR in those with mild disease and normal function the CKD-EPI formula was published in 2009⁽¹⁹⁾. It was developed using pooled data from 10 studies of 8254 subjects with and without kidney disease. The equation has been shown to perform better with less bias and greater accuracy than MDRD at higher GFR^(65, 66).

Many reviews and studies have compared these equations of estimating GFR with each other and with measured GFR – with mixed and somewhat contrasting results. Comparisons are often difficult to make due to various methods of creatinine and GFR analysis, as well as the variable statistical analyses used.

Coresh⁽⁶⁷⁾ reviewed several hundred studies looking to improve accuracy in GFR estimation and found that MDRD formula appeared to be a better estimate of GFR at low ranges but generally underestimated the measured GFR. Both MDRD and Cockcroft-Gault formulae have lower precision at higher GFR levels as they were designed and tested on those with moderate renal disease. When efforts were made to systematically calibrate the creatinine assay the precision and accuracy also improved. Recently several papers have scrutinized CKD-EPI and have found it to have similar accuracy to MDRD below GFR 60 ml/min but improved precision above this level⁽⁶⁸⁾. A contemporary study from Australia⁽⁵³⁾ evaluated 139 patients with an eGFR < 60 mL/min determined by Cr-⁵¹ EDTA clearance compared with the MDRD, Cockcroft-Gault and CKD-EPI formulae. In this group of mainly European individuals with moderate kidney disease, the Cockcroft-Gault formula was found to be more likely to correctly stage actual GFR than the MDRD or CKD-EPI formulae - in contrast to many other studies where at lower GFR the MDRD formulae has been shown to be slightly more accurate⁽⁶⁹⁾. Both Cockcroft-Gault and MDRD formulae significantly underestimated the GFR obtained by isotopic GFR clearances in patients with no kidney disease. Between the two equations the MDRD formula was slightly more accurate⁽⁷⁰⁾ in this group. In contrast, another Australian study found both Cockcroft-Gault and MDRD formulae to be valid although with significant lack of precision. One fifth of their subjects had an estimated GFR more than 30% different to true GFR⁽⁷¹⁾. These inaccuracies contribute to variations of up to 40% in the dose calculated of some medications when using the two formulae^(62, 72). The general consensus appears to be that Cockcroft-Gault is least likely to produce toxic side effects of drug dosing for patients because of the underestimation at higher GFR. It follows that patients will receive lower doses of medications if modification of dosing is made utilizing the Cockcroft-Gault equation rather than the widely reported MDRD estimate of renal function. This is supported by Stevens⁽⁷³⁾ who found 99% of patients would potentially receive a greater dose of antibiotics when the MDRD eGFR was used,

with 11-29% discordance between MDRD and Cockcroft-Gault formulae. This study suggested that the MDRD equation was more accurate than the Cockcroft-Gault equation and this has led to recommendations that the MDRD equation could be used for pharmacokinetic studies and dose adjustments with the caveat that as more accurate estimating equations are developed these should be used. In recent years both Australia and New Zealand have moved to CKD-EPI based laboratory reporting of eGFR given its greater accuracy across a wide range of population.

Efforts are being made to find alternate indicators of GFR given that plasma creatinine has been shown to be inaccurate when used alone and the issues associated with calculating the GFR easily and accurately in clinical practice. One potential candidate is Cystatin-C which appears to be a good marker of GFR as its serum level appears to be less dependent on age, sex and muscle mass than creatinine. Unfortunately while it appears to show promise as a more accurate estimate of GFR than creatinine alone⁽⁷⁴⁾, it has not consistently been shown to be a perform better than the current eGFR equations⁽⁷⁵⁾. Additionally, it is not yet fully validated or commercially available. It is also likely to be prohibitively expensive, costing 2-4 dollars per test compared to 2-10 cents for creatinine. Likewise; iohexol clearance whilst attractive in that no radiation exposure is required and the marker is inexpensive and sensitive has not been used widely mainly due to the cost prohibitions of the assay to measure it.

1.5.2 Tubular secretion and reabsorption

1.5.2a Anion secretion in the proximal tubule

Anion secretion in the proximal tubule occurs via several transporters – and more are being discovered each year. The gold standard for evaluation has been para-amino hippuric acid (PAH) clearance, although probenecid and uric acid have also been used as a marker of anion secretion in some studies⁽⁷⁶⁾. At low dose steady

state PAH is completely removed by glomerular filtration (20-30%) and tubular secretion, which has allowed its classic use as a marker of renal plasma flow. As well as this, if given as a low dose bolus it can be used to determine proximal tubular secretion given PAH is primarily secreted by the renal tubules via the organic anion transporter 1 (OAT1) and is not reabsorbed⁽⁷⁾. Using this method, Gross and colleagues determined that the PAH anion secretion is 359 ± 134 mL/min⁽²⁰⁾. Clinical grade PAH was very difficult to source despite an international search and for this reason an alternative marker of proximal tubular anion transport was sought.

Early animal studies found that the clearance of PAH and uric acid was equal and related^(77, 78). Uric acid (UA) is a non-proteinaceous compound of nitrogen and the end product of purine metabolism. The human adult generates about 5.95 mmol (1 gram) of uric acid daily from metabolism of food, biosynthesis and catabolism of tissue. 70-80% is eliminated by the kidneys and the remaining by the gastrointestinal tract. Humans lack the enzyme uricase to break down uric acid and therefore renal excretion is the critical determinant of systemic urate concentrations. Normal serum concentrations range from 120-420 μ mol/L, with about 5 mmol of uric acid being excreted per day.

The tubular secretion of urate was first discovered in 1950 by Berliner⁽⁷⁹⁾ with a three component mechanism described by Gutman and Yu in 1961⁽⁸⁰⁾. This mechanism entails uric acid being passively and freely filtered at glomerulus, 99% of which is actively reabsorbed in the proximal tubule, followed by 50% re-secreted back into the proximal tubular lumen. A fourth component was determined by Diamond and Paolino⁽⁸¹⁾ in the early 1970's - comprising of post-secretory reabsorption of 80% of that which is secreted. Consequently, uric acid excretion in urine is approximately 10% of the amount filtered. This pathway is the site of several drug interactions as evidenced by pyrazinamide, which competitively inhibits tubular secretion, and probenecid that competitively inhibits post-secretory reabsorption via the OAT4 transporter⁽⁸²⁾. The reabsorption pathway has been

shown in recent studies to be principally mediated by a voltage sensitive uric acid anion transporter (URAT1) that is a member of the OAT family⁽⁸³⁾. This was discovered in 2003 and initially named GLUT9 - as it is considered a possible link between the metabolic syndrome, uric acid handling and sodium reabsorption in the proximal convoluted tubule⁽⁸²⁾. URAT1 is located in the apical membrane of the proximal tubular epithelium and is inhibited by losartan, probenecid and benzbromarone – hence their uricosuric action. Other anion transporters in the proximal tubule also appear to contribute to urate secretion including OAT 1 and 3 on the basolateral membrane, and OAT 2 and 4 on the apical membrane.

Since this discovery several studies have used uric acid to indicate tubular anion handling. Hansen⁽⁸⁴⁾ and Kamar⁽⁸⁵⁾ both used uric acid as a measure of proximal tubular anion function when evaluating cyclosporine effects. Uric acid has also been used in conjunction with lithium by Krusell⁽⁸⁶⁾ and Manunta⁽⁸⁷⁾ to assess proximal tubule secretion. Cappuccio⁽⁸⁸⁾ investigated the relationship between uric acid metabolism and proximal tubular sodium handling in the Olivetti study and suggested that there is a role for endogenous uric acid excretion to be used as a marker of proximal tubular anion function. The Olivetti heart study was reviewed by Strazzullo⁽⁷⁶⁾ and found that proximal, but not distal, tubular sodium reabsorption is associated with uric acid. Subsequently, Ochiai⁽⁸⁹⁾ used uric acid excretion to estimate tubular secretion in heart failure as a marker of hypoxic stress. Based on these papers it appears that uric acid clearance can be utilized to estimate tubular anion secretion with some accuracy and reproducibility. Acknowledgement is made of the influences of angiotensin II and volume, as well as the interactions and actions of other portions of the nephron complex. As an endogenous marker there should be no interactions with other markers used to evaluate tubular function.

1.5.2b Cation secretion in the proximal tubule

As opposed to the active secretion of organic anions, organic cation secretion occurs largely by passive diffusion in the proximal tubule. However, some organic cations are handled by cationic transporters against a concentration gradient. The enantiomers of pindolol have been used to assess cationic secretion previously⁽⁹⁾. This non-selective beta-blocker is an organic base comprising of a racemic mixture of R and S enantiomers. Pindolol is rapidly and well absorbed (95%) from the gastrointestinal tract with 90% bioavailability. It is 40% bound to plasma protein and is secreted by organic cation transporters (OCT). The single dose peak plasma concentration occurs 1-2 hours after ingestion with a half-life of 3-4 hours but hemodynamic effects for 24 hours. The half-life is increased in CKD and in the elderly up to 9-16 hours. Metabolism occurs predominantly in the liver (primarily the R enantiomer) and metabolites are excreted by the kidney, with a smaller proportion of approximately one third of the total dose excreted unchanged in the urine (primarily S enantiomer). Despite these low urinary excretions, racemic pindolol stereo-selective excretion has been safely and effectively used by MacLauchlan, Gross and Tett^(7, 8) to evaluate OCT function in their studies.

1.5.2c Reabsorption

In addition to proximal tubular secretion, reabsorption of substances from the glomerular filtrate occurs at the level of the proximal tubule via both active and passive mechanisms. Fluconazole, commonly used in the prophylaxis and treatment of fungal infections has been previously used as marker of proximal tubule reabsorption due to its pharmacological profile^(7, 8, 90). It is well absorbed within 2 hours of ingestion with an oral bioavailability of $\geq 90\%$. Peak plasma levels occur within 0.5-1.5 hours with a half-life of 30 hours under zero order kinetics and it is predominantly (80%) eliminated unchanged via the renal route. Fluconazole is only weakly protein bound (11%) and is freely filtered by the glomeruli. Although it

is renally eliminated, it has also been shown that fluconazole undergoes tubular reabsorption in the kidneys^(7, 91). Previous studies by Gross et al^(8, 9) have successfully used a single 100 mg dose of oral fluconazole to evaluate tubular reabsorption by measurement of plasma and urinary concentrations.

1.6 Drugs and the aging kidney

The kidney makes a major contribution to drug excretion from the human body. It is also responsible for a variable amount of direct drug metabolism and for some medications this may be a significant part of their metabolic fate⁽⁹²⁾. The net renal excretion of a drug is related to the physico-chemical properties of the drug, its metabolites and the extent to which the drug undergoes glomerular filtration, tubular secretion and reabsorption. In relation to the non-biotransformation function of the kidney, there is considerable heterogeneity along the nephron, particularly in the proximal tubule, which in turn influences drug handling⁽⁹³⁾.

Serum creatinine is the basis for clinically utilised estimates of GFR. However, tubular secretion of creatinine proportionately increases as GFR falls and is also affected by certain drugs such as trimethoprim and cimetidine which directly alter tubular handling of creatinine by competitive transport. This results in alterations of serum creatinine independent of GFR, further adding to the inaccuracy of creatinine clearance as a marker of renal function. Examples of this occur in patients who are on high dose cotrimoxazole, who have evidence of impaired creatinine clearances despite being known to have normal kidney function^(94, 95). Similarly, in patients with human immunodeficiency virus (HIV) infection⁽⁹⁶⁾ the renal clearance of fluconazole is impaired despite a normal creatinine clearance, suggesting altered tubular reabsorption possibly due to the effect of the disease and/or retroviral drugs⁽⁹⁰⁾. Therefore, altered tubular function can potentially occur with any drug that is principally eliminated by renal tubular secretion.

The elderly have heightened susceptibility to drug toxicity. This is partially due to altered clearance mechanisms often associated with reduced renal excretion⁽⁴⁹⁾. Moreover polypharmacy is very common amongst elderly patients due to comorbid disease. In addition to the effect of aging on pharmacokinetics, the pharmacodynamics are also modified with altered sensitivity and response to drugs such as benzodiazepines, opiates, beta-blockers and psychotropic medications⁽⁴⁹⁾. This can lead to harmful sequelae including narcosis and postural hypotension. Acute and chronic kidney disease can affect the handling and excretion of many drugs directly. There is also emerging evidence that kidney disease itself has a significant effect on non-renal clearances of many drugs⁽⁹⁷⁾ by uraemic effects on uptake and efflux transporters in the gut and liver. Therefore, in the setting of significant renal impairment, consideration needs to be given to dose adjustment of all drugs, especially those with narrow therapeutic windows (i.e. digoxin)⁽⁹⁸⁾. There should also be some adjustment of dose based on the knowledge of the specific drug pharmacokinetics including fraction excreted unchanged (fU).

Thus with the complexities of drug pharmacokinetics and dynamics as well as inaccuracies in determining kidney function in the elderly, we may be incorrectly dosing our elderly patients. Clinicians are trained to commence medications for the elderly at a low dose and titrate up slowly to avoid side effects. Aymanns et al⁽⁴⁹⁾ challenges this premise instead proposing that, particularly with respect to antibiotics and chemotherapy drugs, we should “hit hard and fast”. This is in order to overcome a larger volume of distribution associated with a higher percentage of body fat to muscle mass with aging⁽⁴⁹⁾ before dose adjustment for renal function is commenced. Accumulation of drug, with its subsequent side effects, requires time therefore this should not be of paramount concern at initiation of therapy when immediate therapeutic levels are important. Following the commencement of therapy, dose adjustment can be made by increasing the interval between doses, but not reduction of dose itself, in order to retain a therapeutic effect.

1.7 Elimination of drugs by the kidney

The net renal excretion is the result of glomerular filtration, tubular secretion and subsequent reabsorption.

1.7.1 Glomerular filtration

Drug clearance by glomerular filtration is a passive process that is dependent on the proportion of unbound drug in plasma and renal blood flow. Unbound drug is freely filtered and subsequent elimination is dependent upon whether or not there is any tubular reabsorption and/or secretion. There is accumulating evidence that, for many drugs, changes in glomerular filtration rate do not correlate well with changes in renal drug handling^(51, 96).

1.7.2 Tubular secretion

The renal handling of many drugs are the result of interactions with, and/or transport via, organic anionic transporters (OAT) and organic cationic transporters (OCT) in the renal tubules⁽⁹⁷⁾. Both are saturable, energy-dependent systems that transport against a concentration gradient, independent of protein binding. The activity of these transporters may be altered both with aging and GFR⁽⁸⁸⁾.

Anion secretion is coupled to sodium gradient via OAT1-4 and URAT1 transporters for exit and egress into cells. This is the known site for the excretion of beta-lactam antibiotics, NSAIDs, some anti-viral therapies, many diuretics and radio-contrast media. Competition for these pathways can be used clinically e.g. probenecid competitively inhibits anion secretion via OAT to achieve higher blood levels of penicillin with longer duration of action. Another example is frusemide which is 98% protein bound and exerts its activity at the sodium potassium 2 chloride channel (NKCC) on the apical membrane of ascending loop tubular cells.

To enter the urine, it undergoes uptake at the basolateral aspect of the proximal tubular epithelium via OAT, diffuses down a concentration gradient and then exits the tubular cells via another OAT on the apical surface into the tubular lumen⁽⁹⁹⁾. Previous research has indicated that the activity of these transporter mechanisms may be reduced with aging and GFR^(49, 51), nonetheless whether this is a direct linear response is unknown.

Organic cation secretion occurs by organic cation transporters usually linked to the sodium hydrogen (Na/H) transporters and once taken up into the cell, elimination is largely by passive diffusion. Examples of substances handled by these transporters are: creatinine, trimethoprim and cimetidine. Often there is competitive interaction for these transporters as noted previously by the example of trimethoprim increasing the plasma creatinine despite an unchanged GFR.

In addition the proximal tubular epithelial cells have the multi-drug carrier protein, P-glycoprotein, which acts as an efflux pump for removing drugs out of cells.

1.7.3 Tubular reabsorption

Tubular reabsorption of drugs can be an active or passive process influenced by the molecular weight, lipophilic and ionization status of the drug, as well as the urinary pH. Active reabsorption occurs mainly for glucose, vitamins and amino acids usually coupled with sodium transport. Passive reabsorption only occurs in unionized and nonpolar drugs with sufficient lipidophilicity to cross the tubular membrane. Tubular reabsorption can occur both in the proximal and more distal portions of the nephron, which limits the accuracy of endogenous markers to assess tubular reabsorption activity. Fluconazole has been shown to be a useful marker of tubular reabsorption as stated prior⁽⁷⁾.

1. 8 Summary and introduction to this study

Renal drug elimination is a product of the processes of glomerular filtration, tubular secretion and reabsorption. Current recommendations for modification of drug dosing, in the setting of kidney impairment (such as in the British National Formulary⁽¹⁰⁾) are on the basis of changes in the estimated GFR from the original Cockcroft and Gault formula⁽¹¹⁾. As discussed above, these formulae and other measures of GFR have inherent limitations and all assume that there is an obligate decline as a result of the aging process *per se* and changes in tubular function occur in parallel to changes in glomerular filtration rate. However, the published evidence to support these assumptions is limited and with methodological flaws. Furthermore there is scarce and often conflicting evidence with respect to changes in kidney function in normal healthy aging with many studies investigating heterogeneous groups with no control for comorbidities^(20, 21). Conventional measures of renal function such as creatinine clearance may not detect alterations in the integrity of the tubular function and hence may not detect altered renal clearance of a drug.

It has been suggested that to accurately assess renal pharmacokinetics, the varied functions of the nephron need to be assessed with appropriate markers⁽⁷⁾. A method to simultaneously determine each of the renal elimination pathways has been investigated by the Queensland group of Tett, Gross and McLachlan⁽⁷⁻⁹⁾. Their single dose, drug cocktail has advantages for clinical use and allows direct comparisons between the elimination pathways for that individual. The cocktail comprising of sinistrin (for GFR), para-amino hippuric acid (for renal plasma flow and tubular anion secretion), pindolol (for cation secretion) and fluconazole (tubular reabsorption) was evaluated and found to be safe, without interaction and significant side effects and furthermore was representative of those pathways. The organic base pindolol, is a known marker of cation transport in the kidney. Its interaction was confirmed previously by co-administration of the known OCT inhibitors trimethoprim and cimetidine, which reduce the transport of pindolol^(7, 100).

Fluconazole, an anti-fungal, is largely reabsorbed by the proximal tubule and can be used to assess the degree of tubular reabsorption rates by measuring the percentage that is excreted in the urine.

Analytical issues in the development of this drug cocktail were reported in 2001⁽⁸⁾. Using a single volunteer, the marker drugs were administered and analysed for interaction and detection in urine and plasma. Recovery in plasma and urine was greater than 90% and no interference from other markers was found. Repeated freeze thaw of samples did not influence the assays. The co-efficient of correlation of standard linear curves was >0.99, meeting the acceptance criteria for analysis. Overall analysis found the assays to be “sensitive, specific, robust, accurate and precise” and also simple to use^(7, 9).

This drug cocktail was then developed and validated in 12 young, healthy male volunteers with an average age of 24 years⁽⁷⁾. Subjects were given 440mg PAH and 2500mg sinistrin intravenously together with 100mg oral fluconazole and 15mg oral racemic-pindolol. Again, no interaction between the markers was noted, and there were no significant side effects. Fluconazole renal clearance was reliably estimated by sampling for 24 hours compared with 120 hours – making the method simpler to administer. The enantiomers of pindolol were analysed separately, which demonstrated that the S-enantiomer is handled by tubular cation secretion, whereas the R-enantiomer clearance is by non-renal metabolism. Whilst there is a small risk of side effects and potential risks of adverse events with this drug cocktail, these are limited by the small and single dose used although there is some potential for hypotension with this dose of pindolol in the elderly.

The principles and potential clinical application of assessing alterations in renal elimination pathways with this drug cocktail were reviewed by Tett et al in 2003⁽⁹⁾ stating that when the pathways have been characterised in a given population, the implications for assessing tubular function and more accurate drug dosing for the

individual can be assessed. To date this approach has not been utilised to investigate if tubular function declines in parallel with reduced GFR.

This thesis sets out to address two important and related issues using a modified protocol based on the studies performed by Tett and colleagues⁽⁷⁻⁹⁾ for simultaneously measuring glomerular filtration and proximal tubular function.

First: is a decline in glomerular filtration rate reflective of changes in tubular function when considering modification of drug dosing? Can we use estimates of GFR to accurately adjust doses of medications handled primarily by tubular function?

Second: is the change in eGFR associated with aging due to the physiological process of aging alone or is it associated with pathological changes in kidney function related to co-morbidities that are frequently present in the elderly?

Chapter 2

Methods

2.1 Introduction

The study was approved by the Northern Y Regional Ethics Committee in January 2010 (Protocol number NTY/10/12/103), and was registered with the Australian and NZ Clinical Trials Registry number: ACTRN 12611000035921 as an observational, screening, cross-sectional, prospective trial.

The primary aims were:

- To evaluate the relationship between isotopic GFR and tubular transporters
- To examine the function of any age related decline in filtration and tubular transporters and the influence of comorbid pathology; both with and without established CKD
- To examine the accuracy of estimating equations for GFR in our population in order to fully investigate the notion that renally cleared drugs can be accurately dosed adjusted based on estimates of GFR by the common equations in clinical use.

Subjects were recruited largely from a newspaper advertisement supplemented by colleagues and clinic attendees. The initial approach involved a brief explanation of the study and an information sheet was given. A follow-up telephone call was made to ascertain continued interest, and if the subject agreed, a screening interview was undertaken.

Inclusion criteria comprised of:

- Community dwelling and able to travel to and from study laboratory
- Aged 20-40 or over 65 years
- Physically able to complete 24 hour urine collection
- If over 65 years
 - With and without established CKD where creatinine <105 or $>125\mu\text{mol/L}$
 - With and without co-morbid disease

There were several exclusion criteria which comprised of:

- Inability to give consent
- Significant asthma (potential reaction to beta-blockade)
- Pregnancy
- Known allergy to medication class
- The concomitant use of drugs known to interact with tubular transport:
 - Thiazide diuretics: discontinued for 4 days prior to testing
 - Probenecid
 - Atenolol
 - Cimetidine
 - Current antibiotics: required a 2 week washout period

2.2 Subjects

The source population of Dunedin is approximately 120,000 (NZ Census 2006) with 6% Maori, 5% Asian and 2% Pacific Islanders. 13% are aged over 65 with 3.8% older than 80 years.

To assess the effect of aging, a control group of younger subjects aged 18-40 was evaluated to compare to those aged over 65.

To assess the effect of pathology; groups were stratified according to creatinine level and co-morbidities that are known to directly affect renal function such as significant cardiovascular disease, diabetes and hypertension. Plasma creatinine was used as the primary classification of renal function – (despite known issues) – as eGFR equations inherently define renal function according to age. A serum creatinine concentration less than 105 $\mu\text{mol/L}$ was taken as being indicative of normal function and above 125 $\mu\text{mol/L}$ of abnormal function (where incidentally the calculated eGFR was $< 60 \text{ mL/min}$). These levels were chosen to reflect previous studies and local laboratory normal ranges where a creatinine of greater than 110 $\mu\text{mol/L}$ is considered to be elevated. To ensure a clear distinction between groups with and without chronic kidney dysfunction, for convenience, a level of less than 105 $\mu\text{mol/L}$ was chosen for the “normal” renal function groups. No potential subjects were excluded on the basis of a serum creatinine between 105 and 125 $\mu\text{mol/L}$ – this occurred conveniently.

A total of 40 subjects were recruited and stratified into 4 groups.

Group 1: “Young” controls: aged 20-40 years, serum creatinine concentration less than 105 $\mu\text{mol/L}$, and no significant pathology. n=10.

Group 2: “Elderly”: aged over 65 years, serum creatinine concentration less than 105 $\mu\text{mol/L}$, and no significant pathology. n=11.

Group 3: “Elderly pathology”: aged over 65 years, serum creatinine concentration less than 105 $\mu\text{mol/L}$. One or more co-morbidity such as hypertension, diabetes or heart disease. n=11.

Group 4: “Elderly CKD”: age over 65 years, plasma creatinine concentration greater than 125 $\mu\text{mol/L}$. One or more co-morbidity such as hypertension, diabetes or heart disease. n=8.

As there are few published studies investigating renal drug handling in the over 65 age group and multiple markers it was not possible to undertake a further power calculation from the original study design by Gross et al⁽⁷⁾. This study assessed 12 male subjects with an average age of 24 and was sufficiently sensitive (with 80% power) to detect 21% change in S-pindolol renal clearances, and a 20% change in fluconazole renal clearance with an α error of 0.05 and β error of 0.2.

2.3 Protocol

The screening interview was utilized to record a medical history, gain consent, answer questions, and perform baseline blood and urine tests, along-with anthropometric measurements. All subjects were weighed on the same scales and height was recorded. Written consent was obtained prior to commencement of the study. Baseline blood samples were taken to evaluate haematocrit (for estimation of blood volume – although acknowledgement is made it can be affected by other factors such as anaemia, cardiovascular, renal and respiratory disease), serum albumin (for protein binding capacity) and serum creatinine concentration. These baseline samples were tested at Southern Community Dunedin Laboratory where creatinine is analysed by isotope dilution mass spectrometry (IDMS). Dipstick urinalysis was undertaken to screen for proteinuria and haematuria and a sample sent for an albumin/protein:creatinine ratio if greater than 1+ positive for protein. One subject subsequently withdrew due to a newly diagnosed, unrelated illness.

Subjects were instructed to discontinue thiazide diuretics for 4 days prior to testing and withhold morning medications on day of test in order to reduce potential hypotension.

On the study day, participants attended the research clinic at 0800 hours following a light breakfast. The blood pressure and heart rate was recorded, with consent reaffirmed prior to commencement of the study. Two cannulas were inserted intravenously on both upper limbs with one for administration of Cr⁵¹ EDTA which was subsequently removed and the other cannula was used for withdrawing blood samples. The subjects were then instructed to empty their bladder and baseline bloods were taken for a zero reference of assays, uric acid and creatinine prior to marker administration. They were also advised to drink freely over the first 4 hours of the study and collect all urine for 24 hours.

Participants then received the three drugs that served as markers of nephron function at time zero under medical supervision:

- Fluconazole 100 mg (as 50 mg x 2 capsules) orally (Pacific Pharmaceuticals, Auckland, NZ)
- rac-Pindolol 15 mg orally (as 5 mg x 3 tablets: total of 7.5 mg each isomer) (apopindolol, Apronex NZ Ltd)
- Cr⁵¹EDTA iv 2 mL by volume (GE Healthcare, NZ)

Oral medications were administered with at least 250mls of filtered water.

Blood samples were then obtained at seven time points over the following 24 hours for analysis. 15-20 mL samples were taken from the cannula (into 10 mL heparinized tubes after discarding 3 mL residual) at the following times post drug administration: 15-30 minutes, 30-60 minutes, 90 minutes and 2, 4, 6 and 24 hours. The actual times of administration were recorded along with the actual times of blood and urine collection to calculate clearances accurately.

The plasma concentrations were analysed for:

- fluconazole
- pindolol: R and S enantiomers
- Cr⁵¹EDTA (2,4,6,24 hours)
- Creatinine
- Uric acid
- Additional samples were taken for storage

The cannula was subsequently flushed with 2-5 mL normal saline (sodium chloride 0.9%, Demo S.A. Greece) and locked with 1 mL of heparin (50 iu in 5 M mL normal saline (10 iu/mL) (Pfizer, Australia)). The sample was then centrifuged at 2500 rpm at 4° centigrade (°C) for 10 minutes before the plasma was pipetted into 2.5 mL Eppendorf tubes for analysis. The plasma samples were frozen at -20 °C for storage until analysis was undertaken.

The Cr⁵¹EDTA for GFR was stored and prepared in the Department of Nuclear Medicine, Radiology Department under the licence of Professor Terry Doyle using established Dunedin Hospital protocols (see Appendices A and B). Administration was by volume method using 2 mL of isotope and administered using aseptic technique. An isotope standard was prepared for each bottle of Cr⁵¹ EDTA isotope as a concentration of 1 mL in 1000mL tap water, with 2mL aliquots used for counting in duplicate. 10 mL serum samples were obtained at 2, 4, 6 and 24 hours following isotope administration (recorded to nearest minute) then centrifuged for 10 minutes at 2500 rpm. 2 mL aliquots were manually counted in duplicate using a well counter (Wiper TM single well wipe test counter, Laboratory Technologies Inc., Illinois, USA) with weekly Caesium calibration as per Departmental practice. Prior to each test, the background radiation was quantified for 20 minutes to automatically compensate and avoid contamination. Testing was then completed - counts per minute for each set of samples were recorded, along with height and weight for normalization to body surface area (using the Du-Bois and Du-Bois

method⁽¹⁰¹⁾). Calculation of the isotope GFR was performed by the slope-intercept method on Excel software (Nuclear Medicine Department protocol, Dunedin Hospital). Both 6 and 24 hour programmes were calculated with best-fit result taken as the most accurate.

An Excel spread sheet was used to calculate the Cockcroft Gault⁽¹¹⁾ estimate of creatinine clearance: $eCrCL = (140 - \text{age}) \times \text{weight (kg)} / \text{serum creatinine} \times 72$ and the results for females were then multiplied by 0.85 as per the formula. Three variations of this formula were evaluated: actual body weight (ABW) (as described by Cockcroft and Gault and used in most clinical practice), lean body weight (LBW) and corrected for body surface area (BSA). LBW was calculated using the Hume formula^(102, 103) where males: $9270 \times WT \text{ (kg)} / 6680 + 216 \times BMI \text{ (kg m}^{-2}\text{)}$ and females: $9270 \times WT \text{ (kg)} / 8780 + 244 \times BMI \text{ (kg m}^{-2}\text{)}$. BSA was corrected using the DuBois and DuBois method⁽¹⁰¹⁾ where $BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$ where the weight is in kilograms and the height is in centimetres. The MDRD and CKD-EPI formulae both are corrected for BSA using this method. Estimations of GFR using all three formulae were also calculated via the KDOQI website calculator for confirmation http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm.

Timed urine samples were collected over the 24 hour period (0-3, 3-6, 6-24 hours) to determine clearances. Urinary volume was recorded to the nearest 5 mL. Aliquots of 1.4 mL were pipetted into 2.5 mL Eppendorf tubes and frozen at -20°C degrees for analysis of urinary concentration of the following:

- fluconazole
- pindolol: R and S enantiomers
- uric acid
- creatinine
- additional samples for storage

Subjects were encouraged to report any side effects. Monitoring of postural blood pressure (BP) via symptom enquiry was undertaken with BP and HR measurements frequently recorded with each blood sample. Lunch was supplied and free fluids were encouraged.

After 2 hours the participants were free to come and go from the study area between sampling but were encouraged to stay in the vicinity for accuracy of sample times and in case of unexpected delayed side effects. They were allowed home after the 6 hour test, then to return the following morning for the 24 hour blood sample and delivery of the overnight urine collection. Enquiry was made regarding delayed side effects, and the cannula was removed prior to discharge.

2.4 Side effects

Two subjects reported mild side effects most likely related to beta blockade with tiredness and nausea – one a male in his late thirties and the other a female in her seventies – neither had been exposed to beta-blockade prior. Both recovered by the 6 hour time period. Several younger subjects reported a mild decline in exercise tolerance later in the day of the study – once again most likely attributable to the beta blocker medication.

2.5 Assay methods and Statistical Analysis

Plasma and urine creatinine and uric acid concentrations were measured on a Cobas-c-311 autoanalyser (Roche-Hitachi, Tokyo Japan). Plasma and urine fluconazole and the pindolol enantiomer concentrations were measured by validated HPLC assays^(104, 105) in collaboration with the Department of Human Nutrition Laboratory, Otago University). The inter-assay variation for R and S pindolol was 7.3% and 8.5% respectively, and for fluconazole it was 3.04%.

The clearances of the endogenous and exogenous markers were quantified by a pharmacokinetic analysis of plasma and urinary drug concentrations using standard formulae. This analysis was based on the application of standard nonlinear mixed effects modelling techniques using the software NONMEM. The model was developed from both plasma and urinary drug concentrations measured over time by Professor Stephen Duffull (unpublished - School of Pharmacy, Otago University, Dunedin – detailed in Appendix D). These modelling methods provided an estimate of the average clearance via each of the mechanisms of filtration, organic anion and cation secretion, and reabsorption in our sample population, in addition to the between patient variability for the groups of elderly and young subjects. The influence of age on the clearance mechanisms has been assessed in the modelling process and the relationships between the indices of renal drug handling were then investigated using linear regression analysis. In addition, the individual estimates of the parameters for each clearance mechanism have been compared between groups using the unpaired t-test.

Statistical analyses of the measured and estimated GFR were additionally analysed, using Students t-tests and descriptive statistics via Excel and SPSS (version 18; SPSS Inc., Chicago, Ill, USA). Isotopic GFR (measured or mGFR) was considered the standard measure, with the mean difference between estimated and measured GFR interpreted as bias, and precision from the standard deviation of the bias for each of the estimating equations. Linear regression analyses were completed using a SPSS programme.

Chapter 3

Results

3.1 Demographics

Subject demographics are presented in Table 2 with body weight and adjusted body surface area remarkably similar between groups. Group 4 with CKD has a higher proportion of males and are older than the two other groups over 65 (Groups 2 and 3). This reflected sampling bias but not the actual gender distribution of CKD in NZ and Australia where approximately 25% of males and 16% of females over the age of 65 have established CKD⁽¹⁷⁾. The majority of participants were New Zealand Europeans with only one Maori and one Asian participant representing the source population.

Table 2: Demographics

Characteristics	Total n=40	Group1 n=10	Group 2 n=11	Group 3 n=11	Group 4 n=8
Male:Female	27:13	6:4	6:5	6:5	7:1
Age (years)	63.6 (20)	31.5 (7.6)	72.7 (5.7)	70.3 (3.3)	82 (4.9)
Weight (kg)	75.0 (14)	75.7 (11.3)	68.0 (15.3)	80.0 (14.4)	76.8 (13.2)
BMI (kg/m ²)	26.9 (3.8)	25.2 (2.7)	24.7 (3.6)	28.2 (3.9)	27.7 (4.0)
BSA (m ²)	1.9 (0.1)	1.9 (0.1)	1.7 (0.1)	1.9 (0.1)	1.9 (0.9)
Creatinine (µmol/L)	97.7 (32.4)	84.2 (9.2)	83.8 (13.6)	83.6 (10.3)	153.1(30.8)
mGFR (mL/min/1.73m ²)	96.9 (38.7)	130.8 (20.9)	93.8 (18.5)	106.9(19.1)	36.5 (7.9)

Values are expressed as the mean and (standard deviation). mGFR=measured GFR by isotope clearance.

The comorbidities recorded for Group 3 were hypertension on 1 or 2 medications and ischaemic heart disease. Group 4 had a higher burden of vascular disease with peripheral vascular disease, heart disease and controlled heart failure. Two subjects in Group 4 had diabetes. All subjects were community dwelling and independent in self-cares.

3.2 Correlation between GFR and Tubular Function

Glomerular filtration rate (mGFR) (as measured by isotopic evaluation) was compared with markers of proximal and distal tubular function. Assessment was then made to determine whether this relationship was modified by age and impaired kidney function. All clearances were standardized to litres per hour (L/h).

3.2.1 GFR

The standard isotopic GFR was used to measure tubular function against. Values are given in mL per minute to allow clinical correlation, and litres per hour to allow comparison with tubular clearances.

Table 3: GFR clearance

GFR L/h		GFR mL/min/1.73m²	
Mean	5.82	Mean	96.92
Median	6.52	Median	108.61
Standard Deviation	2.32	Standard Deviation	38.72
Minimum	1.42	Minimum	23.61
Maximum	10.30	Maximum	171.70
95% Confidence level	0.74	95% Confidence Level	12.38

The wide range of GFR values reflects the demographic variations of the subjects ranging from a healthy 20 year old to an 86 year old with established chronic kidney and vascular disease.

3.2.2 Creatinine Clearance

The 24 hour creatinine clearance (CrCL) was calculated using UV/P Values are again given in L/h and mL/min.

Table 4: Creatinine clearance

Cr CL L/h		Cr CL mL/min	
Mean	5.23	Mean	87.19
Median	5.52	Median	91.92
Standard Deviation	1.95	Standard Deviation	32.43
Minimum	1.18	Minimum	19.61
Maximum	8.33	Maximum	138.85
95% Confidence Level	0.62	95% Confidence Level	10.37

Figure 1: Comparison of GFR and Creatinine clearances

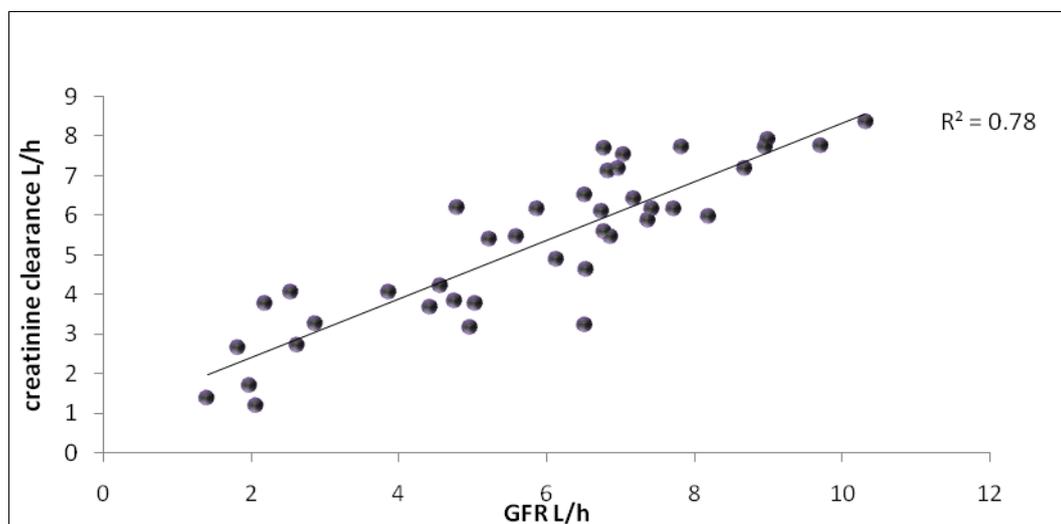


Figure 1 shows the association between measured GFR and Creatinine clearance. As predicted, there is a close association between these two indices which is significant (slope 0.74, $p < 0.001$), with the R^2 value of 0.78.

3.2.3 Proximal Tubule Cation Transport

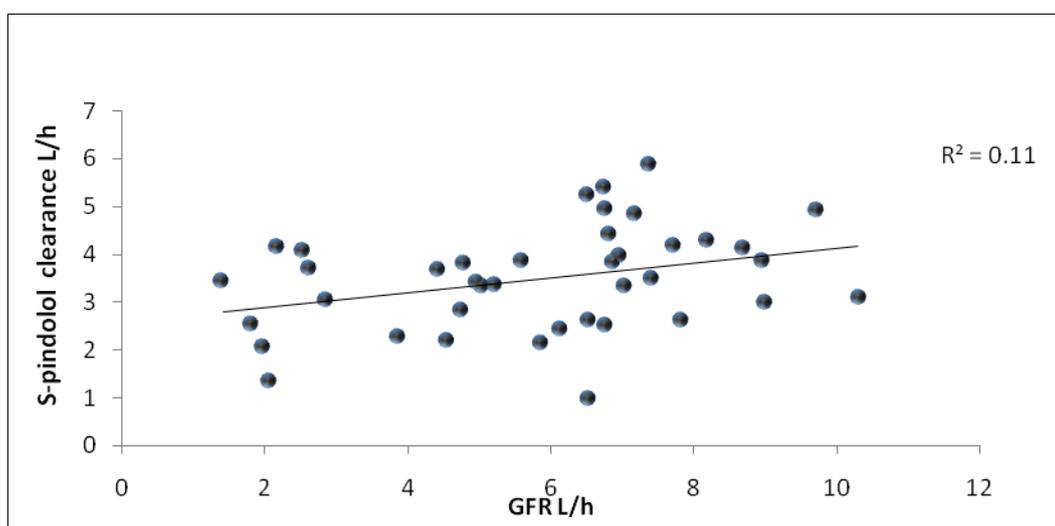
Cation transport was measured by the clearance of the S enantiomer of pindolol which is primarily renally excreted.

Table 5: S-Pindolol Clearance

S-Pindolol L/h	
Mean	3.54
Median	3.46
Standard Deviation	1.18
Minimum	1.07
Maximum	6.51
95% Confidence Level	0.38

Figure 2 shows the poor correlation found between GFR and proximal tubule cation clearance, as measured by S-pindolol clearance ($R^2 = 0.11$).

Figure 2: Comparison of GFR and S-Pindolol clearances



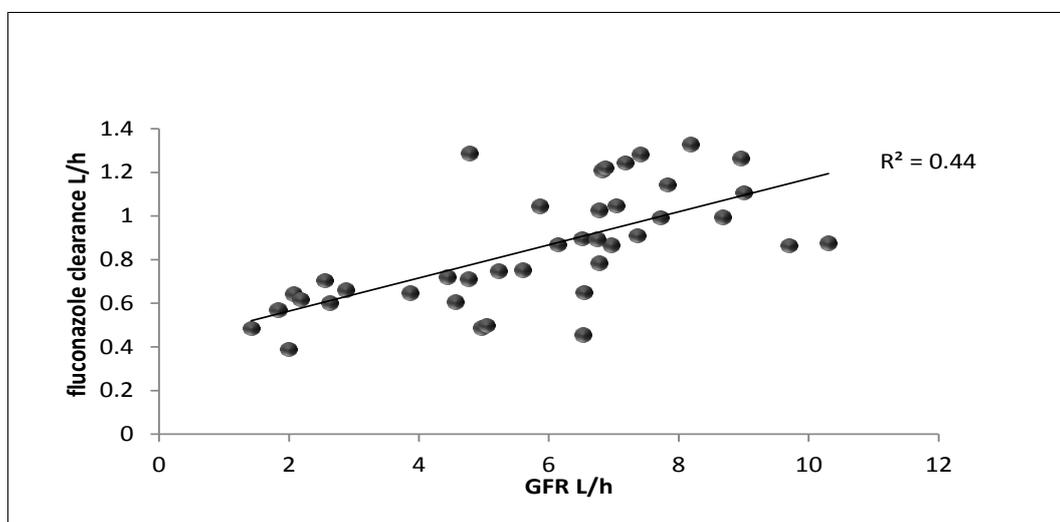
3.2.4 Tubular reabsorption

Fluconazole clearance was the drug probe marker used to measure tubular reabsorption. As shown in Figure 3, fluconazole clearance shows a moderate association with GFR ($R^2 = 0.44$).

Table 6: Fluconazole Clearance

Fluconazole L/h	
Mean	0.85
Median	0.87
Standard Deviation	0.27
Minimum	0.39
Maximum	1.33
95% Confidence Level	0.09

Figure 3: Comparison of GFR and Fluconazole clearances



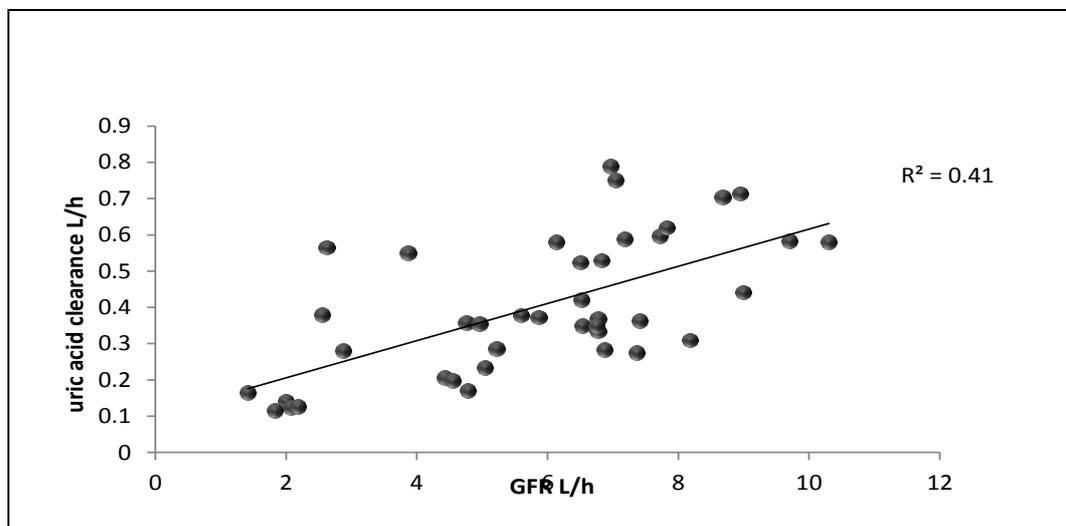
3.2.5 Proximal Tubule Anion Transport

Uric acid (UA) clearance, as a marker of proximal tubule anion transport, demonstrated a moderate correlation (Figure 4) with GFR ($R^2 = 0.41$) and is similar to the relationship found with fluconazole clearance.

Table 7: Uric acid Clearance

Uric acid L/h	
Mean	0.40
Median	0.37
Standard Deviation	0.19
Minimum	0.12
Maximum	0.79
95% Confidence Level	0.06

Figure 4: Comparison of GFR and Uric acid clearances



3.3 Influence of Age and Pathology

The clearances in L/h for all markers and comparisons by groups are shown in Table 8 below.

Table 8: Clearances of markers. Clearance L/h (SD). Normalized to GFR: ratio of probe clearance to GFR with (standard deviation).

	Group 1	Group 2	Group 3	Group 4
GFR	8.26 (1.25)	5.63* (1.11)	6.41 (1.14)	2.19 (0.47)
Fluconazole	1.00 (0.14)	0.81* (0.25)	0.96 (0.31)	0.59 (0.10)
Normalized to GFR	0.12	0.14	0.15	0.27
Pindolol S	4.01 (0.91)	2.89* (1.11)	3.91**(0.94)	3.04 (1.00)
Normalized to GFR	0.49	0.51	0.60	0.17
Uric acid	0.57 (0.15)	0.40* (0.14)	0.37 (0.16)	0.24 (0.16)
Normalized to GFR	0.07	0.07	0.06	0.11

* $p < 0.05$ Group 1 and 2: AGE

** $p < 0.05$ Group 2 and 3: PATHOLOGY

To assess the influence of age alone Group 1 (young, normal renal function and no pathology) was compared to Group 2 (age >65, normal renal function and no pathology). The clearances of all markers were found to be significantly lower when age alone was considered.

To assess the influence of pathology or co-morbid disease such as hypertension alone Group 2 was compared with Group 3 (age > 65, normal renal function and mild pathology – predominately hypertension). When considering the effect of pathology alone only pindolol clearance was significantly different. Of additional interest is that those in Group 3 had generally greater clearances (aside uric acid) than their elderly counterparts without co-morbid disease in Group 2.

Group 4 with documented CKD and co-morbid pathology clearly demonstrated reduced GFR and tubular function for all markers.

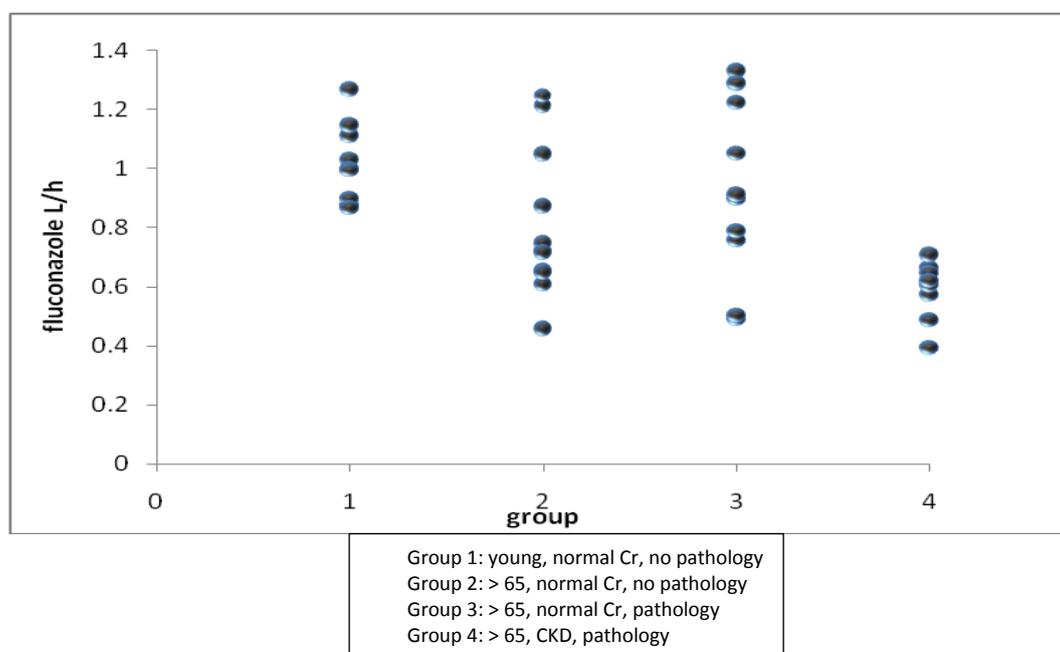
The calculated clearances normalized to GFR which are also shown in Table 8. This shows that the three groups with normal creatinine had very similar results indicating similar relative contributions of GFR and tubular function to overall clearance. However, Group 4 had greater influence of tubular function to overall clearances. When the coefficient of variation is calculated for each group (that is standard deviation of the mean / mean multiplied by 100 to give a percentage) the results are: group 1 - 100.74%, group 2 - 97.94%, group 3 - 107.98% and group 4 - 44.15%. This is indicated the expected wide variation in result of the groups with greater clearances compared to the group 4 with lower clearances. Therefore further inferences are not able to be made with these clearances normalized to GFR.

Calculation of the FE (fraction excreted unchanged) was found to be 27% in our study vs. 29% in the original study by Gross et al⁽⁷⁻⁹⁾ which indicates that the renal clearances are paralleled between this study and Gross's study.

3.3.1 Tubular reabsorption: Fluconazole

Fluconazole clearance by age and group is shown in Figure 6 below. Overall, the mean fluconazole clearance was 0.85 L/h (SD 0.27). There were significant differences in the mean clearances by age alone between Groups 1 and 2 which reflect the changes seen in GFR. Of interest, in the group with pathology (Group 3) a minor but significant reduction in tubular reabsorption between this group and the young group 1 (1.0 ± 0.14 L/h versus 0.96 ± 0.31 L/h ($p < 0.045$)) was seen. However, the elderly group without pathology in Group 2 had significantly lower clearances than their young counterparts and those of similar age with disease. The increased GFR seen in Group 3 removed any significant difference in tubular reabsorption. Clearances in those with CKD (Group 4) were predictably lower at 0.59 ± 0.10 L/h.

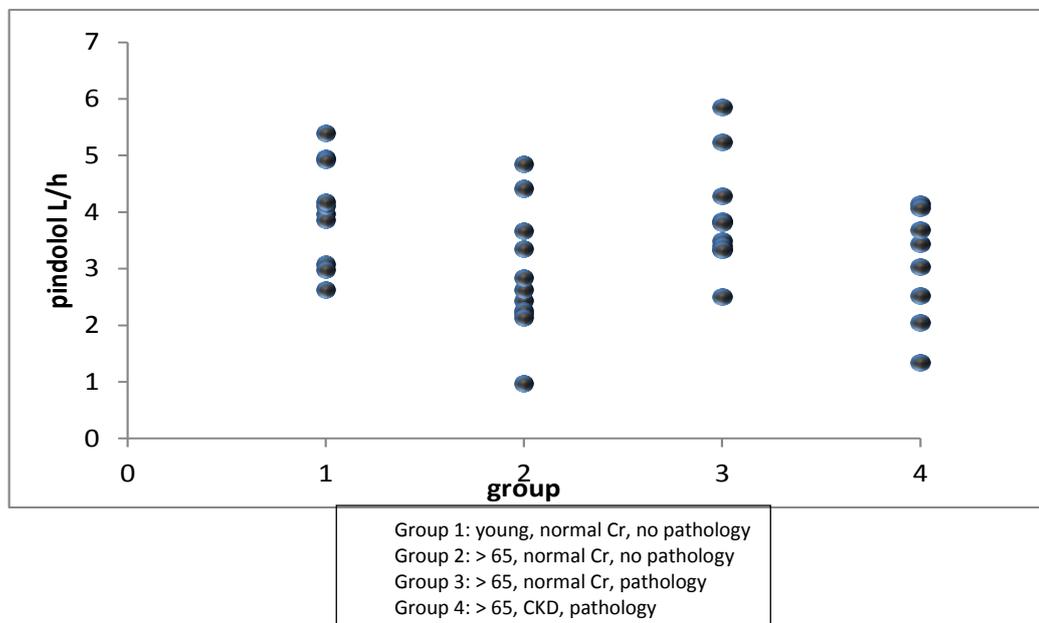
Figure 5: Clearance of Fluconazole by Group



3.3.2 Proximal Tubule Cationic Transport: Pindolol

The overall clearance of S-pindolol was 3.54 L/h (1.18 SD). Although proximal cation transport correlated poorly with GFR, there was a small but significant reduction in S-pindolol clearance due to age alone (Group 1 and 2: $p = 0.02$). In a similar pattern to GFR and fluconazole there was a greater average clearance for Group 3 than that of Group 2 who have co-morbid disease ($p=0.03$).

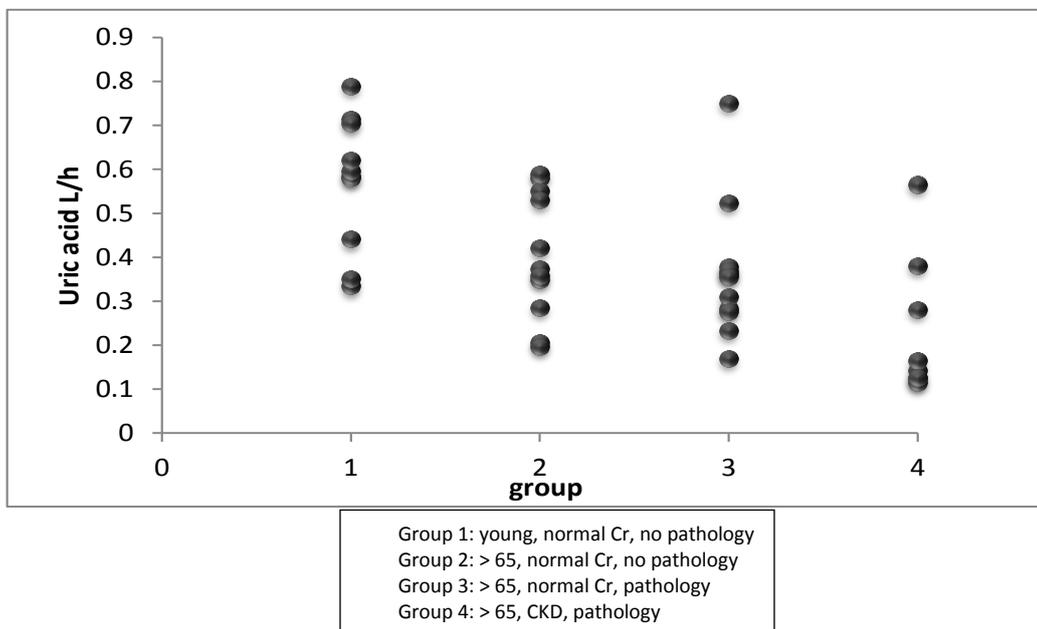
Figure 6: Clearance S-Pindolol by Group



3.3.3 Proximal Tubule Anionic Transport: Uric acid

The total clearance of uric acid over the groups was 0.4 L/h (0.19 SD). There was a significant difference in uric acid clearances based on age alone but not with co-morbid disease (Group 1 and 2: $p=0.02$, Groups 2 and 3: $p=0.55$). Not unexpectedly uric acid clearances were reduced in those with CKD (Group 4).

Figure 7: Clearance of Uric acid by Group



3.4 Glomerular Filtration Rate

3.4.1 GFR vs. age

The plots of GFR as measured by Cr⁵¹ EDTA clearance compared with age are shown below in Figure 8. The groups were similar in body mass (BMI: 25, 25, 28 and BSA: 1.9, 1.74, and 1.9 respectively for groups 1, 2, 3) although there was a small significant difference in weight, but not BMI and BSA between Group 3 and Group 2 (p=0.036). There was no significant difference between Group 3 and Group 1 (p=0.08) in body mass.

For those with normal renal function based on plasma creatinine (average creatinine of 84 $\mu\text{mol/L} \pm 1.9$ SE) (Groups 1, 2 and 3) there was a discernible decline in GFR with age at a value of around 0.83 mL/min/year despite having a similar plasma creatinine level. As the groups were not greatly dissimilar this decline in GFR is less likely to be attributable to changes of muscle mass alone. With the addition of the older age group with chronic kidney disease the decline in GFR is predictably greater at 1.33 mL/min/year. The data is missing a spread across the middle years which may skew this association to the right.

Figure 8 a: Correlation between GFR and age in those with normal renal function

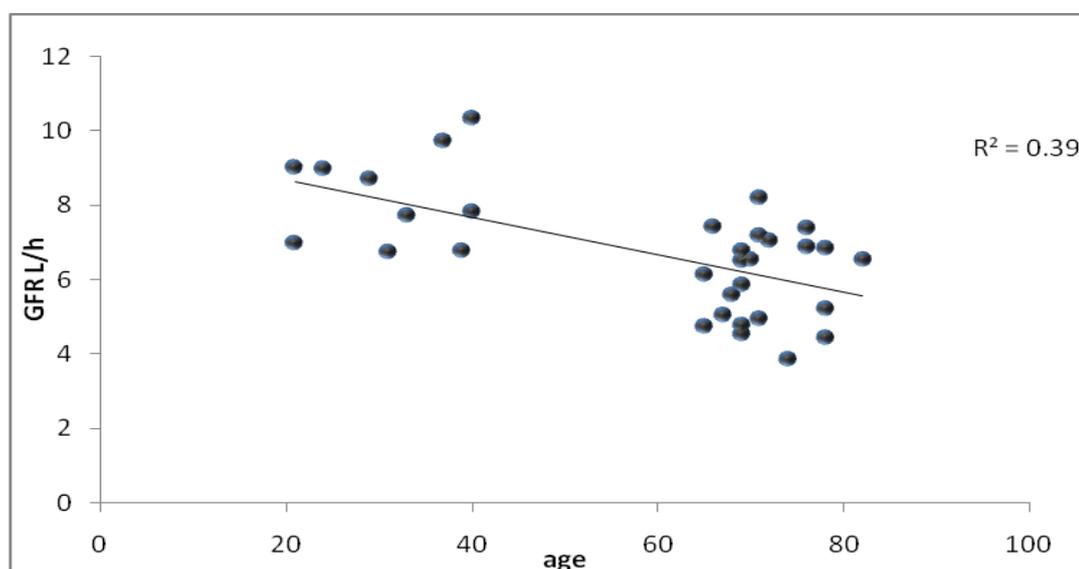
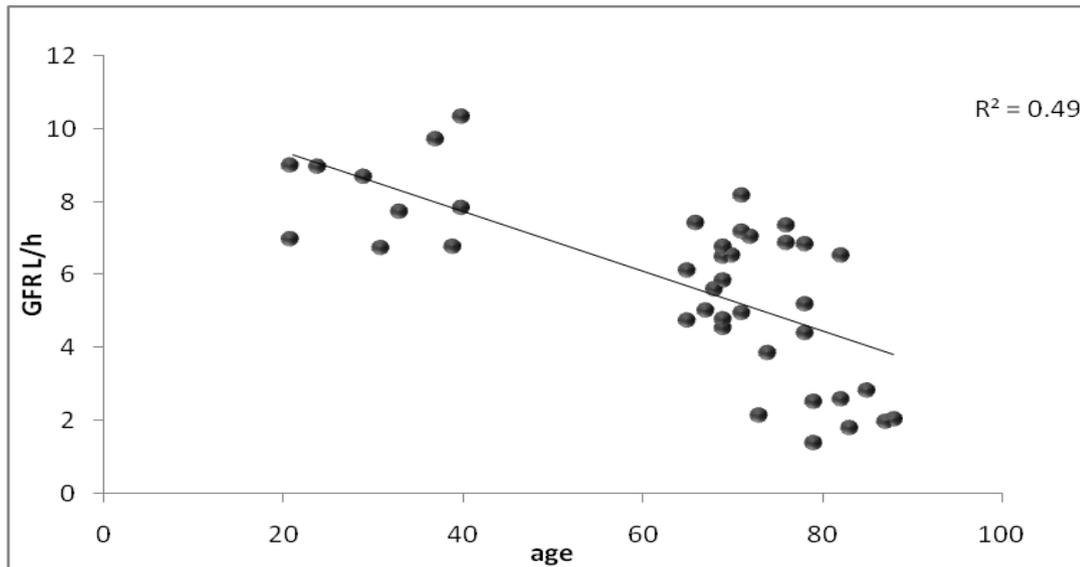


Figure 8 b: Correlation between GFR and age (total subjects)



3.4.2 Accuracy of estimating GFR equations vs. measured GFR:

Influence of age and pathology.

The estimated GFRs for each group are shown in Table 9, together with the isotopic measurement of GFR. There was a considerable range of eGFR over the population from 26.04 mL/min to 114.21 mL/min. Table 10 shows the overall mean bias (difference between average estimated and isotopic GFR) was least using the CKD-EPI equation, followed by the Cockcroft-Gault formula adjusted to actual body weight (ABW) and body surface area (BSA). The Cockcroft-Gault calculation adjusted for lean body weight (LBW) presented the greatest bias across all elderly groups. In the three groups with normal renal function by isotope measured GFR (>90ml/min), the various equations significantly underestimated measured GFR by 12-41% or 20-50 mL/min.

Table 9: Measured (mGFR) and estimated GFR by Group and equation.

Equation	Total	Group 1	Group 2	Group 3	Group 4
CG ABW	73.48 (31.9)	103.30 (11.5)	61.68 (12.9)	78.45 (20.0)	37.13 (11.7)
CG BSA	68.22 (25.8)	114.21 (21.1)	60.94 (8.6)	71.04 (12.0)	34.3 (7.6)
CG LBW	54.94 (23.5)	85.81 (15.7)	47.21 (8.6)	55.61 (11.2)	26.04 (8.4)
MDRD	66.51 (18.0)	85.29 (10.4)	67.64 (9.0)	71.91 (10.6)	38.63 (7.7)
CKD-EPI	70.08 (21.7)	95.30 (11.2)	69.27 (9.6)	73.73 (9.8)	37.63 (7.5)
m GFR	96.92 (38.7)	130.76 (20.9)	93.78 (18.5)	106.86 (19.1)	36.54 (7.9)

Mean and standard deviation in mL/min.

For the younger Group 1, with an average age of 31.5 years and a mean GFR of 131 mL/min (20.9 SD), all equations significantly underestimated measured GFR. For this group only, the Cockcroft-Gault formula adjusted for ABW was most accurate, with the least accurate formula the MDRD (Table 10).

In the group with moderate CKD (Group 4) with an average isotopic GFR of 36.74 mL/min (7.9 SD), the calculated eGFR equations were more reliable, with the CKD-EPI formula demonstrating lowest bias of 1.85 mL/min. This was closely followed by MDRD at 2.92 mL/min with both equations predicting mGFR renal function with 100% accuracy (Table 11).

Table 10:

Bias (mean difference from measured to estimated GFR), precision (standard deviation of bias) and accuracy of equations (within +/- 30% mGFR) of equations.

		CG ABW	CG BSA	CG LBW	MDRD	CKD-EPI
Group 1	bias	32.65	36.14	52.53	54.44	42.44
	precision	19.53	20.37	26.82	25.11	24.88
	accuracy	90%	30%	90%	50%	70%
Group 2	bias	32.1	32.84	46.57	26.14	24.51
	precision	18.52	17.52	17.9	15.55	15.83
	accuracy	36%	0%	27%	64%	64%
Group 3	bias	33.28	35.82	51.25	34.95	33.13
	precision	18.83	22.31	20.21	19.21	19.72
	accuracy	55%	9%	18%	27%	45%
Group 4	bias	7.66	5.17	10.5	2.92	1.85
	precision	4.58	3.17	7.3	1.64	1.85
	accuracy	50%	50%	63%	88%	88%
Total	bias	27.67	28.95	42.13	31	26.83
	precision	19.34	21.24	24.99	24.82	22.6
	accuracy	43%	25%	73%	58%	63%
Correlation to GFR R²		0.57	0.69	0.60	0.67	0.72

CG: Cockcroft Gault formulae, ABW: actual body weight, BSA: body surface area, LBW: lean body weight, MDRD – modified diet & renal disease formula.

For the age groups greater than 65 years with a normal creatinine (Groups 2 and 3) all the formulae underestimated mGFR substantially (Table 10). The MDRD and the CKD-EPI formulae displayed greatest accuracy although still with a 25-35 mL underestimation of measured GFR. Despite this, the MDRD and CKD-EPI equations are both reasonably accurate in CKD but overall less correct for those with normal renal function at any age. This leads to many subjects with normal renal function being wrongly classified with having more significant kidney disease than exists. As demonstrated in Table 11, this is most evident in those with normal renal function (especially in those who are younger) being misclassified as having stage 2 kidney disease (GFR 60-90 mL) despite having a measured GFR > 90 mL/min and no proteinuria. In those with normal renal function (Groups 1, 2 ,3) the laboratory reported equations of CKD-EPI and MDRD only correctly predicted the correct stage of renal disease (according to K/DOQI⁽¹²⁾) 18-40% of the time. The most recent formula CKD-EPI was more accurate in groups 2 and 3 above 65 years of age with normal function. It is acknowledged with the small numbers per group that these results may be inaccurate.

Table 11: Percentage of Group correctly staged by eGFR equation

	Group 1	Group 2	Group 3	Group 4
CG ABW	36	18	80	25
CG BSA	18	18	70	75
CG LBW	0	0	50	20
MDRD	18	36	30	100
CKD-EPI	18	27	40	100

CG: Cockcroft Gault , ABW: actual body weight, BSA: body surface area, LBW: lean body weight.

Bland-Altman⁽¹⁰⁶⁾ analysis (Figure 10 shown in Appendix C) was used to examine this relationship further by visually demonstrating the substantial underestimation of GFR of all equations when compared to higher measured GFR values. This

analysis confirms that the CKD-EPI is most representative of mGFR with $R^2=0.722$, followed by the MDRD $R^2=0.672$, and the CG (Cockcroft Gault) BSA at $R^2=0.686$. Interestingly CG LBW ($R^2=0.604$) performed better than CG alone ($R^2=0.574$) which reflects the improved accuracy in the younger subjects. For those above the age of 65 years the CKD-EPI was the most accurate equation demonstrating underestimation bias of 22 mL/min and correlation to GFR of 0.73. For those without established CKD, all equations aside from the Cockcroft Gault adjusted to lean body weight, demonstrated similar under estimation of 33-5 mL/min with moderate correlation to mGFR $R^2= 0.34-0.37$.

As was seen with the other cationic marker pindolol, those elderly subjects with similar creatinine level but the addition of mild pathology (Group 3 vs. Group 2) had a higher average mGFR. Again this may represent hyperfiltration in mild disease before established nephron damage occurs and CKD ensues.

3.4.3 Influence of gender

In this study, there was a non-significant difference in mGFR between genders, despite the estimating equations assumption of meaningful distinctions requiring adjustment which have then been incorporated within the respective formulae.

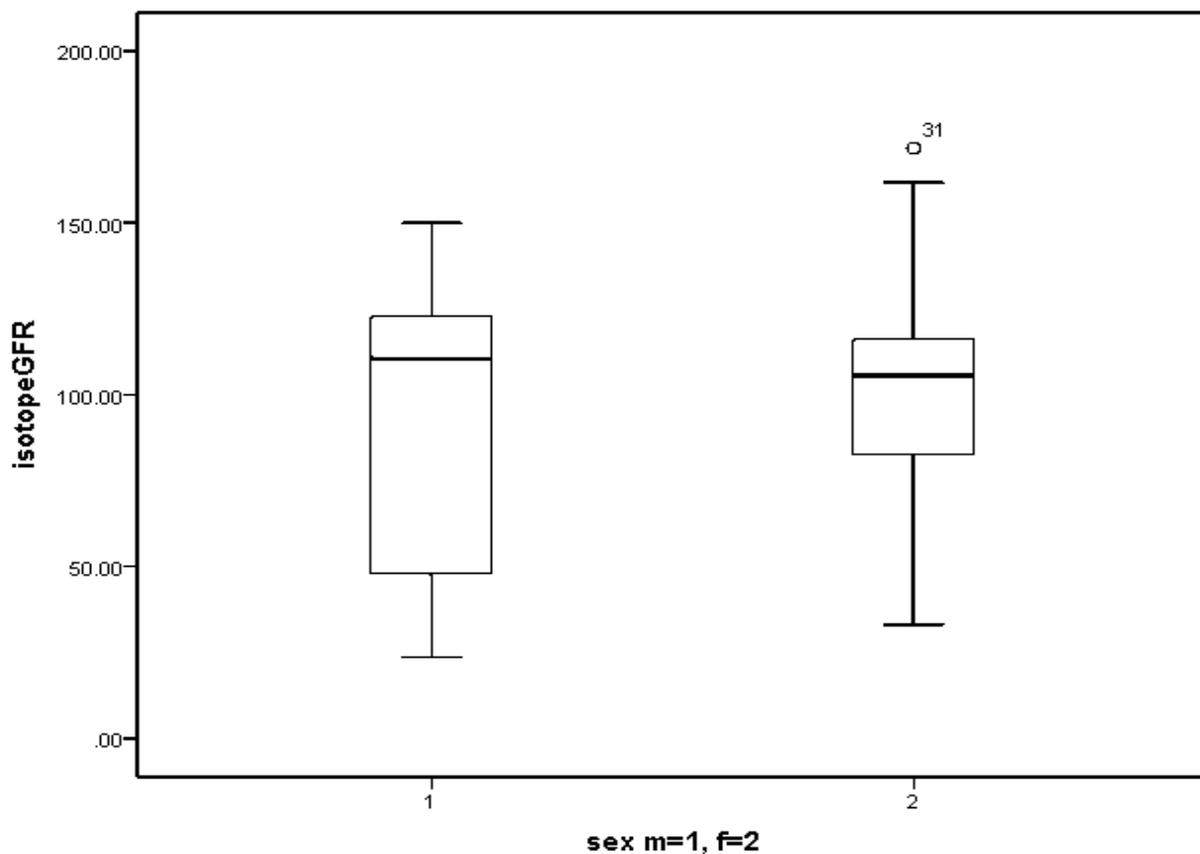
Analysis of the entire group of subjects showed males to have a lower average GFR (92 mL/min (39.8 SD) than females (105 mL/min (36.4 SD) – with some bias due to the largely male CKD Group 4. If this group are removed from the analysis there was a non-significant difference in the average mGFR of males 112.8 mL/min compared to females at 110.9 mL/min (Figure 9).

In the Groups 1, 2 and 3 with normal creatinine levels, for males the estimated GFR ranged from 66.9 to 89.1 mL/min and was generally lower for females at 55.2 to 76.6 mL/min. The Cockcroft-Gault ABW was the most accurate equation for males with a mean bias (difference compared to the measured GFR) of -23.7 mL/min. For

females, the Cockcroft-Gault ABW, Cockcroft-Gault BSA and CKD-EPI formulae all performed remarkably evenly with an average under-estimation of -34 mL/min.

Therefore, interpretation of an estimated GFR for those with normal renal function, especially for females, needs to take into account the considerable inaccuracies between these estimates and the measured GFR.

Figure 9: Box plot of gender differences in mGFR (normal renal function)



To summarize these findings:

- Good correlation between CrCl and mGFR
- Weak, if any, correlation between proximal tubular cation clearance and GFR
- Moderate correlation between tubular drug reabsorption and GFR
- GFR and all clearances are reduced by age alone
- CKD is not only associated with reduction in GFR but also of all aspects of tubular transport, although to a lesser degree with cationic transport
- Possible additional effect of hypertension/co-morbid pathology on cationic transport but not on other markers in the elderly
- Substantial underestimation of mGFR occurs with all estimating equations for those without established CKD.

Chapter 4

Discussion

Drug elimination from the kidneys is a combined product of the processes of glomerular filtration, tubular secretion and reabsorption. Conventional measures of renal function, such as glomerular filtration rate or creatinine clearance, may not detect alterations in the integrity of the tubular function and hence do not necessarily detect altered renal clearance of a drug. Recommendations in formularies such as the British National Formulary (BNF)⁽¹⁰⁾ for modification of drug dosing in the setting of kidney impairment are generally on the basis of changes in the calculated creatinine clearance using the Cockcroft & Gault formula⁽¹¹⁾. Current clinical practice is to determine renal function by estimating glomerular filtration rate (eGFR) and then adjusting the dose based on this result and the fraction of the drug excreted unchanged. This is on the assumption that a change in GFR reflects a parallel change in tubular function. All eGFR formulae use age as a variable, thus automatically assuming that the elderly have a degree of kidney impairment. This often places them into a defined category of chronic kidney disease even though, in the healthy this assumption may not be valid as shown in the sentinel Baltimore Longitudinal Study of Aging (BLSA) where approximately one third of the participants, all of whom were aged over 65 years, did not demonstrate a reduction of measured GFR with age⁽²⁰⁾.

This thesis set out to address two important and related enquires:

First: is a decline in glomerular filtration rate reflective of changes in tubular function when considering modification of drug dosing? Can estimates of GFR be used to accurately adjust doses of medications handled primarily by tubular

function? In order to fully answer this question an assessment of the accuracy of several common estimating equations were made.

Second: given that incorporation of age into the estimated GFR equations automatically adjusts eGFR, is any decline in mGFR accompanying aging due to the physiological process of senescence alone or associated with pathological changes secondary to co-morbid disease? What is the relationship of physiology vs. pathology with respect to renal proximal tubule function?

4.1 Relationship between proximal tubular function and GFR with reference to drug dosing

To address the first question, a modified protocol for simultaneously measuring glomerular filtration rate and proximal tubular function was employed⁽⁷⁻⁹⁾. This demonstrated a moderate correlation between GFR and proximal tubular anion transport ($R^2=0.41$) and reabsorption ($R^2=0.44$) (Figures 3, 4) implying that where the drug is handled by anion transport and/or has significant tubular reabsorption modification of drug clearance based on GFR is reasonable. However, there was only a poor correlation demonstrated between GFR and cation clearance ($R^2=0.11$) (Figure 2).

Organic cation secretion occurs via organic cation transporters which are frequently linked to Na/H sodium hydrogen transporters and once taken up into the cell, elimination into the urine is largely by passive diffusion. Examples of substances handled by cation transporters are: creatinine, trimethoprim, cimetidine, and metformin and there can be competitive interaction for these transporters. Drug dosing may be adjusted downward because of an increase in serum creatinine arising, not from a true decline in GFR, but altered creatinine handling from the competitive transport between creatinine and cationic handled medications such as trimethoprim. This may lead to inadequate dosing. Furthermore the results of this

study reveal initial dosing of basic/cationic drugs may be inadvertently low based on the assumption of parallel clearance by eGFR when it appears to show essentially steady clearances over a wide range of GFR. This requires more detailed pharmacokinetic drug studies to clearly elucidate the extent of the potential interactions between cationic drugs and tubular creatinine handling.

This study shows the relevance and importance of the investigations of drug handling in the elderly. Drug-dosing schedules in the popular formularies⁽¹⁰⁾ utilise calculated GFR values alone to modify dosing according to kidney function. The findings presented in this study suggest that such global recommendation for dose reduction according to eGFR should be treated with some caution especially where cationic tubular handling is the major component of elimination. Given that many drugs are weak organic bases (cations), using eGFR to determine kidney function may lead to withdrawal of a medication due to the erroneous assumption a parallel decline of cationic secretion to GFR with aging. Metformin is an example of a drug primarily handled by cation transport and adjusted on the basis of renal function. It is the drug of choice in controlling hyperglycaemia in type 2 diabetics with proof of improved mortality and morbidity⁽¹⁰⁷⁾. Drug formularies⁽¹⁰⁾ recommend that metformin be withdrawn when an individual has developed CKD stage 3 or when the creatinine is greater than 150 $\mu\text{mol/L}$ based on the rare occurrence of lactic acidosis, which almost always occurs in the setting of acute kidney injury and dehydration. Taking into account the results of this study, basic medications such as metformin may potentially be safely used in patients with more significant kidney disease than was previously believed.

With respect to the method of simultaneously evaluating several pathways of renal function via a drug cocktail, and interpreting the results, there is a potential for the index drugs to interact with another. This may, in turn, alter the marker's own clearances or that of another drug. McLachlan and colleagues investigated this thoroughly and did not demonstrate any significant interactions between the

drugs⁽⁸⁾. With the modified protocol used there were no apparent interactions between pindolol, fluconazole and uric acid. Therefore the observed values measured ought to reflect the attributes of tubular function under investigation.

4.2 Accuracy of eGFR in the elderly

Addressing the second question, this small study of a largely European population reaffirms previous studies findings^(67, 69) that creatinine-based predicting equations for GFR can be significantly inaccurate. The use of these eGFR formulae in individuals with normal renal function of any age - especially for those in the younger age groups, significantly underestimated mGFR. This then inaccurately identifies the individual who does not have structural or functional renal dysfunction as having chronic kidney disease (according to international guidelines⁽¹²⁾). In those with normal function, the results display the significant underestimation of GFR using any equation of around 30 mL/min (Table 10). For the group with chronic kidney disease, traditionally defined with an estimated GFR less than 60 mL/min, the accuracy of the estimations improved. This is not unexpected given that these formulae were largely derived from study groups with chronic kidney disease. Although small, this study adds to the interpretation of estimating equations across and within populations at subjects with a range of ages, gender and baseline creatinine.

All three main estimating equations have been derived from Northern American populations^(11, 108, 109) and not formally validated for the New Zealand population although in a comparable Australian population, Jones^(110, 111) has shown the MDRD, CKD-EPI and Cockcroft-Gault equations to be validated. This was despite one fifth of subjects having a true GFR greater than 30% above the estimate, similar to values seen in this study's New Zealand group (Table 10). As in this study, Jones found that adjusting for lean body weight was less accurate than using actual body weight

when using the Cockcroft Gault formula. Likewise they found in established kidney disease that the MDRD equation tends to underestimate GFR and Cockcroft Gault overestimates the measured GFR with a small actual and relative bias. In contrast, recent work by Brown and colleagues⁽¹¹²⁾ examined the performance of Cr⁵¹ EDTA GFR against the three derived formulae for GFR, again in an Australian population with mild-moderate kidney disease. They found the Cockcroft-Gault equation corrected with body surface area to be more accurate than both the other formulae. This highlights the often disparate results from comparable studies in similar populations. The recent suggestion from Brown et al⁽⁵⁹⁾ to compare ranges for lean or ideal body weight to body surface area, as opposed to an absolute value, appears the most useful strategy to move forward.

Extensive reviews have shown that the MDRD equation appears to underestimate GFR in younger people, women and those with plasma creatinine concentrations in the normal range – overestimating the prevalence of CKD⁽¹¹³⁾. Of interest, in the AusDiab study the “prevalence” of CKD reduced from 13.1 to 11.5% when the CKD-EPI formula was used in comparison to MDRD equation⁽¹¹³⁾ reflecting the greater accuracy of CKD-EPI. Following the conclusion of this study New Zealand laboratories have moved to the CKD-EPI formula⁽¹⁹⁾ for standard reporting of eGFR due to its greater accuracy for the general population⁽⁵³⁾. This study confirmed this CKD-EPI formula to be the most accurate estimate of renal glomerular filtration across a range of ages and renal function by displaying the least bias between the measured and estimated GFR (Table 10) and highest correlation to GFR $R^2=0.72$. For the total subject group of this study the Cockcroft Gault equation adjusted to BSA demonstrated the second most accurate bias behind the CKD-EPI formula. The previously reported MDRD₄⁽¹³⁾ formula closely followed but was less accurate for those with normal renal function of any age. Noteworthy in the younger group of subjects, the Cockcroft-Gault formula⁽¹¹⁾ equations incorporating actual body weight and adjusting for body surface area were more accurate than both of the newer formulas. Both of these Cockcroft-Gault formulae

are significantly more accurate and robust than adjusting for lean body weight consistent with other studies⁽⁵⁹⁾. The additional information gained from calculating the body surface area did not improve the Cockcroft Gault formula accuracy above that using actual body weight in this subject group. This may reflect the normal to mildly overweight anthropometry of the study group where adjustment for lean/ideal body weight is less likely to improve the accuracy of the creatinine generating capacity of lean muscle mass.

To reflect gender associated differences in proportion of lean body mass the derived equations for estimating GFR incorporate an adjustment for gender. In our cohort of normal individuals, i.e. with a normal measured GFR and plasma creatinine <105 $\mu\text{mol/L}$, the difference in measured GFR attributable to gender was not as great as one would expect from the estimating equations. The average mGFR differed by 13 mL/min between males and females in the total group was reduced to 2.1 mL/min if those with established CKD were excluded. This result compares favourably with work by Granerus⁽⁵⁶⁾ and more recently Soares⁽¹¹⁴⁾ who showed a similar non-significant difference in actual GFR between genders. There were insufficient females in the CKD group to make any meaningful comparisons.

The findings from this thesis study replicate the results of previous studies^(21, 53, 56) that demonstrate the isotopic measured GFR is lower in healthy elderly individuals than young subjects despite having a comparable plasma creatinine concentration. Comparing the younger age group with the older group, accepting the limitations of a cross-sectional study, there is a decline in GFR of 0.8 mL/min/1.73 m² per year despite the same average creatinine. In the older age group with normal plasma creatinine concentrations, the cohort with hypertension/mild vascular disease unexpectedly had a slightly higher GFR compared to the normal older age group. This may reflect the impact of an increased mean arterial pressure sensed at the glomeruli leading to a degree of hyperfiltration such as that seen in early diabetes⁽¹¹⁵⁾. The lack of consistent deterioration of GFR with normal aging suggests

that an individual's chronological age does not necessarily predict a physiological decline in renal function⁽⁴³⁾. It remains unknown whether or not the fall in GFR seen in the healthy older age group is a physiological re-setting associated with age alone. Regrettably this study is too small and of inappropriate design to be able to evaluate this decline in subgroups but it was surprising the substantial decline in GFR seen for those without co-morbid disease indicating a clear physiological aging component. Clearly more studies, following well defined older populations over time, are required to answer the question of whether GFR truly declines as a function of age alone and the relative contribution of comorbid pathology.

Looking forward to the future clinical use of GFR and the estimating formulae holds many challenges. This study, along with many other studies, reports the accuracy of eGFR by focussing on the precision of a point estimate in time. The suggestion to evaluate the decline in eGFR over time for the individual⁽¹¹⁶⁾ rather than repeated use of an absolute value focuses on the more important aspect of deteriorating renal function. This enables the clinician to review the patient's long term function in order to accurately assess and treat the individual patient over time. It may also be feasible to test tubular function in a longitudinal fashion using a similar protocol to allow much more precise modification of drug doses. Clinical information such as this will allow much more accurate dose adjustment according to any actual decline in drug handling, which may be disparate from that assumed using Bricker's theory of a parallel decline of tubular and GFR function with aging.

4.3 Strengths and weakness of the study

Despite the limitation of small size and selection bias, this study reaffirms other published studies which indicate that co-morbid disease influences the rate of decline of renal function with aging^(4, 6, 20, 43, 44). In addition this study found that GFR

and all aspects of proximal tubular function do not decline at similar rates. This speaks against the assumptions inherent to the intact nephron theory.

A major strength of the study was the focus on specific aspects of renal tubular function in the elderly, particularly as it applies to drug handling, as this is the group often most affected by both drug side effects and chronic kidney disease. Further strengths of this study include the use of a sole researcher to perform all of the clinical investigations along with the measurement and calculations of the Cr⁵¹ EDTA clearances which reduced inter-tester variation. Similarly, the analyses of plasma and urinary markers were undertaken in a single evaluation by a single laboratory, again reducing inter-test variability.

It is accepted that the numbers of subjects are relatively modest; however the individuals within each group were reasonably homogenous aside from the gender differences in the group with CKD. Ideally, it would have been beneficial to have greater numbers of Maori and other ethnic groups although recruitment correctly reflects the largely NZ European ethnicity of the local population. The aetiology of chronic kidney disease in the older age group was primarily related to hypertensive nephrosclerosis and associated cardiovascular disease. Two individuals with CKD had type 2 diabetes mellitus as a contributing factor to their diagnosis of CKD. The impact of diabetes and altered tubular function would be the subject of a separate thesis; however the small numbers probably did not influence the results dramatically.

To more accurately determine the effects of CKD alone versus CKD combined with age, it would have been beneficial to have a younger cohort of CKD patients aged matched to the younger healthy normal controls. In addition, a cohort between the younger and older age groups i.e. aged 40-65 years, would provide superior strength to the analyses related to age. This is likely to be the focus for a planned subsequent study.

Using the slope-intercept method⁽⁵⁴⁾ to determine GFR from Cr⁵¹ EDTA clearances inherently over-estimates GFR which may explain the potential variation between estimated and measured GFR evident in participants with normal renal function, especially in the younger age group. However, from a clinical perspective it is the local standard, with many published studies using similar isotopic method for comparison to mGFR.

With regard to the drug cocktail method employed for this study, although the renal clearance of S-pindolol is only 20 %, Tett and colleagues have previously validated this as an accurate method to assess renal tubular cation transport. Consequently, although the renal clearances are low, in the absence of an alternative marker, this variability is acceptable. In the original drug cocktail⁽⁷⁻⁹⁾, para-aminohippurate (PAH) was used as the marker of proximal tubular anion transport. Despite an extensive international search, pharmaceutical grade PAH was no longer available for clinical use. Uric acid was used as an alternative marker of anion clearances although interpretation of clearances could be considered difficult due the nature of its handling by the kidney. Uric acid clearance is a composite of a 4 stage process⁽¹¹⁷⁾ although secretion is the net end product and has been used in previous studies to assess anion renal clearance⁽⁸⁸⁾. Overall the renal clearances in our cohort were similar to those of the original study by Gross and colleagues⁽⁷⁾ supporting a degree of accuracy and reproducibility between both studies.

4.4 Summary and Conclusions

This thesis demonstrates that the use of estimating equations for GFR to adjust medication doses for those drugs primarily handled by tubular mechanisms can be significantly inaccurate. These arise from the imprecisions associated with the use of estimating equations where age is the primary variable (especially in those with

normal renal function) and additionally from the finding that not all aspects of tubular function are closely associated with any decline of GFR in aging.

The relationship of age and renal function has been examined. In this study population deterioration in GFR with age was seen across the group. This signal remained when those with established CKD were removed where an average fall of approximately 0.8 mL/min/1.73 m² per year was seen despite similar creatinine levels. The results from this study demonstrate the creatinine blind area where renal function declines before the creatinine rises due to enhanced tubular secretion. This in itself demonstrates the disparity between GFR and tubular function – at least with respect to the cationic transport for creatinine. These outcomes would indicate that GFR does decline with age alone as part of normal cellular and organ senescence. Whether this represents a disease process is controversial taking into consideration the international diagnostic criteria for CKD are based on eGFR thresholds rather than recognition of any secondary consequences of renal disease such as anaemia and bone mineral disorder.

This study reviewed the impact of comorbid disease and found that mild vascular disease or hypertension does impact GFR. However contrary to the assumption that a further decline in GFR would result, this study found a non-significant elevation in GFR with comorbid pathology despite similar baseline creatinine level. This appears to occur in the early stages of disease before CKD develops with structural and functional sequelae and most likely represents a degree of hyperfiltration. As the two diabetic subjects in the study population were in the group with established CKD this is unlikely to be the cause for this finding.

A significant decline of clearances with age alone was seen in all three aspects of renal tubular function. In addition, cationic secretion was significantly elevated with the addition of mild systemic hypertension and vascular disease in a similar pattern to GFR. Interestingly tubular reabsorption was maintained at near normal levels until recognized CKD occurs. Consequently it appears that both

physiological and pathological processes impact on the decline of renal function with aging with a thought-provoking finding of an apparent hyperfiltration and/or hyperperfusion process occurring in early disease. This observation has not been previously well documented in the literature outside diabetic patients. Accordingly the results of this study may contribute to enhanced understanding of how renal function alters with aging and pathology.

The premise of the intact nephron theory that all aspects of renal drug elimination decline at similar rates has been examined by a method to simultaneously measure four aspects of renal drug elimination. The study found a moderate relationship between glomerular filtration, tubular anion secretion and reabsorption. However, cationic transport was not well correlated with GFR raising the concern of underdosing of some important medications such as antibiotics and chemotherapy drugs if GFR is used to adjust dose. For this reason where cationic drug clearances do not parallel changes in eGFR, it is important for the clinician to be cognisant of drug physicochemical properties before making an assumption about renal clearances.

A particular strength of this study was the range of ages and kidney function examined with the drug cocktail. Typically these types of studies are undertaken in a small number of young, healthy and usually male volunteers. The method was generally well tolerated with the small amount of subjects experiencing side effects to the beta blocker generally in the younger age group. This study provides a unique insight into the alterations in drug elimination in the elderly and is particularly relevant to medicine with the increasing proportion of elderly in the general population. Many of the elderly patients seen in daily medical practice require multiple medications with potential interactions and significant side effects affecting morbidity and sometimes mortality. An understanding of factors contributing to this, with the ultimate goal of making more accurate clinical

decisions on drug dosing to avoid under and overdosing of the vulnerable elderly is important to all physicians.

Furthermore, this study reaffirmed the imprecision of the estimating equations for GFR in clinical use. The bias is most substantial and relevant in subjects with normal renal function of any age. Without the knowledge of the underestimation of, on average, 30 mL/min of GFR in the healthy patient of any age with normal renal function, clinicians may mislabel their patients as having kidney disease based on an inaccurate estimation of GFR. The CKD-EPI formula was the most precise estimate of mGFR overall although all the equations were significantly more accurate in those with established chronic kidney disease with smaller biases. This is not surprising taking into account that the older formulae were derived from populations with CKD. This study also revealed discrepancies between actual and anticipated differences in GFR between genders. Therefore the young, female individual with normal renal function is the most disadvantaged by the use of eGFR formulae to measure her renal function. For this group the older Cockcroft Gault offers the most precise estimate of GFR.

As a consequence of the inaccuracy and inconsistencies of formulae for estimating GFR, adjustment of drug dosing using eGFR alone may lead to significant underdosing with potential therapeutic consequences. For example, as most antibiotics are renally cleared, a reduction in dosing due to a presumed decline in GFR may lead to sub-therapeutic concentrations and inadequate treatment for serious infections.

In conclusion; this thesis demonstrates that the use of estimating equations for GFR to adjust medication doses for those drugs primarily handled by tubular mechanisms can be significantly inaccurate. This arises not only from the imprecision associated with the use of estimating equations (especially in those with normal renal function) but also from the findings that not all aspects of tubular function are closely associated with GFR in aging. The use of a drug cocktail to

simultaneously measure four aspects of renal drug elimination pathways displayed the relationship between these pathways and the implications associated with alterations in aging. Applied in healthy volunteers of varied ages and baseline renal function, this method provides a unique insight into the alterations in drug elimination in the elderly. This study also illustrates the impact of mild co-morbid pathology in the elderly with a normal creatinine. Clearances closer to those of the younger and healthy group were found due, most likely, to hyperfiltration and hyperperfusion before kidney disease is established and recognized. Future studies on drug handling in the elderly, with particular reference to the relationship to GFR and cationic transport are required. Supplementary research to investigate the handling of metformin is planned to enhance the understanding of pharmacokinetics in aging and declining renal function in order to inform a more accurate prescribing regime focussed on the elderly. This study contributes to a developing understanding of how renal function alters changes with aging and pathology with a particular focus on improving drug dosing in this burgeoning and vulnerable group in our population.

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Appendices

Appendix A:

GFR Information for Staff Taking Blood Samples – Radiology (Otago)

Pharmaceutical - The injection given is ^{51}Cr -EDTA.

Side effects - There are no side effects to the injection and the patient can eat and drink as per normal.

Precautions - Obviously the patient should not undergo dialysis during the course of the examination.

To avoid contamination of blood samples, and possible erroneous results, it is preferable that the injection is given into a vein separate from the site where the blood samples will be taken.

E.g. Injection into a peripheral vein and blood samples taken from a Hickman's line or Portacath. Or IV injection in the left arm, blood samples from the right arm. **The blood samples must not be diluted.** Therefore if samples are being taken from a Hickman's line or IV line then withdraw and discard 1ml of blood before taking sample.

Blood samples - Taken at 2, 4 and 6 hours post injection and occasionally at 24+hrs post injection.

We will provide the blood tubes for the samples, as the hospital does not supply 10ml tubes.

A full 10ml sample is needed to gain enough plasma to detect the ^{51}Cr adequately. We can make do with less but this decreases the reliability of the result. **Please label the tubes with the patients' name and the time the blood sample was taken. It is more important to note the actual time than to take the samples at exactly 2, 4 and 6 hours.**

Once all the samples are taken return them to Nuclear Medicine. If the samples are returned before 1615hrs then we will be able to have a result for the next morning. After this time the results will not be available till the next afternoon.

Time of 2hr sample
Time of 4hr sample
Time of 6hr sample
Time of 24hr sample

^{51}Cr -EDTA lot Number
<u>Injection Volume (2ml)</u>
Time of Injection
Patient Height
Patient Weight

Appendix B:

Protocol for Glomerular Filtration Rate (GFR) - NM Radiology (SDHB, Otago)

Follow this procedure to image the kidney to estimate their glomerular filtration rate.

Associated Policy Responsibilities of Nuclear Medicine Staff in Administration of Radiopharmaceuticals (Otago) (21074).

Introduction This determination analyses the rate of plasma disappearance of ^{51}Cr -EDTA after a single intravenous injection from which the GFR can be calculated.

For patients suffering from severe renal failure; it may be necessary to extend the sampling time to 24 hours to maintain an accurate estimate of T1/2.

Indications Suspected renal failure.

Examination time 6 hours. Patient is free to leave department between injection and blood samples.

Preparation Patient must be well hydrated.

Radio pharmaceutical & dose ^{51}Cr -EDTA 2ml (approx. 6MBq)

Actions

1. Prepare patient dose:
 - Draw up the ^{51}Cr -EDTA in 2ml volume.
 - Change needle.

2. Prepare standard dose:
 - Draw up 1ml of ^{51}Cr -EDTA.
 - Change needle.

Note: Only need to make standard once for each lot number.

3. Record patient's height and weight accurately.

4. Inject patient by direct venous puncture.
5. Ensure syringe is well flushed.
6. Document exact time of injection.
7. Add standard dose to 1000mL water in a volumetric flask.
8. Withdraw 10mL blood samples from a different vein than that injected into at the following times post injection.
 - 2 hours
 - 4 hours
 - 6 hours
 - 24 hours if required

Document time of sample accurately.

9. Centrifuge all samples for 10 minutes at 1500 - 2000 rpm.
10. Pipette 2 ml aliquots in duplicate of standard, patient samples and water (BG) into suitably labelled counting tubes.
11. Pipette 2 ml aliquots into suitably labelled counting tubes in duplicate of:
 - Standard
 - Patient samples
 - Water (background)
12. Count on ^{51}Cr setting for 20 minutes per sample.

Record the results as counts per minute.

Include standard in duplicate and background counts on spreadsheet. **Note:** Do not calculate GFR using total counts.

13. Calculate the GFR using the GFR spreadsheet on the PC.

Important point to remember:

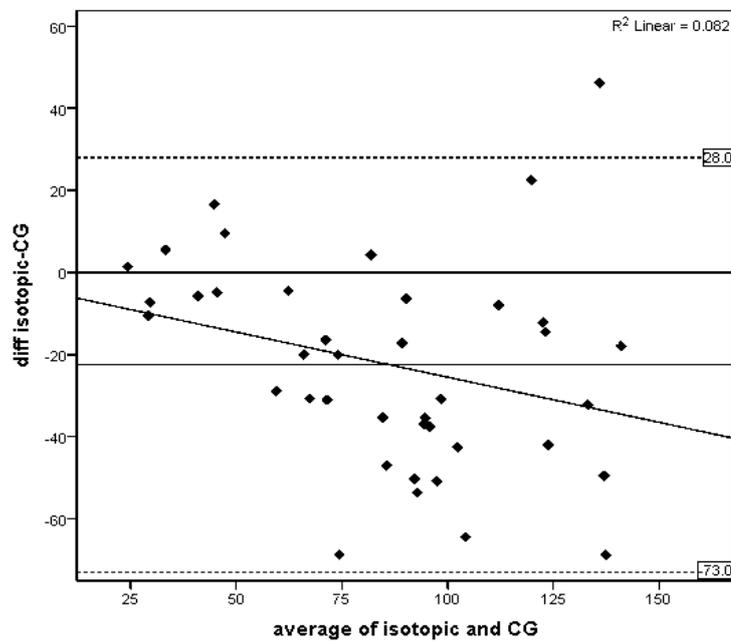
Any extravasation at the time of injection, or failure to correctly record the volume injected, will decrease the accuracy of the test, as will inaccurate sampling times.

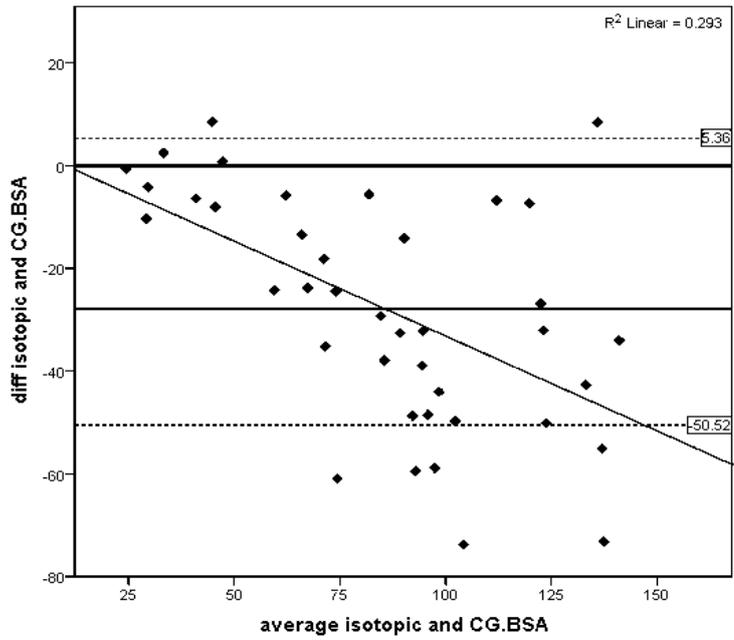
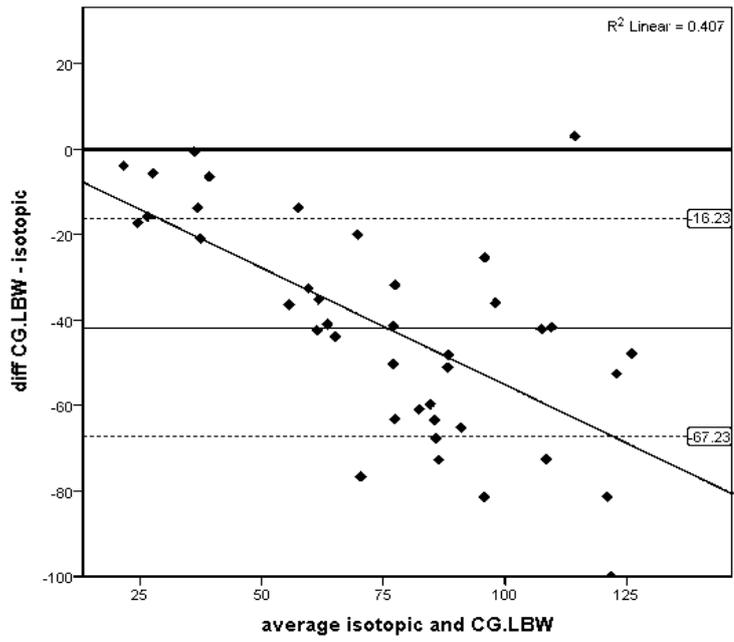
Appendix C:

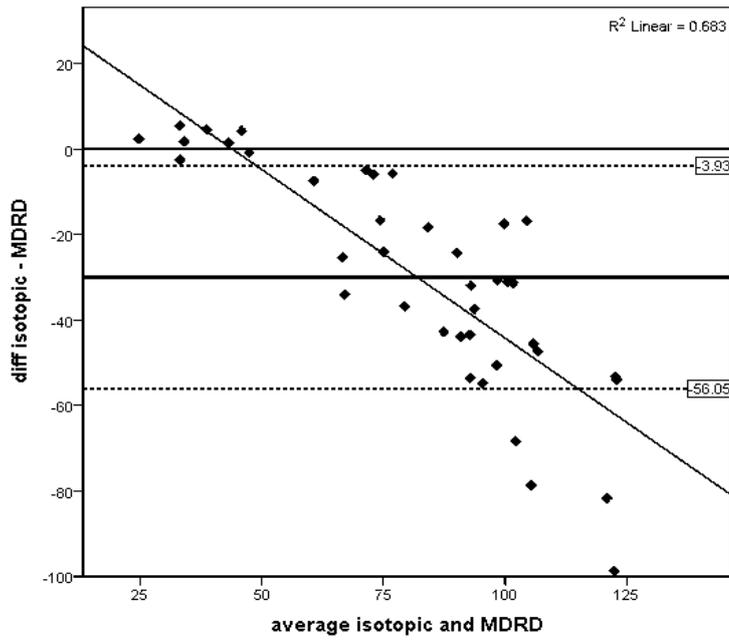
Bland Altman plots

Figure 10: Bland-Altman correlations between measured GFR and estimating equation. (Cockcroft-Gault (CG) ABW, CG BSA, CG LBW, MDRD and CKD-EPI)

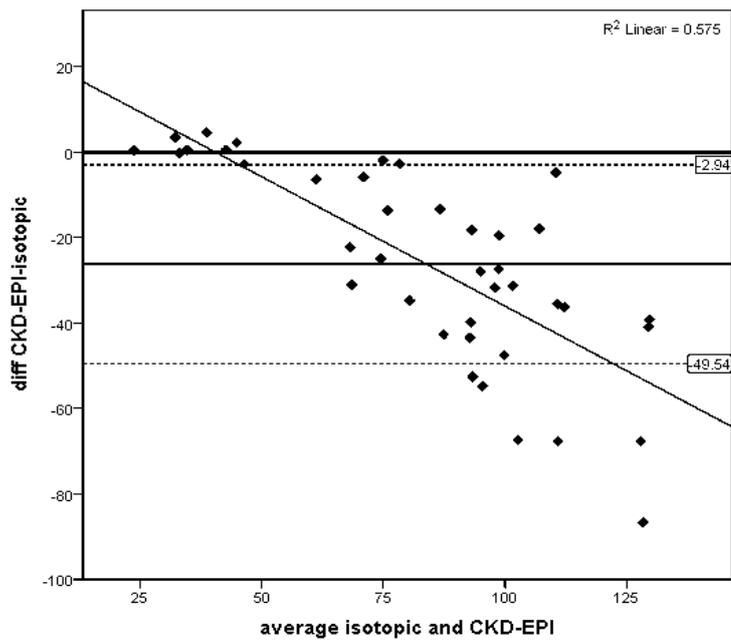
The horizontal axis reflects the average of the isotopic and estimated GFR and the vertical axis the difference between the two methods. The mean and zero line are solid; the dotted line represents the limits of agreement (95% confidence).







1



Appendix D

NONMEM method of clearances:

Population analysis of the renal substrates s-pindolol and fluconazole.

The renal substrates s-pindolol and fluconazole were analysed using a nonlinear mixed effects modelling framework within the NONMEM software (ver 7.2.0). The methodology was the same for both substrates and a generic approach is described here. For both substrates both plasma concentrations and urine amounts and volumes were available. Since the purpose of this analysis was to get estimates of clearance for each individual then quantification of the influence of covariates was not considered in this work. The assay details and limits of quantification are provided in the thesis. No concentrations were censored based on the limit of quantification.

Model building

Model building was conducted in NONMEM using the FOCE option with INTERACTION. One, two and three compartment models were considered and the best model selected based on statistical criteria (see Model Selection). The models were parameterised in terms of clearances (CL, Q1, Q2) and volumes (V1, V2, V3), where CL is clearance, Q1 is the first intercompartmental clearance (etc), V1 is the volume of distribution of the central compartment, V2 the volume of distribution of the 2nd peripheral compartment (etc). The absorption of the substrate from the gut was assumed to follow a first-order process, zero order and lag-time models were also considered and choice based on fit of the model to the data. The residual error was modelled as additive, proportional or a combined error model. The combined error structure was given as

$$y_{i,j} = f(\theta_i, D_i, t_{i,j}; \eta_i) \cdot e^{\varepsilon_{1,i,j}} + \varepsilon_{2,i,j},$$

where θ_i is a $p \times 1$ vector of parameter values for the i^{th} individual, D_i is the dose for the i^{th} subject, $t_{i,j}$ the time of the j^{th} observation for the i^{th} subject. The residual error is shown here for the combined model with a structure:

$$\begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \end{bmatrix} \sim N(0, \Sigma),$$

and Σ a 2×2 vector of residual variances.

Between-subject variances were considered for estimation for all fixed effects parameters (e.g. CL, V1, V2 ...). The model for the between-subject variance was given,

$$\theta_i = \beta e^{\eta_i} \text{ and}$$

$$\eta_i \sim N(0, \Omega).$$

In this work only the diagonal elements of the BSV matrix (Ω) were considered for estimation.

Model selection

Model selection was based on a likelihood ratio test determined by a decrease in the objective function value (OFV) provided by NONMEM. For comparison of nested models, the OFV is approximately, asymptotically χ^2 distributed with degrees of freedom equal to the number of parameters difference between successive models. A difference of 3.84 units for 1 degree of freedom is at the critical value where $P=0.05$. Since covariates were not considered in this analysis then forward selection and backward elimination procedures were not considered. Model selection was also based on stability and plausible parameter estimates. Stability was determined as convergence to the same solution for dispersed initial parameter values. Plausible parameter estimates was based on positive values of clearance and

volume parameters with at least 1 volume parameter value exceeding plasma volume.

Model evaluation

The model was evaluated based on goodness of fit criteria which included residual and weighted residual plots. Since the model was not intended to be used for predictive purposes then more sophisticated model evaluation techniques such as visual or numerical predictive checks were not considered.

The final NONMEM control files for fluconazole and s-pindolol are provided.

fluconazole NONMEM control file

```
$PROB FLUCONAZOLE
$INPUT ID GRP WT AGE SEX CR AMT TIME DVID DVA MDV CMT DV

$DATA ..\Data_fluc_v3.csv IGNORE=#

$SUBR ADVAN6 TOL=6
$MODEL COMP=(GUT) COMP=(PLASMA) COMP=URINE

$PK

; COVARIATE MODEL
TVCL=THETA (1)
TVV=THETA (2)
TVKA=THETA (3)
LAG=THETA (4)
FE=THETA (5)

; MODEL FOR RANDOM BETWEEN SUBJECT VARIABILITY
CL=TVCL*EXP (ETA (1))
V=TVV*EXP (ETA (2))
KA=TVKA*EXP (ETA (3))
ALAG1=LAG*EXP (ETA (4))

; SCALE CONCENTRATIONS
S2=V

; REPARAMATERISATION
K20=CL/V

$DES
DADT (1) =-KA*A (1)
DADT (2) =KA*A (1) -K20*A (2)
DADT (3) =FE*K20*A (2)

$ERROR
FP=A (2) /V
FU=A (3)

IF (DVID.EQ.1) THEN
  Y=FP+EPS (1)
ELSE
  Y=FU+EPS (2)
ENDIF

IPRDP=FP
IPRDU=FU

$THETA
(0,0.7) ; CL
(0,40) ; V
(0,3) ; KA
(0, 0.25) ; LAG
(0, 0.8, 1) ; FE

$OMEGA BLOCK (2)
0.1
0.01 0.1
```

```
$OMEGA  
  0.1  
  0.1
```

```
$SIGMA  
  0.1  
  1
```

```
$EST MAX=9999 SIG=3 PRINT=10 METHOD=COND INTER NOABORT  
$COV
```

```
$TABLE ID TIME CMT DVID IPRDP IPRDU  
ONEHEADER NOPRINT FILE=fluc_1cpt_lag_cov_add_urine.fit
```

```
$TABLE ID TIME WT AGE SEX AMT CL V KA  
ONEHEADER NOAPPEND NOPRINT FILE=FLUC2.fit
```

s-pindolol NONMEM control file

```
$PROB PINDOLOL S
$INPUT ID GRP WT AGE SEX CR AMT TIME DVID DVA MDV CMT DV

$DATA ..\Data_pinds_v3.csv IGNORE=#

$SUBR ADVAN6 TOL=6
$MODEL COMP=(GUT) COMP=(PLASMA) COMP=(PERIP) COMP=URINE

$PK

; COVARIATE MODEL
TVCL=THETA (1)
TVV2=THETA (2)
TVKA=THETA (3)
LAG=THETA (4)
TVV3=THETA (5)
TVQ=THETA (6)
FE=THETA (7)

; MODEL FOR RANDOM BETWEEN SUBJECT VARIABILITY
CL=TVCL*EXP (ETA (1) )
V2=TVV2*EXP (ETA (2) )
KA=TVKA*EXP (ETA (3) )
ALAG1=LAG*EXP (ETA (4) )
V3=TVV3
Q=TVQ

; SCALE CONCENTRATIONS
S2=V2

; REPARAMATERISATION
K20=CL/V2
K23=Q/V2
K32=Q/V3

$DES
DADT (1) =-KA*A (1)
DADT (2) =KA*A (1) -K20*A (2) -K23*A (2) +K32*A (3)
DADT (3) =
K23*A (2) -K32*A (3)
DADT (4) =FE*K20*A (2)

$ERROR
FP=A (2) /V2
FU=A (4)

IF (DVID.EQ.1) THEN
Y=F*EXP (EPS (1) ) +EPS (2)
ELSE
Y=FU+EPS (3)
ENDIF

IPRDP=FP
IPRDU=FU

$THETA
(0,30) ; CL
(0,170) ; V
(0,1.5) ; KA
(0, 0.25) ; LAG
```

```
(0, 10) ; V3  
(0, 5) ; Q  
(0, 0.2, 1) ; FE
```

```
$OMEGA BLOCK(2)
```

```
0.1  
0.01 0.1
```

```
$OMEGA
```

```
0.1  
0.1
```

```
$SIGMA
```

```
0.1  
0.1  
0.1
```

```
$EST MAX=9999 SIG=4 PRINT=10 METHOD=COND INTER NOABORT
```

```
$COV
```

```
$TABLE ID TIME CMT DVID IPRDP IPRDU
```

```
ONEHEADER NOPRINT FILE=pinds_2cpt_cov_lag_urine.fit
```

```
$TABLE ID TIME WT AGE SEX AMT CL V2 KA
```

```
ONEHEADER NOAPPEND NOPRINT FILE=PINDS2.fit
```