# Chapter 14
# Nutrient metabolism in peripheral tissues

*A. Danfær*

**This chapter describes:**

- How nutrients are distributed among body tissues
- How nutrients and metabolites are transported into and out of tissue cells
- The organisation of intracellular biochemical pathways that supply substrates and energy for synthesis and degradation of protein, lipid and bone in the peripheral tissues
- How energy efficiency in protein and lipid retention can be theoretically estimated

## 1. Introduction

This chapter deals with the molecular basis for growth in the large peripheral masses of the body, i.e. muscle, adipose and bone tissues. The peripheral tissues are those “outside” the splanchnic bed, which comprises the portal drained viscera (PDV: digestive tract, pancreas, spleen, and omental fat) as well as the liver. This means that the net release of nutrients from the splanchnic bed is available for use in peripheral tissues.

The overall objective of the chapter is to provide the reader with an understanding of the biological processes responsible for conversion of dietary nutrients into tissue mass.

## 2. Nutrient flows through the pig body

An overview of the major nutrient flows from feed to body tissues is presented in Figure 14.1. The net release of amino acids, glucose and SCFA (short chain fatty acids, i.e. acetate, propionate and butyrate) from PDV is transported to the liver with the portal blood. Amino acids and glucose are absorbed from the small intestine (see Chapters 8 and 9), whereas most of the SCFA formed by fermentation of fibrous carbohydrates is absorbed from the large intestine (see Chapter 8). Absorbed fatty acids are taken up as chylomicrons by intestinal lymph and transported with the lymphatic system to the peripheral circulation, thus surpassing the liver (see Chapter 10).

The liver takes up nutrients both from the portal vein and from the peripheral blood via the hepatic artery, while nutrients exported from the liver enter the peripheral circulation via hepatic veins and the vena cava (see Chapter 13). In fed pigs, a small part of the glucose supplied with portal blood is taken up by the liver and stored as glycogen or oxidized, but most of the glucose passes to the peripheral circulation (see Chapter 13). In fasted animals, glucose is released from degraded glycogen, can be synthesized from keto acids and lactate, and is exported from the liver.

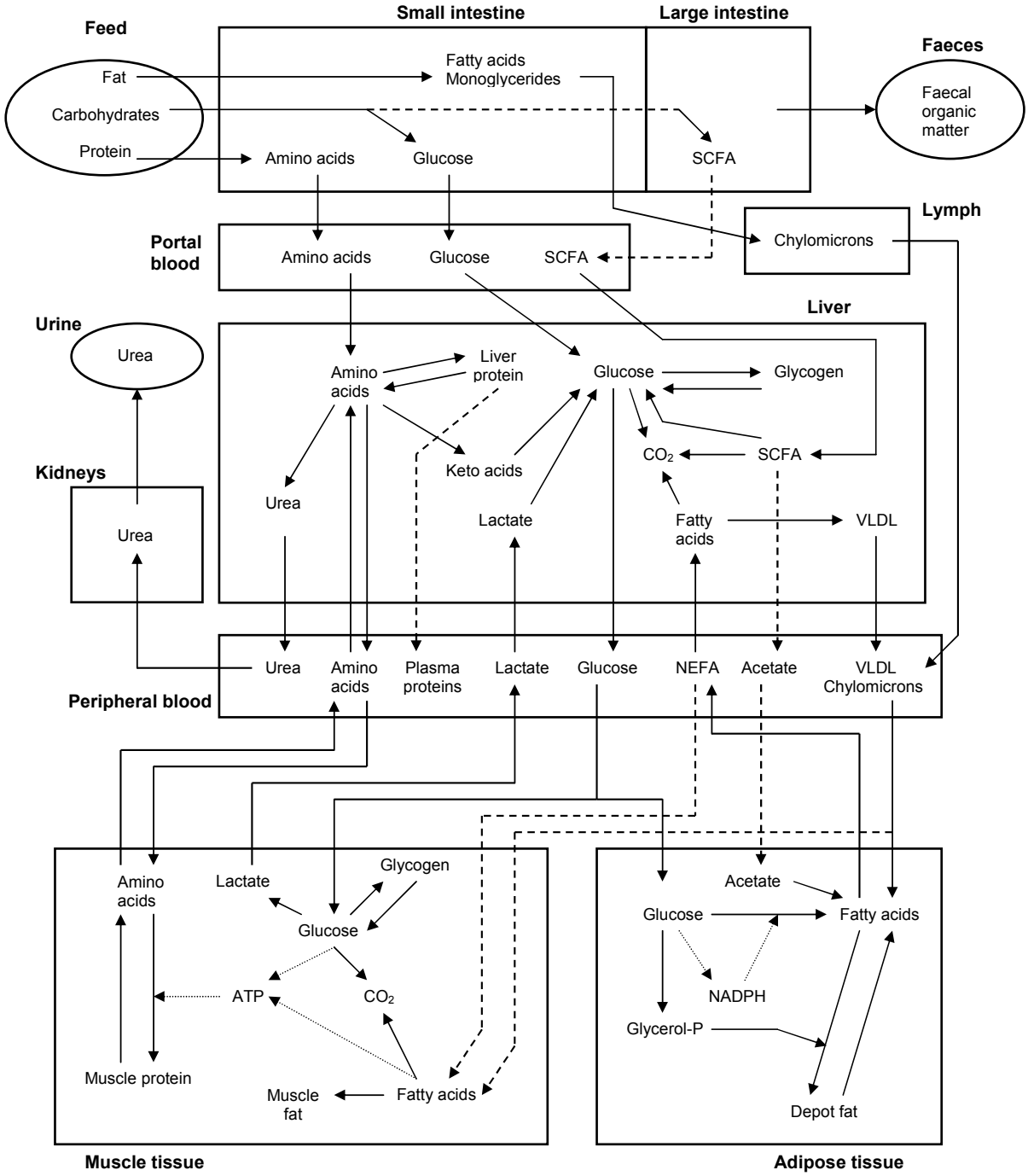



**Figure 14.1. An outline of nutrient digestion, interorgan transport, and tissue metabolism in the pig.**

Amino acids are taken up into the liver from the portal as well as from the peripheral blood. Metabolic fates of hepatic amino acids include protein syntheses, transaminations into other amino acids, and deamination into keto acids. Some of the synthesized proteins are domestic liver proteins and some are exported. Degraded liver protein also contributes to the hepatic pool of free amino acids. In growing animals in which the liver mass is increasing, the rate of protein synthesis exceeds the rate of degradation. Nitrogen from deaminated amino acids is trapped into urea, ex-

ported, and secreted via the kidneys into the urine. As described in Chapter 13, there are large differences among the individual amino acids with regard to the extent of their metabolism in the liver. For some of them, notably the branched chain and other essential amino acids, the major fraction is not metabolized, but exported to the peripheral circulation.

Most of the propionate and butyrate is metabolized in the liver by oxidation, but some propionate can be used for glucose synthesis. Acetate passes to the peripheral circulation. Non-esterified fatty acids (NEFA) are taken up by liver cells from the arterial blood and can be oxidized to $CO_2$ or incorporated as triacylglycerol in very low density lipoproteins (VLDL) for export.

Circulating amino acids are taken up by all peripheral tissues, but in growing pigs, the largest fraction is used in skeletal muscles for protein synthesis. Muscle protein retention is the difference between protein synthesis and degradation (proteolysis), and some amino acids from degraded protein are transported back to the peripheral blood. Muscle glucose can be temporarily stored in glycogen or be oxidized to $CO_2$ providing energy in the form of ATP, which is required for protein synthesis and muscle contractions. Glucose can also be metabolized anaerobically to lactate, which in turn is released to the extracellular fluid and transported to the liver, where it is converted back to glucose by gluconeogenesis. This journey of lactate completes the so called Cori cycle. Fatty acids are taken up by muscle tissues both from the circulating NEFA pool and from lipoprotein particles, such as chylomicrons and VLDL, by action of the enzyme lipoprotein lipase. Intracellular fatty acids are used as fuel providing ATP, and as substrates for synthesis of intramuscular fat.

Of the circulating nutrients and metabolites shown in Figure 14.1, only amino acids, glucose, acetate and chylomicrons originate directly from the diet. Urea and VLDL are produced in the liver, lactate comes from muscles (although some lactate may be absorbed from the stomach), and NEFA is released to the extracellular fluid from adipose tissues as a result of lipolysis. Lipid retention in adipose tissue is - like protein retention - the difference between synthesis (lipogenesis) and degradation (lipolysis). The substrates for lipogenesis are fatty acids and glycerol-P. Fatty acids are taken up from circulating lipoproteins with the aid of lipoprotein lipase, but are also synthesized from glucose and acetate. Glucose provides both carbon and energy in the form of NADPH for fatty acid synthesis, and is also the substrate for synthesis of glycerol-P. The individual metabolic pathways taking place in muscle, adipose and bone tissues are described in more detail in later sections of this chapter.

As indicated in Figure 14.1, there is a heavy traffic of nutrients and metabolites between and into different tissues. One level of transport is circulation with blood and lymph (common transport), and another level is membrane transport whereby individual molecules are transferred into or out of tissue cells (individual transport). Body growth is a result of increases in mass of the large body compartments, i.e. muscle, adipose and bone tissues. Figure 14.1 shows that the metabolism in muscle and adipose tissues is based on largely the same circulating nutrients, and the composition of growth is therefore dependent on the nutrient partition between these compartments. This partition is regulated by variations in rates of blood flow to different tissues, and by variations in rates of nutrient cellular uptake. The chemical structure of cell membranes discriminates between different kinds of nutrients with regard to their ability to cross these membranes. Therefore, different transport mechanisms exist so that the transfer of various nutrient molecules in and out of cells can proceed at sufficiently high rates.

## 3. Mechanisms of membrane transport

Membranes surrounding cells (plasma membranes) are built as a bilayer of phospholipids with the polar, hydrophilic glycerol-phosphate heads facing the aqueous extracellular and cytoplasmic sides, while the non-polar, hydrophobic tails of fatty acids turn inside against each other. Cholesterol molecules are inserted between the phospholipid tails, thus contributing to create a lipophilic zone at the interior of the membrane. Furthermore, various types of proteins are embedded in the

phospholipid bilayer: transmembrane proteins extend fully across the membrane, while peripheral proteins are more loosely attached to the outer or inner surface of the membrane. Some carbohydrates are linked to either membrane proteins (forming glycoproteins) or to membrane lipids (forming glycolipids) at the outer surface, i.e. towards the extracellular space. One function of glycoproteins is to participate in the body's immune response.

This membrane structure works as a tool to control the passage rate of nutrients and ions from the extracellular to the intracellular space and vice versa. Passage of substances across membranes takes place by passive or active transfer mechanisms. Passive transport is not energy-dependent, whereas active transport requires the input of energy from some outside source, such as the high-energy phosphate bond of ATP. Very small molecules and those that are lipid-soluble can cross directly through the phospholipid bilayer. Larger or less lipid-soluble molecules are excluded from crossing the bilayer unless the cell has a specific mechanism for their transport. For these molecules, membrane proteins are the usual mediators of transport. Very large lipophobic molecules must enter and leave cells in vesicles. According to this, transport mechanisms can be described as diffusion, protein-mediated transport, or vesicular transport. By transepithelial transport, molecules are transferred through intact cells, i.e. crossing two membranes. In the following, these different modes of transport are briefly described.

### *3.1. Diffusion*

Diffusion is a passive transport process as it only uses the kinetic energy possessed by all molecules. Molecules diffuse down a concentration gradient from higher to lower concentration, and net movement of molecules occurs until the concentration is equal on both sides of a membrane. Diffusion directly across the phospholipid bilayer of a membrane is called **simple diffusion**. The rate of diffusion depends on the ability of the diffusing molecule to dissolve in the lipid bilayer of the membrane, and, as a rule, only lipids, steroids, and small lipophilic molecules can move across the membrane by simple diffusion. Hydrophilic substances dissolve in water and are lipophobic, i.e. they do not readily dissolve in lipids. For this reason, the hydrophobic lipid core of the cell membrane acts as a barrier that prevents hydrophilic molecules from crossing. Ions move in response to combined electrical and concentration gradients, i.e. electrochemical gradients.

### *3.2. Protein-mediated transport*

The majority of molecules in the body is either lipophobic or electrically charged and therefore cannot cross membranes by simple diffusion. These substrates cross membranes with the help of membrane proteins, i.e. mediated transport. **Facilitated diffusion** is protein-mediated transport that is passive and moves molecules down their concentration gradient. Sugars and some amino acids are examples of molecules that enter or leave cells using facilitated diffusion. A family of carrier proteins, known as the GLUT transporters, transfer glucose and related hexose sugars across membranes. The transported molecules move down their concentration gradient; the process requires no energy input, and net movement stops at equilibrium. Cells in which facilitated uptake takes place can avoid reaching equilibrium by keeping the substrate concentration low inside the cell. With glucose, this is accomplished by phosphorylation of glucose to glucose-6-P, the first step of glycolysis. As an important feature in glucose metabolism and homeostasis, specific GLUT transporters are restricted to different tissues: GLUT2 transports glucose and other hexoses, and is located in the liver as well as in the transporting epithelium of the intestine and kidneys; the GLUT4 transporters are regulated by insulin and are located in adipose and skeletal muscle tissues; GLUT5 transports fructose and is located in intestinal epithelium. When the blood glucose concentration is high, i.e. just after feeding, GLUT2 transporters on hepatocytes move glucose into these cells. During fasting when blood glucose levels decrease, liver cells convert their glycogen stores to glucose, and when the increasing intracellular concentration exceeds the blood glucose concentration, glucose leaves the cells via the reversible GLUT2 transporters, thus increasing the blood glucose level so that GLUT4 proteins can facilitate high rates of glucose uptake in muscle and adipose tissues.

*3.3. Active transport*

If protein-mediated transport requires energy from an outside source and moves a substance against its concentration gradient (an uphill reaction), the process is known as active transport. The energy for active transport comes either directly or indirectly from the high-energy phosphate bond of ATP. In primary (direct) active transport, the energy to push molecules against their concentration gradient comes directly from the hydrolysis of ATP. Secondary (indirect) active transport uses potential energy stored in the concentration gradient of one molecule to push other molecules against their concentration gradient. A substrate to be transported binds to a membrane carrier, and the carrier then changes conformation and releases the substrate into the opposite compartment. Active transport differs from facilitated diffusion because the conformation change in the carrier protein requires energy input.

An example of a **primary active transport** mechanism is the sodium-potassium pump, which is probably the single most important transport protein in animal cells because it maintains the $Na^+$ and $K^+$ concentration gradients across the cell membrane. This transporter is arranged in the cell membrane so that it pumps 3 $Na^+$ out of the cell and 2 $K^+$ into the cell for each ATP consumed. The sodium concentration gradient, with $Na^+$ concentration high in the extracellular fluid and low inside the cell, is a source of potential energy that the cell can harness for other functions. **Secondary active transport** uses the kinetic energy of one molecule moving down its concentration gradient to push other molecules against their concentration gradient. One secondary active transporter is the $Na^+$-glucose symporter (SGLT), which binds to both $Na^+$ and glucose on the extracellular fluid side. Sodium binds first and causes a conformational change in the protein and creates a high-affinity binding site for glucose. When glucose binds to the SGLT, the protein changes conformation again and creates a channel to the intracellular side. Sodium is released as it moves down its concentration gradient. The loss of $Na^+$ from the protein changes the binding site for glucose back to a low-affinity site, so glucose is released and follows $Na^+$ into the cytoplasm. The net result is the entry of glucose into the cell against its concentration gradient, coupled with the movement of $Na^+$ into the cell down its concentration gradient. However, the $Na^+$ concentration gradient is originally created at the expense of ATP.

GLUT carriers are found on all cell membranes, but $Na^+$-glucose symporters are restricted to cells that bring glucose into the body from the external environment. GLUT transporters move glucose into or out of the cells depending on the concentration gradient. In contrast, the SGLT transporters can only move glucose into the cell because it must follow the $Na^+$ gradient. Consequently, SGLT transporters are found on epithelia, such as intestinal mucosal and kidney tubular cells that bring glucose into the body from the external environment.

Amino acids cross membranes with the aid of several protein-mediated transport systems:

- *system ASC* has specificity for neutral amino acids, especially serine, alanine, proline, cysteine and threonine;
- *system A* also has specificity for neutral amino acids, especially glycine, alanine, proline and methionine;
- *system L* has specificity for branched chain amino acids, phenylalanine, methionine and histidine;
- *system Gly* has specificity for glycine, proline and hydroxyproline;
- *system N* has specificity for histidine, asparagine and glutamine;
- *system* $Ly^-$ has specificity for glutamate and aspartate; and
- *system* $Ly^+$ has specificity for the basic amino acids (lysine and arginine), histidine and cysteine.

Systems ASC, A, Gly, N and $Ly^-$ are dependent on active transport via the $Na^+$-$K^+$ pump, while L and $Ly^+$ systems seem to work via facilitative rather than active processes [40], [5]. As described, several of the individual amino acids can be transported by more than one system, and most of the essential amino acids, such as the branch-chained, phenylalanine, methionine, histidine, lysine

and cysteine, are transported by facilitated diffusion (passive transport) with systems L and $Ly^+$. So it seems that the energy cost of transport is lower for essential than for non-essential amino acids.

### *3.4. Vesicular transport*

Molecules and particles that are too large to cross a cell membrane are transported inside small hollow granules or vesicles covered by a membrane. **Endocytosis** is a method to import large molecules through which a small part of the cell membrane encloses the substance to be taken up from the extracellular fluid, and transfers it into the cytoplasm. Endocytosis is an active process that requires energy from ATP. The opposite of endocytosis is **exocytosis** by which intracellular vesicles move to the cell membrane, fuse with it and then release their contents into the extracellular fluid. In regulated exocytosis, the process usually begins with an increase in intracellular $Ca^{++}$ concentration, which acts as a signal. Exocytosis, like endocytosis, requires energy in the form of ATP. In many endocrine cells, hormones are stored in secretory vesicles in the cytoplasm and released in response to a signal from outside the cell.

### *3.5. Transepithelial transport*

Molecules entering and leaving the body across an epithelium must pass two membranes. Movement across epithelial cells, i.e. transepithelial transport, uses a combination of active and passive transport. The surface of the epithelial cell that faces the lumen of an organ is called the apical membrane. This membrane is separated from the remainder of the cell membrane and from adjacent cells by tight junctions. The three surfaces of the cell that face the extracellular fluid are collectively called the basolateral membrane. Certain transport proteins, such as the $Na^+$-$K^+$-ATPase, are found almost only on the basolateral membrane, whereas others, like the $Na^+$-glucose symporter, are located in the apical membrane. This polarized distribution of transporters results in the one-way movement of certain molecules across the epithelium. Transport of material from the lumen to the extracellular fluid is called absorption, which is the opposite of secretion, i.e. the movement of material from the extracellular space to the lumen.

For example, transepithelial movement of glucose involves three transport systems, because the glucose concentration is higher in the epithelial cell than in the extracellular fluid and in the lumen of the kidney or intestine. First, the secondary active symporter transports glucose with $Na^+$ from the lumen into the epithelial cell at the apical membrane. Secondly, sodium moves out by primary active transport via $Na^+$-$K^+$-ATPase and thirdly, glucose leaves the cell by facilitated diffusion.

Molecules like proteins that are too large to be moved by membrane proteins can be transported across the cell by **transcytosis**, which is a combination of endocytosis, vesicular transport through the cell, and exocytosis. Transcytosis makes it possible for large proteins to move across an epithelium and remain intact. It is the means by which newborn piglets absorb maternal antibodies in sow milk.

## 4. Metabolism in skeletal muscle tissues

From a perspective of growth, protein turnover (synthesis and degradation) is the most fundamental process in muscle tissues. From the animal's view, the ability of muscles to contract and to relax is vital. Both protein turnover and muscle contractions are processes that require energy in the form of ATP, which is supplied by oxidation of fatty acids and glucose (Figure 14.2).

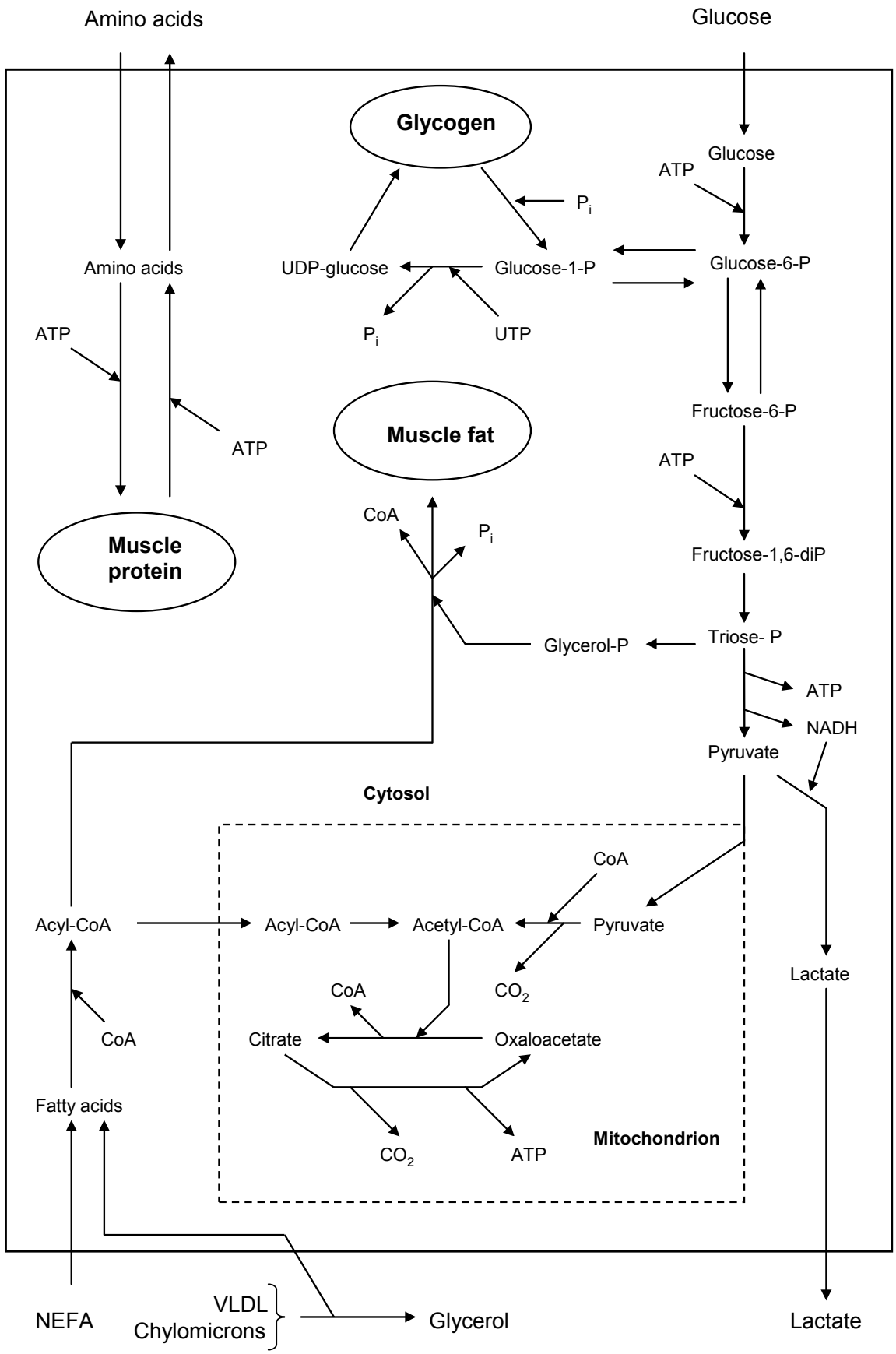



**Figure 14.2. Muscle tissue metabolism of major organic nutrients.**

*4.1. Protein synthesis*

As this chapter deals with the molecular basis for growth, it would be worthwhile to summarize the major biochemical steps in protein synthesis. The nucleic acid, DNA, in the cell nucleus contains the information necessary for construction of specific proteins characterized by the length and amino acid sequence of the peptide chain. The DNA molecule consists of two long strings, each of them built of pentose units (deoxyribose) linked together by phosphate groups. One of four nitrogenous bases (purines: adenine and guanine, and pyrimidines: cytosine and thymine) is attached to each pentose moiety with a C-N bond. Pairs of bases, one from each string, are linked to each other by hydrogen bonds so that the entire molecule forms a double helix. A purine is always linked to a pyrimidine and vice versa: adenine (A) pairs only with thymine (T), and guanine (G) pairs only with cytosine (C). Three of these bases next to each other on a string (a triplet) form a codon and thus, $4^3 = 64$ different codons are possible. The codons bring information about the projected amino acid sequence to the protein synthesis machinery outside the cell nucleus, and this is done via messenger RNA (mRNA).

By the process of **transcription**, a specific region of DNA (a gene) opens its double helix so that a complementary string of mRNA can be synthesized. The RNA molecule is a nucleic acid built as DNA except that the sugar component is ribose instead of deoxyribose, it is a single string, and the pyrimidine thymine is replaced by another pyrimidine, uracil (U). The sequence of codons in mRNA is determined by the corresponding sequence in DNA because of the rule for pairing the bases: A with U and G with C. RNA polymerase, the enzyme responsible for the transcription, is able to distinguish between the two strands of the open DNA helix and binds only to one of them, the so-called sense strand. The two strands are distinguishable because they run in opposite directions, i.e. the chain of the sense strand starts at carbon 3' of the pentose ring structure, and the chain of the other strand starts at carbon 5'. Of the 64 possible triplets, one (AUG) is a start codon that determines the beginning of a transcribed sequence, and three other codons (UAA, UAG and UGA) act as signals for termination of the sequence. Thus, when RNA polymerase reaches a stop codon, the transcription ends and the mRNA strand is released.

Once formed, the functional piece of mRNA leaves the nucleus and enters the cytosol to govern **translation**, i.e. the linking of amino acids into protein. Two other forms of RNA participate in the translation: ribosomal (rRNA) and transfer RNA (tRNA). The mRNA molecule binds to a ribosome, which is a two-unit particle of proteins and rRNA. The smaller of the two subunits (40 S) binds mRNA, and then adds the larger subunit (60S) to form the ribosome-mRNA complex on which protein synthesis takes place. This assembly of mRNA and ribosomes is facilitated by a complex of different proteins (initiation factors) that in turn is stimulated by insulin and IGF-I. During translation, the mRNA codons are matched with the proper amino acids by means of tRNA molecules. One region of each tRNA contains an anticodon, which is a sequence of three bases complementary to an mRNA codon, and another region of the tRNA molecule is bound to a specific amino acid.

As translation begins, tRNA anticodons bind to the complementary codons of the mRNA string on the ribosome complex. For example, a tRNA with the anticodon UUC carries lysine and binds to the complementary AAG codon on mRNA, which is one of two codons for lysine. If the next codon on mRNA is, for example, GGU that codes for glycine, a tRNA with anticodon CCA carries glycine to the protein machine and binds to the GGU sequence of mRNA. The incoming amino acid (glycine) is connected to its predecessor (lysine) with a peptide bond. Lysine is then released from its tRNA, and the empty tRNA is in turn released from mRNA. In this way, the peptide chain is growing until it is terminated by attachment of the last amino acid. Then the mRNA, the peptide, and the ribosomal subunits separate from each other, and the free peptide chain is subjected to **post-translational modifications**. These can be folding into various three-dimensional shapes, creation of cross-linkages between different parts of the peptide chain, cleavage into fragments, attachment of other molecules (e.g. sugars in glycoproteins), and assembly with other peptides into polymeric proteins. The properties of a specific protein depend on its primary structure, i.e. the amino acid sequence, as well as on its post-translational changes.

The energy requirement in protein biosynthesis is supplied by hydrolysis of at least four high-energy phosphate bonds per peptide bond formed; the precise number will differ between different proteins. The four ribonucleoside triphosphates: ATP, GTP, UTP and CTP are used as precursors in RNA synthesis, and hydrolysis of the high-energy bond in pyrophosphate (PPi) drives the reactions. Activation of an amino acid with its tRNA uses two moles of ATP. The start of the translational processes is formation of the initiation complex, which requires one mole GTP. Elongation of the started peptide chain uses one mole GTP for each incoming amino acid, and translocation, i.e. release of the empty tRNA and exposure of the next codon on the mRNA strand, costs another mole of GTP. Thus, the consumption of four (2 ATP and 2 GTP) high-energy phosphate bonds can be ascribed to each peptide bond, and, in addition to this, high-energy bonds are expended to maintain the protein synthesis machinery (RNA synthesis and formation of initiation complex).

### *4.2. Protein degradation*

As shown in Figure 14.2, skeletal muscle proteins are subjected to degradation by proteolysis. In fact, all body proteins undergo degradation, but proteolytic processes are not as well described or understood as the pathways of protein synthesis. Three intracellular proteolytic systems possess the potential to degrade myofibrillar proteins in muscle tissues: the lysosomal, the $Ca^{2+}$-dependent, and the ATP-ubiquitin-protease system [1], [2].

#### *4.2.1. Lysosomes*

Lysosomes are intracellular organelles enclosed by a membrane and containing 40-60 different hydrolases, including cathepsins. These proteases have endopeptidase (cathepsins D and L) or exopeptidase (A and C) activity or have both activities (B and H). A membranous $H^+$/ATPase pump maintains the acidic environment inside lysosomes (pH 4.5-5.0) at which most cathepsins and other hydrolases have optimum activity. Different transport systems are responsible for the entry of protein substrates and for the release of free amino acids from the degraded proteins to the cytosol. There seems to be a low level of lysosomal activity in skeletal muscles, and its overall contribution to catabolism in this tissue is probably small, except in the case of tissue injury.

#### *4.2.2. Calpains*

Calpains are $Ca^{2+}$-dependent proteases in the cytosol. This system has been extensively studied in domestic meat animals because of its putative relationship with post-mortem proteolysis and hence with meat quality. The calpains constitute a large family of cysteine proteases that require $Ca^{2+}$ for activation, in either millimolar or micromolar concentrations. Calpastatin is a specific inhibitor of calpains. The significance of this system to overall muscle protein breakdown is unclear, but it has been suggested that calpains are rate-limiting for the release of filaments from the myofibrillar structure of muscles.

#### *4.2.3. Ubiquitin-proteases*

Muscle protein catabolism appears to be mediated primarily by the ATP dependent ubiquitin-proteasome system, which is a cytosolic proteolytic mechanism that works in all eukaryotic cells. It is responsible for the breakdown of most short-lived and long-lived proteins, including myofibrillar proteins. Ubiquitin is a peptide of 76 amino acids and when it is linked to a protein substrate, the protein is marked for degradation. Ubiquitin is linked at the expense of ATP to an activating enzyme (E1); the protein substrates are selected by other enzymes (E2 and E3 families) and attached to chains of activated ubiquitin moieties. These poly-ubiquitinylated proteins are then degraded by large protein complexes with proteolytic activity (proteasomes). The degradation process itself also needs ATP as an energy source.

Protein breakdown is not random, but specific and well controlled. Insulin seems to decrease proteolysis possibly by inhibition of the ubiquitin pathway, but insulin may also affect other

pathways of protein degradation by decreasing mRNA levels for cathepsin D and m-calpain. The existence of the different degradation systems makes it possible to strictly regulate the access of different protein substrates to a suitable degradation pathway. However, it is unclear if myofibrillar proteins are broken down to free amino acids by one degradation mechanism or if more pathways are interconnected and work in concert. The precise consumption of ATP in protein degradation is also uncertain. Values from 0.5 to 1.0 mol ATP per mol hydrolysed peptide bond have been used in simulation models [21], [12].

### *4.3. Energy metabolism*

The major fuels in muscle tissues are fatty acids and glucose. The supply of **fatty acids** comes from circulating lipoproteins (chylomicrons and VLDL) and from non-esterified fatty acids (NEFA) that are circulating as bound to plasma proteins, primarily albumin. Fatty acids released from lipoproteins by action of the endothelial enzyme lipoprotein lipase, mix in the interstitial fluid with fatty acids from the NEFA pool. Glycerol from triacylglycerols in the lipoproteins is withheld in circulation and taken up in other tissues that have glycerol kinase activity, primarily the liver, where it can be used for esterification of fatty acids or used for gluconeogenesis. The free fatty acids are dispersed in the interstitial fluid at a very low concentration due to their nonpolar chemical structure. The cellular uptake is probably by simple diffusion, which means that the intracellular fatty acid concentration must be still lower than the extracellular one. The intracellular concentration is kept low by immediate activation of incoming fatty acids with coenzyme A (HS-CoA):

$$\underset{\text{(fatty acid)}}{CH_3(CH_2)_n(CO)O^-} + HS\text{-}CoA + 2\,ATP^{4-} + H_2O \rightarrow \underset{\text{(acyl-CoA)}}{CH_3(CH_2)_n(CO)\text{-}S\text{-}CoA} + 2\,ADP^{3-} + 2\,P_i^{2-} + H^+,$$

where n is an even, positive number; (CO) denotes a C=O bond; and $P_i^{2-}$ denotes inorganic phosphate, $HPO_4^{2-}$.

This activation costs 2 moles of ATP per mol fatty acid, which makes the reaction practically irreversible. Acyl-CoA can be esterified in the cytosol with glycerol-P to triacylglycerol and incorporated into the pool of intramuscular fat. However, the major route for acyl-CoA is oxidation in the mitochondria. Long-chain acyl-CoA molecules do not easily cross the mitochondrial inner membrane, so they exchange CoA for carnitine, and the resultant acylcarnitine molecules can then penetrate the mitochondrial membranes via protein carriers (antiports) that also transport carnitine in the opposite direction. Inside the mitochondrial matrix, acylcarnitine is changed back to acyl-CoA and carnitine is returned to the cytosol.

The further metabolism of acyl-CoA involves β-oxidation to acetyl-CoA and oxidation of acetyl-CoA in the citric acid cycle. The β-oxidation is a recurring sequence of reactions (oxidation, hydration, oxidation and thiolysis) whereby the fatty acid chain is shortened by two carbon atoms in each sequence with concomitant formation of $FADH_2$, NADH and acetyl-CoA:

$$\underset{\text{(acyl-CoA)}}{CH_3(CH_2)_n(CO)\text{-}S\text{-}CoA} + 0.5n\,(HS\text{-}CoA + H_2O + NAD^+ + FAD) \rightarrow$$
$$(0.5n+1)\,\underset{\text{(acetyl-CoA)}}{CH_3(CO)\text{-}S\text{-}CoA} + 0.5n\,(NADH + H+ + FADH_2).$$

In the citric acid cycle, acetyl-CoA reacts with oxaloacetate ($C_4H_2O_5^{2-}$) to yield citrate ($C_6H_5O_7^{3-}$), which during the remaining steps of the cycle gives away two moles of $CO_2$ and is oxidized back to oxaloacetate. Energy released by the oxidations is trapped in GTP, NADH and $FADH_2$:

$$(0.5n+1)\,(CH_3(CO)\text{-}S\text{-}CoA + 2\,H_2O + GDP^{3-} + P_i^{2-} + 3\,NAD^+ + FAD) \rightarrow$$

$$(0.5n+1)\,(2\,CO_2 + HS\text{-}CoA + GTP^{4-} + 3\,NADH + 2\,H^+ + FADH_2).$$

Summation of these reaction schemes gives:

$$CH_3(CH_2)_n(CO)O^- + (0.5n\text{-}1)\,(ADP^{3-} + P_i^{2-}) + (1.5n+3)\,H_2O + (2n+3)\,NAD^+ + (n+1)\,FAD \rightarrow$$
(fatty acid)

$$(n+2)\,CO_2 + (0.5n\text{-}1)\,ATP^{4-} + (2n+3)\,NADH + (1.5n+3)\,H^+ + (n+1)\,FADH_2.$$

Note that GTP formed in the citric acid cycle is converted to ATP: GTP + ADP ↔ ATP + GDP.

Oxidation of the reduced co-enzymes, NADH and $FADH_2$, in the respiratory chain at the inner mitochondrial membrane releases energy for the formation of high-energy phosphate bonds in ATP. Thus, in oxidative phosphorylation, an electron-transfer potential of NADH and $FADH_2$ is converted to a phosphate-transfer potential with oxygen as the ultimate electron acceptor and with ADP as the phosphate group acceptor. Oxidation of one mole of NADH yields 3 moles, and oxidation of $FADH_2$ yields 2 moles of ATP:

$$(2n+3)\,(NADH + H^+) + (n+1.5)\,O_2 + (6n+9)\,(ADP^{3-} + P_i^{2-} + H^+) \rightarrow (2n+3)\,(NAD^+ + H2O) + (6n+9)\,(ATP^{4-} + H_2O)$$

and

$$(n+1)\,FADH_2 + 0.5\,(n+1)\,O_2 + (2n+2)\,(ADP^{3-} + P_i^{2-} + H^+) \rightarrow (n+1)\,(FAD + H_2O) + (2n+2)\,(ATP^{4-} + H_2O).$$

The general equation for oxidation of a saturated, even-chained fatty acid is then:

$$CH_3(CH_2)_n(CO)OH + (1.5n+2)\,O_2 + (8.5n+10)\,(ADP^{3-} + P_i^{2-} + H^+) \rightarrow$$

$$(n+2)\,(CO_2 + H_2O) + (8.5n+10)\,(ATP^{4-} + H_2O).$$

The average chain length of circulating fatty acids in pigs has been estimated as 17.4 mol C [9], and by using the above equation, we can calculate that oxidations of palmitate ($C_{16}$, n = 14) and stearate ($C_{18}$, n = 16) release enough energy to produce 129 and 146 mol ATP, respectively, i.e. elongation of a saturated fatty acid with two carbon units gives extra 17 mol ATP when oxidized.

Unsaturated fatty acids are more oxidized than saturated fatty acids. For each double bond, there are two electrons and two protons fewer in the molecule, which means that one fewer FAD can be reduced to $FADH_2$. This is because the first step in β-oxidation of a saturated acyl-CoA is oxidation by an acyl-CoA dehydrogenase, whereby a double bond is created and FAD is reduced:

$$CH_3(CH_2)_nCH_2CH_2(CO)\text{-}S\text{-}CoA + FAD \rightarrow CH_3(CH_2)_nCH{=}CH(CO)\text{-}S\text{-}CoA + FADH_2.$$
(acyl-CoA) (enoyl-CoA)

Thus, complete oxidation of an unsaturated fatty acid yields 2 mol ATP less per mol double bond than a saturated fatty acid with the same chain length. For example, oxidation of oleic acid with one double bond ($C_{18:1}$) gives 146-2 = 144 ATP.

Some fatty acids have an odd number of carbon atoms in the chain. The β-oxidation of these acids proceeds as already described, and the last step therefore produces one acetyl-CoA and one 3-carbon compound, propionyl-CoA ($CH_3CH_2(CO)$-S-CoA). This compound is carboxylated to methylmalonyl-CoA, which in turn is isomerized by methylmalonyl mutase to succinyl-CoA ($^-O(CO)(CH_2)_2(CO)$-S-CoA), an intermediate of the citric acid cycle. The carboxylation step requires 2 mol ATP per mol propionyl-CoA. Succinyl-CoA is further metabolized in the citric acid cycle via fumerate and malate to oxaloacetate with concomitant production of GTP, NADH and $FADH_2$. The net

reaction is:

$$\underset{\text{(propionyl-CoA)}}{CH_3CH_2(CO)\text{-S-CoA}} + CO_2 + 4\,ADP^{3-} + 4\,P_i^{2-} + 2\,H^+ \rightarrow \underset{\text{(oxaloacetate)}}{^-O(CO)(CO)CH_2(CO)O^-} + HS\text{-CoA} + 4\,ATP^{4-} + H_2O.$$

Thus, metabolism of propionyl-CoA furnishes the citric acid cycle with oxaloacetate and produces ATP.

**Glucose** is taken up from the extracellular fluid by facilitated diffusion involving the transport proteins, GLUT1 and GLUT4 (Figure 14.2). Of these transporters, GLUT4 is stimulated by insulin, whereas GLUT1 is not. The glucose concentration gradient across the cell membrane is created by an irreversible phosphorylation of the incoming glucose molecules at the expense of ATP. The product, glucose-6-P, is isomerized to fructose-6-P, which is further phosphorylated to fructose-1,6-diP. This metabolite is then split by the enzyme aldolase into two triose-P compounds, i.e. glyceraldehyde-3-P and dihydroxyacetone-P, which are interconvertible and oxidized to pyruvate:

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} + ATP^{4-} \rightarrow \underset{\text{(glucose-6-P)}}{C_6H_{11}O_6\text{-}\mathbf{P}^{2-}} + ADP^{3-} + H^+, \qquad \text{where } \mathbf{P}^{2-} \text{ denotes } -PO_3^{2-}$$

$$\underset{\text{(fructose-6-P)}}{C_6H_{11}O_6\text{-}\mathbf{P}^{2-}} + ATP^{4-} \rightarrow \underset{\text{(fructose-1,6-diP)}}{C_6H_{10}O_6\text{-}\mathbf{P}_2^{4-}} + ADP^{3-} + H^+$$

$$C_6H_{10}O_6\text{-}\mathbf{P}_2^{4-} \leftrightarrow \underset{\text{(dihydroxyacetone-P)}}{C_3H_5O_3\text{-}\mathbf{P}^{2-}} + \underset{\text{(glyceraldehyde-3-P)}}{C_3H_5O_3\text{-}\mathbf{P}^{2-}}$$

$$\underset{\text{(glyceraldehyde-3-P)}}{2\,C_3H_5O_3\text{-}\mathbf{P}^{2-}} + 4\,ADP^{3-} + 2\,P_i^{2-} + 2\,NAD^+ \rightarrow \underset{\text{(pyruvate)}}{2\,C_3H_3O_3^-} + 4\,ATP^{4-} + 2\,H_2O + 2\,NADH.$$

The sum of these reactions gives an overall equation for the anaerobic glycolysis taking place in the cytosol (Figure 14.2):

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} + 2\,ADP^{3-} + 2\,P_i^{2-} + 2\,NAD^+ \rightarrow \underset{\text{(pyruvate}_{cyt}\text{)}}{2\,C_3H_3O_3^-} + 2\,ATP^{4-} + 2\,H_2O + 2\,NADH + 2\,H^+.$$

The further aerobic metabolism takes place inside the mitochondria. Pyruvate is a small molecule that is able to cross mitochondrial membranes and enter the matrix, where it is converted by oxidative decarboxylation to acetyl-CoA, which is completely oxidized in the citric acid cycle:

$$\underset{\text{(pyruvate}_{mit}\text{)}}{2\,C_3H_3O_3^-} + 2\,HS\text{-CoA} + 2\,NAD^+ \rightarrow \underset{\text{(acetyl-CoA)}}{2\,CH_3(CO)\text{-S-CoA}} + 2\,CO_2 + 2\,NADH$$

$$2\,CH_3(CO)\text{-S-CoA} + 2\,ADP^{3-} + 2\,P_i^{2-} + 4\,H_2O + 6\,NAD^+ + 2\,FAD \rightarrow$$

$$4\,CO_2 + 2\,HS\text{-CoA} + 2\,ATP^{4-} + 4\,H^+ + 6\,NADH + 2\,FADH_2.$$

Thus, from one mole of glucose, 2 mol NADH are formed in the cytosol, while 8 mol NADH and 2 mol $FADH_2$ are formed inside the mitochondria. NADH cannot cross the mitochondrial membrane, and the high-energy electrons in this compound must enter the mitochondria in another carrier molecule. The three carbon compounds, dihydroxyacetone-P and glycerol-3-P, are small enough to penetrate the membranes, so dihydroxyacetone-P is reduced to glycerol-3-P by NADH in the cytosol, and glycerol-3-P is oxidized by FAD inside the mitochondria:

$2\ C_3H_5O_3\text{-}\mathbf{P}^{2-} + 2\ NADH + 2\ H^+ \rightarrow 2\ C_3H_7O_3\text{-}\mathbf{P}^{2-} + 2\ NAD^+$ (in cytosol)
(dihydroxyacetone-P) (glycerol-3-P)

$2\ C_3H_7O_3\text{-}\mathbf{P}^{2-} + 2\ FAD \rightarrow 2\ C_3H_5O_3\text{-}\mathbf{P}^{2-} + 2\ FADH_2$ (in mitochondria).

The net result of this glycerol phosphate shuttle is that reducing equivalents (electrons and protons) in cytosolic NADH are transferred with glycerol-3-P to mitochondrial FAD and reduce it to $FADH_2$. The shuttle is operating at the expense of energy as only 2 mol ATP are harvested per mol $FADH_2$ instead of 3 mol ATP per mol NADH. Thus, a total of 8 NADH and 4 $FADH_2$ are processed by oxidative phosphorylation resulting in 8·3 + 4·2 = 32 ATP. Finally, the total oxidation of glucose can be summarized:

$C_6H_{12}O_6 + 6\ O_2 + 36\ ADP^{3-} + 36\ P_i^{2-} + 36\ H^+ \rightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP^{4-} + 36\ H_2O.$

Some glucose is used as substrate for the synthesis of glycerol-3-P to esterify long-chain fatty acids (Figure 14.2):

$0.5\ C_6H_{12}O_6 + ATP^{4-} \rightarrow C_3H_5O_3\text{-}P^{2-} + ADP^{3-} + H^+$
(dihydroxyacetone-P)

$C_3H_5O_3\text{-}P^{2-} + NADH_{cyt} + H^+ \rightarrow C_3H_7O_3\text{-}P^{2-} + NAD^+_{cyt}$
(glycerol-3-P)

$C_3H_7O_3\text{-}P^{2-} + 3\ CH_3(CH_2)_n(CO)\text{-S-CoA} + H_2O \rightarrow CH_2O(CO)(CH_2)_nCH_3 + 3\ HS\text{-CoA} + P_i^{2-}$
(acyl-CoA)
$CHO(CO)(CH_2)_nCH_3$
$CH_2O(CO)(CH_2)_nCH_3$
(triacylglycerol)

The energy cost of **lipogenesis** is then 1 mol ATP and 1 mol $NADH_{cyt}$ per mol triacylglycerol added to the pool of intramuscular fat.

Plasma concentrations of glucose and insulin are increased postprandially (1-2 hours after feeding) as compared with concentrations preprandially in pigs fed twice daily [41], see also Chapter 8. This means that the supply and cellular uptake of glucose is much higher after than just before feeding [20], [8], and that the glucose availability in muscles can exceed the glucose requirement for ATP production etc. during the early hours after feeding. In this situation, the surplus glucose is stored as **glycogen**, which can be broken down again to glucose during periods of short supply (Figure 14.2). Glycogen synthesis is an energy-requiring process that involves use of the high-energy compound, uridintriphosphate (UTP). Glucose-6-P is isomerized to glucose-1-P, which in turn reacts with UTP. The activated glucose is then built into glycogen by the enzyme glycogen synthase:

Glucose-6-$\mathbf{P}^{2-} \leftrightarrow$ glucose-1-$\mathbf{P}^{2-}$

Glucose-1-$\mathbf{P}^{2-} + UTP^{4-} + H_2O \rightarrow UDP^{2-}$glucose $+ 2\ P_i^{2-}$

$Glycogen_n + UDP^{2-}glucose \rightarrow glycogen_{n+1} + UDP^{3-} + H^+$

$UDP^{3-} + ATP^{4-} \leftrightarrow UTP^{4-} + ADP^{3-}.$

When glucose is needed in the cells at a higher rate than it is taken up from the extracellular space, breakdown of glycogen (glycogenolysis) comes into play. Several enzymes take part in this process. Phosphorylase works only on straight chains of glucose units linked by α-1,4-glycosidic

bonds and not at the branch points with α-1,6 bonds. A transferase and an α-1,6-glucosidase (debranching enzyme) straighten the chains at branch points and pave the way for phosphorylase to remove one glucose unit at a time from glycogen:

$Glycogen_{n+1} + P_i^{2-} \rightarrow glycogen_n + glucose\text{-}1\text{-}\mathbf{P}^{2-}$.

Summation of the reaction schemes for glycogen synthesis and glycogenolysis shows that the energy cost for glucose-6-P to be incorporated into glycogen and released again is one ATP. However, this is only true for about 90% of the glycosidic bonds, namely the α-1,4 bonds cleaved by phosphorylase. The remaining 10% are α-1,6 bonds that are cleaved by hydrolysis giving rise to free glucose, and one ATP is therefore used to phosphorylate each of these glucose molecules [36], [5]. According to this, the overall efficiency of storage and release of glucose-6-P can be estimated as (37-1.1)/37 ~ 97%, recalling that one mol glucose-6-P yields 37 mol ATP when oxidized completely.

In some tissues, such as the liver and the kidneys, glucose-6-P can be hydrolyzed into free glucose by the enzyme glucose-6-phosphatase: glucose-6-$\mathbf{P}^{2-}$ + $H_2O$ → glucose + $P_i^{2-}$. This is why the liver and the kidneys are able to export glucose to the peripheral circulation and thereby contribute to maintain glucose homeostasis, which is vital for those tissues that are dependent on glucose as a fuel. Glucose-6-phosphatase is not active in muscle tissues, and hence released glucose-6-P cannot leave muscle cells, but is destined to be metabolized within the tissue.

Although glycogen turnover is energetically efficient, it still costs energy to store glucose in glycogen and release it again. Hence, it could be costly if glycogen synthesis and breakdown both proceed at high rates at the same time. Protein turnover is expensive in energy terms, but this is a necessary cost because, with time, proteins are damaged or modified and need to be renewed or repaired in order to maintain their proper function. In contrast to this, glycogen is merely a depot, and both its synthesis and breakdown are tightly regulated so that these processes practically never occur simultaneously. The regulation is achieved by a series of phosphorylations of key enzymes. The crucial trick is that phosphorylation deactivates glycogen synthase, while it activates phosphorylase. The two major hormones controlling glycogen turnover are adrenalin and insulin. Release of adrenalin from the adrenal medulla is stimulated by impulses in the sympathetic nervous system in response to low glucose plasma levels and in response to emergencies (fight or flight situations, coldness). Adrenalin binds to its β-receptors on muscle cells and thereby activates a membranous G protein that in turn activates adenylate cyclase (Figure 14.3). This enzyme converts ATP to cyclic AMP (cAMP) that activates a cAMP-dependent protein kinase, and this kinase phosphorylates both the active form of glycogen synthase as well as the inactive form of another kinase, phosphorylase kinase. Phosphorylation of glycogen synthase makes it inactive and blocks glycogen synthesis, while phosphorylase kinase is made active by phosphorylation. Phosphorylase kinase uses ATP to phosphorylate the inactive b-form of phosphorylase into an active a-form, which then attacks the glycogen structure and starts glycogenolysis. Adrenalin also binds to α-receptors on the cell membrane, and this binding creates a signal to increase the intracellular $Ca^{2+}$ concentration. This increase activates a $Ca^{2+}$-dependent protein kinase, and the cascade of enzyme phosphorylations just described is initiated resulting in glycogen breakdown. Muscle contractions are also triggered by the release of $Ca^{2+}$ and hence, muscular work and glucose supply from glycogen are coordinated by a transient increase in the cytosolic $Ca^{2+}$ concentration.

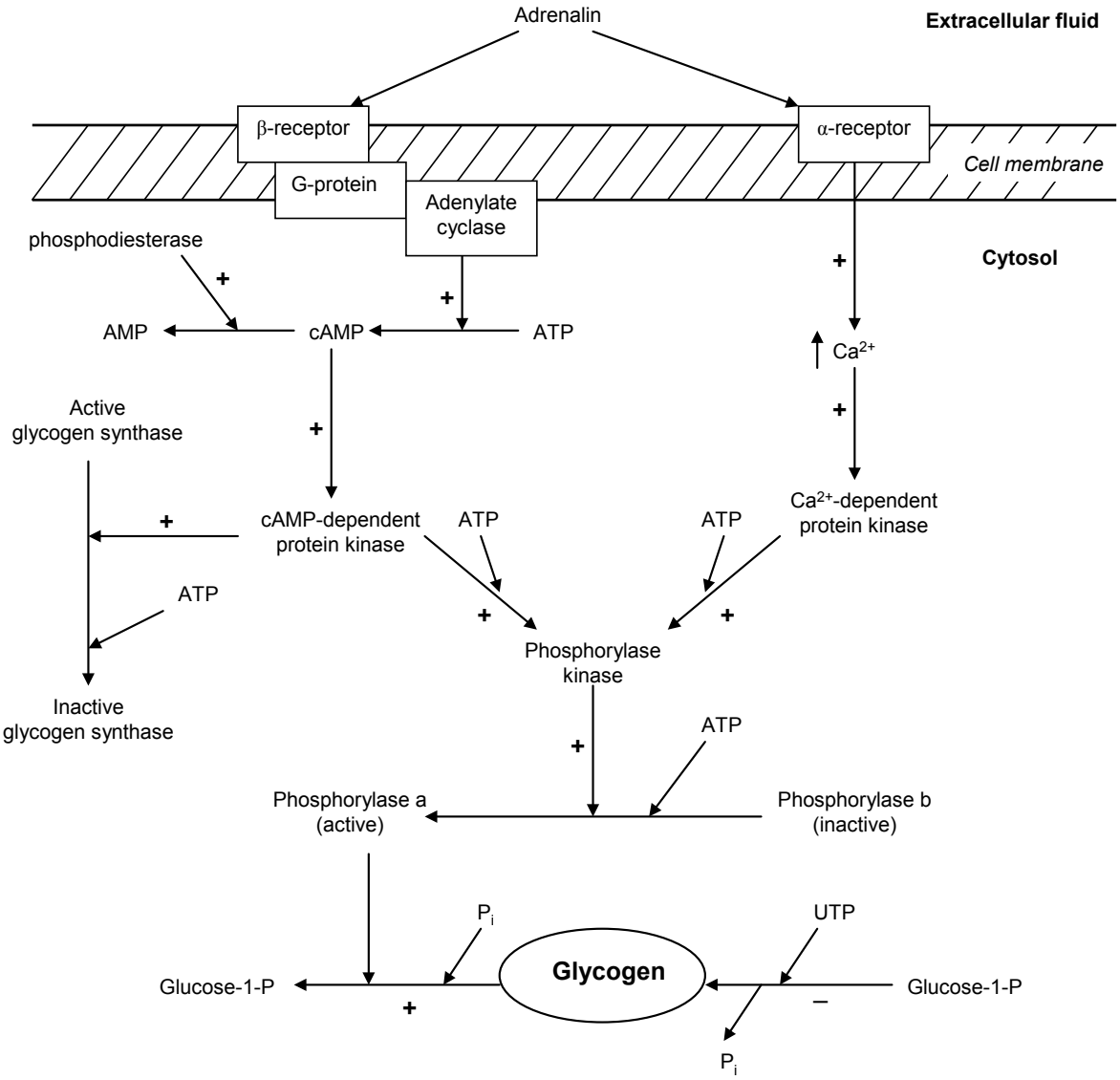



**Figure 14.3. Adrenergic regulation of glycogen breakdown in muscle tissues.**

In situations where the pigs are peaceful, comfortable and well-fed with high plasma levels of glucose and insulin, signals from the adrenergic receptors disappear and the intracellular concentration of cAMP decreases. This leads to inactivation by dephosphorylation of an inhibitory protein, which in its active form inhibits a dephosphorylating enzyme, phosphoprotein phosphatase. Hence, at low cAMP levels, this phosphatase is active and catalyzes the removal of phosphoryl groups from three enzymes: the active phosphorylase kinase, the active a-form of phosphorylase, and the inactive form of glycogen synthase. Dephosphorylation of the first two enzymes makes them inactive and stops glycogenolysis, while dephosphorylation activates the synthase and starts glycogen synthesis. Moreover, insulin stimulates the synthesis of glycogen, possibly by activation of phosphodiesterase, an enzyme that lowers the concentration of cAMP by converting it to AMP.

**Muscular work** needs energy in the form of ATP. The ATP concentration in muscle tissue is low, i.e. the ATP pool is small and can support intensive muscular contractions for only a few seconds [5]. Creatine phosphate is another compound with a high-energy bond that can furnish the ATP pool by phosphorylation of ADP: creatine-$\mathbf{P}^{3-}$ + $ADP^{3-}$ + $H^{+}$ ↔ creatine$^{-}$ + $ATP^{4-}$.

However, the pool of creatine phosphate is only 3-4 times as large as that of ATP and must be restored by phosphorylation of creatine in the reverse reaction as shown above. Some ATP can also be generated by the myokinase reaction: 2 $ADP^{3-}$ → $ATP^{4-}$ + $AMP^{2-}$, but sustained muscular work requires a continuous supply of ATP from glucose and fatty acid metabolism. Fatty acids yield

ATP by aerobic oxidation in the mitochondria, and so does glucose beyond the level of pyruvate, but ATP can be produced more rapidly by anaerobic glycolysis in the cytosol when pyruvate is reduced to lactate:

$$\text{Glucose} + 2\,ADP^{3-} + 2\,P_i^{2-} + 2\,NAD^+ \rightarrow 2\,\text{pyruvate} + 2\,ATP^{4-} + 2\,H_2O + 2\,NADH + 2\,H^+$$

$$\underset{\text{(pyruvate)}}{2\,C_3H_3O_3^-} + 2\,NADH + 2\,H^+ \rightarrow \underset{\text{(lactate)}}{2\,C_3H_5O_3^-} + 2\,NAD^+.$$

Anaerobic glycolysis is the dominant pathway in white, fast-twitch muscle fibres designed for rapid and violent contractions that need immediate supply of energy from a large reservoir of glucose. This is why glycogen is primarily stored in white, glycolytic muscles. The advantage of glycolysis is that it rapidly produces ATP, but the disadvantage is that it yields only 2 mol ATP per mol glucose compared with 36 mol ATP by total oxidation. The produced lactate can decrease the intracellular pH and cause muscle fatigue as its production rate can exceed its outflow rate to the extracellular fluid. Once in the peripheral circulation, lactate is transported to the liver, where it completes the Cori cycle as substrate for glucose synthesis (Figure 14.1).

The so-called red muscles with high proportions of slow-twitch, oxidative and mitochondria-rich type I fibres respond more slowly and have a long latency. They have posture-maintaining functions (e.g. longissimus dorsi and some leg muscles) and their energy metabolism relies primarily on oxidation of fatty acids.

ATP is of course not only an energy source for muscle contractions, but also for other energy-requiring processes in muscle tissues, primarily protein turnover and active transport across membranes.

### *4.4. Amino acid catabolism*

Apart from their role as substrates for protein synthesis, amino acids also take part in energy metabolism. Branch-chained amino acids are not metabolized to any great extent in the liver and they may be available to muscle tissues in quantities that exceed the requirement for protein synthesis. These and other surplus amino acids then serve as energy-yielding substrates, but for amino acids to be oxidized, they must loose their amino groups ($-NH_3^+$). Unlike the liver, muscle tissues cannot synthesize urea and therefore have to get rid of the surplus nitrogen by other mechanisms involving transamination and deamination. Transaminases are enzymes that catalyze the transfer of an α-amino group to an α-keto acid. For example, alanine transaminase uses pyruvate as the amino group acceptor:

$$\underset{\text{(α-amino acid)}}{R\text{-}CH(NH_3)^+(CO)O^-} + \underset{\text{(pyruvate)}}{CH_3(CO)(CO)O^-} \rightarrow \underset{\text{(α-keto acid)}}{R\text{-}(CO)(CO)O^-} + \underset{\text{(alanine)}}{CH_3CH(NH_3)^+(CO)O^-}.$$

The α-keto acid is further metabolized and oxidized, while alanine is exported to the extracellular fluid, taken up by the liver, deaminated and used as a glucogenic substrate (Figure 14.1). This completes the alanine cycle: glucose (liver) → glucose (muscle) → pyruvate → alanine → alanine (liver) → pyruvate → glucose. The lost amino group from alanine in the liver is used in synthesis of another nonessential amino acid or is built into urea and excreted with the urine. It is important that the intracellular concentration of $NH_4^+$ is kept very low because at concentrations above 1 mM, the equilibrium of the glutamate dehydrogenase reaction: $\alpha\text{-ketoglutarate}^{2-} + NH_4^+ + NADPH + H^+ \leftrightarrow \text{glutamate}^- + NADP^+ + H_2O$, is towards the synthesis of glutamate. This will impair the function of the citric acid cycle as one of its intermediates, $\alpha\text{-ketoglutarate}^{2-}$, is removed from the cycle.

Another route for muscle nitrogen disposal (although of minor quantitative importance) is the glutamine cycle. The amino acid to be catabolised looses its amino group by transamination to α-ketoglutarate ($^-O(CO)(CH_2)_2(CO)(CO)O^-$). The products are the corresponding keto acid and glutamate ($^-O(CO)(CH_2)_2CH(NH_3)^+(CO)O^-$), which in turn is deaminated by glutamate dehydrogenase

using $NAD^+$ as electron and proton acceptor. This reaction regenerates α-ketoglutarate, and the amino group reacts with another glutamate to synthesize glutamine ($NH_2(CO)(CH_2)_2CH(NH_3)^+(CO)O^-$); the enzyme involved is glutamine synthase that uses ATP as the driving force. The produced glutamine leaves the muscle tissue and is transported to the kidneys, where it is deaminated to glutamate by glutaminase: glutamine + $H_2O$ → glutamate$^-$ + $NH_4^+$. The ammonium ion is excreted from the body in urine, and glutamate is returned with the peripheral circulation to the muscles thus completing the glutamine cycle as shown by the overall balance:

α-amino acid + $NAD^+$ + $ATP^{4-}$ + 2 $H_2O$ → α-keto acid$^-$ + $NH_4^+$ + NADH + $ADP^{3-}$ + $P_i^{2-}$ + 2 $H^+$.

## 5. Metabolism in adipose tissues

Adipose tissues have several functions in the body: as subcutaneous insulation and protection layers, as physical protection of internal organs, and as labile energy stores. Large amounts of lipid can be deposited in adipose tissues because adipocytes are capable of both hypertrophy and hyperplasia postnatally (see Chapter 3). Mature adipocytes contain from 70 to 90% lipid depending on dietary energy supply [27]. The amount of fat in adipose tissue is determined by rates of lipid synthesis (lipogenesis) and lipid degradation (lipolysis). In normally fed pigs, the lipid content in the body increases during the entire growth period (see Chapter 3), i.e. the rate of lipogenesis exceeds that of lipolysis. Substrates for lipogenesis are activated fatty acids and glycerol-3-P, and products of lipolysis are fatty acids and glycerol. The latter is not reused in the tissue, but exported to the extracellular fluid. The substrate pool of fatty acids has three sources: cellular uptake from circulating lipoproteins, fatty acids released from depot fat, and fatty acids synthesized de novo in the tissue. Glycerol-3-P is also synthesized in situ from glucose (Figure 14.4).

### *5.1. Fatty acid synthesis*

The major substrate for fatty acid synthesis is glucose taken up from the peripheral circulation by facilitated diffusion. Like in muscle tissues, both GLUT1 and GLUT4 transport proteins are present to mediate the uptake, and as only GLUT4 is regulated by insulin, the ratio of the active forms of those two transporters determines the sensitivity of adipose tissue to insulin. In growing pigs fed ad lib, the rate of body lipid retention exceeds that of body protein retention from 2-3 months of age and onwards (see Chapter 3). Based on this observation, it may be hypothesized that the relative abundance of GLUT1 and GLUT4 in muscle and adipose tissues is age-dependent, so that the proportion of GLUT4 is decreasing with age in muscles, but not in adipose tissues or at least not to the same extent.

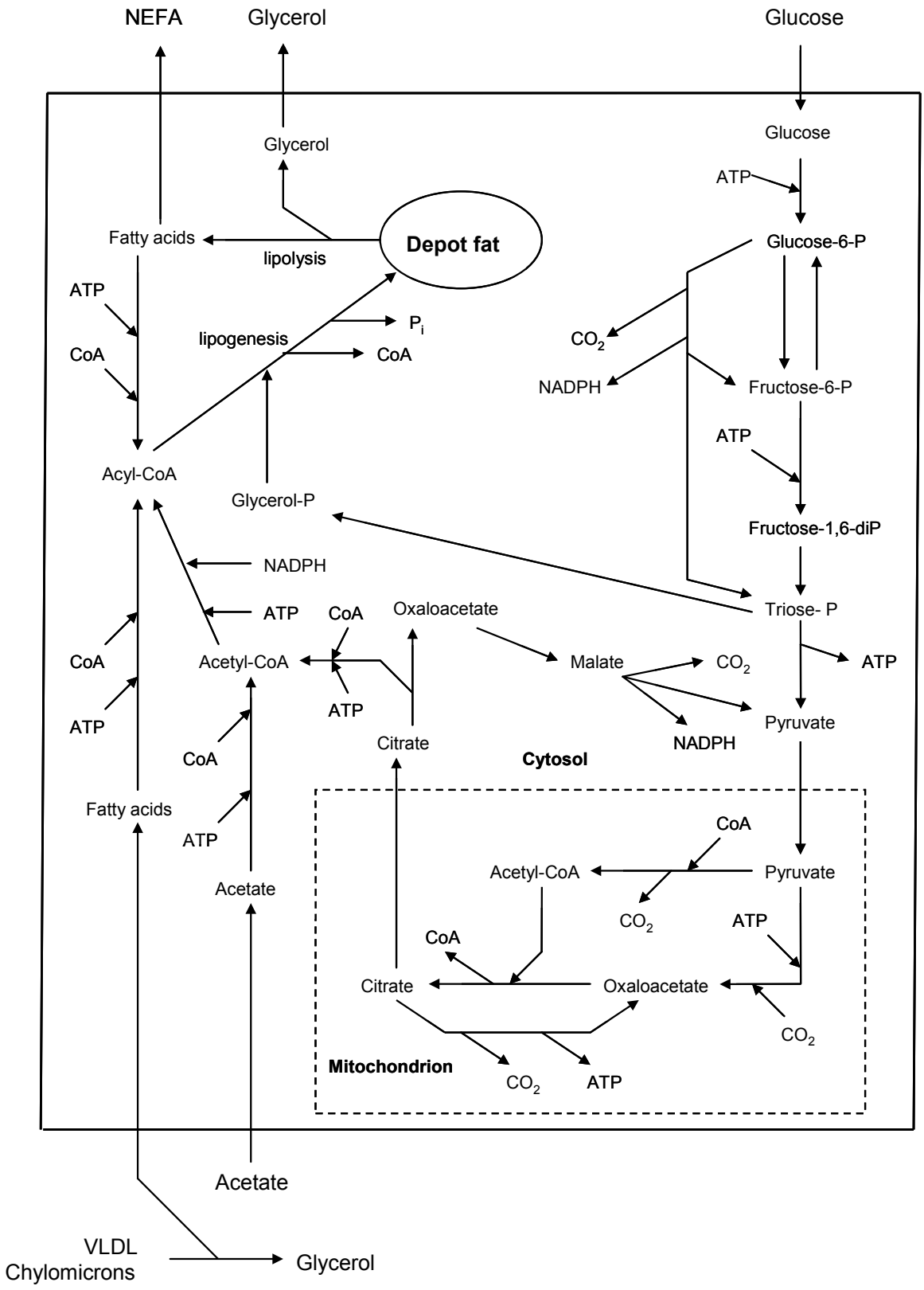



**Figure 14.4. Carbohydrate and lipid metabolism in adipose tissues.**

The synthesis of fatty acids takes place in the cytosol (Figure 14.4) and is in principle a linkage of acetyl-CoA units, but the reactions involved are not just the reverse of the β-oxidation sequence. Acetyl-CoA derived from glucose via pyruvate is produced inside the mitochondria, but is not able to cross the mitochondrial membranes. This obstacle is overcome by its reaction with oxaloacetate to citrate, which passes through the membranes to the cytosol, where it is cleaved by citrate lyase

into oxaloacetate and acetyl-CoA. In this way, the citric acid cycle delivers a key intermediate to the cytosol. The pathways starting with glucose can be written as follows:

$$\text{Glucose} + 2\ ADP^{3-} + 2\ P_i^{2-} + 2\ NAD^+ \rightarrow 2\ \text{pyruvate}^-_{cyt} + 2\ ATP^{4-} + 2\ H_2O + 2\ NADH + 2\ H^+$$

$$2\ \text{pyruvate}^-_{mit} + 2\ \text{HS-CoA} + 2\ NAD^+ \rightarrow 2\ \text{acetyl-S-CoA}_{mit} + 2\ CO_2 + 2\ NADH$$

$$\underset{(\text{acetyl-CoA}_{mit})}{2\ CH_3(CO)\text{-S-CoA}} + 2\ {}^{-}O(CO)(CO)CH_2(CO)O^- + 2\ H_2O \rightarrow \underset{(\text{citrate}_{mit})}{2\ C_6H_5O_7^{3-}} + 2\ \text{HS-CoA} + 2\ H^+$$

$$2\ \text{citrate}^{3-}_{cyt} + 2\ \text{HS-CoA} + 2\ ATP^{4-} \rightarrow 2\ \text{acetyl-CoA}_{cyt} + 2\ \text{oxaloacetate}^{2-}_{cyt} + 2\ ADP^{3-} + 2\ P_i^{2-}.$$

The cytosolic oxaloacetate is further metabolized via malate to pyruvate and regenerated in the mitochondria (see later). The net result is that 1 mol of glucose yields 2 mol acetyl-CoA available in the cytosol for fatty acid synthesis:

$$\text{Glucose} + 2\ \text{HS-CoA} + 2\ NAD^+_{cyt} + 2\ NAD^+_{mit} \rightarrow 2\ \text{acetyl-CoA}_{cyt} + 2\ CO_2 + 2\ NADH_{cyt} + 2\ NADH_{mit} + 4\ H^+.$$

This translocation of acetyl-CoA from the mitochondria to the cytosol costs 2 mol ATP per mol glucose as the ATP produced in glycolysis to pyruvate is used by the citrate lyase reaction, i.e. cleavage of citrate to oxaloacetate and acetyl-CoA. Two of the four NADH are produced in the cytosol and two are produced in the mitochondria.

The rate-limiting step in fatty acid synthesis is the carboxylation of acetyl-CoA to malonyl-CoA catalyzed by acetyl CoA carboxylase using ATP as the driving energy force:

$$\underset{(\text{acetyl-CoA})}{CH_3(CO)\text{-S-CoA}} + HCO_3^- + ATP^{4-} \rightarrow \underset{(\text{malonyl-CoA})}{{}^{-}O(CO)CH_2(CO)\text{-S-CoA}} + ADP^{3-} + P_i^{2-} + H^+.$$

The fatty acid chain is synthesized by action of fatty acid synthase, a multi-enzyme complex that governs a series of repeated reactions: condensation, reduction, dehydration and reduction. Substrates for this enzyme system are acyl units coupled to an acyl carrier protein (ACP) instead of acyl units coupled to coenzyme A. Thus, to start the sequence of reactions, acetyl-CoA and malonyl-CoA are converted to their respective ACP derivatives, then fatty acid synthase takes over and as the first step, acetyl-ACP reacts with malonyl-ACP yielding a four-carbon unit (acetoacetyl-ACP) and CO2. During the next steps, the four-carbon ketoacyl-ACP is reduced by NADPH, dehydrated and reduced once more by NADPH:

1. Condensation step

$$\underset{(\text{acetyl-ACP})}{CH_3(CO)\text{-S-ACP}} + \underset{(\text{malonyl-ACP})}{{}^{-}O(CO)CH_2(CO)\text{-S-ACP}} + H^+ \rightarrow \underset{(\text{acetoacetyl-ACP})}{CH_3(CO)CH_2(CO)\text{-S-ACP}} + CO_2 + \text{HS-ACP}$$

2. First reduction step

$$CH_3(CO)CH_2(CO)\text{-S-ACP} + NADPH + H^+ \rightarrow \underset{(\text{3-hydroxybutyryl-ACP})}{CH_3(CHOH)CH_2(CO)\text{-S-ACP}} + NADP^+$$

3. Dehydration step

$$CH_3(CHOH)CH_2(CO)\text{-S-ACP} \rightarrow \underset{(\text{crotonyl-ACP})}{CH_3CH{=}CH(CO)\text{-S-ACP}} + H_2O$$

4. Second reduction step

$CH_3CH=CH(CO)\text{-}S\text{-}ACP + NADPH + H^+ \rightarrow \underset{\text{(butyryl-ACP)}}{CH_3(CH_2)_2(CO)\text{-}S\text{-}ACP} + NADP^+.$

In the next round of the cyclic sequence, a new malonyl-ACP is formed as described above and reacts with butyryl-ACP in the condensation step, which is followed by steps 2-4 resulting in a six-carbon unit. The cycle is then repeated five times more until a 16-carbon unit is obtained, i.e. palmityl-ACP. The cycle stops at this stage because palmityl-ACP cannot be used further as a substrate for the condensing enzyme; instead it is converted to palmityl-CoA. The overall stoichiometry starting with glucose is then calculated as:

$4\ glucose + HS\text{-}CoA + 7\ ATP^{4-} + 14\ NADPH + 8\ NAD^+_{cyt} + 8\ NAD^+_{mit} \rightarrow$

$palmityl\text{-}S\text{-}CoA + 8\ CO_2 + 7\ ADP^{3-} + 7\ P_i^{2-} + 14\ NADP^+ + 8\ NADH_{cyt} + 8\ NADH_{mit} + 9\ H^+.$

The end-product of the fatty acid synthase pathways, palmityl-CoA, can be further metabolized by elongation or desaturation in the endoplasmic reticulum. The elongation is performed with acetyl-CoA by reactions as described for β-oxidation, but in the opposite order (condensation, reduction, dehydration, reduction) and with NADPH as the reducing agent. The net equation is:

$\underset{\text{(palmityl-CoA)}}{CH_3(CH_2)_{14}(CO)\text{-}S\text{-}CoA} + CH_3(CO)\text{-}S\text{-}CoA + 2\ NADPH + 2\ H^+ \rightarrow$

$\underset{\text{(stearyl-CoA)}}{CH_3(CH_2)_{16}(CO)\text{-}S\text{-}CoA} + HS\text{-}CoA + H_2O + 2\ \ NADP^+.$

In the process of desaturation, molecular oxygen is used as an acceptor for two pairs of electrons and protons from the acyl-CoA and from NADPH, which is required as a co-reductant:

$\underset{\text{(stearyl-CoA)}}{CH_3(CH_2)_{16}(CO)\text{-}S\text{-}CoA} + NADPH + H^+ + O_2 \rightarrow \underset{\text{(oleyl-CoA)}}{CH_3(CH_2)_7CH=CH(CH_2)_7(CO)\text{-}S\text{-}CoA} + NADP^+ + 2\ H_2O.$

Acetate derived from microbial fermentation in the hindgut is also a substrate for fatty acid synthesis, although of minor quantitative importance except in sows on high fibre diets. Acetate is taken up in adipocytes from the circulation and activated in the cytosol with CoA at the expense of 2 mol ATP per mol (Figure 14.4). This route for fatty acid synthesis is the dominant one in ruminants in which acetate is the major nutrient supplied from the digestive tract, and in which the activities of citrate lyase and malic enzyme are very low both in adipose and mammary tissues.

As shown above, the reducing equivalents (electrons and protons) used in fatty acid synthesis are provided by NADPH. The two major sources of cytosolic NADPH production are the malic enzyme reaction and the pentose phosphate pathway. Oxaloacetate produced in the cytosol by cleavage of citrate is reduced to malate, which in turn is oxidized by the malic enzyme to pyruvate. This three-carbon compound crosses the mitochondrial membranes and regenerates mitochondrial oxaloacetate by carboxylation, thereby bringing back the lost intermediate to the citric acid cycle (Figure 14.4):

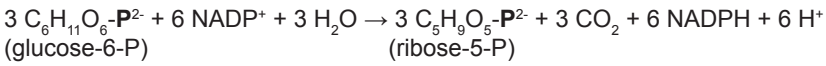
$\underset{\text{(oxaloacetate}_{cyt})}{^-O(CO)(CO)CH_2(CO)O^-} + NADH + H^+ \rightarrow \underset{\text{(malate}_{cyt})}{^-O(CO)(CHOH)CH_2(CO)O^-} + NAD^+$

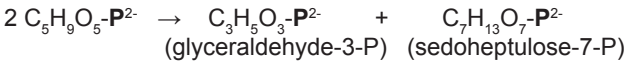
$^-O(CO)(CHOH)CH_2(CO)O^- + NADP^+ \rightarrow \underset{\text{(pyruvate}_{cyt})}{CH_3(CO)(CO)O^-} + CO_2 + NADPH$

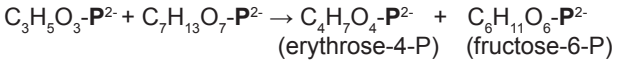
$Pyruvate^-_{mit} + CO_2 + ATP^{4-} + H_2O \rightarrow oxaloacetate^{2-}_{mit} + ADP^{3-} + P_i^{2-} + 2\ H^+.$

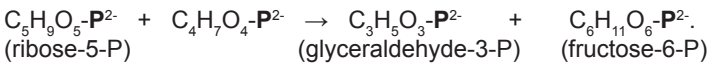
Thus, the recycling of oxaloacetate across the mitochondrial border results in consumption of NADH and ATP as well as production of NADPH, which is used for fatty acid synthesis.

In the pentose phosphate pathway, NADPH is generated as glucose-6-P is oxidized to the five-carbon compound, ribose-5-P. This is the oxidative branch of the pathway, and it is followed by a series of transketolase and transaldolase reactions whereby two hexose phosphates and one triose phosphate are formed from three pentose phosphates:

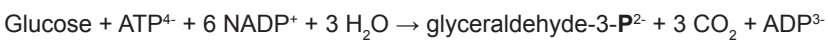


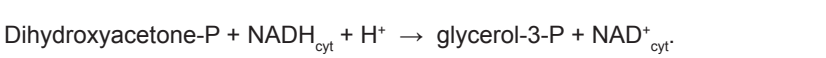




The two moles of fructose-6-P thus formed can be converted back to two moles of glucose-6-P by phosphoglucose isomerase, so only 1 mol glucose-6-P is used. However, if we start with glucose, we must add 1 mol ATP. The net outcome of the pathways is then:




As we have seen, the synthesis of 1 mol palmityl-CoA from 4 moles glucose via 8 moles acetyl-CoA needs 14 moles NADPH. Eight of these are generated by the malic enzyme reaction when 8 moles oxaloacetate are returned from the cytosolic to the mitochondrial compartment. The remaining 6 moles NADPH are produced from 1 mol glucose in the pentose phosphate pathway as just shown.

The final step in fat formation is esterification of acyl-CoA molecules with glycerol-3-P ($C_3H_7O_3$-**P**$^{2-}$), i.e. lipogenesis (Figure 14.4). The glycerol moiety is synthesized from glyceraldehyde-3-P ($C_3H_5O_3$-**P**$^{2-}$) via dihydroxyacetone-P:

The glyceraldehyde-3-P is derived from glycolysis or from the pentose phosphate pathway. The formation of triacylglycerol is governed by a complex of enzymes, triacylglycerol synthase. First, two acyl-CoA are attached with ester bonds to carbon 1 and 2 of glycerol-3-P to yield phosphatidate, which is dephosphorylated to a diacylglycerol that in turn accepts the third acyl-CoA:

$$C_3H_7O_3\text{-}\mathbf{P}^{2-} + R_1(CO)\text{-S-CoA} + R_2(CO)\text{-S-CoA} \rightarrow \begin{array}{l} CH_2O(CO)R_1 \\ | \\ CHO(CO)R_2 \\ | \\ CH_2O\text{-}\mathbf{P}^{2-} \end{array} + 2\ \text{HS-CoA}$$

(phosphatidate)

$$\begin{array}{l} CH_2O(CO)R_1 \\ | \\ CHO(CO)R_2 \\ | \\ CH_2O\text{-}\mathbf{P}^{2-} \end{array} + H_2O \rightarrow \begin{array}{l} CH_2O(CO)R_1 \\ | \\ CHO(CO)R_2 \\ | \\ CH_2OH \end{array} + P_i^{2-}$$

(diacylglycerol)

$$\begin{array}{l} CH_2O(CO)R_1 \\ | \\ CHO(CO)R_2 \\ | \\ CH_2OH \end{array} + R_3(CO)\text{-S-CoA} \rightarrow \begin{array}{l} CH_2O(CO)R_1 \\ | \\ CHO(CO)R_2 \\ | \\ CH_2O(CO)R_3 \end{array} + \text{HS-CoA.}$$

(triacylglycerol)

Let us now estimate the total cost of substrates and metabolic energy to synthesize an average triacylglycerol from glucose. The average chain length of fatty acids in adipose tissue is assumed to be 17.4 mol C [9], which approximately corresponds to one palmitic ($C_{16:0}$), one oleic ($C_{18:1}$) and one mol stearic acid ($C_{18:0}$).

From the foregoing reaction schemes, we can calculate the balance of matter and energy in the synthesis of these fatty acid CoA derivatives from glucose. Recycling of oxaloacetate and oxidation of $NADH_{mit}$ is included in the balance:

$$13\ \text{glucose} + 3\ \text{HS-CoA} + 14\ O_2 + 31\ ADP^{3-} + 31\ P_i^{2-} + 21\ \text{NADPH} + 52\ H^+ \rightarrow$$

$$\text{palmityl-S-CoA} + \text{oleyl-S-CoA} + \text{stearyl-S-CoA} + 26\ CO_2 + 31\ ATP^{4-} + 21\ NADP^+ + 82\ H_2O.$$

The total need for reducing power in the synthesis of these three fatty acids is 47 NADPH, and 26 NADPH are supplied in the malic enzyme reaction. The remaining 21 mol NADPH shown in the above balance must be delivered by metabolism of 3.5 mol glucose in the pentose phosphate pathway, which in addition yields 3.5 mol glyceraldehyde-3-P. If we assume that 1.75 mol glyceraldehyde-3-P is used for glycerol-3-P synthesis, and the remaining 1.75 mol is oxidized to pyruvate (see Figure 14.4), the $NADH_{cyt}$ produced by this oxidation is used up in the synthesis of glycerol-3-P as shown in the balance:

$$3.5\ \text{glyceraldehyde-3-}\mathbf{P}^{2-} + 3.5\ ADP^{3-} + 1.75\ P_i^{2-} + 1.75\ H^+ \rightarrow$$

$$1.75\ \text{glycerol-3-}\mathbf{P}^{2-} + 1.75\ \text{pyruvate}^- + 3.5\ ATP^{4-} + 1.75\ H_2O.$$

Summation of reactions in the pentose phosphate pathway, synthesis of glycerol-3-P, oxidation of glyceraldehyde-3-P to pyruvate, and complete oxidation of pyruvate gives:

$$3.5\ \text{glucose} + 4.375\ O_2 + 26.25\ ADP^{3-} + 28\ P_i^{2-} + 5.25\ H^+ + 21\ NADP^+ \rightarrow$$

$$1.75\ \text{glycerol-3-}\mathbf{P}^{2-} + 15.75\ CO_2 + 26.25\ ATP^{4-} + 21\ H_2O + 21\ \text{NADPH}.$$

Now we can write the net reaction for lipogenesis from glucose:

16.5 glucose + 18.375 $O_2$ + 57.25 $ADP^{3-}$ + 58 $P_i^{2-}$ + 57.25 $H^+$ →

triacylglycerol + 0.75 glycerol-3-$\mathbf{P}^{2-}$ + 41.75 $CO_2$ + 57.25 $ATP^{4-}$ + 102 $H_2O$.

The surplus glycerol-3-P is used to esterify fatty acids taken up from the peripheral circulation. Note that 2 moles of ATP are required for activation of one mol fatty acids with CoA. We assume the same average fatty acid composition as above:

0.75 (glycerol-3-$\mathbf{P}^{2-}$ + palmitic acid + oleic acid + stearic acid) + 4.5 $ATP^{4-}$ + 3 $H_2O$ →

0.75 triacylglycerol + 4.5 $ADP^{3-}$ + 5.25 $P_i^{2-}$ + 4.5 $H^+$.

Finally, the overall balance for the formation of one mol triacylglycerol from de novo fatty acids and of 0.75 mol triacylglycerol from blood fatty acids with average fatty acid composition can be stated:

16.5 glucose + 0.75 (palmitic acid + oleic acid + stearic acid) + 18.375 $O_2$ + 52.75 $ADP^{3-}$ + 52.75 $P_i^{2-}$ + 52.75 H+ →

1.75 triacylglycerol + 41.75 $CO_2$ + 52.75 $ATP^{4-}$ + 99 $H_2O$.

**Lipolysis** of deposited fat is a hydrolytic process in which lipases split the ester bonds between glycerol and fatty acids (Figure 14.4):

Triacylglycerol + 3 $H_2O$ → glycerol + $palmitate^-$ + $oleate^-$ + $stearate^-$ + 3 $H^+$.

Of the products of lipolysis, glycerol cannot be phosphorylated and reused in adipose tissue because of very low glycerol kinase activity, and it is therefore exported and metabolized in tissues that possess glycerol kinase activity, mainly the liver where it can be used for synthesis of glucose or for esterification of fatty acids. Most of the non-esterified fatty acids (NEFA) are also exported and used in other tissues, mainly the muscles, but some fatty acids from degraded fat are activated with CoA in the adipocytes and reused for lipogenesis. It should be kept in mind that free fatty acids are detergents and as such poisonous to the cell. Therefore, free fatty acids are immediately transported out of the cell, or they are bound to proteins or to CoA.

### *5.2. Regulation of lipid metabolism*

The main enzyme in lipolysis is a hormone-sensitive lipase that primarily acts on the first two ester bonds, while the third bond can also be hydrolyzed by a hormone-insensitive monoacylglycerol lipase. The hormone-sensitive lipase is activated by phosphorylation, which is catalyzed by a protein kinase. This kinase is activated by cAMP, which in turn is increased by the action of adenylate cyclase in much the same way as described previously for glycogenolysis. High cellular levels of cAMP also lead to inactivation of acyl-CoA glycerol transferase, the enzyme that creates the first two ester bonds in the process of lipogenesis. Synthesis of cAMP from ATP is a response to binding of adrenalin and other compounds with β-adrenergic activity, e.g. β-agonists. Thus, in situations with increased adrenalin secretion, lipolysis is stimulated and lipogenesis is held back, thereby providing fatty acids as a fuel for muscular work.

Insulin is the primary anabolic hormone that affects adipose tissue as it increases both glucose uptake and the activity of lipoprotein lipase. Furthermore, insulin inhibits lipolysis probably by decreasing the level of cAMP as described in the section concerning muscle tissue metabolism. Growth hormone decreases lipid retention (e.g. [37]) and its effect seems to be a direct one (i.e.

not mediated by IGF-I) on lipogenesis rather on lipolysis [14], [15] as growth hormone downregulates the sensitivity of adipocytes to insulin [24], [16].

In addition to hormonal regulation, lipid retention is also affected by metabolic regulation of enzyme activities. High cytosolic concentrations of citrate stimulate acetyl-CoA carboxylase and thereby the rate of fatty acid synthesis, i.e. a feed-forward regulation. However, products of the fatty acid synthase reactions, long-chain acyl-CoAs, inhibit acetyl-CoA carboxylase, the transfer of citrate from mitochondria to the cytosol, as well as NADPH generation in the pentose phosphate pathway (by inhibition of glucose-6-P dehydrogenase, which catalyses the initial step in the formation of ribose-5-P). Thus, fatty acid synthesis from glucose is strongly reduced when pigs are given high-fat diets providing an ample supply of blood fatty acids to the tissue. As we have just estimated, lipid synthesis from glucose results in a surplus production of ATP, which is a controlling factor both in glycolysis and in the citric acid cycle. High concentrations of ATP inhibit phosphofructokinase (fructose-6-P → fructose-1,6-diP), citrate synthase (acetyl-CoA + oxaloacetate → citrate), and isocitrate dehydrogenase (isocitrate → α-ketoglutarate), i.e. feed-back regulations. Therefore, fatty acid synthesis from glucose is a self-limiting process as also pointed out by Flatt [19]. The reduced activity of isocitrate dehydrogenase implies that the carbon flow in the citric acid cycle beyond citrate is very low [25]. This is confirmed by our calculations, which show that of the 99 moles glucose carbon (16.5 × 6) used for lipid synthesis, only 5.25 moles carbon (1.75 × 3) are oxidized to $CO_2$ in the citric acid cycle, i.e. around 5%. As there seems to be both physical and metabolic limitations as to how much fat can be deposited in individual adipose cells, we may ask how pigs can continue to retain lipid in appreciable amounts until maturity (see Chapter 3). The explanation is probably that pigs are able, until late in life, to increase the number of adipocytes in their body when fed ad lib with high-energy diets.

Although lipid synthesis from glucose leads to production of ATP, it is still an energy-requiring process because the substrates (16.5 glucose + 0.75 (palmitate + oleate + stearate)) contain more biochemical energy than the products (1.75 triacylglycerol + 52.75 ATP). If we calculate the energy as ATP equivalents, we have:

| | |
|---|---|
| Substrates | |
| Glucose: 16.5 × 36 = | 594.00 ATP |
| Fatty acids: 0.75 × (129 + 144 + 146) = | 314.25 ATP |
| | 908.25 ATP |
| Products | |
| Glycerol: 1.75 × 20 = | 35.00 ATP |
| Fatty acids: 1.75 × (129 + 144 + 146) = | 733.25 ATP |
| ATP: | 52.75 ATP |
| | 821.00 ATP |

Thus, the energetic efficiency in lipogenesis from glucose and fatty acids is very high, i.e. 100×821/908.25 = 90%.

## 6. Metabolism in bone tissues

Bone is a special form of connective tissue made of a collagenous matrix in which crystals of calcium phosphates are embedded. Bones have various purposes in the body, such as protection of vital organs (e.g. brain, heart and lungs), as attachment points for skeletal muscles to allow locomotion, and as support of the body against gravity. In addition to these physical functions, bones serve as reservoirs of exchangeable calcium and phosphorus, thereby playing a vital role in maintaining Ca and P homeostasis. Thus, bones are far from “dead” tissues, but are subjected

to continuous degradation (resorption) and rebuilding. This turnover of bones is a means of tissue repair as well as a way to buffer the extracellular concentration of Ca and P.

The structural protein in the bone matrix is mostly collagen, and the major part of the mineral content is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, which is a mixture of calcium phosphate, $Ca_3(PO_4)_2$, and calcium hydroxide, $Ca(OH)_2$. Sodium and small amounts of magnesium and carbonate are also present. Most bones are built of an outer layer of compact tissue surrounding an inner layer of spongy or trabecular bone. The long leg bones, e.g. humerus and femur, have a cavity of bone marrow inside the trabecular layer. About 75% of the bone mass is compact tissue, which is more dense, but less metabolically active than trabecular tissue. Three main cell types are present in bones (see Chapter 3). **Osteoblasts** are the bone-forming cells that synthesize and secrete collagen to create a matrix around them. Alkaline phosphatases associated with osteoblasts hydrolyze phosphate ester bonds so that the local concentration of phosphate increases and calcium phosphates will precipitate. As the matrix in this way calcifies, the cells become **osteocytes**, i.e. mature bone cells. **Osteoclasts** are multinuclear cells that erode previously formed bone. Proton pumps ($H^+$-dependent ATPases) in the osteoclast membrane acidify a small area of the bone tissue around the osteoclast. This lowering of pH to approximately 4 will dissolve hydroxyapatite, and proteases secreted from osteoclasts degrade the collagen matrix.

Longitudinal bone growth takes place in the epiphyseal plates, i.e. narrow bands of actively proliferating cartilage that separate both ends (epiphyses) from the shaft (diaphysis) of long bones. The width of the epiphyseal plates is positively related to the rate of bone growth and is increased by actions of growth hormone and IGF-I. The synthesis of bone collagen is also stimulated by IGF-I. Sex hormones exert some regulatory effects on bone growth, but their modes of action are unclear. Castrated male pigs grow to almost the same size at maturity as entire males, but females have smaller mature size than males (Danfær, unpublished). Oestrogen secretion during menstrual cycles may be responsible for the earlier cessation of bone growth in females as oestrogens have inhibiting effects on the proliferating activity of the epiphyseal plates. At epiphyseal closure when the epiphyses unite with the diaphysis, linear bone growth ceases. In human females, increase in height and in length of hands and feet often stops at puberty, or shortly after, as a result of oestrogen secretion. However, in female pigs, the body continues to grow both in height and length for a long time after puberty occurs. The epiphyseal plates in the humerus bone of female pigs close at 2-2½ years of age (Danfær, unpublished). This would suggest that the balance between IGF-I and oestrogen around puberty is different in humans and pigs.

### *6.1. Regulation of calcium and phosphorus flow in the body*

The body content of Ca and P is approximately 0.7 and 0.5% of body weight, respectively (Danfær, unpublished). Almost all of the body Ca (~99%) and most of the body P (~85%) is contained in bone tissues. There are two pools of bone calcium; a readily exchangeable reservoir and a much larger, more stable pool of slowly exchangeable calcium. The smaller of these pools participate in the short term homeostatic regulation of plasma Ca concentration, while the larger pool is involved in bone development and remodelling by constant Ca retention and resorption. Intracellular Ca has many important regulatory functions, e.g. in muscle contraction, nerve cell activity, hormone secretion, and enzyme activation. Intracellular phosphate is a component of nucleotides, nucleic acids as well as phosphorylated metabolites and enzymes. In order to maintain a stable supply for these vital functions, the extracellular Ca concentration is tightly controlled at around 2.5 mM, while the concentration of P is more variable, 1.5-2.7 mM. Two hormones bear the main responsibility for the homeostatic regulation of Ca and P plasma levels: parathormone (PTH) from the parathyroid glands and calcitonin (CT) from the so-called C cells in the thyroid gland. The modes of action in this regulatory system are outlined in Figure 14.5.

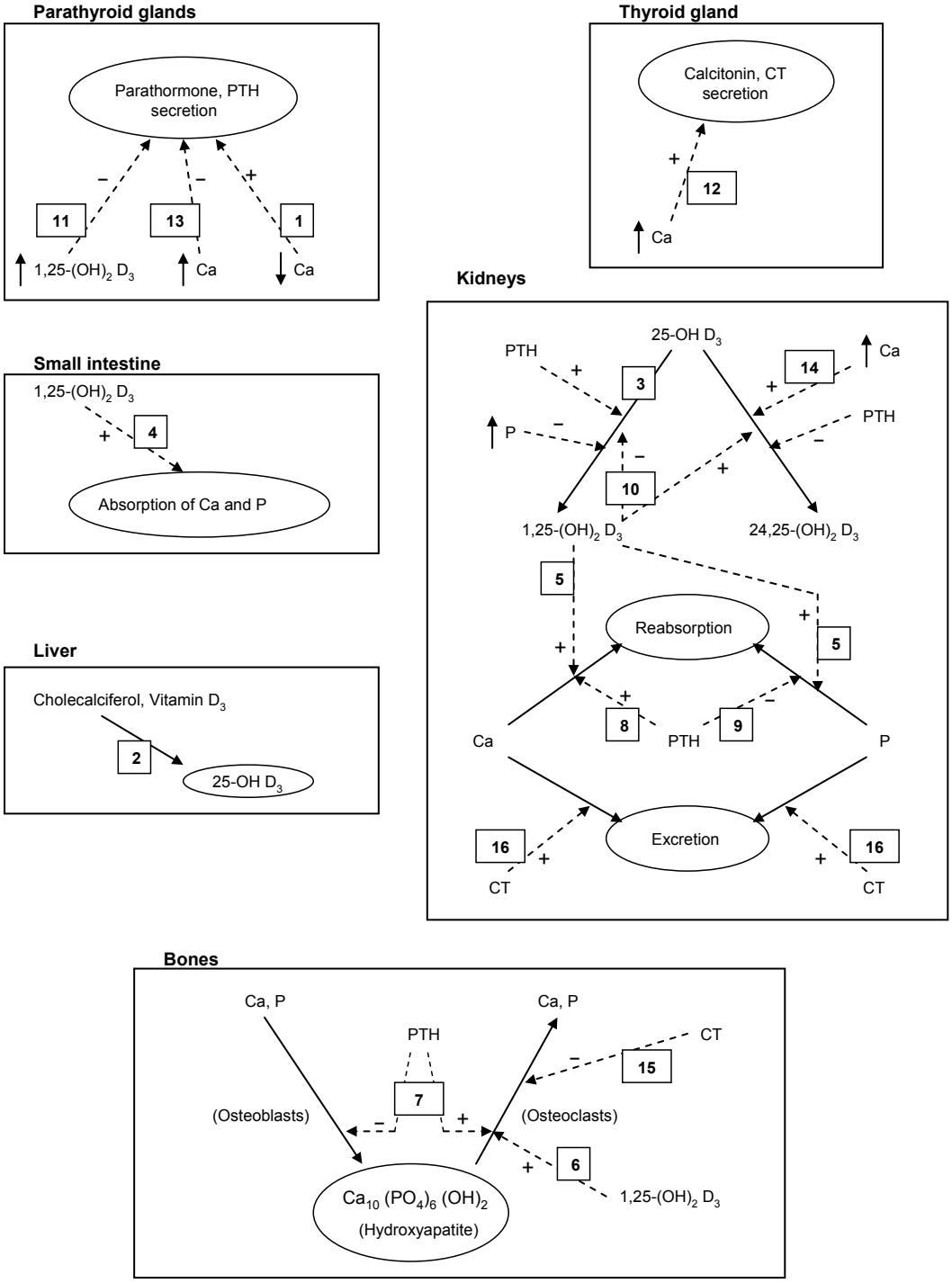



**Figure 14.5. Hormonal and feed-back regulations of calcium and phosphorus metabolism.**

In periods of rapid bone growth, Ca is retained in bone tissues at a rate that may exceed the rate of Ca absorption from the digestive tract. Then, the extracellular concentration decreases, and this decrease in Ca concentration triggers secretion of PTH from the parathyreoidea **(1)**, see Figure 14.5. The regulatory effects of PTH are both direct and indirect via vitamin $D_3$ (cholecalciferol). Vitamin $D_3$ is converted in the liver to 25-hydroxycholecalciferol (calcidiol or 25-OH $D_3$) **(2)**. This metabolite is further hydroxylated upon stimulation by PTH in the proximal tubules of the kidneys to the more active compound, 1,25-dihydroxycholecalciferol (calcitriol or 1,25-$(OH)_2$ $D_3$) **(3)**. Actions of calcitriol are aimed at an increase of Ca and P availability: it promotes intestinal absorption **(4)**, increases reabsorption in the kidneys **(5)**, and resorption from bones **(6)**. The more direct effects of PTH are to enhance resorption and to slow down retention of Ca and P in bone tissues **(7)**, to increase Ca **(8)** and decrease P **(9)** reabsorption in the kidneys. It is important to prevent an ex-

tracellular concentration of P that is too high in order to avoid precipitation of calcium phosphates. The overall result of these hormonal responses is therefore an increase in the extracellular Ca concentration.

As part of the control system, several feed-back mechanisms exist. Calcitriol slows down the rate of its own synthesis and stimulates the formation of 24,25-$(OH)_2$ $D_3$, which is a less active metabolite **(10)**. A high concentration of phosphate also decreases the formation of 1,25-$(OH)_2$ $D_3$. Furthermore, high levels of 1,25-$(OH)_2$ $D_3$ will decrease the secretion of PTH **(11)**.

The Ca concentration increases when its rate of absorption exceeds its net removal from the extracellular fluid. This will stimulate secretion of the thyroid hormone, CT **(12)**, decrease the secretion of PTH **(13)**, and enhance the synthesis of 24,25-$(OH)_2$ $D_3$ in the kidneys **(14)**. CT decreases the activity of osteoclasts, which reduces the resorption of bones and the release of Ca and P to the extracellular space **(15)**. Finally, CT stimulates the tubular excretion of Ca and P in the kidneys **(16)**. Thus, these responses to a high plasma level of Ca tend to lower the concentration.

As the extracellular concentrations of Ca and P are held within narrow limits, the rate of bone mineralization and thereby growth rate does not seem to be regulated by a direct push effect. Instead, the invariable plasma concentration of especially Ca secures a stable supply to the bone tissues, while growth hormone and IGF-I stimulate the use of nutrients for synthesis of collagen and hydroxyapatite. The average retention rates are 7-8 g Ca/d and 5-6 g P/d in fast growing pigs during the period from 25 to 100 kg body weight (Danfær, unpublished) as also found by Fernández [17]. It has been estimated that the resorption rates of Ca and P are in the order of 10 and 25%, respectively, of what is retained daily in pigs of 35 kg body weight [18]. If these estimates are used for the growth period 25-100 kg, the resorption rates can be calculated as ~0.7 g Ca/d and ~1.2 g P/d, which is of the same magnitude as the extracellular pool sizes of Ca and P in 60 kg pigs. A large amount of Ca is filtered in the kidneys, and 98-99% of this is reabsorbed corresponding to a daily amount that is 5-10 times higher than the extracellular pool size. Thus, small changes in the percentages of resorption from the bones and reabsorption in the kidneys could have a strong influence on Ca and P plasma concentrations unless these are carefully controlled.

## 7. Energy utilization in growth

The energy efficiency of body protein and lipid retention can be expressed as the ratio of retained energy (net energy for growth) to metabolizable energy for growth, i.e $k_g = NE_g/ME_g$. Net energy for growth can be separated into net energy for protein retention ($NE_p$) and net energy for lipid (fat) retention ($NE_f$), i.e. $NE_g = NE_p + NE_f$. The utilizations of ME for protein and for lipid retention in growing pigs have been estimated in numerous balance and respiration trials resulting in values of 0.4-0.6 for $k_p = NE_p/ME_p$ and 0.75-0.8 for $k_f = NE_f/ME_f$ as reviewed by van Es [39] and Mc Donald et al. [26].

In previous sections of this chapter, we elucidated the biochemical background for protein and lipid synthesis including the energy requirements for these syntheses. In this section, we will calculate theoretical energetic efficiencies of growth based on the presented metabolic pathways as well as on the extent of protein and lipid turnover. The energy efficiency of growth depends on the ratio of retention to synthesis of the body components, protein and lipid, as $NE_g$ represents the energy content (heat of combustion) in deposited protein and lipid, while $ME_g$ is the energy content of substrates used in retentions plus energy used as fuel for the syntheses. Therefore, theoretical estimation of efficiencies for growth requires quantitative knowledge of both retention and synthesis rates of body protein and lipid during the growth period.

The fractional rate of body protein synthesis ($FRS_p$ = % of the protein mass per day) in rats, pigs and other farm animals is high at birth and declines exponentially with age [40], [32], [13]. The fractional rate of protein synthesis in pigs can be described quantitatively from data on protein turnover [30], [33], [34], [4], [35] as $FRS_p(t) = 3.5 + 19.5 \times \exp(-0.01 \times t)$, where t is age of the pig in days. The absolute rate of synthesis is then calculated as $FRS_p(t) \times P(t)/100$ (kg/d), where P(t) is mass (kg) of the body protein pool at age t.

**Table 14.1.** Equations for prediction of body weight, body protein and body lipid mass (kg) in ad lib fed pigs from weaning to maturity [11].

| **Body weight:** $W(t) = W_b \times W_m/(W_b^d + (W_m^d - W_b^d) \times \exp(-c \times t))^{1/d}$ | | | |
|---|---|---|---|
| | **Males** | **Females** | **Castrates** |
| $W_b$, kg | $8.32 \times 10^{-20}$ | $7.63 \times 10^{-18}$ | $5.03 \times 10^{-19}$ |
| $W_m$, kg | 472.23 | 375.06 | 448.60 |
| c | $4.88 \times 10^{-3}$ | $5.17 \times 10^{-3}$ | $4.79 \times 10^{-3}$ |
| d | -0.4680 | -0.5004 | -0.4847 |
| **Body protein:** $P(t) = P_b \times P_m/(P_b^d + (P_m^d - P_b^d) \times \exp(-c \times t))^{1/d}$ | | | |
| | **Males** | **Females** | **Castrates** |
| $P_b$, kg | $7.09 \times 10^{-4}$ | $4.40 \times 10^{-6}$ | $1.15 \times 10^{-5}$ |
| $P_m$, kg | 77.73 | 58.96 | 59.43 |
| c | $6.40 \times 10^{-3}$ | $7.04 \times 10^{-3}$ | $6.40 \times 10^{-3}$ |
| d | -0.3853 | -0.4326 | -0.4674 |
| **Body lipid:** $L(t) = L_b \times L_m/(L_b^d + (L_m^d - L_b^d) \times \exp(-c \times t))^{1/d}$ | | | |
| | **Males** | **Females** | **Castrates** |
| $L_b$, kg | $2.45 \times 10^{-10}$ | $4.22 \times 10^{-3}$ | $1.01 \times 10^{-7}$ |
| $L_m$, kg | 145.04 | 145.57 | 224.28 |
| c | $5.28 \times 10^{-3}$ | $6.68 \times 10^{-3}$ | $3.97 \times 10^{-3}$ |
| d | -0.3202 | -0.2161 | -0.3709 |

$W_b$, $P_b$, $L_b$: mass at birth (t=0); $W_m$, $P_m$, $L_m$: mass at maturity; t: age in days.

Body weights and body compositions of growing pigs have been measured from birth to maturity in comprehensive slaughter experiments (Danfær, unpublished), see Chapter 3. From these data, equations of body weight, body protein and body lipid as related to age of the pigs were developed for three genders [11]. Some of these equations valid from weaning to maturity are presented in Table 14.1. Differentiation with respect to time of the equation for body protein, P(t), leads to an equation for the rate of protein retention: $dP(t)/dt = P(t) \times (1 - (P(t)/P_m)^d) \times c/d$ (for definition of parameters, see Table 14.1). With the equations given, we can calculate the rates of protein synthesis and retention at any time from weaning to maturity as shown by the following example.

Let us choose a male pig at 90 days of age. Its body weight, body protein mass and rate of body protein retention is estimated as 52.8 kg, 9.47 kg and 0.197 kg/d, respectively. The energy content in the retained protein ($NE_p$) is calculated by use of the heat of combustion value of body protein: 23.7 MJ/kg [7]. The metabolizable energy ($ME_p$) is the heat combustion value of the substrate amino acids (AA) plus the energy value of ATP consumed in protein synthesis and degradation. The average heat combustion value of AA is 2.7 MJ/mol [39] corresponding to a molecular weight of protein-bound AA of 2.7 × 1000/23.7 = 114. The ATP cost in protein synthesis and degradation is assumed to be 5 and 1 mol per mol AA, respectively. Most ATP in the body is produced by oxidation of glucose and fatty acids. The combustion heats of glucose and palmitate are 2.80 and 10.03 MJ/mol, respectively [7], and the ATP yields are 36 and 129 mol per mol oxidized glucose and palmitate, respectively. The result in both cases is an energy value of 0.078 MJ/mol ATP. With this information, the efficiency of protein retention can be calculated:

| | |
|---|---|
| Rate of protein synthesis: (3.5 + 19.5 × exp(-0.01 × 90)) × 9.47/100 = 1.082 kg/d ~ 1082/114 = | 9.49 mol AA/d |
| Rate of protein degradation: 1.082 – 0.197 = 0.885 kg/d ~ 885/114 = | 7.76 mol AA/d |
| Net energy: ($NE_p$): 0.197 × 23.7 = | 4.67 MJ/d |
| Energy in substrates: | 4.67 MJ/d |
| ATP used in synthesis: 9.49 × 5 × 0.078 = | 3.70 MJ/d |
| ATP used in degradation: 7.76 × 0.078 = | 0.61 MJ/d |
| Metabolizable energy ($ME_p$): 4.67 + 3.70 + 0.61 = | 8.98 MJ/d |
| $k_p = NE_p/ME_p$ = 4.67/8.98 = | 0.52 |

Thus, 52% of ME available for protein retention is deposited in body protein. The ratio of protein retention to protein synthesis in this example is 0.197/1.082 = 0.18, but from the equations presented here (see also Table 14.1) it is clear that at maturity, the rate of retention approaches zero while the rate of synthesis is still rather high and approaches 2.7 kg/d. Thus, the ratio between the two processes approaches zero and so does the utilization factor, $k_p$. This shows that the energy efficiency of protein retention depends heavily on the age of the pig.

The body lipid mass and the rate of lipid retention in our 90-day-old male pig are 6.97 kg and 0.189 kg/d, respectively, as estimated by use of the parameter values presented in Table 14.1. To the knowledge of the author, very few data are available on lipid turnover rates in growing pigs. However, in one study [14], [15], fractional rates of lipid synthesis, degradation and retention were estimated in 80 kg pigs as 2.3, 0.8 and 1.5% of the body lipid mass per day, respectively, i.e. the ratio of retention to synthesis was 0.65. If we assume that the fractional rate of lipid synthesis declines exponentially with age in the same manner as for protein, we can describe the fractional rate: $FRS_f(t) = a + b \times \exp(-c \times t)$. The parameter values can be estimated as a = 2.0, b = 4.7 and c = 0.016 by using the information in Table 14.1 (lipid parameters), by using the value 0.65 for the ratio retention:synthesis at 80 kg, by assuming that this ratio is 0.8 at 50 kg body weight, and further by assuming that $FRS_f(t)$ is 2%/d at maturity. In the section on lipid metabolism, we estimated the substrate use to be 16.5 moles glucose and 2.25 moles preformed fatty acids for the synthesis of 1.75 moles triacylglycerol with a fatty acid composition of palmitate, stearate and oleate in equal amounts. This gives a molecular weight of 861.4 g/mol triacylglycerol. The heat of combustion values are 2.80, 1.66 and 10.86 MJ/mol for glucose, glycerol and an average fatty acid (mean of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$), respectively [7]. We also estimated that 52.75 mol ATP is formed during synthesis of 1.75 mol triacylglycerol. This ATP is assumed to be hydrolyzed to ADP in other processes, and the heat energy of this hydrolysis is about 0.052 MJ/mol ATP [23]. The combustion heat energy of triacylglycerol is close to the sum of the energies in the components, glycerol and fatty acids: (1.66 + 3 × 10.86) × 1000/861.4 = 39.7 MJ/kg, a value very close to the energy value of human body fat [7]. In calculation of ME for lipid deposition, we must include heat combustion energies of the substrates as well as the heat produced by ATP hydrolysis, but subtract the heat energy in glycerol and fatty acids released by lipolysis:

| | |
|---|---|
| Rate of of lipid synthesis: (2.0 + 4.7 × exp(-0.016 × 90)) × 6.97/100 = | 0.217 kg/d |
| Rate of lipolysis: 0.217 – 0.189 = | 0.028 kg/d |
| Net energy ($NE_f$): 0.189 × 39.7 = | 7.50 MJ/d |
| Energy in substrates: 0.217 × 1000/(1.75 × 861.4) × (16.5 × 2.80 + 2.25 × 10.86) = | 10.17 MJ/d |
| Hydrolysis of produced ATP: 0.217 × 1000/(1.75 × 861.4) × 52.75 × 0.052 = | 0.39 MJ/d |
| Energy in products of lipolysis: 0.028 × 1000/861.4 × (1.66 + 3 × 10.86) = | 1.11 MJ/d |
| Metabolizable energy ($ME_f$): 10.17 + 0.39 – 1.11 = | 9.45 MJ/d |
| $k_f$ = = $NE_f/ME_f$ = 7.50/9.45 = | 0.79 |

Thus, 79% of ME available for lipid retention is deposited in body lipid. The utilization of ME for growth ($k_g$) is: 100 × (4.67 + 7.50)/(8.98 + 9.45) = 66%.

In Table 14.2, utilization efficiencies of ME for protein retention, lipid retention and total growth are calculated for males, females and castrates at different body weights by use of the principles and assumptions described above.

**Table 14.2.** Theoretically estimated utilization of metabolizable energy for growth, k = NE/ME, in ad lib fed pigs at different body weights.

| | Males | | | | Females | | | | Castrates | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Body weight, kg | Age, d | Protein, $k_p$ | Lipid, $k_f$ | Total, $k_g$ | Age, d | Protein, $k_p$ | Lipid, $k_f$ | Total, $k_g$ | Age, d | Protein, $k_p$ | Lipid, $k_f$ | Total, $k_g$ |
| 25 | 60 | 0.59 | 0.84 | 0.71 | 58 | 0.57 | 0.85 | 0.68 | 59 | 0.55 | 0.82 | 0.69 |
| 50 | 88 | 0.52 | 0.80 | 0.66 | 88 | 0.49 | 0.81 | 0.65 | 88 | 0.48 | 0.78 | 0.65 |
| 75 | 113 | 0.48 | 0.76 | 0.63 | 115 | 0.44 | 0.78 | 0.63 | 114 | 0.44 | 0.75 | 0.62 |
| 100 | 135 | 0.45 | 0.73 | 0.61 | 140 | 0.41 | 0.75 | 0.61 | 138 | 0.40 | 0.72 | 0.60 |
| 200 | 227 | 0.35 | 0.60 | 0.50 | 253 | 0.28 | 0.56 | 0.48 | 235 | 0.30 | 0.59 | 0.51 |
| 300 | 339 | 0.23 | 0.42 | 0.36 | 435 | 0.11 | 0.26 | 0.22 | 361 | 0.18 | 0.42 | 0.37 |
| 400 | 531 | 0.08 | 0.19 | 0.16 | - | - | - | - | 609 | 0.04 | 0.19 | 0.16 |

This table clearly shows that the energy utilization in growth of body components decreases with age because rates of retention decrease faster than rates of synthesis towards maturity. At 300 kg body weight, the efficiencies in females are much lower than in males and castrates because at this body weight, females are closer to maturity than the other two genders. Thorbek [38] measured utilization of ME for growth in 12 castrated male pigs from 20 to 90 kg body weight in four experimental series. From these 48 balance and respiration trials, average k-values at the mean body weight (about 55 kg) were estimated as: $k_p$ = 0.48, $k_f$ = 0.77 and $k_g$ = 0.67. The corresponding k-values calculated in Table 14.2 for 50 kg castrates are very close to these in vivo data, i.e. 0.48, 0.78 and 0.65, respectively. It could be expected that the theoretically estimated $k_g$ value (0.65) is lower than found in the experiments (0.67), because the ratio $NE_p$:$NE_g$ is higher (0.33) in the calculated example than the experimental value (0.28) found by Thorbek [38].

## 8. Summary and implications

The intention with the present chapter was to focus on the biochemical and metabolic background of growth of the large body masses: muscle, adipose and bone tissues. Nutrient metabolism in the pregnant uterus and in the mammary gland is dealt with in other chapters of this book.

As an introduction to the message of the chapter, the flow of organic nutrients from the feed through the pig body is outlined in the text and depicted in Figure 14.1. Due to the structure of cell membranes, different mechanisms exist for transport of individual nutrients and metabolites into and out of tissue cells. These mechanisms are described in some detail in order to shed light on the molecular basis for nutrient partition between tissues and regulation of cellular uptake. Growth is the difference between synthesis and degradation of body mass, and the biochemistry of the major intracellular metabolic pathways involved in turnover and growth of body protein and lipid is presented in the main sections of the chapter and illustrated in Figures 14.2-14.4. In description of the structure and growth of bone tissues, regulations of calcium and phosphorus metabolism are emphasized and shown in Figure 14.5. Finally, theoretical utilization efficiencies of metabolizable energy for growth are estimated at different body weights for males, females and castrated male pigs. These estimations are based partly on experimental data concerning protein and lipid retention and turnover rates; partly on the metabolic energy cost (ATP) of protein and lipid turnover; and partly on some assumptions regarding an age-dependent decline of the turnover rates. The calculated energetic efficiencies (k-values) are very close to experimental values for castrates at about 50 kg body weight, and show further that the utilization of metabolizable energy for protein ($k_p$) and lipid ($k_f$) deposition decreases with age and approaches zero at maturity (Table 14.2).

In the context of pig growth biology, what is the usefulness of the information presented here? Hopefully, it can be of value to the theoretically interested reader, who may wonder how a growing animal, like the pig, can organize the conversion of dietary chemical constituents into body mass in a highly efficient way. One example of an interesting question could be how synthesis and breakdown of muscle glycogen are prevented to occur at the same time, thereby avoiding unnecessary waste of metabolic energy (Figure 14.3). Another example is the apparent paradox that although fatty acid synthesis from glucose is a self-limiting process, pigs can get very fat at maturity when fed low fat diets ad lib. Furthermore, the reader may find it convenient to follow the major metabolic pathways at the molecular level without consulting biochemistry textbooks.

Feed evaluation is a discipline in applied animal science and, traditionally, feed evaluation systems have been based on chemical composition of the feed, digestibility coefficients of individual nutrient fractions, and empirical equations describing utilization of digestible and metabolizable nutrients. These systems have served the pig industry for many decades as useful tools to design diets for different categories of pigs. However, these systems also have shortcomings, e.g. they are static and assume additive feed values. As described in the present chapter, the intermediary metabolism, which ultimately determines the rate of growth, is affected by numerous regulatory factors and nutrient interactions, and furthermore, utilization of metabolizable energy for growth is age-dependent. The justification of static and additive properties of feed evaluation systems can therefore be questioned. The real value of a diet should be an expression of the pig performance obtained with that diet, i.e. a specific diet can have different feed values when fed to growing pigs of different age or even to pigs of the same age, but different gender. Thus, an ideal feed evaluation system for growing pigs should be based on quantitative, dynamic descriptions of the major biological processes leading to body growth, i.e. a set of mathematical equations for nutrient flow rates like those shown in Figure 14.1. Several dynamic and mechanistic simulation models of nutrient digestion and utilization in pigs have been published [6], [29], [31], [3], [10], [28], [22], but the potential of such models as predictive tools of pig performance and thereby feed value has not yet been fully exploited.

# 9. References


1. **Attaix, D., Combaret, L. & Taillandier, D.** (1999) Mechanisms and regulation in protein degradation. In: Protein metabolism and nutrition (eds. G.E. Lobley, A. White and J.C. MacRae). Proc. VIIIth Internat. Symp. Protein Metabolism and Nutrition, EAAP Publ. No. 96. Wageningen Pers, 51-67.

2. **Baracos, V.E.** (2005) Whole animal and tissue proteolysis in growing animals. In: Biology of Metabolism in Growing Animals (eds. D.G. Burrin and H.J. Mersmann). Biology of Growing Animals Series, vol. 3. Elsevier, Amsterdam, 69-82.

3. **Bastianelli, D. & Sauvant, D.** (1997) Modelling the mechanisms of pig growth. Livest. Prod. Sci. 51, 97-107.

4. **Bergen, W.G., Johnson, S.E., Skjaerlund, D.M., Babiker, A.S., Kent Ames, N., Merkel, R.A. & Anderson, D.B.** (1989) Muscle protein metabolism in finishing pigs fed ractopamine. J. Anim. Sci. 67, 2255-2262.

5. **Bezkorovainy, A. & Rafelson Jr., M.E.** (1996) Concise Biochemistry. Marcel Dekker, Inc., New York, 624 pp.

6. **Black, J.L., Campbell, R.G., Williams, I.H., James, K.J. & Davies, G.T.** (1986) Simulation of energy and amino acid utilisation in the pig. Res. Develop. Agric. 3, 121-145.

7. **Blaxter, K.** (1989) Energy Metabolism in Animals and Man. Cambridge University Press, Cambridge, 336 pp.

8. **Danfær, A.** (1980) Protein- og aminosyreomsætning hos voksende grise [Protein and Amino Acid Metabolism in Growing Pigs] Licentiatafhandling, Den kgl. Veterinær- og Landbohøjskole, København, 160 pp.

9. **Danfær, A.** (1999) Carbohydrate and Lipid Metabolism. Chapter 14 in: A Quantitative Biology of the Pig (ed. I. Kyriazakis). CABI Publishing, Wallingford, 333-362.

10. **Danfær, A.** (2000) A pig model for feed evaluation. Chapter 30 in: Modelling Nutrient Utilization in Farm Animals (eds. J.P. McNamara, J. France and D.E. Beever). CABI Publishing, Wallingford, 393-408.

11. **Danfær, A.** (2009) Vækst, vækstens sammensætning og foderudnyttelse hos danske grise fra fødsel til udvokset størrelse (forsøgsresultater) [Growth, composition of growth as well as feed utilization in Danish pigs from birth to maturity (experimental results)]. In: Kompendium til Tema Husdyrvidenskab 1 (ed. M.O. Nielsen). Det Biovidenskabelige Fakultet, Københavns Universitet, 190-206.

12. **Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J. & Volden, H.** (2006) The Nordic Dairy Cow Model, Karoline - Description. Chapter 32 in: Nutrient Digestion and Utilization in Farm Animals (eds. E. Kebreab, J. Dijkstra, A. Bannink, W.J.J. Gerrits and J. France). CABI Publishing, Wallingford, 383-406.

13. **Davis, T.A. & Fiorotti, M.L.** (2005) Regulation of skeletal muscle protein metabolism in growing animals. In: Biology of Metabolism in Growing Animals (eds. D.G. Burrin and H.J. Mersmann). Biology of Growing Anmals Series, vol. 3. Elsevier, Amsterdam, 37-68.

14. **Dunshea, F.R., Harris, D.M, Bauman, D.E., Boyd, R.D. & Bell, A.W.** (1992a) Effect of porcine somatotropin on nonesterified fatty acid and glycerol metabolism in growing pigs. J. Anim. Sci. 70, 132-140.

15. **Dunshea, F.R., Harris, D.M, Bauman, D.E., Boyd, R.D. & Bell, A.W.** (1992b) Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in growing pigs. J. Anim. Sci. 70, 141-151.

16. **Etherton, T.D., Louveau, I., Sørensen, M.T. & Chaudhuri, S.** (1993) Mechanisms by which somatotropin decreases adipose tissue growth. Amer. J. Clin. Nutr. 58 (Suppl.), 287S-295S.

17. **Fernández, J.A.** (1995a) Calcium and phosphorus metabolism in growing pigs. I. Absorption and balance studies. Livest. Prod. Sci. 41, 233-241.

18. **Fernández, J.A.** (1995b) Calcium and phosphorus metabolism in growing pigs. III. A model resolution. Livest. Prod. Sci. 41, 255-261.

19. **Flatt, J.P.** (1970) Conversion of carbohydrate to fat in adipose tissue: an energy-yielding and, therefore, self-limiting process. J. Lip. Res. 11, 131-143.

20. **Freeman, C.P., Noakes, D.E. & Annison, E.F.** (1970) The metabolism of glucose, acetate, palmitate, stearate and oleate in pigs. Br. J. Nutr. 24, 705-716.

21. **Gill, M., Thornley, J.H.M., Black, J.L., Oldham, J.D. & Beever, D.E.** (1984). Simulation of the metabolism of absorbed energy-yielding nutrients in young sheep. Br. J. Nut. 52, 621-649.

22. **Halas, V., Dijkstra, J., Babinszky, L., Verstegen, M.W.A. & Gerrits, W.J.J.** (2004) Modelling of nutrient partitioning in growing pigs to predict their anatomical body composition. 1. Model description. Br. J. Nutr. 92, 707-723.

23. **Lehninger, A.L.** (1975) Biochemistry, 2. edn., Worth Publishers, Inc., New York, 1104 pp.

24. **Magri, K.A., Adamo, M., Leroith, D. & Etherton, T.D.** (1990) The inhibition of insulin action and glucose metabolism by porcine growth hormone in porcine adipocytes is not the result of any decrease in insulin binding or insulin receptor kinase activity. Biochem. J. 266, 107-113.

25. **Martin, B.R.** (1987) Metabolic Regulation: A Molecular Approach. Blackwell Scientific Publ., Oxford, 299 pp.

26. **McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A.** (2002) Animal Nutrition, 6. edn. Pearson Education Ltd., Harlow, 693 pp.

27. **Mersmann, H.J. & Smith, S.B.** (2005) Development of white adipose tissue lipid metabolism. In: Biology of Metabolism in Growing Animals (eds. D.G. Burrin and H.J. Mersmann). Biology of Growing Anmals Series, vol. 3. Elsevier, Amsterdam, 275-302.

28. **Milgen, J. van, Quiniou, N. & Noblet, J.** (2000) Modelling the relation between energy intake and protein and lipid deposition in growing pigs. Anim. Sci. 71, 119-130.

29. **Moughan, P.J., Smith, W.C. & Pearson, G.** (1987) Description and validation of a model simulating growth in the pig (20-90 kg liveweight). N. Zeal. J. Agric. Res. 30, 481-489.

30. **Mulvaney, D.R., Merkel, R.A. & Bergen, W.G.** (1985) Skeletal muscle protein turnover in young male pigs. J. Nutr. 115, 1057-1064.

31. **Pomar, C., Harris, D.L. & Minvielle, F.** (1991) Computer simulation model of swine production systems: 1. Modelling the growth of young pigs. J. Anim. Sci. 69, 1468-1488.

32. **Riis, P.M.** (1983) The pools of tissue constituents and products: proteins. Chapter 5 in: Dynamic Biochemistry of Animal Production (ed. P.M. Riis). Elsevier, Amsterdam, 75-108.

33. **Sève, B., Reeds, P.J., Fuller, M.F., Cadenhead, A. & Hay, S.M.** (1986) Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early-weaning: possible connection to the energy metabolism. Reprod. Nutr. Dévelop. 26, 849-861.

34. **Sève, B., Ballèvre, O., Ganier, P., Noblet, J., Prugnaud, J. and Obled, C.** (1993) Recombinant porcine somatotropin and dietary protein enhance protein synthesis in growing pigs. J. Nutr. 123, 529-540.

35. **Simon, O.** (1989) Metabolism of proteins and amino acids. In: Protein Metabolism in Farm Animals (eds. H.-D. Bock, B.O. Eggum, A.G. Low, O. Simon and T. Zebrowska). VEB Deutscher Landwirtschaftsverlag, Berlin and Oxford University Press, Oxford, 273-366.

36. **Stryer, L.** (1981) Biochemistry, 2. edn. W.H. Freeman & Co., San Francisco, 949 pp.

37. **Thiel, L.F., Beerman, D.H., Krick, B.J. & Boyd, R.D.** (1993) Dose-dependent effects of exogenous porcine somatotropin on the yield, distribution, and proximate composition of carcass tissues in growing pigs. J. Anim. Sci. 71, 827-835.

38. **Thorbek, G.** (1975) Studies on Energy Metabolism in Growing Pigs. Report 424, National Institute of Animal Science, Copenhagen, 159 pp + appendices.

39. **van Es, A.J.H.** (1980) Energy costs of protein deposition. Chapter 12 in: Protein Deposition in Animals (eds. P.J. Buttery and D.B. Lindsay). Butterworths, London, 215-224.

40. **Waterlow, J.C., Garlick, P.J. & Millward, D.J.** (1978). Protein Turnover in Mammalian Tissues and in the Whole Body. Elsevier, Amsterdam, 804 pp.

41. **Wood, J.D., Gregory, N.G., Hall, G.M. & Lister, D.** (1977) Fat mobilization in Pietrain and Large White pigs. Br. J. Nutr. 37, 167-186.