Biochemistry #02

The biochemical basis of skeletal muscle and bone disorders

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Greetings everyone, ladies and gentlemen…

The biochemical basis of skeletal muscles, bone disorders and diseases

Muscular disorders can be due either to:

- Muscular dystrophy
  - Becker and Duchenne muscular dystrophy
- Metabolic myopathies
  - Muscle glycogen storage diseases
  - Fatty acid oxidation diseases

Whereas bone disorders are related to:

- Imbalance in bone remodeling

**Muscular dystrophy**

Dystrophin is a rod-shaped cytoplasmic protein, and a vital part of a protein complex that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix through the sarcolemma, and so has a critical role in muscular development. This type of muscular dystrophy is related to deletion mutations in the dystrophin gene.

The N and C terminal domains are the most essential regions needed for proper functioning of dystrophin protein, therefore mutations in those regions causes the defective protein to completely lose its function = *Duchenne muscular dystrophy (DMD)*. Whereas mutations in the spectrin-like domain have a very minimum effect, comparative with mutations in the N and C termini, leaving the dystrophin protein partially functional = *Becker muscular dystrophy (BMD)*

In both cases patients suffer from late walking, but due to the difference in the defective protein structures produced, different phenotypes are presented by the patients.
I. **Becker muscular dystrophy (BMD)**

In-frame deletion of a large region of the spectrin-like repeats, as in one form of BMD (shown by the large white rectangle) shortens the molecule but retains the important N- and C-terminal domains. BMD is a less-severe disease (patients are still walking after 12 yrs).

II. **Duchenne muscular dystrophy (DMD)**

In contrast, in a case of DMD, a smaller out-of-frame deletion (the deletion is shown as a small white rectangle and the out-of-frame sequence is shown as a cross-hatched rectangle) results in a protein lacking the dystrophin C-terminal domain; DMD is a more-severe disease (patients are not walking at 12 yrs).

The severity of the diseases is not correlated with the size of the deletion. Although the mutation deletion in DMD (C-terminal deletion) is much smaller, yet it resulted in a much more defective dystrophin and a severe case of muscular dystrophy, compared to BMD (spectrin-like domain deletion). Deletion in N terminal is not discovered yet, but it would probably resemble DMD.
**Metabolic myopathies**

A Heterogeneous group of disorders, sharing the common feature of inadequate production of cellular energy, due to defect in muscular metabolism.

**Review on muscle energy metabolism and metabolic pathways**

Under normal circumstances, energy for skeletal muscle function (in the form of ATP) is derived from:

- Muscle stored glycogen (via glycogenolysis)
- Blood glucose
- Free fatty acids (via beta oxidation)

The majority of fuel for muscle is provided by carbohydrates, which are in the form of glycogen. Whereas energy supplied by lipids comes in the form of free fatty acids.

In the cytosol, glycogen gets degraded down to glucose, by an enzyme called glycogen phosphorylase. Then through the process of glycolysis (aerobic pathway), glucose is then oxidized to pyruvate. While in anaerobic conditions, glucose is converted to lactate. Inside the mitochondria, pyruvate is then decarboxylated to Acetyl-Coenzyme A (acetyl-CoA, which is a high energy compound) by pyruvate dehydrogenase complex, similarly, fatty acid oxidation (FAO) through beta oxidation pathway provides another source of acetyl-CoA.

Acetyl-CoA then enters the Krebs cycle (TCA), undergoes oxidation, to generate reduced electron carriers (NADH and FADH$_2$) which are strong reducing powers.

NADH and FADH$_2$ undergo oxidative phosphorylation, delivering electrons to the mitochondrial electron transport chain, thus driving the production of energy and generating ATP.

\[
\begin{align*}
\text{NADH} & \rightarrow 3\text{ATP} \\
\text{FADH}_2 & \rightarrow 2\text{ATP}
\end{align*}
\]

Genetic mutations causing defects in enzymes of any of the steps involved in this complex metabolic pathway, can lead to an insufficient supply of ATP and an inability to sustain normal muscle function, finally resulting in diseases concerning energy production in muscles.
I. Muscle glycogen storage disease

Glycogen storage diseases may also be related to liver.

- **Type V (McArdle Disease)**

  Deficiency in glycogen phosphorylase, results in accumulation of glycogen in the muscle and eventually destroying it.

  Confirmatory tests:
  - Sampling glycogen phosphorylase and assessing its activity
  - DNA testing by amplifying the gene of the enzyme and testing for known mutations that cause defects in the enzyme.

Biochemical markers:
- An elevated CK (normal = 30 - 220 U/L)
  
  *Note: Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phosphocreatine kinase is expressed by various tissues and cell types. CK catalyses the conversion of creatine by utilizes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CK enzyme reaction is reversible and thus ATP can be generated from PCr and ADP. The mitochondrial creatine kinase (CKm) is present in the mitochondrial intermembrane space, where it regenerates phosphocreatine (PCr) from mitochondrial generated ATP and creatine (Cr) imported from the cytosol.*

- Mild elevations of aspartate aminotransferase (15 – 41 U/L), and alanine aminotransferase (normal = 17 – 63 U/L)

- Urine-analysis is negative for myoglobin

- **Type VII**

  Deficiency in phosphofructokinase, which is an essential enzyme in glycolysis.

Biochemical markers:
- Hemolytic anemia
- Myogenic hyperuricemia (increase in blood uric acid)
- Accumulation of normal glycogen in muscle, and abnormal glycogen
Q) Why does uric acid level increase in deficiency of phosphofructokinase?

The classical form of GSDVII is the most common form. Its features usually appear in childhood. This form is characterized by muscle pain and cramps, often following moderate exercise; strenuous exercise can lead to nausea and vomiting. During exercise, muscle tissue can be abnormally broken down, releasing a protein called myoglobin. This protein is processed by the kidneys and released in the urine (myoglobinuria). If untreated, myoglobinuria can damage the kidneys and lead to kidney failure. Some people with the classical form of GSDVII develop high levels of a waste product called uric acid in the blood (hyperuricemia) because the damaged kidneys are unable to remove uric acid effectively.

II. Fatty Acid Oxidation Disorders

FAO (beta-oxidation of fatty acids) is the major source of energy during periods of sustained, low-intensity exercise or prolonged fasting.

Exercise intolerance and myoglobinuria are the most common presenting features.

The major disorders of lipid metabolism that present with isolated myopathy include:

- Carnitine palmitoyltransferase II (CPT II) deficiency
- Long-chain acyl-CoA dehydrogenase (LCHAD) deficiency
- Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency

The severity of the disease is related to the type of mutation.

Fatty acids (as Acyl-CoA) located in the cytosol get transported into the mitochondrial matrix by binding them to a carrier called carnitine, which is mediated by CPT1 enzyme found on the outer mitochondrial membrane. The Acyl-carnitine complex passes through the intermembrane space into the matrix, where it gets hydrolysed back to carnitine and Acyl-CoA, which is mediated by CPT2 enzyme found on the inner membrane.

The fatty acid is now ready for beta oxidation.

If any of the previous enzymes is defective, then no further transport of fatty acids into the matrix, no beta oxidation, lack of energy for exercise, this is why exercise is intolerable in CPT deficiency.
Q) How many ATP molecules produced per 1 molecule of Acetyl CoA completely oxidised to CO₂ and water?

Ans) Each TCA round produces 1 ATP directly, 3 NADH and 1 FADH₂. The yield is 3 ATP for each NADH oxidized and 2 ATP for each FADH₂ oxidized. So using those values, you would produce 12 ATP for each acetyl-CoA oxidized.

**Bone Remodelling**

Bone remodelling is the balance between bone resorption and bone formation. It maintains a healthy skeleton by removing of old bone and replacing it with new bone.

Imbalance in bone remodelling results in metabolic bone disease.

Bone remodelling can be measured by biochemical markers of bone resorption and formation.

Metabolic bone disorders:

- Osteoporosis
- Paget’s disease of bone.
- Osteomalacia in adults and Rickets in children
- Osteogenesis imperfecta

**I. Osteoporosis**

Osteoporosis is characterized by “weak bones”, It is a disease where decreased bone strength increases the risk of a broken bone.

The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation.

Oestrogen deficiency increases bone resorption… as well as other factors affecting bone resorption such as PTH and Vitamin D

Lab blood tests:

- PTH
- 25(OH) vitamin D
- PO₄
Biochemical markers of bone (collagen) formation:

- Bone specific alkaline phosphatase
- Osteocalcin
- Aminoterminal propeptide of type 1 collagen

Biochemical markers of bone (collagen) resorption:

- Serum C telopeptide
- Urinary N-telopeptide

**II. Osteomalacia**

Osteomalacia is characterized by "soft bones" caused by impaired bone metabolism primarily due to:

- Inadequate levels of available phosphate, calcium, and vitamin D
  
  OR

- Because of resorption of calcium

The impairment of bone metabolism causes inadequate bone remineralization.

Laboratory findings:

- Low vitamin D concentration in blood serum
- Low serum and urinary calcium
- Low serum phosphate
- Elevated serum alkaline phosphatase (due to an increase in compensatory osteoblast activity)
- Elevated parathyroid hormone (due to low calcium)
III. **Paget’s disease of bone**

It is a disease of osteoclast, characterized by accelerated rate of bone remodelling. It is of both genetic and environmental causes.

Laboratory findings:

- Specific alkaline phosphatase, is usually elevated in these patients
- Elevated procollagen type I N-terminal propeptide (PINP), serum C-telopeptide (CTx), urinary N-telopeptide (NTx), and urinary hydroxyproline
- Serum calcium and phosphorus are normal in most patients

IV. **Osteogenesis imperfecta (OI)**

Osteogenesis imperfecta (OI) is not due to bone remodelling imbalance as the previous bone diseases, but it is the result of a mutation in one of the two polypeptide genes (alpha1) that carries instructions for making type 1 collagen (the major protein in bone and skin).

The mutation may result in either a change in the:

- Structure of type 1 collagen molecules (quality)
- Number of collagen molecules made (quantity)

Either of these changes results in weak fragile bones that fracture easily.

Edited by: Raya al-Rashdan

*The End*

“What's dead may never die, but rises again harder and stronger”