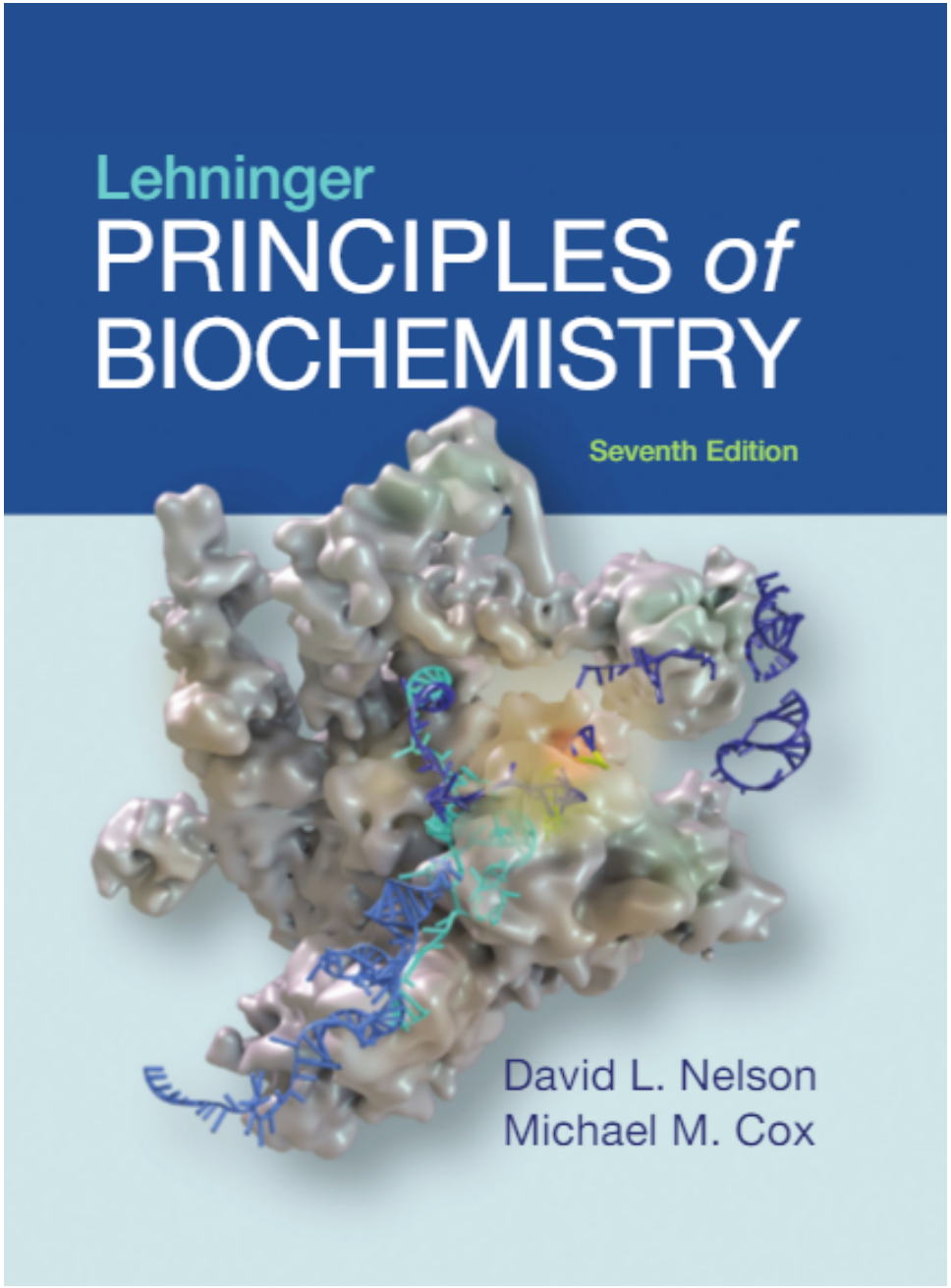
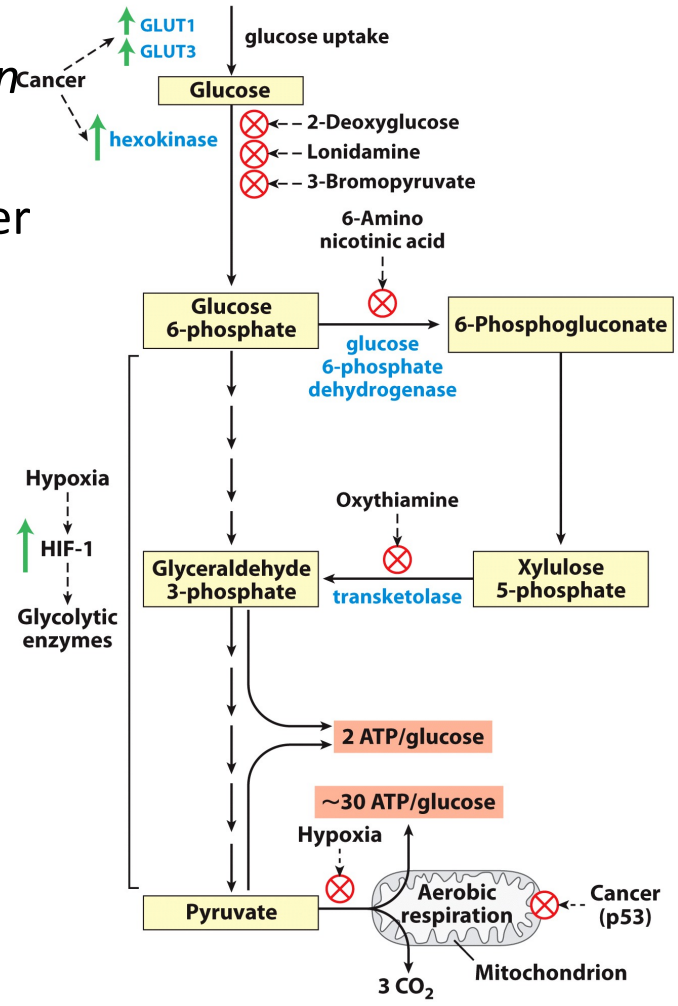
14| Glycolysis, Gluconeogenesis, and the Pentose Phosphate Pathway



Glycolysis occurs at elevated rates in tumor cells

- ***Warburg effect:*** tumor cells carry out glycolysis at a much higher rate than normal cells *even when oxygen is available (~10x)*
- In general, the more aggressive the tumor, the greater is its rate of glycolysis
- HIF-1 (hypoxia-inducible transcription factor) stimulates the production of at least 8 glycolytic enzymes and glucose transporters when the oxygen supply is limited
- HIF-1 also stimulates the production of VEGF (which stimulates angiogenesis)
- Overreliance of tumors on glycolysis suggests a possibility for anticancer therapy: deplete ATP from cancer cells by blocking glycolysis
- PET scans take advantage of the high uptake of glucose by tumor cells. Used to pinpoint cancers



Box 14-1 figure 1
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Glucose uptake is deficient in type 1 Diabetes Mellitus

- Glucose uptake into cells is mediated by GLUT family
- GLUT1 & GLUT2 (hepatocytes) and GLUT3 (brain neurons) are always present in the plasma membrane of these cells
- GLUT4 (skeletal and cardiac muscles and adipose) only move to the plasma membrane in response to an insulin signal
- Patients with type 1 DM have too few β cells in the pancreas (cannot synthesize enough insulin) → heart, muscles and fat tissues cannot uptake glucose → hyperglycemia (after carb-rich meals)
- Fat cells turn to fat metabolism to provide alternative energy → formation of ketone bodies
- In untreated type 1 DM ketoacidosis is common and is life-threatening
- Reversed by insulin injection

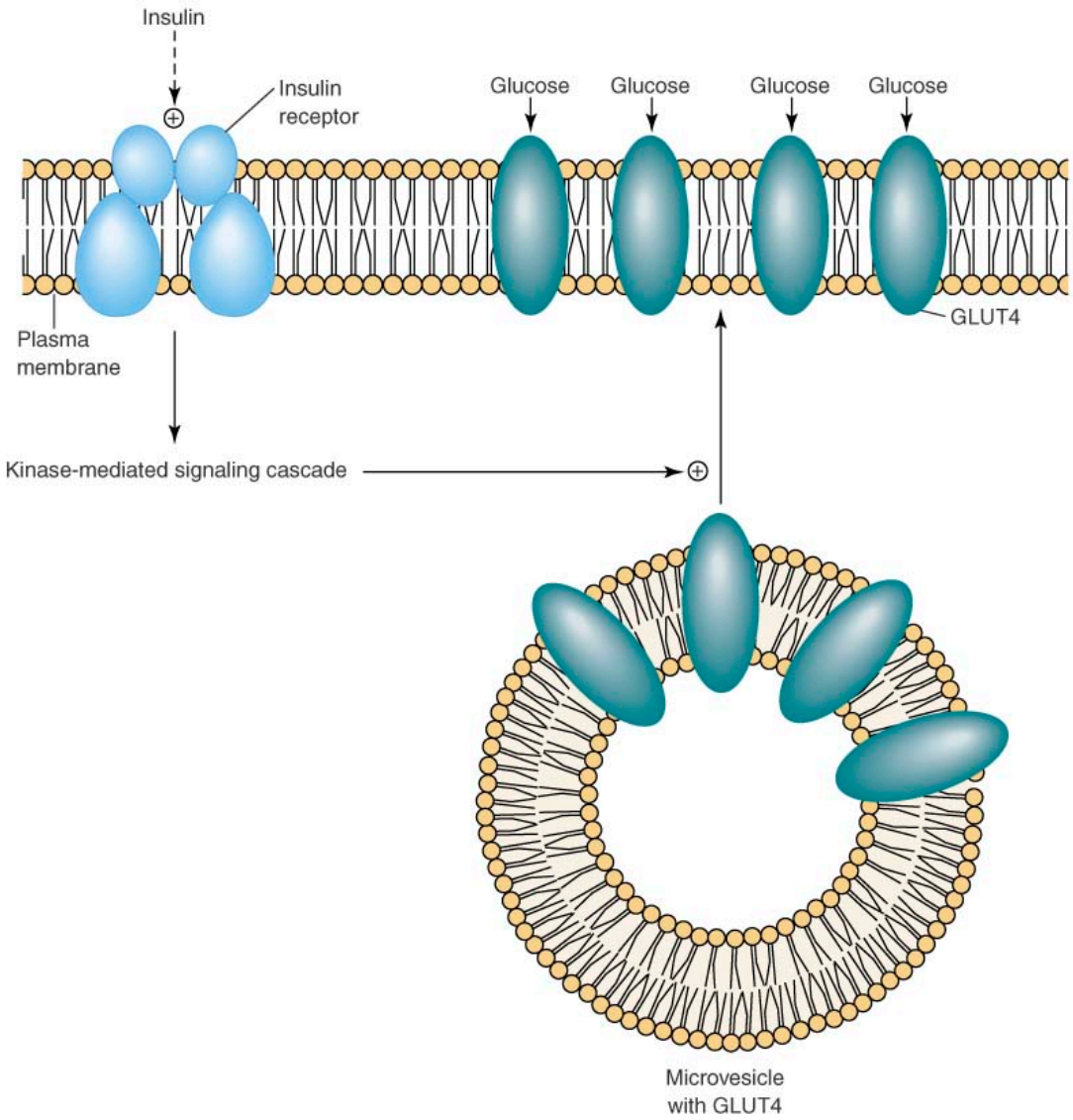

Figure 15.5 Insulin stimulates glucose uptake by adipose tissue and muscle by increasing the number of glucose transporters (GLUT4) in the plasma membrane.

Feeder Pathways for Glycolysis

Oxidation of Multiple Carbohydrates Involves Glycolysis

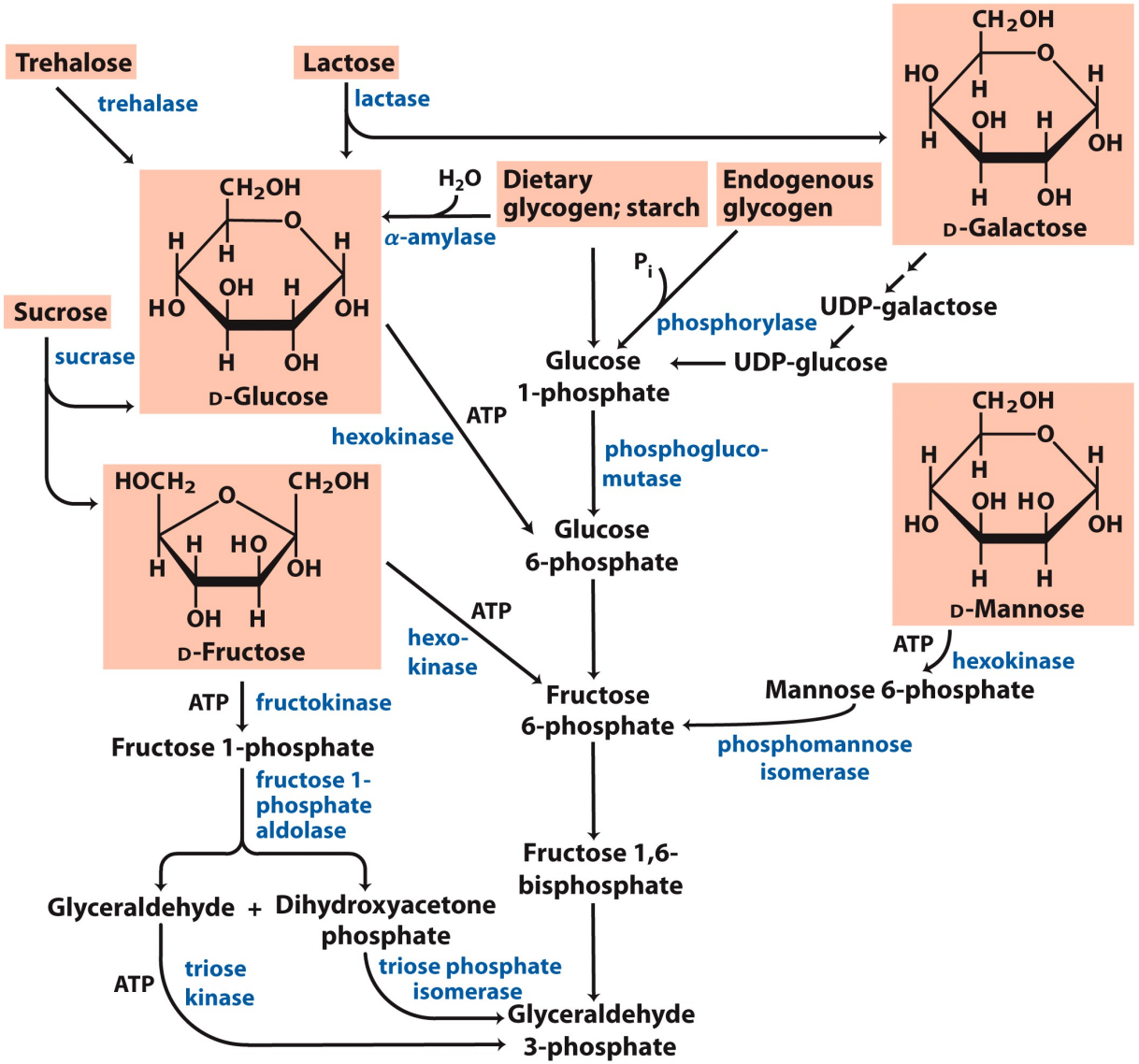

Figure 14-11
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Feeder Pathways for Glycolysis

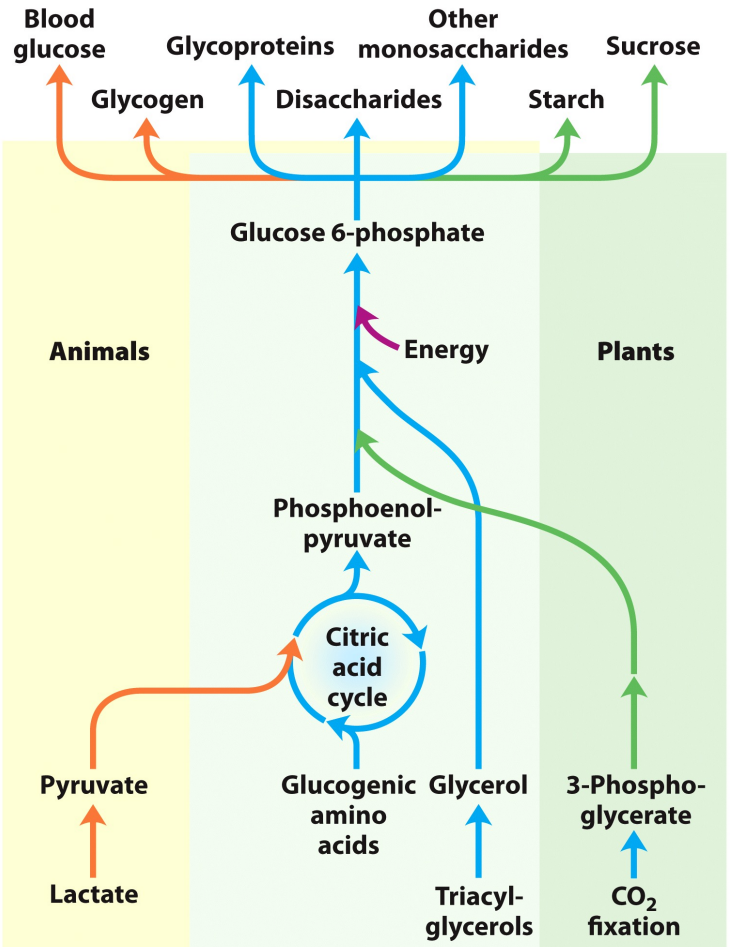
- Glucose molecules are cleaved from endogenous glycogen by glycogen phosphorylase (*phosphorolysis*)
  - Yielding glucose-1-phosphate
- Dietary starch and glycogen are cleaved by α-amylase to produce oligosaccharides and subsequently maltose and maltotriose in the small intestine, by pancreatic α-amylase (*hydrolysis*)
- Disaccharides are hydrolyzed
  - Lactose: glucose and galactose *(lactose intolerance?)*
  - Sucrose: glucose and fructose
  - Fructose, galactose, and mannose enter glycolysis at different points

Gluconeogenesis: Precursor for Carbohydrates

- Brain and nerve cells, RBC, renal medulla, testes an embryonic tissue use only glucose as the energy source
  - 120 g of glucose daily (brain)

- Synthesizing glucose from noncarbohydrate precursors – ***gluconeogenesis***

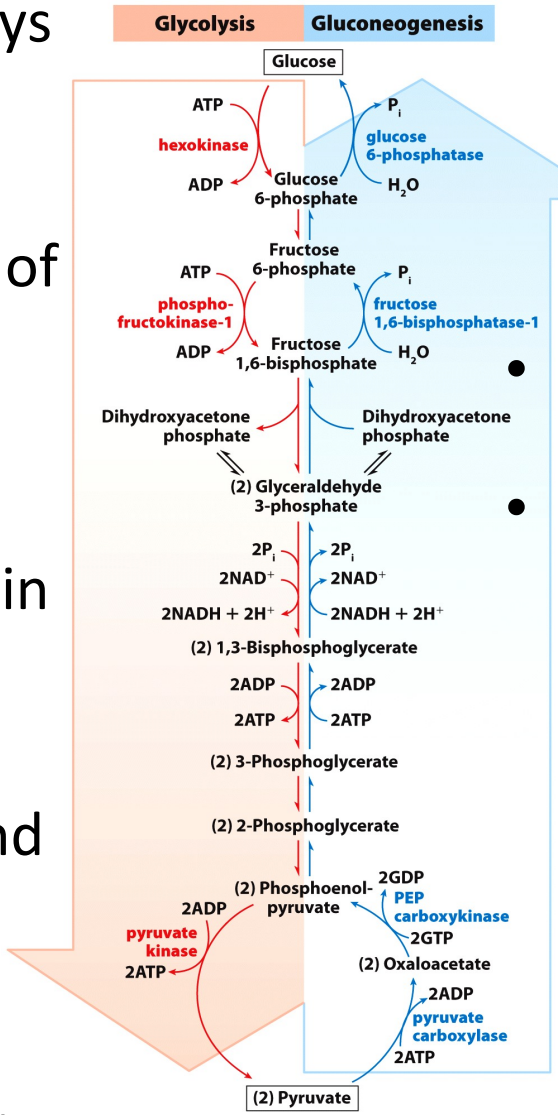
- In mammals, occurs in the liver (mainly) and in renal cortex



Notice that mammals cannot convert fatty acids to sugars.

Glycolysis vs. Gluconeogenesis

- Not identical pathways running in opposite directions
- 7 of the 10 reactions of gluconeogenesis are the reverse of glycolysis
- Both are irreversible in cells
- Both occur in the cytosol (reciprocal and coordinated regulation)



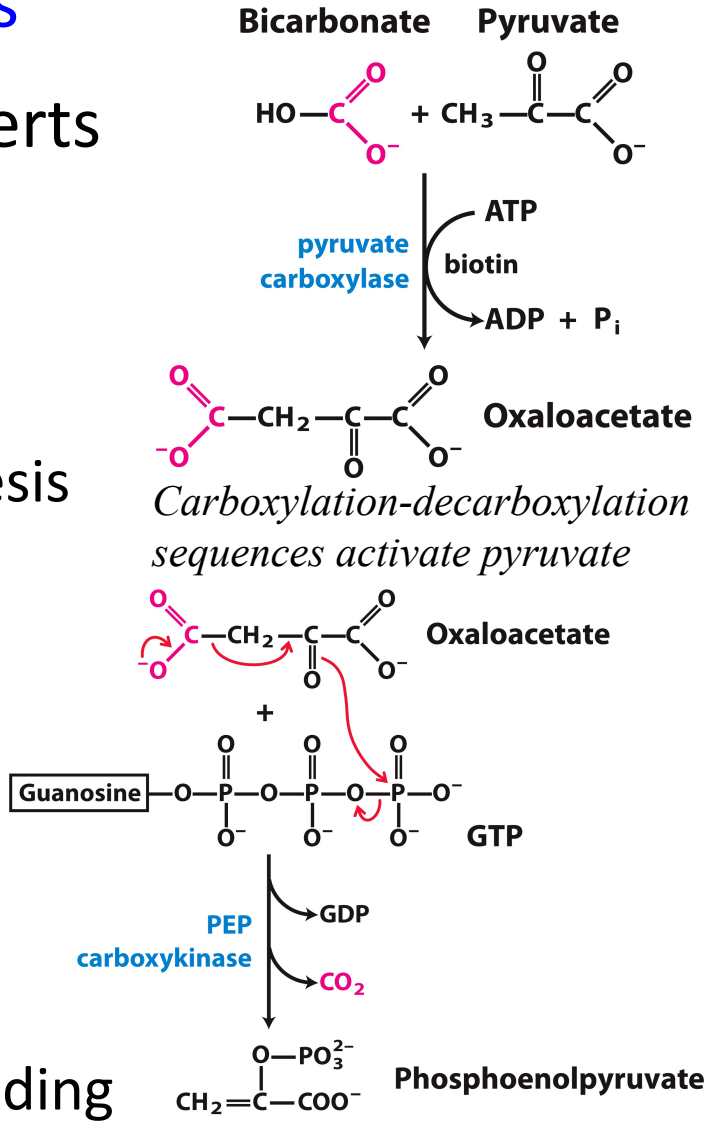
- Opposing pathways that are both thermodynamically favorable
  - Operate in opposite direction
    - end product of one is the starting cpd of the other
- Reversible reactions are used by both pathways
- Irreversible reaction of glycolysis must be bypassed in gluconeogenesis
  - Highly thermodynamically favorable, and regulated
  - Different enzymes in the different pathways
  - Differentially regulated to prevent a futile cycle

Glycolysis occurs mainly in the muscle and brain.

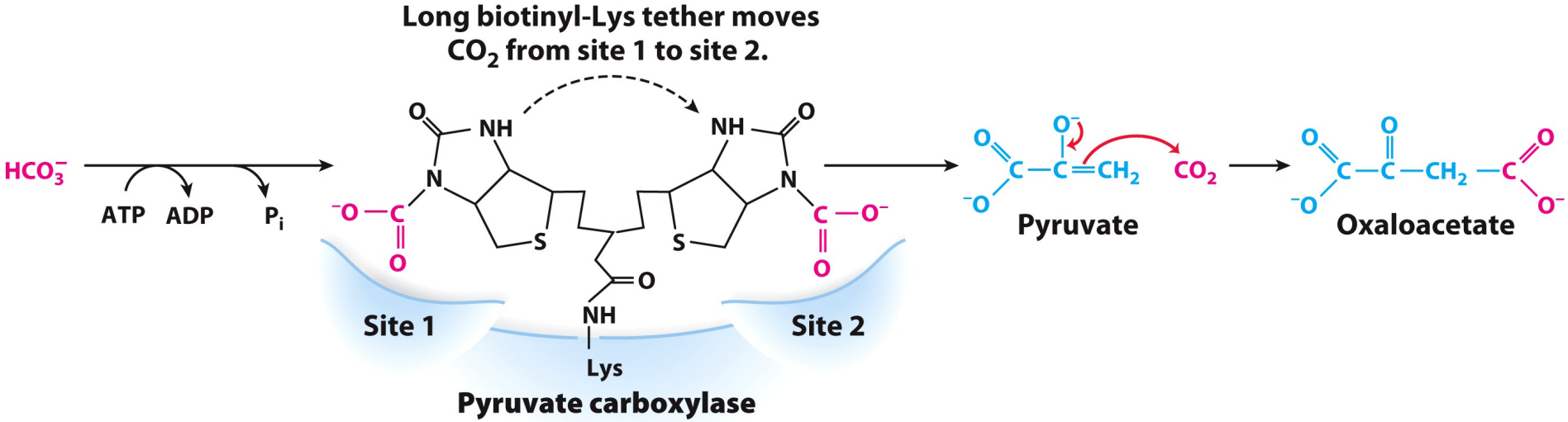
Gluconeogenesis occurs mainly in the liver.

Pyruvate to Phosphoenolpyruvate

- Requires two energy-consuming steps
- First step, pyruvate carboxylase converts pyruvate to oxaloacetate
  - Carboxylation using a biotin cofactor
  - Requires transport into mitochondria
  - First regulatory enzyme in gluconeogenesis (acetyl CoA is +ve effector)
- Second step, phosphoenolpyruvate carboxykinase converts oxaloacetate to PEP
  - Phosphorylation from GTP and decarboxylation
  - Occurs in mitochondria or cytosol depending on the organism



Biotin is a CO_2 Carrier



- Biotin is covalently attached to the enzyme through an amide linkage to the ε-amino group of a Lys residue
- The reaction occurs in two phases (at two different sites):
- At catalytic site 1, bicarbonate ion is converted to CO_2 at the expense of ATP. CO_2 reacts with biotin, forming carboxybiotinyl-enzyme
- The long arm carries the CO_2 of carboxybiotinylenzyme to catalytic site 2 on the enzyme surface, where CO_2 is released and reacts with the pyruvate, forming oxaloacetate
- The general role of flexible arms in carrying reaction intermediates between enzyme active sites

Malate dehydrogenase

- No transporter of oxaloacetate in mitochondria
- OA must be reduced to malate by mitochondrial malate dehydrogenase using NADH

  $OA + NADH + H^+ \leftrightarrow$ L-malate + NAD^+

- Very low [OA] makes the $\Delta G \sim 0$ despite the high $\Delta G'^o$
- In cytosol, L-malate is reoxidized producing NADH

  L-malate + $NAD^+ \rightarrow$ OA + NADH + H^+

- $[NADH]/[NAD^+]_{mito} > [NADH]/[NAD^+]_{cyto}$ 10^5x
  cytosolic NADH is consumed in gluconeogenesis, glucose production cannot continue unless NADH is available. Moving malate from mito to cytosol moves also NADH equivalents to allow the process to occur

Overall bypass reaction

- OA + GTP ←→ PEP + CO_2 + GDP (PEP carboxykinase)
- Reversible under cellular conditions: formation of one high energy phosphate is balanced by the hydrolysis of another
- Pyruvate + ATP + GTP + HCO_3^- ←→ PEP + CO_2 + ADP + GDP + P_i $\Delta G'^o$ = 0.9 kJ/mol
- ΔG for the reaction ~ –25 kJ/mol because the actual cellular [PEP] is very low ➔ the reaction is irreversible in vivo

First Gluconeogenic Steps Travel Through Mitochondria

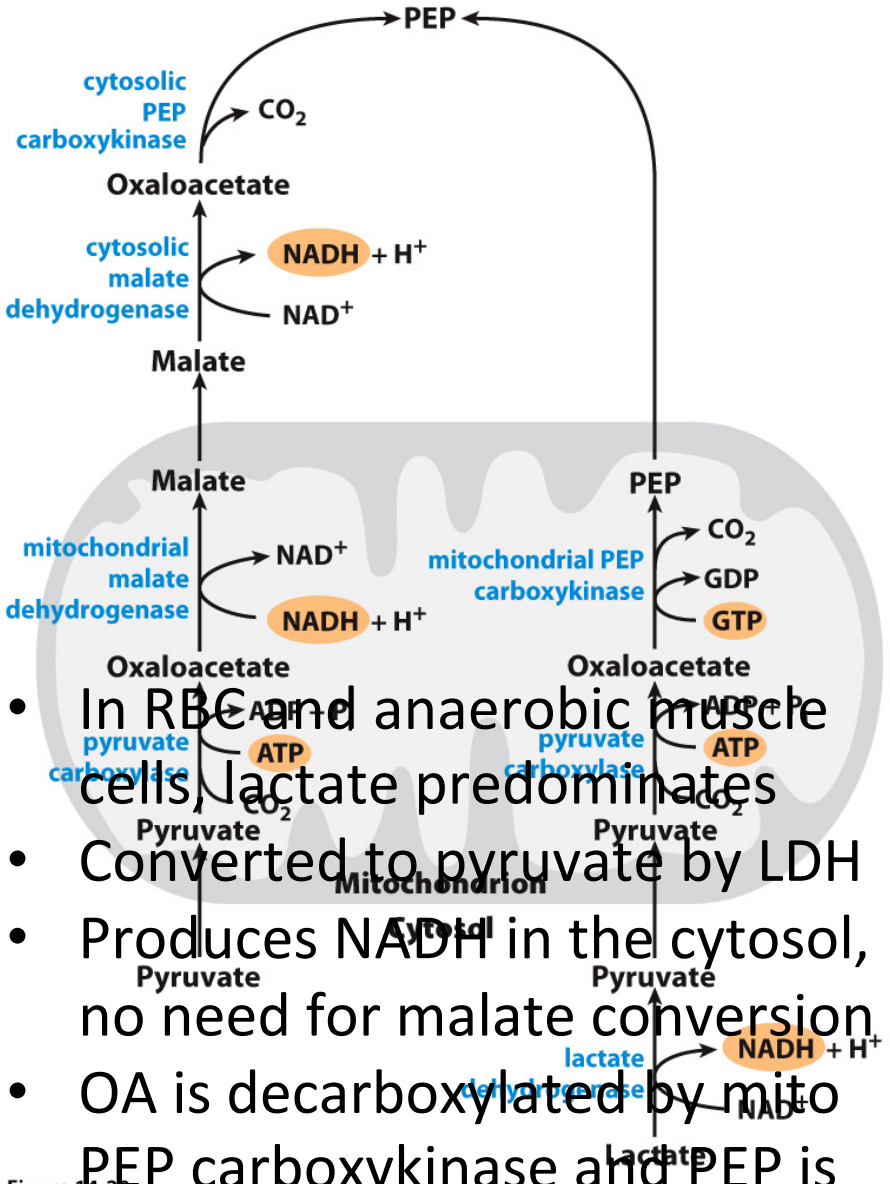


Figure 14-26
Lehninger Principles of Biochemistry, Seventh Edition

- In RBC and anaerobic muscle cells, lactate predominates
- Converted to pyruvate by LDH
- Produces NADH in the cytosol, no need for malate conversion
- OA is decarboxylated by mito PEP carboxykinase and PEP is exported from mito

- The mitochondrial inner membrane is selectively permeable: Malate, PEP, and pyruvate can cross via transporters, while oxaloacetate cannot escape.
- Oxaloacetate can be utilized in the citric acid cycle (Kreb's cycle) if needed.
- Oxaloacetate can be converted to PEP or malate to allow transport to cytosol for gluconeogenesis.

First Gluconeogenic Steps Travel Through Mitochondria

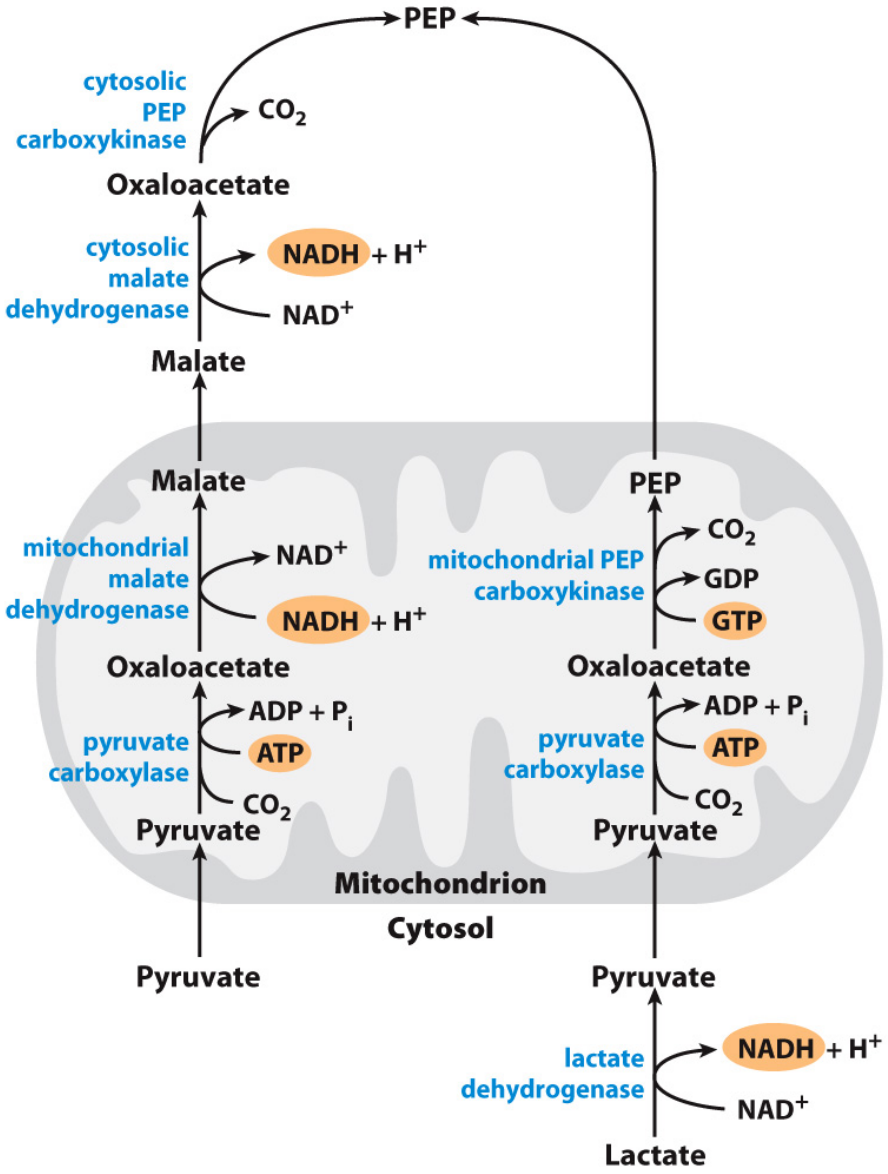


Figure 14-20
Lehninger Principles of Biochemistry, Seventh Edition

- In RBC and anaerobic muscle cells, lactate predominates
- Converted to pyruvate by LDH
- Produces NADH in the cytosol, no need for malate conversion
- OA is decarboxylated by mito PEP carboxykinase and PEP is exported from mito

Additional Bypasses

- Catalyze reverse reaction of opposing step in glycolysis
- Are irreversible themselves

- Fructose 1,6-bisphosphate → Fructose 6-Phosphate
  - By fructose bisphosphatase-1 (FBPase-1)
  - Coordinately/oppositely regulated with PFK
  - cleaves phosphate with water
  - DOES NOT generate ATP

- Glucose 6-phosphate → Glucose
  - By glucose 6-phosphatase
  - cleaves phosphate with water
  - DOES NOT generate ATP
  - Enzyme found in hepatocytes, renal medulla and intestinal epithelial cells, NOT anywhere else (*if it were found everywhere, … what do you expect would happen?*)

Gluconeogenesis is expensive

2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 4 H_2O →
Glucose + 4 ADP + 2 GDP + 6 P_i + 2 NAD^+

- Costs 4 ATP, 2 GTP, and 2 NADH
- Not the reversal of the conversion of pyr to glc
- Also, there's a need to keep pyruvate inside the cell instead of secreting it outside. Pyruvate has the potential to make more than 10 ATP per full oxidation of pyruvate
- *Physiologically necessary:* Brain, nervous system, and red blood cells generate ATP ONLY from glucose
- Allows generation of glucose when glycogen stores are depleted:
  - during starvation
  - during vigorous exercise
  - can generate glucose from amino acids, but not fatty acids

Precursors for Gluconeogenesis

- Glucose can be produced from all intermediates of the CAC (citrate, isocitrate, α-KG, succinyl-CoA , succinate, fumarate and malate) since all of them can undergo oxidation to OA

- Also, most a.a. can undergo transformations to pyruvate or CAC intermediate, and therefore has the potential to make glucose: i.e. ***glucogenic***
  - Only Leu and Lys are non-glucogenic
  - Ala and Gln are particularly important glucogenic a.a. in mammals

Precursors for Gluconeogenesis

- Animals can produce glucose from sugars or proteins and parts of fat (triacylglycerol)
  - Sugars: pyruvate, lactate, or oxaloacetate
  - Protein: from glucogenic a.a.
  - Glycerol: the breakdown product of fats can be used after a two step reaction. *Glycerol kinase* phosphorylates it and the oxidation of the central C yields dihydroxyacetone phosphate (an intermediate in gluconeogenesis)

- Animals cannot produce glucose from fatty acids
  - Product of fatty acid degradation is acetyl-CoA
  - Cannot have a net conversion of acetyl-CoA to oxaloacetate (2 C that enter the CAC are removed as $2CO_2$)
    - Plants, yeast, and many bacteria can do this (the glyoxylate cycle), thus producing glucose from fatty acids

Pentose Phosphate Pathway

- Glc 6-P has another catabolic fate which leads to specialized products needed by cells
- The main products are NADPH and ribose 5-phosphate
- NADPH is an electron donor
  - Reductive biosynthesis of fatty acids and steroids (liver, adipose, gonads, etc.)
  - Repair of oxidative damage esp. in cells directly exposed to O_2 (RBC, cornea)

- Ribose-5-phosphate is a biosynthetic precursor of nucleotides
  - Used in DNA and RNA synthesis esp. in rapidly dividing cells (skin, bone marrow, tumors, etc.)
  - Or synthesis of some coenzymes (ATP, NADH, $FADH_2$)

Pentose Phosphate Pathway

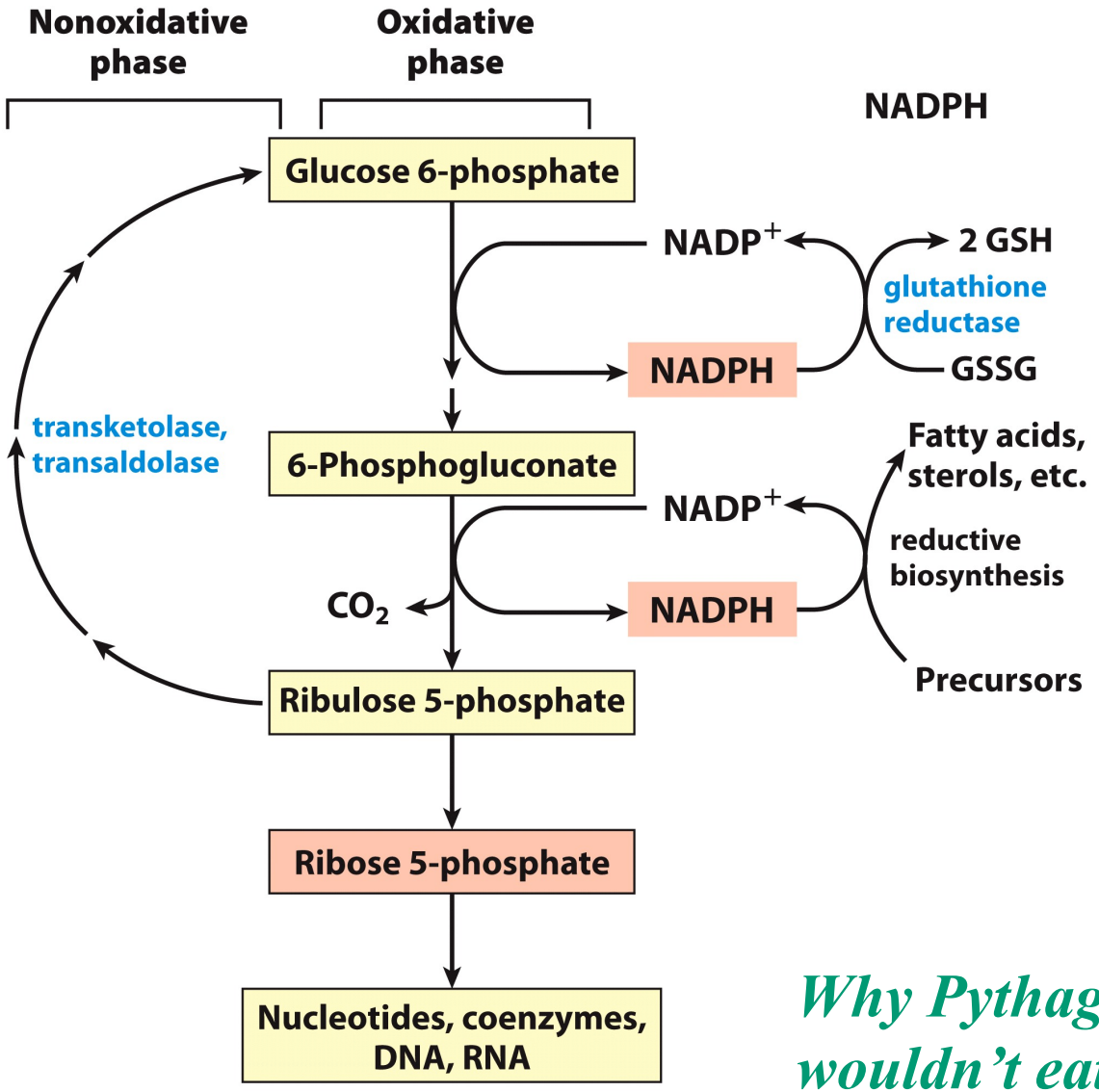


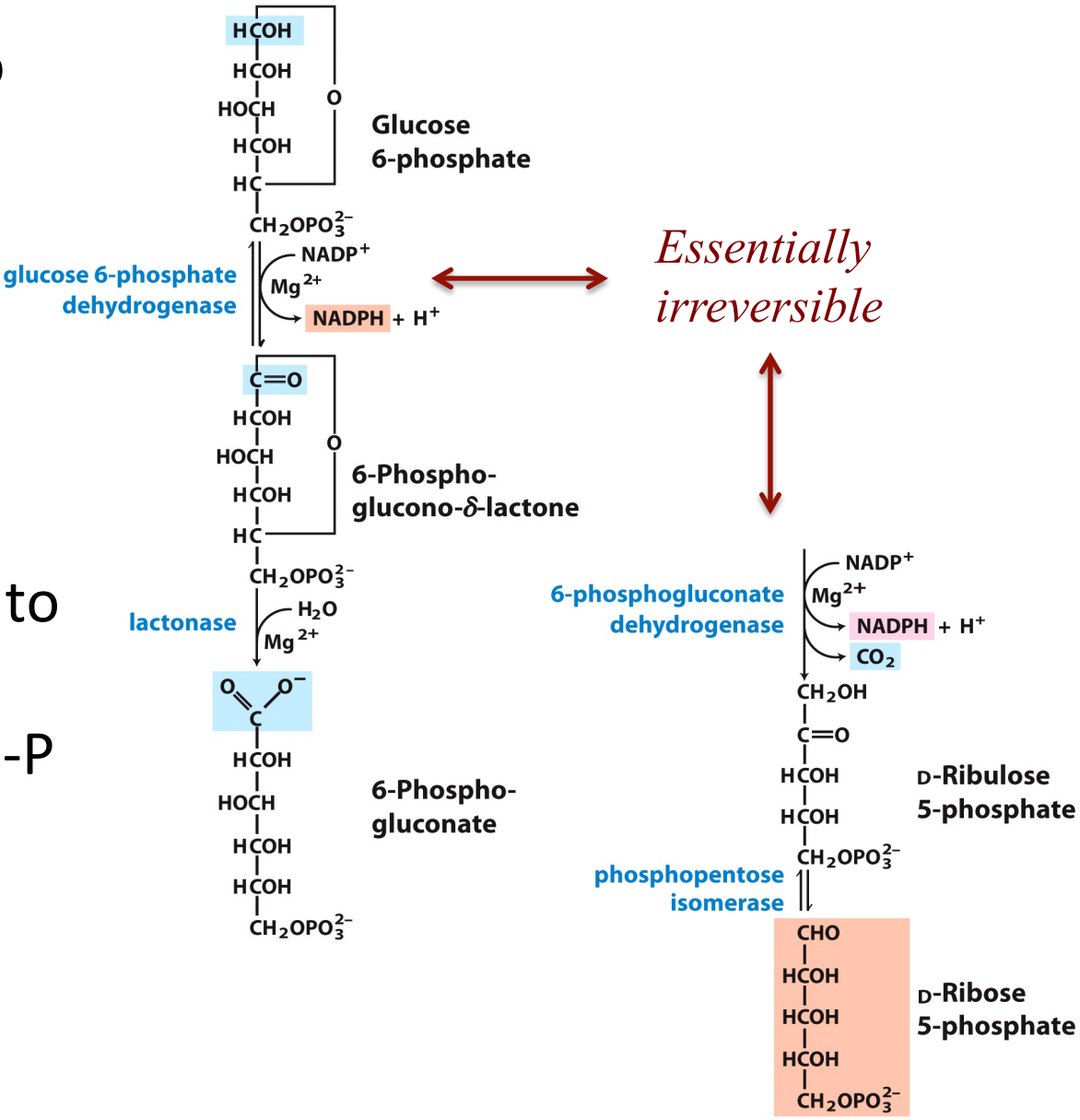
Figure 14-21
Lehninger Principles of Biochemistry, Sixth Edition

Why Pythagoras wouldn't eat falafel???
Box 14-4: self check

Oxidative phase generates NADPH and R-5-P

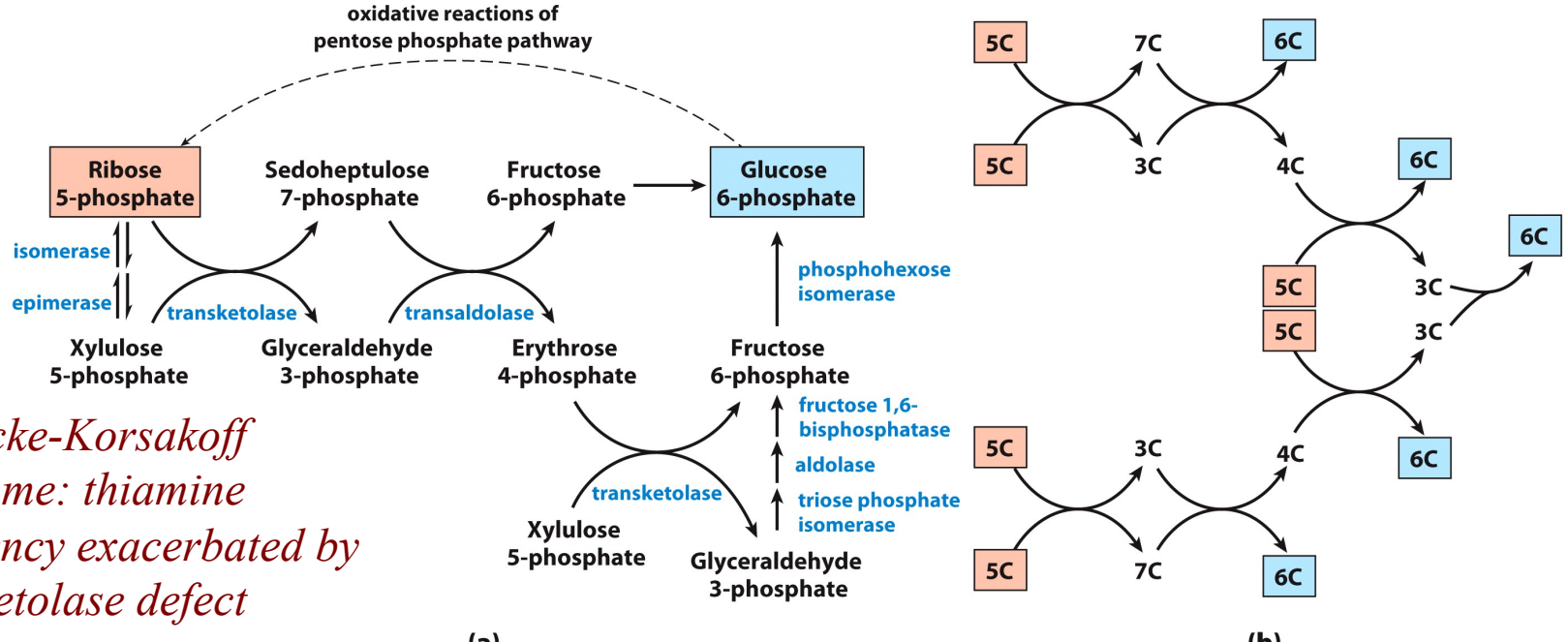
1. Oxidation of G-6-P to δ-lactone by ***G6PD***, reduction of $NADP^+$
2. Lactone hydrolysis by ***lactonase***
3. Oxidation and decarboxylation by ***6-PG dehydrogenase*** to produce ribulose 5-P
4. Formation of ribose 5-P by ***phosphopentose isomerase***

- Pentose pathway ends here in some tissues



Non-oxidative phase regenerates G-6-P from R-5-P

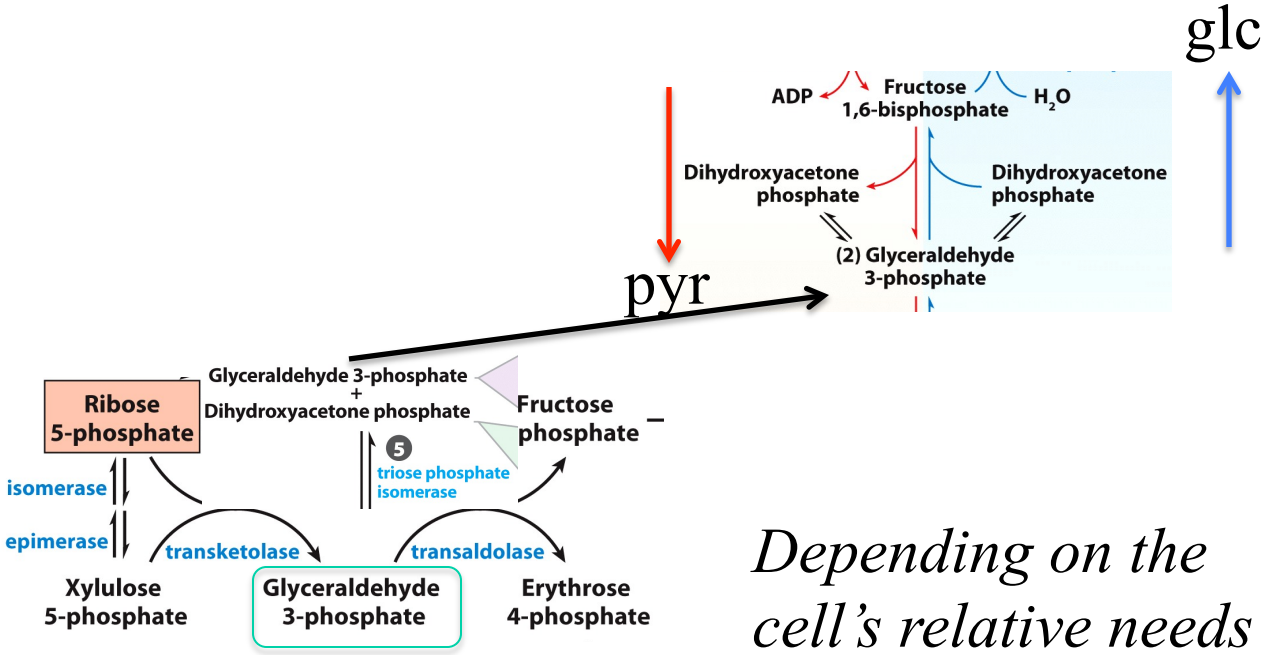
- Used in tissues requiring more NADPH than R-5-P (e.g. liver and adipose)
- Six 5-C sugar phosphates are converted into five 6-C ones, allowing continued G6P oxidation and NADPH production
- Details are not important, but remember the two key enzymes unique in this pathway: **transketolase** and **transaldolase**



Wernicke-Korsakoff syndrome: thiamine deficiency exacerbated by transketolase defect

Glycolysis, gluconeogenesis and pentose phosphate pathway

- All enzymes of PPP are in the cytosol
- Glycolysis, gluconeogenesis and PPP are connected through several shared intermediates and enzymes:



Depending on the cell's relative needs for NADPH, ATP and pentose phosphates

NADPH regulates partitioning into glycolysis vs. pentose phosphate pathway

G6P can enter glycolysis or PPP depending on the current needs to the cell and the concentration of $NADP^+$ and NADPH

When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction, [NADPH] rises and inhibits the first enzyme in the PPP. As a result, more glucose 6-phosphate is available for glycolysis.

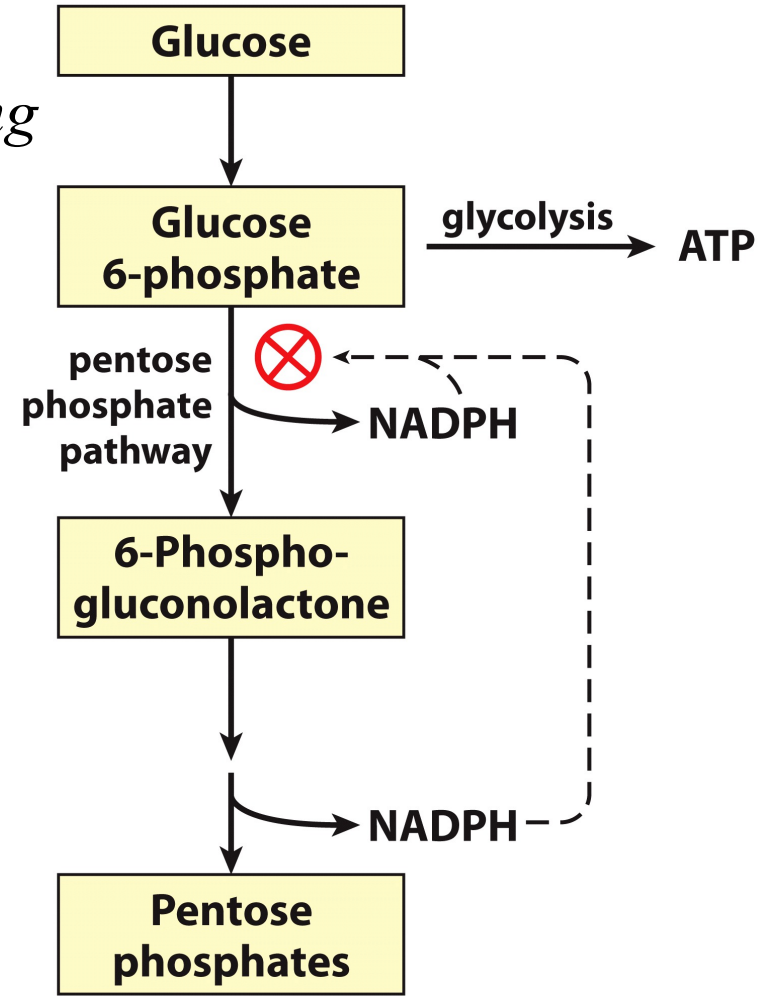


Figure 14-28
Lehninger Principles of Biochemistry, Sixth Edition

Diabetes

- Chronic disease
- Characterized by excessive urine excretion, **polyuria**
- Greek word for “passing through” i.e. urine

- Two main forms:

Diabetes Insipidus

Diabetes Mellitus

Diabetes Insipidus

- Insipidus means “tasteless”. Diabetes insipidus = tasteless urine
- Due to a deficiency of antidiuretic hormone (ADH, aka arginine vasopressin, AVP)
- AVP increases water resorption in kidneys
- Deficiency of AVP can be
  - **Neurogenic:** decrease in AVP release (e.g. due to alcohol intoxication or tumor)
  - **Nephrogenic:** decreased renal sensitivity to AVP (e.g. by mutations of receptors or aquaporins)
- Either neurogenic or nephrogenic → little water retention → excessive output of dilute urine → **diabetes insipidus**, **hypernatremia** (elevated $[Na^+]_{blood}$), **polyuria** (excess urine production), and **polydipsia** (thirst)
- *Has nothing to do with carbohydrate metabolism*

Diabetes Mellitus

- Mellitus means “honey”. Diabetes mellitus = honey urine
- Due to defects in CHO, fats, and/or protein metabolism
- Elevated glucose in the plasma and urine
- Excessive urine excretion is due to ***osmotic diuresis*** (high blood sugar leaking into the urine and taking excess water along with it)
- Two major types:

TYPE 1 and TYPE 2

Type 1 Diabetes Mellitus

- Usually appears in childhood
- Complete absence of insulin production from pancreas due to defective beta cell function (autoimmune)
- Inability of tissues to uptake glucose and continuous gluconeogenesis in liver ➔ **high $[glc]_{blood}$**
- Increased lipolysis in adipose and increased beta oxidation in liver ➔ **ketoacidosis**
- Absence of insulin (TF) will induce lower lipoprotein lipase activity ➔ **hyperchylomicronemia**
- **Body is always in a starved state**
- Exogenous insulin is the only effective medication which doesn't cure it but alleviates clinical symptoms. Must keep changing the dose to match nutritional states

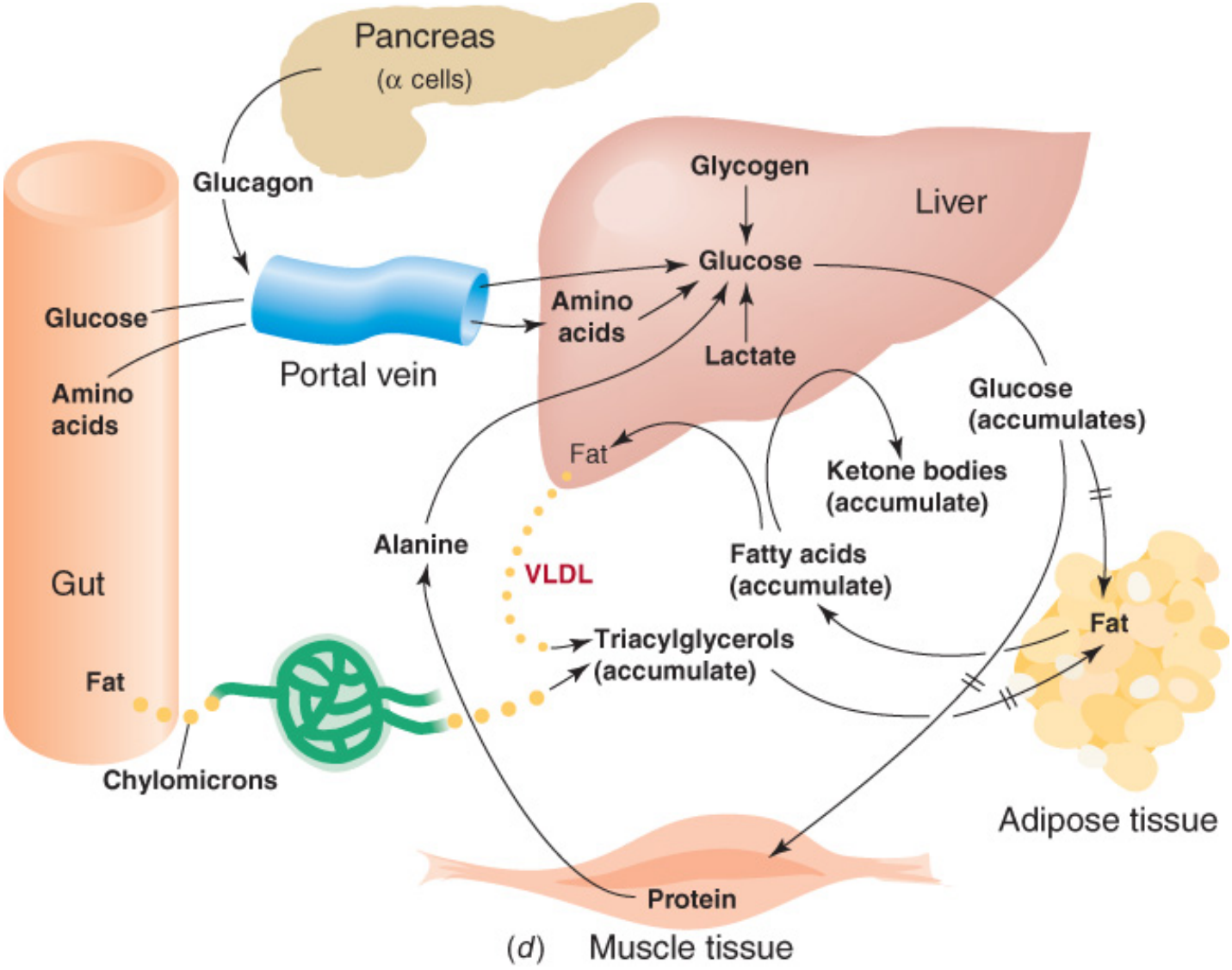


Figure 21.26 Metabolic interrelationships of tissues in type 1 diabetes mellitus.

Type 2 Diabetes Mellitus

- β cell failure and insulin resistance in obese diabetic patients
- Insufficient production of insulin to promote glucose uptake into tissues or to block gluconeogenesis in liver ➔ **hyperglycemia**
- **Ketoacidosis *rarely*** develops of (enough insulin is present to prevent uncontrolled release of fatty acids from adipocytes)
- **Hypertriacylglycerolemia** occurs (increase in VLDL without hyperchylomicronemia because fatty acids are combined in the liver to form TAGs and VLDL)
- Note that concurrent lipogenesis and gluconeogenesis should never occur, yet they occur in type 2 DM because of the ***state of mixed insulin resistance*** and its effects on different pathways (more on that in later chapters)
- To treat: (1) diet and exercise (2) **metformin** (inhibitor of gluconeogenesis) and (3) **insulin injections** (most effective despite insulin resistance)

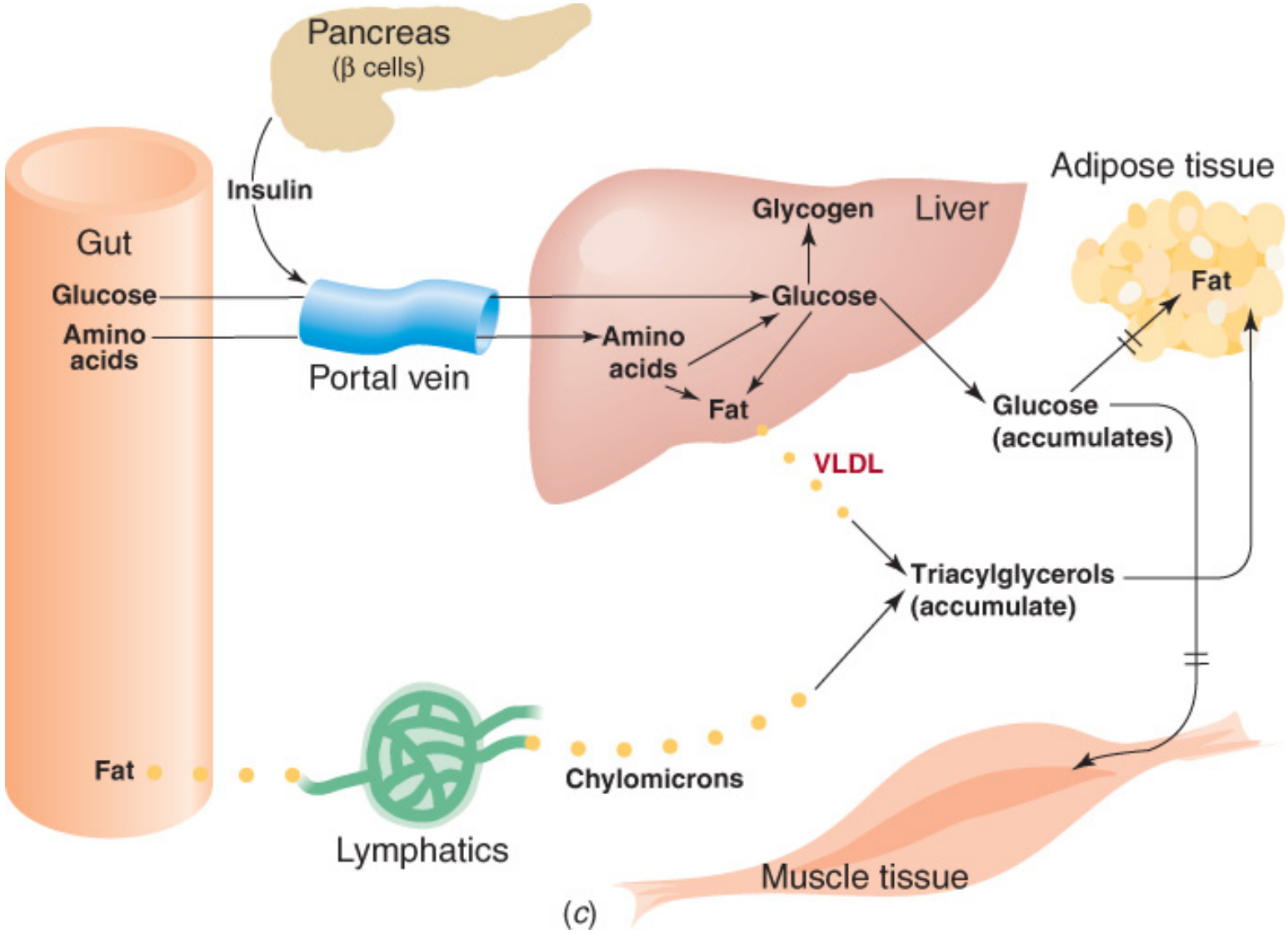
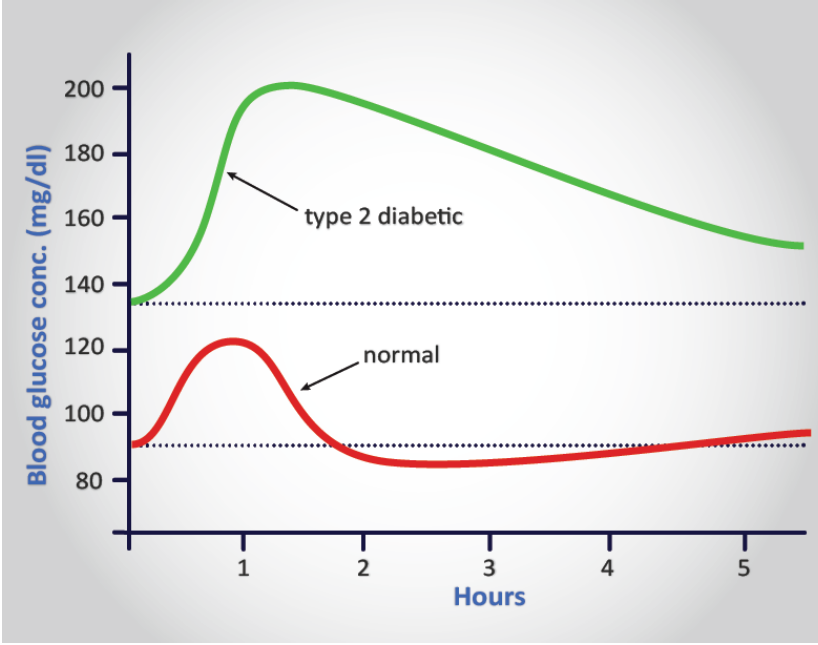


Figure 21.25 Metabolic interrelationships of tissues in type 2 diabetes mellitus.

Type 2 Diabetes Mellitus diagnosis

- OGTT for diagnosis (measuring $[glc]_{blood}$ every 30-60 min for 2-4 h after ingesting 100 g carbohydrate)
- Normal individuals → $[glc]_{blood}$ returns to normal in 2 h
- Diabetics → $[glc]_{blood}$ starts high and remains high for longer periods
- An abnormal OGTT does not mean diabetes in all cases
- Common cold can contribute to abnormal reading
- Fasting blood sugar of more than 126 mg/dL is a better indication of the occurrence of diabetes



Suggested Questions

- **Please solve questions:**

1. **14 (Arsenate poisoning)**
2. **16 (Niacin)**
3. **18 (Clinical symptoms of enzyme deficiency)**
4. **25 (Ethanol affects blood glucose)**
5. **28 (Phloridzin)**

For written answers, I prefer to have them typed in Word. I can accept the assignment in one file sent to my email. For answers that require solving mathematically, you can either type them or write them down and scan them.