Chapter 2
Energy Balance

The body maintains energy balance throughout the daily feed-fast cycles, across periods where energy demands increase due to physical exertion, and when energy supplies are depleted due to nutrient deprivation. During a typical day, several meals are consumed leading to periods of time several hours long after the meals, the fed state, where there is a net energy gain. This situation changes during sleep and during gaps in meal intake where the net energy balance, that is calories in minus calories out, becomes negative. These are the fast states that lead to refeeding. The ability of the body to maintain energy balance is centered about a set of peripheral organs and tissues that digest, convert, ship, and store nutrients and energy, namely,

- Pancreas
- Liver
- Adipose tissue
- Muscle
- Gastrointestinal tract

In the fed state, nutrients – carbohydrates, fat, and amino acids – are taken in, converted, and stored. In response to the increase in circulating glucose, the pancreas secretes insulin that serves as a fed-state start signal to the other peripheral tissues. The liver takes up and stores the glucose as glycogen. Triglycerides are packaged into large, transportable particles; these are synthesized and stored in adipose (fat) tissue, and muscle rebuilds its supply of protein.

In the fast state, nutrients are no longer taken in but instead energy sources residing in peripheral tissues are mobilized and released to maintain energy homeostasis. This state is signaled by glucagon release from the pancreas. The liver now breaks down and releases the stored glucose/glycogen, initiates gluconeogenesis, initiates fatty acid oxidation, and generates ketone bodies as an alternative/supplement to the dwindling glucose supply. Adipose tissue degrades their triglycerides thereby supplying fatty acids and glycerol to other peripheral tissues, muscle utilizes fatty acid oxidation, and ketone bodies are shipped to the brain.

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To properly manage feeding and energy balance, the central nervous system and peripheral tissues communicate continuously with one another. Local signals are sent and received by cells residing in the stomach and intestines, pancreas, liver, adipose tissue, and muscle. Longer ranging signals are sent through the circulation to specific regions in the brain from these peripheral tissues and, in response, feedback regulatory signals are sent from the brain to the peripheral tissues.

The brain exerts considerable influence over feeding. Hormones secreted into the bloodstream by the pancreas, adipose tissue, stomach, and intestines pass through the blood-brain barrier and enter the part of the hypothalamus called the arcuate nucleus. The hypothalamus is a region buried deep in the brain responsible for regulating, for example, eating, drinking, and sleeping. The hormones secreted into the bloodstream by the food-processing and energy-storing organs convey needed information on the amount of stored fat and the energy balance. In response, neurons in the arcuate nucleus send out signals in the form of neuropeptides that instruct other control centers to either stimulate or suppress the appetite.

The central nervous system regulates fat stores to maintain proper energy balance in the body. In order for this to happen, there has to be a feedback mechanism operating between regulatory center(s) and fat stores. The existence of such a mechanism was first postulated by Kennedy in 1953. He noted that in healthy animals, fat stores remain fairly constant in the face of changing conditions and suggested that the hypothalamus might act as a central regulator of energy balance through negative feedback mechanisms much like those described by Norbert Wiener in his 1948 landmark tome on cybernetics. He further suggested that a hypothalamic center might have a key role in the regulation, noting that age-related changes and damage to this region lead to obesity. The discovery that insulin and leptin convey signals to the hypothalamus serves as a confirmation of Kennedy's hypothesis.

Several conditions need to be met for this type of negative feedback regulation to take place. First the signal strengths, as measured by the amounts of insulin and leptin released into the circulation, should be proportional to the amount of fat. Next the amount entering the CNS from the circulation must reflect the amount of body fat, and third in response to increasing signaling there has to be a negative behavioral response – reduced feeding. Under normal healthy conditions in the body, all of these conditions are met for insulin and leptin, but as will be discussed in detail they fail under the unhealthy conditions of the metabolic syndrome.

Alterations in metabolism vary from short-term changes associated with fed-fast states to long-lasting changes in metabolic strategy associated with healthy and disease states. These alterations are made possible by the presence of multiple-layered, overlapping, signaling systems that tightly integrate together growth and metabolism. Some of the regulatory signals arrive at critical control points, typically rate-limiting enzymes, within metabolic pathways. Other signals are conveyed to the nucleus where they induce changes in expression of genes involved in metabolism. The arriving signals encompass not only hormonal and growth factor signals such as insulin sent from cells in other tissues but also local signals indicative of the cell's current nutrient and energy status.

The goal of this chapter is to provide an overview of the signals regulating energy balance sent and received by peripheral tissues and the central nervous system. This overview will illustrate many of the themes mentioned in the preceding paragraph. It will begin with the liver and the reciprocal actions of insulin and glucagon signaling at critical control points for glucose management. It will include a first look at β-oxidation, or fat burning, in muscle tissue, and in doing so examine how this process is influenced by hormonal signals as well as local signals that convey information about the cell's energy status. A critical intracellular sensor of energy status – AMP-activated protein kinase (AMPK) – will be introduced, and the chapter will conclude with an examination of how insulin, leptin, and ghrelin hormonal signals are processed in the hypothalamus.

2.1 Hormonal Signaling by the Endocrine Pancreas

The pancreas consists of two distinct populations of cells. Those belonging to the exocrine pancreas have roles in digestion while those in the endocrine pancreas, the Islets of Langerhans mentioned in Chapter 1, are prominent regulators of glucose and lipid homeostasis and energy balance. These endocrine cells along with populations of endocrine cells strategically situated in other peripheral organs secrete a variety of signaling molecules – hormones and signaling peptides – that act locally to coordinate the actions of the peripheral organs and globally over a long range conveying energy balance information to the central nervous system. These signaling molecules are listed in Table 2.1.

Table 2.1 Pancreatic Hormones

Hormone or signaling peptide	Sending cells	Receiving cells	Physiological function
Glucagon	Pancreatic Islet α-cells	Liver	Stimulates release of glucose into the circulation and conversion of glycogen into glucose (L)
Insulin	Pancreatic Islet β-cells	Liver, muscle, adipose tissue, brain	Storage of glucose (L), uptake of glucose (M, A), storage of fatty acids (A), inhibits breakdown of glycogen (L), satiety signal (B)
Somatostatin	Pancreatic Islet δ-cells	Intestine, stomach, pancreas	Suppresses release of gastrointestinal hormones, inhibits gastrointestinal motility and blood flow (I), secretion of stomach acid (S), inhibits the release of insulin and glucagon (P)

The exocrine pancreas receives signals from the gastrointestinal tract. Recall from the Introduction that secretin was the first hormone discovered. In response to secretin, cells in the exocrine pancreas secrete bicarbonate that buffers low pH conditions, while cholecystokinin (CCK) triggers secretion of digestive enzymes. There are four populations of pancreatic endocrine cells. These are referred to as α-, β-, δ-, and PP-cells. In humans, these cells are scattered throughout the Islet. These cells are associated with other endocrine cells to facilitate paracrine communications and are aligned along the blood vessels. More than half of the cells are β-cells, with α-cells the next most common cell type. These cells respond to changes in glucose levels by either increasing or decreasing secretion of glucagon, insulin, and somatostatin thereby maintaining glucose and lipid homeostasis and energy balance throughout the feeding cycle.

Insulin, a polypeptide hormone secreted by the pancreas is the central regulator of energy balance in the body. It is secreted by Islet β-cells in response to eating as indicated by increased glucose levels released into the circulation from the small intestine. The hormone instructs the liver to take up and store glucose by forming glycogen from it. This hormone keeps blood glucose levels from becoming too high. It does so by increasing glucose uptake by fat and muscle cells and inhibits glucose production by the liver. It also stimulates the synthesis and storage of lipids and inhibits their degradation and release into the bloodstream.

Insulin receptors in muscle and adipose tissue stimulate uptake of glucose by the GLUT4 transporter (Table 2.2). In the liver, insulin stimulates the storage of glucose in the form of glycogen, a storage polymer form of glucose. If the amount of glucose taken up exceeds the storage capacity, the excess is converted to fatty acids that are released into the bloodstream as lipoproteins. Insulin also facilitates the entry of glucose into adipose tissue where it is converted to glycerol. The glycerols together with fatty acids are then used to make triglycerides. In sum, insulin stimulates uptake and burning of glucose (glycolysis) and storage of fatty acids in adipose tissue.

Table 2.2 Glucose transporters and their distribution in the body

Glucose transporter	Distribution
GLUT1	Erythrocytes, endothelial cells of the blood-brain barrier
GLUT2	Liver, pancreatic β-cells, kidney, intestines
GLUT3	Neurons, placenta
GLUT4	Adipose tissue, striated muscle – skeletal and heart

2.2 In Response to Signals from the Pancreas, the Liver Maintains Glucose and Lipid Homeostasis

Once blood glucose levels decline, pancreatic α cells secrete glucagon that instructs the liver to release the stored glucose into the bloodstream. This is accomplished by the conversion of glycogen to glucose (glycogenolysis), by the synthesis of glucose from noncarbohydrate sources (gluconeogenesis),

by the inhibition of glycolysis, and by inhibiting the storage of glucose as glycogen molecules (glycogenesis).

Insulin signals received by the liver launch the opposite set of actions. In response to feeding leading to an elevation in circulating glucose, insulin instructs the liver to take up glucose and store it in the form of glycogen and inhibits the release of glucose into the bloodstream. Insulin is a polypeptide hormone and binds to receptor tyrosine kinases that transduce the insulin signals into the hepatocytes. This pathway will be examined in detail in Chapter 3. A brief sketch at the chain of events leading to the conversion and storage of glucose as glycogen in the liver is presented in Fig. 2.1. As shown in this figure, glucose is converted sequentially to glucose-6-phosphate and from there to glucose-1-phosphate. Two enzymes control the interconversions between glucose-1-phosphate and glycogen. One of these, glycogen synthase (GS), catalyzes the formation of glycogen from glucose-1-phosphate. This enzyme is inactivated when phosphorylated and activated when dephosphorylated. Another enzyme, glycogen phosphorylase (GP), when phosphorylated catalyzes the conversion of glycogen to glucose-1-phosphate. Insulin and glucogen exert their regulatory influences by toggling on and off GS and GP at the correct times in fed-fast cycle.

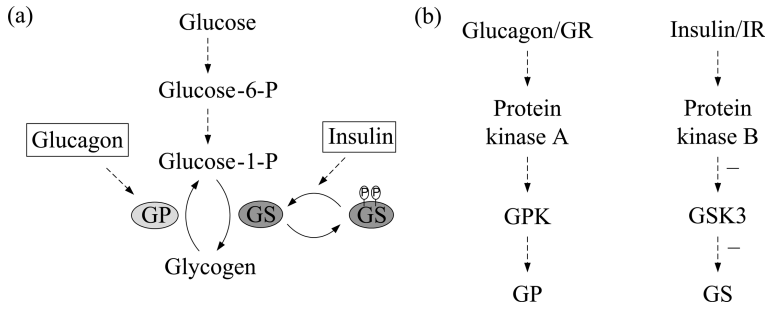

Fig. 2.1 The glycogen synthase shuttle

Phosphorylation and dephosphorylation actions regulate whether glucose is converted to glycogen for storage or whether the converse takes place where glycogen is converted back to glucose for release. Glycogen synthase is the rate-limiting enzyme in the conversion of glucose to glycogen. Insulin promotes the storage process by activating protein kinase B (Akt), which then phosphorylates glycogen synthase kinase-3 (GSK3) thereby inactivating it. GSK3 is the leading kinase that phosphorylates and inactivates GS. Thus insulin promotes the dephosphorylated state of GS leading to activation and glycogen storage. Glucagon exerts its regulatory actions through protein kinase A, which phosphorylates and activates glycogen phorphorylase kinase (GPK), which then phosphorylates and actives glycogen phosphorylase. Glycogen

phosphorylase plays a comparable role to GS operating as the rate-limiting enzyme in the breakdown of glycogen to glucose. The insulin/glycogen ratio thus acts on GS and GP to switch back and forth between the two modes of action (Fig. 2.1).

An illustrative example of how hormonal and internal signals come together to regulate a metabolic program is that of gluconeogenesis in the liver. Here again insulin and glucagon operate in opposition to turn on and off a metabolic process, but in this case the critical control points are those of transcription factors and their cofactors operating in the nucleus. The key regulatory targets in this case are the transcription factor known as FOXO1 and the coactivator referred to as TORC2. Insulin signaling during the fed state prevents these factors from turning on transcription of a pair of enzymes critical for gluconeogensis, namely G6Pase and PEPCK. Glucogon, in contrast, promotes activation of the transcription factors during fasting and stress situations leading upregulation of these key enzymes and increased generation of glucose. Most interestingly, the internal energy-status sensor AMPK can override the hormonally derived pro-gluconeogenesis signals when energy levels in the cell are too low. The insulin signaling pathway is the main topic in Chapter 3 and metabolic reprogramming is the chief subject in Chapter 4. The aforementioned signal transducers and transcription factors will be defined and examined in considerable detail in those two chapters.

2.3 Energy in the Form of Lipids Is Stored and Released When Needed in Adipose Tissue

Adipose tissue is composed of fat-storing cells called adipocytes. There are two types of adipose tissue – white adipose tissue (WAT) and brown adipose tissue (BAT). Fuel in the form of fat is stored in white adipocytes and converted to heat in brown adipocytes. These cells grow and shrink as they gain and lose lipids, which are stored in the form of triglycerides within lipid droplets. The trafficking of lipids and glucose in and out of these cells is regulated by the sympathetic nervous system and by hormones in a manner that parallels the movements of glucose in and out of other tissues.

Insulin stimulates the uptake of lipids and glucose. It does so by triggering the translocation of the glucose transporter GLUT4 and several kinds of the fatty acid transporters from intracellular parking locations to the plasma membrane. Insulin also inhibits lipolysis, the hydrolysis of lipids, the central step required for release of the lipids back into circulation, and stimulates *de-novo* free fatty acid synthesis. As was the case in other tissues, insulin signals are transduced into the cell by means of receptor tyrosine kinase activation leading to PI3K activity and the activation of Akt. The latter stimulates the activity of phosphodiesterase PDE3B, which antagonizes cAMP second messengers thereby repressing lipolysis.

2.4 Adipose Tissue Functions as an Endocrine Organ

Adipose tissue serves as an energy store, provides insulation and cushioning for the body, and presents an inflammatory milieu. Adipose tissue also functions as an endocrine organ, secreting hormones into the bloodstream. The obesity (ob) gene product leptin was identified in 1994 by Jeffrey Friedman and coworkers. The name leptin was coined from the Greek word "leptos" meaning thin. This hormone is secreted into the circulation by adipocytes. Leptin receptors were found shortly thereafter in 1995 in the hypothalamus, in the arcuate nucleus, and other nuclei. Leptin is widely regarded as a long sought for lipostatic factor – the amount of leptin secreted by white adipocytes into the circulation tracks the total amount of body fat present as well as the body mass index (BMI). The more fat tissue the greater the amount of leptin secreted into the bloodstream, and this is accomplished in a slow constitutive manner.

Leptin was just the first of a number of adipose tissue hormones to be found. Its discovery in 1994 was followed by that of adiponectin in 1995/1996. These and still other hormones discovered during the past few years are collectively referred to as adipokines, by analogy to the term cytokines. Some of the adipokines act on cells of the adipose tissue itself; others such as adiponectin binds to receptors in the liver or in skeletal muscle, and still others cross the blood-brain barrier to act on the central nervous system as does leptin. A short list of white adipose tissue hormones and their target organs and tissues is presented in Table 2.3.

Leptin acts not only as a hormone but also as a pleiotropic cytokine. The gastric mucosa of the stomach secretes leptin in an exocrine manner into the lumen of the stomach and in an endocrine manner into the circulation. Whereas adipocyte-secreted leptin acts over a long time scale to regulate energy homeostasis, stomach-associated leptin acts on much short time frame. The amount of stomach-secreted leptin goes up when eating and goes down afterwards. Secreted leptin working together with another peptide, cholecystokinin (CCK), secreted by the small intestine, regulates actions by the duodenum, the part of the small intestine into which the stomach first empties. These signaling peptides regulate stomach emptying and also help control meal size.

Table 2.3 Hormones secreted into the bloodstream by cells in white adipose tissue (WAT)

Hormone or signaling peptide	Sending cells	Receiving cells	Physiological function
Adiponectin	WAT	Liver, muscle	Glucose, insulin, and energy management
IL-6	WAT	Liver, muscle	Energy and insulin management
Leptin	WAT	Brain, muscle	Satiety signal (B)

2.5 Ghrelin Released by Endocrine Cells in the Stomach Acts in Short-Term Feeding and Long-Term Energy Management

Endocrine X/A cells in the stomach secrete ghrelin, a 28 amino acid peptide, into the circulation. This peptide ghrelin has two distinct actions. It binds receptors expressed in the anterior pituitary to stimulate the release of growth hormone (GH). Second, it supplies "need-to-feed" or hunger, signals to the hypothalamus, and regulates energy balance by opposing the actions of leptin.

Growth hormones are secreted by somatotroph cells in the anterior pituitary. Their release is regulated by two hypothalamic peptides – growth hormone releasing hormone (GHRH) and growth hormone inhibiting hormone (GHIH), or somatostatin. The release of GHs can also be stimulated by artificial compounds called growth hormone secretagogues (GHSs). Ghrelin is the natural signaling peptide for these receptors. The existence of receptors for ghrelin in both the anterior pituitary and arcuate nucleus of the hypothalamus provides a coordinating link between growth and feeding.

Ghrelin is produced predominately by the stomach. Its concentration in the circulation increases during fasting and decreases during refeeding. The opposite pattern is seen in the stomach where decreases occur during fasting and increases take place during feeding. This inverse response property is a consequence of the continual secretion of the peptide hormone from the stomach during fasting. Thus, ghrelin is a hunger signal sent by the stomach that acts on the arcuate nucleus (ARC).

Ghrelin supplies orexigenic signals to the same hypothalamic nuclei as leptin. It stimulates the synthesis and release of neuropeptide Y (NPY) and agouti-related protein (AgRP) in the arcuate nucleus thereby stimulating increased food intake. The ghrelin receptor is capable of signaling in the absence of ligand binding and does so about 50% of the time. The circuit mediating feeding behavior is depicted in Fig. 2.9 and will be discussed later in the chapter.

2.6 Satiation Signals Are Sent by Cells in the Gastrointestinal Tract

Several populations of neuroendocrine cells in the gastrointestinal (GI) tract secrete hormones and peptides that coordinate the actions of different parts of the digestive system (Table 2.4). These cells also transmit satiation signals that halt feeding. To accomplish this task, biophysical changes indicative of fullness such as pyloric pressure (the pylorus is the region connecting the stomach to the duodenum), stomach motility and muscle relaxation, and gastric filling are sensed. In response, the neuroendocrine cells send out "stop eating" messages that complement the "start eating" signals conveyed by ghrelin. Two key satiation hormones sent out by the neuroendocrine cells are cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1).

Table 2.4 Hormones/peptides secreted into the bloodstream by cells in the gastrointestinal tract

Hormone or signaling peptide	Sending cells	Receiving cells	Physiological function
Cholecystokinin (CCK)	I-cells of the duodenum, jujunum	Exocrine pancreas	Stimulates release of digestive enzymes
Ghrelin	Stomach (A-cells of the gastric fundus)	Brain	Hunger signal
GLP-1	L-cells of the distal small and large intestine	Endocrine pancreas	Regulates insulin and glucagon release, gastric emptying and secretion
PYY_{3-36}	L-cells of the distal small and large intestine	Brain, I, P	Satiety factor (B), inhibits gut motility (S) and pancreatic secretion (P)
Secretin	S-cells of the duodenum	Exocrine pancreas	Stimulates secretion of bicarbonate

Cholecystokinin is secreted by cells called I cells in response to the presence of fat or protein in the duodenum. These cells are able to sense the presence of nutrients and respond by secreting the hormones. The hormone binds to receptors in the vagal afferents triggering vagus nerve messages to the hindbrain. There are several different bioactive forms of this hormone. The most prevalent forms are designated as CCK8, CCK33, and CCK58. These hormones serve as short-term satiation signals from the upper part of the GI tract. Among their several regional roles is the control of gallbladder contraction; another is the inhibition of gastric emptying.

Glucagon-like peptide 1 functions in a similar role in the lower part of the GI tract (ileum). In response to the presence of carbohydrates and lipids, it adjusts stomach and intestinal motility, thereby contributing to an "ileal brake" to eating. Receptors for this peptide are expressed in the GI tract, pancreas, vagal afferents, brainstem, and hypothalamus. This peptide is also released by neurons in the brain as a feeding inhibitory peptide, in regions involved in energy balance and associated with leptin signaling.

2.7 Brown Adipose Tissue Carries Out Adaptive (Diet-Induced and Cold-Induced, Nonshivering) Thermogenesis

Brown adipocytes are unique to mammals. These cells utilize a protein-uncoupling protein 1 (UCP1) to redirect the electron transport chain to produce heat instead of ATP. Recall that the electron transport chain embedded in the inner mitochondrial membrane (IMM) couples electron flow to the pumping of protein across the membrane from the matrix to the intermembrane space. That is the energy

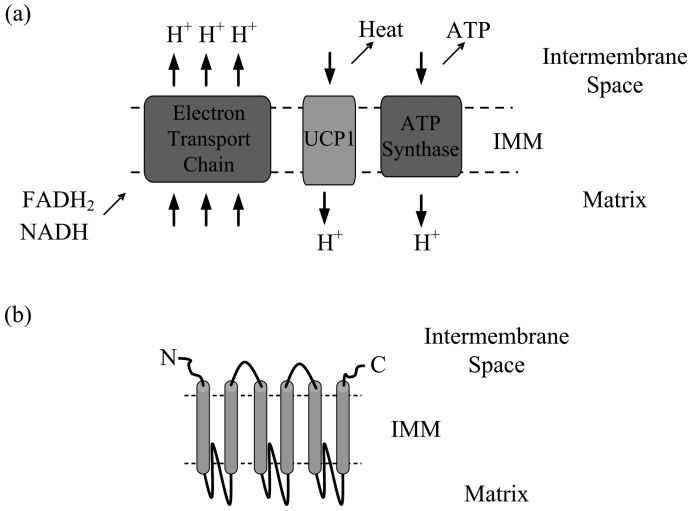

Fig. 2.2 Uncoupling proteins. (**a**) Organization and actions of the electron transport chain, uncoupling protein 1 (UCP1), and ATP synthase in the inner mitochondrial membrane (IMM). (**b**) UCP1 chain topology

derived from the flow of the electrons from Complex I to Complex IV is used to pump protons and establish a proton electrochemical gradient. As shown in Fig. 2.2, the UCP1 protein resides in the IMM and facilitates proton leak in the other direction from the intermembrane space back into the matrix. This step cuts off the last step in oxidative phosphorylation, in which reentry of the protons generates ATP. It bypasses the F0/F1-ATPase generation of ATP in Complex V and in its place produces heat.

Mitochondrial uncoupling proteins (UCPs) belong to a large family of mitochondrial transporters encoded by SLC25 genes. These transport proteins shuttle metabolites across the inner mitochondrial membrane. They form dimers; each chain passes back and forth through the IMM six times with both N and C terminals in the intermembrane space as shown in Fig. 2.2. The founding member of the UCP family, UCP1, is expressed in brown adipose tissue. Other family members, UCP2–UCP5, are broadly distributed and will be discussed further in later chapters.

The signaling route that mediates adaptive thermogenesis is, as follows. Feeding triggers signaling to the CNS, which then responds by sending return signals via the sympathetic nervous system to peripheral tissues such as BAT. These nerves emit norepinephrine, which then binds β-adrenergic receptors on target tissues. In BAT the predominant type of receptor is the β_3-adrenergic receptor. Receptor binding leads to cAMP and PKA activation as it did in WAT. Two sets of changes result from these events in BAT. The first is that CREB gene expression is enhanced leading to expression of UCP1 and increased mitochondrial biogenesis. The second is PKA activation of HSL

and perilipin leads to FFA release from lipid droplets. The FFAs then bind and stimulate UCP1 activity. In addition, the FFAs are metabolized in the mitochondria via β-oxidation and the citric acid cycle to produce reducing agents NADH and $FADH_2$ thereby increasing the rate of oxidative phosphorylation leading through UCP1 activity to enhance heat generation.

2.8 Muscle Cells and β-Oxidation

β-Oxidation of free fatty acids is a major source of energy for the body. It is especially important for skeletal muscle, cardiac muscle, and liver. In liver and heart, it provides some 80% of the total energy production. In liver, it is used to produce ketone bodies, that is, acetone, 3-hydroxybutyric acid (β-hydroxybutyrate) and acetoacetate, all of which are used as extra energy sources by tissues such as the brain.

The β-oxidation process begins with the activation of the FFA through formation of a bond between the carbonyl group of the FFA and the sulfhydryl group of Co-enzyme A (CoA) and leads to formation of acyl-CoA (Fig. 2.3).

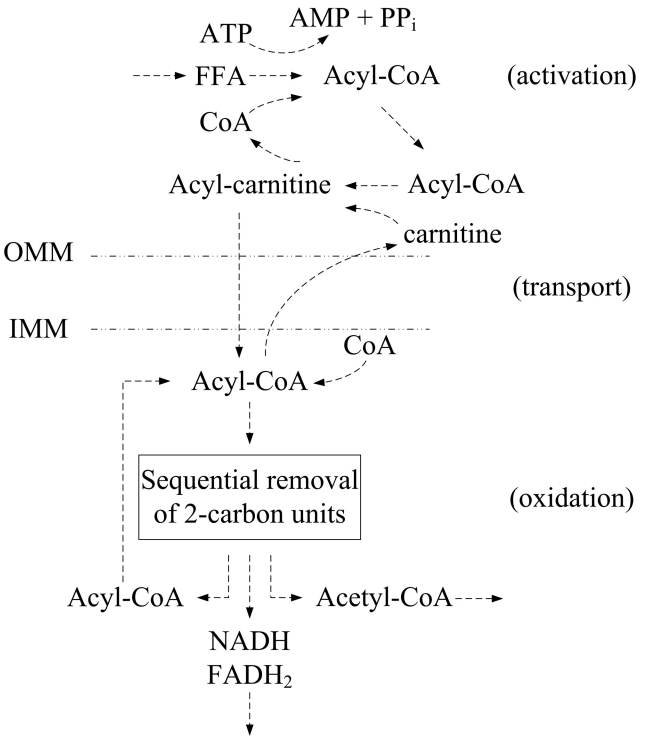

Fig. 2.3 β-Oxidation of free fatty acids illustrating its three stages of activation, transport, and oxidation

This step takes place on the outer mitochondrial membrane (OMM) and is catalyzed by acetyl-coenzyme A synthase (ACS). In the next preparatory step, the acyl-CoA molecules are transported across the OMM and IMM into the mitochondrial matrix by carnitine carrier molecules. This operation is facilitated by carnitine palmitoyltransferase 1 and 2 (CPT1 and 2), the former catalyzing carnitine attachment at the cytosol-OMM and the latter carnitine detachment at the IMM-matrix.

Once inside the matrix, the main steps in β-oxidation begin. In β-oxidation, pairs of carbon atoms are removed from the end of fatty acyl-CoA molecules in a series of enzymatic reactions using NAD^+ and FAD as coenzymes. These generate from each fat molecule one NADH, one $FADH_2$, and an acetyl-CoA. The process takes the name β-oxidation from the observation that the cuts to the acyl-CoA are made at the β-carbon position. The acetyl-CoA molecules output from β-oxidation are used in the Krebs cycle and the electron-rich NADH and $FADH_2$s are used in both the Krebs cycle and in the electron transport chain of oxidative phosphorylation.

The rate-limiting step in β-oxidation is the transport step catalyzed by CPT1. As will be discussed shortly, this step is regulated by intracellular signaling molecules such as AMPK and hormones such as leptin and ghrelin in response to energy needs and nutrient supplies. The output from β-oxidation, acetyl-CoA, is centrally involved in a variety of metabolic processes. It is used by the liver as a precursor in biosynthesis of ketone bodies, and as indicated in Fig. 2.4 can be generated by means of glycolysis as well as through β-oxidation. Acetyl-CoA is also used as the starting point in feedback regulation of β-oxidation. As illustrated in Fig. 2.4, the negative (endpoint) feedback loop terminates at CPT1 – acetyl-CoA is converted to malonyl-CoA, which then inhibits CPT1.

Fatty acid oxidation is regulated in skeletal muscle in response to hormonal input and stress brought on by activities such as exercise. The signaling pathway that responds to these activities is the AMPK pathway that monitors energy status and is activated when increases in energy supplies are needed. As shown in Fig. 2.5, low-energy supplies plus signals from an upstream kinase such as LKB1 activate AMPK. This kinase, in turn, phosphorylates ACC. There are

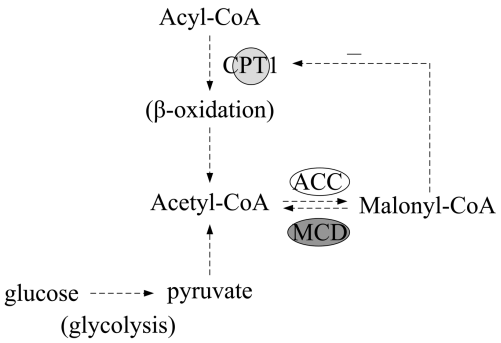

Fig. 2.4 Feedback regulation of β-oxidation. Abbreviations: ACC, acetyl-coenzyme A carboxylase; MCD, malonyl-coenzyme A decarboxylase

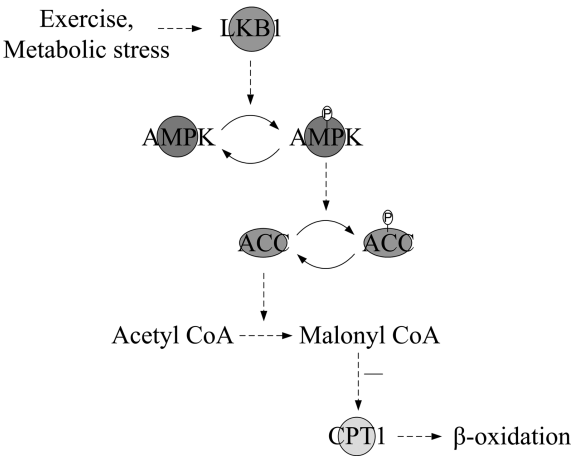

Fig. 2.5 Regulation of beta oxidation by the AMPK, metabolic stress-activated pathway

two ACC isoforms, ACC1 and ACC2. The latter is involved in regulating fatty acid oxidation in skeletal muscle. In the absence of AMPK phorphorylation, unphosphorylated ACC2 stimulates the conversion of acetyl CoA to malonyl CoA, which inhibits CPT1 thereby preventing β-oxidation. This blockage is relieved by AMPK-mediated phosphorylation of ACC2.

Fatty acid oxidation is regulated at the transcriptional level in addition to its regulation at the catalytic level by AMPK and through end-point feedback. Key regulators of fatty acid synthesis are members of the nuclear receptor family that will be examined in Chapter 4. Several members of this family are of particular interest. These include most notably the peroxisome proliferator activated receptors (PPARs) and the associated PPARγ coactivator 1α (PGC-1α) protein. The latter plays a central role in coordinating fatty acid metabolism and the Krebs (TCA) cycle. As will be seen in the next chapters, chronic overfeeding leading to excessive fatty acids and metabolic overload is believed to have a causative role in insulin resistance and Type 2 diabetes (T2D).

2.9 AMPK Is an Intracellular Energy Sensor and Regulator

AMP-activated protein kinase, or AMPK, is the key regulator of energy balance operating at the cellular level. Once AMPK is activated, it acts in several ways to bring energy supply and expenditure back into balance. It both stimulates energy-producing catabolic processes and throttles back energy-using anabolic ones. This is accomplished by phosphorylating key elements of the metabolic pathways and key regulators of the expression of genes involved in metabolism. Representative examples of energy balancing by AMPK in different tissues are presented in Fig. 2.6.

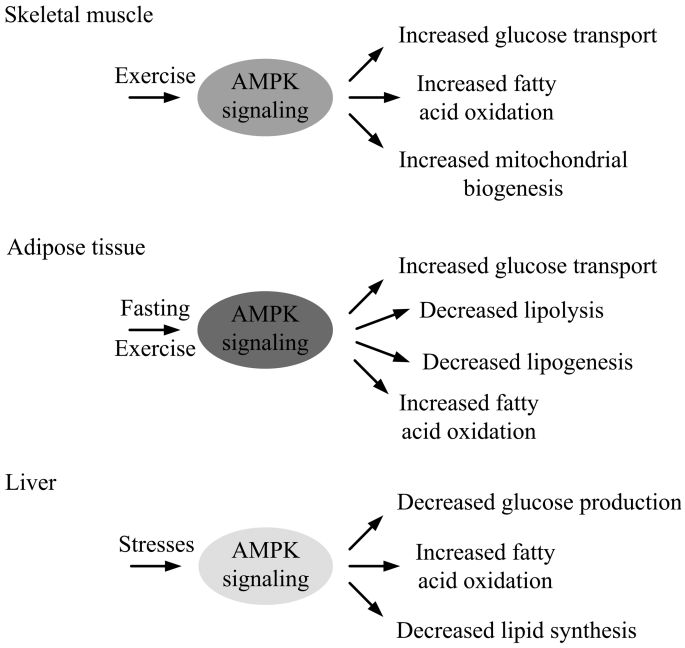

Fig. 2.6 Energy balancing by AMPK in skeletal muscle, adipose tissue, and liver

The first example shown in Fig. 2.6 is skeletal muscle, one of the principal sites of glucose and fatty acid utilization in the body. Stimulation of glucose uptake occurs through AMPK stimulation of the translocation of GLUT4 transporter to the plasma membrane. This occurs through AMPK-catalyzed phosphorylation of AS160, a Rab GTPase activating protein (GAP) involved in GLUT4-bearing vesicle transport (discussed further in Section 3.7). Insulin signaling through Akt also targets AS160, and both routes operate synergistically to promote glucose uptake.

Exercise rapidly depletes the available ATP, and AMPK signal transduction helps maintain ATP supplies in several ways one of which is the stimulation of fatty acid oxidation. This is accomplished through AMPK phosphorylation of the metabolic enzyme acetyl-CoA carboxylase (ACC). This enzyme serves as a key control point in fatty acid oxidation; it catalyzes the conversion of acetyl-CoA to malonyl-CoA, which in turn represses CPT1 activity. This enzyme is the rate-limiting enzyme for transport of long-chain fatty acids into the mitochondria where they undergo β-oxidation as discussed in the previous section. AMPK stimulates β-oxidation by phosphorylating and inhibiting ACC's ability to catalyze the conversion of acetyl-CoA to malonyl-CoA. As indicated in Fig. 2.6, increased fatty acid oxidation is one of the ways that AMPK restores energy balance in other peripheral tissues, not just skeletal muscle.

Another key operation carried out by AMPK is to inhibit protein synthesis, an intensive energy-consuming process. This is accomplished by AMPK phosphorylation of components of the tuberous sclerosis complex (TSC). This complex functions as a key signaling node involved in the signaling pathways regulating cellular growth and metabolism. By phosphorylating TSC2, AMPK prevents activation of the target of rapamycin (TOR) protein, which activates key mediators of mitochondrial biogenesis and protein synthesis. Several signals impinge on AMPK to trigger its regulation of protein synthesis. Leptin activates AMPK as does ischemia, hypoxia, and other stress conditions. In addition to these AMPK relayed signals, the TSC and TOR signaling nodes receive signals from the insulin signaling pathway. The TSC, TOR signaling node, and other key elements of this pathway will be discussed in detail as Chapter 3 where signaling from insulin and its relationship to Type 2 diabetes will be examined.

The liver has a major role in the homeostatic regulation of whole body metabolism and energy balance. As was the case for skeletal muscle, it regulates fatty acid oxidation in adipose tissue and in the liver. Overall, it integrates hormonal and nutrient signals by switching on energy-generating catabolic pathways and turning off ATP-consuming ones. AMPK does so by phosphorylating regulatory proteins and by turning on gene expression.

Metformin is the leading drug in use today against noninsulin dependent diabetes mellitus (NIDDM). Its use is especially recommended for people suffering from obesity and insulin resistance. This drug acts on the liver to reduce sugar levels in the blood and acts on peripheral tissues to increase their sensitivity to insulin. This drug exerts its protective actions in the liver through the AMPK pathway.

2.10 AMPK Is Activated by Upstream Kinases and by Depleted Energy Supply as Indicated by Increased AMP/ATP Ratios

AMP-activated protein kinases, or AMPKs, are serine/theorine kinases. They possess three subunits, a catalytic α-subunit, and a pair of regulatory subunits designated as β and γ. As depicted in Fig. 2.7,the α-subunit contains an N-terminal kinase domain and a C-terminal $\beta\gamma$-subunit-binding domain. The N-terminal domain has a residue, Thr-172, situated in the activation loop, which is phosphorylated by an upstream kinase. Two protein kinases – LKB1 and CaMKK – are capable of phosphorylating AMPK on Thr-172. The CAMKK signaling route is prominent in neurons, while the LKB1 path seems to be the important route in nonneural cells.

The γ-subunit contains a pair of sites adjacent to one another in the C-terminal region that are bound by AMP in a manner that depends on the AMP/ATP ratio. High levels of ATP, indicating plentiful energy supplies, inhibit binding and activation of the AMPK by AMP. In contrast, when energy supplies are depleted,

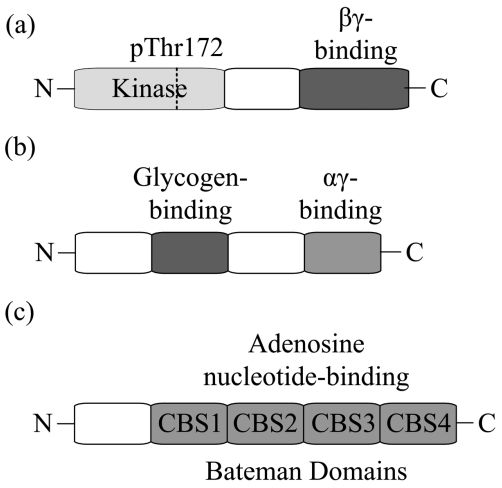

Fig. 2.7 AMPK catalytic and regulatory subunits. (**a**) α-subunit showing the arrangement of the N-terminal kinase domain and a C-terminal βγ-subunit binding domain. (**b**) β-subunit showing the presence of a glycogen-binding domain and an αγ-subunit binding domain. (**c**) γ-subunit. The N-terminal regions vary in length for the three different isoforms. These are followed by four repeats of a sequence referred to as CBS motif. The first two form an AMP/ATP-binding domain and the second pair another AMP/ATP-binding domain

resulting in a higher AMP/ATP ratio, AMP binding can occur. The selection of AMP/ATP rather than ADP/ATP renders the protein highly sensitive to small changes in available energy supply. AMP binding triggers a conformation change that enables the protein to be phosphorylated by upstream kinases such as LKB1. Once this happens the kinase is catalytically active.

The LKB1 protein forms a complex with two regulatory subunits – a kinase-like protein called STE-related adaptor (STRAD) and a scaffolding protein named mouse protein 25 (MO25). These subunits provide cytoplasmic anchorage for the kinase and serve to activate it. The tertiary complex is constitutively expressed but in the absence of AMP binding, dephosphorylation by resident protein phosphatases prevents activation of AMPK. Binding by AMP inhibits dephosphorylation by resident protein phosphatases. Eventually, dissociation of AMP from the γ-subunit leads to dephosphorylation on Thr172 within the catalytic subunit, returning AMPK to an inactive state.

The physical arrangement of the three subunits as determined through X-ray crystallography is depicted in Fig. 2.8. Shown in this figure are results obtained for *Saccharomyces pombe*. Similar findings have been reported for *S. cerevisiae* and human AMPK, but there are also differences in the details among the three AMPK complexes. Overall, one observes that the binding of AMP does not generate large and obvious conformational shift in the complex. Instead AMP binding to the γ-subunit activates the kinase though an allosteric mechanism

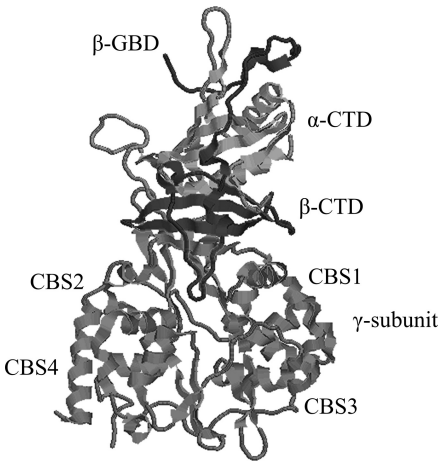

Fig. 2.8 Structure of the AMPK energy sensor determined by means of X-ray crystallography. The figure was prepared using Jmol with atomic coordinates deposited in the PDB under accession number 2OOX

involving inter-subunit interactions that increase the catalytic activities of the α-subunit and inhibit dephosphorylation. It is noteworthy that each of the three subunits is in contact with the other two.

The domain structure of the γ-subunit is quite revealing. The four repeats depicted schematically in Fig. 2.8 are labeled as cystathionine β-synthase motifs 1–4 (CBS1–4). These motifs are arranged as two mirror pairs facing one another. One pair, CBS1 and CBS3, jointly referred to as Bateman domain A faces the other pair, CBS2 and CBS4, termed Bateman domain B. These domains form a deep binding pocket that provides sites for AMP binding, $Mg^{2+}\cdot$ATP binding, and perhaps ADP binding as well. The BCS motifs are also a major site for disease-causing mutations. In these situations, nucleotide binding is disrupted and AMPK signaling is lost.

2.11 The Hypothalamic Network Provides Feedback Signals to Peripheral Tissues

The hypothalamus integrates energy and nutrient signals and based on these signals provides feedback to key peripheral tissues such as the liver. Hormones secreted by cells in pancreas, adipose tissue, stomach, and gastrointestinal tract impinge on neurons in the arcuate nucleus, which function in a sensory role relaying signals from the periphery to second and higher order neurons. Unlike most regions of the central nervous system, the blood-brain barrier of the hypothalamus is reduced so that key hormones and metabolites can pass through.

Insulin and leptin enter by means of saturable transporters; that is, by receptors that chaperone the signaling molecules across the blood-brain barrier. Ghrelin, the third member of the "big-three regulatory hormones" (insulin and

leptin being the other two), is a 28 amino reside acid peptide with an unusual, acyl modification. It too passes through the BBB by means of saturable transport. That these three key signaling molecules pass through the BBB by means of saturable transport is significant – this type of mechanism can be regulated by the BBB and most importantly the amount of hormones that passes across the BBB at high concentrations is limited. In contrast, entry by means of passive diffusion is nonsaturable, that is, it is nonlimiting. Metabolites and other peptides pass through in one of several ways. Some enter by passive diffusion as in the case of free fatty acids while others are aided by transporters as in the case of ketone bodies. Many of the signal peptides secreted by the gut (e.g., $PYY_{3\text{-}36}$) rely on passive diffusion for entry into the hypothalamus.

Two populations of neurons residing in the arcuate nucleus of the hypothalamus are the targets of insulin, leptin, and ghrelin signals. These neurons alter their expression and secretion of neuropeptides either positively or negatively in response to binding by these hormones to their receptors. One population of neurons secretes neuropeptide Y (NPY) and agouti-related protein (AgRP), which stimulate feeding, while the second releases pro-opiomelanocortin (POMC) [α-MSH], which acts opposite to the others by inhibiting feeding behavior. The core feeding circuitry is pictured in Fig. 2.9 and operates in the following manner. When energy intake minus energy expenditure is positive as is the case just following feeding, insulin and leptin satiety signals impinge on the two populations of neurons. They reduce the firing and peptide release from feeding-stimulatory NPY cells and at the same time promote the firing and peptide release from stop-feeding POMC neurons.

Ghrelin hunger signals are received by NPY neurons. The NPY neurons respond in three ways to these signals. First, the NPY neurons inhibit signaling by the POMC neurons through release of the inhibitory neurotransmitter

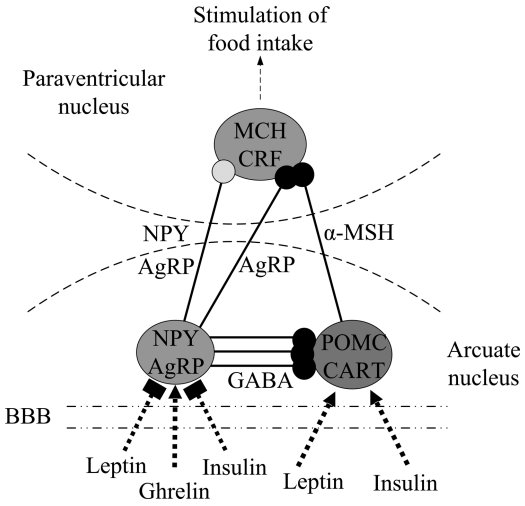

Fig. 2.9 Central hypothalamic regulatory network. (See text for details.)

γ-aminobutyric acid (GABA) at synapses between NPY and POMC. Second, ghrelin receptor activity stimulates release of NPY and AgPR peptides at second-order, MCH/CRF neurons in the paraventricular nucleus (PVN). Third, the NPY neurons send out processes that target the same postsynaptic sites that are used by the POMC neurons and inhibit the effect of α-MSH through release of AgRP. The PVN neurons receive greater input from the NPY stimulatory neurons and reduced input from inhibitory POMC neurons. The PVN neurons, in turn, send out signals that stimulate food intake.

2.12 Leptin Signaling and Regulation of Energy Balance in the Hypothalamus

The 3D structure of leptin as determined by means of X-ray crystallography is shown in Fig. 2.10. As can be seen in this figure, the protein forms four-helix bundle (HA–HD) with a fifth, short helix (HE) serving as a hydrophobic cap. The four-helix bundle is an unusual one. The helices are connected to one another by means of two long loops (L_{AB} and L_{CD}) and one short loop (L_{BC}). These loops give rise to an up-up-down-down topology for the four helices.

This tertiary organization is structurally similar to Class I hematopoietin cytokines, in particular to long-chain helical cytokines such as the pro-inflammatory cytokine IL-6 and IL-11 that signal via receptors sharing a gp130 subunit. Cytokines of this type are pleiotropic in their functions and leptin is no exception to this trend. Leptin receptors have been identified in a variety of central and peripheral tissues. In support of these varied roles, six alternatively spliced forms can be generated. These are designated as Ob-Ra through Ob-Rf. These receptors can be placed into three groups – short, long, and secreted. Short and long receptors have the

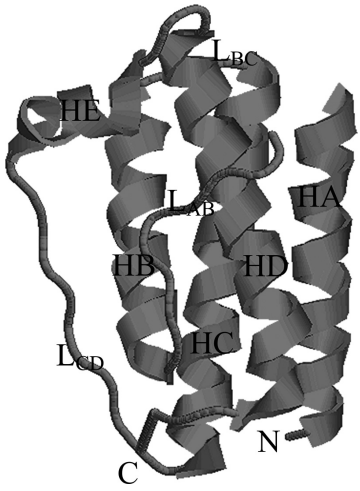

Fig. 2.10 Structure of leptin determined by means of X-ray crystallography. The figure was prepared using Jmol with atomic coordinates deposited in the PDB under accession number 1AX8

same extracellular and transmembrane domains but differ in the length of their cytoplasmic regions. Short forms such as Ob-Ra possess cytoplasmic regions of 30–40 residues while long forms such as Ob-Rb possess 300 residue-long cytoplasmic regions forming a more substantial signaling platform.

Binding of leptin to Ob-Rb, a long form of the leptin receptor, initiates leptin signal transduction leading to alterations in the mix of neuropeptides being released. As is customary for cytokine receptors, the Ob-Rb receptor signals through a Jak-STAT pathway. The Jaks are a family of nonreceptor tyrosine kinases. Members of this family, namely Jak2s, are recruited by the activated receptors, and phosphorylate the receptors at several cytoplasmic sites. These locales function as docking sites for the signal transducer and activator of transcription (STAT) proteins. The STATs, in particular, STAT3s, undergo phosphorylation by the Jaks, dimerize, and translocate to the nucleus where they function as transcription factors (Fig. 2.11). One of the genes upregulated by the STAT3s encodes SOCS3, a negative regulator of leptin signaling that operates through a feedback loop to block tyrosine phosphorylation by the Jak2s and terminate leptin signaling. The appetite-suppressing neuropeptide POMC is upregulated and the appetite-stimulating neuropeptide AgRP is downregulated, both actions supporting leptin's role as a satiety factor.

Leptin signaling is not restricted to Jak-STAT pathway alone but rather operates through multiple pathways. One of the other pathways utilized by leptin is the PI3K pathway. A sketch of this pathway has been included in Fig. 2.11. As indicated in the figure, signals proceed from PI3K to phosphodiesterase 3B (PDE3B) resulting in reduced signaling by cAMP and a corresponding reduction in expression and secretion of the stimulatory neuropeptide NPY. This leptin pathway shares a number of components with the central

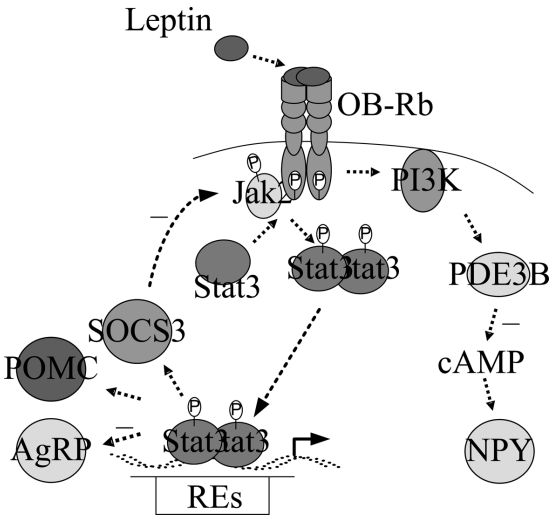

Fig. 2.11 Leptin signal transduction through the Jak-STAT system in the hypothalamus

signaling pathway used by insulin. Because of its importance not only in diabetes but also in cancer, the insulin pathway will be dissected in considerable detail in the next chapter.

2.13 Ghrelin Signaling and Regulation of Energy Balance in the Hypothalamus

Ghrelin modifies the firing rate of NPY neurons, acting via AMPK and β-oxidation-associated metabolites. This regulatory activity occurs in the following manner. Receptor activation in response to ghrelin binding leads to phosphorylation and activation of AMPK. In the next step, AMPK phosphorylates and deactivates acetyl-coenzyme A carboxylase ACC. This enzyme, as shown in Fig. 2.12, catalyzes the formation of malonyl-CoA from acetyl-CoA. Malonyl-CoA is a negative regulator of CPT1 and by deactivating ACC malonyl-CoA is not generated from acetyl-CoA. As a result CPT1 is free to transport acyl-CoA molecules into the mitochondrial matrix where it undergoes β-oxidation. The resulting energy increase supports an increased neural firing rate.

Ghrelin binds growth hormone secretagogue receptors (GHSRs) on NPY neurons in the arcuate nucleus of the hypothalamus. This receptor is a member of a small group of seven-pass receptors that exhibit a high level of constitutive activity. In particular, the GHSR exhibits an activity level in the absence of ligand binding of about 50%. When active it, like other G-protein-coupled receptors, signals via G-proteins. In its inactive form, the G-proteins consisting of a Gα and Gβγ subunits are bound and inactive. Receptor activity leads to dissociation and signaling. Recall that there are four families of Gα subunits. The GHSR utilizes the Gαq family to activate phospholipase C (PLC), which generates the second messengers diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) from phosphatidylinositol 4,5 biphosphate (PIP_2). In this case, IP_3 stimulates the release of Ca^{2+} from the intracellular stores resulting in activation of calcium-dependent serine/threonine kinases such as CaMKIV.

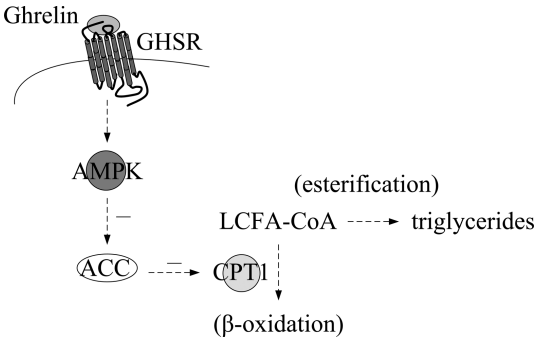

Fig. 2.12 Ghrelin signaling via AMPK to modify the firing of NPY neurons

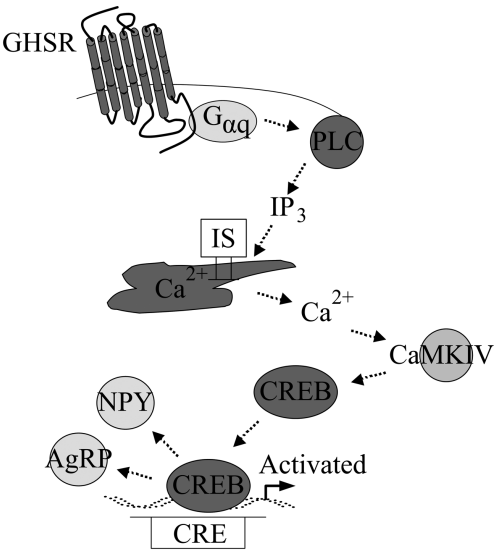

Fig. 2.13 Signaling and gene expression induced by Ghrelin

This kinase phosphorylates and activates CRE-responsive binding protein (CREB) transcription factor leading to upregulation and release of the neuropeptides NPY and AgRP (Fig. 2.13).

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