

ADIPOSE TISSUE: PHYSIOLOGY TO METABOLIC DYSFUNCTION

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Received April 1, 2020

ABSTRACT

Like the obesity epidemic, our understanding of adipocytes and adipose tissue is expanding. Just in the past decade, substantial advances have led to new insights into the contributions of adipose tissue to normal physiology and obesity-related complications, which places adipocyte biology at the epicenter of a global pandemic of metabolic diseases. In addition to detailing the types, locations, and functions of different adipose tissue depots, this chapter will review the secretory capacities of adipose tissue. Arguably one of the most significant discoveries in the last two decades of adipocyte research is that not only do adipocytes release endocrine hormones, but fat cells and adipose tissue secrete a variety of effectors, including exosomes, miRNA, lipids, inflammatory cytokines, and peptide hormones that act in both paracrine and endocrine capacities to impact local and systemic metabolic responses. The origins of adipocytes via progenitor cells and the process of adipocyte development are discussed. Inflammation, metabolically healthy fat, and adipose tissue expansion are also considered. Finally, several emerging research areas in fat cell biology with therapeutic potential in the management patients who are overweight and have obesity are summarized.

INTRODUCTION: AN HISTORICAL PERSPECTIVE ON ADIPOSE TISSUE BIOLOGY

The first published citation referencing adipose tissue (AT) dates to 1837. Subsequent sporadic single AT citations appeared in the literature until the 1940's, including a 1933 publication in *Biochemical Journal* examining the degree of fatty acid unsaturation in human AT in relation to its depth from the skin surface (1). The first year in which two AT-related citations were recorded was in 1942. In 1947, nearly ten AT citations appeared. Adipose tissue remained understudied for decades due to the misconception that it was simply an inert energy storage depot, but recent discoveries of AT's wider role in cell and whole-body signaling have created a scientific renaissance in this field. As of early 2019, over 139,000 citations involving adipocytes or AT are now discoverable.

The earliest recognized function of adipocytes was the storage of energy in the form of triacylglycerols (TAGs). It was not until the mid-1980s that the secretory functions of AT and the production of adipocyte-specific proteins were revealed. At that time, a serine protease named adipisin was shown to be secreted from cultured adipocytes and reported to be reduced in mouse models of obesity compared to lean littermates (2). Acylation stimulating protein, a member of the alternative complement family, was also revealed to be produced by AT (3) and implicated in lipid storage (4). Although the functions of these AT secretory products remain poorly understood, their discovery revealed adipocytes and AT to be significant sources of a variety of protein products, including many endocrine hormones.

Arguably one of the most important of these discoveries was leptin (5), a bona-fide adipocyte-derived hormone that clearly acts not only as an afferent “adipostat” signal of fat mass to central brain centers in the regulation of body weight (5) but also has peripheral actions that impact glucose metabolism (6) and immune function (7).

In addition, adipocytes are also highly sensitive to insulin and involved in the regulation of blood glucose levels. Insulin action on fat cells stimulates glucose uptake and modulates lipid metabolism by increasing the accumulation and decreasing the breakdown of TAGs (and subsequent release of free fatty acids into the circulation) within the adipocyte. The importance of each of these 3 fat cell functions (Figure 1) – lipid storage, secretory function, and insulin sensitivity – is underscored by the demonstration that disruption of any one role has profound systemic ramifications in mice and man that can contribute to a variety of obesity-related metabolic disease states (8).

The first CDC statistics reporting obesity rates over 20% in many US states also appeared in the late 1990's, as did literature from a variety of disciplines showing that obesity, or excess adipose tissue, enhanced the risk of metabolic diseases, particularly type 2 diabetes (T2D). This was a substantial shift in thinking from the previous two decades when AT was not considered to have much importance or

relevance to T2D. In addition to metabolic diseases, obesity is associated with increased risk of 13 types of cancer that account for ~40% of all cancers diagnosed in the United States (9).

Today, obesity and accompanying epidemics of co-morbidities have become global problems. While in 2015–2016 the prevalence of obesity was 39.8% in adults and 18.5% in youth in the USA (10), the World Health Organization (WHO) reports that obesity has nearly tripled across the world since 1975, and in 2016 more than 1.9 billion adults were overweight and over 650 million were obese. Today, with most of the world's population living in countries where overweight and obesity account for more deaths than malnutrition (underweight), excess AT presents a major challenge to chronic disease prevention and health across the planet. This global epidemic can be attributed to advancing economies and the adoption of mechanized transport, urbanization, commercial growth, industrialization, a progressively more sedentary lifestyle, and a nutritional transition to processed foods and high calorie diets over the last 30 years (11). Besides preventing obesity by promoting a healthy lifestyle through diet and exercise, one of the best ways for modern-day physicians and scientists to combat the global menace of obesity is to better understand AT.

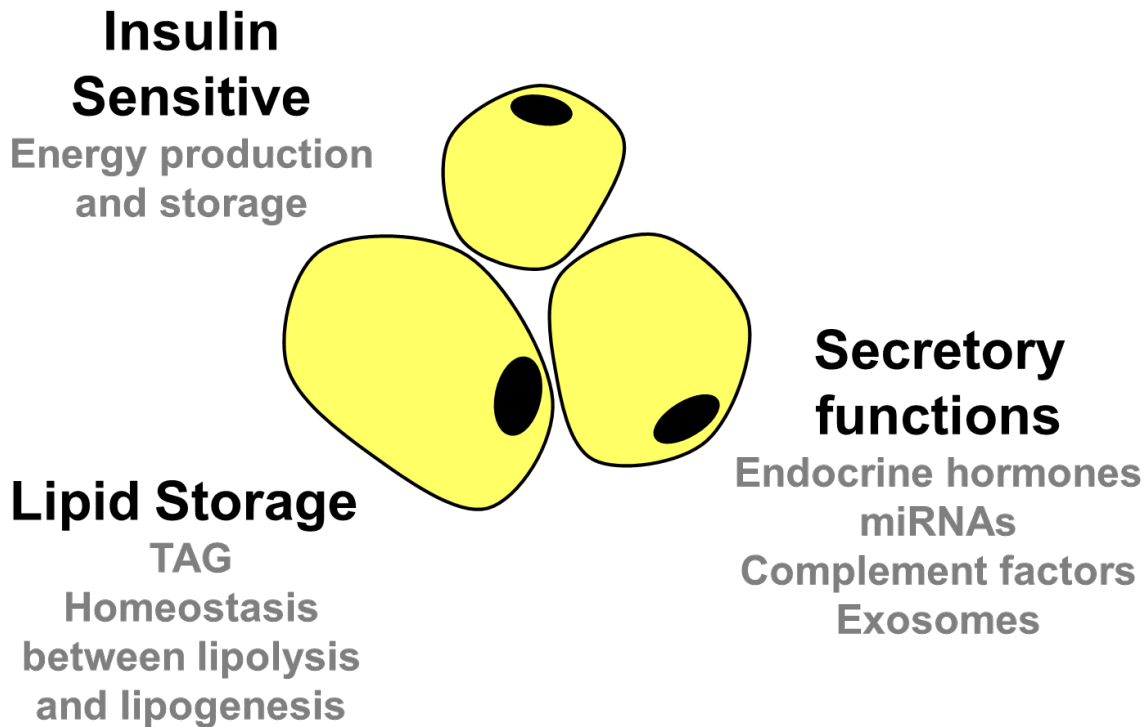


Figure 1. Physiological characteristics of adipocytes. Disruption of any one of these fat cell functions may lead to the development of systemic metabolic dysfunction.

ADIPOCYTE PHYSIOLOGY

Adipocyte Hues – White, Brown, Beige and Pink

Adipose tissue has historically been classified into two types, white adipose tissue (WAT) and brown adipose tissue (BAT), which are visibly distinguishable based on tissue color. The white and brown adipocytes comprising these depots exhibit physiological differences, which give rise to specialized tissue functions. White adipose tissue, which is critical for energy storage, endocrine communication, and insulin sensitivity, comprises the largest AT volume in most mammals including humans. In contrast, BAT is largely present in mammals postnatally and during hibernation. Brown adipose tissue uses energy for non-shivering heat production, which is critical for body temperature maintenance. While BAT was originally thought to only be present in infant humans, imaging studies have revealed metabolically active BAT in the supraclavicular and thoracic regions of adults (12–14). Although women have increased BAT mass and activity over men (14,15), the chance of detecting BAT activity in either sex has been shown to be inversely correlated with

age and body mass index (BMI) (14). Seasonal correlations have also been observed with BAT activity being higher in the winter and lower in the summer, possibly due to either the temperature or, more likely, the photoperiod (14,15). In healthy humans, BAT activity contributes to whole-body fat oxidation and diet-induced thermogenesis (16), supporting a physiological role for this AT depot in adults.

Brown and white adipocytes differ in shape, size, and the intracellular structure of their organelles (Figure 2). White adipocytes are generally spherical in shape and each contains a large, single lipid droplet that pushes all other organelles, including the nucleus, to the cell's periphery. Brown adipocytes contain multiple lipid droplets dispersed throughout a more ellipsoidal-shaped cell that is enriched with iron-containing mitochondria, giving the cell (and the BAT as a whole) a brownish hue. The thermogenic activity of brown adipocytes is conferred by the presence of its numerous mitochondria containing uncoupling protein 1 (UCP-1), a proton transporter that short-circuits the ATP (energy)-generating proton gradient and allows for concurrent heat production as protons flow back into the mitochondrial matrix (17). Brown fat cells typically grow to

15 to 50 μm , while white fat cells have a larger capacity for lipid storage and can expand to nearly 100 μm in diameter (18). The capacity of white adipocytes to expand in number

and size is depot-dependent and is discussed in more detail in the Adipose Tissue Expandability and Metabolic Health section.

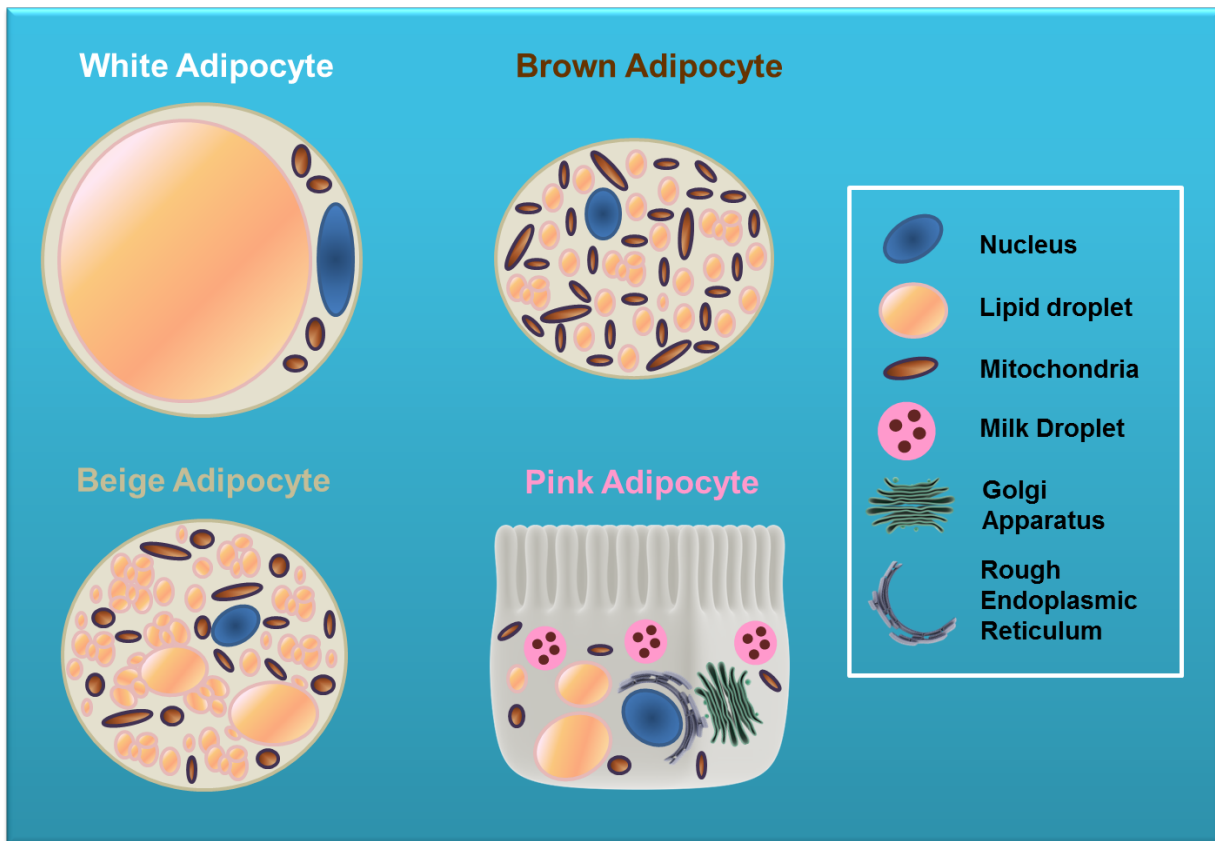


Figure 2. Adipocyte types are described by color hues. The primary characteristic of an adipocyte is its ability to store lipid; white, brown, beige, and pink adipocytes all share this property. However, each type of fat cell is somewhat specialized and has a distinct intracellular distribution of organelles and gene expression profile. All fat cells have Golgi and endoplasmic reticulum, but these organelles make up a more significant portion of pink adipocytes than other adipocyte types.

Recently, two additional adipocyte hues – beige and pink – have been described. Beige adipocytes display characteristics of both brown and white fat cells (Figure 2) and typically develop within subcutaneous WAT from a distinct subset of preadipocytes (19) or via the transdifferentiation of existing white adipocytes (20,21). However, gene expression analyses indicate that beige fat cells represent a distinct type of thermogenic fat cell (19). Beige adipocytes were originally observed to arise in response to cold exposure in rodents (22,23); however, many studies have since identified that diet (24), exercise (25), pre- and post-biotics (26), pharmaceutical agents, numerous plant-based bioactives, and even adipokines (27) can also induce “beiging” or “browning” of WAT, which may

protect against obesity and associated metabolic dysfunction. The “beiging” of WAT is inducible in both mice and humans (28), but this process is more highly observed in mice.

Pink adipocytes were first described in 2014, arising in the subcutaneous WAT of female mice during days 17-18 of pregnancy and persisting throughout lactation. These fat cells appear to derive from white adipocytes that take on epithelial-like features to form milk-secreting alveoli, giving the tissue a pink hue (29). Pink adipocytes are characterized by compartmentalized lipid droplets, cytoplasmic projections, and abundant organelles including mitochondria, peroxisomes, and rough endoplasmic

reticulum, that show a structure more typical of epithelial cells. While reversible transdifferentiation appears to be responsible for the development and disappearance of pink adipocytes during pregnancy, lactation, and post-lactation in rodents (30), it remains uncertain whether or not pink adipocytes form in humans. Notably, loss of a key adipogenic transcription factor within the mammary secretory epithelium creates a pro-breast tumorigenic environment and indicates that the reversible white-to-pink transition might reveal insights into breast cancer biology (29,31). Further investigations into adipocyte plasticity might therefore identify novel therapeutic targets to combat obesity and its pathological consequences, as well as cancer. However, since WAT makes up the largest AT volume in the human body and undergoes the most expansion during obesity, in this chapter we will focus on the roles that white adipocytes and WAT play in normal physiology and metabolic dysfunction.

Adipose Tissue in the Regulation of Lipid Metabolism

Adipose tissue stores body fat as neutral TAGs and represents the chief energy reservoir within mammals. Although many diverse cell types are found in whole AT, adipocytes constitute the largest cell volumes and are the defining AT cell type. White adipocytes are characterized by their large unilocular central lipid droplets (cLDs). However,

the biogenesis of unilocular LDs in adipocytes is poorly understood due to the fragile nature of WAT.

Using live-cell imaging combined with fluorescent labeling techniques, the cytoarchitecture of unilocular adipocytes (Figure 3) and spatiotemporal dynamics of lipid droplet formation have been investigated (32). As shown in Figure 3, cytoplasmic nodules containing micro LDs (mLDs; small green fluorescent protein (GFP)-negative spheres within the cytoplasm) appear on the surface of fat cells, pushed to the edges by the large cLD. Surprisingly, the cytoplasm and organelles do not distribute uniformly around the edge of the cell, but instead form numerous, discrete cytoplasmic nodules connected via a thin layer of GFP-positive cytoplasm. The largest nodule also contains the nucleus, which is surrounded by a thicker layer of cytoplasm. The electron micrograph (Figure 3F) shows the close contacts between mLDs and mitochondria. Furthermore, additional nascent lipid droplets can be visualized budding off from the smooth ER (sER). Studies using a fluorescent-labeled free fatty acid (FFA) analog revealed that exogenously added lipids were rapidly taken up by the fat cell and concurrently esterified to TAG and absorbed by mLDs prior to packaging within the cLD. The lipid transfer followed a unidirectional path from mLD to cLD and provides insight into adipose tissue growth via fat cell hypertrophy (32).

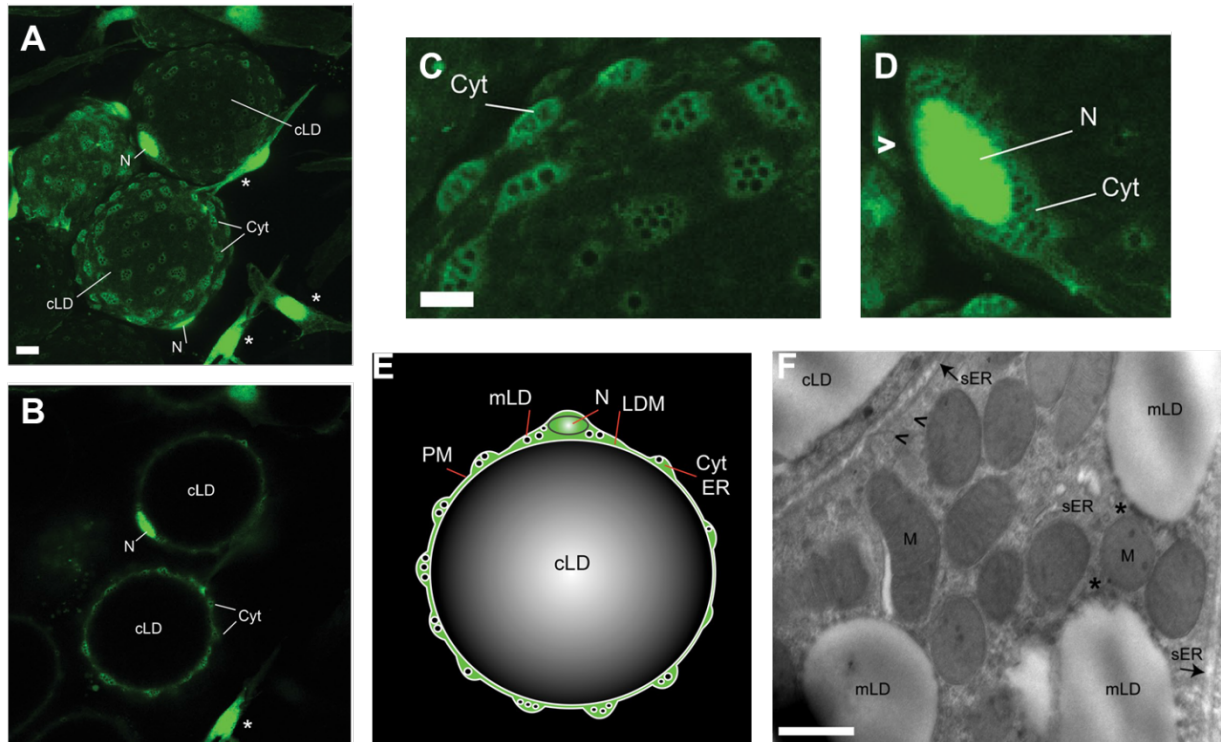


Figure 3. Architecture of primary unilocular adipocytes. Figure adapted from (32). The cytoplasm and nuclei of adipocytes and stromovascular cells were labeled by infecting visceral WAT explants from nonhuman primates with an adenoviral vector encoding enhanced green fluorescent protein (eGFP). Two days after infection, live explants were examined for GFP expression using confocal microscopy. Cellular and subcellular features are labeled: cLD, central lipid droplet; Cyt, cytoplasm; LDM, lipid droplet membrane; mLD, micro-LD; N, nucleus; PM, plasma membrane; sER, smooth ER. (A) GFP-positive unilocular adipocytes (spheres) and stromovascular cells (asterisks) residing in WAT. The image represents the sum of all confocal slices. Bar, 10 μ m. (B) Single confocal section of the image in A. Enhanced magnification of adipocytes containing cytoplasmic nodules (C) and perinuclear cytoplasm (D). (E) Schematic representation a unilocular adipocyte demonstrates that the cLD is a sphere tightly fitted within the cell, whereas the cytoplasm collects in multiple organelle- and mLD-containing nodules. (F) Electron micrograph of a unilocular adipocyte from a visceral WAT explant that was fixed and processed for electron microscopy. Asterisks mark contact sites between mitochondria and mLDs, whereas arrowheads point towards vesicles budding off the ER tubules. Bar, 500 nm.

Adipocytes store TAG under conditions of energy surplus and release fatty acids to supply to other tissues during fasting or times of high energy demand. As such, AT is central to the regulation of systemic lipid metabolism, and nutritional and hormonal cues serve to balance lipid storage and breakdown within the fat cell (Figure 4).

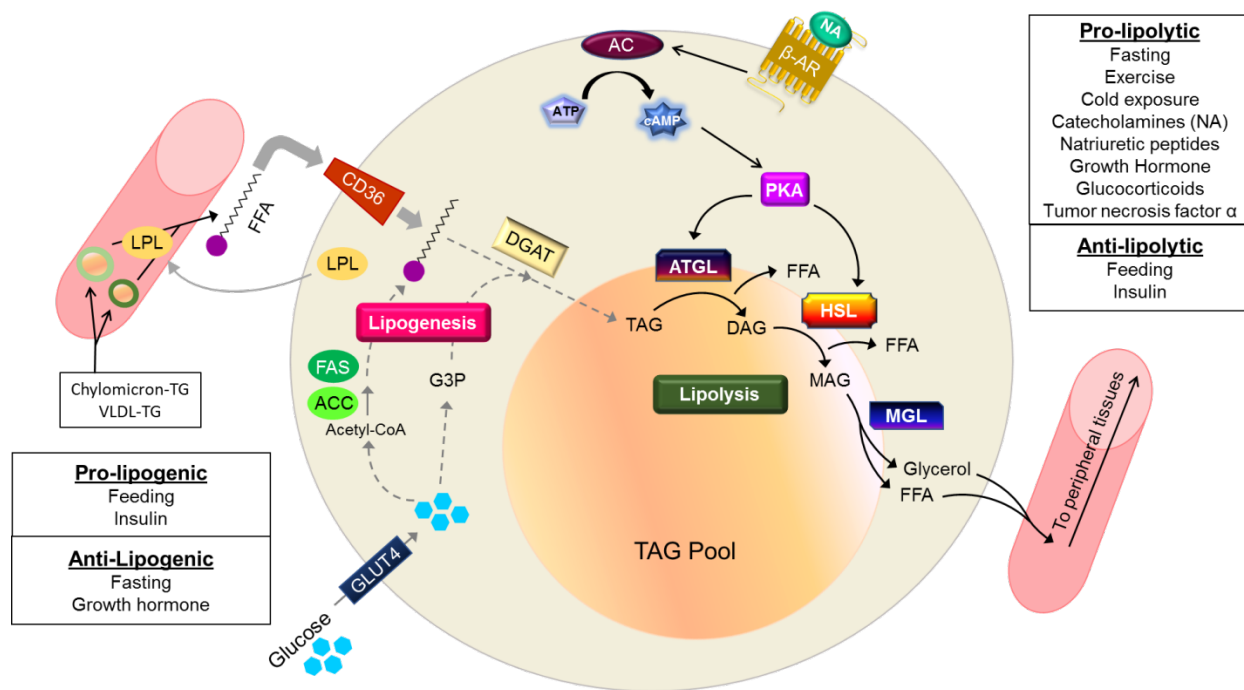


Figure 4. A critical balance between lipogenesis and lipolysis within adipocytes must be established to maintain whole body insulin sensitivity and energy homeostasis. Lipogenesis is shown on the left (gray arrows mark the pathway), whereas lipolysis is shown on the right and is marked by black arrows. Nutritional and hormonal cues regulate both processes. Lipid droplet associated proteins, such as perilipin and comparative gene identification-58 (CGI-58) are not shown but play important roles in lipolysis. CD36 (cluster of differentiation 36) is a fatty acid transporter that facilitates entry of free fatty acids (FFAs) into the cell. Insulin stimulates glucose uptake into fat cells by increasing the localization of the insulin responsive glucose transporter, GLUT4, within the plasma membrane. Other abbreviations: VLDL-TG – triglyceride-containing very low density lipoprotein; LPL – lipoprotein lipase; ACC - acetyl-CoA carboxylase 1; FAS – fatty acid synthase; G3P – glycerol 3 phosphate; DGAT - diacylglycerol acyltransferase; β -AR – β -adrenergic receptor; NA – noradrenaline; AC – adenylyl cyclase; PKA – protein kinase A; ATGL - adipocyte triglyceride lipase; HSL - hormone sensitive lipase; MGL - monoacylglycerol lipase; TAG – triacylglycerol; DAG – diacylglycerol; MAG – monoacylglycerol.

LIPOGENESIS

Adipocytes accumulate lipid via one of two processes (Figure 4). In the first process, under normal daily feeding conditions adipocytes take up dietary lipids from the circulation in the form of FFA's liberated from circulating TAGs via the action of lipoprotein lipase (LPL) (33). Adipocytes secrete LPL, which is transported to the adjacent capillary lumen to catalyze the hydrolysis of FFA's from circulating triglyceride-containing lipoproteins (34,35), such as chylomicrons produced in the small intestine and very low density lipoproteins (VLDLs) synthesized by the liver (36). Adipocytes also take up glucose, which is converted to glycerol and serves as the backbone for the

sequential esterification of fatty acids for form TAG. The final step in TAG synthesis, re-esterification of circulating free fatty acids, mediated by diacylglycerol acyltransferase (DGAT) (37,38). The second process is by *de novo* lipogenesis (DNL) within the adipocytes themselves. Lipogenesis comprises both *de novo* synthesis of fatty acids from acetyl-coenzyme A (acetyl-CoA) and the esterification of these fatty acids to a glycerol backbone producing TAGs (Figure 4). *De novo* lipogenesis can occur in the fasting and fed states (36). Following a meal, especially one high in carbohydrates, excess glucose oxidation yields elevated levels of acetyl-CoA that become substrate to generate fatty acids. This occurs through actions of the DNL enzymes acetyl-CoA carboxylase 1 (ACC1) and fatty acid synthase

(FAS) to convert acetyl-CoA to palmitate, which can then be elongated and desaturated to form other fatty acid species (39).

Surprisingly, in rodents DNL is relatively low in WAT compared to BAT and liver, and it plays an even lesser role in WAT lipid storage in humans under physiological conditions (40,41). Typically, hepatic DNL activity exceeds that of AT and is a more substantial contributor DNL-generated circulating lipids. However, in humans fed high-carbohydrate diets, liver DNL contributes only a small portion of total *de novo* fat biosynthesis, suggesting that AT contributes significantly to whole body DNL when there is a carbohydrate surplus (39,42). Under this condition, adipocyte DNL is usually quite low but has been shown to be important for whole body substrate metabolism (43,44) as inhibition of WAT DNL is associated with insulin resistance (45).

A primary transcriptional regulator of adipocyte DNL is carbohydrate response element-binding protein (ChREBP) (39). Mice lacking AT ChREBP have decreased DNL and insulin resistance (46). The other major DNL regulator in AT is sterol regulatory element-binding protein 1 (SREBP1). Mice with whole body knockout of SREBP1 do not display decreased lipogenic gene expression in AT (45,47), thus supporting ChREBP as the primary lipogenic transcription factor driving AT DNL. However, a new mouse model of inducible, overexpression of insulin-induced gene 1 (Insig1), an inhibitor of SREBP1 activation and transcriptional activity, demonstrated that several acute and chronic white adipocyte-specific compensatory mechanisms are activated to restore adipocyte DNL in the absence of SREBP1 activity (44). Decreased SREBP1 activity prior to this compensation and during conditions where compensation was inactivated result in decreased lipogenic gene expression, impaired whole body glucose tolerance, and elevated lipid clearance (44) suggesting that both SREBP1 and ChREBP play important roles in adipocyte DNL.

Enhanced AT DNL can produce favorable lipid species that may be therapeutically advantageous in the context of obesity and insulin resistance (48). Adipocytes synthesize and secrete a novel family of bioactive lipids, known as the branched fatty acid esters of hydroxyl fatty acids (FAHFAs). Although FAHFAs are found in many tissues, the highest levels are in white and brown AT, and their production is

likely dependent on AT lipogenesis as disruption of adipocyte DNL impairs their synthesis (39,49). Over 1000 structurally distinct FAHFAs have been predicted based on *in silico* analyses and at least 20 FAHFA families have already been identified in mammalian tissues (50). The serum and subcutaneous AT levels of one FAHFA family, palmitic acid esters of hydroxyl steric acids (PAHSAs) have been shown to be higher in insulin-sensitive compared to insulin-resistant individuals (51). In animal models, PAHSAs have been shown to decrease inflammation and enhance whole body insulin sensitivity (39,49). Recent evidence from a mouse model of high-fat diet (HFD)-induced insulin resistance demonstrates that PAHSAs act via both direct and indirect mechanisms to improve insulin sensitivity in multiple metabolic tissues, such as glycolytic skeletal muscle, heart, liver, and AT. In WAT explants, PAHSAs directly inhibit lipolysis and enhance insulin's ability to suppress lipolysis. While PAHSAs can also directly inhibit endogenous glucose production (EGP) in isolated hepatocytes, the decreased AT lipolysis indirectly attenuates EGP because of reduced glycerol (gluconeogenic substrate) delivery to the liver (50).

Additional evidence from humans support a role for increased DNL and ChREBP activity in maintaining metabolic health. These include restoration of DNL in WAT following as bariatric surgery-induced weight loss (52) and reported observations of elevated WAT DNL in other metabolically favorable states including caloric restriction and adaptive thermogenesis (53,54). Collectively, these studies in mice and man support a potential role of WAT DNL in metabolic health.

LIPOLYSIS

Under physiological conditions when metabolic fuels are low and/or energy demand is high, such as fasting, exercise, and cold exposure, adipocytes mobilize their TAG stores via the catabolic process of lipolysis to supply fuel to peripheral tissues (55). Lipolysis is a highly regulated biochemical process that generates glycerol and FFAs from the enzymatic cleavage of TAGs by lipases (36) and can occur in all tissues, although it is most prevalent in AT where the bulk of TAG is stored. As shown in Figure 4, TAGs are broken down into diacylglycerols (DAGs) and monoacylglycerols (MAGs) by the sequential action of adipocyte triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and monoacylglycerol lipase (MGL). At each

step a single FFA is released, and in the final step MGL releases the glycerol backbone from the last FFA. These breakdown products can be re-esterified within the adipocyte or released into circulation to be used by other tissues (36,55), including by the liver for gluconeogenesis (glycerol) and for oxidative phosphorylation by muscle or other oxidative tissues (56).

Lipolysis is controlled by sympathetic nervous system (SNS) input as well as a variety of hormones (55). The best understood of these regulators is the catecholamine, noradrenaline (NA), also known as norepinephrine. Noradrenaline stimulates β -adrenergic receptors (Figure 4), which, in turn, stimulate protein kinase A (PKA) via adenylyl cyclase (AC)-mediated production of cyclic AMP (cAMP). PKA activates the lipolytic action of ATGL and HSL by different mechanisms. Several lipid droplet-associated proteins, such as perilipin 1 (PLIN1) and comparative gene identification-58 (CGI-58) are also important in regulating lipolysis (57,58). Whereas PKA can directly phosphorylate and activate HSL (59–62), it primarily stimulates ATGL activity indirectly by phosphorylating PLIN1. This phosphorylation releases CGI-58 to potentially activate ATGL (58,63,64). Non-adrenergic lipolytic stimuli include glucocorticoids, natriuretic peptides, growth hormone, and tumor necrosis factor alpha (TNF α) (58). These hormones are typically less potent lipolytic inducers than β -adrenergic stimulation and the molecular mechanisms responsible for their lipolytic abilities have not been clearly elucidated. However, some of these hormones clearly utilize different pathways than β -adrenergic signaling with additive or synergistic effects to increase lipolysis (55,58).

After a meal, the post-prandial increase in circulating insulin readily suppresses lipolysis (65) by increasing the activity of phosphodiesterase 3 (PDE3B) and decreasing cAMP levels (58). In the fasting state, insulin levels drop and NA is released, thus promoting lipolysis (66). Physiologically, exercise is another major pro-lipolytic stimulus in humans (58). Growth hormone, along with NA, adrenaline, and cortisol increase with exercise intensity, while insulin levels decrease. These changes culminate in an overall lipolytic response, the magnitude of which depends on exercise intensity and duration (58,67).

When AT becomes insulin resistant, as occurs in patients with diabetes and may also be present in patients with obesity, insulin's ability to inhibit adipocyte lipolysis and reduce serum levels of FFA and glycerol are impaired. As a result, excessive lipolysis leads to increased FFA levels in both the fasted and fed state. Constant exposure of the liver and muscle to these high FFA levels is thought to promote the uptake and ectopic storage of lipids in these tissues (68). Ectopic lipids have been shown to impair insulin signaling, and thus insulin resistance at the level of adipocyte via increased lipolysis may be a major contributor to whole body insulin resistance (69). In addition to impaired insulin responsiveness in fat cells, elevated lipolysis in obesity may be mediated by decreased expression of adipocyte lipid droplet proteins such as PLIN1 and Fsp27/Cidec (fat-specific protein 27/cell death-inducing DFFA-like effector c) (70). These proteins coat the lipid droplet and promote TAG retention via the inhibition of lipolysis, and mice or humans deficient for PLIN1 (71) or Fsp27 (72,73) exhibit lipodystrophy and insulin resistance (70). Interestingly, adipocyte-selective gene deletions or transgenic overexpression mouse models of proteins involved in insulin signaling, glucose and lipid metabolism demonstrate parallel modulation of adipocyte insulin action and systemic insulin sensitivity or glucose tolerance.

In addition to potent anti-lipolytic action (58), insulin also stimulates lipogenesis (74) by activating LPL (activation) and increasing the transcription of lipogenic enzymes (74). Growth hormone antagonizes insulin by promoting lipolysis and inhibiting lipogenesis (36,58). The insulin-sensitizing, anti-inflammatory lipids (PAHSAs) generated during AT DNL and the excess basal lipolysis associated with ectopic lipid deposition and insulin resistance make both AT lipogenesis and lipolysis attractive targets for pharmaceutical intervention. On the other hand, the balance between lipid storage, mobilization, and utilization is homeostatically regulated through a complex interaction of often redundant hormonal signaling, neurological input, and nutrient flow. These intricacies complicate attempts to develop therapies targeting one aspect of lipid metabolism since disrupting the balance between lipolysis and lipogenesis may, in turn, have unanticipated effects on insulin sensitivity and whole-body energy homeostasis.

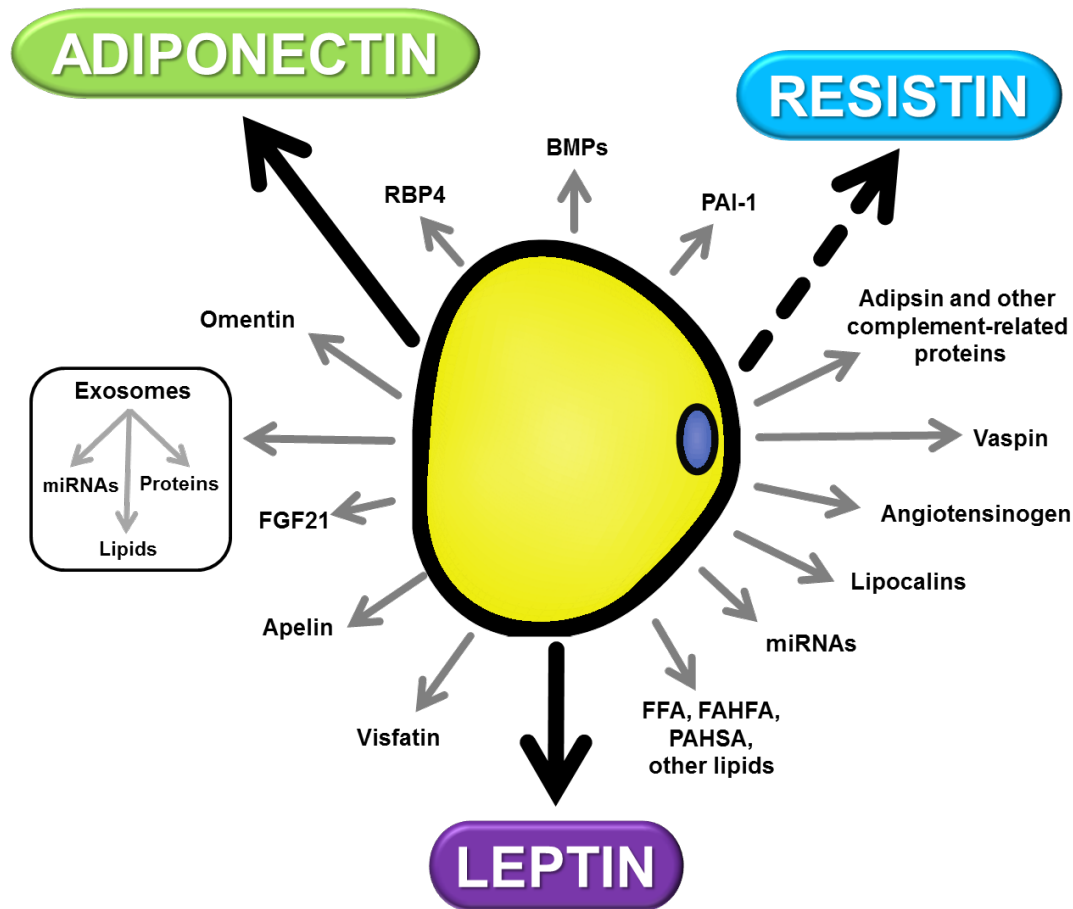


Figure 5. The Adipocyte Secretome. Fat cells express and release numerous protein, lipid, and nucleic acid factors that can act on other nearby or distant tissues within the body in a paracrine or endocrine manner. Leptin, adiponectin, and resistin are highlighted here because they are exclusively secreted from mouse adipocytes, while the other factors can also be secreted from other cell types. The arrow-headed line representing secretion of resistin is dashed since in humans, macrophages, and not adipocytes, primarily produce this adipokine. Abbreviations are RBP4 – retinol binding protein 4, BMPs – bone morphogenetic proteins, PAI-1 – plasminogen activator inhibitor 1, miRNA – microRNA, FFA – free fatty acid, FAHFA - fatty acid esters of hydroxyl fatty acids, PAHSA – palmitic-acid-hydroxy-steric-acid, FGF21 – fibroblast growth factor 21.

Endocrine Properties of Adipose Tissue

Adipocytes and other AT cells secrete a variety of mediators, including exosomes, miRNA, lipids, inflammatory cytokines, and peptide hormones that act in both paracrine and endocrine modes (Figure 5) (75). Although adipocytes secrete a large variety of bioactive molecules with widespread systemic effects contributing to numerous physiological and pathological processes, the autocrine and paracrine actions of these molecules are highly complex, and our understanding of these processes is likely rudimentary. However, substantial progress has

been made studying three endocrine hormones that are almost exclusively produced in adipocytes and function to regulate food intake, the reproductive axis, insulin sensitivity, and immune responses. These hormones are leptin, adiponectin, and resistin, and we review their expression in obesity, their receptors, and effects in target tissues including metabolic actions (Figure 6). While not produced in human adipocytes directly but secreted instead by AT macrophages, resistin has similar functions in mouse and man. The dysregulation of any one of these hormones can contribute to systemic metabolic dysfunction, as well as

to the pathogenesis of chronic metabolic diseases and some types of cancer.

	LEPTIN		ADIPONECTIN	RESISTIN
Expression in Obesity	↑		↓	↑
Receptor(s)	Leptin Receptor (LR) (multiple isoforms)		T-cadherin, AdipoR1, & AdipoR2	TLR4 & CAP1
Target Tissues	Brain & CNS	Hepatocytes & β -cells	Liver, Skeletal Muscle, AT, Bone, Cartilage, Heart	AT, Liver, Endothelium, & Heart
Main Metabolic Actions	↓ Food Intake ↑ Energy Expenditure	Glucose & Lipid Metabolism	↓ Gluconeogenesis, glucose output, lipogenesis & TAG accumulation in liver ↑ Insulin Sensitivity, FAO, & EE in muscle	↑ Insulin Resistance ↓ Adipogenesis & ↑ Proinflammatory response in AT ↓ Glucose uptake & ↑ gluconeogenesis in liver
Other Functions	Reproduction Angiogenesis Bone homeostasis Wound healing Immune Responses Cancer		↓ Liver fibrosis & inflammation ↑ Cell survival ↑ Cardioprotection Reproduction	↑ Vascular Dysfunction ↑ Cell Adhesion ↓ Contractility ↑ Heart Failure

Figure 6. Summary of adipocyte-specific adipokines, and their actions on other tissues. Abbreviations: TLR4 - Toll-like receptor 4; CAP1 - adenylyl cyclase-associated protein 1; AdipoR1 & R2 - Adiponectin receptors 1 and 2; CNS - Central nervous system; FAO – fatty acid oxidation; EE – energy expenditure.

LEPTIN

The first discovered endocrine hormone of adipocyte origin was leptin (5). In 1949 spontaneously occurring obese offspring in a Jackson Laboratories' non-obese mouse colony were determined to be homozygous for a recessive mutation, termed "obese" (*ob*) (76). These *ob/ob* mice appear normal at birth, but soon begin gaining excess fat mass and displaying hyperglycemia and hyperinsulinemia (77). In the 1950s, *ob/ob* mice and their non-obese littermates underwent parabiosis experiments, where two animals are surgically joined (usually by peritoneum or a long bone of the leg) to allow for the exchange of whole blood between them (78). Weight gain was inhibited in *ob/ob* mice parabiosed with non-obese littermates, providing evidence that the *ob/ob* gene product was a circulating factor transferred from the blood of the lean littermate. In 1972, a similar study demonstrated that parabiosis with lean animals not only reduced weight gain in *ob/ob* mice, but also improved hyperglycemia, hyperinsulinemia, and insulin sensitivity (6). Finally, in the 1990s, positional cloning studies identified the product of

the *ob* gene, dubbed leptin, which was derived from the Greek word "leptos" meaning to be thin. Further characterization of leptin revealed that adipocytes were its predominant source (5). Following this discovery, the first directly observed function of leptin was its effect on food intake (79) followed shortly thereafter by demonstration that leptin levels in mice and men strongly correlate with fat mass and play a key role in body-weight (energy) homeostasis as described below.

Leptin Receptor and Signaling

Another spontaneously arising mutant mouse that developed obesity and type 2 diabetes is the diabetes or *db/db* mouse (80). In contrast to *ob/ob* mice, parabiosis of *db/db* mice to wild type littermates did not improve body weight or diabetes, but instead resulted in unhealthy weight loss in the lean littermates, leading investigators to deduce that mice with the *db* mutation lacked a functioning receptor for the *ob* gene but still manufactured a circulating protein that crossed over to the lean littermates to induce anorexia (81). Further confirmation of these hypotheses came when

parabiosis of *ob/ob* with *db/db* mice induced weight loss in the *ob/ob* mouse while the obese state was preserved in the *db/db* mouse (81–83). Although the *db* gene was cloned in 1990 (84), it was not until almost 5 years later (85) following the identification of leptin that the *db* gene was identified to encode the leptin receptor (LR).

The LR is a class 1 cytokine receptor with substantial homology to glycoprotein 130, a plasma membrane receptor that mediates the actions of many cytokines. Unlike other plasma membrane receptors, such as the insulin receptor, the LR lacks intrinsic kinase activity and signals via Janus kinases (JAKs). Six LR isoforms exist, designated LRA–LRf, with LRb being the best characterized. It is the longest LR isoform that is capable of full signaling via the JAK/STAT pathway (86).

Leptin-regulated circuits involved in energy homeostasis have been mapped to distinct yet diverse brain regions (87) expressing the long form of the LR (88). Increased central leptin signaling inhibits food intake and elevates energy expenditure, while leptin deficiency (such as during fasting or starvation) has opposite effects. Expression of the LR has also been detected in peripheral tissues, but the exclusivity of the central leptin circuits to modulate energy intake and expenditure is supported by studies showing that deletion of the long form LR in peripheral tissues had no effects on these processes (89). Leptin levels strongly correlate with fat mass in mice and men (90,91), and as such leptin acts as a sensor of energy stores signaling the availability of body fat to the brain and regulating adipose reserves. However, during obesity the negative feedback loop between increasing leptin levels that signal high energy availability and inhibit food intake becomes disrupted due to the development of leptin resistance (92) — the inability to respond to leptin despite having sufficient or excess levels in circulation during accumulation of excess adipose stores. Although the physiological causes of leptin resistance are not well understood, it has been shown that hyperleptinemia is required for the development of leptin resistance during obesity. When leptin levels of mice are clamped to low levels (similar to lean mice), these clamped mice still develop obesity on HFD, but they do not become leptin resistant (93). The inability to overcome leptin resistance by giving supplemental doses has precluded leptin's use as an anti-obesity therapeutic. Interestingly, leptin resistance that accompanies obesity appears to result from selective impairment of leptin's ability to reduce food intake, while

preserving its other capacity to raise energy expenditure (94). The molecular basis for this phenomenon has not yet been elucidated and remains under active investigation.

Leptin also has central nervous system effects not directly related to energy balance, including modulation of reproduction and thermoregulation. Additionally, research into the role of leptin to mediate anxiety and depression is currently ongoing (95). Leptin can also act peripherally on hepatocytes and pancreatic β -cells to regulate glucose and lipid metabolism independently of its central effects (96). Leptin has also been shown to affect innate and adaptive immunity (7), bone formation (97), bone metabolism (98,99), angiogenesis, and wound healing (88). Skeletal muscle, liver, and intestines have been described as targets for leptin action (100), and some evidence suggests that leptin may also act in an autocrine manner on AT (101). How leptin mediates responses in peripheral tissues is poorly understood and complicated by the existence of its six receptor isoforms, their differential expression across tissues, the pleiotropic nature of leptin's effects, the demonstration of “selective” or tissue-specific leptin resistance, and the complexity of the signaling pathways involved.

Leptin and Cancer

Given the elevated risk for many cancers in patients with obesity in whom leptin levels are also high, it is not surprising that leptin has been implicated in tumorigenesis. Indeed, leptin levels or leptin signaling has been found to be dysregulated in breast, thyroid, endometrial, and gastrointestinal malignancies (102). Ectopic leptin expression in colorectal adenomas increases during the progression to colorectal cancer (103,104) yet associates with a favorable prognosis of the cancer (103). In papillary thyroid cancer, increased circulating leptin levels occur independently of body mass index (BMI), coincide with elevated LR expression on the tumor cells, and associate with aggressive carcinogenesis and poor prognosis (105,106). In contrast, reported associations between leptin levels and endometrial cancer are not maintained when adjusted for BMI, suggesting that leptin is not likely a causative factor in the development of this cancer (107–109).

Studies show that postmenopausal women with obesity have a 20–40% greater risk of developing breast cancer

compared to normal weight women (110). In breast cancer, particularly in high-grade tumors, overexpression of both leptin and LR is associated with cancer progression and poor patient survival. Leptin's ability to stimulate angiogenesis, regulate endothelial cell proliferation, and crosstalk with insulin and human epidermal growth factor receptor 2 (HER2) signaling pathways represent a few of the possible mechanisms by which leptin plays a role in breast cancer (111). As obesity rates continue to rise, it is likely that studies examining the relationship between leptin and cancer will become even more relevant.

ADIPONECTIN

Adiponectin is a unique and extensively studied adipocyte-derived hormone with complex biology. Efforts to identify genes regulated during adipogenesis led to the discovery of

adiponectin in 1995 (112) and 1996 by three separate research groups employing different approaches (113–115). Secreted by adipocytes, adiponectin is characterized by its remarkably high circulating levels reaching plasma concentrations in humans of 2–20 $\mu\text{g/ml}$ (116), values that are more than 1000-fold higher than most other secreted factors (Figure 7). Unlike leptin, adiponectin levels decrease as a function of increasing fat mass in both rodents and humans with obesity (115); thus, they are lower in patients with obesity than those who are lean. Adiponectin's widely reported anti-hyperglycemic, anti-atherogenic, and anti-inflammatory effects have made it an attractive therapeutic target for the treatment of obesity and insulin resistance. However, efforts to develop therapies targeting adiponectin function have been impeded by its complex structure and regulation (117).

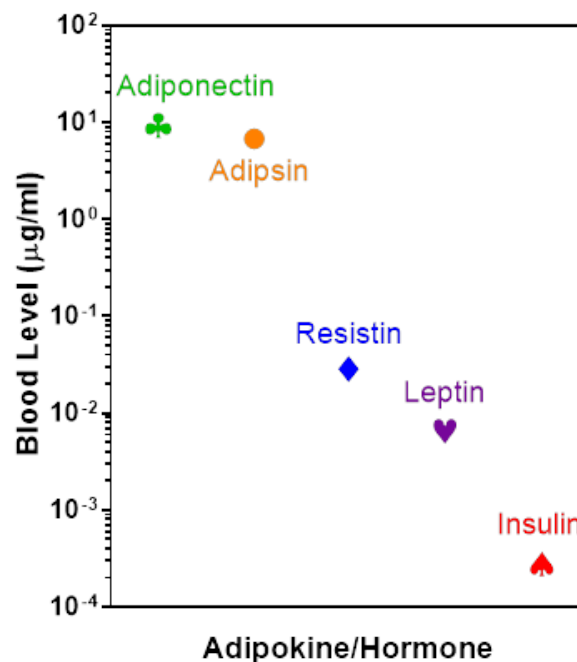


Figure 7. Typical circulating concentrations of select adipokines and insulin for normal weight, healthy humans. Adipokine levels in the blood are several orders of magnitude higher than that of insulin.

Adipocytes secrete different forms of adiponectin: low-molecular weight (LMW) trimers (the most basic form), medium-molecular weight (MMW) hexamers, and high-molecular weight (HMW) oligomers (118), as well as globular adiponectin, a proteolytic fragment of the protein (119,120). In humans, the MMW and HMW oligomers make up most of the circulating adiponectin while the LMW trimer

constitutes less than 30% of serum adiponectin. The HMW oligomer is most closely associated with enhanced insulin sensitivity and reduced glucose levels (121).

Adiponectin signaling is complex and incompletely understood. Three adiponectin receptors have been identified. Adiponectin receptor-1 and -2, referred to as

AdipoR1 and AdipoR2, bind the LMW and globular forms (122). T-cadherin binds HMW adiponectin (123). Both AdipoR1 and AdipoR2 can modulate insulin sensitivity and metabolic gene expression in insulin-responsive tissues, and both receptors have demonstrated roles in the pathophysiology of insulin resistance and T2D (124,125). T-cadherin, which is expressed in a variety of tissues including the liver (126), belongs to a family of cell surface proteins involved in cell-cell interactions (127). Mice lacking T-cadherin accumulate adiponectin in circulation and have a similar cardiovascular phenotype to adiponectin knockout mice (128), suggesting that T-cadherin is the primary effector of cardioprotection by adiponectin (129–131).

Adiponectin enhances fatty acid oxidation through activation of AMP-activated kinase (**AMPK**) (132,133), a cellular energy sensor, which then inhibits acetyl CoA carboxylase, a rate limiting enzyme in **DNL** (134). This, in turn, reduces malonyl-CoA production and enhances fatty acid oxidation. Adiponectin can activate AMPK through two independent pathways, and can also modulate lipid metabolism by increasing mitochondrial density and mitochondrial DNA content (135,136). Adiponectin has diverse effects in many tissues, including bone and cartilage (137), and can act in an autocrine or paracrine manner in AT and other tissues (138). Adiponectin also appears to modulate a wide range of biological processes, including reproduction and embryonic development (139,140). The heart, liver, and skeletal muscle are considered the primary targets for adiponectin action, and adiponectin's prominent insulin-sensitizing effects have been most fully characterized at the mechanistic level in liver and muscle (117) (Figure 6).

The liver performs a critical function in maintaining normal blood glucose levels by releasing glucose (i.e. hepatic glucose output) into circulation in conditions such as fasting, exercise, and pregnancy. Conversely, the ability of the liver to reduce its glucose output when demand is low, as in the fed state, is also crucial to preventing hyperglycemia, and this process is often impaired with obesity and insulin resistance. Adiponectin can robustly reduce plasma glucose levels predominantly by inhibition of hepatic glucose production as opposed to effects on whole-body glucose uptake into cells and glycolysis (141). The importance of adiponectin in regulating glucose output in the liver is underscored by studies showing that mouse models with genetic deletion (142) or overexpression (143)

of adiponectin have impaired or enhanced hepatic insulin sensitivity, respectively. Adiponectin levels are increased by thiazolidinediones (TZDs), which is thought to be the predominant mechanism of action that improves insulin sensitivity and glucose tolerance with this class of medications (142–144). Adiponectin also promotes hepatocyte survival, inhibits hepatic fibrosis and inflammation, stimulates fatty acid oxidation (133,145) and modulates fatty acid uptake and metabolism (146). In patients with nonalcoholic fatty liver disease (NAFLD) who are insulin resistant, low plasma adiponectin levels are associated with the progression of NAFLD and non-alcoholic steatohepatitis (147,148). In summary, adiponectin has beneficial effects in the liver, where it protects against metabolic dysfunction and hepatic diseases (Figure 6).

Skeletal muscle is responsible for up to 80% of insulin-mediated glucose uptake in healthy individuals (149,150). Adiponectin can promote glucose uptake (151,152), enhance fatty acid oxidation (152,153), and enhance insulin sensitivity (154) in cultured muscle cell lines and mouse skeletal muscle. Adiponectin administration to obese, insulin-resistant adiponectin-knockout mice improves skeletal muscle insulin sensitivity (146,155,156). In human myotubes, adiponectin promotes fat oxidation via AMPK activation; this response is impaired in myotubes from patients with T2D and obesity (157). Thus, adiponectin has an important role in skeletal muscle metabolism in humans as well as rodents, and defective adiponectin signaling in skeletal muscle may contribute to insulin resistance.

Finally, in addition to its insulin-sensitizing and glucose-lowering effects in liver and skeletal muscle, adiponectin is also cardioprotective. Low circulating adiponectin levels correlate significantly and independently with coronary artery disease (158), and are considered a risk factor for cardiovascular diseases (CVD) such as hypertension, coronary artery disease, and restenosis (159). The vascular endothelium is believed to mediate some of the cardioprotective effects of adiponectin via AMPK activation and subsequent activation of eNOS (endothelial nitric oxide synthase) (160).

In light of these beneficial functions, adiponectin has significant therapeutic potential in the treatment of T2D, CVD, and NAFLD. Several years ago, small molecule screening efforts produced the first small molecule AdipoR

agonist. ***“AdipoRon”, as it was named, not only recapitulated adiponectin’s effects on AdipoR signaling pathways*** but also had profound anti-hyperglycemia effects in both diet-induced obese mice and a genetic mouse model for diabetes (161). A more recent study has now shown that AdipoRon can also decrease ceramides and lipotoxicity, and mitigate diabetic nephropathy (162). Hence, small molecule activators of adiponectin signaling show promise in the management of obesity-associated metabolic diseases like insulin resistance, NAFLD, and T2D.

RESISTIN

Resistin, the most recently discovered of the major adipocyte-derived hormones, was independently identified by two laboratories. In one case, the gene coding for this novel endocrine factor was identified in a screen for genes inhibited by TZD drugs and was named “resistin” because it induced insulin resistance (163). Another group identified the same gene in a screen for genes expressed exclusively in adipocytes and induced during adipogenesis; they named it ADSF, for adipose tissue-specific secretory factor. In this study, the product of the gene was shown to inhibit differentiation of adipocytes *in vitro* (164), later confirmed in a separate study (165).

Elucidating resistin’s role in physiology has been challenging. While resistin is expressed in both white and brown fat in mice, the various WAT depots (inguinal, gonadal, retroperitoneal, and mesenteric) and BAT exhibit distinct patterns of resistin expression (166). In addition, circulating levels of resistin are directly proportional to its gene expression in some conditions, but inversely proportional in others (167,168). Remarkably, while resistin produces similar metabolic and inflammatory effects in humans and mice, human resistin is predominantly secreted from macrophages, not adipocytes (169–171). The complex regulation of resistin expression and the fundamental differences in resistin biology between species are significant obstacles to fully understanding this hormone’s functions and mechanisms of action in humans.

Resistin interacts with two known receptors: the toll-like receptor 4 (TLR4) and adenylyl cyclase-associated protein 1 (CAP1) (172,173). Resistin signaling through TLR4 contributes to monocyte recruitment and chemokine expression, and is involved in inflammatory responses in

atherosclerosis and acute lung injury (135,174). Both knockout and overexpression studies of CAP1 indicate that this receptor can also mediate proinflammatory effects of resistin (173). Overall, the similarities, differences, and tissue specificity of resistin signaling through TLR4 versus CAP1 remains poorly understood.

Resistin has also been shown to regulate fasting blood glucose levels in mice (175). Elevated levels of circulating resistin are reported in genetic and diet-induced mouse models of obesity (163). Anti-resistin antibody administration improves insulin sensitivity in diet-induced obese mice, and conversely, resistin injection impairs glucose tolerance in normal mice; supporting a causative role of resistin in mediating insulin resistance in mouse models (176). Moreover, both human and mouse resistin have been shown to impair insulin-stimulated glucose uptake in cultured murine myocytes *in vitro* (177). Other studies have shown similar insulin desensitizing effects of resistin in liver and brain (178,179).

The finding that human resistin originates not in adipocytes but in mononuclear lymphocytes raised the possibility that the hormone may have distinct roles in the two species. An elegant mouse model was generated to address this issue, the so-called humanized resistin mouse. In these mice, the endogenous resistin gene (normally expressed in adipocytes) was deleted, and the macrophage-expressed human resistin gene was inserted (180). Data from this study revealed that like murine adipocyte-derived resistin, the humanized resistin induced systemic insulin resistance, adipose tissue inflammation, and elevated circulating free fatty acids in high-fat diet (HFD)-fed mice.

In humans, epidemiological, genetic, and clinical data support a role for resistin in dysfunctional metabolism and related pathologies (181). As in mouse models, serum resistin levels are elevated during human obesity (182,183). Furthermore, high circulating resistin concentrations in humans have been associated with atherosclerosis, coronary heart disease, congestive heart failure, as well as inflammatory conditions including systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis (184–188). Whether the relationship between resistin and insulin resistant states is merely correlative and whether interventions to antagonize resistin action will be of therapeutic value in the treatment of

metabolic or cardiovascular disease in humans remains undetermined.

Cell Types in Adipose Tissue

Besides adipocytes, AT is comprised of endothelial cells, blood cells, fibroblasts, pericytes, preadipocytes, macrophages, and several types of immune cells (189). These non-adipocyte cell types are commonly referred to as the AT stromal vascular fraction (SVF) (Figure 8). Our understanding of the complexity of the cell types present in the SVF and how this milieu is altered by metabolic disease states is an area of active investigation. Cells in the SVF

produce hormones and cytokines that can act in a paracrine manner on adjacent adipocytes. In the early 1990s, it was shown that TNF alpha production was increased in AT during metabolic disease states, in particular, T2D (190). Yet, it wasn't for another ten years that adipose tissue macrophages (ATMs) were identified as the primary cellular source of AT TNF alpha (191). It is now largely accepted that in conditions of obesity and T2D, TNF alpha is produced in ATMs and acts on adjacent adipocytes within AT to promote insulin resistance. Hence, it is important to consider the presence and dynamic interactions of the SVF cells, especially when determining the cellular sources of AT-derived paracrine and endocrine hormones.

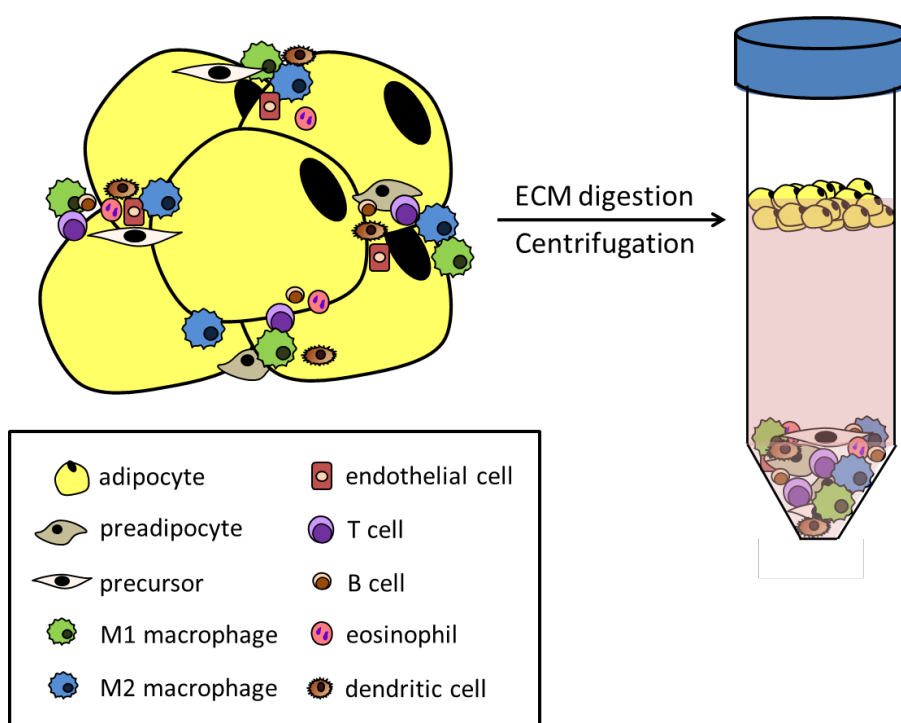


Figure 8. Constituents of adipose tissue (AT). Left: Along with mature, functional adipocytes and precursor cells, many cell types related to vasculature and immune function reside within AT. They perform both physiological and pathophysiological functions by communicating with the adipocytes via secreted factors and scavenging lipid from dying fat cells. The number and diversity of these cell types increases with developing obesity and metabolic dysfunction. Right: The non-adipocyte cells are collectively referred to as the stromal vascular fraction (SVF), and the SVF can be separated from lipid-containing adipocytes by digesting the extracellular matrix (ECM) and centrifuging the cellular mixture. The SVF will form a pellet at the bottom of the tube, while the adipocytes will float and form a visible lipid layer at the top of the aqueous medium. This separation technique is critical to studying the cellular composition of adipose tissue and gaining insight regarding the individual functions of these diverse and distinct cell types under physiological and pathophysiological conditions.

Adipogenesis

To understand how adipocytes contribute to systemic metabolic regulation, it is important to understand their development. Adipogenesis refers to the process by which precursor cells differentiate and become committed to storing lipid and maintaining energy homeostasis as adipocytes. Adipogenesis is regulated by hundreds of factors, including nutrients, cellular signaling pathways, miRNAs, cytoskeletal proteins, and endocrine hormones

such as growth hormone, insulin-like growth factor 1, insulin, and several steroid hormones, as well as cytokines. Generally, pro-inflammatory cytokines inhibit adipogenesis (192), although some cytokines within the same family exert opposing effects (192). Cytoskeletal proteins (193), ECM proteins and their regulators (194), microRNAs (miRNAs) (195), and long noncoding RNAs (lncRNAs) (196) differentially modulate adipogenesis. Dozens of different transcription factors, briefly described below, also regulate adipogenesis (Figure 9).

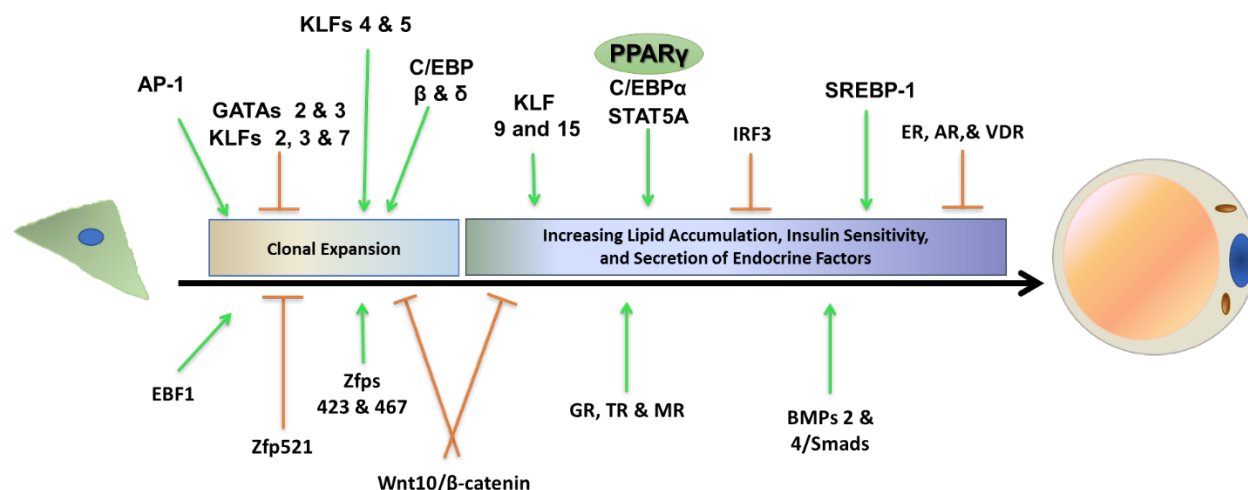


Figure 9. Transcriptional regulation of adipogenesis as determined *in vitro* in a fibroblast-like preadipocyte clonal cell line. Preadipocytes are grown to confluence and become growth arrested. Following induction of differentiation, they re-enter the cell cycle and undergo several rounds of proliferation, a process known as mitotic clonal expansion. At the end of this short proliferative phase, preadipocytes terminally differentiate into adipocytes as they begin synthesizing lipid and assume characteristics of mature fat cells. Numerous transcription factors have been determined to promote (green arrows) or inhibit (orange horizontal ended line) adipogenesis either during clonal expansion or at later stages of terminal differentiation. The timing of activation (i.e. when each transcription factor is turned on and off) is critical to the progression of adipocyte differentiation.

PROMOTORS OF ADIPOGENESIS

The transcription factor peroxisome proliferator activated receptor gamma (PPARγ) is considered the principal adipogenesis regulator (197). Its discovery substantially enhanced our understanding of the adipocyte and its role in metabolic disease. For example, mice with adipocyte-specific PPARγ deletion have decreased AT mass and are insulin resistant (198). In humans, PPARγ gene mutations can also cause lipodystrophy (partial or generalized loss of

fat in the body) and severe insulin resistance (199–201). The discovery of PPARγ as the functional receptor for the insulin-sensitizing TZDs resulted in a significant effort to understand PPARγ action and identify additional agonists. Synthetic TZDs induce weight gain in humans and rodents by increasing fat mass, more so in the subcutaneous adipose depot, which is associated with improved metabolic outcomes. However, this weight gain is also considered as a negative side effect of TZD treatment, especially in the typical patient who has pre-existing obesity. Other adverse

side effects of TZDs, such as bone fractures and heart failure, have spawned the search for structurally distinct PPAR γ ligands capable of inducing unique receptor-ligand conformations with signature affinities for diverse co-regulators (202). Several selective PPAR γ modulators (SPPARMs) with fewer side effects have been identified. These act as partial PPAR γ agonists, alter specific post-translational modifications of PPAR γ , and preserve anti-hyperglycemia effects while minimizing or eliminating the adipogenic effect that leads to increased fat mass via activation of distinct gene profiles that may be cell and tissue specific (203,204). Interestingly, the TZD, rosiglitazone, is capable of improving glucose homeostasis even in the absence of PPAR γ in mature adipocytes (205), suggesting that its adipogenic effects (in addition to its non-adipogenic ones) may also be important for its anti-hyperglycemic action.

In addition to TZDs, PPAR γ binds endogenous lipophilic molecules, including: long chain fatty acids (LCFAs), oxidized or nitrated FAs, prostaglandins, and arachidonic acid derivatives (206). Interestingly, serotonin (5-hydroxytryptamine, 5-HT) has also been shown to be a high affinity agonist for PPAR γ (207). Many of the endogenous PPAR γ ligands enhance adipocyte differentiation and regulate fat cell functions such as lipolysis, glucose uptake, and lipogenesis through PPAR γ -dependent and independent methods (208–214). Overall, these endogenous ligands have low affinity and limited subtype selectivity for PPAR γ relative to other PPARs, suggesting that much remains to be understood regarding this critical adipogenesis regulator. While there is no question that PPAR γ is essential for adipogenesis and lipid accumulation within fat cells, a better mapping of its gene expression profiles in discrete cell and tissue types and with endogenous and synthetic ligands will improve our understanding of AT development and function under both physiological and pathophysiological conditions.

The CCAAT/enhancer-binding proteins (C/EBPs) are widely expressed transcription factors that regulate proliferation and differentiation of various cell types in mammals. Studies *in vivo* and *in vitro* have identified C/EBP isoforms α , β and δ as important regulators of adipogenesis (215). C/EBPs β and δ work together in early adipogenesis to promote fat cell differentiation by inducing expression of C/EBP α and PPAR γ (216). Additionally, the transcription

factors Krox20 and ZNF638 can modulate adipogenesis by affecting C/EBP β function (217,218).

The Signal Transducer and Activator of Transcription (STAT) family of transcription factors was first identified over 20 years ago (219). Both the protein expression of STATs and their ability to regulate gene expression are tissue-specific (220). In AT, STATs regulate gene expression during adipogenesis, and the expression of STATs 1, 3, 5A, and 5B is induced during differentiation of murine and human preadipocytes (221,222). Notably, the ability of STAT5 proteins to promote adipogenesis has been documented by over a dozen independent laboratories using both *in vitro* and *in vivo* approaches (17).

Of the three isoforms of Sterol Response Element Binding Proteins (SREBP-1a, SREBP-1c, and SREBP-2), SREBP-1c is the predominant form expressed in white AT (223,224) and is an important regulator of lipogenesis genes, while SREBP-2 regulates the expression of cholesterol biosynthesis genes (225). Intriguingly, two miRNAs (miR-33a and miR-33b) located within the SREBP genes are highly induced during adipogenesis (226). Although SREBP-1 clearly plays a promoting role in adipogenesis *in vitro*, *in vivo* studies suggest that SREBP-1 is not critical for AT development and/or expansion, perhaps due to compensatory SREBP-2 overexpression (47,227).

Members of the early B-cell factor (EBF) family of transcription factors are characterized for their ability to modulate islet beta-cell maturation and neural development. Three primary members of this family (EBFs 1, 2, and 3) are expressed in fat cells. EBFs 1 and 2 can promote adipogenesis (228,229), and EBF2 can also play roles in determining brown versus white adipocyte identity *in vivo* (230) and the beiging process of adipose tissue in mice (231).

INHIBITORS OF ADIPOGENESIS

The interferon-regulatory factor (IRF) family of transcription factors has functionally diverse roles in the immune system, but also plays a role in adipocyte development. All nine IRF family members are regulated to different degrees during adipogenesis *in vitro*, and some members can repress adipogenesis (232) and contribute to insulin resistance (233). For example, knockdown of IRF3, whose expression is elevated in visceral and subcutaneous AT of

obese mice as well as in subcutaneous AT from humans with obesity and diabetes decreases fat mass and prevents insulin resistance in high fat diet-fed mice (233).

Wingless-related integration site (Wnt) proteins regulate development and cell fate through both autocrine and paracrine signaling (234) by using three well-characterized pathways: the canonical Wnt signaling and the planar cell polarity and Wnt/calcium pathways, which are non-canonical. The canonical pathway is dependent upon the transcription factor, β -catenin (235). Wnt10b is the best studied member of the Wnt signaling family in terms of adipocyte development. In the presence of Wnt10b, β -catenin translocates to the nucleus where it inhibits PPAR γ and C/EBP α activity, thereby impeding adipogenesis (236,237). On the other hand, extracellular antagonists of Wnt/ β -catenin signaling have been reported to promote adipocyte differentiation (238,239).

The GATA family of transcription factors were named based on their ability to bind the DNA sequence GATA (240). Only GATAs 2 and 3 are expressed in preadipocytes residing in white AT (241), and both are repressed during adipogenesis. In fact, GATA2 can directly bind to the PPAR γ promoter to suppress its activity (241). In addition to inhibition of PPAR γ expression, GATAs 2 and 3 can also associate with C/EBPs to disrupt their transcriptional activity (242). GATA3 expression is driven by the canonical Wnt signaling pathway (243,244). Collectively, these studies demonstrate that two GATA proteins can attenuate adipocyte development via multiple transcriptional and signaling pathways.

TRANSCRIPTION FACTOR FAMILIES THAT CAN EITHER PROMOTE OR INHIBIT ADIPOGENESIS

The Krüppel-like transcription factors (KLFs) include 17 members that can either activate or repress transcription. In relation to adipocyte development, KLFs 4, 5, 6, 9 and 15 can promote adipogenesis, while KLFs 2, 3 and 7 repress adipocyte development. Most studies on the roles of KLFs in adipogenesis have been performed *in vitro* using a variety of cell culture models, and have demonstrated that KLFs act in concert with other transcription factors modulate adipogenesis (245).

The transcription factor activator protein 1 (AP-1) consists of Jun proteins (c-Jun, JunB, and JunD), Fos proteins (c-

Fos, FosB, Fra1, and Fra2), ATF and JDP family members, several of which are induced during adipogenesis (222). In humans, a mutation in the c-fos gene that is associated with lipodystrophy has been shown to reduce c-fos activity and adipocyte development (246). Many *in vitro* and *in vivo* studies demonstrate that, like KLFs, AP-1 transcription factors can positively and negatively regulate adipogenesis.

Many of the zinc finger proteins (ZFPs) function as transcription factors with several contributing to adipocyte determination and/or adipogenesis. Zfp423 and Zfp467 can promote adipocyte differentiation by enhancing PPAR γ expression and activity (247,248). In addition to stimulating adipogenesis, Zfp423 can suppress 'beige-like' properties in white adipocytes that are typically associated with improved metabolic health (249). Zfp521 can inhibit Zfp423 to reduce adipocyte development and is also considered a critical regulator of the commitment to either osteogenic or adipogenic lineages (250,251).

The transforming growth factor beta (TGF- β) superfamily encompasses a large number of proteins, including bone morphogenetic proteins (BMPs) (252). BMPs and TGF- β have been reported to be involved in both adipocyte commitment and differentiation (253–255). Specifically, BMPs 2 and BMP4 can promote adipogenesis via the Smad signaling pathway (256) to regulate transcription of target genes such as PPAR γ (257,258). While BMPs are known to promote adipogenesis, *in vitro* and *in vivo* studies demonstrated that TGF- β primarily inhibits fat cell differentiation.

HORMONAL REGULATION OF ADIPOGENESIS

Steroids are prominent regulators of AT development and distribution, and adipocytes express high levels of many steroid hormone receptors. These lipophilic hormones diffuse through plasma membranes, dimerize, and bind to their specific receptors to impart both genomic and non-genomic responses (259,260). Since steroid-bound receptors act as transcription factors, their capabilities should be fully considered in the transcriptional regulation of adipogenesis.

Two types of estrogen receptors, ER α and ER β , are expressed in rat and human preadipocytes, mature adipocytes, and in other AT cells (261–263). Although many studies describing the role of estrogens in AT are

contradictory, most investigations indicate that estrogen inhibits adipocyte differentiation (245) and the adipogenic action of PPAR γ (264). Aromatase is an enzyme found in several tissues, including AT, that aromatizes androgens into estrogens. Both ER α - and aromatase-knockout mice have increased adiposity, suggesting that both estrogen and its receptor can reduce adipocyte development (265,266). Mice lacking ER α have enhanced visceral AT deposition and increased weight gain compared with wild-type mice (267).

Androgen receptors (AR) are also expressed in rodent (268,269) and human AT (270). Similar to estrogen, many studies report contradictory actions of androgens on the differentiation and function of adipocytes. These inconsistent results highlight the importance of accounting for sex-, depot- and organism-specific effects. In studies of human AT, testosterone and the non-aromatizable androgen, dihydrotestosterone, inhibit differentiation of preadipocytes obtained from subcutaneous and omental depots of both men and women, although the magnitude of the inhibitory effect may differ between the sexes (271,272). Overall, most studies indicate that androgens exert inhibitory effects on adipogenesis.

Glucocorticoids (GCs) are well-known promoters of adipocyte development. GCs also promote adipocyte hypertrophy and differentiation of central fat depots that can lead to abdominal obesity and insulin resistance (273). *In vitro* adipogenesis studies include the wide use of the synthetic GC, dexamethasone. Although the mechanisms of action and target genes of GCs involved in adipocyte differentiation are not completely clear, it is known that GCs induce expression of C/EBPs β and δ and that GC-induced C/EBP δ coordinates with C/EBP β to induce PPAR γ expression and adipogenesis (274).

To understand the actions of GCs via the glucocorticoid receptor (GR), it is important consider the enzyme that affects circulating levels of cortisol, the active form of GR's endogenous ligand. 11 β -hydroxysteroid dehydrogenase type 1 (11 beta HSD1) is an enzyme highly expressed in AT and liver that in AT converts inactive cortisone to the active hormone cortisol. Hence, it is not surprising that 11 beta HSD1 mRNA expression and activity is essential for the induction of human adipogenesis and that adipocyte development can be blocked with a 11 beta HSD1 specific inhibitor (275). In addition to inducing the expression of

early adipogenic transcription factors, GCs promote adipocyte development by mechanisms that include suppression of anti-adipogenic factors (Pref-1 and Runx2); anti-proliferative effects on preadipocytes; and sensitizing or 'priming' of human preadipocytes to insulin action (276). Recent attention has focused on the potential contributions of environmental pollutants known as endocrine disrupting chemicals (EDCs) in the development of metabolic diseases. Studies reveal that EDCs can promote adipogenesis through GR activation (277), thereby implicating these compounds in the rising rates of obesity and diabetes.

In addition to regulating water and salt homeostasis, the mineralocorticoid aldosterone and its receptor (MR) have also been shown to play a role in the regulation of adipocyte development. This is important since MR is a high-affinity receptor for both mineralocorticoids and GCs. Aldosterone promotes adipogenesis in an MR-dependent manner (278) and a MR antagonist can inhibit adipogenesis (279). Although GRs and MRs are expressed in AT and thought to mediate cortisol's actions on AT, the levels of GR are several hundred-fold higher than MR in both human preadipocytes and adipocytes (280). Loss of GR, but not MR, blocks the adipogenic capabilities of cortisol in human preadipocytes (280). However, MR expression is higher in omental than in subcutaneous AT, so there could potentially be depot differences in the relative importance of MR and GR in cortisol-induced adipogenesis (280). There could also be differences in the contribution of MR to adipogenesis during obesity when MR and 11 beta HSD1 expression levels are increased, while the GR and 11 beta HSD2 (the enzyme that deactivates cortisol) levels do not increase accordingly (280). Most of the current evidence suggests that the ability of aldosterone to modulate adipogenesis *in vitro* is largely dependent on MR. Additional studies are needed to determine if MR plays a role in adipocyte development *in vivo*.

Vitamin D is another steroid hormone with strong experimental evidence that it can regulate adipogenesis. Unlike most of the water-soluble vitamins that are excreted via urine when in excess, Vitamin D, along with the other fat-soluble vitamins (A, E, and K), can be stored within fat-laden adipose tissue. The vitamin D receptor (VDR) and 1 α -hydroxylase (CYP27B1), the enzyme that activates vitamin D, are expressed in human AT, primary preadipocytes, and newly-differentiated adipocytes (281). The most active form

of Vitamin D, 1, 25-Dihydroxyvitamin D, represses adipocyte differentiation (282,283) and the VDR can block adipogenesis by inhibiting C/EBP β expression (284). Vitamin D-induced inhibition of adipogenesis also involves direct suppression of C/EBP α and PPAR γ (285). Vitamin D and VDR also play a role in the inhibition of adipogenesis of bone marrow stromal cells (286), in part by suppressing the expression of inhibitors of the canonical Wnt/ β -catenin signaling pathway (287). Although vitamin D inhibits adipogenesis in the widely used murine and bone marrow-derived cells, both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D₃ can promote the differentiation of human subcutaneous preadipocytes (281). Overall, a case could be made that concentrations of vitamin D as well as the type of adipocyte precursor determine whether this hormone exerts pro- or anti-adipogenic actions via the VDR.

On the other hand, evidence regarding Vitamin D's role in adipocyte development in humans is controversial and contradictory. According to a systematic review and meta-analysis of 23 studies between 2002 and 2014, overweight or obese subjects exhibit a higher prevalence of Vitamin D deficiency (288). In two double-blind, placebo-controlled randomized clinical trials, Vitamin D-supplemented individuals with healthy overweight or obesity lost significantly more fat mass than the placebo group when fed either a calorie-restriction (289) or weight-maintenance (290) diet for 12 weeks. While decreased fat mass may result from Vitamin-D induced inhibition of adipogenesis, this hypothesis was not directly tested in the studies, and two other longer term studies demonstrated no change in fat mass with Vitamin D supplementation between 14,000 and 20,000 IU per week (291,292).

The relationship between adipocyte development and thyroid hormones has been recognized since 1888 when a report on myxedema proposed that obesity was a requirement for a diagnosis of hypothyroidism (293). The most biologically active form of thyroid hormone, T₃, can induce brown adipocyte differentiation (294). Hyperthyroidism in rodents induces adipocyte hyperplasia, whereas hypothyroidism impedes AT development (295). Overall, studies on the involvement of thyroid hormones in AT development are controversial. While the induction of adipogenesis is differentially regulated by various thyroid hormone receptor (TR) isoforms, studies largely indicate that TRs promote adipogenesis in the majority of model systems (245).

Adipocyte Progenitors

In AT, pools of adipose stem/progenitor cells (APs) exist that can differentiate into mature adipocytes (296,297). At least two distinct progenitor populations give rise to adipocytes: developmental APs and adult APs (296,298). Our understanding of the molecular characteristics of APs has dramatically increased in recent years as discussed below.

APS IN ADIPOSE DEVELOPMENT

AT organogenesis in mice and humans begins during embryogenesis, and ends in the postnatal period for mice and just before birth in humans (296,297,299). AT is widely accepted to be of mesodermal origin (297). However, some of the spatiotemporal and molecular differences observed in formation of different AT depots suggest diverse developmental origins (297,300). Further, white and brown adipocytes, once considered to have common APs are now known to have different origins (297,301,302).

In the generation of white adipocytes, developmental APs express the master adipogenesis regulator PPAR γ but have distinct functional and molecular properties compared to adult APs (298,302). Developmental APs do not contain lipid but express the mature adipocyte markers perilipin and adiponectin, are able to replicate, and are located along the vasculature in developing white adipose tissue (298,302,303). Brown adipocytes can arise from myogenic Myf5-expressing precursors that also give rise to skeletal myocytes (297,302,304). Interestingly, brown-like adipocytes, known as beige adipocytes, emerge in white adipose from Myf5-negative precursors in response to cold or adrenergic stimuli, which suggests that the developmental origins of brown adipocytes and beige adipocytes are different (297,304). Collectively, these findings highlight the complex developmental heterogeneity of APs observed among adipose tissue depots in animals and humans.

APS IN ADULT ADIPOSE TISSUE

The notion that we are born with all the fat cells we will ever have is now considered archaic and inaccurate. Adipose tissue continues to generate new adipocytes throughout the lifespan, with a median adipocyte turnover rate of 8.3 years

(302,305). Adult APs have been found in the SVF of AT depots in both rodents and humans (302,306–308) and are thought to represent an AP pool that contributes to this adipocyte renewal. Flow cytometry techniques that use a variety of cell surface and stem cell markers, have helped identify stromal cells that can undergo adipogenesis (302,306,307,309). These adult APs arise from tissue-resident mesenchymal stem cells, and are a major source of new adipocytes in AT (297,310). Bone marrow-derived APs from the myeloid lineage can also be recruited to AT where they become adipocytes (Figure 10). Bone marrow-

derived adipocytes (BMDAs) are more abundant in female mice and are more frequent in visceral depots (297,311,312). Though BMDAs have been observed in human AT and are increased in patients with obesity, the processes and factors involved in BMDA recruitment to AT remain unclear. Compared to normal adipocytes, BMDAs have reduced expression of lipid metabolism genes and increased pro-inflammatory gene expression, suggesting that they may have negative metabolic effects (297,311,313).

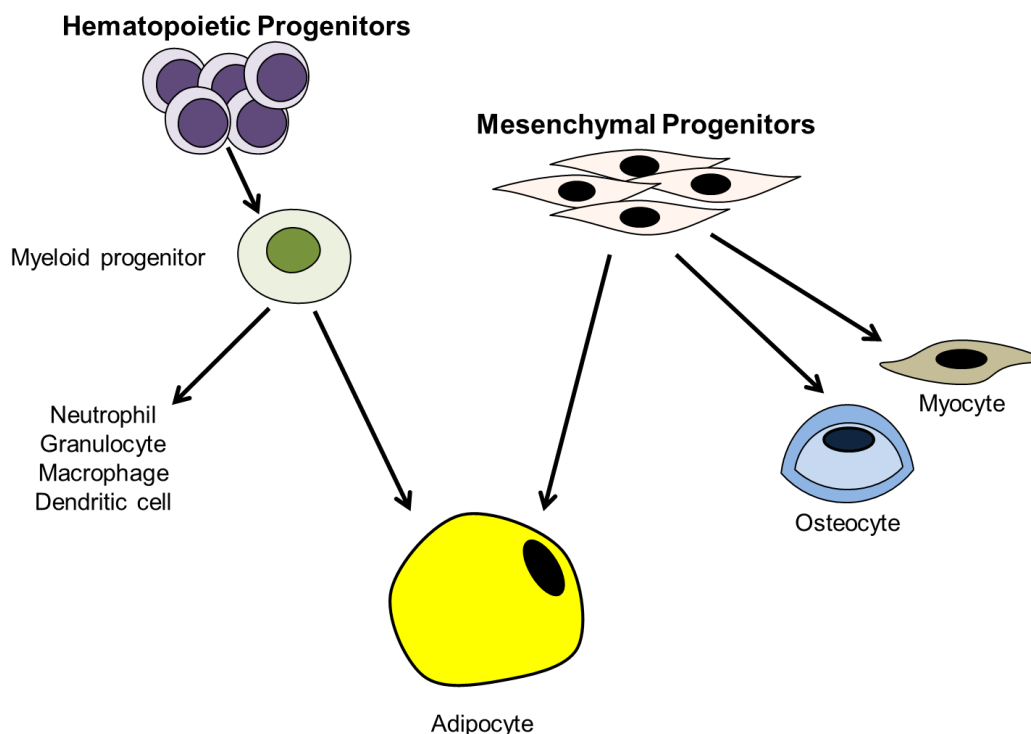


Figure 10. Adipocytes are derived from both resident mesenchymal cells in the stromal-vascular fraction of adipose tissue and hematopoietic progenitors that reside in the bone marrow. In addition to adipocytes, mesenchymal progenitors can form other connective tissue cells, such as myocytes and osteocytes. Myeloid progenitors derived from hematopoietic progenitors in bone marrow give rise to adipocytes as well as neutrophils, macrophages, dendritic cells, and granulocytes.

Most of the information regarding AP proliferation in obesity comes from rodent models. In mice fed high-fat diets to induce obesity, APs form new adipocytes primarily in the visceral depot (299,302). Although limited data report decreased AP proliferation and differentiation capacity from humans with obesity compared to lean individuals (314), convincing evidence for depot-specific AP populations in humans has emerged. Subcutaneous APs were shown to have a higher growth rate and adipogenic potential than

visceral APs, giving rise to more functional adipocytes (315,316). Increasingly sophisticated methods for assessing APs in mice will help facilitate the identification of the origins of all APs for each adipose depot as well as the niches in which they reside.

Adipose Extracellular Matrix: From Normal Development to Fibrosis

An underappreciated influence on AT physiology is the adipose extracellular matrix (ECM). The dynamics and composition of the ECM are critical for proper adipocyte development and function (317). During adipogenesis, there is increased synthesis of laminar ECM constituents and maintenance of peri-adipocyte fibrillar collagens that ultimately allows the adipocyte to embed itself in the basal lamina (317). In the growth phase of adipogenesis, adipocytes require ECM-mediated traction forces to

properly accumulate lipids and increase in size. A number of inhibitors, enzymes, and modifiers contribute to adipocyte ECM maintenance and renewal; these reactions consume a large amount of energy in the mature fat cell (317). In obesity, the ECM expands to accommodate the adipocyte hypertrophy and hyperplasia, and subsequent tissue growth, induced by the increased demand for lipid storage (317–321). This process appears to occur in a similar fashion in both animal models and humans.

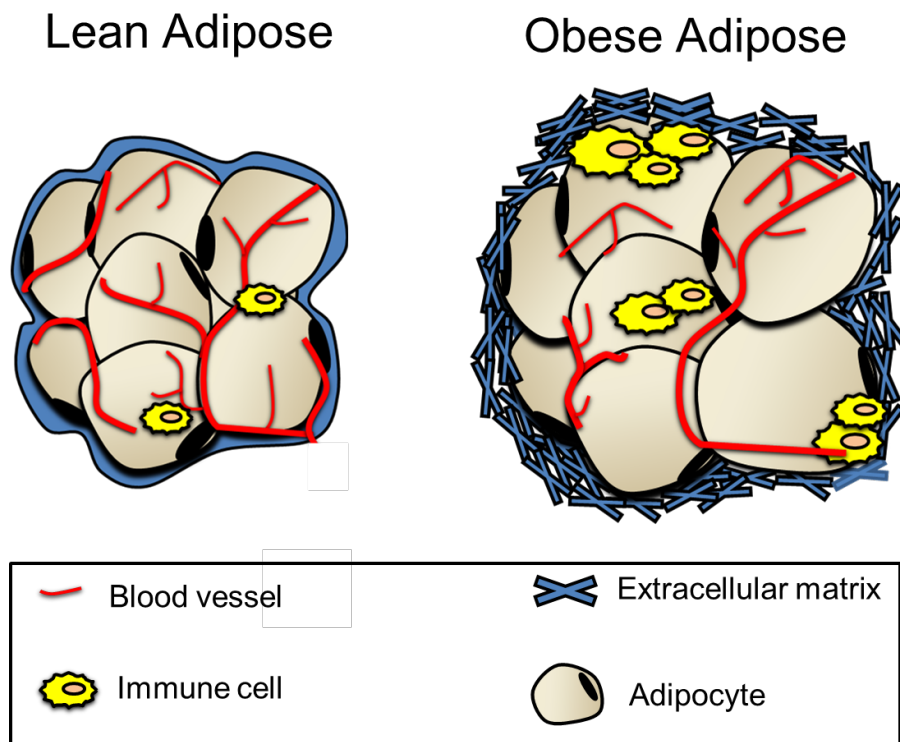


Figure 11. Differences in AT between lean and obese mammals. The AT extracellular matrix (ECM) is important for normal tissue function but can also contribute to its dysfunction. In obesity, accumulation of ECM components can restrict AT expansion, promote inflammation by recruiting immune cells, and impair adipogenesis. These combined effects can worsen insulin resistance.

Adipose tissue expansion during obesity, coupled with immune cell accumulation and hypoxia, can lead to AT fibrosis (Figure 11) (317,319,322). Fibrosis is the excessive accumulation of ECM components, such as collagens, that typically results from an imbalance of the synthesis and degradation of ECM components (319,323). Ultimately, adipocyte dysfunction will result from the decreased ECM flexibility conferred by the accumulation of fibrillar ECM components (317,320,323). Abnormal ECM collagen deposition is associated with immune cell infiltration, which can worsen fibrosis and contribute to AT dysfunction that

often occurs in obesity (319,323). The removal of collagen VI, a major AT ECM component, improves adipocyte function and metabolism in obese mice by both decreasing AT immune cell infiltration and “weakening” the ECM, which allows uninhibited adipocyte hypertrophy (317,318,323). In humans, AT collagen VI expression is increased in obesity, and subjects with higher collagen VI have increased macrophage content and AT inflammation (324). Endotrophin, an adipocyte-derived cleavage product of collagen VI, directly stimulates AT fibrosis and macrophage accumulation, and can lead to systemic insulin resistance

(325). Endotrophin can also cause fibrosis and endothelial cell migration in mammary tumors, leading to tumor expansion and the enhancement of metastatic growth (325,326).

Accumulation of ECM components and increased ECM-receptor signaling are associated with insulin resistance in obesity thought to be mediated by several possible mechanisms. In addition to physically restricting AT expansion, excess ECM components can also increase AT inflammation by interactions with their cell surface receptors (CD44, CD36, and integrins) (320). These ECM-receptor interactions can induce adipocyte death, inhibit angiogenesis, and promote macrophage infiltration and inflammation in adipose tissue, thereby driving insulin resistance (320). Interestingly, these downstream effector pathways of ECM-receptor signaling are similar to those involved in tumor growth and pulmonary fibrosis development.

The ECM has clear roles in the normal development and function of adipocytes, but in excess can also play roles in obesity development and metabolic dysfunction. Our understanding of the adipose ECM has deepened in recent years, but more research is necessary to better delineate how ECM components and their interactions can directly influence AT physiology and pathophysiology. Since many AT cell types produce ECM components, studies to determine the specific contributions of adipocyte-derived ECM components to normal AT function as well as dysfunction will be required.

Rodent versus Human Adipose Depots

Much of the knowledge about the depot-specific characteristics and metabolic profiles of AT has been obtained from rodents. However, the validity of translating studies conducted in rodent fat to humans remains controversial. Relative to humans, rodents have substantially more BAT and rely heavily on this highly-inducible depot to stimulate thermogenesis (327). While BAT activation in rodents has been shown to elicit beneficial effects, including improvements in glucose and lipid metabolism (328,329), BAT function in humans is more controversial. Overall, the majority of studies have reported that the amount of active BAT in humans appears

insufficient to induce meaningful changes in energy metabolism and, thus, is not thought to impact whole-body physiology and metabolic control in humans (330) as described in rodents.

With regard to white AT, notable differences exist with respect to fat depot structure and function between species (18). Humans have subcutaneous depots primarily in the abdominal and gluteal-femoral regions; whereas rodents have subcutaneous fat pads located anteriorly and posteriorly (Figure 12). With regards to location, the inguinal (posterior) fat pad in rodents is considered comparable to the gluteal and femoral depots in humans. Human subcutaneous

abdominal AT can be categorized as superficial SAT or deep SAT (331), which are morphologically and metabolically different. Deep SAT has been reported to be closely related to the pathophysiology of obesity-related metabolic complications, while superficial SAT is more closely related to the protective lower-body SAT (332–334). However, these subcutaneous layers are not present in rodents. In humans, intra-abdominal fat refers to visceral AT, which surrounds the inner organs, and includes omental, mesenteric, retroperitoneal, gonadal, and pericardial depots (335). For most purposes, however, when used in reference to human studies, visceral AT refers to omental and mesenteric depots that are quantified by abdominal computed tomography or MRI scans. On the other hand, visceral fat pads in rodents are classified as perigonadal (epididymal in males and periovarian in females), retroperitoneal, and mesenteric. While the mesenteric fat pad is most analogous to abdominal (visceral) AT in humans, it is not often studied in rodents due to surgical limitations. The perigonadal fat pads are the largest and most the readily assessable fat in rodents; hence, they are most frequently used in mouse studies and cited the most often in the literature as surrogates for human visceral AT. However, humans do not have an AT depot analogous to the rodent perigonadal fat pads. In addition, the omental depot is clearly defined in humans, but in mice it is difficult to detect. Overall, striking anatomical differences in AT distribution exist between rodents and humans, and these differences should be considered when interpreting rodent studies and potentially translating these observations to humans.

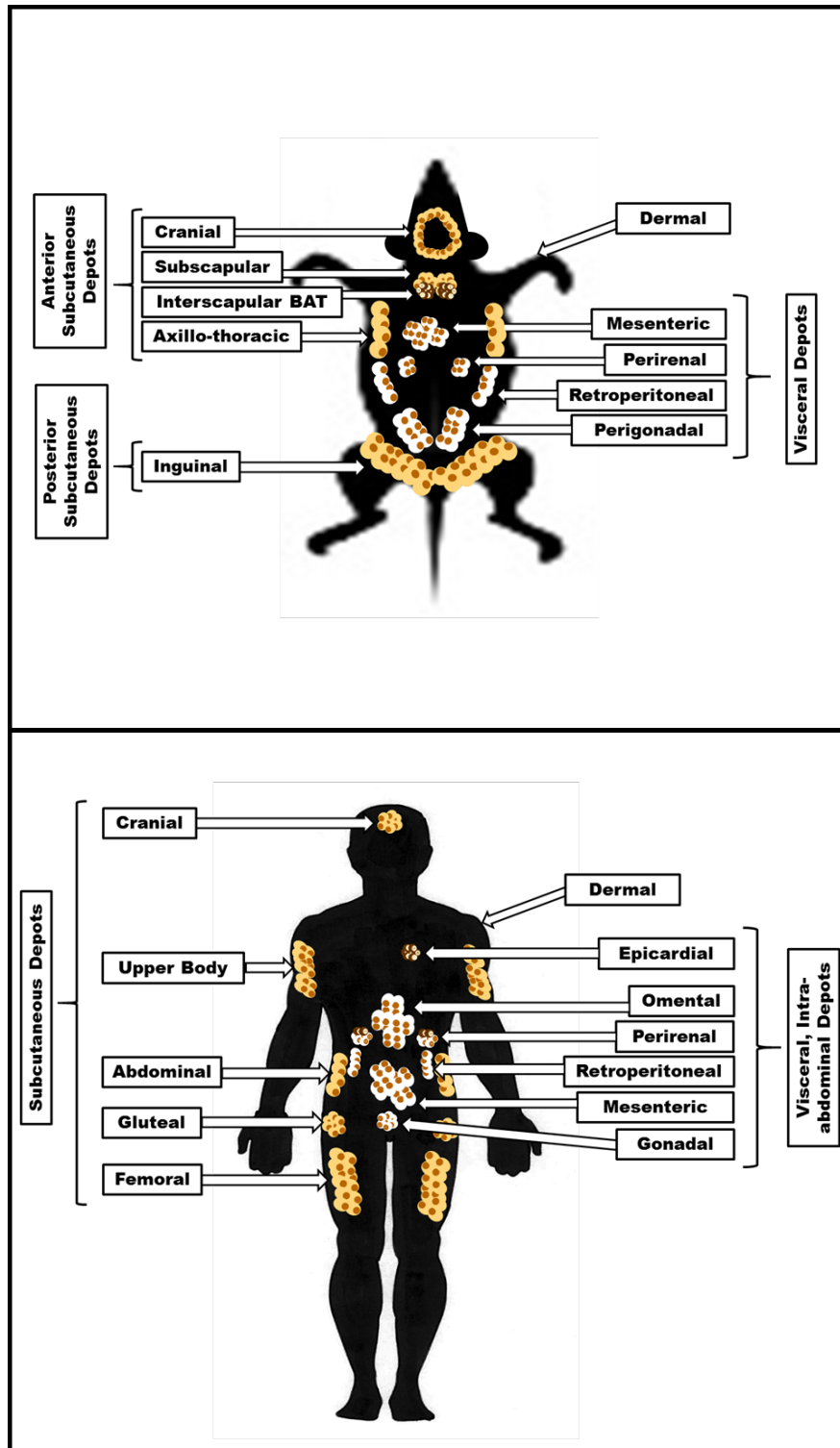


Figure 12. Rodent versus Human AT depots. Several differences exist between rodent and human subcutaneous (SubQ) and visceral AT depots. In the figure SubQ depots are colored as beige, while visceral depots are white, and BAT or BAT-like depots are brown.

It is well-established that the various adipose depots display metabolic heterogeneity and are intrinsically different within

each species. In humans, fat deposition in the upper body, mainly the visceral but also the subcutaneous abdominal

depot, is linked to a higher risk of metabolic dysfunction; while lower body adiposity in the subcutaneous gluteal and femoral regions is associated with lower risk and may even be protective (336). Rodent studies reveal that surgical removal of visceral fat pads improves insulin action, glucose tolerance, and longevity (337,338), while the removal of subcutaneous fat pads can cause metabolic syndrome (339). In addition, subcutaneous, but not visceral, donor AT transplanted into the visceral region of recipient mice improves glucose metabolism (340). In contrast, human studies have shown that the removal of small amounts of omental AT in individuals with obesity provided no metabolic health benefits (341). Likewise, liposuction (~10 kg) of subcutaneous AT in humans neither harmed nor improved the cardio-metabolic profile (342,343). Nevertheless, fat is redistributed from the subcutaneous to visceral depots during aging (344) in conjunction with increasing prevalence of chronic diseases such as hypertension, T2DM, and cardiovascular disease, suggesting that subcutaneous AT may be metabolically beneficial in humans as has been extensively reported in rodents.

Studies of depot-specific expression patterns have enhanced our understanding of the mechanisms underlying abdominal versus gluteal and femoral adiposity (345–347). Unique expression patterns in different adipose tissue depots in mice indicate substantial difference in the expression of homeobox (HOX) developmental genes (348). Not surprisingly, HOX genes exhibit differential expression patterns in human compared to mouse fat depots (346,347). In contrast, structural and hormonal regulators, including collagen VI (349,350) and glucocorticoids (351,352), respectively, that influence fat distribution are similarly associated with AT expansion in both rodents and humans.

Similar to humans (353), female rodents have a higher percentage fat mass relative to males, yet remain more insulin sensitive (354). However, there are many notable sex differences in rodent versus human depots. The inguinal depots of female mice contain mammary glands and the gonadal fat pad is near reproductive tissue, which is not the case in humans. In addition, high-fat diet-induced obesity affect men and women alike, but in many strains of mice females are resistant HFD obesity, unlike male mice (355,356). Furthermore, the periovarian (visceral) fat pad in female mice has been shown to be more insulin sensitive

than the inguinal fat pad (354), which is contrary to human data that indicates in women the gluteal and femoral depots are more insulin sensitive relative to the visceral AT (357).

Current literature suggests that the secretion patterns of adipokines (including leptin, interleukin-6, and tumor necrosis factor α) in the visceral versus subcutaneous depots of humans are relatively similar to that of rodents. Interestingly, lower body AT has been shown to secrete more metabolically favorable adipokines such as adiponectin (358). These observations are similar in rodents studies (340).

While lipolysis can be stimulated in rodents and humans under similar physiological conditions, important biological differences in AT lipolysis among these species have been suggested. The $\beta 1$ and $\beta 2$ adrenergic receptors (AR) are ubiquitously expressed in rodents and humans, while $\beta 3$ -AR expression is confined to white AT in rodents and only marginally expressed in human adipocytes (359). The $\alpha 2$ -ARs are highly expressed in the subcutaneous AT of humans and act to inhibit lipolysis (360), but are absent in rodent adipocytes. Though common factors, including catecholamines, growth hormone, and cortisol, are similar among species in regulating lipolysis, differences in the response to other lipolytic agents have also been reported. Natriuretic peptide induces lipolysis in humans, but not in rodents (361), while adrenocorticotrophic hormone and alpha-melanocyte-stimulating hormone modulate lipolysis in rodent but not human adipocytes (362,363). Therefore, it is important to account for these differences and commonalities in AT lipolysis among species.

Rodent studies are essential to expand our understanding of pathways underlying the associations between fat distribution and metabolic health and disease. Fortunately, there are many shared traits among rodent fat pads and human fat depots. However, given the clear differences in adipose depot location and physiology between the species, interpretation of experimental data and the extrapolation of conclusions drawn from rodent data to humans should be conducted with appropriate caution and caveats.

Dermal Adipose Tissue

A thick layer of adipocytes, historically referred to as subcutaneous AT, underlies the reticular dermis in both

rodents and humans (364). Recent studies have revealed major differences between the adipocytes from this dermal layer and more typical subcutaneous adipocytes found in other locations (364–366). Today, dermal adipose tissue (dWAT) is considered a separate adipose depot that is distinguishable from subcutaneous fat (364). Two unique features of dermal adipocytes in this regard are that they can alter their cellular characteristics and have high turnover rates (366). An additional distinguishing factor for dWAT is its organization. In rodents, dWAT forms several adipocyte layers between the dermis and muscle layer (panniculus carnosus) (367). Human dWAT is present as individual units referred to as dermal cones. These cones are concentrated around pilosebaceous units that functionally interact with each other to form the dWAT structure (366,368). Interestingly, only body regions prone to scarring contain dermal cones (368), indicating a potential role for dWAT in scarring and wound healing. Also, dWAT can regenerate after injury. Following injury, adipogenesis is activated in the proliferative phase of wound healing and dermal adipocytes repopulate the wound (366,367). This is a critical event, as mouse models lacking mature adipocytes cannot recruit the fibroblasts required for wound healing (369–371).

Other identified roles for dWAT include insulation (372), barrier protection from skin infection (373), and hair follicle cycling (374). It is well known that brown adipose tissue (BAT) rapidly responds to cold temperature challenges by mobilizing lipids for heat generation (adaptive thermogenesis), yet dWAT slowly responds to these challenges by thickening/expanding over days to provide an effective layer of insulation (367,372). Mouse models lacking adequate dWAT undergo chronic activation of BAT since the dWAT cannot provide adequate mitigation of body temperature (367). Conversely, obese mice with excess dWAT undergo minimal adaptive thermogenesis (367). The dWAT thickening observed with cold exposure also occurs with bacterial exposure. Adipocytes in dWAT differentiate and become hypertrophic and result in a thicker dWAT layer in response to epidermal *Staphylococcus aureus*. This dWAT adipocyte reaction is also critical for immune response to bacterial invasion (373). Hair follicles go through repeated rounds of death and regrowth, referred to as the hair follicle cycle (367). Robust dWAT expansion is characterized by increased adipogenesis and dermal adipocyte hypertrophy that accompanies the regrowth of hair follicles (374). Conversely, inhibiting adipogenesis

impedes hair follicle regeneration. In several species of mammals, a thickening of the hair coat accompanies dWAT expansion in response to cold exposure (367). In summary, dWAT has distinct roles from subcutaneous AT. Thus far, unlike other AT depots, the contribution of dWAT to metabolic health has not been investigated. Nonetheless, there is clear evidence that dWAT has distinct structures and functions and plays a role in variety of physiological processes.

Epicardial AT

Epicardial AT (EAT) has recently emerged as an important player in the development of cardiovascular disorders (375,376). Notably, EAT is distinct from pericardial fat. While pericardial AT surrounds the pericardium, EAT lies between the visceral pericardium and the myocardium and shares a blood supply with the coronary arteries (375–378). The adipocytes in EAT are smaller than those in other visceral or subcutaneous depots and are outnumbered by preadipocytes; this is thought to be related to the high energy requirement of the heart, which normally favors oxidation of fatty acids over other substrates (376,379). Furthermore, the gene expression and adipokine secretion profiles of EAT are unique from those of other depots (376,380,381).

In normal physiological conditions, EAT behaves like BAT and serves to protect the coronary vessels and myocardium against hypothermia (376,382). In pathologies such as coronary artery disease and type 2 diabetes, EAT can display an extensive pro-inflammatory signature (383–385). Macrophages and mast cells have been shown to infiltrate EAT, undergo activation, and through a cascade of signaling events facilitate lipid accumulation in atherosclerotic plaques (376,384). Pro-inflammatory adipokine secretion from EAT has also been shown to induce atrial fibrosis (381). Further, insulin sensitivity and EAT thickness are inversely correlated, whereas fasting glucose and EAT size are positively correlated, with enlarged EAT depots often found in individuals with type 2 diabetes (376,386,387). These data suggest that EAT functions as a distinct fat depot with important physiological and pathological roles.

METABOLIC DYSFUNCTION ASSOCIATED WITH ADIPOSE TISSUE

Adipose Tissue Expandability and Metabolic Health

White AT retains the ability to expand during adult life to accommodate chronic excess caloric intake. AT expansion is characterized by adipocytes accumulating lipid and growing in size (hypertrophy) or number (hyperplasia or adipogenesis) or increasing in both size and number. Evidence suggests that the capacity of subcutaneous AT to expand as well as the manner of expansion (hypertrophy vs. hyperplasia) can influence cardiometabolic health. This mechanism is thought to underlie the benefits of thiazolidinedione (TZD) medications, which are approved for the treatment of type 2 diabetes (388,389). These PPAR γ agonists stimulate preadipocyte differentiation and the proliferation of adipocytes (390,391), especially in subcutaneous depots as compared to visceral adipose tissue (392), which leads to increased adiponectin levels and improved insulin sensitivity (393,394). Hence, there is a clear rationale to further characterize the mechanisms of AT expansion through adipocyte proliferation in humans that may inform future effective drug therapies.

On the other hand, the presence of enlarged, hypertrophic adipocytes, a lack of hyperplasia, and development of AT inflammation and fibrosis reflect impaired AT expansion and is associated with metabolic derangements (395–398). These observations support the “AT expandability hypothesis”, which postulates that a lack of adipogenesis (or hyperplasia) results in the limited capacity of AT to expand and store lipid, causing ectopic fat accretion and “lipotoxicity” in non-adipose tissues such as skeletal muscle and liver (399–401). The degree of ectopic lipid deposition in the liver and skeletal muscle is a significant determinant of metabolic syndrome (MetS) and the development of T2D and CVD (402).

Other findings do not support the AT expandability hypothesis and indicate that higher adipogenesis does not necessarily denote improvements in metabolic health. These studies report a higher population of small adipocytes (a measure of hyperplasia) in the AT of individuals with insulin resistance and T2D (403–406) and in those with more visceral AT and liver fat (406,407). Experimental overfeeding intervention studies have shown that individuals with smaller adipocytes at baseline have poorer metabolic health outcomes (i.e. impaired insulin sensitivity) in response to substantial weight gain than those with larger adipocytes (408,409). In addition, one *in vivo*

analysis in humans demonstrated that increased hyperplastic expansion correlated with an increased number of metabolic syndrome components (410). Collectively, these data imply an alternative model of impaired AT expansion, as compared to the mechanisms proposed by the AT expandability hypothesis, and suggest that there is not a deficiency in hyperplasia but an abundance of adipocytes with a limited capacity to adequately expand and accommodate lipid, whether large or small. This inability to store excess lipid in AT is thought to be a key feature that leads to metabolic dysfunction.

Although the mechanisms of adipose expansion and its precise role in promoting glucolipid dysregulation remain a matter of debate, all of the aforementioned studies support the view that AT’s capacity to expand is intimately related to metabolic homeostasis, as the failure to store excess lipid appropriately in AT can contribute to many obesity-related complications.

AT Inflammation

A variety of cell types from both the innate and adaptive immune systems have been found in AT (411–413). Though resident AT immune cells are critical to normal adipocyte function in healthy individuals, AT inflammation, as mentioned in several preceding sections, is considered a major contributor to the metabolic dysfunction associated with obesity (413,414).

During nutrient excess as AT expandability reaches its limit, a strong association exists between adipocyte size and adipocyte death (415). In response to adipocyte death, pro-inflammatory macrophages surround dead and dying cells and remove debris from the damaged area. During this process, macrophages acutely produce inflammatory cytokines (413,416). In obesity, this cytokine production often fails to resolve, becomes chronic, and leads to impaired adipocyte insulin signaling, further inflammation, and a continued worsening of AT dysfunction (413,416,417). In a field that is rapidly changing, it is worth mentioning that some degree of inflammatory signaling might be required for normal AT function. The pro-inflammatory cytokines TNF alpha and oncostatin M have been shown to be required for proper AT expansion and maintenance of insulin sensitivity in mice (414,418–420). Although AT inflammation clearly has detrimental effects in obesity, evidence also indicates adaptive and homeostatic

roles for pro-inflammatory signaling in AT expansion and function.

Metabolically Healthy (MHO) versus Metabolically Unhealthy (MUO) Obesity

An estimated 10-30% of individuals with obesity are considered to have “metabolically healthy obesity” (MHO) with favorable metabolic profiles (421). Although there is currently no consensus for parameters used to classify MHO, these individuals are characterized by normal insulin sensitivity, normal fasting glucose levels, low incidence of

hypertension, and blood lipid profiles in the healthy range (422,423) (Figure 13). In contrast, individuals with “metabolically unhealthy obesity” (MUO) have comparable body mass indices (BMI) but develop metabolic aberrations. Factors that distinguish individuals with MHO from MUO (Figure 13) highlight the premise that metabolic health risk is not solely dependent on body weight and are described in more detail below. Understanding these characteristics and potential mechanisms underlying the MHO and the perceived healthy metabolic state of these individuals is an important area of ongoing research.

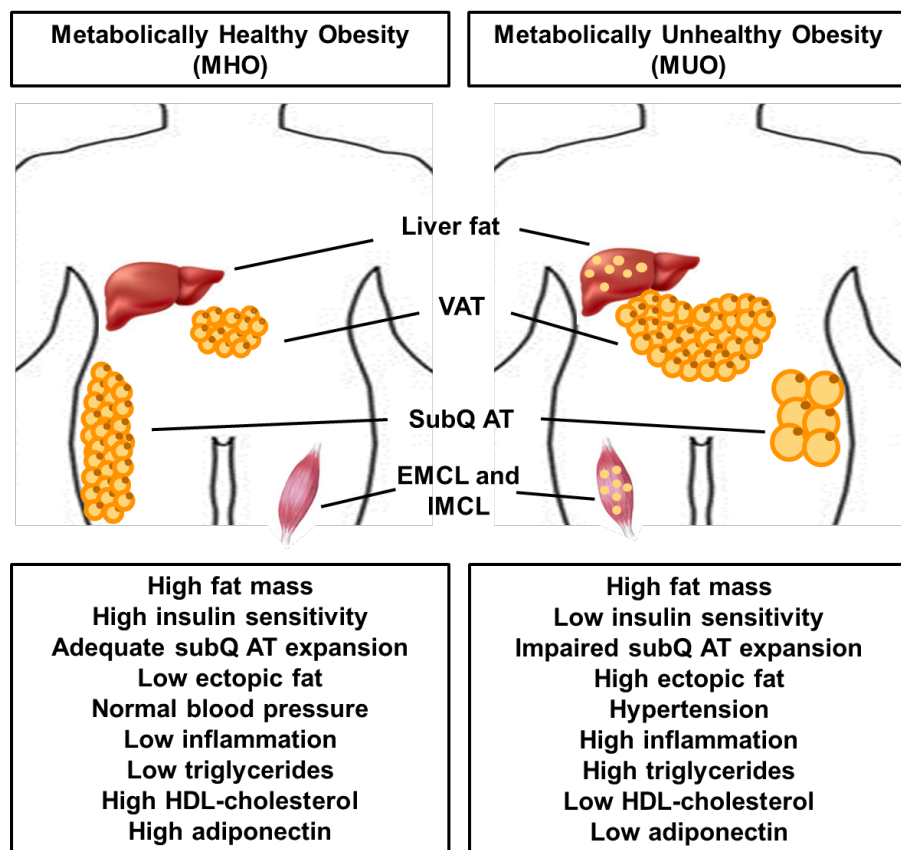


Figure 13. Clinical and biological factors thought to distinguish metabolically healthy obesity (MHO) from metabolically unhealthy obesity (MUO). Abbreviations: VAT – Visceral AT, SubQ AT – Subcutaneous AT, EMCL - extramyocellular lipid; IMCL – intramyocellular lipid; HDL – high density lipoprotein.

Evidence suggests that WAT plays a critical role in the development of MHO vs MUO, as its properties, location, and function are closely linked with cardiometabolic risk. Fat distribution (422), as well as changes associated with AT expansion, including the capacity for adipocyte differentiation (403) and parameters related to ECM remodeling (424), may also contribute to the MHO

phenotype. In addition, adipose-derived circulating factors that impact whole-body metabolism have been implicated in MHO vs MUO differences (425). However, studies have shown that the location of AT, rather than overall obesity, may be a stronger predictor of metabolic health risks (336). The accumulation of upper-body fat, namely visceral AT (VAT) but also subcutaneous abdominal (scABD) adipose

tissue, confers a higher risk of obesity-related disorders (426), while lower-body fat (subcutaneous gluteal and femoral) may be metabolically protective (427). The preserved metabolic function of individuals with MHO may be attributed to significantly lower accumulation of VAT relative to MUO (422,428,429). As described in the previous section, enlarged adipocyte size, independent of adiposity, is positively correlated with the development of insulin resistance and impaired metabolic health (396). MUO individuals have been shown to have larger adipocytes than their MHO counterparts (430,431). Hypertrophic adipocytes may represent the failure of subcutaneous AT to expand and store excess fat, which can ultimately lead to ectopic lipid deposition in non-adipose tissues such as the liver and skeletal muscle (402).

Ectopic lipid accumulation in both the liver and skeletal muscle is of pathophysiological significance as part of the “lipotoxicity” hypothesis and may also impact the varying health risk of MHO vs MUO. Extramyocellular lipid (EMCL) and intramyocellular lipid (IMCL) are postulated to cause defects in insulin signaling and reduce insulin-stimulated skeletal muscle glucose uptake (432). These lipid stores are strong correlates of insulin resistance and are increased in individuals with T2D (402). Paradoxically, increased IMCL is also observed in ‘insulin sensitive’ athletes, which may be attributed to the oxidative capacity of skeletal muscle (433) and increased glucose transport in trained muscle (434). Intrahepatic lipid accumulation strongly associates with impaired insulin-induced suppression of hepatic glucose production, even independently of visceral AT amount, and the development of T2D (435). Ectopic fat in both the liver and skeletal muscle has been shown to be lower in MHO than MUO individuals (422,436,437) (refer to Figure 13).

The differential secretion of pro-inflammatory adipokines has also been proposed as a mechanism underlying the MHO phenotype (438–440) by some investigators, although others have reported conflicting results (441). Nevertheless, studies show reduced macrophage infiltration in MHO (442,443), supporting a reduced inflammatory state in these individuals. Intriguing data implicating potential genetic differences among MHO vs. MUO indicate that specific polymorphisms in genes, including the adiponectin receptor 1 and hepatic lipase, may be associated with the MHO phenotype (436). In addition, genes encoding some proinflammatory cytokines can be more highly expressed in

the adipose tissue of MUO compared with MHO individuals (444,445).

A lingering question that remains unanswered is whether MHO subjects will sustain a healthy metabolic state throughout their lifespan or if they will eventually become MUO. An additional question is if a healthy lifestyle can help to maintain a favorable profile and prevent the transition to MUO. Indeed, longitudinal data clearly show that not all MHO individuals remain metabolically healthy, as up to 30% progress to MUO over a 5-10 year time frame (446–448). Of note, the length of time for follow-up assessments of MHO individuals is an important factor that may have considerable effects on the observed outcomes, because the total number of years as obese and aging can independently increase mortality risk. A major obstacle in advancing the understanding of the MHO phenotype is the manner by which metabolic health is described, including the parameters used to define insulin sensitivity and metabolic syndrome (449). Defining metabolic outcomes based on differing criteria can result in a broad range of reported prevalence, discrepancies regarding the observed characteristics, varied interpretations of health and mortality risks, and disagreement concerning the implications of therapeutic interventions. In addition, the use of the “healthy” descriptor may be misrepresentative of the true medical risks to these individuals, as long-term adverse health outcomes have been observed in individuals with MHO during follow-up years, thus no longer characterizing them as “metabolically healthy” (450,451). Additional long-term prospective studies are necessary to assess features of the MHO phenotype and to observe how the factors discussed above are altered over time. In addition, these studies may reveal if WAT function is a cause or consequence of the MHO and MUO phenotypes.

Lipodystrophy

While excessive adiposity, or obesity, can have adverse health consequences, deficiency of AT mass, as seen in lipodystrophy, can also lead to derangements in glucolipid metabolism. Lipodystrophy encompasses a group of rare, heterogeneous, genetic or acquired disorders characterized by varying degrees of severe reduction or absence of body fat (452). Anatomically, this disease can present as a partial (i.e. localized to certain body areas) or generalized lack of AT. The combined overall prevalence of lipodystrophy is estimated to be 1 in 1,000,000 individuals, with ~1000

patients reported with genetic forms (453). Lipodystrophy associated with highly active antiretroviral therapy for HIV is one of the most common acquired forms worldwide (454). The diagnosis of this disorder mostly relies on clinical criteria. In most cases of generalized lipodystrophy, standard physical examination is sufficient to establish this diagnosis. In contrast, partial lipodystrophy may be represented by mild physical abnormalities and can sometimes be misdiagnosed as common forms of central (abdominal) obesity, suggesting that this form of lipodystrophy may be an underestimated condition (455). Although the pathological basis of most lipodystrophies remains unclear, it is well-accepted that AT dysfunction is a primary determinant of the resulting health consequences in these patients. Limited development and non-expandability of AT and failure of AT to accommodate excess lipid leads to the redistribution and storage of fat ectopically in the liver and skeletal muscle and the development of non-alcoholic fatty liver disease, often severe insulin resistance and type 2 diabetes, hypertriglyceridemia, and associated diseases (456–458).

Markedly reduced levels of leptin and adiponectin may also contribute to the pathology of lipodystrophy. As described above, leptin plays an important role in the regulation of body weight and energy metabolism, and leptin deficiency is common in lipodystrophic patients, due to the lack of AT (452). Low leptin levels can not only impact glucose metabolism (459) but also contribute to increased appetite and excessive caloric intake in these patients (460). Transgenic animal models shed light on the pathology of lipodystrophy and confirm the importance of AT in normal physiology. Fatless rodents, created via AT ablation, display hypertriglyceridemia and ectopic lipid accumulation, along with severe insulin resistance (457,461). In addition, several groups have successfully treated the metabolic derangements in these fatless mice by transplanting AT from wild-type animals (461–464). However, transplantation of AT from leptin-deficient mice did not improve the metabolic abnormalities in fatless mice (465), while leptin administration in the fatless mice ameliorated insulin resistance and hepatic steatosis (466). In humans with total lipodystrophy, leptin treatment also markedly improves the severe hypertriglyceridemia and insulin resistance that accompanies this disorder (467). These studies confirm that both AT and leptin deficiency play a central role in lipodystrophy-associated pathologies.

Adiponectin has insulin-sensitizing and anti-inflammatory effects, and low levels of this adipokine have also been observed in patients with lipodystrophies (468). Recombinant adiponectin, adiponectin analogues (i.e. osmotin), and compounds that upregulate endogenous adiponectin (i.e. TZDs) have all been proposed as treatment approaches for lipodystrophy (469). In a fatless mouse model, treatment with the globular domain of adiponectin significantly improved the hyperglycemia and hyperinsulinemia characteristic of these lipodystrophic mice (146). Interestingly, the insulin resistance observed in these mice was completely ameliorated by treatment with both adiponectin and leptin, but only partially by either adiponectin or leptin alone (146), suggesting that both adiponectin and leptin deficiency may contribute to the insulin resistance in humans with lipodystrophy.

Studies to date support the premise that too little AT, as seen in lipodystrophy, appears to be just as deleterious as too much AT. Emerging data reveal that patients with lipodystrophy may have reduced survival and high mortality at an early age, predominantly due to cardiometabolic complications (470–472). Lipodystrophy has no cure; therefore, the primary treatment option is to improve metabolic outcomes via physical activity and dietary and pharmacological interventions. Conventional insulin-sensitizing agents, such as metformin and TZDs, are often used (453,473), and leptin replacement is also an approved therapy for total congenital lipodystrophy (467). Future investigations to better understand the pathogenesis and the clinical manifestation of lipodystrophy syndromes are essential for the development of improved therapeutics.

Adipose Tissue and Reproduction

While many studies have primarily examined the influence of white AT on the metabolic consequences associated with obesity, less frequently mentioned is the interplay between AT and reproductive health. Nevertheless, it is well established that AT is important for the normal function of the reproductive system, including the production and regulation of sex and reproductive hormones, pubertal development, and the maintenance of pregnancy and lactation (474).

Leptin and adiponectin are the most investigated adipokines as mediators of reproductive health and pathology. Receptors for both leptin and adiponectin have been

identified in all major reproductive tissues, including the testes, placenta, ovaries, oviducts and endometrium (475). Obese mice that are deficient in leptin or the leptin receptor are unable to reproduce (476). Although rare, humans with leptin mutations have been identified, and studies in these individuals have validated the infertility findings in rodents (477).

Leptin administration in rodents was shown to increase luteinizing hormone (LH), follicle stimulating hormone (FSH), and ovarian and uterine weights in females, and testosterone, testicular weights and sperm counts in males (478,479). During human pubertal development, there is a steady increase in leptin, stimulating a rise in testosterone levels and fat-free mass in boys and in estradiol and fat mass in girls (480,481). Adiponectin administration was shown to inhibit gonadotrophin releasing hormone (GnRH), LH, and FSH (482,483) in pigs and increase estrogen and progesterone (484) in rats, while circulating levels in humans have been shown to be associated with serum levels of sex hormones (primarily estrogens), though this correlation was largely mediated by body weight (485).

In humans, leptin levels increase during pregnancy and rapidly fall in the post-partum period (486), and other reports have suggested that adiponectin may influence the amount of gestational weight gain and weight maintenance post-partum, even after adjusting for the sum of skinfold thickness and BMI (487,488). The effects of leptin on fetal development continue to be investigated, and have been suggested to correlate with fetal growth, birth weight, and organogenesis (489). Adiponectin plasma levels were shown to be significantly lower in overweight patients than normal weight women during pregnancy and negatively correlated with progressive gestational age and weight gain (490). In addition, women with low adiponectin concentrations experienced a significantly increased risk of gestational diabetes mellitus (491,492), and large reductions in adiponectin levels during pregnancy may also predict large-for-gestational-age offspring and increased birth weight (493). Interestingly, several studies have shown that higher adiponectin levels are associated with increased conception success in women undergoing assisted reproduction approaches (494). Overall, these studies are consistent with adipose-derived leptin and adiponectin having critical roles in reproductive function.

Many lines of evidence also demonstrate that either insufficient or excessive AT can have detrimental effects on reproductive health. Women with lipodystrophy disorders (see above), are characterized by AT and leptin deficiency and are frequently infertile (495,496). Anorexia nervosa is an eating disorder characterized by very low AT mass that is often accompanied by amenorrhea (absence of at least three menstrual periods in a row) (497). It is estimated that ~38% of women affected by anorexia experience infertility (498). Leptin deficiency is common in these patients (499) and may lead to disruptions in downstream neuroendocrine signaling (500). This was tested when leptin replacement to women with hypogonadotrophic hypogonadism due to anorexia nervosa or excessive exercise was found to restore normal periods (501). Estrogen deficiency in these women results in major implications for bone health, ultimately contributing to increased osteopenia or osteoporosis (502).

Body weight has been shown to predict testosterone levels in men (503); and obesity, specifically central adiposity, is associated with low testosterone levels (504). Increased AT also leads to elevated estradiol, resulting in reduced circulating testosterone through feed-back inhibition of gonadotrophs (504,505). A common medical condition in women at the crossroads of dysfunctional AT and reproduction is polycystic ovary syndrome (PCOS), which in roughly 50% of affected women is associated with increased central obesity and metabolic health risk. PCOS is commonly defined using the consensus of Rotterdam, which requires two of three criteria: polycystic ovaries on ultrasonography, hyperandrogenism, or amenorrhea. Studies of PCOS generally show that adiponectin levels are lower in these patients (506). Another burgeoning area of research is the study of excessive AT and reproductive malignancies, as obesity is known to increase the risk of breast, uterine, cervical, and prostate cancers (507). Studies have reported inverse relationships between leptin and adiponectin levels with breast, endometrial, ovarian, and prostate cancers (508,509).

EMERGING AREAS IN ADIPOCYTE BIOLOGY

Critical considerations in the study of fat tissue are its cellular complexity and heterogeneity. AT depots can exist in close association with other organs and act physiologically as metabolic “sinks” that store excess energy as lipid in a protective manner, or they can promote

systemic metabolic dysfunction by secreting excess lipid or inflammatory adipokines. As the recognition of distinct AT depots increases, so does our understanding of their diversity. A recent review considers the locations and functions of several depots, ranging from facial AT to cardiovascular AT as well as the presence of adipocytes in bone marrow, within and between muscle beds, and joints (510). Currently, we are experiencing a new and exciting period in AT research with the focus shifting toward recognizing neglected AT depots, the expanding types of adipocytes, and the complex developmental and sex-regulated origins of adipocytes. Adipocytes are critical secretory cells that contribute a variety of circulating proteins, including endocrine hormones. Of course, adipocytes also produce lipids and can release genetic material that can have profound systemic functions.

Much remains to be discovered about the types of nerves present in fat tissue and how they vary according to AT type and location. How these AT nerves act to regulate metabolic homeostasis is a current focus of fat cell biology. Recent advances in whole tissue AT imaging and studies on brain-adipose communication suggest we are just beginning to uncover the capabilities and function of AT nerves, and there are many unanswered questions in this field (511). Research on the molecular pathways that connect AT innervation to insulin action in obesity and diabetes may provide insight into our understanding of the pathogenesis of metabolic disease states.

Another developing area of fat cell biology is the effects of exercise on adipocyte function. Recent studies have shown that transplantation of subcutaneous AT from exercise-trained mice improves glucose tolerance and insulin sensitivity in recipient, non-exercised mice (512), and strongly suggest that exercise favorably remodels AT to improve systemic metabolic health. Recently, an AT-derived lipid was shown to increase fatty acid uptake in skeletal muscle (513). The importance of AT to whole-body

energy metabolism is well established; yet, the impacts of different types of endurance or resistance exercise on adipose tissue dynamics remains largely understudied, particularly in the context of obesity and other metabolic disease states.

A newly discovered pathway shows that lipids can be released by adipocytes in the form of exosome-sized, lipid-filled vesicles (514). This process occurs independently of canonical lipolytic pathways, and adipocyte exosomes deliver excess lipid to local macrophages in obesity (514). Other novel pathways of paracrine regulation have also been demonstrated in AT. These paradigm-shifting observations demonstrated that extracellular vesicles (EV) from endothelial cells in adipose tissue can provide lipids and proteins to adjacent adipocytes. This EV communication between endothelial cells and adipocytes within AT is bi-directional and is regulated by fasting/refeeding and in conditions of obesity (515). These very recent observations reveal the highly complex signaling mechanisms that exist in AT.

Though it was once considered a mere energy storage site, AT is now considered an important endocrine organ and site of inflammatory cell signaling that governs not only survival but also plays critical roles in reproduction and in glucometabolic homeostasis. As scientific methods for the study of AT continue to rapidly evolve, so does our understanding regarding the metabolic, biomechanical, immune, and secretory functions of AT in normal physiology and metabolic disease.

ACKNOWLEDGEMENTS

The authors are grateful to Anik Boudreau and Christina Zunica for their assistance in editing and referencing the chapter. This work was supported by National Institutes of Health Grant R01 DK052968.

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