READING GUIDE

PART 1: TRICARBOXYLIC ACID CYCLE AND PYRUVATE DEHYDROGENASE COMPLEX

Objectives

1. Diagram the reactions of the TCA cycle and explain the key points of regulation of the cycle.
2. Describe how pyruvate supplies acetyl-CoA to the TCA cycle and the control of this enzymatic step.
3. Describe the mechanism of arsenic poisoning in the TCA cycle
4. Describe pyruvate dehydrogenase complex deficiency disease and how it is treated.

REACTIONS OF THE TCA CYCLE

Big Picture (Fig. 9.1)

The central function of the TCA cycle is to convert acetate to CO_2 and in the process deliver reducing equivalents to the electron transport chain for production of ATP. In this role, the TCA cycle is the converging point for the metabolism of carbon from sugars, fats and amino acids. It is equally important for the production of building blocks for the synthesis of fats, glucose, amino acids, heme and other derivative compounds, as we shall see in later sections of the course.

Pyruvate dehydrogenase (PDH) (Fig. 9.2,3)

PDH supplies acetyl-CoA from pyruvate to the TCA cycle. It is an important point of regulation for the entry of acetate into the TCA cycle.

How many enzyme activities are found in the PDH complex?

How is PDH regulated? What happens to PDH when pyruvate levels are high? What happens when NADH levels and ATP are high?

What cofactors are needed in the PDH complex?

Is it possible to have a deficiency in pyruvate dehydrogenase? What is the end product of glycolysis in these patients?

How does arsenic poison this enzyme system?

Synthesis of citrate and subsequent conversion to α-ketoglutarate (Fig. 9.4)

How is citrate synthase regulated?

What does aconitase do, in addition to converting citrate to isocitrate? Are you surprised that it has an iron bound in its active site? (Think back to last semester biochemistry.)

Why is conversion of isocitrate to α-ketoglutarate an important step in the TCA cycle?

What are the allosteric regulators of isocitrate dehydrogenase? (Could you have predicted these?)

Conversion of α-ketoglutarate to malate (Fig. 9.5)

What does the α-ketoglutarate dehydrogenase complex have in common with the pyruvate dehydrogenase complex? (think cofactors). How is it different? (think regulation)

What steps in the conversion of α-ketoglutarate to malate contribute to energy production by the mitochondria?

What is different about succinate dehydrogenase compared to the other enzymes or enzyme complexes of the TCA cycle?

Why does the oxidation of malate to oxaloacetate proceed when the ΔGo is positive?

Regulation of TCA cycle (Fig. 9.8)

What steps of the TCA cycle are critical for its regulation? What do these steps have in common?

Can you do a 'back of the napkin' calculation of the ATP derived from each step of the cycle? (use the assumption of 3 ATP per NADH and 2 per $FADH_2$). What does this calculate to per glucose?

Summary (Fig. 9.9)

PART 2: GLUCONEOGENESIS

OBJECTIVES

1. Diagram and describe gluconeogenesis
2. Describe how glycerol, lactate, and certain amino acids are converted into glucose.
3. Describe the mechanism whereby the three irreversible steps of glycolysis are circumvented in gluconeogenesis.
4. Describe the regulation of gluconeogenesis and why it cannot operate concomitantly with glycolysis.
5. Diagram and describe how the Cori cycle operates

GLUCONEOGENIC PRECURSORS

Big picture

Gluconeogenesis is the synthesis of glucose from suitable precursor molecules. Most of the process is simply glycolysis running in reverse. But the three highly energetically favorable reactions in glycolysis need to be bypassed with alternative reactions in gluconeogenesis. These three reactions and their regulation is where most of our attention will be focused.

What are the three precursor molecules that are used in gluconeogenesis?

What is the Cori cycle and why is it important? Fig. 10.2

KEY REACTIONS IN GLUCONEOGENESIS

Conversion of pyruvate to phosphoenolpyruvate Fig. 10.3

The 1-step conversion of phosphoenolpyruvate (PEP) to pyruvate is reversed in 4 synthetic steps. What are they?

What is the role of biotin in pyruvate carboxylase? (remember this role, it will come in handy when we discuss lipid metabolism)

What is the metabolic difference to the cell when PEP carboxykinase in the mitochondria converts oxaloacetate to PEP, with subsequent transport of PEP to the cytosol, versus transporting malate and having PEPCK act in the cytosol to produce PEP? (think redox balance between mitochondria and cytosol)

What molecule serves as a positive allosteric effector of pyruvate carboxylase?

Conversion of fructose 1,6-bisphosphate to fructose 6-phosphate Fig. 10.4,5

What enzyme catalyzes this reaction?

How does the energy state of the cell regulate this step of gluconeogenesis?

What prevents the activities of phosphofructokinase-1 and fructose bisphosphatase-1 from setting up a futile cycle of converting fructose back and forth between the 6-phosphate form and the 1,6-bisphosphate form?

How is glucagon involved in the regulation of this important step of gluconeogenesis?

Conversion of glucose 6-phosphate to glucose Fig. 10.6

Glucose 6-phosphatase is expressed specifically in the liver and kidney. Why would that be?

Where within the cell is glucose 6-phosphatase located?

REGULATION OF GLUCONEOGENESIS

Role of glucagon Fig. 10.5,8,9

What are the three ways, mentioned in Harvey's, that glucagon regulates stimulates gluconeogenesis?

What trans-acting factor would you predict would be involved with the glucagon-induced transcription of the PEPCK gene? (think back……)

Cortisol also up-regulates the PEPCK gene. What transcription factor (hint: ligand activated) would you predict to be involved in this pathway?

What is the role of amino acids in gluconeogenesis? (more on this later…)

What is the source of acetyl-CoA that is necessary to stimulate gluconeogenesis in the liver?

How does acetyl-CoA influence gluconeogenesis?

How does AMP influence gluconeogenesis?

Summary Fig. 10.9