Clearance, RBF, and GFR
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OBJECTIVES:

After studying this lecture, the student should understand:

1. The concepts of renal clearance and clearance ratio.
2. How RBF is autoregulated, including the myogenic mechanism and tubuloglomerular feedback.
3. How RPF is measured with PAH.
4. The difference between true RPF and effective RPF.
5. How to calculate RBF.
6. Characteristics of the glomerular capillary barrier and the factors that restrict filtration.
7. How Starling forces across the glomerular capillary determine GFR.
8. How changes in Starling forces alter GFR.
9. Effects of vasoactive substances on afferent and efferent arterioles and their expected effects on RPF and GFR.
10. How GFR is measured with clearance of inulin.
11. How filtration fraction is calculated.

1. INTRODUCTION TO RENAL PHYSIOLOGY, THE NEPHRON, AND ITS BLOOD SUPPLY

Figure 1. Sagittal and coronal sections of the kidney.
Figure 2. Segments of a superficial and a juxtamedullary nephron.

Note the following segments of the nephron: proximal convoluted tubule, descending limb and thick ascending limb of loop of Henle, distal convoluted tubule, and collecting ducts. Superficial nephrons have their glomeruli in the cortex, and juxtamedullary nephrons have their glomeruli near the junction of cortex and medulla. Juxtamedullary nephrons have much longer loops of Henle.

The juxtaglomerular apparatus is a specialized region with three major components: (1) juxtaglomerular cells surround the afferent arterioles and
secrete renin; (2) macula densa is a specialized region of the early distal tubule that comes in close contact with its own glomerulus; and (3) mesangial cells line the glomerulus, and afferent and efferent arterioles. There are two important functions of the juxtaglomerular apparatus: secretion of renin by juxtaglomerular cells (discussed in the previous lecture) and tubuloglomerular feedback, which will be discussed in this lecture.

The blood supply to the kidney consists of the following components listed (in the direction of blood flow): renal artery, progressively smaller arteries, afferent arterioles, glomerular capillaries site of glomerular filtration), efferent arterioles, peritubular capillaries (surround nephrons, provide nutrient flow and site of reabsorption and secretion), small veins, and renal vein.

The basics of urine production include: (1) glomerular filtration of 180 L/day, which produces an ultrafiltrate of plasma; (2) modification of this ultrafiltrate by subsequent reabsorption and secretion to produce the final urine of 1-1.5 L/day.

II. RENAL CLEARANCE

A. Measurement of renal clearance

\[ C_x = \frac{U_x \cdot V}{P_x} \]

where \( C_x \) is clearance of any substance, \( U_x \) is urinary concentration of the substance, \( V \) is urine flow rate (volume per unit time), and \( P_x \) is plasma concentration of the substance.

Clearance of \( x \) is the volume of plasma cleared of substance \( x \) per unit time. Thus, units of clearance are volume/time (e.g., ml/min or L/min or L/hour or L/day).

Special note: the numerator of the clearance equation (\( U_x \cdot V \)) is equal to excretion rate (see next lecture).

B. Clearances of various substances

Substances with the highest clearances are filtered and secreted. Substances with the lowest clearances are filtered and reabsorbed. Substances that are filtered only (inulin) are glomerular markers and have a clearance equal to the glomerular filtration rate.

C. Clearance ratio

Clearance ratio = \( C_x / C_{inulin} \)

Clearance ratio has no units and expresses the clearance of substance X.
relative to the clearance of inulin (a glomerular marker). Thus, the clearance ratio gives information about the renal handling of substance X. A clearance ratio of 1.0 means that substance X is also a glomerular marker. If the clearance ratio is < 1.0, the substance is either not freely filtered, or is filtered and subsequently reabsorbed. If the clearance ratio is > 1.0, the substance is both filtered and secreted. For future reference, clearance ratio is also called fractional excretion (fraction of the filtered load excreted in urine); this statement will make sense only after we’ve covered reabsorption and secretion.

III. RENAL BLOOD FLOW

A. Autoregulation of RBF

Renal blood flow (and GFR) are kept constant over a wide range of arterial pressures by changing the resistance of the afferent arterioles, a phenomenon called autoregulation. Two mechanisms explain autoregulation:

1. Myogenic hypothesis is familiar from cardiovascular physiology. It states that when arterioles are stretched (i.e., by an increase in \( P_a \)), they contract, leading to an increased resistance. The increased resistance prevents an increase in blood flow that would have otherwise occurred in response to the increased \( P_a \). In the kidney, it is the afferent arterioles that are
stretched, and then contract, in response to increased $P_a$. Thus, an increase in $P_a$ results in constriction of afferent arterioles, which causes an increase in afferent arteriolar resistance that decreases RBF (and GFR) back to normal (i.e., keeps it constant).

2. **Tubuloglomerular feedback**

![Tubuloglomerular feedback](image)

Tubuloglomerular feedback in an autoregulatory mechanism unique to the kidney. When $P_a$ increases, both RBF and GFR *transiently* increase. The increase in GFR leads to increased delivery of solutes and water to the macula densa of the distal tubule. The macula densa senses a component of the increased delivered load (either luminal $\text{Na}^+$ or $\text{Cl}^-$ concentration) and, in response, secretes a vasoactive substance (probably *adenosine*, not *renin*) that causes local constriction of nearby afferent arterioles. This vasoconstriction then decreases RBF and GFR back to normal, i.e., autoregulation.
B. **Measurement of renal plasma flow (RPF) and renal blood flow (RBF).** Renal plasma flow (RPF) is measured with a specific marker substance. Renal blood flow (RBF) is then calculated from the measured RPF (by using hematocrit).

1. **True renal plasma flow.** Renal plasma flow (RPF) is measured by applying the Fick principle of conservation of mass. The amount of a substance entering the kidney (via the renal artery) must be equal to the amount leaving the kidney via the renal vein + ureter (assuming that substance is not synthesized or metabolized by the kidney). The substance that is used to measure RPF is an organic acid, **para-aminohippuric acid (PAH).** PAH is both filtered and secreted by the kidney, thus it has a very high renal clearance; as you will appreciate, its high renal clearance makes it an appropriate marker for measuring RPF. (Incidentally, a constant PAH concentration in blood is maintained by an infusion that replaces what is excreted in urine.)

![Fick Principle for Measuring RPF](image)

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Figure 5. Measurement of renal plasma flow by the Fick principle. PAH, Para-aminohippuric acid; [RA], concentration in renal artery; [RV], concentration in renal vein; [U], concentration in urine; RPF, renal plasma flow.

Amount of PAH entering the kidney = RPF x PAH concentration in renal artery.
Amount leaving the kidney = RPF x PAH concentration in renal vein + urine flow rate (V) x PAH concentration in urine.

Applying the basic premise that PAH in = PAH out, rearranging, and solving for RPF:

\[
\text{“True” RPF} = \frac{[U]_{PAH} \times V}{[RA]_{PAH} - [RV]_{PAH}}
\]

2. **Effective renal plasma flow (clearance of PAH).** Two simplifications can be applied to the equation for RPF above to make it “user-friendly” in humans. (1) Because PAH is both filtered and secreted, it has an extremely high renal clearance. Thus, in one pass of blood through the kidney, almost all PAH that entered in renal arterial blood is excreted in the urine, leaving very little in the renal vein. If we assume that there is no PAH in the renal vein, then we don’t need to measure PAH in the renal vein. (2) We also don’t need to measure PAH in the renal artery because the PAH concentration in renal artery is identical to PAH concentration in systemic venous blood (except for renal venous blood); therefore, we can simply measure PAH in venous plasma, which is called \( P_{PAH} \). By applying these two simplifications, we arrive at:

\[
\text{“Effective” RPF} = \frac{[U]_{PAH} \times V}{[P]_{PAH}} = C_{PAH}
\]

3. Notice that effective RPF is also the clearance of PAH! “Effective” RPF underestimates “true” RPF by about 10% because about 10% of RPF supplies portions of the kidney (e.g., adipose) that have nothing to do with filtration and secretion of PAH; PAH in that small portion of RPF is not excreted in urine, and ends up in the renal vein. In other words, renal vein PAH is not exactly zero (as we had assumed), but it is nearly zero. As a student, you are naturally wondering: when do I calculate effective RPF vs true RPF. If you are given all the values needed for the true RPF equation, then use them. Otherwise, calculate effective RPF.

4. **Calculating renal blood flow**

\[
\text{RBF} = \frac{\text{RPF}}{1 - \text{Hct}}
\]

Hematocrit is the fractional blood volume occupied by blood cells. Thus, 1-Hct is the fractional blood volume occupied by plasma.
5. **Example**

If renal artery PAH concentration is 1.1 mg/ml, renal vein PAH concentration is 0.1 mg/ml, urine PAH concentration is 650 mg/ml, urine flow rate is 1 ml/min, and hematocrit is 0.45, what is the true renal blood flow? (Answer = 1182 ml/min)

6. **What’s the relationship between RPF and RBF?** (To help you picture “things.”)

   Renal plasma flow (RPF) is to renal blood flow (RBF) as plasma is to whole blood. Simple as that. Plasma is the fluid part of whole blood; plasma is 93% water (called plasma water) and 7% plasma proteins. The rest of whole blood is the cells. Fractional volume occupied by RBCs = Hct; fractional volume occupied by plasma = 1 - Hct.

   Why would we want to know renal plasma flow? After all, it's whole blood that flows into the renal artery, not just plasma. The reason we want to know RPF is that's the parameter we can measure with PAH. PAH (the marker for RPF) is dissolved only in plasma, not in RBCs. So...we measure RPF with PAH, and we calculate the RBF from the RPF, by knowing the hematocrit. If we could put a flowmeter on the renal artery, we could measure RBF directly......but we can't, so we use the PAH method.

**IV. GLOMERULAR FILTRATION**

Glomerular filtration is the first step in urine formation. Substances in glomerular capillary blood are filtered across the glomerular capillaries into Bowman’s space (the first part of the proximal tubule). This ultrafiltrate contains water and the small solutes of plasma, but, because of size and charge restrictions of the glomerular barrier, no protein or blood cells.
A. Glomerular capillary barrier consists of three layers: an endothelial cell layer of 70-100 angstroms, which has filtration pores; a basement membrane; an epithelial cell layer with podocytes that attach to the basement membrane by foot processes, interspersed with narrow filtration slits. There are fixed negative charges on the filtration slits and the basement membrane that impede the filtration of large negatively charged solutes like plasma proteins.

Sieving coefficient expresses the ability of a particular solute to be filtered across the glomerular capillary barrier. A sieving coefficient of 1.0 means that there is no restriction, i.e., the substance is freely filtered. A sieving coefficient < 1.0 means the substance does not freely cross the barrier, i.e., is restricted; the lower the coefficient, the more restricted the crossing.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Molecular weight (daltons)</th>
<th>Sieving coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>23</td>
<td>1.0</td>
</tr>
<tr>
<td>K⁺</td>
<td>39</td>
<td>1.0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>36</td>
<td>1.0</td>
</tr>
<tr>
<td>Water</td>
<td>18</td>
<td>1.0</td>
</tr>
<tr>
<td>Urea</td>
<td>60</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>180</td>
<td>1.0</td>
</tr>
<tr>
<td>Inulin</td>
<td>5,200</td>
<td>1.0</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>16,900</td>
<td>0.75</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>68,000</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>69,000</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Molecular weight (and molecular radius) is a major factor that determines filterability. Substances with molecular weight <5500 are freely filtered across glomerular capillaries. For example, even inulin (a large fructose
polymer) with molecular weight of 5200 makes the size “cut-off” and is freely filtered. Above 5500, with increasing molecular weight, filtration is more and more restricted; for example, myoglobin has limited filtration, and normally there are only traces of hemoglobin and albumin in the glomerular filtrate. The other factor that determines filterability is charge on the solute. Because the glomerular barrier is lined with fixed negative charges, solutes that are also negatively charged, such as albumin, are further restricted from filtration. Importantly, some glomerular diseases are characterized by loss of the fixed negative charge on the glomerular barrier, allowing albumin to be filtered and thus present in the urine.

B. Starling forces determine GFR

\[
GFR = K_f \times \text{net ultrafiltration pressure} = K_f \times [(P_{GC} - P_{BS}) - \pi_{GC}]
\]

Starling forces (pressures) across glomerular capillaries are analogous to Starling forces in other capillaries. The nomenclature is changed slightly as follows. Instead of \(P_c\) for capillary hydrostatic pressure, we use \(P_{GC}\). Instead of \(\pi_c\) for capillary colloidosmotic pressure, we use \(\pi_{GC}\). The fluid in Bowman’s space is analogous to interstitial fluid. Finally, there are three, rather than four, Starling forces in glomerular capillaries – assuming no protein is filtered, colloidosmotic pressure in Bowman’s space is zero and can be ignored.

The equation above states that the sum (net) of the Starling forces determines the net ultrafiltration pressure, which is the driving force. Net ultrafiltration pressure, multiplied by the water permeability \((K_f)\) of glomerular capillaries is the GFR. In glomerular capillaries, the net ultrafiltration pressure always favors filtration (never absorption).
Figure 7. Starling forces across the glomerular capillaries. A, Net filtration; B, filtration equilibrium. Arrows show the direction of the Starling pressures; numbers are the magnitude of the pressure (mm Hg); + signs show pressures favoring filtration; - signs show pressures opposing filtration. PGC, Hydrostatic pressure in the glomerular capillary; PBS, hydrostatic pressure in Bowman's space; \( \pi_{GC} \), oncotic pressure in the glomerular capillary.

**Panel A** is a snapshot of the Starling pressures as blood has just entered the glomerular capillary from the afferent arteriole (the pressures are ‘spread out’ across the capillary diagram for readability). At this point, the net ultrafiltration pressure is +16 mm Hg, favoring filtration.

**Panel B** is a snapshot of the pressures as the blood is about to leave the glomerular capillary via the efferent arteriole (again, spread out for readability). What happens along the capillary, i.e., between A and B? When blood enters the capillary, the driving force strongly favors
filtration. Filtration occurs, fluid and small solutes (but not protein or blood cells) are lost from the capillary, causing the protein concentration and oncotic pressure ($\pi_{gc}$) of the glomerular capillary blood to increase. The increase in $\pi_{gc}$ opposes filtration and eventually, there is no net ultrafiltration pressure, no driving force, and glomerular filtration stops (filtration equilibrium). Note that blood leaving the glomerular capillary has a high $\pi_c$ which will be an important fact when we discuss proximal tubule reabsorption.

C. Changes in Starling forces change GFR.

<table>
<thead>
<tr>
<th>Effect</th>
<th>RPF</th>
<th>GFR</th>
<th>Filtration Fraction (GFR/RPF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constriction of afferent arteriole</td>
<td>↓</td>
<td>↓</td>
<td>N.C.</td>
</tr>
<tr>
<td>Constriction of efferent arteriole</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Increased plasma protein concentration</td>
<td>N.C.</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Decreased plasma protein concentration</td>
<td>N.C.</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Constriction of the ureter</td>
<td>N.C.</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

GFR, Glomerular filtration rate; N.C., no change; RPF, renal plasma flow.

Figure 8.

1. Constriction of the afferent arteriole

2. Constriction of the efferent arteriole
3. Increasing plasma protein concentration

4. Constriction of the ureter

5. Simultaneous constriction of afferent and efferent arterioles

Some substances have effects on both afferent and efferent arterioles. Their overall effect on GFR depends on which effect is stronger (i.e., whether $P_{GC}$ increases or decreases). For example, **angiotensin II** constricts both afferent and efferent arterioles, but preferentially constricts efferents. At low concentrations, angiotensin II constricts efferent arterioles more than afferents; thus, RPF decreases, but $P_{GC}$ and GFR increase (or are maintained). This preferential effect of angiotensin II is
important clinically, as it “protected” GFR during high vasoconstrictor states (e.g., response to hemorrhage). **ACE inhibitors** are used to treat hypertension because they dilate arterioles; in the kidney, they preferentially dilate efferent arterioles, thus increasing RPF, but decreasing GFR (potentially dangerous, therefore, in renal failure).

**Summary of effects of vasoactive substances on renal arterioles**

- **Sympathetic nervous system** (α₁ receptors)
  - Vasoconstriction of afferent and efferent arterioles
  - More α₁ receptors on afferent arterioles
  - Decreases RBF
  - Decreases GFR

- **Angiotensin II**
  - Vasoconstriction of afferent and efferent arterioles
  - Efferent more sensitive than afferent arterioles
  - Decreases RBF
  - Increases or maintains GFR

- **ANP**
  - Vasodilation of afferent arterioles
  - Vasoconstriction of efferent arterioles
  - Increases RBF
  - Increases GFR

- **NO**
  - Vasodilation of afferent and efferent arterioles
  - Increases RBF

- **Prostaglandins**
  - Vasodilation of afferent and efferent arterioles
  - Released locally (in kidney) in high vasoconstrictor states
  - Modulate, or offset, vasoconstriction by sympathetic and Angiotensin II
  - Protect RBF and GFR

- **Dopamine (low dose)**
  - Vasodilation of afferent and efferent arterioles
D. Measurement of GFR (clearance of inulin)

\[
\text{GFR} = \frac{[U]_{\text{inulin}} \times V}{[P]_{\text{inulin}}}
\]

Inulin is a "glomerular marker," and its clearance measures GFR. Being a glomerular marker means that inulin is filtered, but neither reabsorbed nor secreted. Inulin is not an endogenous substance, thus it must be administered to measure GFR. Creatinine, however, is endogenous, and it is a near-perfect glomerular marker; therefore, creatinine clearance can also be used to measure GFR.

Serum creatinine concentration and blood urea nitrogen (BUN) can be used to estimate changes in glomerular filtration rate. Both creatinine and urea are normally filtered and then excreted. If GFR decreases, there is less filtration and less excretion of creatinine and urea, and their blood concentrations increase.

E. Why is the clearance of PAH the RPF (effective RPF), while the clearance of inulin is the GFR?

First, different substances have different renal clearances based on how the substance is handled in the kidney. A substance that is filtered only will have a mid-range clearance and be called a glomerular marker; a substance that is filtered and reabsorbed will have much lower clearance; a substance that is filtered and secreted will have a much higher clearance. Clearance is ml/min of plasma cleared, or ridded, of that substance.

To illustrate the difference between what is measured by the clearances of PAH and inulin, let's put some PAH and inulin into the plasma and have that plasma flow into the renal artery and then into the afferent arterioles. 20% of that RPF (containing inulin and PAH) is filtered across glomerular capillaries; the inulin and PAH in that 20% of the RPF will be excreted, i.e., that portion of the RPF is cleared of its inulin and its PAH. The remainder (80%) of the RPF that is not filtered flows into the efferent arterioles and then into peritubular capillaries. The PAH (but not the inulin) in that portion of RPF is secreted and then excreted; thus that portion of the RPF is cleared of its PAH, but not of its inulin.

Bottom lines. The entire (effective) RPF is cleared of its PAH by a combination of filtration and secretion; that is why the renal vein PAH is nearly zero. Only 20% of the RPF is cleared of its inulin by filtration; that 20% is the GFR; the renal vein inulin is NOT zero.
V.  FILTRATION FRACTION

Filtration fraction = \( \frac{\text{GFR}}{\text{RPF}} \)

VI. Filtration fraction is approximately 0.2 or 20%, meaning that 20% of the renal plasma flow is filtered across the glomerular capillaries. The remaining 80% leaves by the efferent arterioles and becomes the peritubular capillary blood flow.

Clearance of inulin = GFR = \( \approx 120 \text{ ml/min} \). Clearance of PAH = effective RPF = \( \approx 600 \text{ ml/min} \). Filtration fraction = \( \frac{120}{600} = 20\% \); that's the 20% that is filtered. It works!

VII. PRACTICE QUESTIONS

1. In a urine sample, inulin concentration is 120 mg/ml and PAH concentration is 1220 mg/ml. In plasma sample, inulin concentration is 1 mg/ml and PAH concentration is 2 mg/ml. Urine flow rate is 1 ml/min. What is GFR, effective RPF, and filtration fraction?

2. If a drug is administered that causes selective vasodilation of the efferent arteriole, what effects would you predict on GFR, RPF, filtration fraction?

3. If a drug is administered that causes selective vasodilation of the afferent arteriole, what effects would you predict on GFR, RPF, filtration fraction?

4. Which of the following would cause an increase in RPF, increase in GFR, and increase in filtration fraction?

   - Angiotensin II
   - ACE inhibitor
   - Activation of sympathetic nervous system
   - ANP

ANSWERS
1. GFR, 120 ml/min; effective RPF, 610 ml/min; filtration fraction, 0.197

2. Decrease, increase, decrease

3. Increase, increase, no change

4. ANP (dilates afferent, which increases RPF and GFR; constricts efferent which increases GFR; thus, GFR will increase more than RPR, which increases filtration fraction)