

Bachelor of Chinese Medicine Year IV

2024 – 2025

BCHM4608 – Fundamentals of Diagnosis (Pathology)

Use of Biochemical Laboratory Tests: **Renal Function Test & Blood Gas Analysis**

26 March 2025

14:30 – 16:20

(T06 – 35)

Sidney Tam

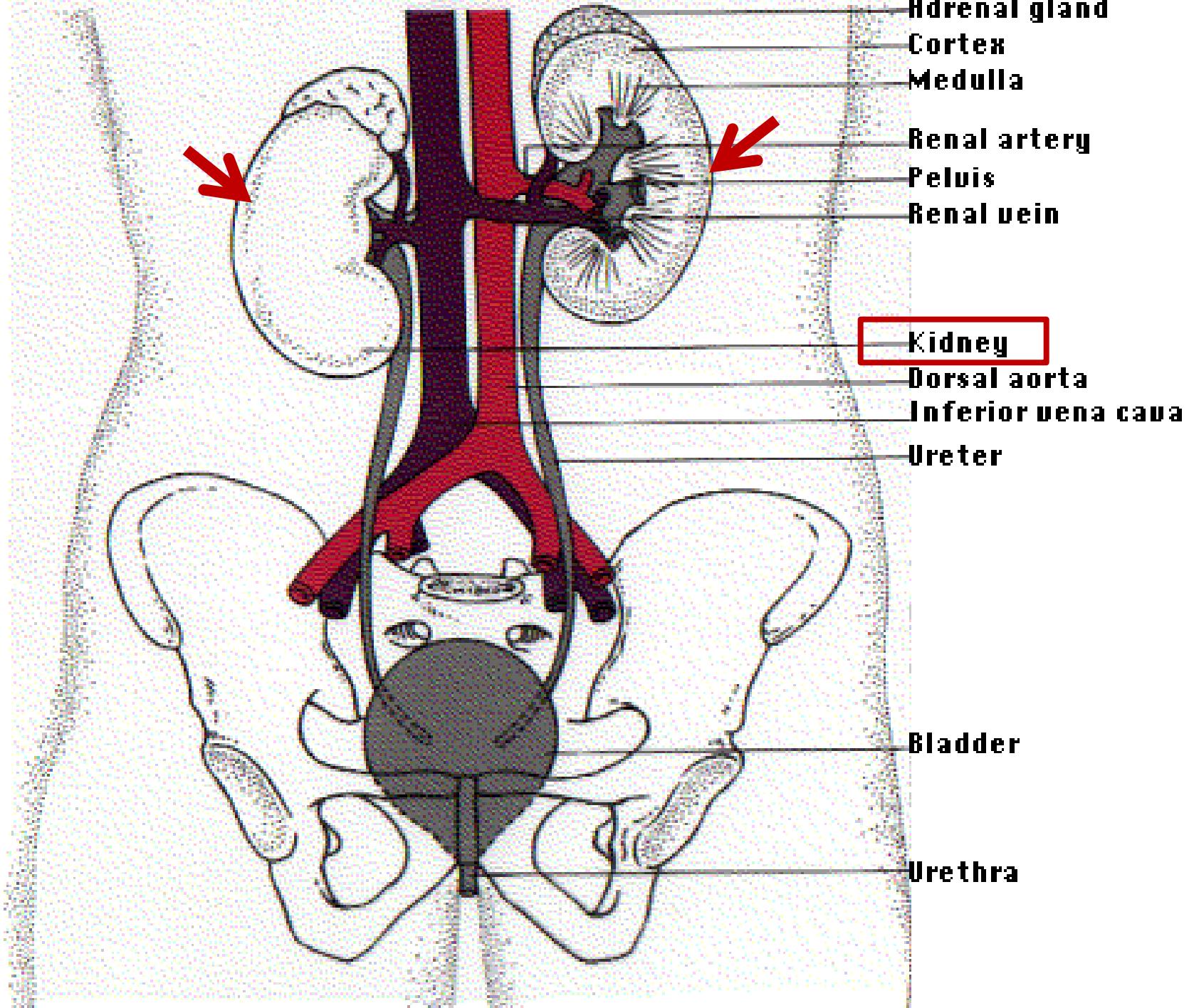
Hon Clinical Professor

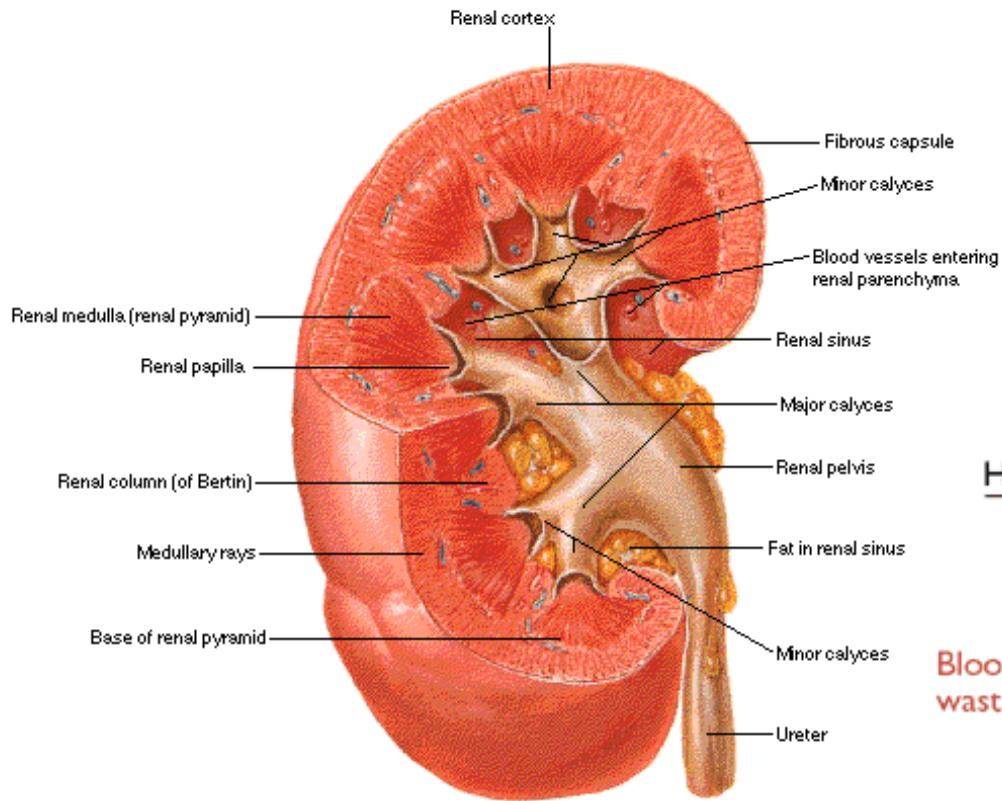
Department of Pathology
The University of Hong Kong

Learning Objectives

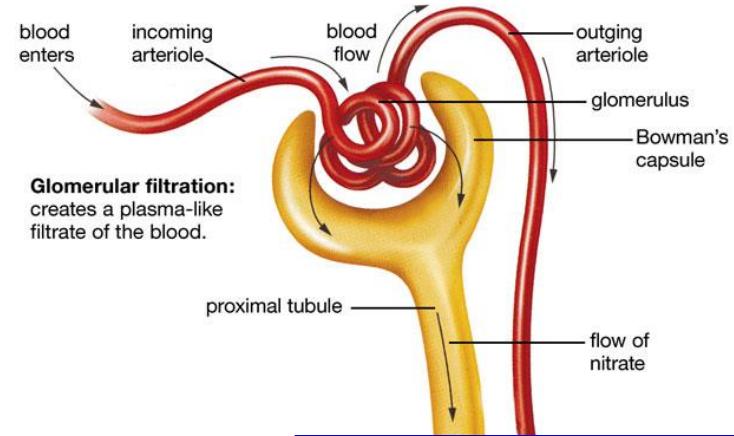
- Understand the basic physiology of water and electrolyte handling by the kidney.
- Understand the role of the kidney in electrolyte and acid-base homeostasis.
- Know what constitute a Renal Function Test (RFT) and a Blood Gas profile and how to interpret these tests in the common clinical settings.
- Recognize the limitations and caveats of RFT for the assessment of Glomerular Filtration Rate (GFR) and renal excretory function.
- Use of the Creatinine-based equations for GFR estimation (eGFR) in clinical practice and their caveats.
- Understand the basic pathophysiology and laboratory investigations of proteinuria.
- Understand the basic principles of acid-base homeostasis and some simple acid-base disorders.
- Enhance and reinforce understanding through case illustrations.

Renal Function Test (RFT)

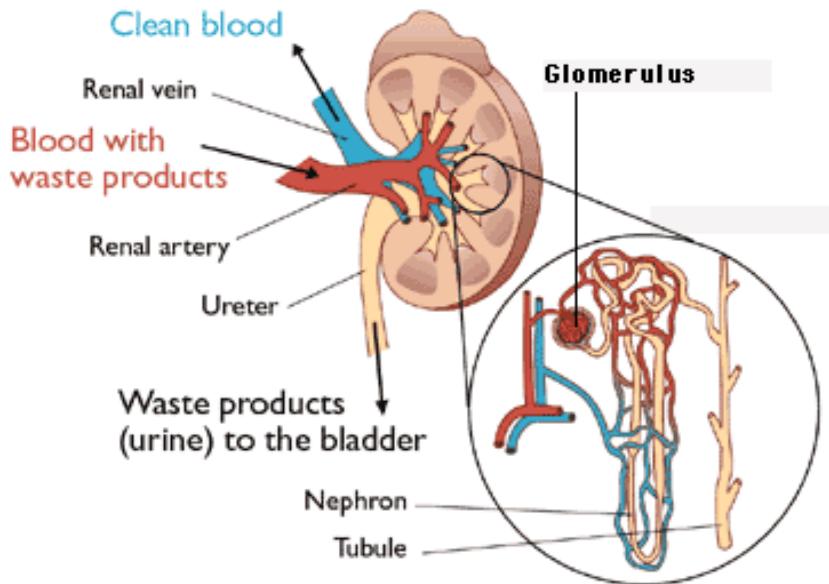




KIDNEY

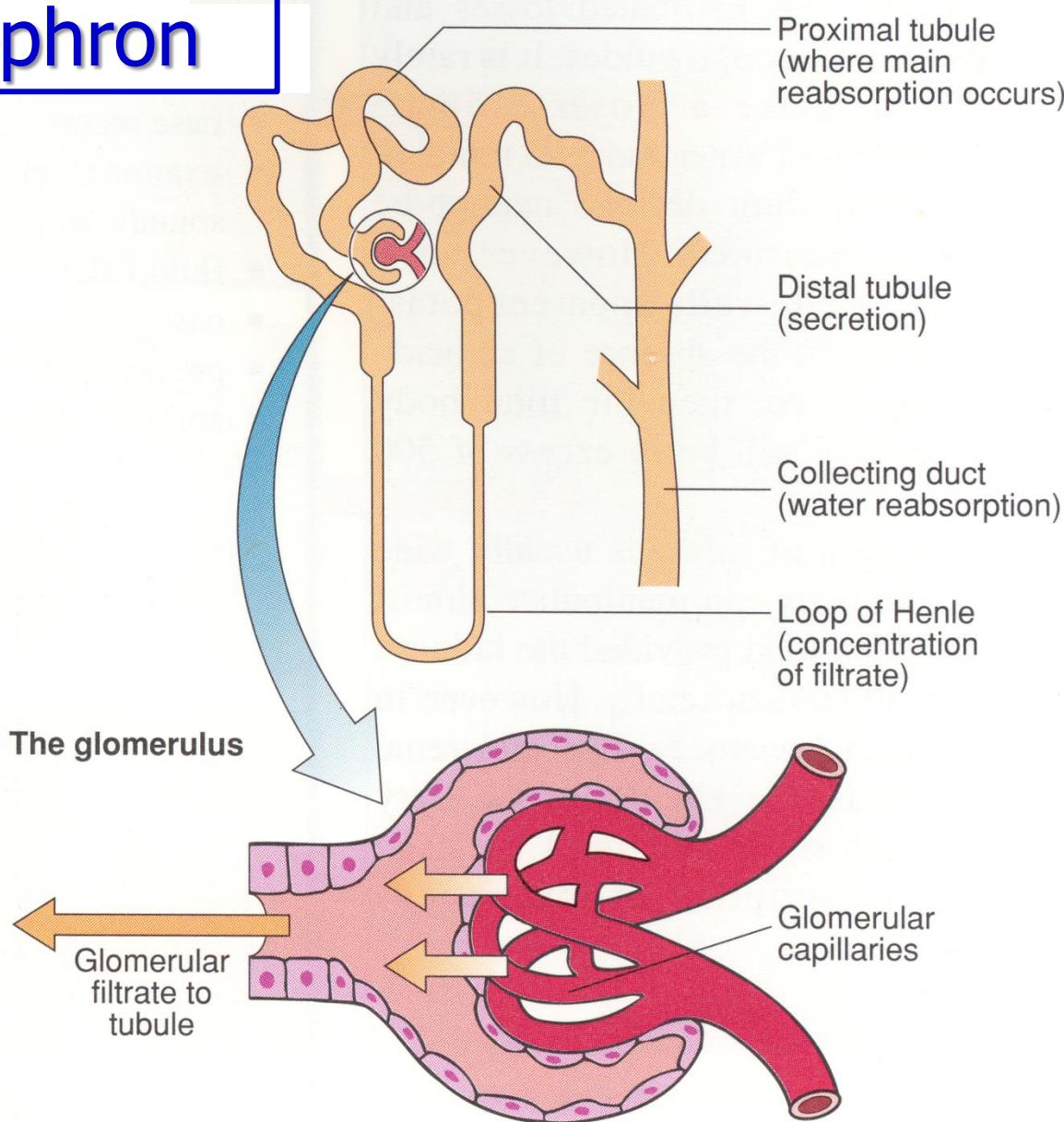


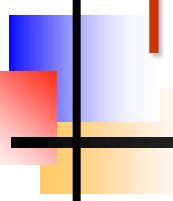
How the kidney works



Nephron

Nephron





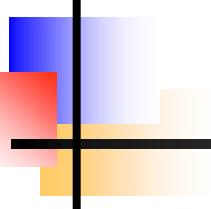
The KIDNEY has 3 Major Functions:

- Regulation of water, electrolyte and acid-base **homeostasis**.
- **Excretion** of waste products of intermediary metabolism, e.g., urea, creatinine, uric acid, phosphate, sulphate and organic acids.
- Production and elaboration of **hormones**, e.g., 1,25-Dihydrocholecalciferol, Erythropoietin (Renal Epo-producing cells, REPs), and Renin (cells in the juxtaglomerular apparatus).



To Do this the Kidney Needs to:

- Prepare an ultrafiltrate.
- Reabsorb valuables (glucose, amino acids, electrolytes, proteins).
- Achieve homeostasis by regulation (effective circulatory volume, acid-base status, blood pressure, electrolyte concentrations).
- Metabolic activities: synthetic, catabolic.
- Synthesis and elaboration of hormones (e.g., Vitamin D, Renin, Erythropoietin).



Some Basic Facts (1)

- Richly perfused organ: 20 - 25 % of cardiac output goes to the kidneys (~0.5% of body weight).
- Each kidney is made up of ~ 10^6 **nephrons** (functional units).
 - **Glomerular** function: Filtering function
 - **Tubular** function: Reclamation of essentials, concentrating glomerular filtrate, secreting metabolic waste products, adjusting secretion / reabsorption of H^+ and HCO_3^- for acid-base homeostasis.



Some Basic Facts (2)

Renal Blood Flow (RBF)
1000 - 1200 mL/min

~20% Cardiac Output

Glomerular Filtration Rate

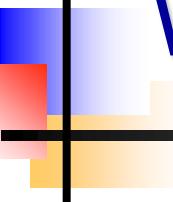
~125 mL/min

Urine Formation
~1.5 L/day

~1 mL/min

~99% H₂O in glomerular ultrafiltrate **reabsorbed by the kidney**

~65% occurs in the proximal renal tubules accompanied by Na⁺ and Cl⁻ reabsorption



What gets Filtered in the Glomerulus?

- **Freely Filtered**

- H₂O
- Na⁺, K⁺, Cl⁻, HCO₃⁻, Ca²⁺, Mg²⁺, PO₄²⁻, etc.
- Glucose
- Urea
- Creatinine
- Inulin (exogenous)

- **Some Filtered**

- β₂-microglobulin
- RBP*
- α₁-microglobulin
- Albumin

- **None Filtered**

- Immunoglobulins
- Ferritin
- Cells

* RBP = Retinol Binding Protein

Reabsorption from Glomerular Filtrate

	<u>% Reabsorbed</u>
Water	99.2
Sodium	99.6
Potassium	92.9
Chloride	99.5
Bicarbonate	99.9
Glucose	100 (< Threshold)
Albumin	95 - 99
Urea	50 - 60
Creatinine	~0 (some tubular secretion when the blood levels is very high)

Renal Function Test Profile (RFT)

- **Sodium** (Na^+)
- **Potassium** (K^+)
- **Chloride** (Cl^-)
- **Bicarbonate** (HCO_3^-) – Not routinely included
- **Urea**
- **Creatinine**
- **eGFR** (derived parameter)

Others: (not routinely included in “RFT” profile)

- **Osmolality** (serum and urine, often interpreted in conjunction with each other)

Queen Mary Hospital

Division of Chemical Pathology
LG238, Block K, 102 Pokfulam Road, Hong Kong
Tel. 22553175 Fax. 28194179

Name: HO, [REDACTED]
(何 [REDACTED])
HKID No: G0289 [REDACTED]
Hosp No: HEPC11
Location: QMH/MED/%HEPC
Sex/Age: M/56Y
Req. Loc.: QMH/MED/GICB
Doctor: PROF. YUEN [REDACTED]



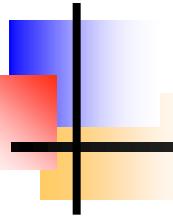
Bed:
DOB: 06/07/1964

Patient Hospital: Queen Mary Hospital

Collect Date :	01/12/20	10/02/21	15/03/21	16/04/21	01/06/21	Ref. Interval	Units
Collect Time :	11:39	09:20	15:38	09:31	14:58		
Request No. :	CC013 [REDACTED]	C2102 [REDACTED]	C3153 [REDACTED]	C4161 [REDACTED]	C601 [REDACTED]		
Remark :	CLD	low phosphate.	low phosphate	low phosphate	CLD		
	Lipid-Low ring ...						

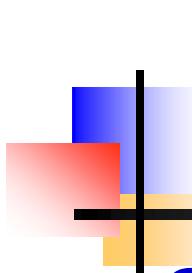
Na	140	142	142	136 - 148	mmol/L
K	3.3 L	3.7	3.6	3.6 - 5.0	mmol/L
Chloride	102	105	100	100 - 109	mmol/L
Urea	5.7	5.2	6.4	3.0 - 8.8	mmol/L
Creatinine	85	98	108	67 - 109	umol/L
Estimated GFR	88 L	74 L	65 L	>90	unit

Renal Function Test



Purposes of Ordering Renal Function Test

- To assess functional capacity of the kidney.
- To diagnose renal impairment.
- To assess the severity and progression of renal impairment.
- To assess the effectiveness of treatment and monitor disease progression.



Renal Function Test

Can be broadly divided into 2 categories:

- Test for Glomerular function -
 - Serum/plasma UREA
 - Serum/plasma CREATININE
 - Clearance tests / GFR estimation
- Tests for Tubular function -
 - Urine concentration / dilution tests
 - Para-aminohippuric acid clearance test
 - Acidification test
- Other important adjunctive test -
 - Urine Proteins

Glomerular Function Assessment



Glomerular Filtration Rate (GFR)

- Renal disease is commonly **asymptomatic** until late or advanced.
- **GFR** (Glomerular Filtration Rate) is the overall best measure of **glomerular function**.

Plasma CREATININE and UREA

- Plasma concentrations of **Creatinine** and **Urea** are used as convenient, but rather insensitive, measures of glomerular function – NB: their levels may remain within reference ranges in the presence of a significant reduction of GFR.

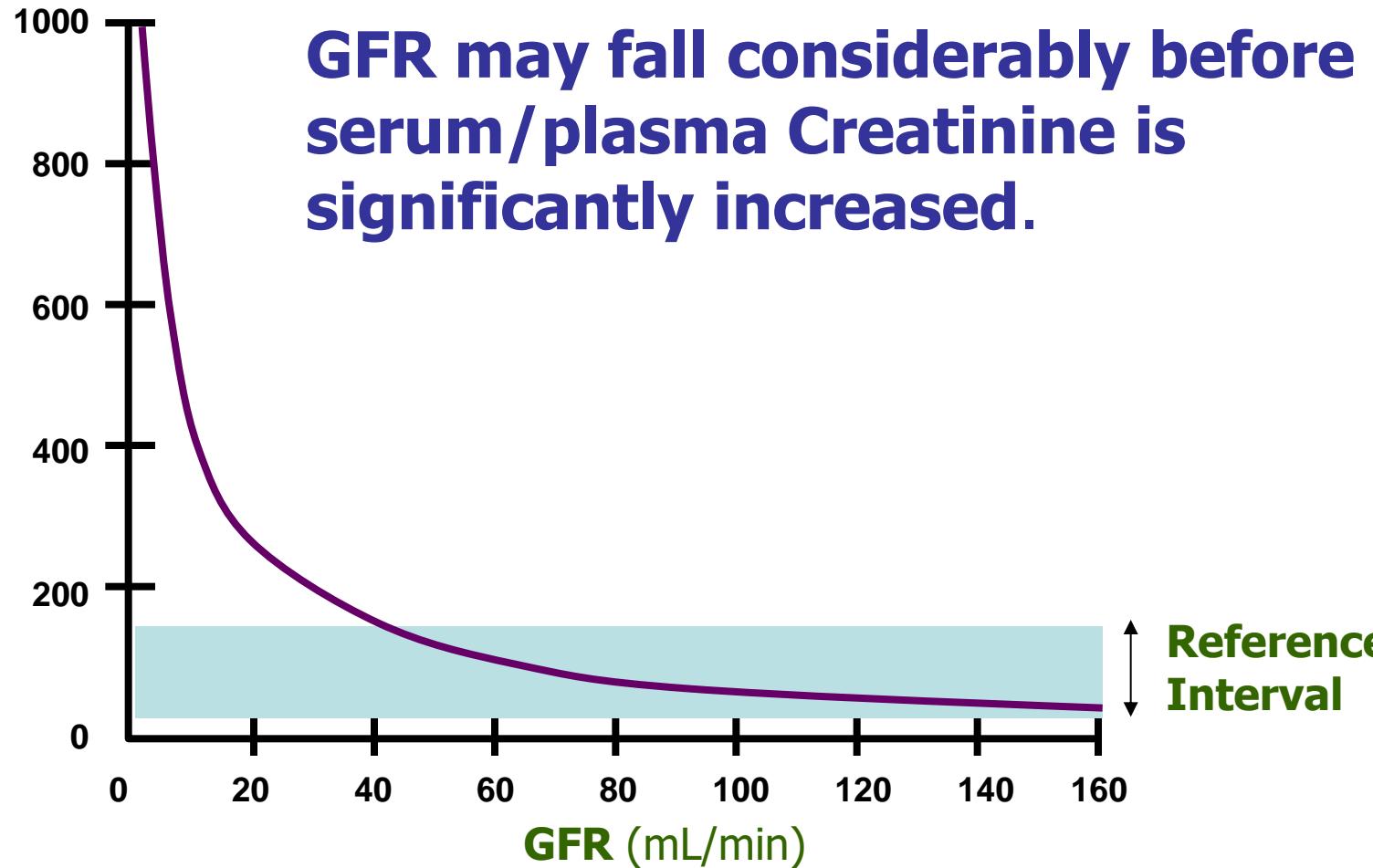
CREATININE

- Creatinine is an end-product of muscle metabolism that is released into circulation at a relatively constant rate.
- Creatinine is **freely filtered** by glomeruli and practically not reabsorbed by renal tubules, but there is some tubular secretion the degree of which increases with rising plasma levels → **Creatinine Clearance** overestimates **GFR** in advanced renal failure where Creatinine concentration is significantly elevated.

Although **Plasma CREATININE** directly reflects **GFR**, it is not always a good indicator of this parameter because:

- Plasma level is dependent on muscle mass.
- Creatinine secretion by the proximal tubule increases as GFR decreases (i.e., in renal failure); some drugs (e.g., cimetidine, trimethoprim) interfere with this secretion, thus affecting plasma Creatinine concentration independent of renal function.
- Various substances interfere with the *Jaffe's Reaction* (the most commonly employed assay methodology in clinical laboratories) causing positive bias (e.g., acetoacetate, cephalothin) and negative bias (e.g., bilirubin).
- Dietary factors e.g., roasted meat contain significant amount of creatinine and ingestion of these can transiently raise the plasma level.

**Serum / Plasma
CREATININE
($\mu\text{mol/L}$)**



**Reference
Interval**

Serum / Plasma CREATININE ($\mu\text{mol/L}$)

1000

800

600

400

200

0

GFR (mL/min)



A



B

Reference
Interval



The Relationship Between Glomerular Filtration Rate (GFR) and Serum Creatinine Concentration

Conditions Affecting CREATININE Independent of GFR

Condition

Mechanism

Spurious or True Elevation:

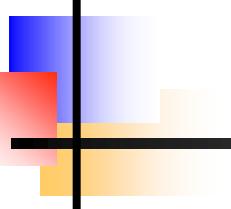
- * Ketoacidosis
- * Drugs: *Cefoxitin, Cephalothin*
- * Ingested cooked meat
- * Drugs: *Aspirin, cimetidine, trimethoprim, amiloride, triamterene, spironolactone*

Non-creatinine chromogen
Non-creatinine chromogen
Gastrointestinal absorption of creatinine
Inhibition of tubular creatinine secretion

Decrease:

- * Increasing age
- * Cachexia/Inanition

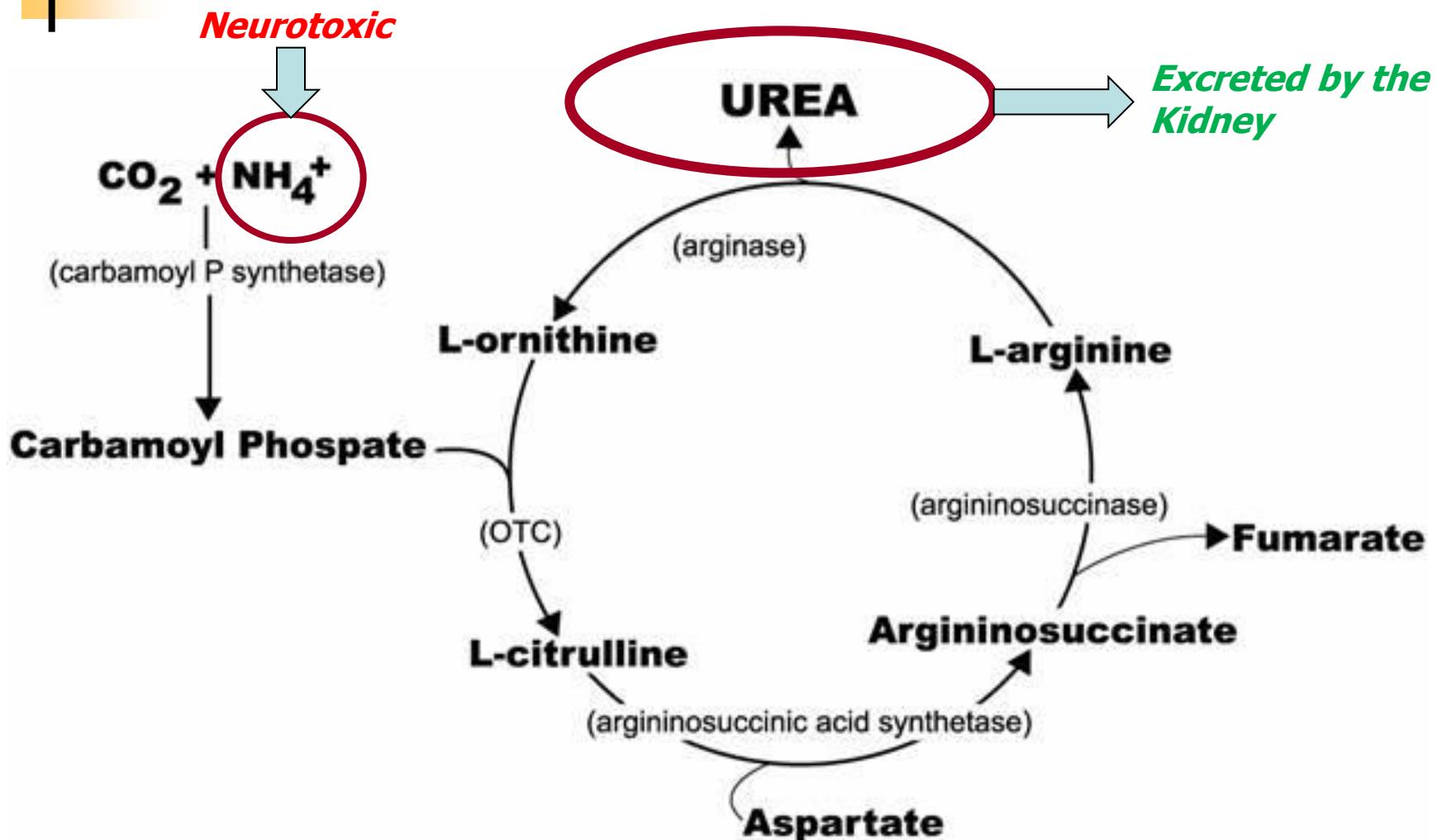
Physiological ↓ in muscle mass
Pathological ↓ in muscle mass



UREA

- Waste product of Amino Acid metabolism.
- Excretory Load dependent on Amino Acid and Protein Intake as well as Net Body Protein Metabolism.
- Filtered freely by the Glomeruli and Diffuses back into the Renal Tubules by a Passive process governed by concentration gradient.
- Clearance dependent on Urine Flow Rate.

Synthesis of Urea



The Urea Cycle

Plasma UREA concentration is a poor indicator of GFR because:

- ↓ production (low protein intake) can lower the plasma [urea] sufficiently to enable a normal plasma level to be associated with significant renal insufficiency.
- GFR has to drop ~ 40% before the plasma [urea] begins to rise above the reference interval.
- ↑ production (e.g., high protein intake) in the face of minor degrees of renal impairment can result in disproportionately high plasma [urea].

Conditions Affecting UREA Level Independent of GFR

↑ UREA

- * High Protein Diet
- * Gastrointestinal Bleeding
- * Tissue Trauma
- * Glucocorticoids
- * Tetracycline

↓ UREA

- * Liver Disease
- * Malnutrition
- * Urea Cycle Defects

Glomerular Filtration Rate (GFR)

- Can be estimated by the rate of clearance of substances from blood that have the following characteristics:

a substance that is **stable** in plasma, **not reabsorbed** from the glomerular filtrate **nor secreted** by the renal tubular cells will be ideal for the estimation of GFR.
- **Creatinine** is an almost ideal endogenous substance in this regard (virtually not reabsorbed but minimally secreted especially when plasma level is high).
- **Creatinine Clearance** is a clinical surrogate for **GFR**.

Creatinine Clearance as an estimation of GFR

Creatinine Clearance (Cr Cl) is calculated as follows:

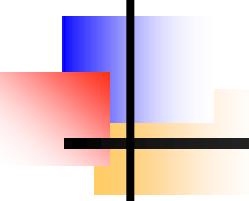
$$\text{Cr Cl} = \frac{U_{\text{Cr}} \times V}{P_{\text{Cr}}}$$

U_{cr} = Urine concentration of Creatinine

P_{cr} = Plasma concentration Creatinine

V = Volume of urine produced over a fixed period (usually a 24-hour collection to minimize fluctuations)

Creatinine Clearance is usually expressed in **mL/min**



Formula-based GFR Predicted from Serum Creatinine

Cockcroft and Gault Formula

Cockcroft DW, Gault MH
Nephron, 1976;16(1):31-41

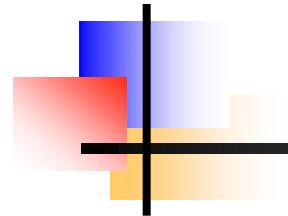
Creatinine, Age, Sex, Race, and Body Weight

4-Parameter MDRD-predicted GFR

Levey AS, et al. Modification of Diet in Renal Disease (MDRD) Study Group.
A more accurate method to estimate GFR from serum creatinine: a new prediction equation. Ann Intern Med 1999;130: 461 - 70.

The 4 Parameters:

Creatinine, Age, Sex, Race



Creatinine Clearance estimation by Cockcroft & Gault Formula

$$\text{Cr Cl (mL/min)} = \frac{(140 - \text{Age}) \times \text{Body Weight}}{814 \times \text{Plasma [Creatinine]}} \times 0.85 \text{ (for female)}$$

Age: Year

Body Weight: Kg

Plasma Creatinine conc: mmol / L

Correlates better with measured values of GFR provided that:

- i. Plasma [creatinine] is not within normal range
 - ii. Renal impairment is not severe and relatively stable
 - iii. No inhibition of tubular secretion of creatinine by medications
-
- Tends to overestimate GFR in advanced renal failure



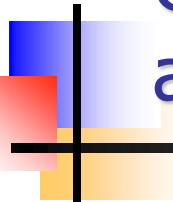
Estimation of GFR (eGFR) in Adults by CKD-EPI Creatinine Equation

- Developed in 2009 by the **Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)** and promulgated by the National Kidney Foundation to replace the MDRD Study Equation.
- **Creatinine** value standardized or traceable to **IDMS** assigned NIST certified reference materials.
- GFR estimated based on the same **4 parameters** as in the MDRD Study Equation, i.e., **Creatinine**, **Age**, **Sex**, and **Race**, but uses a 2-slope “spline” to model the relationship between estimated GFR and serum Creatinine, and a different relationship for Age, Sex and Race.
- More accurate than the MDRD Study Equation, especially for **eGFR >60mL/min/1.73m²**.
- As of **2017**, the **CKD-EPI Equation** has replaced the MDRD Study Equation in the Chemical Pathology Laboratories in the **Hospital Authority** for eGFR.

Ann Intern Med 2009; 130(9):604 - 612

Clinical Situations where Clearance Measurements may be Necessary to Estimate GFR

- Extremes of age and body size
- Severe inanition or obesity
- Disease of skeletal muscle
- Paraplegia and tetraplegia
- Vegetarian diet
- Rapidly changing renal function
- Prior to dosing drugs with significant toxicity that are excreted by the kidneys



Chronic Kidney Disease (CKD) can be staged according to eGFR

- The Kidney Disease Outcomes Quality Initiative (**KDOQI**) of the National Kidney Foundation (**NKF**) established a definition and classification of **CKD** in 2002.
- These guidelines have allowed better communication among physicians and have facilitated intervention at the different stages of the disease.
- The guidelines define **CKD** as either kidney damage or a decreased glomerular filtration rate (GFR) of $< 60 \text{ mL/min}/1.73 \text{ m}^2$ for ≥ 3 months.
- Whatever the underlying etiology, once the loss of nephrons and reduction of functional renal mass reaches a certain point, the remaining nephrons begin a process of irreversible sclerosis that leads to a progressive decline in the GFR.



CKD Staging:

The different stages of CKD form a **continuum**. The stages of CKD are classified as follows

- **Stage 1:** Kidney damage with normal or increased GFR ($>90 \text{ mL/min}/1.73 \text{ m}^2$)
- **Stage 2:** Mild reduction in GFR ($60\text{-}89 \text{ mL/min}/1.73 \text{ m}^2$)
- **Stage 3a:** Moderate reduction in GFR ($45\text{-}59 \text{ mL/min}/1.73 \text{ m}^2$)
- **Stage 3b:** Moderate reduction in GFR ($30\text{-}44 \text{ mL/min}/1.73 \text{ m}^2$)
- **Stage 4:** Severe reduction in GFR ($15\text{-}29 \text{ mL/min}/1.73 \text{ m}^2$)
- **Stage 5:** Kidney failure (GFR $< 15 \text{ mL/min}/1.73 \text{ m}^2$ or dialysis)

CKD Staging:

By itself, measurement of GFR may not be sufficient for identifying stage 1 and stage 2 CKD, because in those patients the GFR may in fact be normal or borderline normal.

In such cases, the presence of one or more of the following markers of kidney damage can establish the diagnosis:

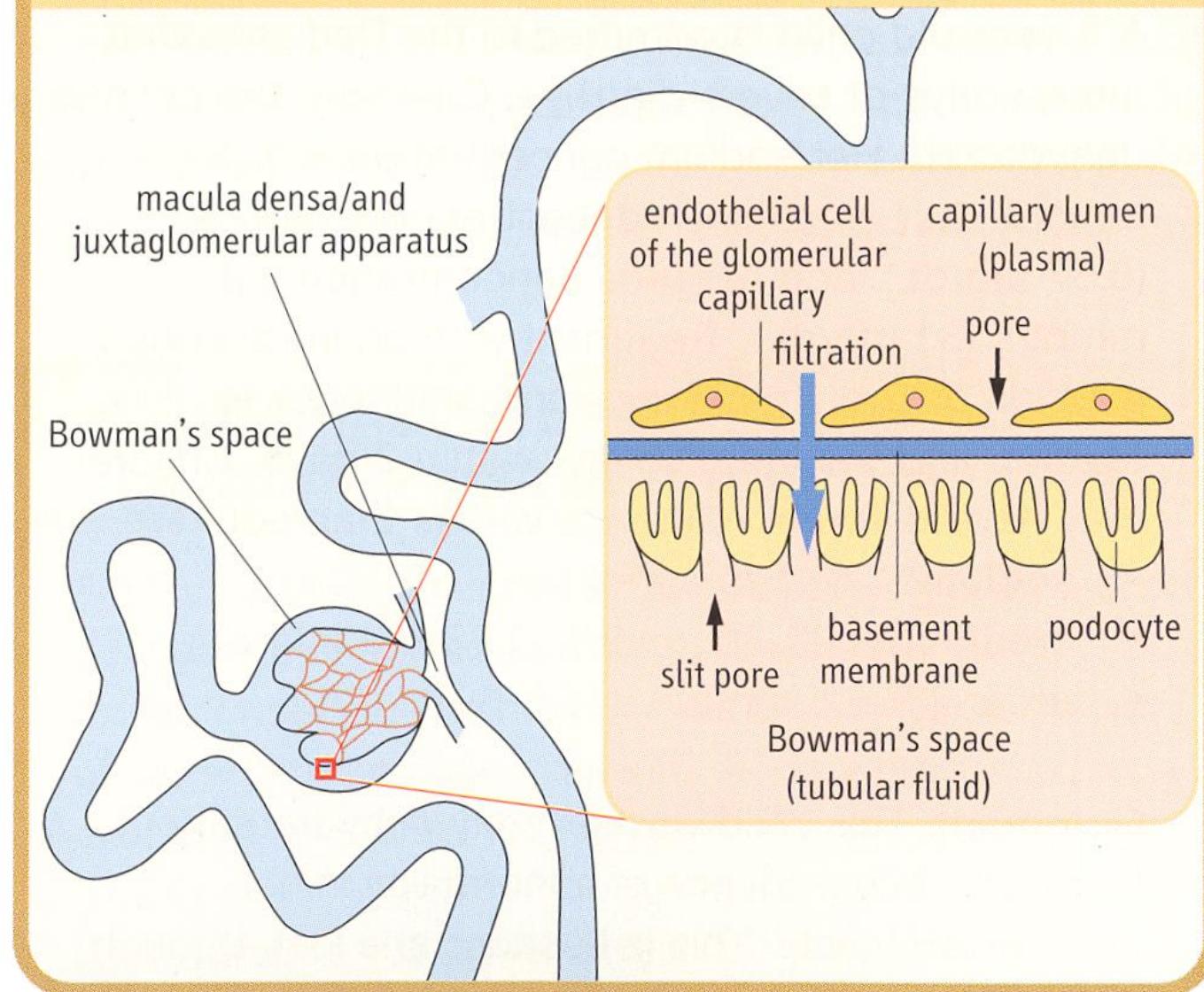
- **Albuminuria** (Albumin Excretion > 30 mg/24 hr or Albumin:Creatinine Ratio > 30 mg/g [> 3 mg/mmol])
- Urine sediment abnormalities
- Electrolyte and other abnormalities due to tubular disorders
- Histological abnormalities
- Structural abnormalities detected by imaging
- History of kidney transplantation in such cases

Urinary Protein Measurement

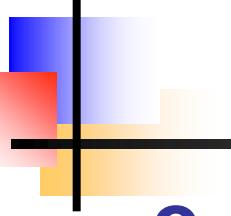
PROTEINURIA (1)

- Filtration through the glomerular basement membrane is dependent on **molecular size** with a cutoff between 20 - 40 Å, corresponding to a protein molecular mass ~30 - 70 kDa.
- **Negatively charged** molecules have lower permeability.
- Small proteins like β_2 -Microglobulin (13.5 kDa) and Lyzozyme (11.5 kDa) are freely filtered but they are almost completely reabsorbed in the proximal tubules.
- Normal daily excretion <150 mg; ~40 - 50% is **Albumin** (67 kDa)

The renal glomerulus

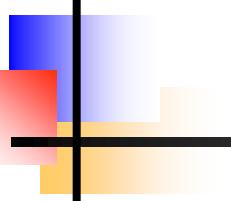


The Glomerular Filtration Barrier



PROTEINURIA: Classification

- **Overload** proteinuria:
 - Bence Jones (multiple myeloma)
 - Myoglobin (crush injury, rhabdomyolysis)
 - Haemoglobinuria
- **Tubular** proteinuria:
 - Mostly **low molecular weight proteins** (not albumin), e.g., Fanconi's syndrome, Wilson's disease, pyelonephritis, cytosis, heavy metal toxicity (Cd, Pb, Hg), galactosaemia.
- **Glomerular** proteinuria:
 - Mostly **albumin** at first, but larger proteins appear as glomerular basement membrane selectivity is lost as disease progresses.



Other Causes of Proteinuria

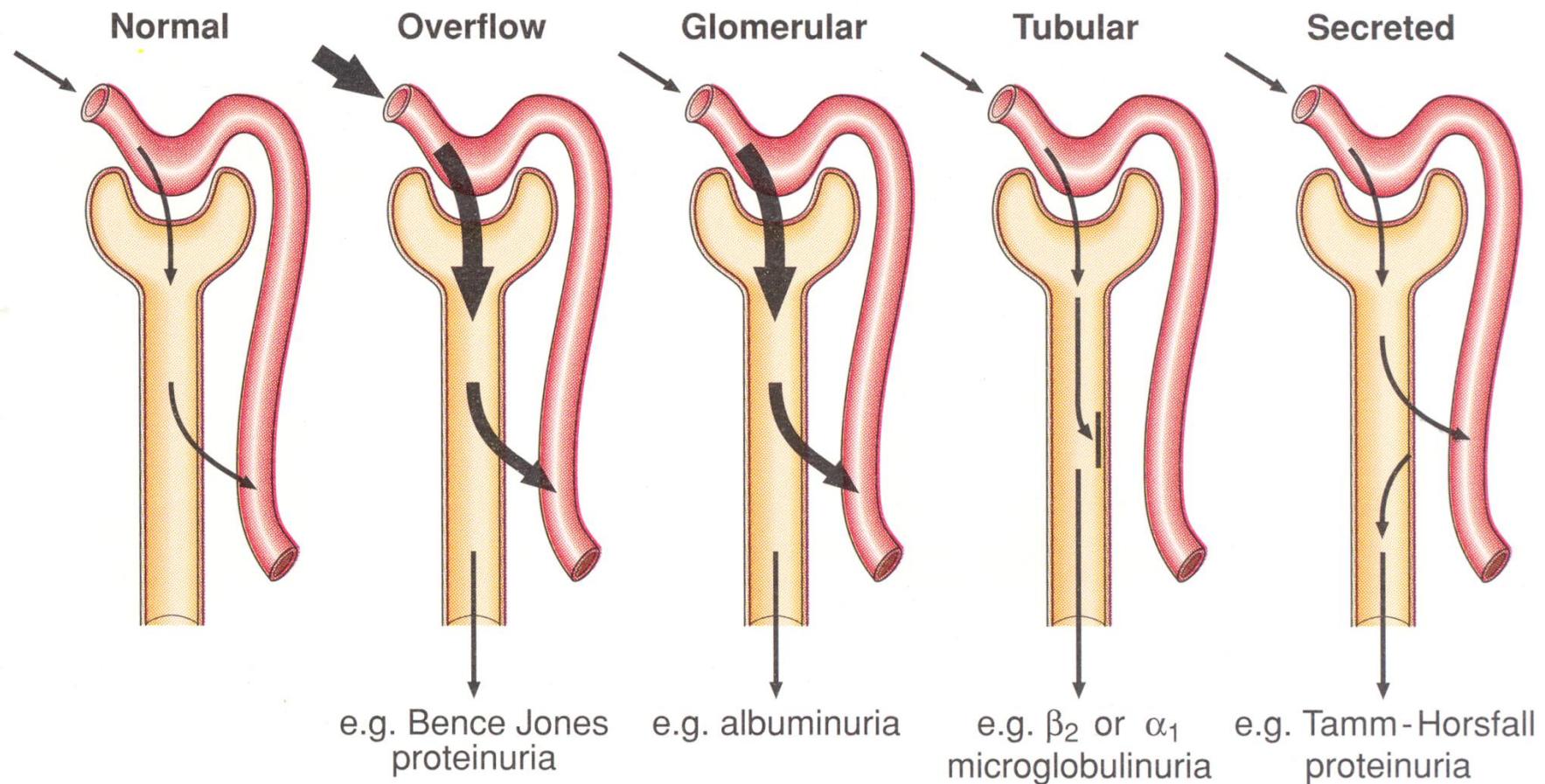
- **Orthostatic proteinuria:**

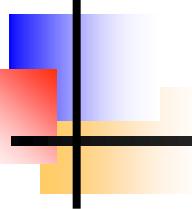
- Protein excretion varies with posture, increasing on standing. Orthostatic proteinuria present in ~ 10 - 20% **healthy subjects** at prolonged upright posture; remits if the subject remains recumbent.

- **Transient proteinuria:**

- Mild to moderate proteinuria may be found in systemic illnesses apparently not related to the kidneys, e.g., high fever, congestive heart failure, and seizures. Such transient proteinuria may also be found in healthy athletes after strenuous exercise and often in urinary tract infection.

Classification of Proteinuria

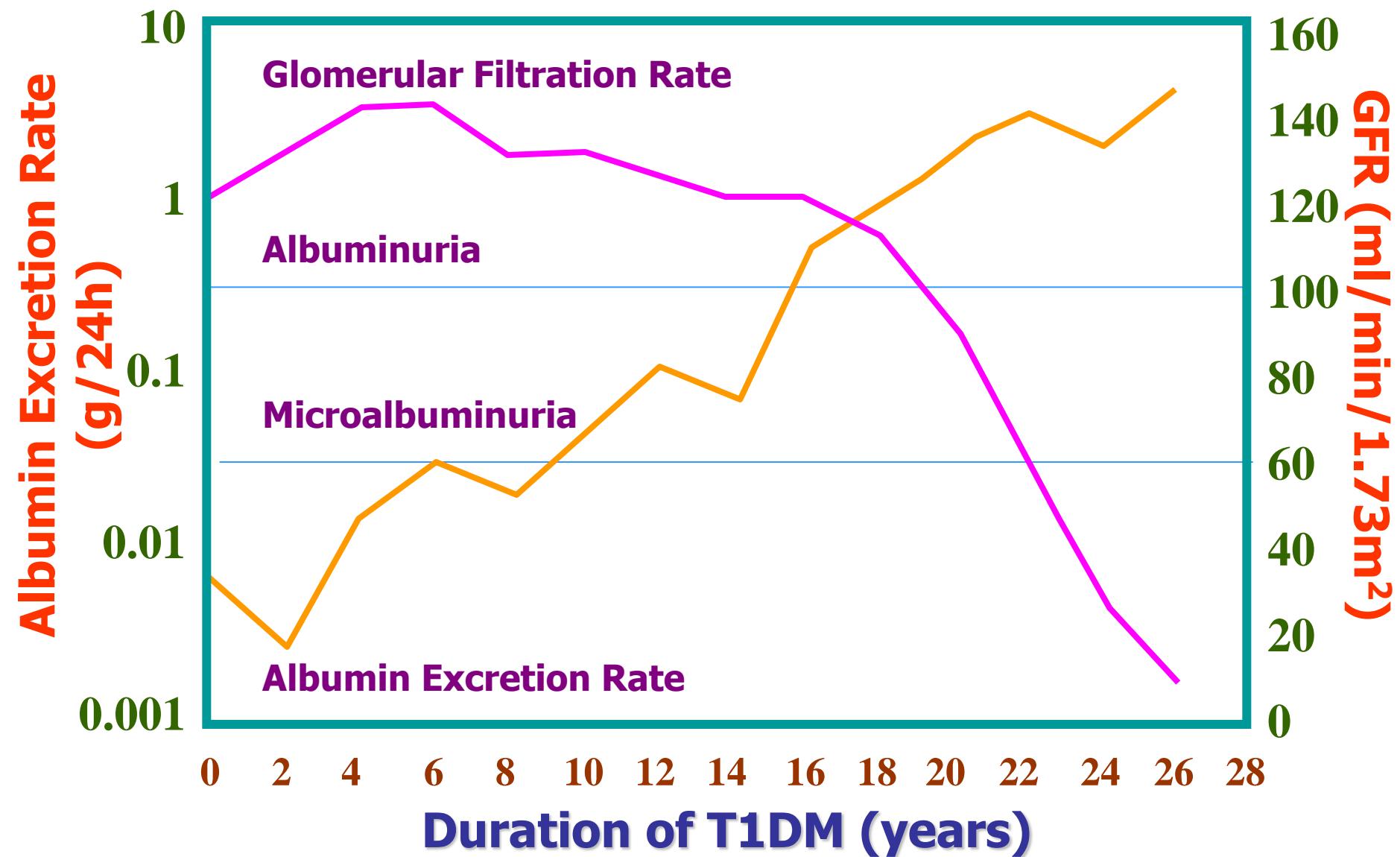




Nephrotic Syndrome

- Proteinuria $>3.5 \text{ gm/day}/1.73 \text{ m}^2$.
- Associated with hypoalbuminaemia, hyperlipoproteinaemia, peripheral oedema.
- Where protein loss is relatively selective for small molecules, larger proteins such as α_2 -macroglobulin are increased in the plasma.
- Glomerular diseases due to, e.g., diabetes mellitus (most common cause), systemic lupus erythematosus, glomerulonephritis (e.g., IgA nephropathy).

Progression of Proteinuria in Diabetes Mellitus



Arterial Blood Gases (ABG, Astrup)



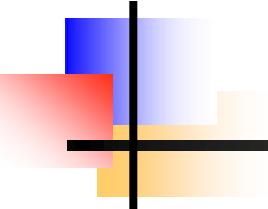
Blood Gas Analysis

Arterial Blood Gas Profile:

- pH
- PO₂
- PCO₂
- Bicarbonate (HCO₃⁻)
- Base Excess (BE)
- O₂ Saturation (%)

Collect Date :	31/12/21	31/12/21	31/12/21	31/12/21	01/01/22		
Collect Time :	16:24	16:24	21:38	23:51	04:07		
Request No. :	CC313 [REDACTED]	CC313 [REDACTED]	C101 [REDACTED]	C101 [REDACTED]	C101 [REDACTED]		
Remark :	hypoglycем ia. Please indicate if this...	hypoglycем ia	hypoglycем ia	hypoglycем ia	hypoglycем ia. Mask	Ref. Interval	Units
					Type =		
					Room	...	

Comment	Below	Below	Below	Room	Air	
Mask Type						
Flow Rate				----		L/min
% inspired O ₂				21		%
pH				7.33	L	7.35 - 7.45
pO ₂				13.7		10.6 - 14.0
pCO ₂				2.4	L	4.7 - 6.0
HCO ₃ ⁻				9	L	22 - 26
Base excess				-15	L	(-4) - (+2)
						mmol/L



Acid-Base Disorders: Diagnosis & Management

- Acid-base disorders can be classified as **acidosis** or **alkalosis**, **compensated** or **uncompensated**, fully or partially compensated.
- The clinical status of the patient and the blood gas results should always match up.
- Management of acid-base disorders should be directed towards the **correction of the underlying illness**.

Henderson-Hasselbalch Equation

$$\text{pH} = 6.1 + \log ([\text{HCO}_3^-] / [0.03 \times \text{PaCO}_2])$$

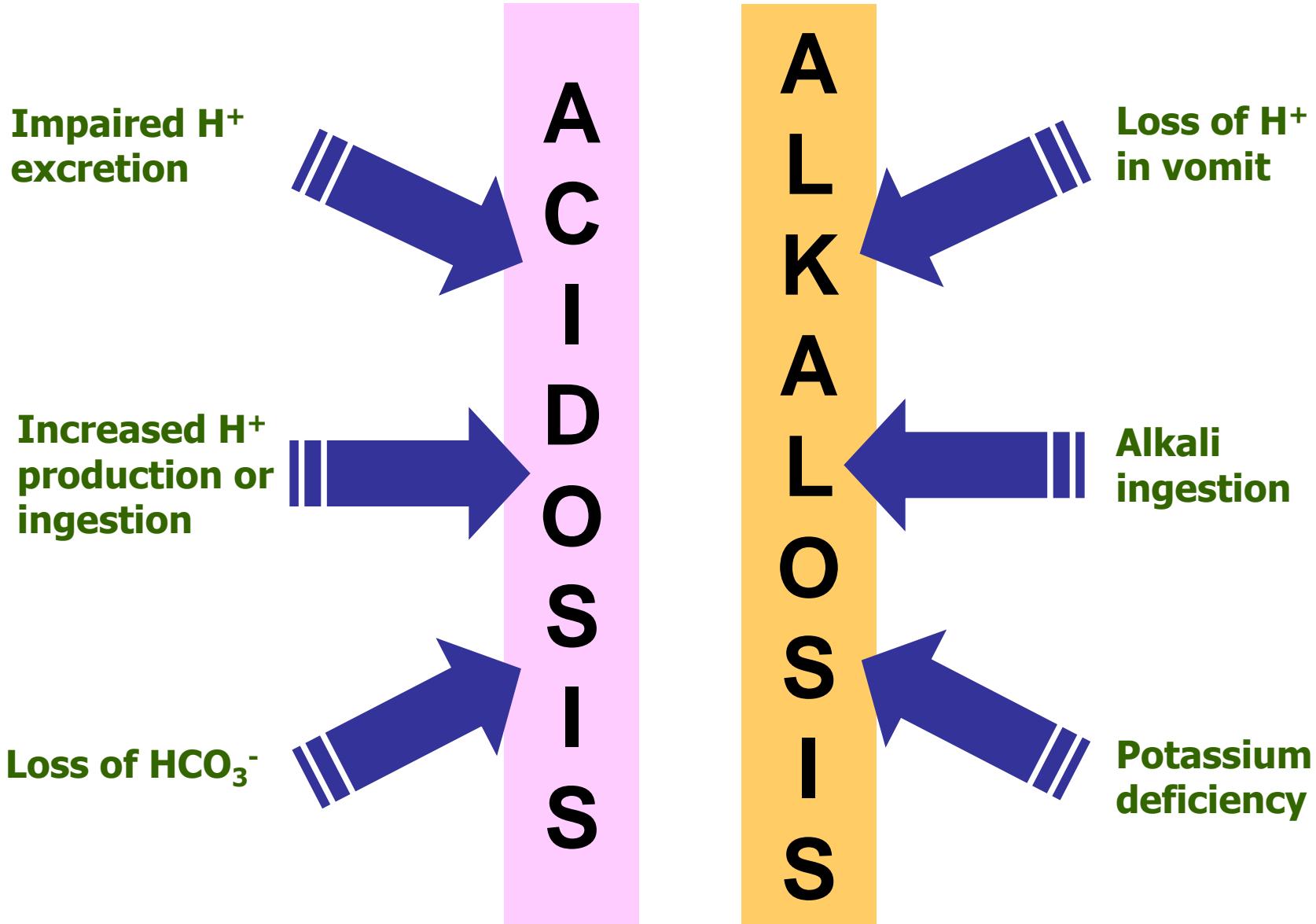
PaCO₂ (mmHg)

[HCO₃⁻] (mEq/L)

6.1 = **pKa** of Carbonic Acid at body temperature

Relationship Between the Arterial pH and H⁺ Concentration in the Physiological Range

pH	[H ⁺] nmol/L
7.80	16
7.70	20
7.60	26
7.50	32
7.40	40
7.30	50
7.20	63
7.10	80
7.00	100
6.90	125
6.80	160

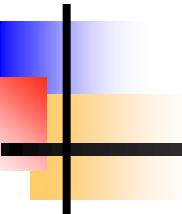


Causes of Metabolic Acidosis and Alkalosis



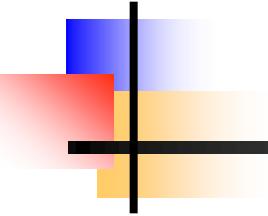
Factors Affecting Blood Gas Results

- **Delayed processing** – falsely low PaO_2 due to oxygen consumption by viable white blood cells *ex vivo*; this can be minimized by prompt delivery of the blood sample to the laboratory in an ice tray.
- **Air bubbles** in syringe – falsely high PaO_2 and falsely low PaCO_2 .
- Body temperature affects blood gas tensions – relevant in febrile or hypothermic patients; body temperature recorded at the time of blood collection helps better interpretation (not a usual practice) .



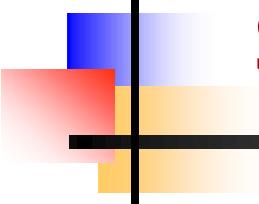
Limitations of Blood Gas Analysis

- Blood gas analysis *per se* cannot yield a specific diagnosis, however it helps differentiating the primary underlying abnormality of an acid-base derangement, i.e., metabolic *vs* respiratory or mixed; acute *vs* chronic; and gauging the severity of the disturbance.
 - e.g., a patient with asthma may have similar ABG values to another patient with pneumonia; a patient with chronic obstructive pulmonary disease and respiratory failure may have similar results to a patient with pulmonary oedema.
- The analysis does not necessarily reflect the degree to which an abnormality actually affects a patient: a low PaO_2 does not necessarily indicate tissue hypoxia, nor does a normal PaO_2 indicate adequate tissue oxygenation. Oxygen utilization is influenced by other factors such as regional blood flow, haemoglobin affinity for oxygen and cardiac output.
- Blood gas analysis cannot be used as a screening test for early pulmonary disease: severe disease may be present before significant changes are seen in blood gases.



Venous Blood Gas Analysis

- It is easier and less traumatic to obtain a venous sample than an arterial sample.
- In some situations analysis of venous blood can provide enough information to assist in clinical decisions.
- In general, the pH, CO_2 and HCO_3^- values are quite similar in venous and arterial blood.
- The main difference is the partial pressure of oxygen (PO_2) in venous blood that is less than half that of arterial blood. Venous blood should not therefore be used to assess oxygenation.



Suggested Supplementary Reading:

- GFR (Glomerular Filtration Rate) - A Key to Understanding How Well Your Kidneys Are Working. *National Kidney Foundation* www.kidney.org.
- Harsten A, Berg B, Inerot S, Muth L. Importance of correct handling of samples for the results of blood gas analysis. *Acta Anaesthesiol Scand* 1988; 32:365-8.
- William AJ. ABC of Oxygen: Assessing and interpreting arterial blood gases and acid-base balance. *BMJ* 1998;317:1213-9.

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