Metabolism & Elimination of Protein Therapeutics

- Protein-based therapeutics is generally subject to the same **catabolic pathways as endogenous proteins.** The **end products** of protein metabolism are thus **amino acids.**
- **Non-metabolic elimination pathways such as renal or biliary excretion** are negligible for most proteins.

Proteolysis

The **metabolic rate for protein degradation generally increases with decreasing molecular weight** from large to small proteins to peptides (**Table below**), but is also dependent on other factors such as:

- Size.
- Charge.
- Lipophilicity.
- Functional groups.
- Glycosylation.
- Secondary and tertiary structure.

Molecular weight	Elimination site	Predominant elimination mechanisms	Major determinant
<500	Blood, liver	Extracellular hydrolysis Passive lipoid diffusion	Structure, lipophilicity
500–1,000	Liver	Carrier-mediated uptake Passive lipoid diffusion	Structure, lipophilicity
1,000–50,000	Kidney	Glomerular filtration and subsequent degradation processes (see Fig. 4)	Molecular weight
50,000–200,000	Kidney, liver	Receptor-mediated endocytosis	Sugar, charge
200,000–400,000		Opsonization	α_2-macroglobulin, IgG
>400,000		Phagocytosis	Particle aggregation

Note: Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability for proteases, aggregation to particles, formation of complexes with opsonization factors, etc. Mechanisms may overlap and endocytosis may occur at any molecular weight range.
Source: After Meijer and Ziegler, 1993.

Table Molecular weight as major determinant of the elimination mechanisms of peptides and proteins.

Sites capable of extensive peptide and protein metabolism are not only limited to the liver, kidneys, and gastrointestinal tissue, but also include blood and vascular endothelium as well as other organs and tissues.

The proteolytic activity of SC tissue, for example, results in a partial loss of activity of SC compared to IV administrated interferon-g.

Gastrointestinal Protein Metabolism

The gastrointestinal tract is a major site of protein metabolism with high proteolytic enzyme activity due to its primary function to digest dietary proteins. Thus, gastrointestinal metabolism of protein drugs is one of the major factors limiting systemic bioavailability of orally administered protein drugs. The metabolic activity of the gastrointestinal tract, however, is not limited to orally administered proteins.

Parenterally administered peptides and proteins may also be metabolized in the intestinal mucosa following intestinal secretion.

At least 20% of the degradation of endogenous albumin, for example, has been reported to take place in the GIT.

Renal Protein Metabolism and Excretion

- The **kidneys are a major site of protein metabolism for smaller sized proteins that undergo glomerular filtration**

- The size-selective cut-off for glomerular filtration is approximately 60 kD, Glomerular filtration is most efficient, however, for proteins smaller than 30 kDa.

Renal metabolism of peptides and small proteins is mediated through three highly effective processes (Fig.). As a result, only minuscule amounts of intact protein are detectable in urine.

1. The first mechanism involves glomerular filtration of larger, complex peptides and proteins followed by: Reabsorption into endocytic vesicles in the proximal tubule. Subsequent hydrolysis into small peptide fragments and amino acids.

2. The second mechanism C glomerular filtration followed by intraluminal metabolism, predominantly by exopeptidases in the luminal brush border membrane of the proximal tubule. The resulting peptide fragments and amino acids are reabsorbed into the systemic circulation.

3. The third mechanism of renal metabolism is peritubular extraction of peptides and proteins from post-glomerular capillaries with subsequent intracellular metabolism.

Peritubular transport of proteins and peptides from the basolateral membrane has also been shown for insulin.

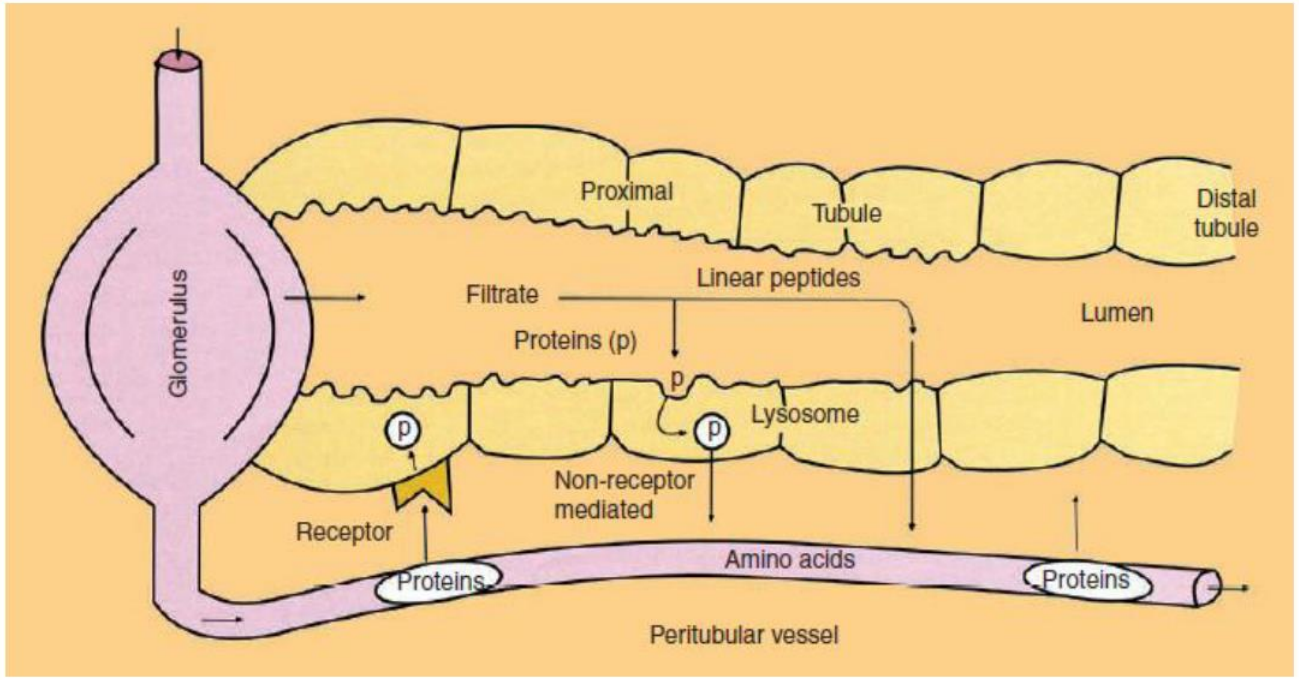

Figure : Pathways of renal metabolism of peptides and proteins: Glomerular filtration followed by either

(a) intraluminal metabolism or

(b) tubular reabsorption with intracellular lysosomal metabolism, and

(c) peritubular extraction with intracellular lysosomal metabolism

Hepatic Protein Metabolism

Aside from renal and gastrointestinal metabolism, the liver may also play a major role in the metabolism of protein therapeutics.

Exogenous as well as endogenous proteins undergo proteolytic degradation to dipeptides and amino acids that are reused for endogenous protein synthesis.

Proteolysis usually starts with endopeptidases that attack in the middle part of the protein, and the resulting oligopeptides are then further degraded by exopeptidases