Study Guide

for

Gropper/Smith’s
Advanced Nutrition and Human Metabolism,
5th Edition

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Introduction to the Study Guide
(Please read this!)

Prior to working on these study questions, it would be best to first review your notes along with the relevant sections of the book (i.e., treat this exercise with study questions like the exam itself). Likewise, don’t just read a question and think “I know that” and move on to the next question. Take the time to write out your answers completely to all of the questions – this will insure that you know the material and it is also good practice to work on writing concise answers.

Example responses to these questions are provided for you so that you can check your work. However, these are based on the lecture notes of the author of this study guide as well as your textbook, and your instructor may take a different perspective on these topics. For instance, your instructor may emphasize different aspects of a topical area, or expect you to refer to information discussed during class that is not in your textbook when answering exam questions. That is why it is important for you to review your class notes and practice answering study questions using the combination of your notes and the textbook.

If you go over your notes, supplemented with the text as need be, and then take the time to completely answer these questions (which includes going back to your notes and the text for areas that give you trouble), this should help you prepare for your exams. Naturally, you should also take advantage of any study questions or outlines provided by your instructor.

Study Hints
Many concepts introduced and covered in the first two chapters will be recurring throughout the course, so it’s important to understand them to minimize future difficulty.

It would be very useful to draw a cell with the basic energy metabolic pathways drawn in, make several copies of your drawing, and then complete it in more detail (important enzymes, regulation by hormones, etc.) for different cell types (liver, intestine, muscle, adipose, brain/other). You can include transport mechanisms as well in addition to the energy pathways. You could also do this twice (2 diagrams for each cell type), the difference being one set is for fed conditions, and one is for fasting.
Study Questions for Chapter 1 – The Cell: A Microcosm of Life

1. Name the various parts of the cell and write a brief (1-3 sentence) definition of each.

2. How do we regulate the function of proteins? Name the three regulation mechanisms and describe, in detail, how they work. How do macronutrients and micronutrients regulate gene expression and thus, protein function (in general; you don’t need to know specific examples at this point)?

3. What is the difference between adaptive and constitutive expression of genes? What is meant by apoprotein vs. holoprotein? What is the importance of knowing these definitions?

4. Describe enzyme kinetics and any relevant terms. How are the inherent kinetic characteristics of an enzyme related to regulation of a metabolic pathway/process?

5. Describe the following concepts/terms and their interrelationship with each other: homeostasis, turnover, differentiation, and apoptosis.
Example Answers for Chapter 1

1. **Mitochondrion** – is an organelle in the cytosol where most of the energy (ATP) is generated from the oxidation of macronutrients. This is accomplished by the Krebs cycle and electron transport chain (i.e., oxidative phosphorylation). It was important for you to state that this is an aerobic (requires oxygen) process.

**Rough Endoplasmic Reticulum** – a cytosolic organelle/membrane, in close proximity to the nucleus, where the mRNA can leave the nucleus, go the ER, and be translated into protein by the ribosomes (“rough”) that are attached to it.

**Phospholipid Bilayer** (plasma membrane) – formed by the alignment of phospholipids to form a cell membrane. It also contains cholesterol and proteins, the latter of which can function as transporters because the lipid bilayer is impermeable to water-soluble (polar) compounds.

**Nucleus** – organelle in the cell that contains DNA (genes, genome). Thus, transcription occurs in the nucleus, after which the mRNA that results must move to the cytosol/RER for translation.

**Cytosol** – the “interior” plasma of the cell; things are not “free-floating,” however, as the microtrabecular lattice keeps many organelles in place.

**Lysosomes/ Peroxisomes** – contain oxidative and digestive enzymes to degrade cell components/waste.

Textbook reference: pages 2-12

2. **Mechanism 1:**

Induction – inducing a gene to be expressed. The transcription and/or translation of the corresponding gene/mRNA is increased. The result is that the abundance of a protein is increased. Remember that induction means increasing abundance by definition, so the abundance of the protein in question will always be increased, not decreased through induction. The protein can be an enzyme, a transporter, etc. Note that induction is slower than posttranslational modification or allosteric regulation.

**Mechanism 2:**

**Posttranslational or covalent modification (PTM)** – no change in the abundance of a protein. A preexisting (posttranslational) protein is covalently modified and thus made either active or inactive. Covalently modifying a protein involves breaking or forming covalent bonds. Phosphorylation, carboxylation, glycosylation, or zymogen activation by breaking a peptide bond are all examples of posttranslational or covalent modification. The protein can be an enzyme, a transporter, etc.

**Mechanism 3:**

**Allosteric regulation** – inhibiting or stimulating the activity of an enzyme. Instead of being covalently modified, the protein is bound to something else—termed a modulator in your text—and
Macronutrients can indirectly result in the induction of protein; that is, a macronutrient is not directly involved in turning on a gene to induce a protein. Rather, hormones, in response to changes in macronutrients, do this— they do this by binding to their receptor on the cell membrane and via signal transduction, send a signal to the nucleus to turn on the expression of a specific protein, or send a signal that results in modification of a protein to make it more or less active, or send a signal to a transporter in the cytosol to move to the membrane so it can be more active. Thus, changes in macronutrients (such as CHOs) result in changes in hormone levels, which in turn can regulate proteins by any of the above mechanisms (NOT just induction).

Micronutrients induce genes directly by entering the cell and binding to DNA to turn on a gene; they directly are involved in covalently modifying a protein to make it more active or inactive; and they can directly bind to an enzyme to allosterically inhibit or stimulate it.

Textbook reference: pages 16-17

3. Adaptive genes or proteins are ones that can be regulated with respect to expression and thus protein synthesis. Specific signals have the ability to induce (“turn on”) these genes, thereby leading to an increased abundance of the protein that is encoded by the gene sequence.

Constitutive (“housekeeping”) genes are expressed at a constant rate, and thus the proteins they encode are also synthesized at a constant rate, not subject to regulation by external signals or factors.

Apo- refers to a protein that is in its inactive state or requires further modification to be active. This modification can be posttranslational (i.e., covalent) modification, or it can involve the binding of specific cofactors (e.g., vitamins, minerals).

Holo- refers to a protein that is fully active and able to carry out its function, whether it is an enzyme, transport protein, or any other type of protein.

Textbook reference: pages 16-17

this results in a change in its conformation. This change affects its enzymatic activity. Typically, things such as substrates, intermediates, or products along the pathway in which the enzyme participates bind to an enzyme (modulators are not other proteins/enzymes).

ATP and ADP are two examples of a modulator. An abundance of ATP, which is the end point of a lot of pathways (glycolysis, TCA cycle), is an indicator that the cell has enough energy. Once the concentration of ATP reaches a certain level, it begins to bind some of the key enzymes in glycolysis and the TCA cycle and inhibits them, decreasing the generation of ATP. In contrast, a greater abundance of ADP relative to ATP indicates a need for energy. When this occurs, ADP binds to these same enzymes. ADP stimulates rather than inhibits these enzymes, increasing the generation of ATP— until ATP concentration reaches the point where it inhibits them again.

A fourth mechanism that is important is translocation— this does not involve changes in gene expression or activating the protein, but simply stimulating the pre-existing transport protein to move from the cytosol to the membrane— an example here is GLUT 4.
4. When plotting the velocity (V) of an enzymatic reaction against the substrate concentration, one sees "saturable" kinetics. That is, at some substrate concentration, the enzyme is functioning at its maximal rate (V_max) and cannot operate any faster. The substrate concentration that results in the enzyme functioning at ½ its maximal rate is called the K_m. K_m can be considered an index of the affinity an enzyme has for its substrate – a high K_m indicates low affinity (i.e., it takes a lot of substrate just to get the enzyme working at half its maximum, thus the enzyme does not have a very strong affinity to bind to the substrate), whereas a low K_m indicates high affinity (i.e., easily binds to the substrate, thus reaches high velocity quickly, at low substrate concentrations). K_m is also important because it can dictate how a substrate is used metabolically. If a substrate (like glucose) has a choice to go down one pathway via enzyme 1 vs. another pathway via enzyme 2, K_max can dictate this. At low substrate concentrations, the enzyme with a high affinity (low K_m) will beat out the other enzyme to bind the substrate and metabolize it. As the substrate concentration increases (for glucose this is around donut number 23), then the high affinity enzyme is already saturated, and the other enzyme can take over, allowing the excess glucose to go down a different pathway (like glycogen synthesis).

The only way to increase V_max is to increase the number of active enzymes, either by induction or by activating pre-existing, but inactive enzymes.

Textbook reference: pages 15-16

5. Homeostasis is the maintenance of body or cellular processes to keep them in a balanced steady-state. However, this is not static – it is very dynamic as everything has a rate of turnover (i.e., a specific lifespan of when they are made vs. when they are degraded). Molecules like proteins turn over; cells turn over; etc. The rate of turnover can vary tremendously, from a few minutes to years. For cells, turnover means replacing old cells with new cells. Cells divide and differentiate into the type of cell they are programmed to become – a process of maturation. After a specific amount of time, cells are programmed to die (and of course be replaced) – this is termed apoptosis. For any of these processes, a disruption in the norm can lead to problems such as disease. Immature red blood cells (not fully differentiated) can result in anemia; a lack of apoptosis can lead to excess growth or cancer. Because homeostasis is dynamic, everything turning over, differentiating, and being replaced, we are constantly in need of "new material" to replace the old – yes, we recycle quite a bit (e.g., 80% of the amino acids from protein breakdown are used to build new amino acids), but we still need, via the diet, a continuous supply of both macronutrients and micronutrients.

Textbook reference: pages 1, 19-20
Study Questions for Chapter 2 – The Digestive System: Mechanism for Nourishing the Body

1. Name the different types of transport mechanisms and describe each. How are they the same? How are they different? What type of nutrients utilize which mechanism and why?

2. What is required for the efficient digestion of carbohydrates? lipids? proteins? Your discussion should include the required organs, regulatory peptides, enzymes, and transport mechanisms.
Example Answers for Chapter 2

1. **Diffusion:**
   - small, lipid-soluble molecules/substrate
   - diffuse through the membrane (no transport protein required)
   - move down a concentration gradient
   - linear kinetics (velocity vs. substrate concentration)
   - some molecules proceed through a protein “pore” rather than the membrane itself – still considered simple diffusion

**Facilitated Diffusion** (aka carrier-mediated):
- larger, more polar compounds transported
- requires a protein to mediate the process, thus...
- is a saturable process kinetically; i.e., a maximal rate of transport is achieved regardless of substrate concentration. The only way to increase this maximum is by having more transporters.
- like simple diffusion, can only proceed down a concentration gradient

**Active Transport:**
- same as above (saturable, polar molecules, protein-mediated, etc.) except...
- requires energy (ATP & Na)
- can transport against a gradient (this is the payoff from investing energy)

**Endocytosis:**
- this involves the cell wall engulfing a substance by surrounding it with the cell membrane.

Textbook reference: pages 51-52 (especially Figure 2.18)

2. **Regulatory Peptides**

**CCK** – secreted by the small intestine into the bloodstream, it stimulates the gallbladder to release bile into the small intestine lumen, and stimulates the pancreas to release both pancreatic enzymes and pancreatic juice into the small intestine lumen.

**Secretin** – same as CCK above except for the action on the gall bladder; secretin is secreted by the small intestine and stimulates the pancreas to release enzymes and juice.

**Gastrin** – released by the stomach and acts on the stomach to release HCl; along with HCl it aids in the activation of pepsinogen to pepsin.

**GIP** – secreted by the small intestine into the bloodstream, it inhibits the action of the stomach (gastrin secretion, contraction, etc.).

**Digestive Enzymes**

**Amylase** – secreted in the mouth (saliva) and by the pancreas (part of pancreatic enzymes) into the small intestine. Most of the amylase we need to digest carbohydrates (i.e., polysaccharides) into smaller saccharides (olio-, di-, and mono-) is pancreatic amylase.
**Pepsinogen** – secreted in this zymogen form in the stomach; activated to pepsin by HCl and gastrin; pepsin is a protease, meaning it breaks down proteins into smaller peptides and amino acids.

**Lipase** – same as amylase above in that it is found in both the mouth and the small intestine via the pancreas; also like the mouth, most of the hydrolysis of lipids (i.e., triglycerides, TGs) into free fatty acids (FAs) and glycerol occurs in the small intestine. Note: 90+% of the lipids you ingest are TGs; lipase does not convert lipids into TGs; for humans, dietary lipids are TGs.

**Trypsin** – one of many proteases that make up part of the pancreatic enzymes which are secreted into the small intestine. Trypsin is released as trypsinogen, which via the action of enteropeptidase is converted into trypsin. Being a protease, trypsin catalyzes the hydrolysis of proteins into smaller peptides and amino acids.

**Dipeptidase** – resides near the brush border (i.e., microvilli of enterocytes) and breaks down peptides into 2 amino acids.

**Disaccharidase** – resides near the brush border (i.e., microvilli of enterocytes) and breaks down disaccharides (e.g., sucrose, lactose, maltose) into 2 monosaccharides (e.g., glucose, fructose, galactose); thus, the specific disaccharidases we are talking about are sucrase, lactase, and maltase.

**Enteropeptidase** – see trypsin above.

**Other Substances**

**HCl** – secreted by the stomach in response to gastrin; its primary function is to aid in digestion, in part by denaturing proteins and activating pepsinogen to pepsin.

**Bile** – is a mixture of bile salts, phospholipids, and other components. Released by the gallbladder (via CCK into the small intestine), its function is to emulsify lipids (i.e., help mix them within the aqueous environment into smaller droplets) by forming micelles. Then, because of the greater surface area of the lipid and the new aqueous environment, lipase from the pancreas can more efficiently break down the lipid (TG into free FAs and glycerol). Bile is not an enzyme – it breaks down lipids in the sense that it transforms large lipid droplets into smaller ones as micelles, but it does not carry out the hydrolysis – that is the function of lipase.

**Pancreatic juice** – composed primarily of water and bicarbonate; released by the pancreas (via CCK and secretin) and in the small intestine it neutralizes the acidity of the chyme due to the HCl in the stomach. Remember, digestive enzymes are proteins – thus, HCl would denature them just like it does dietary proteins.

**Saliva** – secreted by the mouth to lubricate food and aid in swallowing. Also contains amylase and lipase.

**Transport of Nutrients**

For nutrient transport, it depends on if it is water soluble (monosaccharides, amino acids), or lipid soluble.
**Water-soluble:** need transporters to cross the lipid environment of cell membranes, but don’t need transporters to travel in the aqueous environment of the circulation. For digestion, once the water-soluble substance enters the enterocyte via a transporter, it goes into the portal vein (using another transporter to cross that membrane) and is delivered to the liver first. After the liver, it circulates to the rest of the body.

**Lipid-soluble:** don’t need transporters typically to cross membranes (can you think of one exception? β-oxidation), but do need them in the circulation. Once in the enterocyte, lipid-soluble substances are packaged in chylomicrons and released into the lymphatic system, which merges with the general circulation near the heart. Thus, the entire body sees these chylomicrons at the same time – the liver does not get the first shot at them. Note: small FAs (<10-12 carbons in length) follow the same route as water-soluble substances.

Textbook reference: pages 33-52
Chapters 3 & 5 – Carbohydrates & Lipids

The following question / statements/ ideas are meant to help you focus your study time and try to “put things together,” especially when it comes to regulation of CHO and lipid metabolism. Thus, rather than just providing answers, it is a way to rewrite / organize your notes. Don’t just look at a few questions, think you know the answer, and move on—spend time organizing and writing out your answers. A lot of these questions, particularly at the end, are essentially the same in terms of content—the question and or answer is just organized differently or has a different focus.

Structure

1. What are the base structures and nomenclature for carbohydrates and lipids? How are they same? How are they different?
2. How are carbohydrates linked together? How are fatty acids and glycerol linked together?
3. What does the structure of cholesterol look like? What do we use it for?

Digestion, Absorption, Transport

4. Describe the basic processes involved in the digestion, absorption, and transport of both CHOs and lipids. This includes hormones involved, transport requirements, enzymes, other essential compounds, etc.
5. What implications does the nutrient structure have for digestion, absorption, and transport (across membranes and throughout the body)? Answer this for both CHOs and lipids.

Metabolism & Its Regulation

6. What are the various metabolic processes that involve CHOs and lipids (e.g., gluconeogenesis)? You should be able to provide a concise, 1-sentence definition of each one that you list.
7. For each pathway, what are the key proteins (enzymes, transporters, etc.) that are subject to regulation? For each, how is this regulation achieved (allosteric, induction, etc.)? What signals (e.g., hormones, blood glucose) are involved?

“Bigger” Questions that Cover Both CHOs & Lipids

8. For CHOs and lipids, give examples of important proteins that are regulated by induction vs. allosteric vs. posttranslational mechanisms.
9. What are the tissue-specific differences in metabolism? How does CHO metabolism differ in liver vs. muscle and why? How are they the same? How does lipid metabolism differ in adipose and other tissues?
10. For both CHO and lipid metabolism, what occurs under fed vs. fasting conditions? Include the pathways that predominate, how they are regulated, by what signals, etc.
Example Answers for Chapter 3 & 5

1. Both are composed of C, H, and O. For carbohydrates, the base structure is \((\text{CH}_2\text{O})_n\), where in the case of monosaccharides such as glucose, \(n = 6\). For fatty acids, it is \(\text{CH}_3(\text{CH}_2)_n\text{COOH}\) and \(n\) can vary from 1-11 or more (although the most common is \(n = 7-8\) and the FA has 16-18 Cs total). The main difference between the two is solubility (CHOs are water-soluble and lipids are lipid-soluble), and the fact that FAs are more reduced than CHO---thus, when they are oxidized, more energy is derived.

Textbook reference: pages 63-68, 131-134

2. CHO are linked in a linear fashion by \(\alpha\)-1,4 and \(\beta\)-1,4 glycosidic bonds, and by branches (\(\alpha\)-1,6). An important aspect of this is that we have digestive enzymes that can hydrolyze the \(\alpha\) bonds, but not \(\beta\) (with the exception of lactase that can hydrolyze the \(\beta\)-1,4 bond between galactose and glucose that comprises lactose).

The COOH ends of FAs are linked to the OH portion of glycerol by an ester linkage. Glycerol can have 1, 2, or all 3 OH linked to a FA (i.e., mono-, di-, and triglycerides).

Textbook reference: pages 63-68, 134-135

3. Cholesterol is a sterol that is only found in animal products---plants have sterols, but not cholesterol per se. Although we typically think of cholesterol as being bad, it is a precursor for many important compounds, such as vitamin D and sterol hormones. It is essential in that we need to have it, but it is not essential in a dietary sense, as we have the capacity to make what cholesterol we need.

Textbook reference: pages 135-137

4. CHO---digestion begins in the mouth with amylase. Once CHO reach the small intestine, amylase from the pancreas carries out most of the digestion to mono- and disaccharides (remember that chyme entering the small intestine is the signal for the intestine to release CCK and secretin, and they stimulate the pancreas to release pancreatic juice and enzymes). At the brush border, disaccharidases (lactase, maltase, sucrase) complete the digestion of any remaining disaccharidases---end result is that you now have monosaccharides (glucose, galactose, and fructose) ready to be absorbed. Active transport is used for glucose and galactose; fructose is not well characterized, but likely facilitated. After entering the enterocyte, monosaccharides go to the portal vein and liver prior to the rest of the body circulation. The presence of monosaccharides in the bloodstream stimulates pancreatic insulin to be released, which in turn stimulates glucose uptake in muscle and adipose tissue, glycolysis/TCA, and glycogenesis, and down regulates gluconeogenesis.

Lipids (TGs mainly)---as with CHO, lipase from the mouth and pancreas (same hormonal signals for release) are the main digestion processes occurring. CCK also stimulates the gallbladder to release bile, a necessary emulsifier to facilitate the action of pancreatic lipase via micelle formation. Once the micelle delivers the FAs and glycerol to the enterocyte and they diffuse in, they are reassembled into TGs, packaged into chylomicrons, and put into the lymphatic system to be delivered to the entire body following the merging of the lymphatic with the general circulation near the heart.
Chylomicrons, with the help of lipoprotein lipase, deliver FAs and glycerol to tissues. Insulin again is the primary signal to stimulate lipogenesis.

Textbook reference: pages 68-76, 140-149

5. Being water-soluble, CHO's require a transporter to cross membranes, and go to the liver prior to the rest of the body. For lipid substances, they can diffuse across membranes, but require transporters (lipoproteins) in the circulation (lymphatic system first, then bloodstream). Lipids also require bile to emulsify the dietary lipid and enhance the action of digestion via micelle formation.

Textbook reference: pages 68-76, 140-149

   In the mitochondria and under aerobic conditions, carbohydrates and fatty acids can enter the Krebs cycle as acetyl-CoA and undergo complete oxidation to CO$_2$ + H$_2$O, generating the potential energy sources NADH, FADH$_2$, and GTP.

**Glycolysis** (textbook reference: pages 82-85)
The anaerobic oxidation of one glucose molecule to two pyruvate molecules that occurs in the cytosol.

**Lipolysis** (textbook reference: pages 157-158)
Hydrolysis of triglycerides to free fatty acids and glycerol (adipose); fatty acids can be bound to albumin and transported to various tissues where they undergo β-oxidation in the mitochondria to yield acetyl-CoA and energy.

**Glycogenesis** (textbook reference: pages 78-80)
Synthesis of a linear and branched glucose polymer (glycogen) from excess glucose in liver and muscle.

**Gluconeogenesis** (textbook reference: pages 97-99)
Synthesis of glucose form non-carbohydrate precursors, such as amino acids, lactate, and glycerol, that occurs in both the mitochondria and cytosol of the liver (and to some extent the kidney).

**Lipogenesis** (textbook reference: pages 161-167)
Synthesis of fatty acids from acetyl CoA in the cytosol (e.g., liver) and subsequent formation of triglycerides from fatty acids and glycerol in adipose tissue.

**Glycogenolysis** (textbook reference: pages 80-82)
Breakdown of glycogen into glucose (liver) and glucose-6-phosphate (muscle).

**Hexosemonophosphate shunt** (textbook reference: pages 95-97)
Metabolism of glucose, as glucose-6-phosphate, into a pentose (ribose), important for nucleic acid synthesis, and also generates NADPH as a by-product, which in turn is important in anabolic reactions such as the synthesis of fatty acids.
Ketosis (textbook reference: pages 159-160)
In the liver, after fatty acids are oxidized (β-oxidation) to acetyl-CoA in the mitochondria, if carbohydrate levels are limiting, the acetyl-CoA is diverted from the TCA cycle towards the synthesis of ketone bodies (acetoacetate, β-OH-butyrate, acetone) as alternative forms of energy.

7. See responses below.

8. There are many possible examples, so just a few are listed below.

Allosteric – ATP, ADP, NAD, and NADH are modulators for allosteric enzymes. Typically, ATP and NADH allosterically inhibit enzymes involved in energy-generating pathways, whereas NAD and ADP positively regulate this same enzymes. Other examples include TCA cycle enzymes (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase). End products often exert feedback inhibition (acetylCoA carboxylase is inhibited by palmitate, and HMG-CoA reductase is inhibited by cholesterol).

Posttranslational mechanisms – an example is that the enzyme phosphatase, after being stimulated by insulin, dephosphorylates glycogen synthase to activate it. This promotes glycogen synthesis. Phosphorylase kinase, which is stimulated by glucagon (liver) or epinephrine (muscle), phosphorylates the enzyme phosphorylase b. This generates phosphorylase a, the active form that breaks down glycogen to glucose-1-phosphate. These hormones stimulate (rather than induce) the enzyme that renders a second enzyme active or inactive through posttranslational modification (i.e., phosphorylation or dephosphorylation).

Induction – there are many examples. Insulin induces several enzymes, including glucokinase (not hexokinase); PFK; lipoprotein lipase; and acetyl-CoA carboxylase. Glucose-6-phosphatase, PEPCK, and TG lipase are induced by glucagon.

Textbook reference: pages 99-102, 167-168

9. Glycogenesis in either muscle or liver is signaled by the release of insulin in response to high blood glucose. Insulin induces glucokinase to increase glucose metabolism to glucose-6-phosphate in the liver and stimulates glycogen synthase phosphatase. Glycogen synthase phosphatase then dephosphorylates glycogen synthase. The latter results in glycogen synthase activation and glycogen synthesis. The same mechanism occurs in muscle, except that hexokinase rather than glucokinase increases glucose metabolism to glucose-6-phosphate (hexokinase is not induced by insulin).

Glycogenolysis in the liver is signaled by the release of glucagon in response to low blood glucose. Glucagon stimulates glycogen phosphatase kinase, which converts glycogen phosphorylase b to glycogen phosphorylase a, its phosphorylated and active form. This results in the breakdown of glycogen, ultimately to glucose-6-phosphate. Glucagon also induces glucose-6-phosphatase in the liver. This enzyme converts glucose 6-phosphate to glucose, which can then be released into the blood. Glycogenolysis in muscle is signaled by the release of ephinephrine in response to low blood glucose. Again, this stimulates glycogen phosphatase kinase, ultimately resulting in the breakdown of glycogen to glucose-6-phosphate, which is metabolized through glycolysis/the TCA cycle to generate energy. For muscle, there is no induction of glucose-6-phosphatase. Thus, glucose derived from liver glycogen is shared with the entire body, whereas energy derived from muscle glycogen is available only to the muscle.
Gluconeogenesis, which occurs primarily in the liver (it also occurs to some extent in the kidney), involves the conversion of non-CHO precursors into glucose. Gluconeogenesis is signaled by the action of glucagon in response to low blood glucose. Glucagon induces pyruvate carboxylase, PEPCK, fructose bisphosphatase, and glucose-6-phosphatase, enzymes necessary for the conversion of precursors such as AAs, lactate and glycerol into glucose, which is released into the bloodstream.

Glycolysis is the cytosolic and anaerobic oxidation of glucose to pyruvate that occurs in all tissues/cells. Under fed conditions of high blood glucose, secretion of insulin induces glucokinase, PFK, and pyruvate kinase. Glucokinase - PFK, and pyruvate kinase operate in the liver. PFK, pyruvate kinase and hexokinase (which is not induced by insulin) operate in other tissues.

We obtain lipids (namely TG) from the diet, and they are packaged in chylomicrons in the enterocyte and delivered to other tissues for energy or storage. For lipogenesis (under fed conditions), the liver can also synthesize fatty acids from acetyl-CoA, with acetyl-CoA carboxylase, the initial step in FA synthesis, being induced by insulin, as well as stimulated allosterically by elevations in citrate concentrations. The FAs are esterified into TG and packaged into VLDL for delivery. Chylomicrons or VLDL, both containing TGs, deliver their contents to other tissues for energy or to adipose tissue for storage. In either case, lipoprotein lipase is present on the tissue membrane to hydrolyze the TGs inside to FAs, so they can cross the membrane and enter the cell. For adipocytes, the FAs are reassembled into TGs for storage. Insulin (fed state; high blood glucose) induces lipoprotein lipase and inhibits TG lipase (which breaks down stored TGs to FAs inside the adipocyte).

Under fasting conditions, lipolysis occurs, owing to low blood glucose and the action of glucagon. Glucagon induces TG lipase – the resulting FAs in the adipocyte can leave the cell and are transported by albumin to tissues for use as energy (recall that this means that the FA has to be transported into the mitochondria, a process that requires carnitine, and then can undergo β-oxidation to acetyl-CoA).

Textbook reference: pages 78-99, 157-167

10. The high blood glucose characteristic of a fed state triggers the release of insulin into the blood by the pancreas. Glucose uptake, glycolysis/TCA, glycogenesis, and lipogenesis are all promoted by insulin through various mechanisms such as translocation, enzyme stimulation, or enzyme induction. Conversely, the low blood glucose characteristic of a fasted state triggers the release of glucagon into the blood by the pancreas. Gluconeogenesis, lipolysis, and glycogenolysis are all promoted by glucagon. During exercise and stress conditions, epinephrine also promotes glycogenolysis in muscle.

In both fed and fasting states, these pathways are also self-regulating in the sense that high concentrations of products (e.g., ATP, citrate, NADH) allosterically inhibit many enzymes involved in their production. Likewise, high concentrations of substrates (e.g., ADP, NAD) allosterically stimulate these same enzymes.

Textbook reference: pages 78-102, 157-168
Study Questions for Chapter 6 – Protein

The following questions / statements/ ideas are meant to help you focus your study time and try to “put things together.” Thus, rather than just providing answers, it is a way to rewrite / organize you notes. Don’t just look at a few questions, think you know the answer, and move on—spend time organizing and writing out your answers. You should contact your instructor if you have any questions, problems, etc. as you work on this.

Structure, Digestion, Absorption, and Transport

1. What is the base structure for proteins and amino acids? Discuss primary, secondary, tertiary, and quaternary levels of protein structure.

2. How are amino acids linked together? How are proteins digested and absorbed? What components are important for protein vs. CHO vs. lipid? (E.g., bile is not required to digest proteins.)

3. What makes an amino acid essential versus non-essential? Conditionally essential? Dispensable vs. indispensable?

4. What implications does amino acid/protein structure have for digestion? What implications does this have for amino acid catabolism (i.e., using amino acids for energy)?

Metabolism & Its Regulation

5. How do we synthesize proteins from amino acids? What are the functions of amino acids? What do we use amino acids for besides proteins?

6. What reactions are important in the metabolism of amino acids? Why?

7. What aspects of protein/amino acid metabolism occur in the liver? How is the liver unique? How is muscle unique? How do muscle and liver work together with respect to amino acid metabolism (particularly during fasting)? What is the role of the kidney?

8. What is “protein turnover”? How is it regulated to favor synthesis over degradation?
Example Answers for Chapter 6

1. The base structure for proteins is amino acids and for amino acids (composed of C, H, O, & N) it is a central carbon that has a COOH group, a NH₂ group, and an “R” group—R can be a number of things ranging from a H molecule to an aromatic ring, and the R group is what distinguishes one amino acid from the other. Thus, we can have neutral, acidic, basic, branched-chain, sulfur, and aromatic amino acids. The linear sequence of amino acids is its primary structure; secondary structure involves the hydrogen bonding, and tertiary structure (“3D”) is the conformation the protein forms, based on the various interactions between the amino acids and their R groups. Quaternary structure refers to the distinct subunits, sometimes identical or sometimes different, that associate together to form the final functional protein complex. Disulfide (-S-S-) bonds play a big role in protein structure, particularly at the tertiary level, to give a protein its correct conformation, as well as at the quaternary level by linking two or more subunits together.

Textbook reference: pages 182-184

2. The amino acids in proteins are connected by a peptide bond—that bond must be hydrolyzed in dietary proteins so the amino acids can be absorbed. Because there are a variety of amino acids linked together, there are a variety of proteases to handle all the different combinations of amino acids forming the bonds. In the stomach, HCl is released which denatures the protein, and the pepsinogen is converted into pepsin (all of this initiated by gastrin release by the stomach owing to food entering)—pepsin partially digests the protein. Once in the small intestine, numerous proteases (zymogens) are converted to their active forms to hydrolyze the peptide bonds. These proteases are part of the pancreatic enzymes that are released via secretin/CCK stimulation. Ultimately, amino acids and dipeptides are absorbed via active transport into the enterocyte and carried via the portal vein to the liver.

Textbook reference: pages 188-193

3. Essential means that we cannot synthesize the amino acid endogenously (or at least not enough of it) and thus it must be obtained from the diet. Non-essential amino acids can be made (e.g. in the liver) from other amino acids and keto acids. Conditionally essential amino acids are those that can be made from other amino acids (cysteine from methionine; tyrosine from phenylalanine) provided there is enough methionine or phenylalanine in the diet to spare for this process—if not, then cysteine and tyrosine become essential under that condition. Disposable and indispensable are simply other terms for non-essential and essential, respectively.

Textbook reference: pages 187-188

Because of structure, what implications does this have for digestion? Because of structure, what implications does this have for amino acid catabolism (i.e., using amino acids for energy)?

4. Like CHOs, amino acids are water-soluble and thus need a membrane transporter and initially they travel to the liver via the portal vein before reaching the rest of the body. The fact that amino acids contain nitrogen means that when they are catabolized for energy, the nitrogen must be converted
into urea and eliminated from the body. The conversion to urea as a safe means to dispose of the nitrogen is important, as free nitrogen (ammonia) in the bloodstream is toxic.


5. Amino acids are linked together by peptide bonds to form a protein, via ribosomes based on the mRNA sequence which is derived from the DNA sequence. Some functions of proteins or N-containing compounds derived from amino acids are:

**Proteins** – enzymes, transport, receptors, structure, antibodies, hormones, neurotransmitters, storage, energy

**N-containing compounds:**
- glutathione – a tripeptide (glu-cys-gly) that functions as an antioxidant that scavenges free radicals
- creatine – as creatine phosphate is a “storage” form of energy in muscle; it is derived from three amino acids, but is not a tripeptide
- carnitine – required to transport fatty acids into the mitochondria for β-oxidation
- purines/pyrimidines – bases (A,G,C,T,U) that, along with ribose or deoxyribose, form the structure of nucleic acids (DNA, RNA)
- others include thyroid hormone, neurotransmitters (serotonin, norepinephrine, dopamine), and choline (for the synthesis of phosphatidylcholine and acetylcholine)

Textbook reference: pages 179-182, 205-207

6. **Transamination**:

\[
\text{amino acid}_1 + \text{ketoacid}_2 \xrightarrow{\text{TM}} \text{ketoacid}_1 + \text{amino acid}_2
\]

e.g., alanine + α-ketoglutarate \(\equiv\) pyruvate + glutamate

Transamination provides carbon skeletons from amino acids that can be used for energy (like pyruvate when the example reaction goes left to right, or α-ketoglutarate when it goes right to left). Transamination is also important in the synthesis of non-essential amino acids (like glutamate or alanine).

**Deamination:**

\[
\text{amino acid} \Rightarrow \alpha\text{-ketoacid} + \text{NH}_3
\]

e.g., glutamate \(\Rightarrow\) α-ketoglutarate + NH₃

Deamination allows the NH₃ generated through transamination to be changed into a form that can be used in urea synthesis and excreted.

Most transamination is associated with gluconeogenesis, and the majority occurs in the liver with some occurring in the kidneys as well. The exception is BCAAs, which undergo transamination in the muscle. The liver is the site of urea synthesis.

Textbook reference: pages 208-211
7. **Liver:**

- Synthesize proteins (and N-containing compounds): including proteins for use within the liver, such as enzymes and other hepatic proteins, and proteins for secretion into the blood, such as albumin and lipoproteins. N-containing compounds that are made within the liver from amino acids include glutathione, carnitine, and creatine.
- Catabolize amino acids: this involves the production of carbon skeletons and glutamate, as well as the synthesis of non-essential amino acids from AAs or NEAAs, through transamination. Carbon skeletons are used in gluconeogenesis or directly for energy. Glutamate can be used as it is or become part of another reaction. Glutamate can participate in urea synthesis as an intermediate that donates an amine group.
- Some amino acids (BCAAs) remain in the circulation as they are, and are not metabolized by the liver.
- Synthesize N-containing compounds that are not proteins (see above)
- Urea synthesis is unique to the liver; thus during fasting when amino acids are being used for energy, the nitrogen must be delivered to the liver for synthesis of urea, which is then secreted into the circulation and excreted by the kidney.
- Under normal conditions, the liver is the primary site for gluconeogenesis; the kidney can aid in this process under conditions of high gluconeogenesis (like prolonged lack of food).
- The liver is a primary site for ketogenesis; fatty acids that are delivered to the liver from adipose tissue can undergo β-oxidation to acetyl-CoA, which can be used to synthesize ketone bodies (and secreted for energy use by other tissues) under conditions when the lack of CHO prevents the acetyl-CoA from entering the TCA cycle.

**Muscle:**

- Catabolism of branched chain amino acids.
- Alanine generation (from pyruvate): the alanine is transported to the liver for conversion to glucose through gluconeogenesis, and then the liver sends this glucose back to the muscle for energy. Since the muscle cannot synthesize urea, it also uses alanine (and glutamine) to transport nitrogen to the liver so that it can be converted to urea and excreted. During periods of fasting when protein is catabolized for energy, this means of disposing of nitrogen assumes special importance.
- Glutamine is formed by the addition of an amine group to glutamate, which is generated through transamination. Glutamine can be transported to the liver, where the amine groups are converted to urea, secreted into the circulation, and filtered out for excretion by the kidneys in the urine.

**Kidney:**

- A second tissue (to liver) for gluconeogenesis
- Urea, synthesized in the liver, goes to the kidney to be excreted in the urine

Textbook reference: pages 222-229

8. **Protein turnover is the continuous dismantling and synthesis of proteins within the body which allows it to adapt to changes and deal with the limited lifespan of individual proteins. Most (80%) of the dismantled proteins are used to make new proteins; the remainder (20%) are catabolized for energy (via transamination/ gluconeogenesis and deamination/ urea). Dietary proteins are necessary in order to replace this 20% that is “lost” from the body.**
Enzymes involved in protein synthesis have a lower $K_m$ than those involved in catabolism, and thus the synthesis enzymes “win out” when amino acids from protein degradation become available. (The AAs are synthesized into new proteins instead of catabolized.) However a high concentration of AAs resulting from over-abundance can saturate the synthesizing enzymes, allowing the catabolizing enzymes to go to work on the extra AAs.

Textbook reference: pages 232-234
Study Questions for Chapter 7 – Integration and Regulation of Metabolism and the Impact of Exercise and Sport

The following questions / statements/ ideas are meant to help you focus your study time and try to “put things together”; this is especially true with the section on metabolic integration as it involves how CHO, lipid, and protein metabolism is integrated in the body across tissues. Thus, rather than just providing answers, it is a way to rewrite / organize you notes. Don’t just look at a few questions, think you know the answer, and move on—spend time organizing and writing out your answers.

“Bigger” Questions that Cover Metabolic Integration (Proteins, CHO, & Lipids)

1. For CHO, lipid, and protein metabolism, what occurs under fed vs. fasting conditions? What signals regulate metabolic pathways, which pathways are favored vs. inhibited, and how are they regulated? (I.e., you should have at least a couple examples of specific proteins that are altered in order to regulate a given pathway.) If you already answered this question, really it is just a matter of adding proteins to your answer.

2. How does our fuel use/ metabolism change through the fed-fasted-starvation cycle? How is this the same/ different across tissues? What happens as we progress from a condition of fasting to starvation? (“We get skinny” is not an answer!!)

Impact of Exercise

3. What is the respiratory quotient? What does it tell you and why is it important?

4. How do our energy sources (i.e., systems to provide us energy) change as a function of exercise duration and intensity? (This is not a glucose vs. fatty acids vs. amino acids question—all of these contribute to providing energy, but we have different systems to convert them to energy.)
Example Answers for Chapter 7

1. Many, many examples, such as:

   Under fed conditions, high blood glucose leads to insulin secretion by the pancreas, which in turn induces expression of the key glycolytic enzyme phosphofructokinase in all cells, thereby increasing glycolysis.

   In the fasted state, low blood glucose leads to glucagon secretion by the pancreas, which in turn induces PEPCK in the liver to enhance gluconeogenesis, thereby increasing blood glucose levels.

   Textbook reference: pages 251-254, 256-261

2. Fed state:
   Insulin secretion in response to high blood glucose signals increases in: glycolysis, glucose uptake (via GLUT 4 translocation), glycogenesis, lipogenesis, and protein synthesis.

   Postabsorptive/fasting state:
   Glucagon secretion in response to low blood glucose and/or epinephrine signal increases in: glycogenolysis, gluconeogenesis, urea synthesis, protein degradation, and a limited amount of lipolysis.

   Starvation state:
   Glucagon secretion in response to low blood glucose and/or epinephrine signal increases in: lypolysis and ketogenesis. At this point, the body’s glycogen stores have been exhausted and there is a need to spare protein by slowing its catabolism.

   Prolonged starvation state:
   Glucagon secretion in response to low blood glucose and/or epinephrine signal increases in: gluconeogenesis and protein degradation. Fat stores have now been depleted, leaving body protein as the only source of energy. Even visceral proteins will be catabolized in the effort to forestall death.

   Textbook reference: pages 256-261

3. Respiratory quotient (RQ) = volume of CO₂ expired / volume of O₂ used.

   The RQ indicates the volume of oxygen consumed in the complete oxidation of a macronutrient into carbon dioxide during energy production. Lipids, for example, both require more oxygen for oxidation to CO₂ and release more energy as compared to other macronutrients, because they are in a more reduced state. Carbohydrates, on the other hand, are in a partially oxidized state (contain many OH groups), so they require less oxygen for complete oxidation.

   For CHO: \( RQ = 1.0 \)
   For lipid: \( RQ = 0.7 \)
   For protein: \( RQ = 0.8 \)

   Textbook reference: pages 265-266
4. **Creatine phosphate (CP)-ATP system** – phosphate is stored in muscle as CP, which can be used to generate ATP from ADP, providing quick energy for high-intensity exercise of very brief duration.

- **Lactic acid system** – anaerobic glycolysis generates ATP from glucose or glycogen, and the pyruvate is converted to lactate. This system functions simultaneously with the CP-ATP system, but can be sustained for a somewhat longer duration.

- **Aerobic system** – glycolysis of glucose/glycogen, followed by entry of pyruvate into the TCA cycle instead of conversion to lactate, in the presence of oxygen. This produces a greater amount of energy than simple glycolysis, and permits the use of fatty acids as well as glucose. The aerobic system occurs simultaneously with the other two systems, and eventually becomes the major contributor of energy as activity continues.

Textbook reference: pages 266-270
Study Questions for Chapters 9 & 10 – Water-Soluble Vitamins & Fat-Soluble Vitamins

The following questions / statements should help you see not only the specifics of a given vitamin, but how they are similar to and different from each other. Also, it is important to keep in mind the big picture—that is, understanding the role vitamins have in metabolism and how they carry out their functions.

Structure, Digestion, Absorption, and Transport

1. What are the similarities and differences between how we absorb, transport, metabolize, store, and excrete water-soluble vs. fat-soluble vitamins? What components of the digestive system are needed for each?

2. For a number of vitamins, we have more than one form that we get from the diet. How does this relate to the requirements of a given RDA? Think of some specific examples (both fat- and water-soluble) to support you answer.

Function, Requirement, and Assessment

3. What are the active forms of each vitamin? How do the functions of vitamins differ? How are they the same? Name the general type of reactions each vitamin participates in. What specific enzymes and metabolic pathways would be affected for a given vitamin deficiency?

4. In general, in what ways do we assess vitamin status? Which method is the best and why? (You should know assessment for each vitamin.)

5. How do vitamin A and vitamin D alter the expression of genes? For each vitamin, what is the active form, what proteins are required, how mechanistically is this accomplished, and what is the end result?

6. What is the role of vitamin K? What is the importance of the vitamin K cycle?

“Bigger” Questions

7. Discuss the involvement of water-soluble vitamins in the metabolic pathways for each of the macronutrients.

8. How does function relate to deficiency for each vitamin (especially fat-soluble vitamins)?

9. Classify vitamins based on their potential to regulate proteins, such as enzymes. Which vitamins can regulate proteins by induction? Do any of them regulate proteins via posttranslational modification? Allosterically? Provide examples for each of these mechanisms.
Example Answers for Chapters 9 & 10

1. This is basically a water-soluble vs. lipid-soluble question (although there are exceptions for both). Water-soluble vitamins require a carrier for absorption; they travel via the portal vein to the liver first. There is little storage of water-soluble vitamins (exception here is B12), and thus we need to consume them on a regular basis; typically they are not toxic; they are readily excreted in the urine. Lipid-soluble vitamins will be absorbed via micelle formation, diffusion, chylomicrons, lymphatics, and then the general circulation. They need transporters in the circulation (or any aqueous environment). They are stored, and thus we can consume more intermittently; but this also means that they can accumulate and be toxic.


2. This usually results in having to use the term equivalents, to highlight the fact that there are multiple forms or sources of a vitamin that result in differences in absorption, metabolism, etc.—thus, to compare the dietary intake on a weight basis, we need a way to account for these differences. Examples: folate, dietary folate equivalents (DFEs); vitamin A, retinol activity equivalents (RAEs); and niacin, niacin equivalents (NEs). For folate, this is because folate that is present due to fortification is in the monoglutamate form, which is absorbed better than the polyglutamated form found in foods naturally. This results in about a 2-fold difference. For vitamin A, it is a retinyl ester (animal sources) vs. carotenoid (plant sources) issue—retinyl esters are better absorbed than carotenoids, and of the carotenoids that are absorbed, only some are converted into retinol. For β-carotene, this results in needing 12x more than if it were retinyl esters. For niacin, it relates to the fact that tryptophan, from dietary protein, can be converted to niacin, but it takes 60 mg of trp to result in 1 mg niacin.


3. Niacin’s active form is NAD(H) or NADP(H), and it participates in oxidation-reduction reactions. A niacin deficiency would affect TCA cycle reactions, and enzymes such as alpha-ketoglutarate dehydrogenase.

Biotin (biotin is the active form) participates in carboxylation reactions, so a deficiency would affect fatty acid synthesis (acetyl CoA carboxylase), odd-chain fatty acid degradation/oxidation (propionyl CoA carboxylase), and gluconeogenesis (pyruvate carboxylase).

Pantothenate’s active form is coenzyme A, and it participates in reactions by activating substrates or intermediates, and is involved in protein acetylation. A pantothenate deficiency would affect glycolysis, the TCA cycle, fatty acid synthesis, and oxidation (acetyl CoA, malonyl CoA, etc.).

Riboflavin’s active form is FAD(H2), and it participates in oxidation-reduction reactions. A riboflavin deficiency would affect the TCA cycle, fatty acid beta-oxidation, and glutathione reduction.

Vitamin B6’s active form is PLP, and it participates in transamination, transsulfuration, and decarboxylation reactions. A B6 deficiency would affect gluconeogenesis (conversion of amino acids
to ketoacids), synthesis of heme and neurotransmitters, and conversion of homocysteine to cystathionine and then to cysteine.

Thiamin’s active form is TPP or TDP, and it participates in oxidative decarboxylation reactions. A thiamin deficiency would affect conversion of pyruvate to acetyl CoA, conversion of α-ketoglutarate to succinyl CoA, and the function of HMS as transketolase.

Vitamin C (vitamin C or ascorbate acid is its active form) participates in hydroxylation reactions, so a deficiency would affect synthesis of collagen, carnitine and neurotransmitters.

Vitamin K’s active form is dihydrovitamin KH₂, and it participates in carboxylation reactions. A vitamin K deficiency would affect activation of clotting proteins (conversion of prothrombin to thrombin) and bone proteins (osteocalcin).

Folate’s active form is tetrahydrofolate polyglutamates, and it participates in one-carbon transfer reactions. A folate deficiency would affect synthesis of DNA (uridylate to thymidylate) and conversion of homocysteine to methionine.

Vitamin B₁₂’s active form is cobalamin, and it participates in methyl group transfer reactions. A B₁₂ deficiency would affect the enzyme methionine synthase, which is involved in the reaction that combines 5-methyltetrahydrofolate with homocysteine to yield methionine and tetrahydrofolate. It is also involved in the conversion of methylmalonyl CoA to succinyl CoA, a step in the oxidation of odd-chain fatty acids.

Vitamin E’s active form is alpha-tocopherol, and it participates in oxidation-reduction reactions. A vitamin E deficiency would allow increased peroxidation of lipids by free radicals (which are normally scavenged by vitamin E).

Vitamin D’s active form is 1,25-(OH)₂ cholecalciferol, and it participates in calcium homeostasis reactions. A vitamin D deficiency would affect (reduce) calcium absorption by enterocytes, renal reabsorption of calcium, and bone resorption.

Vitamin A’s active forms are retinol, retinal and retinoic acid. It participates in reactions related to reproduction, maintenance of vision (visual cycle), and cell growth/differentiation, so a deficiency would affect all of these aspects of physiology negatively. The retinoic acid form of vitamin A activates nuclear receptors (RAR, RXR). These nuclear receptors bind to responsive elements (RARE) present in specific genes.


4. One method to assess vitamin status is using a “load test,” which is suitable for vitamins that are known to be required at a specific point in a metabolic pathway. A large quantity (load) of substrate is supplied in order to challenge the pathway. Normal metabolism of the substrate indicates the presence of the vitamin. Re-routing of the substrate to an alternate pathway (products of which will appear in urine) or a build-up of the intermediate compound formed prior to the metabolic step requiring the vitamin (and its excretion in urine) indicate a lack of the vitamin.
For example, vitamin B₆ is required for conversion of tryptophan to niacin. With B₆ deficiency, tryptophan enters an alternative pathway and is instead converted to xanthurenic acid, which can be detected in urine. B₆ is also required for catabolism of methionine to cysteine, but in this case, a deficiency results in a build up of the intermediate compound cystathionine, which can be detected in urine.

Another method to assess vitamin status is to measure the activity of an enzyme for which the vitamin is a cofactor. First, a sample is taken from an individual and the specific enzyme’s activity in the sample is measured, usually in the RBC. Then, its activity is measured a second time following introduction of exogenous vitamin into the test tube. An increase in the enzyme’s activity after the vitamin is added indicates a deficiency within the person’s body (i.e., a portion of the total enzyme was originally inactive because they lacked the needed cofactor).

For example, the enzyme transketolase requires the TPP form of thiamin as its cofactor to be active. The activity of the enzyme transketolase in a sample of red blood cells, after they have been lysed, can be measured before and after the introduction of exogenous TPP, and a thiamin deficiency can be diagnosed when an enzyme activity increase of greater than 25% occurs. This is called the erythrocyte transketolase activity (ETKA) test. In the case of riboflavin, a similar test is performed on the enzyme glutathione reductase, which requires FAD as a cofactor. If the ratio of enzyme activity in the FAD-added sample to activity in the unmodified sample is greater than 1.4 (40% increase), a riboflavin deficiency can be diagnosed.

There are many ways to address the question of which test is best, depending on which advantages/disadvantages are most heavily weighted; for instance, you could consider how expensive or simple to perform each test is, etc. A major advantage for both kinds of test is that they are functional tests for assessment of vitamin status.


5. You should know in detail how vitamin A and vitamin D alters the expression of genes – what is the active form, what proteins are required, how mechanistically it is accomplished, and what the end result is.

5. Vitamins A and D alter gene expression through induction—increasing the abundance of a certain protein by increasing the expression (specifically, the transcription) of the genes responsible for its synthesis. Vitamin D attaches to vitamin D binding protein (VDBP) in an intestinal cell’s cytosol and is taken into the nucleus, where it is bound to vitamin D receptor (VDR). Another protein called retinoid X receptor (RXR) binds to VDR to form a complex (VDR-RXR) that can bind to vitamin D response element (VDRE). VDRE is found in the promoter region of the gene that codes for CaBP, calcium transporters, and is activated by VDR-RXR, resulting in increased transcription of CaBP. This increased transcription of CaBP increases its production, which in turn increases the rate of calcium transport into the enterocyte.

The retinoic acid form of vitamin A participates in a similar series of events. It is conveyed into the nucleus by CRABP (protein in the cytosol), binds to a retinoic acid receptor (RAR), and then to RXR to form the RAR-RXR complex. Binding of this complex to the retinoic acid response element (RARE) located on particular genes increases their transcription, and thus the production of proteins encoded by those genes.
Vitamins D and A regulate gene expression only in certain types of cells, such as the enterocytes that produce CaBP (vitamin D). A gene is expressed by manufacture of mRNA for that gene—transcription—and movement of that mRNA to the nucleus for translation into a protein. Minerals such as zinc and copper regulate gene expression by the same mechanism, but with different response elements. (Iron, however, interacts with response elements located on mRNA instead of genes [DNA], so it regulates gene expression at the translational level in the cytosol rather than at the transcriptional level in the nucleus.)


6. Vitamin K, as dihydrovitamin KH₂, participates in the carboxylation of glutamate residues in proteins involved in coagulation. The addition of a COO⁻ group to the glutamate residue in a given protein allows it, along with the other COO⁻ group already part of the glu side chain, to bind Ca²⁺. The binding of calcium activates the protein. One example is the conversion of prothrombin (inactive) to thrombin (active). All of these proteins that require vitamin K and hence carboxylation to be active are part of the coagulation process and represent a form of PTM—we are altering the function of coagulation proteins, in this case activating them, by forming covalent bonds with a carboxy group, without changing the abundance of the protein. Following a reaction, vitamin K must be recycled back to its reduced dihydrovitamin KH₁ form—this requires a series of reactions (vit K cycle). This is also how many anticoagulants (e.g., warfarin) work—by inhibiting this cycle, a functional deficiency of vitamin K ensues and thus blood clotting is impaired.

Textbook reference: pages 411-414

7. **Carbohydrates**: thiamin, as TPP, for the enzymes pyruvate dehydrogenase & α-ketoglutarate dehydrogenasel transketolase (with the HMS); niacin, as NADH, for glycolysis and the TCA cycle, as NADPH from HMS; riboflavin, as FADH₂ from the TCA cycle; and pantothenate as CoA.

**Lipids**: niacin as NADH and riboflavin as FADH₂ for fatty acid oxidation, which produces acetyl CoA (pantothenate); niacin, riboflavin, biotin propionyl CoA carboxylase) and vitamin B₁₂ (methylmalonyl CoA mutase) for odd-chain fatty acid oxidation, which produces acetyl CoA (pantothenate); thiamin, as TPP, for the TCA cycle.

**Protein**: vitamin B₆ for transamination of amino acids to ketoacids during gluconeogenesis; biotin (pyruvate carboxylase) when the ketoacid thus produced is pyruvate, for gluconeogenesis; NAD, FAD, TPP, and pantothenate (CoA with succinyl CoA) for the TCA cycle, which metabolizes ketoacids produced from amino acids with more than 3 carbons.


8. For many of the vitamins involved in energy metabolism, there is an impairment in those pathways when the vitamin is deficient. Folate is required for DNA synthesis—a lack of folate results in anemia due to an inability to synthesize mature red blood cells. One will also see anemia with a lack of B₉, but this is due to insufficient synthesis of heme, which involves the vitamin (thus resulting in a microcytic anemia, not megaloblastic anemia as seen with folate deficiency). For vitamin K, deficiency results in lack of blood clotting; for vitamin A, vision problems, beginning with night blindness; for vitamin D, impaired calcium homeostasis which can result in bone abnormalities; for
vitamin E, red blood cell hemolysis due to membrane fragility owing to lipid peroxidation. This is not an exhaustive answer, just some examples to think about.


9. For examples of induction, see the discussion of induction by vitamins D and A in the answer to #5.

Posttranslational modification means altering the function of an existing protein (but not increasing its quantity) by forming a covalent bond. The coenzyme A form of pantothenic acid activates proteins by adding an acetate group (CH₃COO⁻) to them via a covalent bond, a process known as the acetylation of proteins.

Posttranslational modification also occurs as part of one step in collagen synthesis. In this case, rather than increasing the synthesis of collagen, it serves to modify its structure so that it is able to form cross-links with other collagen molecules and thus become strong, active collagen. This structural change is accomplished through hydroxylation of the lysine or proline residues in collagen (this is the posttranslational modification step), a reaction catalyzed by an enzyme that must be regenerated by vitamin C. Fe⁺⁺, a cofactor contained in this hydroxylation enzyme, is oxidized to Fe+++ after the reaction, and must be reduced to Fe⁺⁺ by vitamin C to restore the enzyme’s functionality. Similar hydroxylation reactions that are dependent on vitamin C follow the same pattern, but because the substrate being hydroxylated in these other reactions is not a protein, they do not represent posttranslational modification.

Vitamin K is involved in increasing the activity of several blood coagulation proteins, and the covalent modification of bone proteins. Vitamin K participates in the carboxylation of glutamate residues in these proteins, which allows them to bind Ca²⁺ and hence become more active.

Allosteric regulation is achieved through the binding of certain substances to enzymes within a pathway; these substances either inhibit or activate the enzymes. ATP acts in this manner by binding to and inhibiting a major glycolytic enzyme, phosphofructokinase (PFK), to slow glycolysis when energy is abundant. Vitamins that can regulate enzymes allosterically include niacin and pantothenic acid.

The NADH form of niacin inhibits enzymes such as pyruvate dehydrogenase, KFK, or α-ketoglutarate dehydrogenase when its concentration increases, as it does after a meal. As part of acetyl CoA, pantothenic acid promotes the activity of pyruvate carboxylase when it is present in high levels, which results when a lot of pyruvate (from glycolysis) is converted to acetyl CoA through the action of another enzyme, pyruvate dehydrogenase. Pyruvate carboxylase diverts pyruvate away from the oxidation pathway to the gluconeogenesis pathway, reducing the amount of acetyl CoA being generated.

Study Questions for Chapters 11 & 12 – Macrominerals & Microminerals

The following questions pertain to the several of the major and trace minerals (zinc, copper, iron, and a few others). You might also want to practice answering some of the questions from the vitamins chapter of this study guide (e.g. how levels are assessed in the body) for the minerals.

1. What are the similarities and differences between how different minerals are absorbed, transported and stored in the body? What factors can have an impact on these processes for minerals? How does this relate to the statement: “…minerals are essential, but potentially toxic…”?

2. Discuss how zinc and copper regulate the expression of metallothionien. Make sure to name the various proteins/ response elements involved. Do the same for iron and regulation of transferrin receptor/ ferritin. What are the similarities in regulation between zinc/copper and iron? What are the differences?

3. In general, what are minerals used for? Name at least a few examples for each general function you listed. How do these functions relate to the symptoms exhibited during deficiency?

4. The deficiency of one mineral can result in the deficiency of other minerals and/ or vitamins – what are some examples?
Example Answers for Chapter 11 & 12

1. A number of minerals such as sodium (electrolytes) are readily absorbed and easily excreted. However, many others, such as Fe, Zn, and Ca, are not readily absorbed and have the potential to accumulate in tissues as they are not readily removed from the body, but rather are stored. Storage of minerals is beneficial because they serve to meet our needs when intake is low, but exceeding the body’s capacity to safely store a mineral can result in toxicity. There are also a number of minerals that are not essential, but nonetheless can get into the food supply and become toxic because they accumulate in tissues (e.g., Pb, Hg). Thus, nutrient minerals are essential (although some are easily absorbed and others are not), but too much of a mineral that cannot be stored safely can lead to many problems.

Other dietary components can impact absorption, both positively and negatively. For example, vitamin C and low iron status can help iron absorption, but other compounds such as phytate can inhibit iron absorption. In addition, many minerals can compete with others for absorption and inhibit each other.


2. The body needs to get iron into cells, as it is essential as a cofactor for a number of proteins; however, we need to store it in a safe form because free iron (Fe\(^{++}\)) can be toxic. Transport of iron into cells requires the transferrin receptor, and storage requires the protein ferritin. As one would predict, we need more transferrin receptor when iron concentrations in the cell are low, and less when concentrations are high. The opposite is true for ferritin – we want more ferritin when iron levels in the cell increase, but less when they are low. Thus, transferrin and ferritin are regulated in a divergent fashion, and in response to intracellular iron concentrations. A key protein to accomplish this is iron regulatory protein (IRP). IRP is a cytosolic protein that binds to the iron responsive element (IRE) present in the 5’ untranslated region of ferritin mRNA, and multiple IREs exist on the 3’ untranslated region of transferrin receptor mRNA. When intracellular iron concentrations are low, IRP is activated, wherein it binds to the IRE – in the case of ferritin, this inhibits its translation, and for transferrin receptor, it stabilizes the message, resulting in an increase in translation. The opposite happens as iron concentrations increase – IRP doesn’t bind to IRE, allowing ferritin translation to increase (thus providing the means to store the excess iron), and transferrin mRNA to be degraded faster (thus decreasing the amount of iron entering the cell).

For zinc and copper, excess can be toxic so we want a storage protein and its synthesis to be responsive to zinc and copper concentrations in the cell. As zinc and copper increase, they activate the nuclear metal binding protein, which in turn binds to the metal response element present in the promoter region of metallothionein, a storage protein for these two minerals. So, this mechanism is conceptually similar to vitamins A and D, whereas iron is a posttranscriptional mechanism.

Textbook reference: pages 476-479, 491-493, 495, 500-501

3. The electrolytes (Na, K, Cl) are involved in water balance, neural/ muscular function, cellular ionic balance, etc. Most of the other minerals are cofactors for proteins in which they play an enzymatic, structural, or transport role. Zn is a cofactor for many enzymes and also plays a structural role for
some transcription factors, like RAR. Iron is well known as having a transport role with hemoglobin, but is also part of a number of enzymes (heme-containing and iron-containing). Ca (along with P) and F play structural roles in bone/teeth, and other minerals that may be part of a protein can have a structural role (zinc for superoxide dismutase). Depending on the protein or structure being affected, there is a clear correspondence with deficiency symptoms (anemia, Fe and Zn; bone damage; etc.).


4. One example of this is iron and copper. Ceruloplasmin is a copper transport protein that also functions as a ferrioxidase – i.e., an enzyme that oxidizes ferrous iron to ferric iron, the latter being the form that binds to transferrin and is transported throughout the body. Thus, a lack of copper can result in iron deficiency. Many minerals do compete for absorption, and thus too much of one can lead to a deficiency of the other. Minerals can affect vitamins if they are involved in some way in their synthesis or absorption. An example here is conjugase – a zinc-containing enzyme that is required for folate absorption (it removes the polyglutamate residues on food folate – necessary as only the monoglutamate form is absorbed). Thus, if zinc is low, the activity of this enzyme is reduced, and the ability to convert food folate to a form that is more readily absorbed is impaired.

Textbook reference: 483, 494, 503; also 439-440, 449-450, 453, 455, 495, 511, 515, 524, 528, 531