CHAPTER 10

Adipose tissue and lipid metabolism

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1. Introduction

The development of adipose tissue and the biochemistry of the adipocyte are research areas that have intrigued investigators for decades. Originally considered as simply a storage organ for triacylglycerol, interest in the biology of adipose tissue has increased substantially within the last decade, coming to the forefront in areas such as molecular genetics, endocrinology and neurobiology. Recent advances have demonstrated that the adipocyte is not a passive lipid storage depot but a dynamic cell that plays a fundamental role in energy balance and overall body homeostasis. Moreover, the fat cell functions as a sensor of lipid levels, transmitting information to a neural circuit affecting hunger, satiety and sleep.

This chapter will focus on the biochemistry of the adipocyte. Adipocytes make up approximately one-half of the cells in adipose tissue, the remainder being blood and endothelial cells, adipose precursor cells of varying degrees of differentiation, and fibroblasts. The reader is referred to excellent reviews by G. Ailhaud (1992) and P. Cornelius (1994) that focus exclusively on the differentiation process. While touching on adipose cell biology, this chapter will focus on the biochemistry of triacylglycerol metabolism.

2. Adipose development

2.1. Development of white and brown adipose tissue in vivo

The study of white adipose tissue (WAT) development in mammals has been facilitated by the use of experimental animal models. Rodents, guinea pigs, rabbits, pigs, as well as humans, have all been evaluated for the development of white adipose tissue. In general, WAT is not detected at all in mice or rats during embryogenesis, but in pigs and humans it is evident during the last third of gestation. In humans, small clusters of adipocytes are present that increase in size during gestation. Larger clusters of fat cells are associated with tissue vascularization and a general increase in cluster size is positively correlated with larger blood vessels. Paracrine/autocrine factors play a significant role in both capillary growth and adipose conversion.

After birth, sex- and site-dependent differences in fat deposits are well known in humans and several animal species. The diet plays a critical role in the degree of lipid...
filling within an adipocyte. However, controversy surrounds the question of new fat cell
development following a long-term fast. In general, starvation conditions lead to a loss
of adiposity and some apparent diminution in the number of fat cells. Refeeding restores
lipid levels and the apparent number of adipocytes. Consequently, fasting/refeeding
typically has little effect on the number of adipocytes in the body. It is generally
accepted that adipose precursor cells are present throughout life and that removal of
adipocytes, either by diet or surgical methods, will ultimately result in a restoration of
adipose levels.

In contrast to white adipose tissue, brown adipose tissue (BAT) develops during fetal
life and is morphologically and biochemically identifiable at birth. Using the uncoupling
protein 1 as a BAT marker (specific for brown adipose tissue mitochondria, see Section
3.6), brown fat development has been shown to occur maximally during the last third
of gestation. Two conditions have been shown to enhance the development of brown
fat hyperplasia in rodents: cold-acclimation and hyperphagia. Both conditions result in
a metabolic demand for high-energy expenditure, either in the form of increased heat
need or increased metabolism. Brown adipose tissue is common in rodents, camels and
hibernating animals such as bears and marmots. The oxidation of triacylglycerol stores
in brown adipose tissue depots during hibernation or fasting provides certain animals
with a source of water and energy during nutrient deprivation. In humans, although still
somewhat controversial, it is generally accepted that brown fat is not present to any
significant extent and that white adipose tissue carries out the body’s energy storage
functions.

In general it is assumed that WAT and BAT develop from different immediate
precursor cells. However, a common precursor for both cannot be ruled out and the
possible transformation of BAT into WAT has been considered. The conversion from
BAT to WAT would be correlated with a decrease in BAT-specific gene products such as
the uncoupling protein. However, the reverse does not appear to take place. That is, cold-
adapted rodents do not lose WAT and redevelop BAT in response to a low-temperature
challenge.

2.2. In situ models of adipose conversion

Ailhaud and colleagues have described the adipoblast to adipocyte conversion as a
multistep process initiating with the determination of pluripotent proliferative cells to
the adipocyte pathway (Table 1). To better characterize the differentiation process and
examine the molecular basis of adipose development, a number of murine, hamster, and
rat model cell lines (3T3-L1, 3T3-F442A, 10T1/2, Ob1771) have been established. In
general, committed preadipocytes express few markers associated with mature fat cells
and are still capable of DNA replication and cell division. Hormonal stimulation by
IGF-1 (or high concentrations of insulin), the addition glucocorticoids (e.g., dexametha-
sone), as well as a phosphodiesterase inhibitor (isobutylmethylxanthine), are frequently
used to induce terminal differentiation in culture [1]. Following differentiation the
mature adipocytes exhibit a massive triacylglycerol accumulation and are responsive to
hormonal stimulation.

For decades, brown fat metabolism has been studied with tissue explants. While
white adipose tissue is important for the storage of energy in the form of triacylglycerol, brown fat functions to dissipate energy in the form of heat through the action of a specific mitochondrial proton transporter, the uncoupling protein 1. While the 3T3-L1 or 3T3-F442A cell lines provided a convenient method to study white adipose metabolism, similar brown fat models were, until recently, lacking. However, by expressing the SV40 early genes under control of the strong fat-cell specific adipocyte fatty acid-binding protein (aP2) promoter in transgenic animals, brown fat tumors developed due to t-antigen-induced oncogenesis [2]. Such tumors were used to derive hibernoma cell lines (rapidly growing brown fat cells) exhibiting the properties of brown fat. The brown fat hibernomas express the mRNA for the uncoupling protein upon stimulation with cAMP, cAMP analogs, or a variety of β2 and β3-receptor agonists. Such cell lines have been extremely useful for the study of brown fat gene expression and metabolism.

2.3. Transcriptional control during development

To characterize the molecular basis for differential gene expression, a number of laboratories have identified transcription factors regulating genes expressed in adipocytes. Of those genes most actively studied, the adipocyte fatty acid-binding protein (aP2) gene and the insulin-stimulatable glucose transporter gene (GLUT4) have proven particularly useful. The aP2 gene is expressed in an adipose-specific manner and is up-regulated at least 50-fold as a consequence of adipose conversion [3]. The aP2 gene is regulated by glucocorticoids, insulin, and polyunsaturated fatty acids while the insulin-stimulatable glucose transporter is regulated primarily by insulin, cAMP and fatty acids [4,5]. Using these and other adipose genes as templates, three different transcription factor families have been identified as critical components of the adipocyte differentiation program.

2.3.1. C/EBP family of transcription factors

The CCAAT/enhancer-binding proteins (C/EBP) are a family of transcription factors strongly implicated in the control of genes involved in intermediary metabolism. Origi-
nally cloned by McKnight and colleagues, the C/EBPs are leucine-zipper transcription factors, a family of proteins whose sequences are characterized by the presence of a basic region followed by a leucine-rich motif. Leucine-zipper proteins are capable of forming coil–coil interactions with other similar types of factors. As such, the C/EBPs form homo- and heterodimers with other family members, thereby allowing for their binding to cis-regulatory elements within the promoter/enhancers of genes regulated by C/EBPs.

A number of genes involved in adipose lipid metabolism are regulated by the C/EBP family of transcription factors. Binding sites for the C/EBP proteins reside within the promoters of the aP2, stearoyl-CoA desaturase and insulin-stimulatable glucose transporter genes [4]. Transient transfection studies have revealed that the C/EBP sites within the promoter of the glucose transporter gene are functional and that these transcription factors play the central role in regulating the expression of the gene in differentiated adipocytes. Expression of antisense C/EBPα RNA in 3T3-L1 preadipocytes blocked the expression of C/EBPα and concomitant expression of several adipocyte genes including the insulin-stimulatable glucose transporter and adipocyte fatty acid-binding protein. Moreover, in such antisense C/EBPα-expressing cells, the accumulation of cytoplasmic triacylglycerol was blocked, suggesting that a global inhibition of genes expressing proteins of adipose lipid metabolism was occurring. Consistent with a central role for C/EBPα in lipid metabolism, mice bearing a targeted disruption in the C/EBPα allele fail to accumulate triacylglycerol in both adipose and liver.

Three members of the C/EBP family of transcription factors are expressed in adipocytes: α, β and δ. The temporal expression of the three isoforms during 3T3-L1 differentiation suggests that the C/EBP genes are subject to exquisite regulatory controls. For example, within the C/EBPα promoter resides a C/EBP binding site that suggests that C/EBPβ and/or C/EBPδ may be responsible for the activation of expression of the C/EBPα gene. In addition, insulin regulates the transcription of the C/EBPα, β, and δ genes in fully differentiated 3T3-L1 adipocytes. Insulin addition to 3T3-L1 adipocytes represses the expression of C/EBPα while inducing the expression of C/EBPβ and C/EBPδ. Furthermore, glucocorticoids reciprocally regulate expression of the C/EBPα and δ genes in 3T3-L1 adipocytes and white adipose tissue [6]. This observation may provide a mechanistic connection between the accumulation of central adipose tissue and hypercortisolemia associated with Cushing’s syndrome.

2.3.2. PPAR/RXR family of transcription factors

While the C/EBP family of transcription factors has been implicated as central to the control of gene expression in the differentiated adipocyte, a different family of DNA-binding proteins is apparently instrumental in regulating the differentiation of preadipocytes into mature fat cells. With the adipocyte fatty acid-binding protein gene as a template, Spiegelman and colleagues employed transgenic animal technology to map the region of the adipocyte fatty acid-binding protein gene necessary and sufficient to direct the expression of a chloramphenicol acetyl transferase transgene in a fat-cell-specific manner. Surprisingly, they found that while the region of DNA necessary for C/EBP action was essential for regulation in the mature adipocyte, a distinct 518
bp enhancer region some 5.4 kb upstream of the start of transcription was required for fat-cell-specific expression. By using DNA gel mobility shift analysis, an adipose-specific factor (ARF6) was identified which bound to a DNA element (ARE6) within the upstream enhancer. Importantly, the ARE6 element exhibited sequence similarity to the consensus nuclear hormone response elements. The ARE6 DNA element was similar to that which bound a heterodimer between the nuclear retinoid X receptor (specific for 9-cis retinoic acid ligands) and the peroxisome proliferator activated receptor (PPAR).

PPARs belong to a family of nuclear transcription factors that function in a ligand-dependent manner. Once bound by a ligand, the receptors heterodimerize with retinoid X receptors (RXR). They can activate transcription in target genes by recognizing and binding to specific DNA recognition elements termed peroxisome proliferator activated receptor response elements, PPRE (direct repeat of AGGTCA spaced by one or two nucleotides). This PPRE element is identical to the ARE6 DNA element originally identified by Spielgelman and his colleagues. To date, three different PPAR isoforms α, δ/β and γ, and splice variants have been identified that are encoded by separate genes [7]. The tissue-specific expression pattern of these transcription factors is indicative of their function in those tissues [8].

PPARα target genes involved in fatty acid catabolism (β and ω oxidation pathways) and is most abundant in liver although also found in kidney, heart and brown adipose tissue [8]. The δ isoform is most widely distributed, and is found in a variety of tissues including heart, kidney, brain, intestine, muscle, spleen, lung and adrenal. The γ isoform is the most highly restricted in its expression pattern with primary sources being adipose, macrophage and mammary tissue. Alternate promoter usage coupled with differential mRNA splicing result in two closely related PPARγ isoforms that differ by only 30 amino terminal amino acid residues [9]. The γ1 isoform is found in adipose tissue and to a lesser extent in liver, kidney and heart. The γ2 isoform is found almost exclusively in white adipose tissue. During the adipocyte differentiation program, the level of expression of the three different PPAR isoforms is temporally regulated. Low expression levels of PPARα in white adipose tissue as compared to brown adipose tissue suggests that its role in differentiation is a minor one.

In cultured cell lines, PPARγ, which is expressed during the late stages of adipocyte differentiation, has been shown to be the most adipogenic of the three isoforms. A balance between the expression of PPARγ and retinoic acid receptor forms (RAR and RXR) controls heterodimer formation during adipogenesis [7]. Some of the genes regulated by PPARγ in adipose tissue include adipocyte fatty acid-binding protein (aP2), lipoprotein lipase, fatty acid transport protein (FATP1), acyl-CoA synthetase, stearoyl-CoA synthetase, and phospho-enol pyruvate carboxykinase [10].

A variety of hydrophobic ligands including polyunsaturated and oxidized fatty acids have been suggested as natural ligands for PPARs. Moreover, the antidiabetic glitazone drugs are targeted towards PPARs [11]. Some eicosanoids, in particular, 15-deoxy Δ12,14-prostaglandin J2, 15 HETE and 9- and 13-hydroxyoctadecadienoic acids derived from linoleic acid (Chapter 13), have been identified as potent natural ligands for PPARγ [2]. Other arachidonic acid metabolites derived from the lipoxygenase pathway, namely 8-S-hydroxyeicosatetraenoic acid (8SHETE) and leukotriene B4 have also been suggested as ligands for PPARα [8]. The expression of PPARγ in adipose tissue
is upregulated by hormones like insulin and glucocorticoids while cytokines, tumor necrosis factor α in particular, have been shown to decrease the expression of PPARγ and C/EBPα [8].

The PPARγ activity is regulated in concert with a nuclear coactivator termed PGC-1. While PGC-1 was originally believed to function only in white and brown adipocytes, PGC-1 is also expressed in hepatocytes where it plays a primary role in controlling the expression of genes involved in gluconeogenesis.

2.3.3. SREBP family of factors
Sterol regulatory element-binding proteins (SREBPs) belong to a family of transcription factors that regulate genes involved in cholesterol and fatty acid metabolism [10]. SREBPs consist of three major isoforms: SREBP1a, 1c (ADD1) and 2. The SREBP1 gene has two alternate promoters which result in the synthesis of two proteins (SREBP1a and 1c) of different lengths. SREBP1a is a more potent activator of transcription because of its longer acidic amino terminal transactivation domain and its capacity to induce a wider range of target genes. SREBP2 is encoded by a separate gene.

SREBPs are basic helix-loop-helix leucine zipper (bHLH-LZ) DNA-binding proteins. These proteins have dual DNA specificity. They can recognize and bind to inverted repeat sequences known as E box motif (5’-CANNTG-3’) as well as direct repeat sterol regulatory elements. SREBPs contain a tyrosine residue within their basic domain which confers a conformation change within the protein allowing for the recognition of sterol regulatory elements or related sites [12].

SREBPs are unique transcription factors because they are initially synthesized as precursor membrane-bound proteins present in the endoplasmic reticulum and the nuclear envelope. Both their amino and carboxyl terminals are cytosolic. Cleavage at two specific sites within the amino terminal by proteases releases the transcription activation and DNA-binding domain which represents the mature SREBP [12]. The cleavage process is regulated by the cellular concentration of sterol which directly regulates the activity of the proteases and by a chaperone protein, SREBP-cleavage activating protein (SCAP), which is involved in trafficking the precursor SREBPs to the Golgi network for cleavage [13,14] (see Chapter 15).

All cultured cell lines express SREBP1a and 2 [12]. Liver and adipose tissue express predominantly SREBP 2 and 1c. Overexpression of SREBP1a in cultured cells and animal livers results in an increase in the expression of cholesterol and fatty acid metabolism genes. An increase in the levels of cholesterol and triacylglycerols was observed in the liver; however, there was no change in the serum levels of these metabolites. A decrease in the mass of triacylglycerols present in white adipose tissue was also noted [14].

Overexpression of SREBP1c in adipose cells led to the development of hyperglycemia, fatty liver and an increase in the levels of serum triacylglycerols while overexpression of SREBP2 led to a significant increase in the accumulation of primarily cholesterol. In mature adipocytes, insulin has been shown to upregulate the expression of SREBP1c which can then induce the expression of genes involved in lipogenesis, e.g. lipoprotein lipase and fatty acid synthetase [15] and possibly enzymes necessary for synthesis of an endogenous PPARγ ligand.
Targeted disruption of the SREBP1 gene (lack of SREBP1a and c) has resulted in negligible effects on white adipose tissue mass and function possibly due to the upregulation of SREBP2. No difference in the expression of genes involved in fatty acid catabolism or triacylglycerol synthesis was observed [10]. SREBP2 knockout mice are embryonic lethal.

3. Biochemical aspects of lipid metabolism

3.1. Lipid delivery to adipose tissue

The primary function of adipose tissue is to serve as a storage site for the excess energy derived from food consumption. This energy can then be utilized by the organism to fulfill subsequent metabolic requirements during times of little or no consumption. In the case of white adipose tissue, these requirements entail efficient storage of large amounts of energy in a form that can be mobilized readily to supply the needs of organs and tissues elsewhere in the body. Lipids, particularly fatty acids, are an exceptionally efficient fuel storage species. The highly reduced hydrocarbon tail can be readily oxidized to produce large quantities of reduced coenzymes and subsequently ATP. At the same time, the very hydrophobic nature of the fatty tail precludes concomitant storage of excess water that would increase the mass and spatial requirements of the organism considerably. Also, the relatively straight, chain-like structure of the fatty acid permits dense packing of many molecules into each cell, maximizing the use of storage space available. Brown adipose also stores energy in lipid form, but more frequently produces heat by oxidizing fatty acids within the adipocyte, rather than supplying free fatty acids for use by other cell types.

3.2. Fatty acid uptake and trafficking

At the adipose tissue beds, fatty acids are liberated from triacylglycerol-rich lipoproteins through the action of lipoprotein lipase (Chapter 20). Released fatty acids are bound by albumin and are the donors of lipid for fatty acid uptake. Two schools of thought have dominated hypotheses dealing with fatty acid uptake. First, local protonation of fatty acids due to the relative acidity at the plasma membrane, coupled with the low aqueous solubility of fatty acids at neutral pH and high permeability of fatty acids into the hydrophobic environment of the plasma membrane, creates a sufficient driving force for diffusion across the outer and inner leaflets of the membrane. A second, and drastically different viewpoint, is that there are protein cofactors called lipid transporters that facilitate the transfer process.

Several studies by Hamilton and others have demonstrated that fatty acids can diffuse through membranes at rates, and with properties, consistent with biological transport. Adsorption, flip-flop, and desorption of fatty acids at the plasma membrane functions to transfer protons across the membrane. Acidification of micelles, blood neutrophils, and hepatocytes accompanies fatty acid uptake, consistent with a diffusional mechanism and indicates that protein-mediated transfer is not necessary.
In contrast, a number of plasma membrane fatty acid-binding proteins have been identified and argued to facilitate transbilayer flux. Abumrad and colleagues have identified a murine homologue of human cell surface antigen CD36 as a lipid-binding protein involved in lipid transport and termed the protein fatty acid translocase. Fatty acid translocase is also found in muscle and cardiac myocytes and has been genetically linked to hyperlipidemia and hypertension. Lodish and co-workers have identified the fatty acid transporter family of plasma membrane proteins and used expression cloning to demonstrate fatty acid transport activity. FATP1 and FATP4, exhibit very long-chain acyl-CoA synthetase activity suggesting that esterification of very long-chain fatty acids at the plasma membrane may be coupled to uptake.

Once inside the cell, free fatty acids are minimally soluble in the aqueous cytoplasm. The charged carboxylate group provides enough electrostatic hindrance to prevent association with the neutral triacylglycerols, whereas the hydrocarbon tail reduces solubility in water. At high enough concentrations fatty acids exert a detergent-like effect that would disrupt membranes and/or they could cluster together in micelles in the crowded cytoplasm. To alleviate this problem, the adipocyte and other lipid-metabolizing cell types have evolved intracellular fatty acid-binding proteins, a family of small, soluble, highly abundant proteins that bind and sequester free fatty acids [16]. Adipocytes express two fatty acid-binding proteins, the products of the FABP4 and FABP5 genes.

The adipocyte fatty acid-binding protein (aP2) has become a paradigm for in vitro studies of protein–lipid interactions. Ligands for this protein are long-chain (>14 carbon) fatty acids and/or retinoic acid. Its small size (~15 kDa), high solubility and stability have facilitated purification and characterization of many features of the protein. Crystal structures of the adipocyte fatty acid-binding protein have been solved at high resolution for wild type and site-directed mutant forms, both in the absence and presence of bound ligands. Despite a widely varying degree of primary sequence similarity, the intracellular lipid-binding proteins as a family share a virtually superimposable tertiary structure consisting of ten antiparallel β-strands arranged in a flattened barrel [16] (Fig. 1). A single lipid ligand is bound inside the barrel within a large interior water-filled cavity, and held in place by the concerted effect of general surface contacts and specific electrostatic interactions between highly conserved cavity residues and the ligand’s polar head group.

3.3. Glucose transport and the generation of the triacylglycerol backbone

The immediate backbone precursor for acylglycerol formation is primarily glycerol 3-phosphate, derived from glycolysis or glycerol genesis within adipocytes. Fat cells express specific glucose transporters on the plasma membrane to ensure a ready supply of glycolytic intermediates for triacylglycerol synthesis. There are two types of glucose transport proteins in adipose: GLUT1 and GLUT4 [17]. Both are structurally similar with 12 membrane-spanning α-helices with intracellular amino and carboxyl termini. Both proteins are expected to have a large, hydrophilic intracellular loop separating transmembrane domains six and seven, as well as an extracellular loop containing N-glycosylation site(s) demarcated by transmembrane domains one and two. The
majority of GLUT1 has been shown by Cushman and co-workers to be present in the plasma membrane of cells unstimulated by insulin, constitutively facilitating transport of glucose down a concentration gradient. However, the bulk of insulin-stimulated glucose transport results from the activity of GLUT4. In the basal state, GLUT4 is largely found in small, intracellular vesicles, but rapidly translocates to the plasma membrane following insulin stimulation. In addition, insulin promotes a change in the rate of intracellular GLUT4 recycling and trafficking which results in a net 10- to 15-fold stimulation of hexose transport in response to insulin [11]. Once inside the cell, facilitative transport of glucose by GLUT1 and GLUT4 is rendered unidirectional by the action of hexokinase. Glucose 6-phosphate can only proceed to the glycolytic pathway because adipocytes do not express significant levels of glucose 6-phosphatase.

3.4. Fatty acid and triacylglycerol biosynthesis

Adipocytes readily convert the products of glycolysis into fatty acids via the de novo biosynthetic pathway (Chapter 6). Briefly, surplus citrate is transported from the
mitochondrion and cleaved to produce cytoplasmic acetyl-CoA. Cytoplasmic acetyl-CoA is acted upon by acetyl-CoA carboxylase that produces malonyl-CoA. The next steps of the fatty acid biosynthetic pathway are carried out by the multifunctional fatty acid synthase enzyme that utilizes NADPH to catalyze multiple condensations of malonyl-CoA with acetyl-CoA or the elongating lipid, eventually generating palmitate.

In adipocytes, two pathways exist for the production of phosphatidic acid. In one, glycerolphosphate is sequentially esterified with two acyl-CoAs to produce 1-acylglycerolphosphate and 1,2-diacylglycerolphosphate. In the second pathway, dihydroxyacetone phosphate is esterified with acyl-CoA to produce acyl dihydroxyacetone phosphate. An acyl dihydroxyacetone phosphate reductase subsequently produces 1-acylglycerolphosphate that leads to the production of 1,2-diacylglycerolphosphate. The resultant phosphatidic acid is dephosphorylated generating 1,2-diacylglycerol and triacylglycerol formed through the activity of diacylglycerol acyltransferase (DGAT).

The DGAT catalyzed reaction is crucial for it represents a branch point for hydrocarbon flow towards either the triacylglycerol or phospholipid pathways. However, this view has been modified through the production of DGAT null mice. DGAT-deficient mice are viable, synthesize triglycerols normally and are resistant to diet-induced obesity. This implies that either alternate biosynthetic pathways are being utilized or additional DGAT isoforms are present in adipose cells. To address this, Farese and colleagues have identified a second DGAT (DGAT2) with kinetic properties distinct from the original DGAT (now termed DGAT1). Moreover, additional DGAT-like sequences are present in the murine and human genome suggesting an unappreciated complexity in diacylglycerol metabolism.

### 3.5. Triacylglycerol mobilization

Lipolysis refers to the process by which triacylglycerol molecules are hydrolyzed to free fatty acids and glycerol. During times of metabolic stress (i.e. during fasting or prolonged strenuous exercise when the body’s energy needs exceed the circulating nutrient levels), the adipocyte’s triacylglycerol droplet is degraded to provide free fatty acids to be used as an energy source by other tissues. Numerous stimuli are capable of eliciting the lipolytic response in adipocytes. However, ultimately the same pair of enzymes, hormone-sensitive lipase and monoacylglycerol lipase, is responsible for catalyzing the hydrolysis of the triacylglycerol ester bonds.

Complete hydrolysis of triacylglycerol involves the breakage of three ester bonds to liberate three fatty acids and a glycerol moiety (Fig. 2). The same enzyme, hormone-sensitive lipase, is responsible for facilitating hydrolysis of the esters at positions 1 and 3 of the triacylglycerol. A second enzyme, 2-monoacylglycerol lipase, catalyzes hydrolysis of the remaining ester to yield a third free fatty acid and glycerol. Glycerol must be shuttled back to the liver for use in oxidation or gluconeogenesis. Glycerol has no alternative fate in the adipocyte; adipocytes do not express a glycerol kinase and so are unable to reuse glycerol. Glycerol is effluxed out of adipocytes via an aquaporin-type of transport molecule. Mono- and diacylglycerols can be re-esterified by the endoplasmic reticulum acyltransferases. During a lipolytic stimulus, re-esterification is thought to be minimized so that the net direction of these reactions is toward lipolysis.
Fig. 2. Schematic representation of the key steps in lipolysis. Triacylglycerol and diacylglycerol depots, surrounded by lipid-associated proteins such as perilipin, are hydrolyzed by hormone-sensitive lipase (HSL) releasing fatty acids (FA) and generating monoacylglycerol. Monoacylglycerol lipase catalyzes the last step in lipolysis formation of fatty acid and glycerol. Glycerol is released from the adipocyte while fatty acids can be bound by intracellular lipid-binding proteins (FABP) and shuttled out of the cell, to signaling depots, or to sites of re-esterification by acyl-CoA synthetase (ACS) and subsequent metabolism or regeneration of triacylglycerol. PM, plasma membrane; P, phosphorylation.

However, under maximal lipolytic conditions, substantial recycling of fatty acids occurs such that on average about two fatty acid molecules are released per glycerol molecule. Outside the adipocyte, fatty acids are immediately bound to serum albumin and carried in the bloodstream to the liver, muscle and other tissues for oxidation.

To avoid futile cycling of fatty acids (and concomitant loss of large amounts of energy), and to maintain proper energy balance between storage and expenditure, triacylglycerol synthesis and hydrolysis are carefully regulated. This regulation is present on several levels, including hormonal secretions from the endocrine system, neurotransmitter secretions from the sympathetic nervous system, intracellular G-protein-mediated signal cascades, gene expression, post-translational modification and product inhibition (Fig. 3). The proximal target of regulatory action is the enzyme responsible for initiating fatty acid mobilization: hormone-sensitive lipase (HSL).

HSL catalyzes the rate-limiting step in lipolysis and is regulated through the stimulation of β-adrenergic receptors by catecholamines or, more recently discovered, by natriuretic peptides [18] and the activation of cAMP-dependent protein kinase. HSL is an 84 kDa protein modeled to be organized into N-terminal (1–300) and C-terminal (300–767) domains. The regulatory and catalytic activity of the enzyme lies within
Fig. 3. Activation of lipolysis via adrenoreceptor-coupled systems. Binding of lipolytic agonists to β-adrenoreceptors (β1, β2 and β3) couples to the G-protein which in turn activates adenylyl cyclase (AC) thereby producing cAMP. cAMP activation of protein kinase A (cAMP PK) results in phosphorylation and activation of hormone-sensitive lipase (HSL) and perilipin and subsequent translocation of HSL to and movement of perilipin around the lipid droplet. cAMP PK also phosphorylates and activates the cGMP-inhibited cAMP phosphodiesterase (PDE), providing a feedback system to lower intracellular cAMP. α2-adrenoreceptor activation results in coupling with Gi and a decrease in adenylyl cyclase activity. Dynamic interplay between β and α2 adrenoreceptors regulates the activity of adenylyl cyclase and, therefore, cAMP PK.

the predicted α/β hydrolase fold of the C-terminal domain. Three residues (Asp703, His733 and Ser423) form the catalytic triad. Phosphorylation of the C-terminal domain results in a modest increase in specific activity of the enzyme and is correlated with a translocation of the protein from the cytoplasm to the surface of the lipid droplet.

Multiple phosphorylation sites on HSL have been identified, and its regulation by phosphorylation/dephosphorylation makes it unique among lipases. Two phosphorylation sites originally thought to be basal and regulatory sites [19], have been extensively examined. Ser565 is a basal phosphorylation site, while Ser563, once thought to be the site of activation, is now of unknown function. More recently two additional serine phosphorylation sites have been mapped and linked to translocation. Ser659 and Ser660 have been determined to be regulatory sites essential for the activation of HSL and mutation of these sites inhibits the translocation of HSL to the lipid droplet [20].

Phosphorylation and translocation of HSL to the droplet surface is thought to be functionally linked to association with one or more droplet-associated proteins. Present at the lipid droplet are a number of proteins, including perilipins, ADRP, lipotransin, and adipophilin. These proteins are thought to form a functional complex surrounding the lipid droplet, both protecting it from uncontrolled hydrolysis and assisting in amassing lipid. Phosphorylation of perilipin is correlated with hormone-stimulated lipolysis suggesting that phosphorylated perilipin has reduced affinity for droplets and dissociates from the surface concomitant with HSL association. Consistent with this
model, perilipin null mice are found to have constitutively active HSL, are hyperphagic, but maintain normal body weights, suggesting that perilipin acts as a barrier to HSL at the droplet surface. Perilipin null mice also reverse the obese phenotype when bred into db/db mice.

The N-terminal domain of HSL has recently been shown to be a docking domain for interaction with the adipocyte fatty acid-binding protein (aP2). Using a combination of yeast two hybrid analysis co-immunoprecipitation, and deletion analysis, it has been shown that the adipocyte fatty acid-binding protein forms a complex with the N-terminal domain. Moreover, the FABP stimulates the activity of HSL and relieves product inhibition by fatty acids. In experiments using aP2 null mice, lipolysis is markedly reduced while fatty acids accumulate intracellularly suggesting that the fatty acid-binding protein docks onto HSL, binds a product fatty acid and facilitates the intracellular trafficking of lipolyzed fatty acids in an efflux shuttle.

Multiple lines of HSL null mice have been developed that exhibit somewhat different characteristics. However, there are some generalizations that can be made. HSL-deficient mice exhibit reduced, but not eliminated hormone-stimulated lipolysis. This suggests that there may be additional lipases linked to hormone stimulation although compensatory mechanisms not normally associated with adipose tissue in vivo have not been ruled out. The HSL null mice are sterile due to the lack of neutral cholesterol ester hydrolase activity inherent to HSL and essential for spermatozoa production.

3.5.1. Catecholamines and adrenoreceptors in adipocytes
The catecholamines epinephrine and norepinephrine (adrenaline and noradrenaline) originate in the inner medullar region of the adrenal glands. Stimulation of the adrenal by the sympathetic nervous system leads to secretion of catecholamines into the bloodstream. In addition, adipose tissue is itself directly innervated by the sympathetic nervous system. Various types of metabolic stress trigger the sympathetic nervous system to release its neurotransmitter, norepinephrine, directly into adipose, where its effects on the adipocyte are mediated by specific plasma membrane adrenoreceptors. Rapid reflex responses are primarily stimulated by the sympathetic nervous system, whereas more long-term (i.e. on the scale of hours, days, and weeks) and/or basal effects are subject to regulation by catecholamine secretion.

The effects of catecholamines and the mechanisms that mediate them have been extensively studied in adipocytes. Adipocytes express a combination of five different adrenoreceptor isoforms: $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, and $\beta_3$ [21]. Lipolysis is signaled by $\beta$-adrenergics. An anti-lipolytic signal is transduced by the $\alpha_2$-adrenergics, and the $\alpha_1$-adrenergics are involved in a separate pathway. In short, although lipolysis is the observed outcome of catecholamine stimulation, it is the steady state result of competition between two opposing pathways triggered by the same signal.

The mechanisms of signal transduction are reasonably well known. Binding of catecholamines to the $\beta$-adrenoreceptors activates adenyl cyclase via a stimulatory G-protein (Gs) (Fig. 3). Adenyl cyclase catalyzes the conversion of ATP to cAMP. cAMP binds the regulatory subunit of protein kinase A, releasing the active catalytic subunit. Active protein kinase A in turn phosphorylates the HSL and perilipins which increase translocation to and from the triacylglycerol droplet, respectively. The same signal
bound to the $\alpha_2$-adrenoreceptor affects an inhibitory G-protein ($G_i$), which inhibits the activity of adenylyl cyclase. Disappearance of cAMP eventually causes cAMP to dissociate from the regulatory subunit of protein kinase A, which then inactivates the catalytic subunit by reassociation. In the absence of continued phosphorylation, dephosphorylation mediates reverse translocation of HSL and perilipins to the basal state.

With simultaneous activation of opposing pathways, the relative contribution of each receptor type becomes very important. Small mammals, such as rats and hamsters, express mainly $\beta_1$ and $\beta_3$ while rats express very little of the $\alpha_2$ isotype. Large mammals (e.g., humans and monkeys) express almost exclusively $\beta_1$ and $\beta_2$ and a significant amount of $\alpha_2$ receptor. It appears that the $\beta_3$ receptor is expressed to a greater extent in brown adipocytes than in white adipocytes.

A second observed pattern of receptor regulation was demonstrated by the use of agonists and antagonists for each receptor isotype. At very low agonist concentrations, only $\alpha_2$-receptor activity is observed (i.e., anti-lipolysis). As the agonist concentration is increased, $\beta_1$ becomes active and initiates lipolysis. Only under much more stimulatory agonist conditions do $\beta_3$ receptors become active. $\beta_2$, in animals that express it, seems to be active under conditions more similar to $\beta_1$. Affinity for ligands and level of expression of receptors are two methods utilized by adipocytes to regulate catecholamine effects. The interplay between the various isotypes is responsible for the adrenergic balance of lipolysis and anti-lipolysis. In general, $\alpha_2$-mediated anti-lipolysis modulates resting adipocyte activity, whereas during stress-induced norepinephrine release, increased binding to the $\beta$-adrenergics overcomes the $\alpha_2$ inhibitory effect and $\beta$-mediated lipolysis prevails.

3.5.2. Glucagon

Although catecholamines are perhaps the strongest physiological lipolytic stimulus, other hormones also play an important role in mediating energy balance. One such hormone is glucagon that is one of three polypeptide hormones secreted by endocrine tissues located within the pancreas. Glucagon is secreted into the circulation in response to low blood glucose levels and the result of its action is mobilization of stored energy.

Stimulation by glucagon takes place by a virtually identical pathway to stimulation by catecholamines. Glucagon binds extracellularly to a specific seven-transmembrane-domain receptor, activating adenylyl cyclase via a stimulatory G-protein. Protein kinase A is subsequently activated and phosphorylates HSL, which begins to hydrolyze triacylglycerol stores. Protein kinase A also phosphorylates (and activates) enzymes in the glycogen degradation pathway, and inhibits de novo fatty acid synthesis by phosphorylation of acetyl-CoA carboxylase in concert with AMP-activated protein kinase (Chapter 6).

Because the same regulatory pathway is activated, the same feedback mechanisms used to modulate chronic catecholamine effects are equally significant for prolonged glucagon stimulation. At some level of cAMP production, the cAMP response element-binding protein transcription factors become phosphorylated by protein kinase A and upregulate cAMP-responsive gene expression leading to increased receptor expression.
However, protein kinase A phosphorylation of the cell surface receptors leads to uncoupled G-protein activity and heterologous desensitization to both the glucagon and catecholamine signals. It is also relevant that protein kinase A can phosphorylate and thereby activate cGMP-inhibited phosphodiesterase [22], which cleaves cAMP and probably helps modulate its effects to minimize desensitization.

3.5.3. Steroid and thyroid hormone
In addition to the major metabolic regulators in adipocytes (catecholamines, glucagon, and insulin), many diverse types of hormones have effects on adipocyte metabolism. The most notable results are effected by glucocorticoids, sex steroids, and thyroid hormones.

Glucocorticoids are steroid hormones secreted by the adrenal cortex in response to stress or starvation. Glucocorticoids display a permissive effect on lipolysis stimulated by catecholamines. Glucocorticoid response elements have been observed in the upstream regions of \( \beta_1 \) and \( \beta_2 \) adrenoreceptor genes and, in fact, an increase in numbers of expressed \( \beta \)-receptors in response to glucocorticoids has been reported. Glucocorticoid response elements have also been identified in the upstream regions of the C/EBP family of transcription factors. Additionally, the activity of the stimulatory G-protein is enhanced by glucocorticoids. These effects are consistent with the finding that adrenalectomy reduces G protein and mRNA levels, and that subsequent administration of a glucocorticoid such as dexamethasone can restore those levels. Glucocorticoids, therefore, probably ensure maintenance of catecholamine-induced lipolysis by enhancing transcription of the genes involved in that signal cascade.

Sex steroids (primarily estrogen in females, which is synthesized by the ovaries, and testosterone in males, synthesized by the testes) like glucocorticoids, also affect gene transcription by binding to nuclear Zn-finger transcription factors that recognize steroid response elements. In female rats, ovariectomy was shown to diminish lipolysis by decreasing the effectiveness of the adenylyl cyclase catalytic activity. Lipolysis was restored to normal levels in these animals by administration of estrogen but not progesterone. Castrated male rats exhibited decreased lipolysis that appeared to be caused both by defective adenylyl cyclase catalysis and a decreased number of \( \beta \)-adrenergic receptors, again implying desensitization to catecholamines. Normal lipolytic levels could be restored by administration of testosterone.

The circulating thyroid hormones thyroxine and its more potent derivative triiodothyronine are secreted from the thyroid gland in response to hypothalamus/pituitary stimuli. The effect of elevated thyroid hormone is increased lipolysis, which appears to be mediated by an increase in \( \beta_1/\beta_2 \)-adrenergic receptor expression and a decrease in inhibitory G-protein expression. These alterations effectively sensitize the adipocyte to catecholamine stimulation.

3.5.4. Insulin and anti-lipolysis
Pancreatic \( \beta \) cells secrete the polypeptide hormone insulin in response to elevated blood glucose levels (hyperglycemia). Insulin is the most important physiological stimulus for energy storage. Its effect directly counteracts the effects of glucagon and the catecholamines. Insulin receptors are found in many diverse cell and tissue types, not the least significant of which is adipose.
The insulin receptor is an integral membrane protein that functions as a tetramer composed of two $\alpha$ and two $\beta$ subunits. The $\beta$ subunits each span the plasma membrane once, and the $\alpha$ subunits are covalently attached to the $\beta$ subunit extracellularly by disulfide bonds. The insulin binding site is external. The intracellular domains contain many tyrosine phosphorylation sites and the receptor is itself a tyrosine kinase. Ligand-binding induces autophosphorylation of several intracellular domains, activating the kinase activity of each $\beta$ subunit. A complex series of interactions follows in which the insulin receptor phosphorylates some of its substrates directly (insulin receptor substrate-1, IRS-2) or recruits various adaptor proteins such as Shc and Grb2 that transmit the insulin signal [23].

Insulin-binding to the adipocyte insulin receptor simultaneously stimulates lipogenesis and inhibits lipolysis. Insulin action effectively clears fatty acids and glucose from the blood both by increasing uptake and storage, and by decreasing mobilization of stored energy. The mechanisms by which these effects are accomplished are highly complex and have not been entirely elucidated although some aspects of the process are clear. The insulin receptor tyrosine kinase is capable of inducing phosphorylation and activation of the cGMP-inhibited phosphodiesterase and several protein serine-phosphatases (most likely protein phosphatases 1, 2A and 2C). Phosphatidyl inositol 3-kinase has been demonstrated as an essential component in the insulin-stimulated activation of the cGMP-inhibited phosphodiesterase but, as previously mentioned, protein kinase A can also fulfill this role in the absence of insulin. Thus, insulin inhibits the cAMP cascade (including activation of hormone-sensitive lipase) through cleavage of cAMP and direct dephosphorylation of protein kinase A-activated substrates. Dephosphorylation also activates acetyl-CoA carboxylase, the enzyme that catalyzes the first committed step in de novo fatty acid synthesis, and fatty acyl-CoA synthetase, the first enzyme in the triacylglycerol synthetic pathway. Glucose transport is stimulated via GLUT4 translocation to the plasma membrane, and lipoprotein lipase secretion (and, therefore, fatty acid uptake) is enhanced. In addition, insulin reduces dramatically the number of cell surface $\beta$-adrenergic receptors, which further desensitizes the adipocyte to lipolytic stimuli.

The concerted insulin-induced actions of fatty acid/glucose uptake and triacylglycerol synthesis reduce blood glucose. Eventually the diminished glucose levels signal the pancreas to stop secreting insulin and initiate secretion of glucagon. Intermediate stress such as fright or strenuous exercise is capable of stimulating lipolysis via sympathetic nervous system secretion of norepinephrine. In well fed, resting adipocytes, insulin effects are supported and strengthened by the anti-lipolytic action of $\alpha_2$-adrenergics. Additional anti-lipolytic influence is exerted by adenosine and certain prostaglandins (notably prostaglandin E2 in mature adipocytes and prostacyclin in preadipocytes). Adenosine effects are modulated through the A1 adenosine receptor, a member of the family of purinergic receptors identified in various tissues. Prostaglandins also bind specific cell surface receptors (Chapter 13). Both types of receptors are known to act through an inhibitory G-protein, and so transduce a signal similar to insulin. Thus, the delicate balance between energy storage and mobilization is ensured by complex (and differential) interplay of many regulatory systems and factors.
Table 2

Comparison of major features of white and brown adipose tissue

<table>
<thead>
<tr>
<th>Major feature</th>
<th>White adipose</th>
<th>Brown adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascularization</td>
<td>Some, limited</td>
<td>Extensive</td>
</tr>
<tr>
<td>Distribution</td>
<td>Extensive, many sites</td>
<td>Restricted</td>
</tr>
<tr>
<td>Sympathetic innervation</td>
<td>Some, limited</td>
<td>Extensive</td>
</tr>
<tr>
<td>Fatty acid role(s)</td>
<td>Synthesis, storage, signaling</td>
<td>Storage, oxidation, signaling</td>
</tr>
<tr>
<td>Uncoupling protein</td>
<td>Present</td>
<td>Highly expressed</td>
</tr>
<tr>
<td>Thermogenesis</td>
<td>Present</td>
<td>Highly developed</td>
</tr>
<tr>
<td>Insulin effects</td>
<td>Extensive</td>
<td>Extensive</td>
</tr>
<tr>
<td>Adrenoreceptors</td>
<td>Primarily α3, β1, β2, β3</td>
<td>Primarily α1, β1, β3</td>
</tr>
<tr>
<td>Droplet size</td>
<td>Large, single</td>
<td>Small, multiple</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Few</td>
<td>Many, densely packed</td>
</tr>
</tbody>
</table>

3.6. Brown fat lipid metabolism

White and brown adipocytes are obviously labeled as a result of their difference in color. The variation in appearance between these tissues is a direct reflection of the very different role each performs in the organism and results from specific morphological differences on a cellular level. Whereas the purpose of white fat is to store and release energy in the form of free fatty acids, the essential function executed by brown fat is the expenditure of fatty acid-derived energy for maintenance of the organism’s thermal stability (Table 2).

Brown fat derives its color from extensive vascularization and the presence of many densely packed mitochondria (due to the heme cofactors in the mitochondrial enzyme cytochrome oxidase). Brown fat is traversed by many more blood vessels than is white fat. These blood vessels assist in delivering fuel for storage and oxidation, and in dispersing heat generated by the numerous mitochondria to other parts of the body. Brown adipocytes differ in appearance from white adipocytes by the presence of many small triacylglycerol droplets, as opposed to a single large droplet (i.e. multilocular, rather than unilocular). Regulation of brown fat activity is accomplished primarily through the action of the sympathetic nervous system. The blood vessels and each individual brown adipocyte are directly innervated by sympathetic nervous system nerve endings that exert control by release of norepinephrine. Stimulation by the sympathetic nervous system in response to external temperature decrease is essential to the maintenance of brown fat function, and atrophy occurs when regular sympathetic nervous system activity declines [24].

Brown adipocytes also differ from white adipocytes at the molecular level. The major adrenergic receptor subtype expressed by brown adipocytes is the β3, but α1 and β1 are also found. The most notable difference between brown and white adipocytes is the production of uncoupling protein by the former and its relative absence in the latter [21,24]. Brown adipocytes also express a type II 5'-deiodinase enzyme, which converts the thyroid hormone thyroxine to its more potent form, triiodothyronine, and are capable of secreting triiodothyronine into circulation.

Uncoupling proteins confer to the brown adipocyte the ability to catabolize fatty acids
Fig. 4. Thermogenesis in the brown fat mitochondrion. The major fuel pathways of brown fat are represented. Triacylglycerol levels are balanced by the processes of lipogenesis (1) and lipolysis (2). When excess fuel is present, or when heat is needed, fatty acids produced by lipolysis are activated with CoA and transferred to the mitochondrion. Via β-oxidation (3), the long-chain fatty acids are degraded to acetyl-CoA and reduced coenzymes (NADH and FADH₂). The coenzymes transfer their reducing equivalents across the mitochondrial inner membrane (4) against the concentration gradient. Typically the proton gradient is dissipated by the action of the proton-ATPase (5) which uses the energy to drive ATP synthesis. However, brown fat mitochondria possess the uncoupling protein (6), which allows for proton transport across the membrane, down the concentration gradient, with the change in free energy lost as heat. The uncoupling protein is positively regulated by fatty acids and inhibited by purines.

inefficiently (that is, without the usual concomitant ATP production) and dissipate the heat generated by this excessive catabolic activity to other tissues via the bloodstream (Fig. 4). This process is known as thermogenesis and is characterized on two levels. Obligatory thermogenesis occurs in all cell types as the result of ubiquitous nominal inefficiencies in metabolism. Facultative thermogenesis occurs specifically in response to stimuli such as cold (non-shivering thermogenesis in adipose, shivering thermogenesis in muscle) or overfeeding (diet-induced thermogenesis). Facultative thermogenesis, particularly non-shivering thermogenesis, is the specific role of brown adipose. It should be noted that uncoupling protein isoforms are expressed ubiquitously throughout the body suggesting that thermogenesis and/or proton dissipation are common regulatory themes.

3.6.1. Triacylglycerol synthesis and storage
Brown adipose tissue, in its cold/epinephrine-activated state (as opposed to an atrophied or quiescent state), demonstrates increases in blood flow, lipoprotein lipase activity, triacylglycerol synthesis, 5'-deiodinase activity, and triiodothyronine-enhanced uncoupling protein gene expression. The processes of fatty acid uptake and triacylglycerol synthesis are essentially the same in both brown and white fat. However, norepinephrine release by the sympathetic nervous system in acute cold exposure stimulates brown adipose tissue to enhance expression and secretion of lipoprotein lipase to its sites in the vascular
epithelium. Lipoprotein lipase releases fatty acids from passing chylomicrons and very low-density lipoprotein (Chapter 20), causing an influx of fatty acids into the brown adipocytes.

Free fatty acids and norepinephrine inhibit acetyl-CoA carboxylase. Increased fatty acid uptake, lipolysis and esterification occur simultaneously in brown adipose tissue. BAT fatty acyl-CoA synthetase and acyltransferases, associated primarily with the endoplasmic reticulum, catalyze triacylglycerol formation as in white adipose. Triacylglycerol synthesis is decreased during fasting, and increases sharply in an insulin- and norepinephrine-dependent fashion, upon refeeding. Probably the increased triacylglycerol synthesis is required by active brown adipocytes to accommodate the enhanced fuel influx, which is in turn required for thermogenesis. Concomitant synthesis of triacylglycerol and degradation of fatty acids probably constitutes a futile process that is itself thermogenic.

3.6.2. Fatty acid oxidation, bioenergetics and thermogenesis

Fatty acids utilized by BAT for thermogenesis are derived from several sources including dietary triacylglycerol (via chylomicrons), very low-density lipoprotein triacylglycerol from the liver, free fatty acids from white adipose bound to circulating albumin, hydrolysis of internal acyl-CoA molecules, and hydrolysis of internal triacylglycerol stores by HSL. In fact, the capacity of BAT for lipolysis actually exceeds its capacity for thermogenesis, such that it becomes an exporter of fatty acids at very high norepinephrine concentrations. Norepinephrine stimulates HSL via β₁ and β₃ adrenoreceptors as described for white adipose (Section 3.5.1). Increased synthesis of thyroxine 5'-deiodinase, responsible for increased levels of triiodothyronine and uncoupling protein, is mediated by α₁ adrenoreceptors [21,24].

Non-shivering thermogenesis is induced by heat loss when the temperature of the environment is significantly below the temperature of the organism. Thermogenesis can be suppressed by fever, exercise, and environmental temperatures similar to body temperature. Heat generation occurs through the dissipative relaxation of the proton gradient independent of ATP production (Fig. 4). The uncoupling protein facilitates proton movement down a concentration gradient across the mitochondrial membrane without simultaneous production of ATP by the proton-dependent ATP synthetase. Since the rate of ATP synthesis is usually the limiting factor of respiration and is dependent on utilization of energy from proton movement along the gradient, dissipation of the gradient by uncoupling protein uncouples oxidation from its rate limitations. Unlimited oxidation produces the large amounts of heat that are distributed by brown adipose during thermal distress.

The original uncoupling protein (termed UCP1) was discovered nearly 25 years ago, and is now recognized to be one member of a burgeoning UCP multigene family. The proteins are found in several tissues and across species. UCP1 is expressed predominantly in brown fat and is a ~306 amino acid (~33 kDa) protein predicted to span the inner mitochondrial membrane several times, projecting its C-terminus into the intermembrane space [25]. Its activity is regulated by free fatty acids, which interact with the protein in the membrane and probably lower the membrane potential for proton translocation, facilitating gradient dissipation. Uncoupling protein also has
a highly pH-dependent C-terminal purine nucleotide binding site that may serve as a regulator of the protein’s activity as well. Small changes in pH drastically affect ADP- and ATP-binding to this site, and ADP/ATP-binding has been shown to inhibit proton translocation in reconstituted phospholipid vesicles.

The oxidative fuel for thermogenesis is exclusively fatty acids even if glucose is available. This is interesting because insulin facilitates uptake of large amounts of glucose during thermogenesis — much more than the cell requires for synthesis of glycerol backbones. During thermogenesis, norepinephrine activates key regulated glycolytic enzymes such as phosphofructokinase and pyruvate dehydrogenase, thus upregulating glycolysis as well as fatty acid oxidation. It has been postulated that upregulation of glycolysis may be essential for ATP production by substrate-level phosphorylation. Since ATP synthesis is uncoupled from oxidation, the cell’s ATP requirements must be met another way. In addition, the cell continues to utilize large amounts of reduced cofactors to produce heat. These too can be replenished by an elevated glycolytic rate.

The transcription of uncoupling protein mRNA is upregulated by norepinephrine activation of adrenoreceptors and increases in cAMP. This upregulation can be enhanced by the presence of triiodothyronine and abolished if the deiodinase activity of the cell is inhibited. BAT has a nuclear receptor for triiodothyronine that functions as a transcription factor, and probably binds upstream of the uncoupling protein gene to activate transcription. The presence of both thyroid response elements and cAMP response elements is likely to be required.

4. Molecular cell biology of adipose tissue

4.1. Energy balance and basal metabolic rate

The balance of food intake and energy expenditure is critical for survival. Each organism represents a unique energy equation based upon its feeding habits, exercise patterns, body composition, and environmental conditions. The net result of the organism’s solution to this equation determines its basal metabolic rate (BMR), which is defined in the laboratory setting as the output of a metabolite per unit time, measured at rest after an overnight fast.

Intake and storage of fatty acids must be counterbalanced by an equivalent expenditure of stored energy to maintain constant body mass. The capacity of an organism for expenditure is indexed by its BMR. So, what factors determine individual BMR? Prolonged exposure to harsh environmental conditions, such as extreme cold, leads to an elevated BMR in rodents via thermogenesis and dissipation of heat by brown adipose. Starvation or semi-starvation lowers BMR while regular strenuous and/or prolonged exercise enhances BMR. Resting muscle metabolizes primarily fatty acids, so lean body mass enhances BMR in the fed state. Obesity also elevates BMR due to the grossly increased lipolytic rates observed for an enlarged, excessively proliferated adipose tissue. However, the increased lipolysis is nonetheless overbalanced by consumption in this syndrome. Also, adipocytes of obese individuals proliferate more readily in culture than
adipocytes derived from lean individuals, suggesting fat tissue may form more easily in obesity. Increased body mass in the absence of a concomitant increase in fat-free (i.e. metabolically active) mass does not enhance BMR, since futile cycling of fatty acids between the triacylglycerol-esterified and non-esterified states is energetically not very costly. The distribution of fat, however, is a relevant factor for BMR. Sex hormones, for example, tend to direct proliferation of abdominal (visceral) adipocytes in males, but preferentially direct deposition of adipose to the lower body (gluteal–femoral region) of females. Visceral adipose displays inherently reduced sensitivity to the anti-lipolytic effects of insulin and, therefore, elevated lipolysis that contributes to higher BMR, whereas lower body adipose tends to the opposite. In addition, female adipocytes produce and secrete estrogen, which stimulates further production of preadipocytes, amplifying the estrogen effect on adipose deposition. The net effect, as in obesity, is an increased body cell mass independent of fat-free mass and a decreased BMR in females relative to males of comparable mass.

4.2. The hypothalamus-adipocyte circuit and the ob gene

In lean individuals, energy balance is maintained by equilibrium between consumption and expenditure. The central control for this complex mechanism is localized in the brain, and specifically to the hypothalamus. The idea that fat cell-derived secreted proteins were responsible for regulating energy intake was first proposed in the 1950s. Studies of mouse models for obesity led to the hypothesis that the adipocyte was capable of sensing when its stores were replete and transmitting a signal to that effect. The theory was expanded as several secreted products have been identified that link the biology of the fat cell to energy homeostasis (Fig. 5). The most notable of these products is leptin, the product of the \textit{ob} gene.

There are five mouse models of obesity identified as resulting from single gene mutations (Table 3). Of these five, \textit{ob}, and its protein product leptin, have received the most attention. Administration of leptin to obese \textit{ob/ob} mice halts hyperphagia and reduces adiposity without dramatically adverse effects on overall body homeostasis [27]. This adipocyte-derived hormone circulates at levels that are proportional to the body fat content and has been extensively investigated for its role in regulation of body fat stores, food intake, satiety and energy expenditure. As part of its mechanism of action, leptin traverses the blood–brain barrier into cerebrospinal fluid and binds to

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Allele</th>
<th>Protein</th>
<th>Physiological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal yellow</td>
<td>Ay</td>
<td>Agouti</td>
<td>Antagonist of melanocortin receptors</td>
</tr>
<tr>
<td>Obese</td>
<td>ob</td>
<td>Leptin</td>
<td>Hormone — regulates food intake</td>
</tr>
<tr>
<td>Diabetes</td>
<td>db</td>
<td>Leptin receptor</td>
<td>Expressed in brain and peripheral tissues — binds leptin</td>
</tr>
<tr>
<td>Fat</td>
<td>fat</td>
<td>Carboxypeptidase E</td>
<td>Processing and sorting neuropeptides and prohormones</td>
</tr>
<tr>
<td>Tubby</td>
<td>tub</td>
<td>Tubby protein</td>
<td>Possible transcription factor in hypothalamus</td>
</tr>
</tbody>
</table>
receptors (products of the \textit{db} gene) on the hypothalamus, thereby controlling feeding behavior. Although the brain expresses the highest number of leptin receptors there are also leptin receptors ubiquitously expressed in peripheral tissues like adipose tissue [28] mammary gland, ovarian follicles, placenta and bone [29]. Therefore, fluctuations in leptin levels not only affect metabolism as a whole but also have a wider impact on reproduction, bone formation and the immune system. Leptin receptors are members of the class I cytokine receptor family and are linked to JAK-STAT signaling systems [28].

The short-term regulation of leptin expression is influenced by a number of factors that regulate C/EBP\(\alpha\) and PPAR\(\gamma\) activity on the leptin promoter. Consumption of a high fat diet, insulin, glucose and glucocorticoids induce \textit{ob} expression [29], while fasting, cold exposure, \(\beta_3\) agonists, catecholamine stimulation and thiazolidinediones all decrease leptin expression [28,29]. Leptin works to suppress appetite by affecting the synthesis and/or action of a complex system of neuropeptides including neuropeptide \(Y\), MCH, POMC, alpha-MSH, all of which directly or indirectly influence food intake.

Insulin is an additional factor that has been implicated in energy homeostasis. Insulin, although an anabolic hormone when acting directly upon liver, muscle, and adipose, mediates a completely opposite effect via the brain. Ventromedial hypothalamic lesions in rodents are associated with obesity and hyperphagia [26]. Administration of insulin directly to the ventromedial hypothalamus of rats has been shown to cease feeding and
initiate weight loss, effects that disappeared when insulin was removed. In addition, feeding was stimulated by direct administration of anti-insulin antibodies to this region. Moreover, the presence of insulin receptors in the hypothalamus and correlation of plasma insulin levels to body mass provide additional support for the putative involvement of insulin in regulation of food intake and body fat stores. One possible vehicle for this proposed insulin-signaling mechanism is the hypothalamic neurotransmitter neuropeptide Y. Administration of neuropeptide Y directly to the hypothalamus strongly stimulates feeding, an effect opposite from that of insulin. Synthesis and release of neuropeptide Y are known to be stimulated by caloric deprivation and there is evidence that insulin may reduce neuropeptide Y mRNA levels. However, it is evident that insulin, whose effects are widespread in various tissues, is not specific enough to solely exert appetite control on the time scale in which satiety and cessation of feeding occur.

4.3. Cytokine control of adipose lipid metabolism

Adipocytes have profound sensitivity to tumor necrosis factor-α (TNF-α), interferons α, β and γ, and interleukins 1, 6 and 11 [30] (Fig. 5). In general, such cytokines inhibit lipogenesis and triacylglycerol storage by adipocytes, activate lipolysis and antagonize insulin. Additionally, many cytokines interfere with proliferation of preadipocytes in 3T3-L1 or 3T3-F442A cell lines and/or diminish adipogenesis in vivo.

TNF-α is expressed at high levels in adipose tissue derived from several genetically defined rodent models of obesity and insulin resistance. In db/db obese mice, TNF-α expression is elevated but not the expression of other cytokines such as interleukin-1 or interleukin-6. Measurements of insulin receptor tyrosine kinase activity in obese fa/fa rats shows a reduced function, which could be restored by administering soluble TNF-α receptor to sequester the secreted TNF-α protein. TNF-α interferes with insulin signaling by affecting the phosphorylation of the insulin receptor, thus contributing to the development of insulin resistance in these animals. In cultured 3T3L1 cells, TNF-α inhibits the expression of C/EBPα and PPARγ, thus leading to reduced expression of genes that are involved in triacylglycerol accumulation and metabolism. Interleukin-6 is secreted by white adipose tissue under basal conditions and its expression is increased by TNF-α and by other conditions linked to wasting disorders (cancer, cachexia, HIV).

TNF-α and the interferons decrease lipogenesis, down-regulating the mRNA levels of the key lipogenic enzymes acetyl-CoA carboxylase and fatty acid synthase; the interferons diminish mRNA levels of fatty acid synthase but not of acetyl-CoA carboxylase. TNF-α, interleukin-1, and some interferons also increase lipolysis, although the mechanism is probably post-transcriptional, since Northern blot analysis shows that TNF-α and the interferons decrease the level of HSL mRNA [30].

The adipocyte is involved in expressing and secreting a diverse range of factors (Table 4) which have a direct impact on adipocyte metabolism. These biomolecules have varied physiological functions in other target tissues thus placing the adipocyte as a central player in regulating energy homeostasis overall.
Table 4
Summary of several adipocyte secreted factors

<table>
<thead>
<tr>
<th>Adipose secreted factor</th>
<th>Expression in the obese state</th>
<th>Function in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylation stimulating protein (ASP)</td>
<td>Decreased</td>
<td>Stimulates triacylglycerol synthesis</td>
</tr>
<tr>
<td>Adiponectin/AdipoQ/Acrp 30</td>
<td>Decreased</td>
<td>Associated with insulin resistance</td>
</tr>
<tr>
<td>Adipsin</td>
<td>Decreased</td>
<td>Activation of alternative complement pathway</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>Increased</td>
<td>Regulates blood pressure</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>Increased</td>
<td>Proliferation/mediates effects of growth hormone</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Increased</td>
<td>Immune response/glucose and lipid metabolism</td>
</tr>
<tr>
<td>Leptin</td>
<td>Increased</td>
<td>Energy expenditure, reproduction satiety factor</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor</td>
<td>Increased</td>
<td>Cardiovascular function/wound healing</td>
</tr>
<tr>
<td>Prostaglandin E₂</td>
<td>Increased</td>
<td>Antilipolytic, suppresses cAMP production</td>
</tr>
<tr>
<td>Prostaglandin F₂</td>
<td>Increased</td>
<td>Inhibits adipogenesis</td>
</tr>
<tr>
<td>Prostaglandin I</td>
<td>Increased</td>
<td>Adipogenic in preadipocytes</td>
</tr>
<tr>
<td>Transforming growth factor β</td>
<td>Increased</td>
<td>Involved in proliferation, differentiation and apoptosis</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>Increased</td>
<td>Contributes to insulin resistance and type 2 diabetes</td>
</tr>
</tbody>
</table>

5. Future directions

Obesity is an increasing worldwide public health concern. In the United States, six out of ten adults are overweight according to a recent National Health and Nutrition survey and approximately, 300,000 deaths annually are a direct result of obesity or complications that result from the disease. While obesity may seem on the surface to simply be the excess consumption of food compared to energy expenditure, it is a complex metabolic disorder centered on adipose lipid metabolism and cellular signaling systems linked to it. Adipocytes are now generally accepted to be a complex cell type involved in generating a number of signals which include cytokines, hormones and growth factors that not only affect itself and the neighboring cells but also impact target tissues involved in energy metabolism. When Albert Lehninger authored the second edition of his famous text Biochemistry in 1975, the topic of adipose tissue was given one and a half thin pages while discussions of liver metabolism occupied four such pages. That was not too bad considering that discussions of the metabolic functions of skeletal muscle or brain were given less than one page. At the time, Lehninger’s text was the most common entry point for students interested in the topic of adipose metabolism. In the last two decades our appreciation and awareness of adipocytes as a dynamic cell type with connections to both the endocrine and nervous systems has increased dramatically. As described within, adipocytes play the central role in maintenance of the energy balance. As such, pathophysiologic conditions such as obesity and non-insulin-dependent diabetes mellitus, two of the most common disease states of the Western Hemisphere, bring adipose tissue to center stage.

Over the next decade, more transgenic and knockout mouse models will be developed to test hypotheses concerning regulation of adipose triacylglycerol metabolism (L. Chan, 2000; C. Londos, 2001) and its relationship to the overall energy balance. In addition, known genetic alleles linked to control of lipid synthesis will be explored in detail.
providing additional insights into control points affecting lipid storage and oxidation (K. Reue, 1999). The combination of natural variants and laboratory model systems provides a wealth of experimental systems designed to explore lipid metabolism. Adipocytes will continue to be a favorite experimental system for the analysis of hormone action. The major effects of insulin on fat-cell metabolism have made adipocytes the system of choice for probing the mechanistic basis of insulin-stimulated glucose transport, adrenergic receptor activation of lipolysis and the connections of cytokine biology to wasting diseases. Genomic and proteomic technology platforms now support the analysis of fat cells under a variety of hormonal, metabolic and genetic backgrounds. The ability to integrate complex gene expression patterns with protein analysis into a comprehensive view of lipid metabolism and its control is within reach. As such, it remains to be seen in 20 to 25 years how many pages fat cell metabolism will warrant in the modern textbooks.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACS</td>
<td>acyl-CoA synthetase</td>
</tr>
<tr>
<td>ADRP</td>
<td>adipose differentiation-related protein</td>
</tr>
<tr>
<td>alpha-MSH</td>
<td>alpha-melanocyte-stimulating hormone</td>
</tr>
<tr>
<td>aP2</td>
<td>adipocyte fatty acid-binding protein</td>
</tr>
<tr>
<td>ARE</td>
<td>adipocyte response element</td>
</tr>
<tr>
<td>ARF</td>
<td>adipocyte regulatory factor</td>
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<tr>
<td>BAT</td>
<td>brown adipose tissue</td>
</tr>
<tr>
<td>bHLH-LZ</td>
<td>basic helix–loop–helix leucine zipper</td>
</tr>
<tr>
<td>BMR</td>
<td>basal metabolic rate</td>
</tr>
<tr>
<td>C/EBP</td>
<td>CCAAT/enhancer-binding protein</td>
</tr>
<tr>
<td>DGAT</td>
<td>diacylglycerol acyltransferase</td>
</tr>
<tr>
<td>FABP</td>
<td>fatty acid-binding protein</td>
</tr>
<tr>
<td>FATP</td>
<td>fatty acid transport protein</td>
</tr>
<tr>
<td>GLUT</td>
<td>glucose transporter</td>
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<tr>
<td>HETE</td>
<td>hydroxyeicosatetraenoic acid</td>
</tr>
<tr>
<td>HSL</td>
<td>hormone-sensitive lipase</td>
</tr>
<tr>
<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
</tr>
<tr>
<td>IRS-1</td>
<td>insulin receptor substrate-1</td>
</tr>
<tr>
<td>MCH</td>
<td>melanin-concentrating hormone</td>
</tr>
<tr>
<td>NUC1</td>
<td>nuclear transcription factor 1</td>
</tr>
<tr>
<td>PDE</td>
<td>phosphodiesterase</td>
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<tr>
<td>PGC-1</td>
<td>nuclear receptor coactivator 1</td>
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<tr>
<td>PKA</td>
<td>cyclic AMP-dependent protein kinase A</td>
</tr>
<tr>
<td>POMC</td>
<td>pro-opiomelanocortin</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PPRE</td>
<td>peroxisome proliferator-activated receptor response elements</td>
</tr>
<tr>
<td>RXR</td>
<td>retinoid X receptor</td>
</tr>
<tr>
<td>SCAP</td>
<td>SREBP-cleavage activating protein</td>
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</tbody>
</table>
SRE  sterol regulatory elements  
SREBP  sterol regulatory element-binding proteins  
SV40  simian virus 40  
TNFα  tumor necrosis factor  
UCP  uncoupling protein  
WAT  white adipose tissue

References


