The organs of the human body were created to perform ten functions among which is the function of the kidney to furnish the human being with thought.

Leviticus Rabba 3 Talmud Berochoth 61b
Objectives

• How do you interpret the tests of kidney function? What is the importance of eGFR?

• Assessment of Chronic Kidney Disease.

• Lithium and Kidney function. Pathophysiological changes linked to clinical importance.
Kidneys working!
How do we measure changes in kidney function?
Stages of CKD – a clinical continuum

- **Stage 1**: Kidney damage or mild reduction GFR
  - Proteinuria
  - GFR >90 mL/min

- **Stage 2**: GFR 60-90 mL/min

- **Stage 3**: GFR 30-60 mL/min

- **Stage 4**: GFR <30 mL/min

- **Stage 5**: RRT = Dialysis or transplant

- **S CR**

GFR
Chronic Kidney Disease (CKD)

Increased risk – 1:3

Australia or NZ

Healthy Adult Community

Data from AusDiab

CKD – 1:7

Dialysis or Transplant 1:1400
How much CKD in Australia?
16% of Adults >25yrs have protein or blood in urine or moderately severe reduction in kidney function*

*MDRD based eGFR
eGFR

• Limitations:
  Initially derived from MDRD study.
  Accuracy around stage 3a CKD
eGFR40 -59 ml/min/1.73m²

• Inaccurate above 60 ml/min/1.73m²

• Improved with the CKD-EPI formula
  addition of proteinuria sub groups

• Underestimates GFR compared to Cockcroft & Gault (tends to over estimate GFR).
Referral Information

• Renal function for past 5 years (where possible)
• Urine analysis
  - albumin / creatinine ratio*
  – protein/ creatinine ratio (>23g/mol)
• Blood pressure control & medications

• * differences to what is measured.
eGFR

• A Major determinant is AGE
• Lab variation in plasma creatinine +/- 15umol/l
• No estimation related to weight (standardised to BSA 1.73m\(^2\)) (muscle bulk) – source of creatinine correction factor for African-Americans but not for Māori / Pacific People.
Renal Function: Age-related changes.

Elseviers et al, Lancet 1987; 1 : 457
Impact of Age

• Male with a plasma creatinine of 100 umol/l
• Age 65 – eGFR = 68 ml/min/1.73m²
• Age 75 – eGFR = 63 ml/min/1.73m²

• Less variation with greater degree of renal impairment.
• What is senescence versus CKD?
65 year old male: On long term Lithium therapy
Please review deteriorating kidney function.
Do you stop his Lithium?
CKD in the setting of Lithium Therapy.

- 65 year male – 20 yr history of a bipolar disorder
  Lithium therapy for 15 years (drug monitoring 0.4 – 0.8 mmol/l)
- Polydipsia and polyuria – 4.5l/day
- Overnight fluid deprivation – urine osmolality 468 mosom/kg
  dDAVP – max urine osmolality 528 mosom/kg
- Do you stop his lithium? Will discuss this in the next section.
No change in eGFR over 3 years.
Variation around a mean.
Calculated creatinine clearances 22 – 26 ml/min.
Interpreting eGFR

• Extra creatinine values last few years very useful.
• Important to add other variables proteinuria and blood pressure control.
• Why do we get these fluctuations in eGFR?
Progressive Nephropathy

• Overall in CKD
  • Lose the protective vasodilation or vasoconstriction
  • Kidney senses these highs and lows in perfusion pressure
  • Creatinine does rise and fall as you correct this
  • (a linear relationship almost)
  • Pre-glomerular pressure response lost and post-glomerular compensation lost
Renal blood flow not protected vascular bed.

Hypertension 160/96
eGFR 46 ml/in

110/70 Hypotension for patient eGFR 30ml/min
Arteries stiffen

• Long standing arterial pulsation:
• Medial degeneration
• Direct effects on matrix proteins, collagen and elastin
  Elastin fibres break – reduplication
  Detachment smooth muscle fibres
  Increased VSMC
• Calcification
  Oxidative stress AGE add to stiffness
Arteriosclerosis and Pulse Wave Velocity

Systole

Diastole
Pulse Wave Forms

Shape – force felt by artery.
Older stiff vessel – reflected wave early
↑SBP ↓DBP
Increased pressure –
Heart brain kidney

SE Greenwald J Pathol
2007
Impact of vascular stiffness

• Heart
  - impaired diastolic perfusion
  - diastolic dysfunction
  - additional impact of coronary calcification

• Brain
  - increased risk stroke

• Kidneys
  - arteriolar sclerosis, glomerulosclerosis
Arteriolosclerosis

Interstitial fibrosis, glomerulosclerosis and tubular atrophy

Compensatory glomerular hypertrophy
Kidneys reflect vascular injury

• In patients with CKD Stage 3
  Hypertensive nephrosclerosis is a major aetiology.
• Impact of proteinuria – marker of endothelial injury
• Blood pressure control – main focus of therapy.
Impact of vascular stiffness

- Heart
  - impaired diastolic perfusion
  - diastolic dysfunction
  - additional impact of coronary calcification
### Prevalence of LVH in kidney failure

<table>
<thead>
<tr>
<th>Renal Insufficiency</th>
<th>CCr ml/min</th>
<th>Prevalence LVH</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Population</td>
<td></td>
<td>17%</td>
</tr>
<tr>
<td>Mild</td>
<td>75-50</td>
<td>27%</td>
</tr>
<tr>
<td>Moderate</td>
<td>50-25</td>
<td>31%</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;25</td>
<td>45%</td>
</tr>
<tr>
<td>Start Dialysis</td>
<td></td>
<td>75%</td>
</tr>
</tbody>
</table>

*Levin et al, AJKD 1999;34:125-134*
LV disease predicts survival on dialysis

![Graph showing survival probability over months for different LV conditions](image-url)

Parfrey and Foley, 1999
Intramyocardial fibrosis and calcification
Figure 1 | Joint contribution of CKD and hypertension to cardiac risk. CKD, chronic kidney disease; GFR, glomerular filtration rate.
“bubbles appearing on the surface of the urine indicate renal disease with a prolonged course”
Assessment of Proteinuria

- Proteinuria influenced by
  - Exercise
  - Obesity
  - Hypertension
- Early morning urine – before physical activity
- Orthostatic benign proteinuria.
Fig 1 Time to clinical outcomes by proteinuria and kidney dysfunction

From the CARE Study: Tonelli, M. et al. BMJ 2006;332:1426
Fig 2  Adjusted risk of all cause mortality according to proteinuria and kidney dysfunction

From the CARE Study: Tonelli, M. et al. BMJ 2006;332:1426
CKD predicts rate of all cause death (age standardized/100 person years) – HMO population

Median Follow-up = 2.8 yrs

Take Home Points

• eGFR is an estimate, not an absolute value.
• Hypertension is a major component of Kidney disease
• Contributes to progression of CKD.
• Most patients with CKD die of CVD
• Any referral for CKD must have eGFR, Blood Pressure and urinalysis.
• Proteinuria and hypertension are major prognostic factors – require more aggressive treatment.
• Proteinuria conveys a greater risk for CVD.
• Frequently require 3 drug therapy – drug type.
“Look deep into nature and you will understand everything better” Albert Einstein
CKD in the setting of Lithium Therapy.

• 65 year male – 20 yr history of a bipolar disorder
  Lithium therapy for 15 years (drug monitoring 0.4 – 0.8 mmol/l)
• Polydipsia and polyuria – 4.5l/day
• Overnight fluid deprivation – urine osmolality 468 mosom/kg
dDAVP – max urine osmolality 528 mosom/kg
• Do you stop his lithium? Will discuss this in the next section.
Lithium and the Kidney

- Effective drug in management of bipolar – unipolar disorders.
- Major side effect – renal manifestations
- 60% have evidence of impaired renal concentrating ability
- 20 – 30% significant polyuria & polydipsia.
- Short term reversible
- Long term irreversible functional lesion associated with an interstitial fibrosis
Lithium Transport

• Lithium is excreted by the kidneys
• Lithium can substitute for sodium on several transport proteins providing entry into cells. Several pathways: Na/H exchange (NHE3) and sodium co-transport (Proximal tubule) ENaC – cortical collecting tubule
• Pathways for lithium out of cells are more limited: Na/H exchange 1?
Action of Lithium in the Kidney

- Lithium enters CCD via ENaC
- Inhibition AVP stimulated phosphorylation of AQP2
- Limited trafficking AQP2 to apical membrane
- Impaired water reabsorption
- Clinically polyuria.

AQP2 in human kidney

Urinary concentrating ability in individuals with mood disorders.

- Is it truly a drug effect of lithium?
- Role of psychogenic polydipsia?
- Role of washout medullary gradient by polyuria alone?
- Drug-induced polydipsia? (serotonergic vs non-serotonergic drugs)
Demographics: cross-sectional study of dDAVP-stimulated urinary concentrating ability. Impact of psychotropic agents

<table>
<thead>
<tr>
<th></th>
<th>Lithium</th>
<th>Lithium naive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female: male</td>
<td>36: 19</td>
<td>31: 6</td>
</tr>
<tr>
<td>Age - mean (range)</td>
<td>48 (20 – 68)</td>
<td>33 (16 – 66)</td>
</tr>
<tr>
<td>Duration - lithium therapy</td>
<td>22 (11 -28 years)</td>
<td></td>
</tr>
<tr>
<td>Alternative + supplementary Drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonergic agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI’s</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Tricyclics</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>No serotonergic effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOIs</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Anti-epileptics</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Anti-psychotics</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>
Baseline data after overnight water deprivation in the lithium naïve and lithium treated group

<table>
<thead>
<tr>
<th></th>
<th>Lithium naïve</th>
<th>Lithium Uosm &gt; 750mosm/kg</th>
<th>Lithium Uosm 750-300mosm/kg</th>
<th>Lithium Uosm &lt; 300 mosm/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33 (15-66)</td>
<td>43 (20 - 68)</td>
<td>51 (30 – 68)</td>
<td>43, 54</td>
</tr>
<tr>
<td>Duration lithium therapy</td>
<td></td>
<td>13.5 ± 2.4 years</td>
<td>20.8 ± 2.7 years</td>
<td>25, 28 years</td>
</tr>
<tr>
<td>Baseline plasma Osm.</td>
<td>290 ± 1</td>
<td>292 ± 1</td>
<td>296 ± 2</td>
<td>298, 314</td>
</tr>
<tr>
<td>Baseline plasma [Li]</td>
<td></td>
<td>0.73 ± 0.06 mmol/l</td>
<td>0.83 ± 0.05</td>
<td>0.71, 1.06</td>
</tr>
<tr>
<td>Baseline plasma [Cr]</td>
<td>82 ± 3 umol/l</td>
<td>83 ± 3</td>
<td>95 ± 5</td>
<td>135, 116</td>
</tr>
<tr>
<td>Baseline plasma [AVP]</td>
<td>4.3 ± 0.6 pmol/l</td>
<td>6.5 ± 0.8</td>
<td>6.3 ± 0.9</td>
<td>14.7, 18.2</td>
</tr>
</tbody>
</table>
Urinary osmolality, and AQP2 excretion grouped according to the length of exposure to lithium, compared to Lithium-naïve individuals,

The reduction of impaired urinary concentrating ability, and decreased urinary AQP2 excretion correlated with the duration of lithium therapy ($r = 0.63$ for AQP2)

Bedford et al. CJASN 2008
Actions of amiloride on urinary concentrating ability and urinary aquaporin 2 (AQP2) excretion in human volunteers on lithium therapy

• Early treatments to ameliorate lithium-induced polyuria utilized the ENaC channel blocker, amiloride (Battle et al. NEJM 1985)

• What impact does this have on urinary AQP2?

• No RCTs of amiloride on lithium-induced NDI.
## Amiloride and Lithium therapy
### A randomised placebo controlled trial

- Double blind placebo controlled trial. Cross-over design.
- 6 week run-in period on their normal lithium medication,
- Amiloride or placebo (5mg 1\textsuperscript{st} week and increased to 10mg 2\textsuperscript{nd} week)
- 6 weeks of a placebo or amiloride treatment, 6 weeks washout and then a final 6 weeks of placebo or amiloride.
- Measurements as for previous clinical studies – standard overnight dDAVP stimulation test at start and end of each treatment arm.

<table>
<thead>
<tr>
<th>Female : Male</th>
<th>Age (years)</th>
<th>Duration of lithium therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 : 2</td>
<td>58 (37 – 71)</td>
<td>20 (8 – 34)</td>
</tr>
</tbody>
</table>
Amiloride caused a significant increase in maximal urinary osmotic pressure and a significant but lesser increase in AQP2 excretion.

Bedford et al. CJASN 2008
Osmolytes in the renal medulla of rat with lithium-induced NDI given drinking water containing 0.2 mmol-1 amiloride.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>60 mmol Lithium</th>
<th>lithium+ amiloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inositol (mmol kg-1 protein)</td>
<td>221 ± 35</td>
<td>85 ± 10**</td>
<td>179 ± 8§ §</td>
</tr>
<tr>
<td>Sorbitol (mmol kg-1 protein)</td>
<td>35 ± 9</td>
<td>3 ± 1**</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>GPC (mmol kg-1 protein)</td>
<td>352 ± 80</td>
<td>91 ± 20**</td>
<td>231 ± 38§ §</td>
</tr>
<tr>
<td>Betaine (mmol kg-1 protein)</td>
<td>69 ± 11</td>
<td></td>
<td>84 ± 10</td>
</tr>
<tr>
<td>Urea (mmol kg-1 protein)</td>
<td>2868 ± 624</td>
<td>480 ± 117**</td>
<td>2132 ± 184§ §</td>
</tr>
<tr>
<td>Osmol (mosm/kg)</td>
<td>1211 ± 90</td>
<td>287 ± 19**</td>
<td>1132 ± 154</td>
</tr>
</tbody>
</table>

mean ± SEM, **p<0.01, *p<0.05 compared to control values; § § p<0.01, § p<0.05 compared to lithium only values.
AQP2 Expression Renal Inner Medulla

Control
Lithium 7 weeks
Lithium 7 weeks + Amiloride 3 weeks

37kDa
29kDa

AQP2 western blot

Control Lithium Lithium + amiloride

Bedford et al. AJP Renal 2008
Mechanisms of chronic lithium injury
Lithium nephrotoxicity

• 65 male – 20 yr history of a bipolar disorder
  Lithium therapy for 15 years (drug monitoring 0.4 – 0.8 mmol/l)
• Polydipsia and polyuria – 4.5l/day
• Plasma creatinine 233 umol/l
• Renal biopsy – diffuse interstitial fibrosis.
Lithium-induced ‘microcysts’ in renal cortex
Lithium-induced interstitial fibrosis after 15 years therapy
Lithium nephrotoxicity

• Presne and colleagues (kidney Int 2003)
• 74 patients with lithium-induced nephropathy, 12 of whom reached ESRD.
• The degree of interstitial fibrosis on renal biopsy was related to duration of therapy and cumulative dose of lithium.
• if estimated creatinine clearance was $\leq 40$ ml/min, stopping lithium did not alter the progression to ESRD
• Is there a point of no return where fibrosis continues to progress despite suppression of the triggering toxic event?
Lithium nephrotoxicity

• Presne and colleagues (kidney Int 2003)
• 74 patients with lithium-induced nephropathy, 12 of whom reached ESRD.
• The degree of interstitial fibrosis on renal biopsy was related to duration of therapy and cumulative dose of lithium.
• if estimated creatinine clearance was ≤ 40 ml/min, stopping lithium did not alter the progression to ESRD
• Is there a point of no return where fibrosis continues to progress despite suppression of the triggering toxic event?
• Do you stop his Lithium therapy?  NO.
Does Amiloride modify chronic lithium-induced fibrosis

• Rats – Lithium-induced NDI after 4 weeks
• Amiloride 0.2mmol in drinking water + lithium for a further 5 months compared to control & lithium alone.
• Tissue for immunohistochemistry, western blotting and RT-PCR
Results - Staining for fibrosis - Masson’s trichrome

Control  Lithium  Lithium + amiloride

Scale bar = 100 µm

% cortical area stained
CTGF expression
Summary
Amiloride down-regulates fibrosis but not morphological changes.
Lithium induces progressive morphological changes along with interstitial fibrosis. Not a classical macrophage mediated fibrosis
Speculative mechanisms
Alterations adenylyl cyclase – intracellular phosphorylation
Inhibition of GSK3 β and upregulation of β catenin and the Wnt canonical pathway
Acknowledgements:

Funding – HRC (NZ) NKF(NZ) Otago Medical Research Foundation, University of Otago Research Grant,

Collaborators - A/Prof John Leader & Dr Jenny Bedford
PhD students: Rena Jeng, Priya Kalita, Leunie van der Tholen

www.otago.ac.nz/kidney
What is man, when you come to think upon him, but a minutely set, ingenious machine for turning, with infinite artfulness, the red wine of pinot noir into urine?

Apologies to Karen Blixen C19th writer.