Laboratory Evaluation of Kidney Function

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- This speaker has nothing to disclose.

Objectives

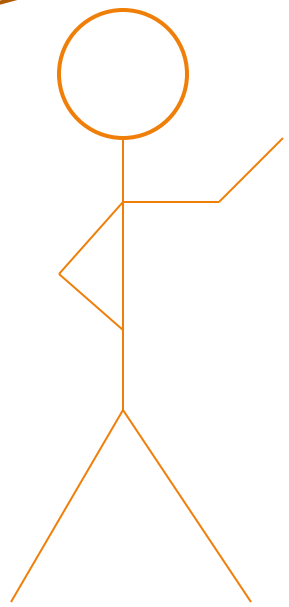
- At the end of this presentation, participants should be able to:
  - Diagram the functional unit of the kidney.
  - Compare and contrast the markers used to evaluate glomerular filtration rate.
  - List criteria for staging chronic kidney disease.

Outline

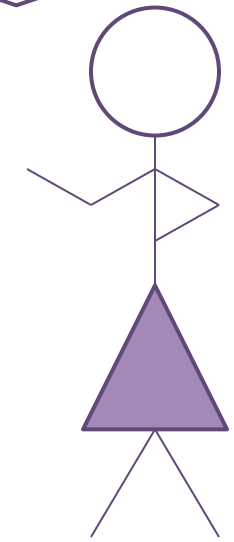
- Review of the kidneys
  - Anatomy
  - Physiology
  - Pathophysiology
- Assessment of kidney function in chronic disease
  - Laboratory tests
  - Clinical practice guidelines

Case Study

My doctor just told me I have stage 2 kidney disease.



My doctor just told me I have stage 3 kidney disease!

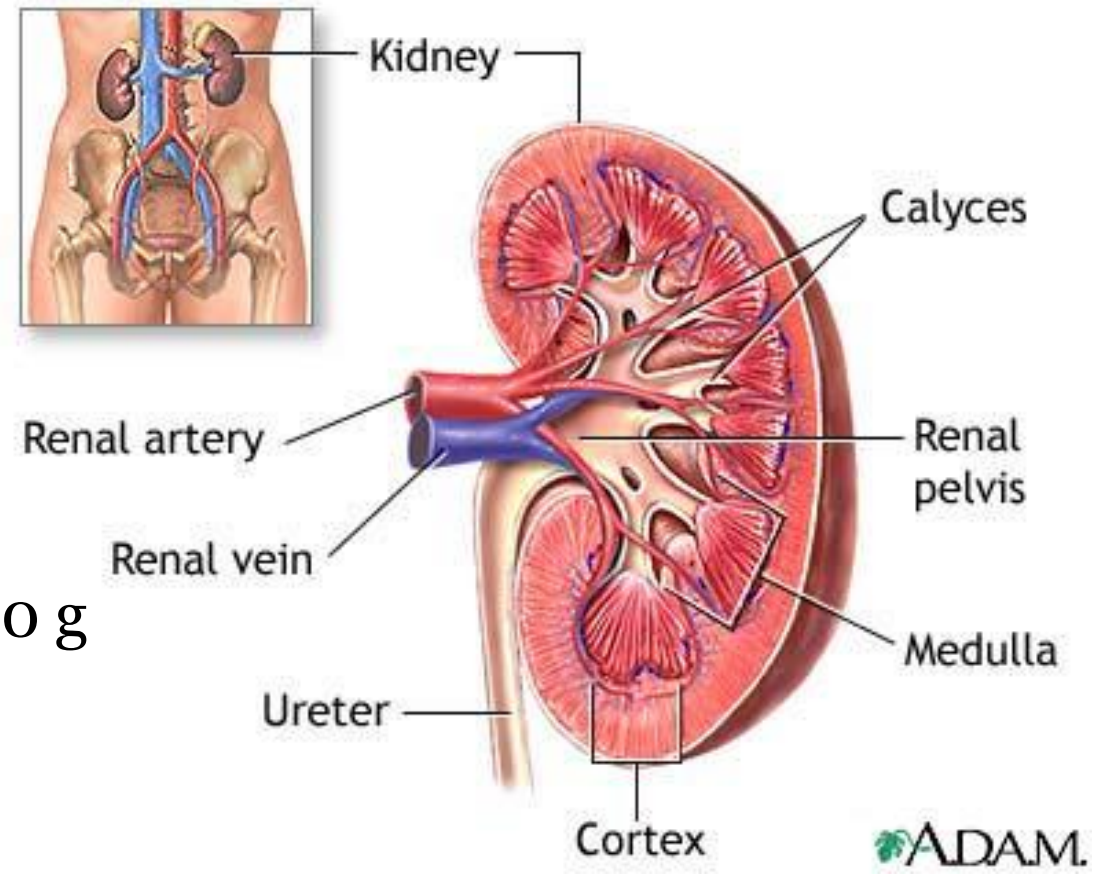


Anatomy and Physiology

Overview of Kidneys

- Functions:
  - Regulation of homeostasis
    - Electrolytes
    - Water
    - Acid-base balance
  - Remove waste and toxins from the body
    - Filtration and excretion
  - Synthesize hormones

Anatomy

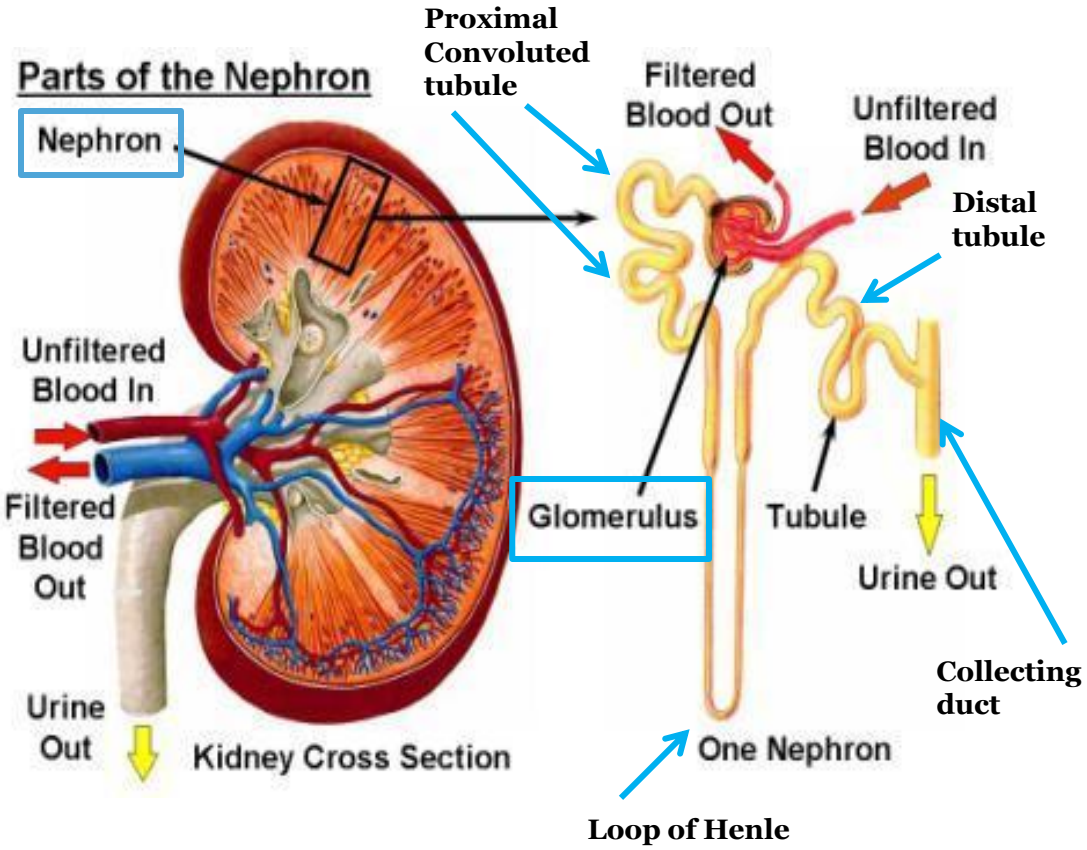


- Size: ~12 cm long and ~150 g
- Receive 25% of cardiac output
  - Cardiac output ~3 L/min
  - Kidneys ~0.75 L/min
- Filters 180 L per day
- Produces urine
  - 0.4-2 L per day

http://www.umm.edu/patiented/articles/what_kidney_stones_000081_1.htm

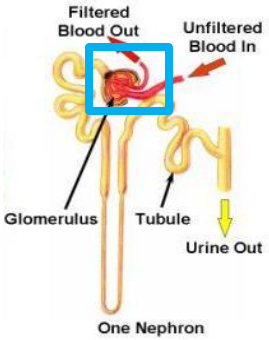
Nephron

- Functional unit of the kidney
  - 0.6 – 1.5 million per kidney
- Composed of:
  - Glomerulus
  - Proximal tubule
  - Loop of Henle
  - Distal tubule
  - Collecting duct

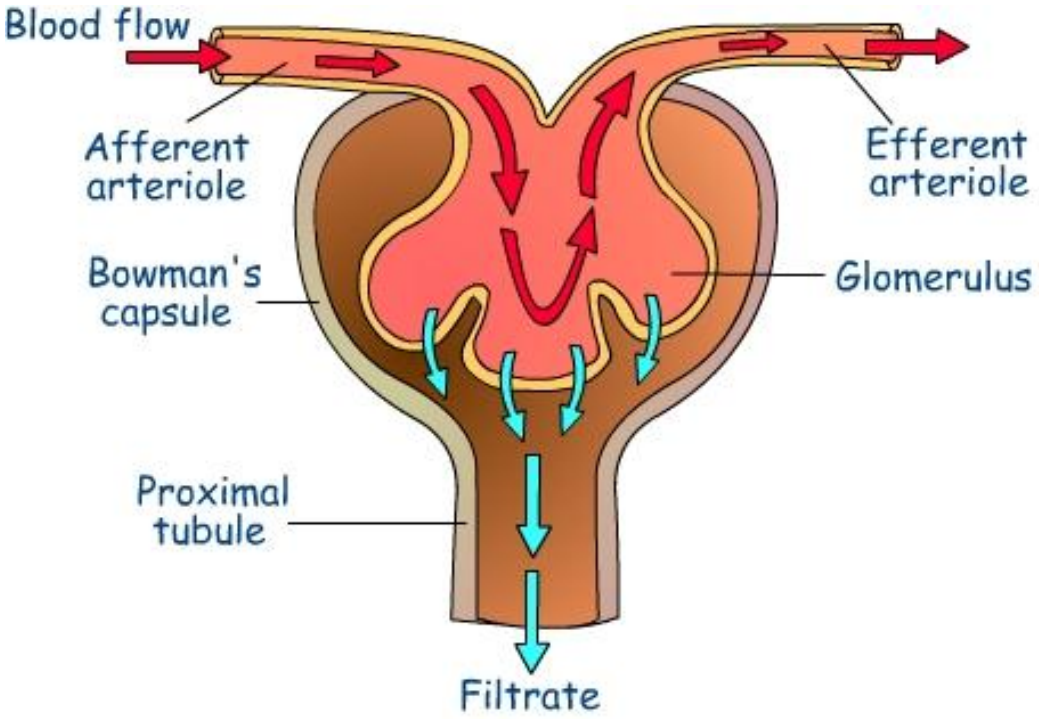


http://www.unckidneycenter.org/kidneyhealthlibrary/glomerulardisease.html

Glomerulus



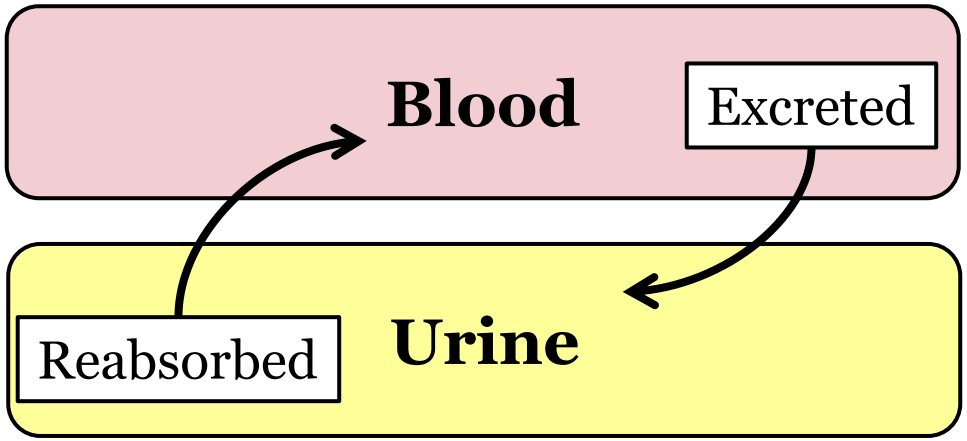
- Function: to filter plasma to form an ultrafiltrate
- Size exclusion
- Charge exclusion



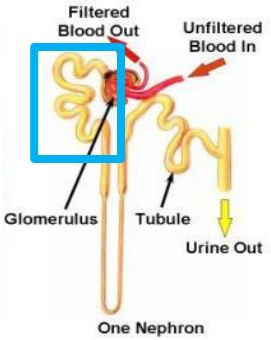
http://robbwolf.com/2011/06/16/clearing-up-kidney-confusion-part-deux/

Ultrafiltrate: Reabsorption and Excretion

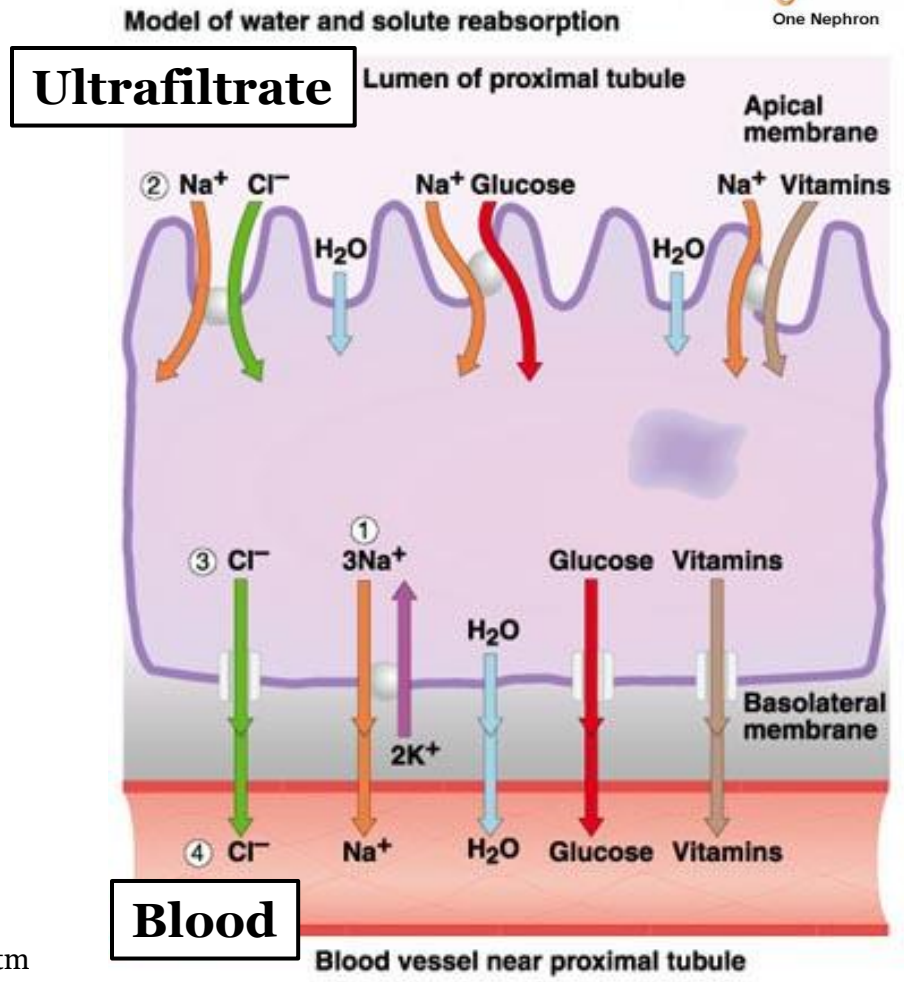
- Ions/electrolytes
  - Na^+, K^+, Cl^- , Ca^{2+}, PO_4^{3-}, Mg^{2+}, SO_4^{2-}, HCO_3^-, H^+
- Water
- Small molecules
  - Glucose
- Waste products
  - Creatinine, urea



Proximal Convoluted Tubule



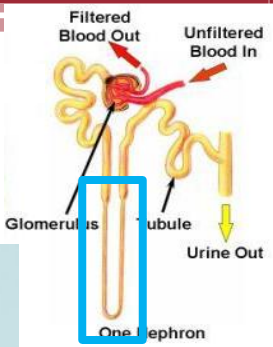
- The most metabolically active part of the nephron
  - 60-80% of reabsorption
- Driving force is active transport of Na^+
  - Water follows Na^+
- Filtrate volume decreases

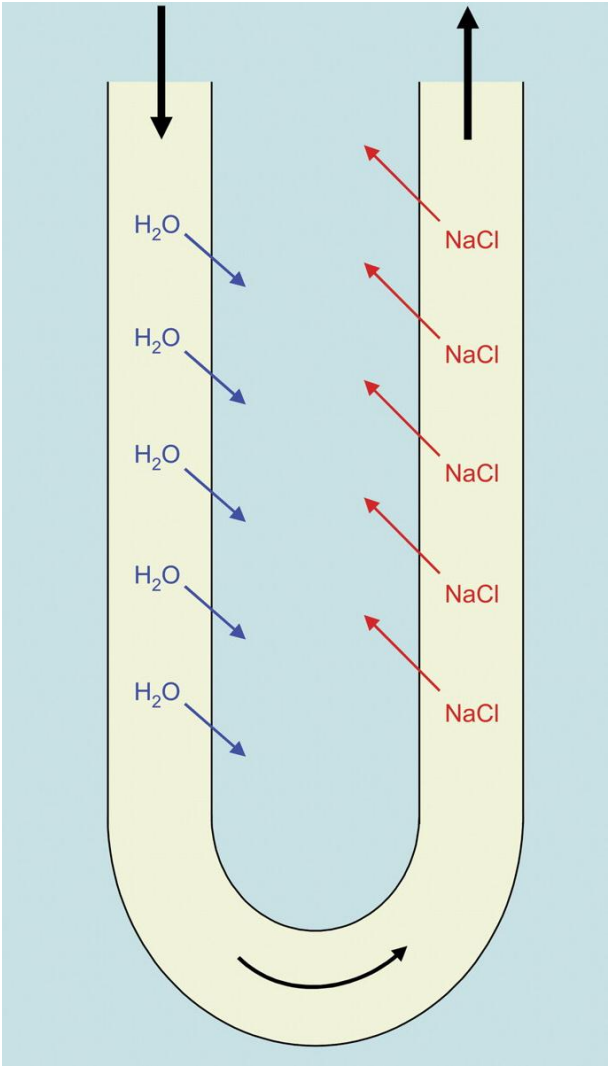


http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect21.htm

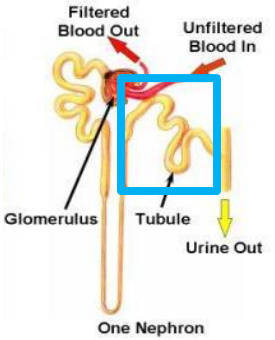
Loop of Henle

- Descending limb
  - Permeable to water
  - Impermeable to solutes (Na^+, Cl^-)
- Ascending limb
  - Impermeable to water
  - Permeable to solutes (Na^+, Cl^-)



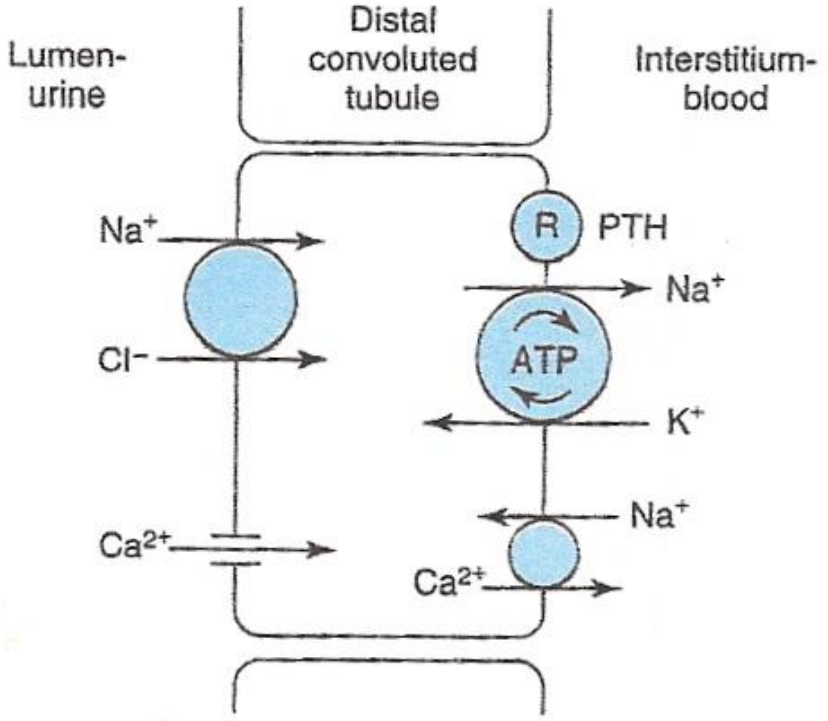


Physiol Rev 2007;87:1083-1112



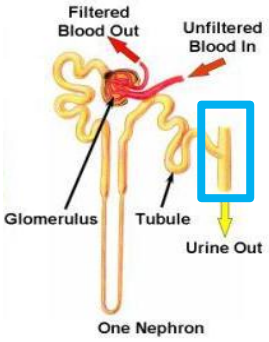
Distal Convoluted Tubule

- Reabsorption of Na^+
  - Active transport
  - Cl^- follows Na^+
  - Water
- Reabsorption of Ca^{2+}
- Excretion of K^+

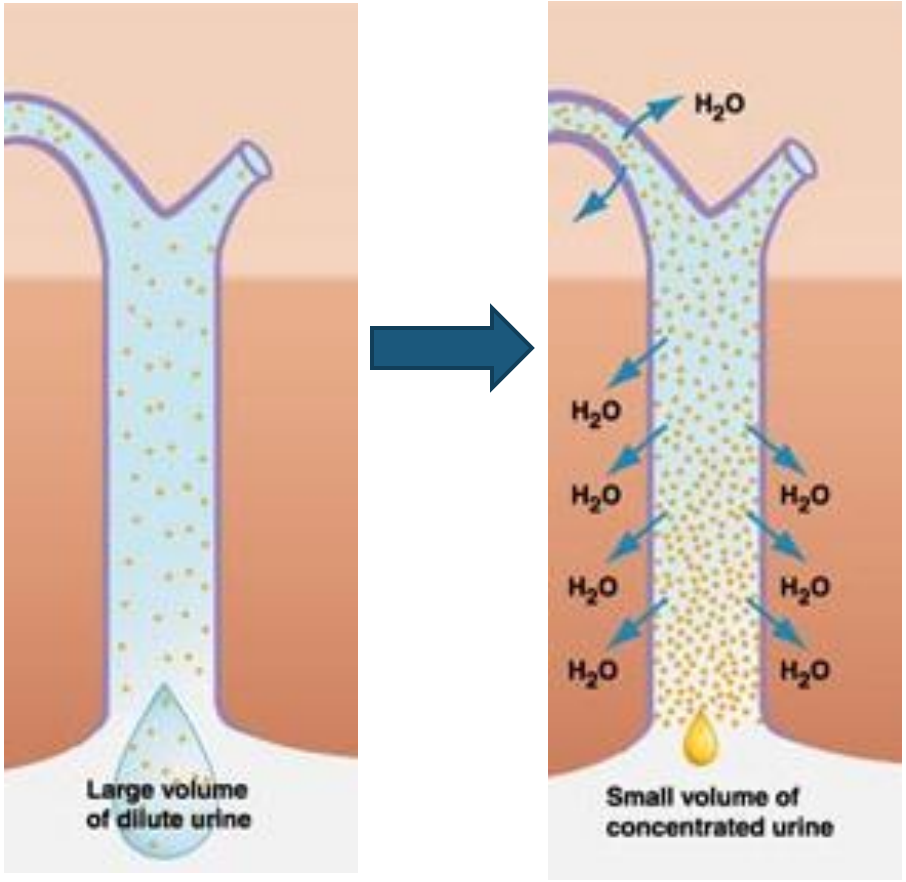


http://www.uky.edu/~mtp/Diuretic_Drugs.htm

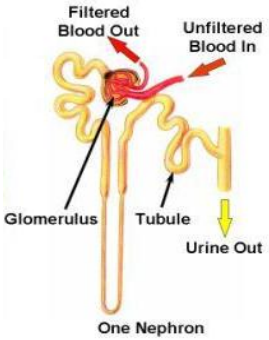
Collecting Duct



- Determines final concentration of urine
- Normally impermeable to water reabsorption
  - Responds to external signals



http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect21.htm



Final Product: Urine

- Healthy urine:
  - 0.4-2 L/day
  - Clear, amber colored
  - pH 5.0-6.0
  - Osmolality: 50-1400 mOsms/kg
  - Protein: 50-80 mg/day
    - Albumin: <30 mg/day
  - Glucose: <0.5 g/day

Pathophysiology

Chronic Kidney Disease (CKD)

- A progressive decline in kidney function
  - Decreased filtration
  - Progresses to end stage renal disease
    - Dialysis or kidney transplant

Definition: a decreased glomerular filtration rate or signs of kidney damage that persist >3 months

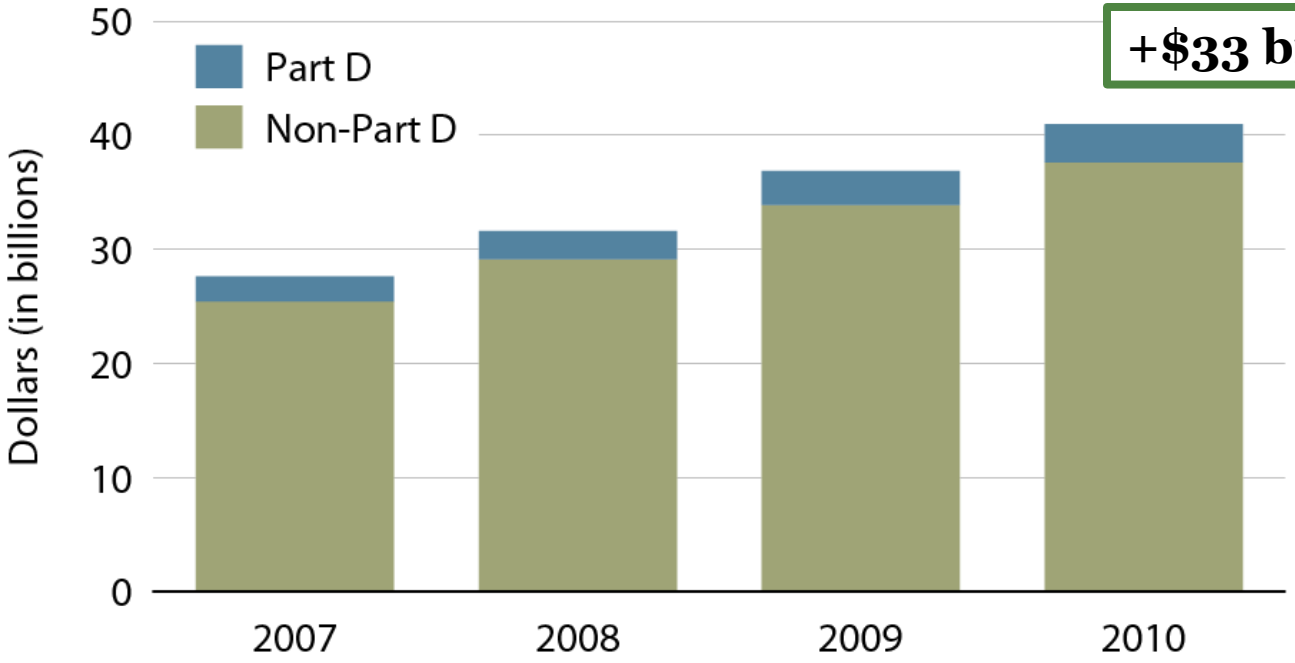
- Occurs over many years
  - Often asymptomatic

Chronic Kidney Disease (CKD)

- >26 million Americans have CKD

- Risk factors include:
  - Diabetes
  - Hypertension
  - Family history of kidney disease

Overall Medicare Part D & non-Part D costs in patients with CKD, by year

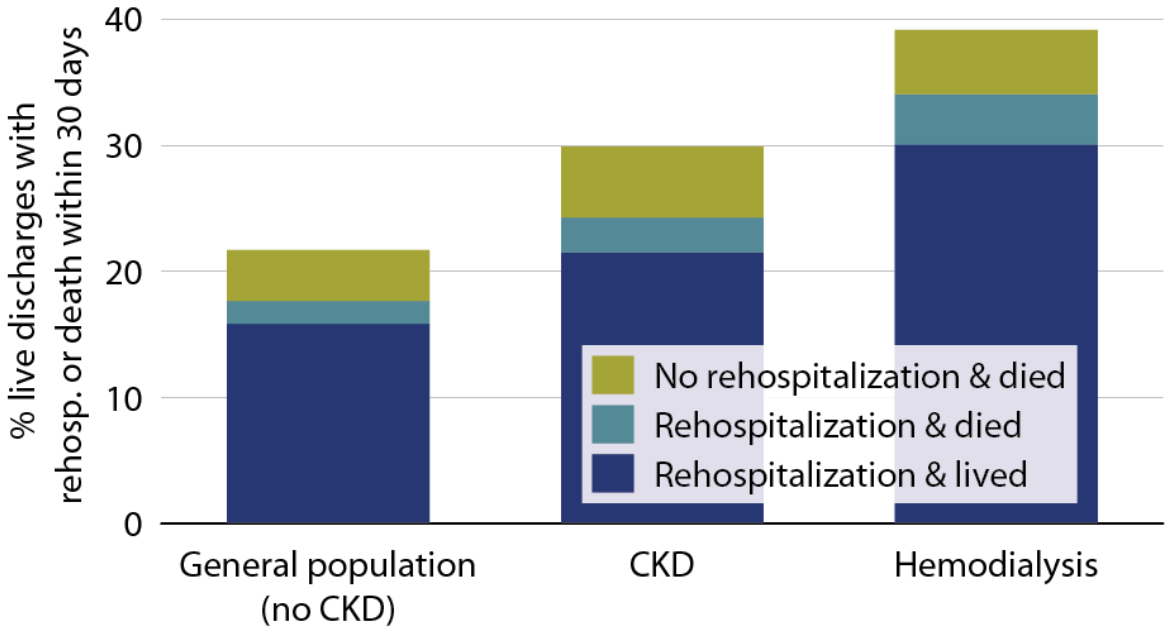


+$33 billion for ESRD

Point prevalent Medicare CKD patients age 65 & older.

USRDS 2012 ADR

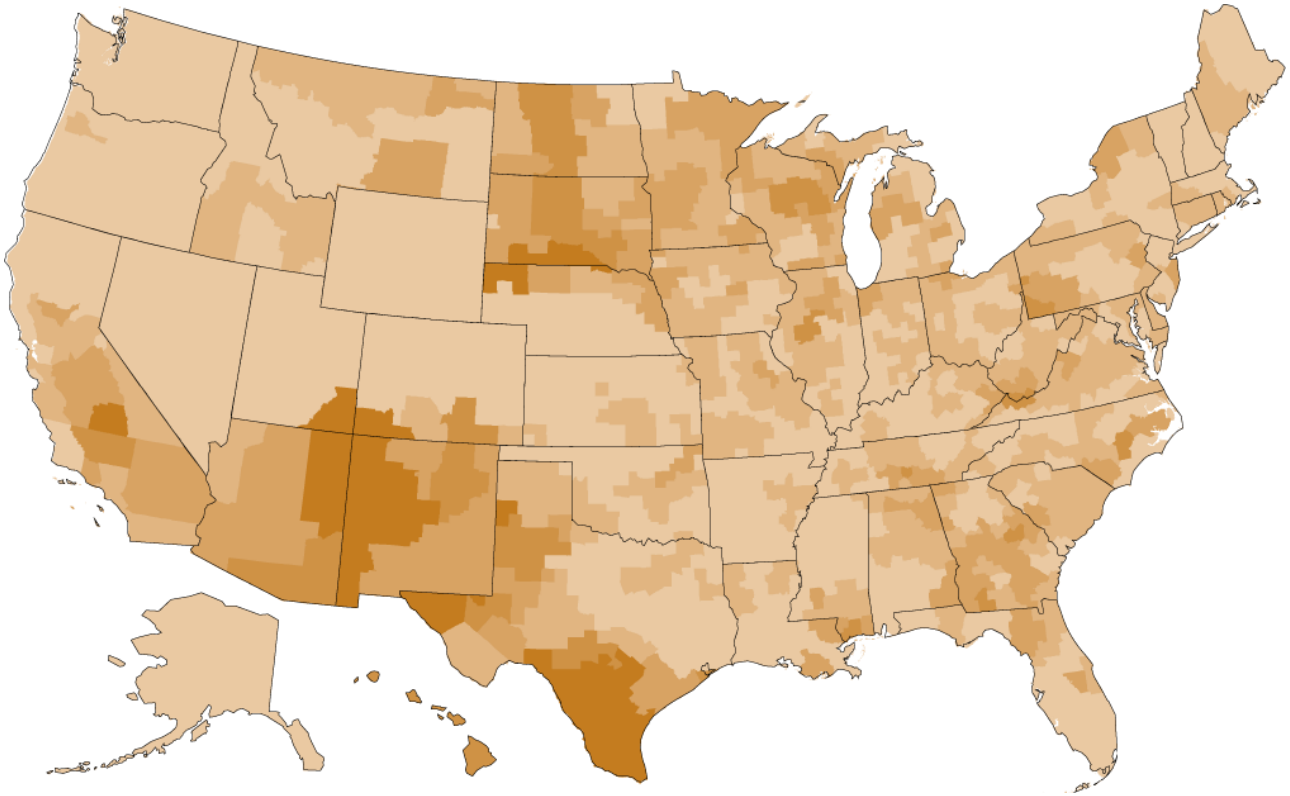
All-cause rehospitalization or death within 30 days after live hospital discharge, 2010



January 1, 2010 point prevalent Medicare patients, age 66 & older on December 31, 2009, unadjusted. Includes live hospital discharges from January 1 to December 1, 2010.

USRDS 2012 ADR

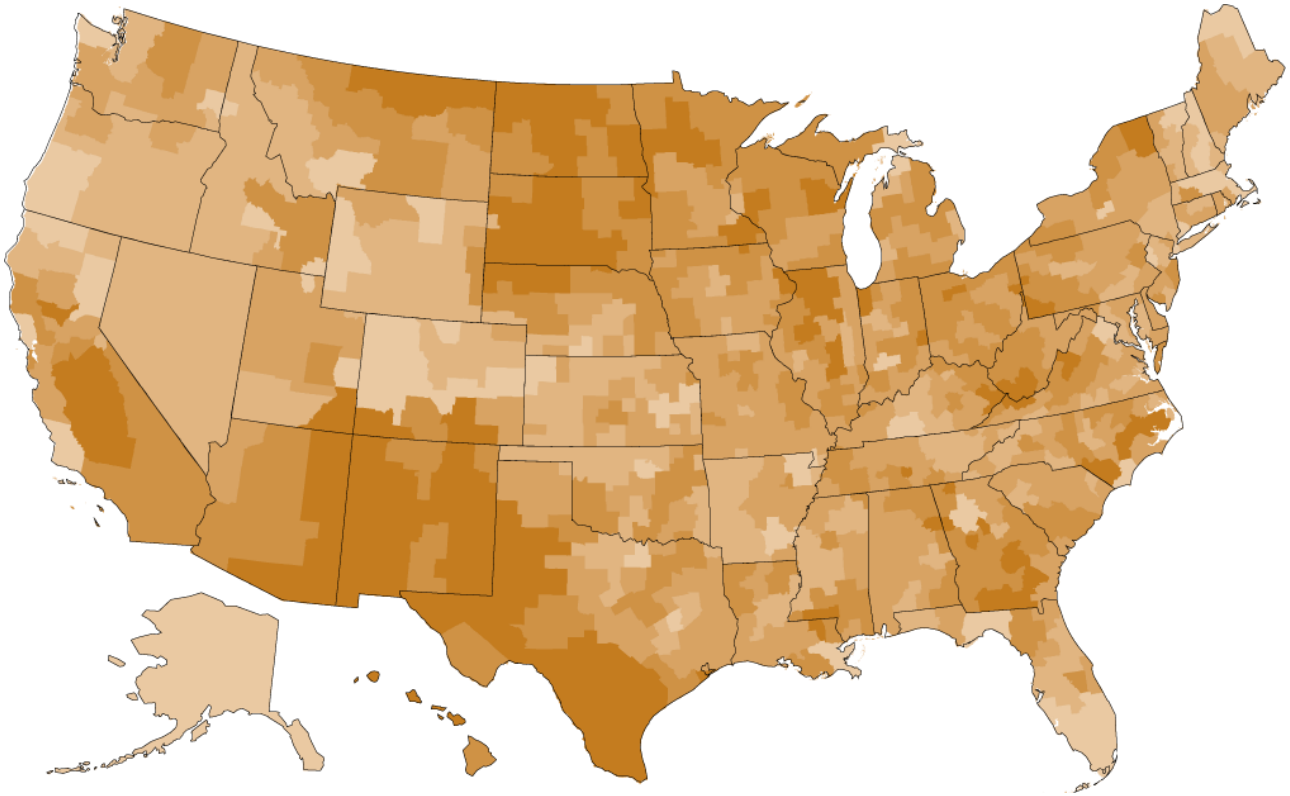
Geographic variations in adjusted prevalent rates (per million population), 1997



December 31 point prevalent ESRD patients. By HSA; rates adjusted for age, gender, & race. Excludes patients residing in Puerto Rico & the Territories.

USRDS 2009

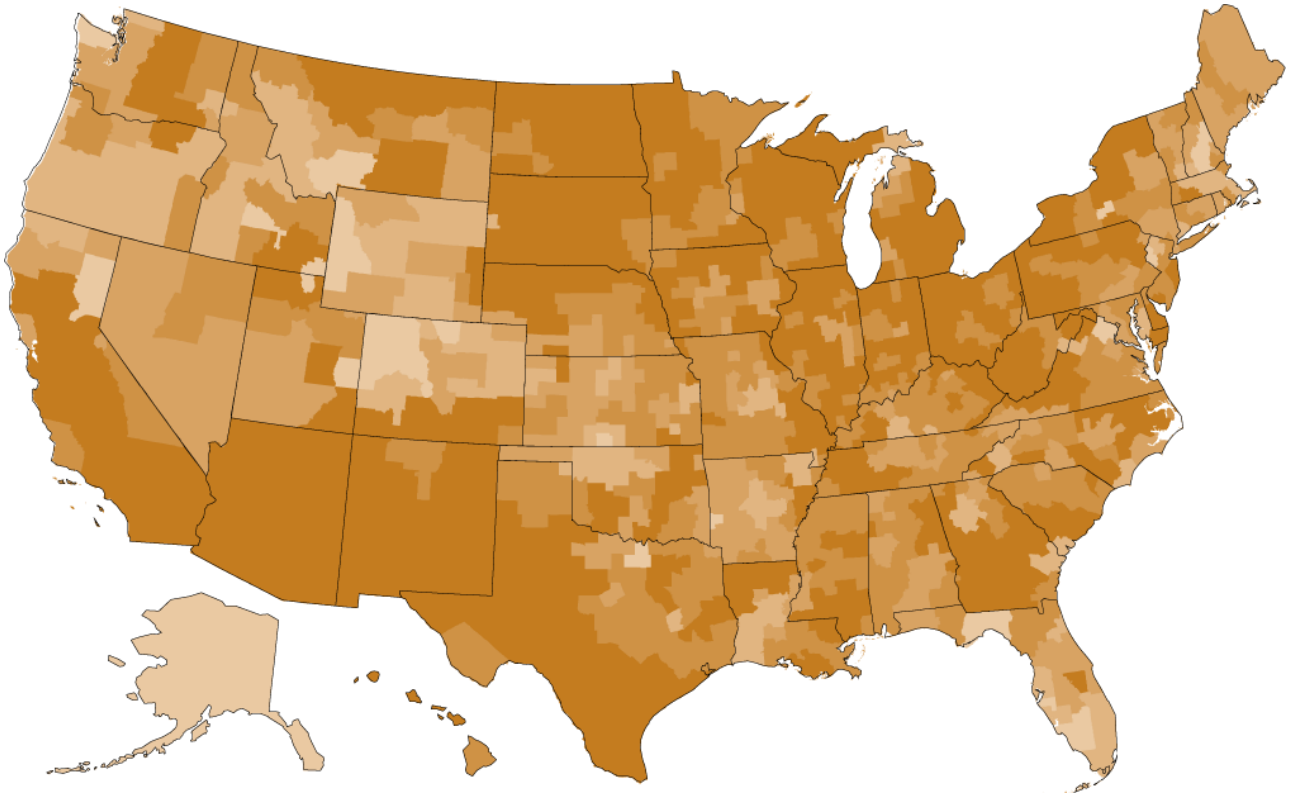
Geographic variations in adjusted prevalent rates (per million population), 2002



December 31 point prevalent ESRD patients. By HSA; rates adjusted for age, gender, & race. Excludes patients residing in Puerto Rico & the Territories.

USRDS 2009

Geographic variations in adjusted prevalent rates (per million population), 2007



December 31 point prevalent ESRD patients. By HSA; rates adjusted for age, gender, & race. Excludes patients residing in Puerto Rico & the Territories.

USRDS 2009

The Role of the Laboratory

Tests to evaluate kidney function

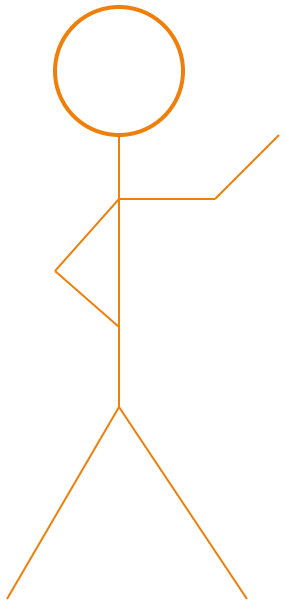
Tests of Kidney Function

- Creatinine
- Glomerular filtration rate
- Urine albumin
- Cystatin C
- Urinalysis
  - Dipstick
  - Microscopic
- Blood urea nitrogen (BUN)
- Osmolality
- Urine protein
- Urine protein electrophoresis
- Kidney stone assessment
  - Calcium, citric acid, uric acid, oxalate

Creatinine

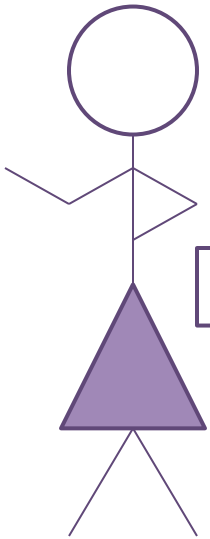
Case Study

My serum creatinine is 1.1 mg/dL.



*Stage 2

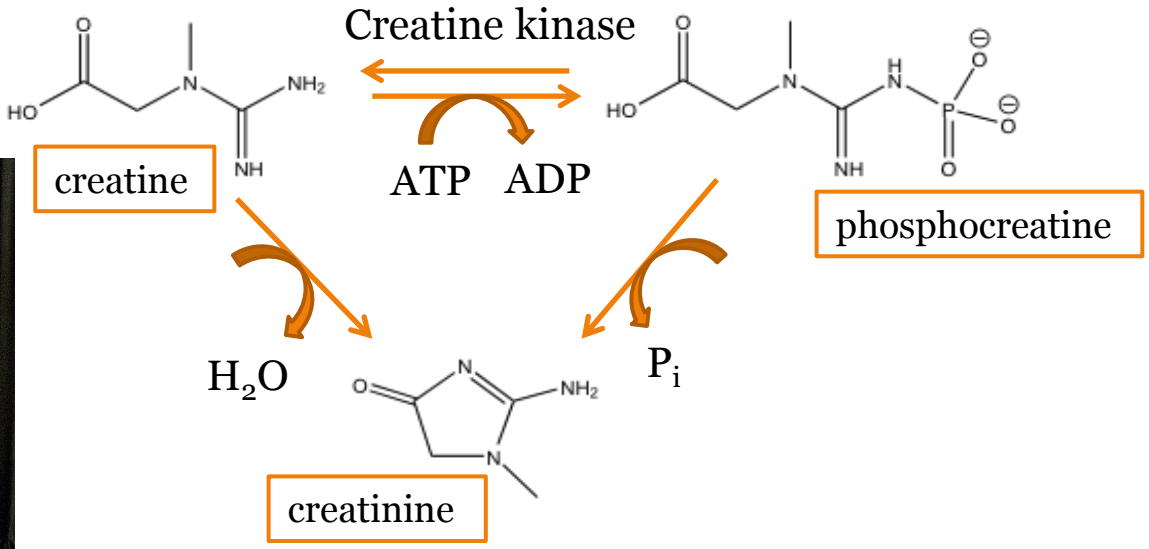
My serum creatinine is also 1.1 mg/dL. Why do I have stage 3 kidney disease?

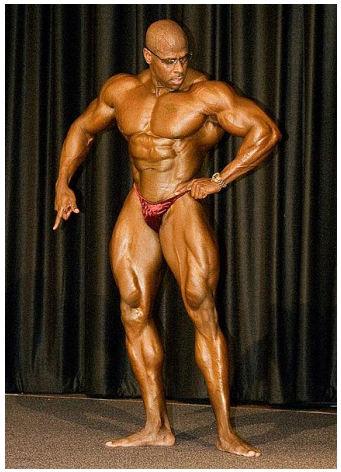


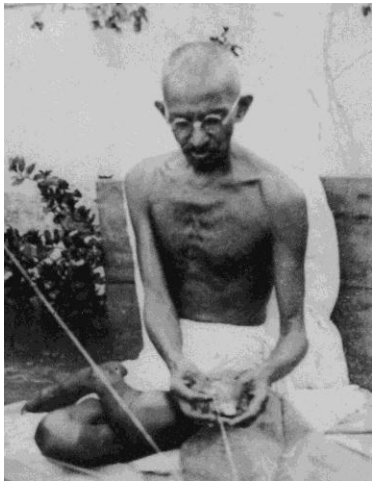
*Stage 3

Creatinine

- Creatinine – a waste product of creatine
  - In muscles, phosphocreatine is used as an energy source

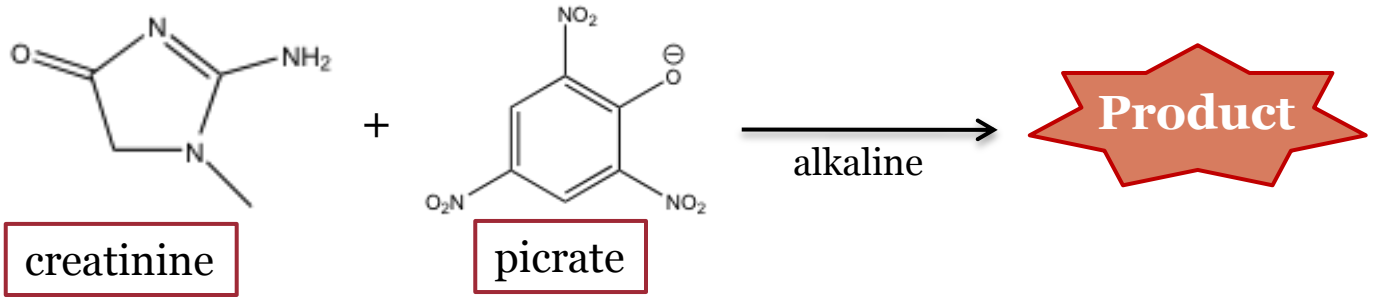






Creatinine Assay

- Jaffe Reaction/Alkaline Picrate



- Interferences
  - Protein, glucose, bilirubin, hemoglobin
  - Acetoacetate – diabetic ketoacidosis

Enzymatic Reaction: Creatininase and Creatinase

$$\text{Creatinine} + H_2O \xrightarrow{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + \text{ATP} \xrightarrow{\text{Creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{\text{Sarcosine oxidase}} \text{Formaldehyde} + \text{Glycine} + H_2O_2$$

$$\text{Indicator (reduced)} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Indicator (oxidized)} + 2H_2O$$

- Fewer interferences
- More expensive, less widely available

Glomerular Filtration Rate

Glomerular Filtration Rate (GFR)

- Glomerular filtration rate (GFR)
  - GFR = rate (mL/min) at which substances in plasma are filtered through the glomerulus
  - Best indicator of overall kidney function
  - Can be measured or calculated using a variety of markers

GFR and Chronic Kidney DIsease

- National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI)
  - 2002 Clinical Practice Guidelines for Chronic Kidney Disease

Stage	Description	GFR (mL/min/1.73 m²)
1	Kidney damage with normal or ↑ GFR	≥90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure	<15 (or dialysis)

Markers for GFR

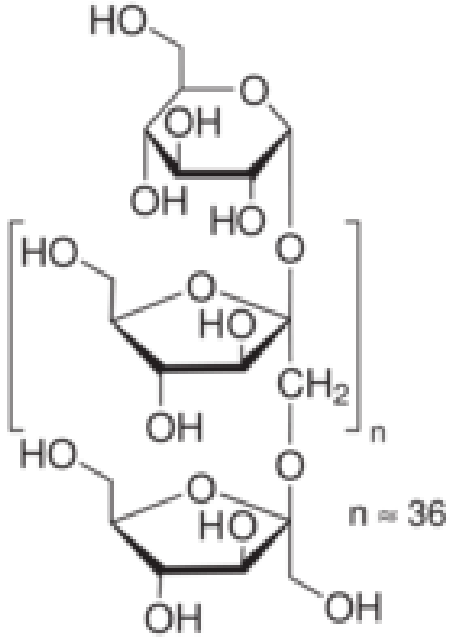
- Ideal characteristics:
  - Freely filtered at the glomerulus
  - No tubular secretion or reabsorption
  - No renal/tubular metabolism
- Exogenous or endogenous
  - Exogenous – not normally present in the body
    - Inulin
  - Endogenous – normally present in the body
    - Creatinine
- Radiolabeled or non-radiolabeled

Direct Measures of GFR: Clearance

- C = (U x V)/P
  - C = clearance
  - U = urinary concentration
  - V = urinary flow rate (volume/time)
  - P = plasma concentration
- Clearance = GFR

Inulin Clearance

- Gold standard for renal clearance
  - Freely filtered at glomerulus
  - No tubular metabolism
  - No tubular reabsorption or secretion
- Protocol
  - IV infusion
  - Blood samples
  - Urine catheter
- Limitations
  - Expensive, hard to obtain
  - Difficult to assay
  - Invasive



Creatinine to Calculate GFR

- Advantages
  - Endogenous
  - Produced at ~constant rate per day
  - Routinely measured
  - Freely filtered at glomerulus
    - Inversely related to GFR
  - Not reabsorbed or metabolized by renal tubules
  - Assays are standardized
- Disadvantages
  - Estimate of GFR
  - Is secreted by renal tubules
    - ~10%
    - Secretion increases as kidney function decreases

Estimated Glomerular Filtration Rate (eGFR)

- MDRD equation
  - GFR (mL/min/1.73 m^2) = 175 x $(S_{Cr})^{-1.154}$ x $(age)^{-0.203}$ x 0.742 x 1.210

If female

If African American

- Study group:
  - Primarily caucasian
  - Patients with kidney disease (mean GFR = 40 mL/min/1.73 m^2
- Limitations
  - Less accurate in patients with normal GFR
    - Often reported ">60 mL/min/1.73 m^2"
  - May be less accurate in some other ethnicities

Ann Intern Med. 2006;145(4):247.

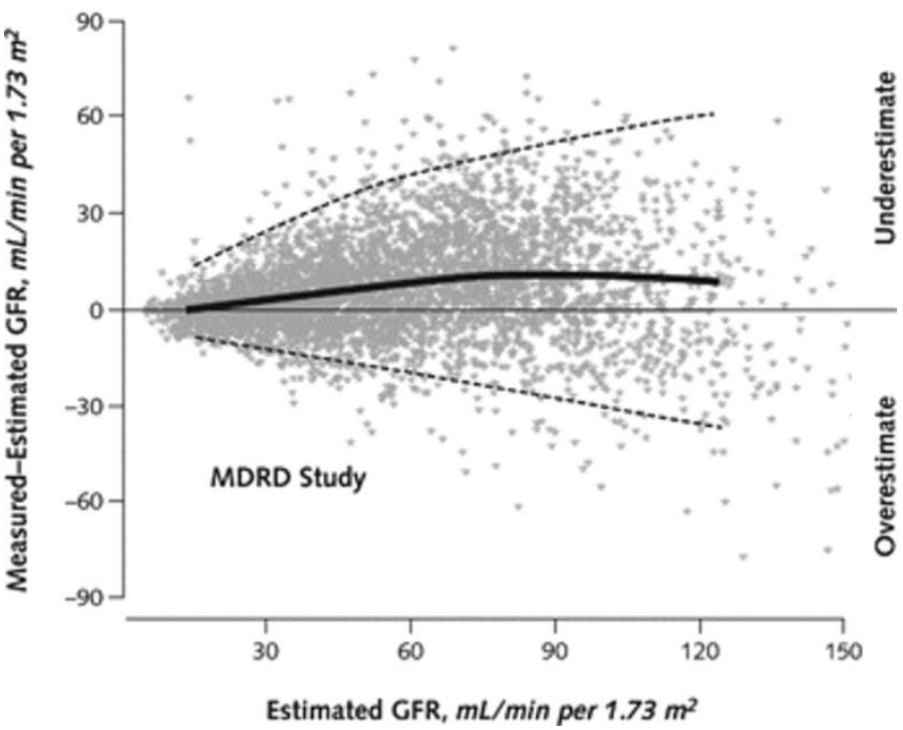
Estimated Glomerular Filtration Rate (eGFR)

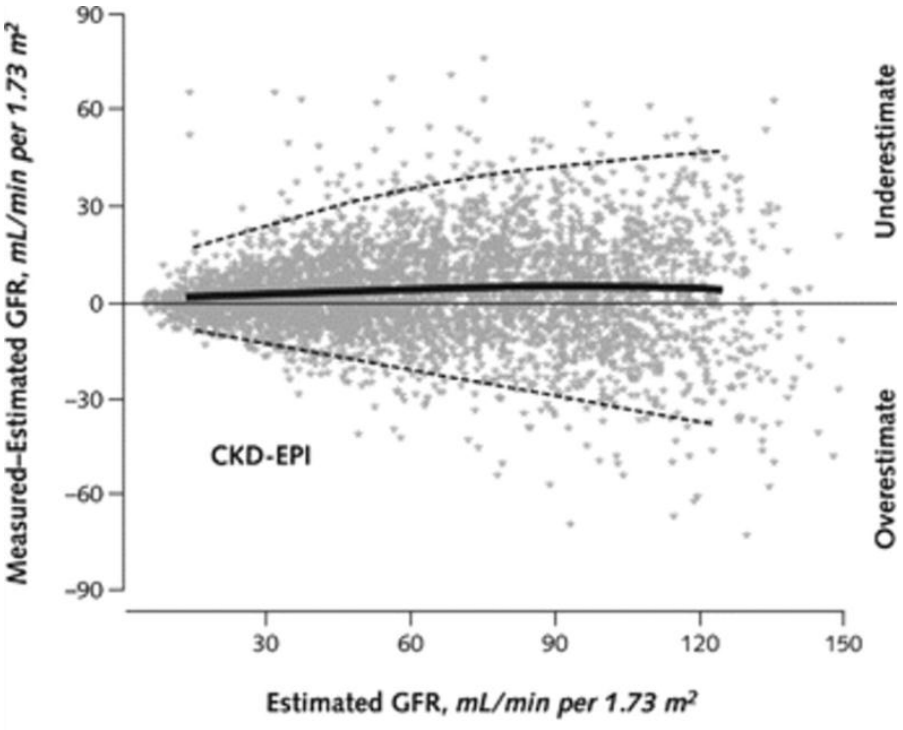
- CKD-EPI equation:
  - $GFR = 141 \times \min(SCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{age} \times 1.018 \times 1.159$

    If female — If African American
    - SCr = serum creatinine (mg/dL)
    - κ = 0.7 (female) or 0.9 (male)
    - α = -0.329 (female) or -0.411 (male)
    - Min = minimum of SCr/κ or 1
    - Max = maximum of SCr/κ or 1
- Study population
  - Patients with and without kidney disease
- Performance
  - Similar to MDRD equation at lower GFR
  - Improved performance at higher GFR
- Limitations
  - More recent equation
  - Not in widespread use

Ann Intern Med 2009; 150:604

MDRD vs CKD-EPI



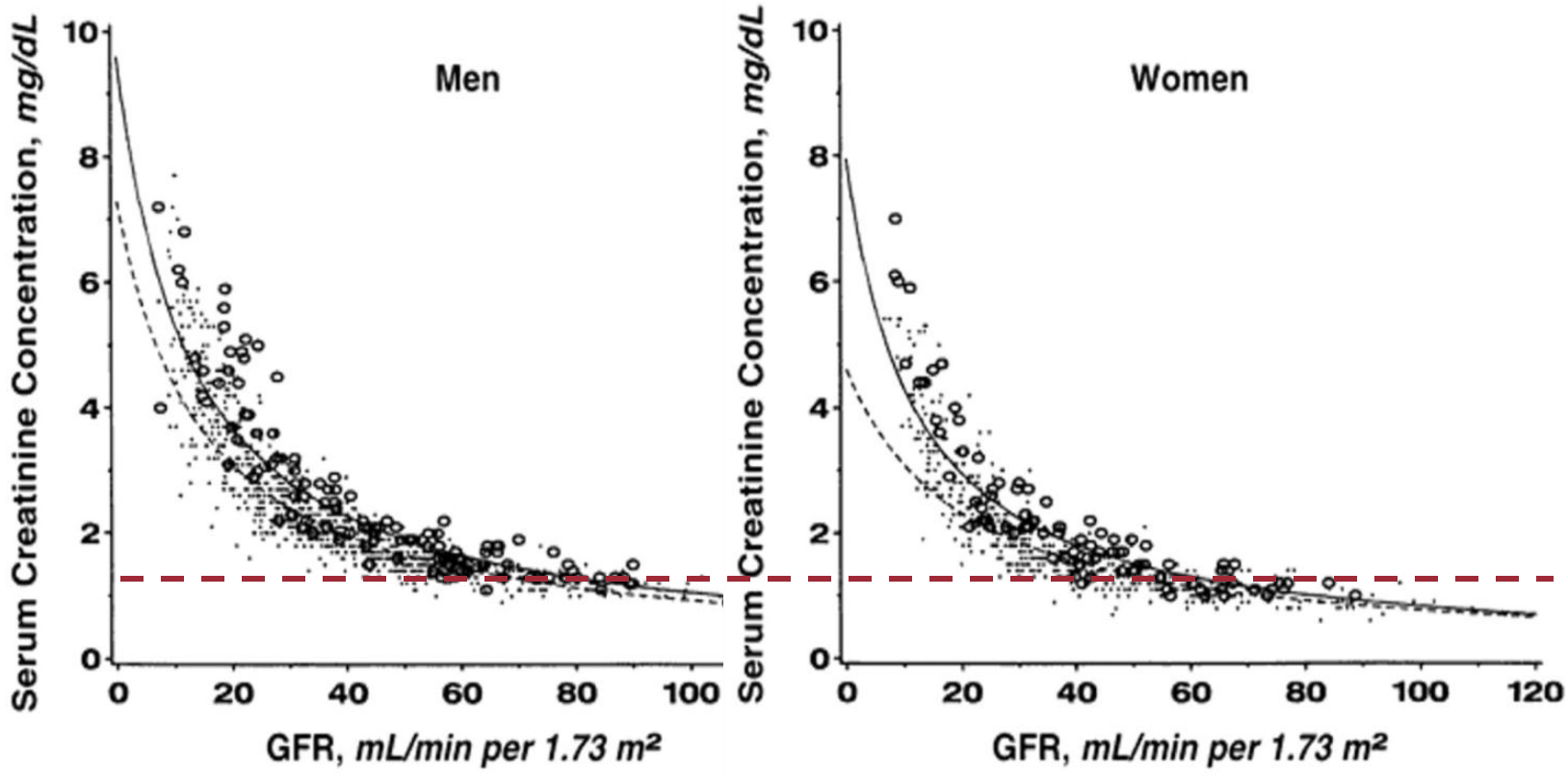


Ann Intern Med 2009; 150:604

Creatinine Considerations

- Creatinine is related to muscle mass
  - eGFR calculations may be influenced by:
    - Age
    - Sex
    - Race
    - Body builders
    - Body habitus (amputees)
    - Vegetarian/recent ingestion of cooked meat

Serum Creatinine vs GFR

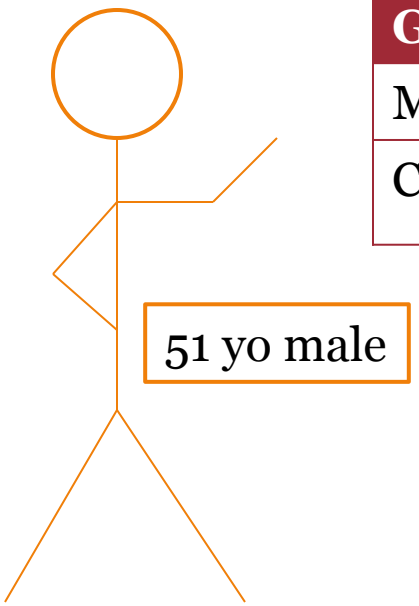


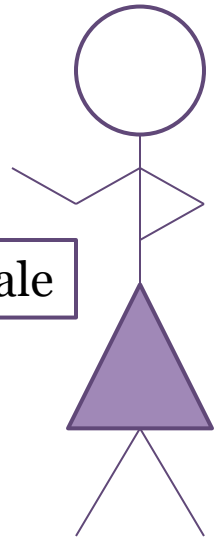
Ann Intern Med. 1999;130(6):461-470.

Case Study

- Calculate GFR (SCr = 1.1 mg/dL)
  - http://www.nephron.com/MDRD_GFR.cgi

GFR Calculations	His	Hers
MDRD (mL/min/1.73 m²)	71	51
CKD-EPI (mL/min/1.73 m²)	77	56



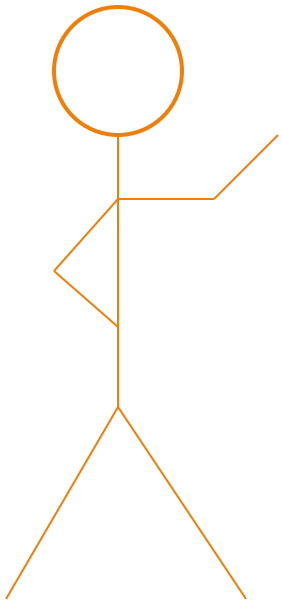


Urine Albumin and Protein

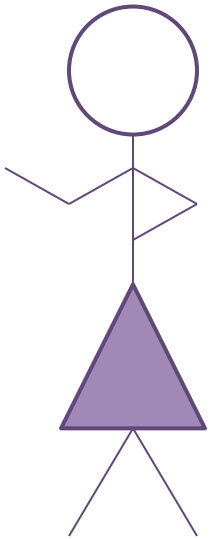
Case Study

My urine ACR is 120 mg/g.

*Stage 2
*S_{cr} 1.1 mg/dL
*eGFR = 77 mL/min/1.73 m^2



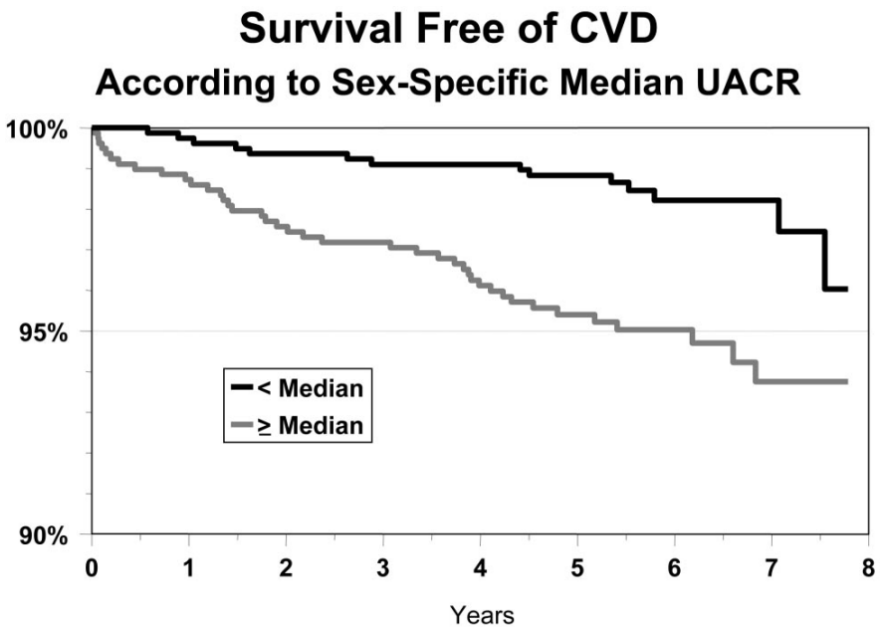
Well my urine ACR is only 40 mg/g.



*Stage 3
*S_{cr} 1.1 mg/dL
*eGFR = 56 mL/min/1.73 m^2

Albuminuria: Beyond Kidney Disease

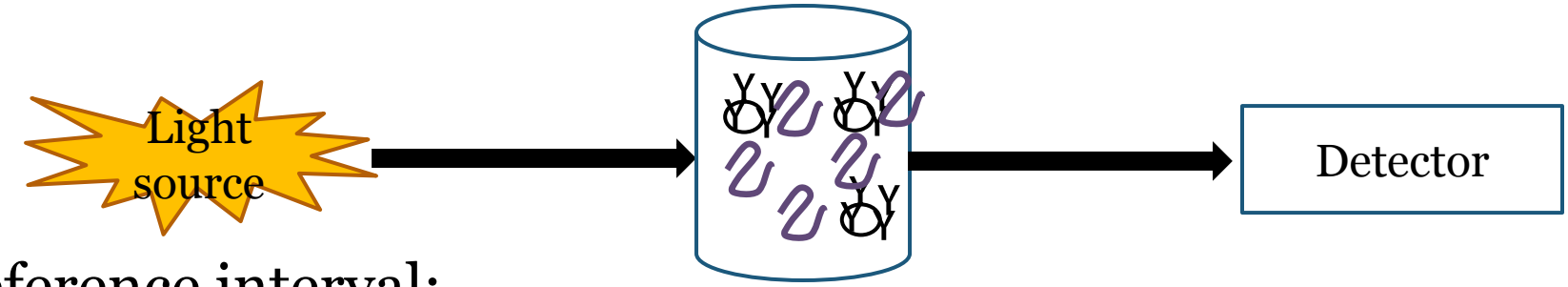
- Albuminuria = the presence of albumin in urine
- Albuminuria is an independent marker for cardiovascular disease morbidity and mortality
  - Non-diabetic
  - Non-hypertensive



Circulation 2005; 112:969

Urine Albumin

- Immunoturbidimetric or nephelometric



- Reference interval:
  - <30 mg/d
  - Albuminuria = Albumin to creatinine ratio (ACR) >30 mg/g
- Limitations
  - No reference method
  - No reference material for urine albumin
    - Recommended standardization against serum albumin reference material (CRM 470)

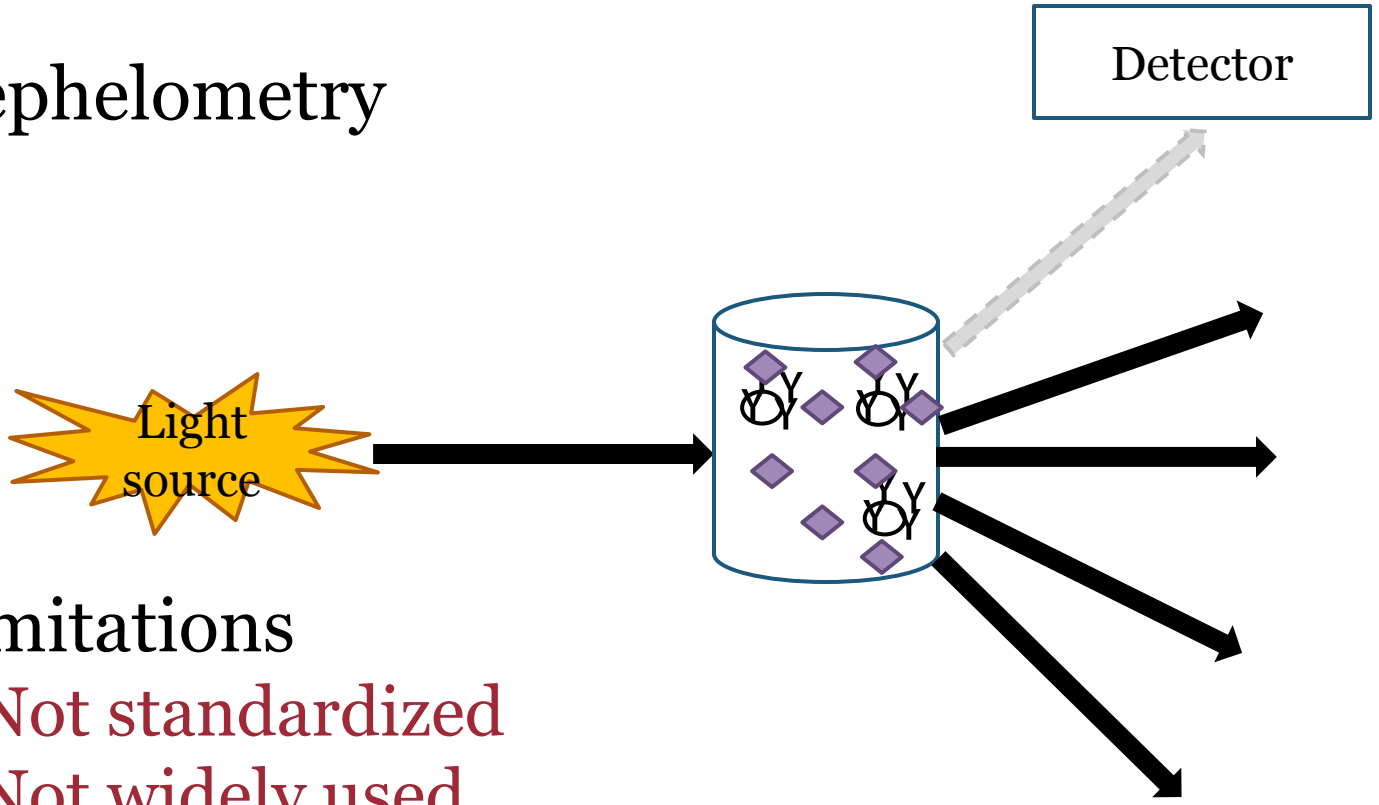
Serum Cystatin C

Cystatin C

- ~13 kD protein
  - Cysteine protease inhibitor
  - Produced by all nucleated cells
    - Constant production rate
- Freely filtered by glomerulus
  - No tubular secretion or reabsorption
- Is metabolized by the tubules
- Serum concentrations are unaffected by:
  - Muscle mass, diet

Cystatin C Assay

- Nephelometry



- Limitations
  - Not standardized
  - Not widely used
  - Expensive – compared to creatinine

Estimated Glomerular Filtration Rate (eGFR)

- CKD-EPI cystatin C equation
  - $GFR = 133 \times \min(SCysC/0.8, 1)^{-0.499} \times \max(SCysC/0.8, 1)^{-1.328} \times 0.996^{age} \times 0.932$

    If female
    - SCysC = serum cystatin C (mg/L)
    - Min = minimum of SCysC/0.8 or 1
    - Max = maximum of SCysC/0.8 or 1

- Limitations
  - Extensively metabolized by the renal tubules
  - New equation, not widely used

New Engl J Med 2012; 367(1):20

Clinical Practice Guidelines

Kidney Disease Improving Global Outcomes

- KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease
  - *Kidney Int Suppl* 2013; 3(1).
  - Sponsor: National Kidney Foundation

KDIGO 2012 Clinical Practice Guideline: Staging of CKD

GFR Category	GFR (mL/min/1.73 m²)	Terms
G1	≥90	Normal or high
G2	60-89	Mildly decreased
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

Kidney Int Suppl 2013; 3(1).

KDIGO 2012 Clinical Practice Guideline: Albuminuria

- "The term microalbuminuria should no longer be used by laboratories."
  - ~30-300 mg/day of albumin

Category	Albumin to creatinine ratio (mg/g)	Terms
A1	<30	Normal to mildly increased
A2	30-300	Moderately increased
A3	>300	Severely increased

Kidney Int Suppl 2013; 3(1).

KDIGO 2012 Clinical Practice Guideline: Assessing CKD

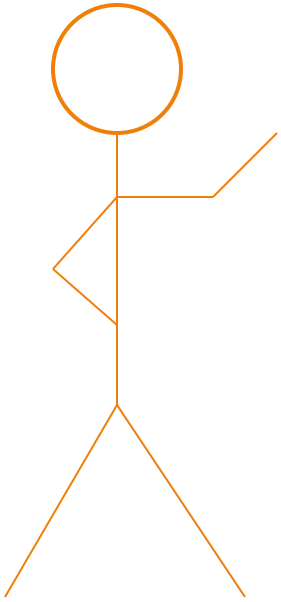
Prognosis of CKD by GFR and Albuminuria Categories: KDIGO 2012

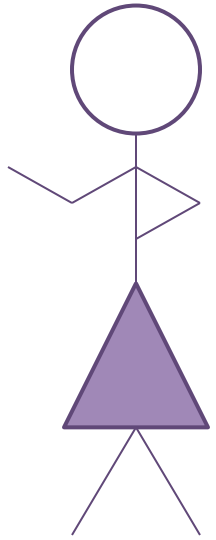
GFR categories (ml/min/1.73 m²) Description and range				Persistent albuminuria categories Description and range		
				A1	A2	A3
				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
	G1	Normal or high	≥90			
	G2	Mildly decreased	60-89			
	G3a	Mildly to moderately decreased	45-59			
	G3b	Moderately to severely decreased	30-44			
	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Kidney Int Suppl 2013; 3(1).

Case Study

	His	Hers
Age	51	56
Serum creatinine (mg/dL)	1.1	1.1
CKD-EPI eGFR (mL/min/1.73 m^2)	77	56
ACR (mg/g)	120	40
Kidney Disease Stage	2	3





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	G4	Severely decreased	15-29			
	G5	Kidney failure	<15			

Kidney Int Suppl 2013; 3(1).

Therapeutic Interventions

Recommended Management: CKD

- Blood pressure regulation
  - ACE-inhibitors
- Diet
  - Low protein (<0.8 mg/kg/day)
  - Low salt (<2 g/day)
- Blood glucose control
  - HbA_{1c} = ~7.0%
- Supplements:
  - Vitamin D
  - Bicarbonate

Kidney Int Suppl 2013; 3(1).

Summary

- The nephron is the functional unit of the kidney
  - The glomerulus is a key regulator of filtration rate and filtration selectivity
- Progression of chronic kidney disease is commonly monitored using glomerular filtration rate and albuminuria
- Numerous laboratory methods exist to evaluate glomerular filtration rate
  - Exogenous vs endogenous markers
  - Direct vs calculated
- KDIGO 2012 Clinical Practice Guidelines:
  - 6 stages of chronic kidney disease based on GFR
  - 3 stages of albuminuria

References

- Arnlov, J. et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: The Framingham Heart Study. *Circulation* 2005; 112(7):969.
- ARUP SOP's: Creatinine, Total Protein Urine, Microalbumin Urine, Cystatin C Serum
- Burtis, C.A., et al. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th Edition. 2012. St. Louis, MO: Elsevier Saunders.
- CDC, www.cdc.gov
- Inker, L.A. et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. The New England Journal of Medicine 2012; 367(1):20.
- KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int Suppl* 2013; 3(1).
- Kumar, V., et al. *Robbins and Cotran Pathologic Basis of Disease*. 8th Edition. 2010. Philadelphia, PA: Saunders Elsevier.
- Levey, A.S. et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of Internal Medicine* 1999; 130(6)
- Levey, A.S., et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Annals of Internal Medicine* 2006; 145(4):247.
- Levey, A.S., et al. A new equation to estimate glomerular filtration rate. *Annals of Internal Medicine* 2009; 150(9):604.
- Lopez-Novoa, J.M., et al. Common pathophysiological mechanisms of chronic kidney disease: therapeutic perspectives. *Pharmacology & Therapeutics* 2010; 128:61.
- National Kidney Foundation, www.kidney.org
- NIDDK, http://kidney.niddk.nih.gov/kudiseases/pubs/kustats/
- UpToDate: Inker, L.A. and Perrone, R.D. Assessment of kidney function.
- U.S. Renal Data System, USRDS 2012 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2012
- Wu, A.H.B Tietz Clinical Guide to Laboratory Tests. Fourth Edition. 2006. St. Louis, MO: Saunders Elsevier

Questions?