REVIEW ARTICLE

Adipose Tissue Biology: An Update Review

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Abstract

BACKGROUND: Obesity is a major health problem in most countries in the world today. It increases the risk of diabetes, heart disease, fatty liver and some forms of cancer. Adipose tissue biology is currently one of the "hot" areas of biomedical science, as fundamental for the development of novel therapeutics for obesity and its related disorders.

CONTENT: Adipose tissue consists predominantly of adipocytes, adipose – derived stromal cells (ASCs), vascular endothelial cells, pericytes, fibroblasts, macrophages, and extracellular matrix. Adipose tissue metabolism is extremely dynamic, and the supply of and removal of substrates in the blood is acutely regulated according to the nutritional state. Adipose tissue possesses the ability to a very large extent to modulate its own metabolic activities including differentiation of new adipocytes and production of blood vessels as necessary to accommodate increasing fat stores. At the same time, adipocytes signal to other tissues to regulate their energy metabolism in accordance with the body's nutritional state. Ultimately adipocyte fat stores have to match the body's overall surplus or deficit of energy. Obesity causes adipose tissue dysfunction and results in obesity – related disorders.

SUMMARY: It is now clear that adipose tissue is a complex and highly active metabolic and endocrine organ. Understanding the molecular mechanisms underlying obesity and its associated disease cluster is also of great significance as the need for new and more effective therapeutic strategies is more urgent than ever.

KEYWORDS: Obesity, Adipocyte, Adipose Tissue, Adipogenesis, Angiogenesis, Lipid Droplet, Lipolysis, Plasticity, Dysfunction.

Introduction

Obesity is increasing in an epidemic manner in most countries and constitutes a public health problem by enhancing the risk for cardiovascular disease and metabolic disorders such as type 2 diabetes (1,2). Owing to the increase in obesity, life expectancy may start to decrease in developed countries for the first time in recent history (3). The factors determining fat mass in adult humans are not fully understood, but increased lipid storage in already developed fat cells (adipocytes) is thought to be most important (4,5).

Adipose tissue has now moved centre stage in obesity research, there having been a revolution in our understanding of the biological role of the tissue over the past decade. Indeed, adipose tissue biology is currently one of the 'hot' areas of biomedical science – principally because it is now recognized as a major endocrine and signalling organ (6).

White adipose tissue is part of what Cinti has termed 'the adipose organ', which consists of two functionally distinct tissues – brown and white adipose tissue. Brown adipose tissue is specialized for heat production by non-shivering thermogenesis, and in this tissue the stored lipid droplets serve primarily as a fuel for the production of heat. In white adipose tissue, on the other hand, the stored triacylglycerols provide a long-term fuel reserve for the animal (6).

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In addition to fuel storage, white adipose tissue can act as a thermal insulator and protect other organs from mechanical damage. Two further features of the tissue should be highlighted. First, unlike most other organs, white fat is distributed in multiple depots in the body, both subcutaneously and internally, and clusters of adipocytes may also be located adjacent to, or embedded in, other organs such as the lymph nodes and skeletal muscle. A second important feature is that adipose tissue is not made up simply of mature adipocytes, which store the lipid, but contains a variety of other cells (e.g. fibroblasts, endothelial cells, macrophages) which constitute around 50% of the total cellular content (6).

Adipocyte number is a major determinant for the fat mass in adults. However, the number of fat cells stays constant in adulthood in lean and obese individuals, even after marked weight loss, indicating that the number of adipocytes is set during childhood and adolescence. Approximately 10% of fat cells are renewed annually at all adult ages and levels of body mass index. Neither adipocyte death nor generation rate is altered in early onset obesity, suggesting a tight regulation of fat cell number in this condition during adulthood. The high turnover of adipocytes establishes a new therapeutic target for pharmacological intervention in obesity (7).

The fat mass can expand by increasing the average fat cell volume and/or the number of adipocytes. Increased fat storage in fully differentiated adipocytes, resulting in enlarged fat cells, is well documented and is thought to be the most important mechanism whereby fat depots increase in adults (4,5).

The generation of adipocytes is a major factor behind the growth of adipose tissue during childhood, but it is unknown whether the number of adipocytes changes during adulthood (8).

The difference in adipocyte number between lean and obese individuals is established during childhood (7,8) and the total number of adipocytes for each weight category stays constant during adulthood. The small variation in adipocyte number for each BMI category demonstrates that this is a stable cell population during adulthood. This may indicate that the number of adipocytes is set by early adulthood with no subsequent cell turnover. Alternatively, the generation of adipocytes may be balanced by adipocyte death, with the total number being tightly regulated and constant (7).

Adipocytes can be generated from adult human mesenchymal stem cells and pre-adipocytes in vitro (9) and may undergo apoptosis or necrosis(10-12), but it is unclear whether adipocytes are generated in vivo (9).

Although the study by Spalding KL, et al show that the adipocyte number is static in adults, they also demonstrate that there is remarkable turnover within this population, indicating that adipocyte number is tightly controlled and not influenced by the energy balance. Thus, a tight regulation of adipocyte number, together with mechanisms maintaining their energy balance, may contribute to why obese individuals have difficulties maintaining weight loss (7).

Adipose tissue could play a crucial part in buffering the flux of fatty acids in the circulation in the postprandial period, analogous to the roles of the liver and skeletal muscle in buffering postprandial glucose fluxes. Adipose tissue provides its buffering action by suppressing the release of non-esterified fatty acids into the circulation and by increasing triacylglycerol clearance (13).

Adipose tissue buffering of lipid fluxes is impaired in obesity through defects in the ability of adipose tissue to respond rapidly to the dynamic situation that occurs after meals. It is also impaired in lipodystrophy because there is not sufficient adipose tissue to provide the necessary buffering capacity (13).

Much of Frayn's thesis would fit with the idea that, as adipocytes enlarge with fat storage, their efficiency as 'metabolic buffers' decreases. In this context, it is interesting that the thiazolodinedione insulin sensitizers act, via the nuclear receptor PPARγ, to stimulate adipocyte differentiation and to increase the number of small adipocytes (15,16). It could well be that the new, smaller adipocytes thus formed act as powerful 'buffers', avidly absorbing fatty acids in the postprandial period. Indeed, it has been proposed that an inability to differentiate new adipocytes as required for storage of excess energy underlies the development of Type II diabetes (17).

Adipose tissue metabolism is extremely dynamic, and the supply of and removal of substrates in the blood is acutely regulated according to the nutritional state. Adipose tissue possesses the ability to a very large extent to modulate its own metabolic activities, including differentiation of new adipocytes and production of blood vessels as necessary to accommodate increasing fat stores. At the same time, adipocytes signal to other tissues to regulate their energy metabolism in accordance with the body's nutritional state. Ultimately adipocyte fat stores have to match the body's overall surplus or deficit of energy (14).

Adipose tissue is now recognised as a highly active metabolic and endocrine organ. Great strides have been made in uncovering the multiple functions of the adipocyte in cellular and molecular detail (14).

Obesity

The development of obesity is dependent on the coordinated interplay of adipocyte hypertrophy (increased fat cell size), adipocyte hyperplasia (increased fat cell number), and angiogenesis. Evidence suggests that adipocyte hyperplasia, or adipogenesis, occurs throughout life, both in response to normal cell turnover as well as in response to the need for additional fat mass stores that arises when caloric intake exceeds nutritional requirements (18).

Recent reports have suggested that disruptions in sleep patterns, often linked to our '24-h' lifestyle, are associated with increased body fat and altered metabolism, although the cause–effect relationship for these associations has yet to be elucidated. Abnormal sleep/wake patterns likely alter intracellular circadian clocks, which are molecular mechanisms that enable the cell/tissue/organism to anticipate diurnal variations in its environment. The environment may include circulating levels of nutrients (e.g. glucose, fatty acids and triglycerides) and various hormones (e.g. insulin, glucocorticoids). As such, alterations in this molecular mechanism, in particular within the adipocyte, likely induce metabolic changes that may potentiate disrupted metabolism, adipose accumulation and/or obesity. Although diurnal variations in adipokines and adipose tissue metabolism have been observed, little is known regarding the molecular mechanisms that influence these events (19). Circadian clocks are defined as a set of proteins that generate self-sustained transcriptional positive and negative feedback loops with a free-running period of 24 h (20).

There are three major components to the circadian clock (i) input signals (zeitgebers or timekeepers) which reset the circadian clock; (ii) the circadian clock mechanism itself and (iii) the output from the clock (which manifests at the level of altered gene and protein expression, metabolism and/or function, depending upon the cell/organ) (19).

The role of the peripheral circadian clock mechanism within the adipocyte represents an exciting new field of study in pursuit of the causes of increasing obesity prevalence. Elucidation of the link between the adipocyte-specific circadian clock and obesity may have profound implications on the timing of obesity therapies (19).

One of the most important recent developments in obesity research is the emergence of the concept that obesity is characterized by chronic mild inflammation – paralleling the situation with other diseases. The basis for this view is that the circulating level of several cytokines and acute phase proteins associated with inflammation is increased in the obese. As adipocytes secrete a number of cytokines and acute phase proteins, it is considered that the expanded adipose tissue mass contributes, either directly or indirectly, to the increased production and circulating levels of inflammation-related factors in obesity. In other words, the state of inflammation in adipose tissue in obesity leads to an increased production and release of inflammation-related factors (6).

Previous studies have demonstrated that the enlargement of adipocytes is associated with substantial changes in metabolic functions, e.g. in lipid metabolism (21,22). It has been hypothesized that such alterations may contribute to the health risks of obesity. Recently, adipocyte size in the sc abdominal depot was identified to be a significant predictor for the future development of diabetes mellitus type 2 (23). Adipocytes are known to release a variety of factors, including cytokines, chemokines, and many other biologically active molecules, commonly called adipokines. These secreted products may be involved in the development of a chronic low-grade inflammatory state, which may represent the "common soil" for the pathogenesis of the metabolic and cardiovascular complications of obesity (24,25).

Obesity is linked to a variety of metabolic disorders, such as insulin resistance and atherosclerosis. Dysregulated production of fat – derived secretory factors, adipocytokines, is partly responsible for obesity – linked metabolic disorders (26).

Adipogenesis

Adipose tissue is the only organ in the body that can markedly alter its total mass from 10 – 20 kilograms in the normal state to several hundreds kilograms in subjects with monogenic disorders of obesity. After obesity is established, the adipose mass can be reduced to normal a few years after bariatric surgery or following a successful change in caloric intake/physical activity. How is this plasticity of adipose tissue possible? The adipose tissue mass can expand in two different ways. Pre – existing fat cells can accumulate more lipids so the volume of the cell increases. Alternatively, new fat cells are made from progenitor cells in the stroma of adipose tissue such as mesenchymal cells or already commited precursor cells (preadipocytes) (27).

It was demonstrated that about 10% of the total fat cell pool is renewed every year due to constant generation of new fat cells. However, this production is counterbalanced by an equal rate of fat cell death keeping the total amount

of fat cells constant overtime in adulthood, even marked body weight reduction. This turnover process was found to be much accelerated among obese subjects who have a two times higher rate of generation of new fat cells in spite of the same relative death rate of old fat cells as lean subjects. Thus, there is a marked ongoing adipogenesis in adult humans which may be an important factor for weight gain and difficulties in retaining weight loss after slimming (27).

Both adipocyte hypertrophy and hyperplasia occur during normal growth phases and during the development of obesity(8,28,29). Hypertrophy often precedes hyperplasia in a cyclic manner. Hyperplasia, herein referred to as ''adipogenesis,'' represents the complex process by which new fat cells are developed from adipocyte precursor fat cells called preadipocytes. Adipogenesis involves two major events – the recruitment and proliferation of preadipocytes followed by their subsequent differentiation into mature fat cells (31-33). ''Proliferation'' refers to the process by which preadipocytes replicate so as to increase fat cell number, whereas ''differentiation'' refers to the process by which undifferentiated, proliferating fibroblast-like preadipocytes become permanently cell cycle – arrested, spherical, lipid-filled and functionally mature fat cells (31). Differentiation is accompanied by dramatic alterations in cell shape as well as by molecular changes that lead to dramatic increases in the ability of the cell for lipid synthesis and increases in hormonal responsiveness specific to the specialized role of the adipocyte in energy homeostasis (33-35).

During embryonic development of adipose tissue, the cell-fate of pluripotent precursor stem cells is at first restricted by largely unknown mechanisms to multipotent mesenchymal stem cells (MSCs). Of mesodermal origin, MSCs can differentiate into a number of tissues including adipose tissue, cartilage, bone and muscle tissue (36). Once MSCs are directed toward the adipocyte lineage via a poorly understood process called ''determination,'' fibroblastic-appearing preadipocytes with the capacity or adipocyte differentiation are formed (Fig 1).

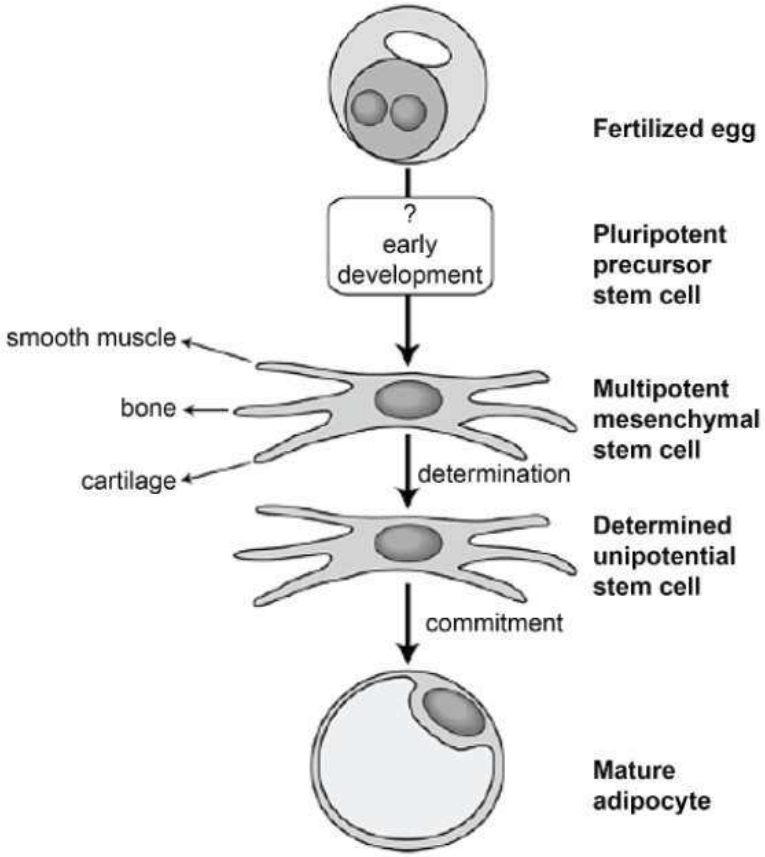

Fig 1. From egg to mature adipocyte (Adapted with permission from Avram MM et al, J Am Acad Dermatol 2007).

Recently, much attention has been given to the hormones, cytokines, and growth factors that modulate preadipocyte proliferation and adipocyte differentiation in positive or negative ways (31,34,37-40). Much of what is known about these stimulatory and inhibitory regulators has been gleaned from studies using in vitro models of adipogenesis. Widely expressed and secreted by adipocytes, (90) IGF-I is considered the most essential and universally effective paracrine adipogenic signal (31,42). IGF-I promotes both preadipocyte differentiation (43-45) and proliferation (44,46,47).

It has been shown that, both IGF-I and insulin, critical for early adipogenic events, are necessary for adipocyte differentiation induction in vitro and in vivo (43-48). Both hormones mediate their adipogenic effect via a common mechanism, the IGF-I receptor pathway, which represents a complex signaling cascade initiated by activation of the tyrosine kinase IGF-I receptor at the cell surface (45). Once triggered, the IGF-I receptor then, in turn, phosphorylates insulin receptor substrates, which subsequently activate distinct downstream signal transduction pathways with impact on adipogenic gene transcription, such as the up-regulation in the expression of PPARγand C/γBPα (49).

Glucocorticoids produce rapid and transient changes in transcription of adipogenic genes during the early stages of differentiation after hormonal induction (50). Most notably, DEX has been shown to induce the expression of C/EBPd in 3T3-L1 cells (50,51), which, in turn, triggers pathways for C/EBPa-driven differentiation (52,53). DEX also stimulates adipocyte differentiation of human osteoblastic (stromal) stem cells in bone marrow, thereby shifting the balance from bone formation to fat production in the skeletal system and providing a mechanism for steroid – associated osteoporosis. In concert with adipose tissue differentiation in adipose tissue, glucocorticoid-induced adipogenesis from bone marrow stromal cells is mediated through a reaction cascade initiated by transcriptional upregulation of C/EBPd by DEX (55).

The long list of external signals with negative impact on adipogenesis, including growth factors, proinflammatory cytokines, and nutritional signals, have been uncovered (39). In some cases, the inhibitory response is stage dependent. Tumor necrosis factor-α not only inhibits adipocyte differentiation, but also mediates dedifferentiation (10) and apoptosis (56) of mature fat cells. Retinoic acid, the main active form of vitamin A, inhibits both adipocyte proliferation and differentiation (57). Effects of retinoic acid on fat are mediated via retinoic acid receptors, retinoic acid receptor and retinoid X receptor, both of which are expressed by adipocytes (58).

Intracellular molecular signals and pathways that control adipogenesis have only recently been identified. The extracellular signal-regulated kinase (ERK) pathway, a subset of mitogen-activated protein kinase, plays a particularly prominent role in adipocyte differentiation (59). Preferentially activated by mitogens, such as insulin, ERK kinases provide a bridge between various extracellular signals and the intracellular response. Activation of the ERK pathway yields both positive and negative effects at different stages throughout adipogenesis (59). Stimulation of the ERK pathway is required for the proliferative phase of adipogenesis (60), but must be down-regulated thereafter for effective differentiation. Postmitotic clonal expansion stimulation of the ERK pathway leads to the phosphorylation of PPARγ, which subsequently decreases its transcriptional activity and inhibits adipocyte differentiation (61).

A recently described group of inhibitory proteins, CUP/AP-2α isoforms 1, 3, and 4 (62), Sp1 (63), CHOP-10 (64) and Pref-1 (also known as Dlk1) (65) are expressed by undifferentiated preadipocytes and must be coordinately down-regulated or functionally inactivated after the induction of adipogenesis in order for adipocyte differentiation to proceed. It has been postulated that this class of proteins may function as gatekeepers in vivo, maintaining the preadipocyte phenotype until hormonal and nutritional conditions are supportive for adipocyte differentiation.

An additional gate-keeping system recently described involves the transcription factorsGATA-2 and GATA-3, members of the GATA family of transcription factors, all of which share highly conserved zinc-finger DNA binding domains (66). Unlike the C/EBPs, PPARγ2, and CREB, transcription factors that positively affect adipogenesis, GATA-2 and GATA-3, specifically expressed in white and not brown adipose tissue in vivo, have an inhibitory role in the molecular control of the preadipocyte – adipocyte transition (67). Expression of GATA-2 and GATA-3, restricted to preadipocytes, is subsequently down-regulated upon adipocyte differentiation. Constitutive expression of both GATA-2 and GATA-3 suppresses adipocyte differentiation, thereby trapping cells in a preadipocyte stage, an effect mediated, in part, through direct inhibition of PPARγ transcriptional activity (67)1 and interference with C/EBP function via protein-protein interactions (68).

Intense interest in the mechanisms that drive adipose tissue development has uncovered a particularly important relationship between dietary FAs and adipocyte differentiation in recent years. It is currently well established that diets high in fat induce proliferation and adipocyte differentiation in rodents, thereby increasing

adipose tissue mass and the tendency for obesity (69-71). All dietary FAs, however, do not mediate a universal and consistent proadipogenic effect. Rats fed diets rich in omega-3 PUFAs, and in particular diets consisting of large quantities of fish oil (72), demonstrate decreased adipose tissue growth and reduced tendency toward obesity (72-75). In vitro and in vivo evidence shows that PUFAs limit adipocyte differentiation as well as the size of lipid droplets formed in new adipocytes (74,76,77).

At present we do not know which factor(s) regulate(s) turnover in humans. However, a protein previously known because of its role in bone formation, tartrate resistant acid phosphatase (TRAP), could also be involved in this process. TRAP is produced by macrophages as a pro-enzyme that previously was thought to be biologically inactive; after release from macrophages it is cleaved into a biologically active form (78,79).

Some years ago it was demonstrated that obesity is accompanied by a low grade inflammation of adipose tissue with macrophage infiltration as the most prominent histological feature of the inflammation (80,81). It is believed that this inflammation is a defense mechanism against further fat accumulation, because the inflammatory reaction leads to release of cytokines and chemokines in adipose tissue which inhibits adipogenesis and induces insulin resistance of the fat cells (80-82). However, Arner's findings with TRAP (83) and the demonstration of phagocytosis be macrophages in human obese adipose tissue targeting large adipocytes (12) shed new light on the role of macrophages in obese adipose tissue. These cells may keep up the high turnover rate of adipocytes by stimulating adipogenesis through TRAP and by killing old fat cells through phagocytosis. The fact that adipocyte turnover is very dynamic in man and that the body seems to keep the total number of fat cells fixed in adulthood has important implications for both the development of obesity and the ability to maintain a lower body weight after weight loss (27).

The adipose tissue stroma contains blood vessels and other cell types. It is well documented that among the stromal cells, there are preadipocytes that can be induced to differentiate into adipocytes in vitro (84-86). Recent studies have also demonstrated that obesity induces macrophage infiltration of the stroma of adipose tissue (80,87) and that inhibition of angiogenesis reduces adipose tissue mass (88-91). These findings strongly suggest that stromal cells and blood vessels play key roles in adipogenesis and obesity. However, little is known about how adipogenesis proceeds in vivo or the significance and mechanism of the interactions between stromal cells, vascular cells, and adipocytes (92,93).

MicroRNAs (miRNAs) are short noncoding RNAs that regulate gene expression by binding to target mRNAs, which leads to reduced protein synthesis and sometimes decreased steady-state mRNA levels. Although hundreds of miRNAs have been identified, much less is known about their biological function. Several studies have provided evidence that miRNAs affect pathways that are fundamental for metabolic control in higher organisms such as adipocyte and skeletal muscle differentiation (94).

Potential regulators of adipogenesis include microRNAs (miRNAs), which encode an abundant class of ~22 nucleotide evolutionarily conserved RNAs that control gene expression at the posttranscriptional level by targeting mRNAs for degradation or translational repression or both (95-97).

Xie's results provide the first experimental evidence for miR-103 function in adipose biology. The remarkable inverse regulatory pattern for many miRNAs during adipogenesis and obesity has important implications for understanding adipose tissue dysfunction in obese mice and humans and the link between chronic inflammation and obesity with insulin resistance (98).

The gap junction is an intercellular membrane channel and mediates direct exchange of cytoplasmic small molecules such as ions, cyclic nucleotides, inositol triphosphates, and other small molecules of <1 kDa between adjacent cells (99,100). Several studies have reported that gap-junctional communication (GJC) is required for the differentiation of myoblasts and osteoblasts (101-107).

The major component of gap junctions is Cx43 that exists in almost all tissues (108). Consistent with the loss of GJC during adipogenesis, down-regulation of Cx43 expression is observed in the murine marrow-derived stromal cell line, H-1/A, when the cells were induced to differentiate into adipocytes (109). Therefore, GJC would seem to play some important roles in an early stage of adipocyte differentiation, especially in mitotic clonal expansion. Study by Yanagiya et al show that the blockade of GJC or Cx43 during an early stage of differentiation inhibited mitotic clonal expansion and adipocyte differentiation (110).

Until recently, it was thought that all adipocytes are derived from a common precursor. It seemed almost obvious that different types of fat cells are closely related. In contrast to white adipose tissue, brown adipose tissue plays an active role in energy expenditure, oxidizing fatty acids produced by triglyceride hydrolysis to generate heat. As the name suggests, brown adipose tissue appears different from white adipose tissue, largely because of the increased number of mitochondria Also, brown adipocytes contain multiple small lipid droplets, whereas

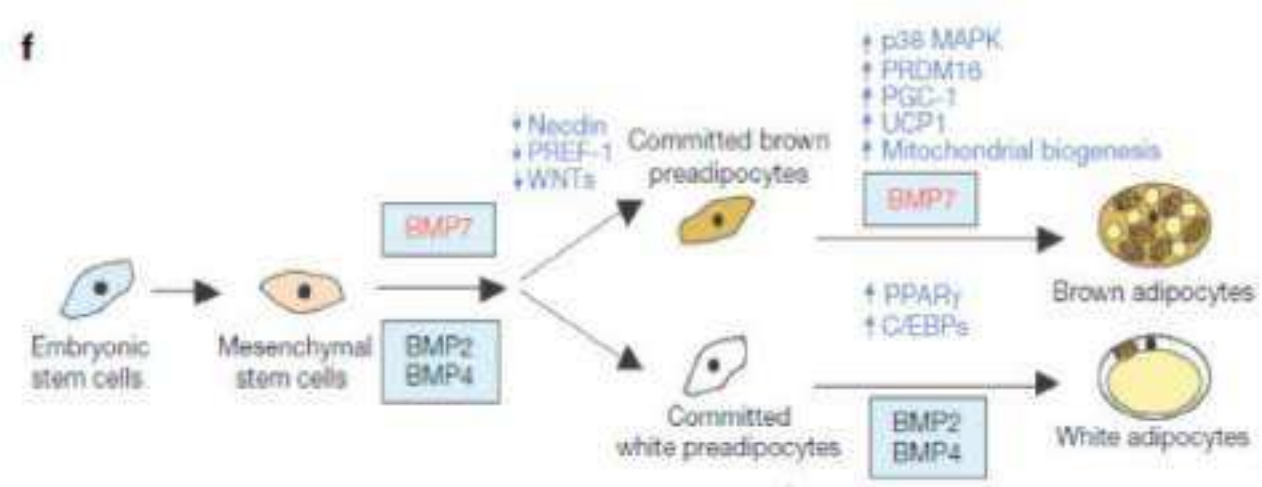

Fig 2. Proposed model for the role of BMPs in determination of brown versus white adipocyte development (Adapted with permission from Tseng et al, Nature 2008).

white adipocytes usually have a single large lipid droplet. The thermogenic oxidation of fatty acids is facilitated by uncoupling protein–1, which is expressed specifically in brown adipocytes (111).

The link between brown fat and skeletal muscle was ed by the observation that gene-expression profiles wn adipose tissue share features characteristic of skeletal muscle (112).

Many proteins found in brown fat cells — especially those involved in fat uptake from the blood, intracellular fat transport and mitochondrial fat combustion — are more similar to those found in muscle than in white adipose tissue. So the more one thinks about it, the more reasonable it seems that muscle and brown fat cells are closely related. And perhaps we must now look at a brown fat cell as an 'adiponyocyte' — that is, as a muscle cell that has accumulated fat — rather than as a variety of white fat cell (113).

The central question that must now be addressed is whether BAT function significantly impacts energy balance and human obesity. Classic experiments in rodents have shown that BAT is activated and proliferates in response to overfeeding (114). This so-called "diet-induced adaptive thermogenesis" is an apparent compensatory mechanism to limit excess weight gain and obesity. Overfeeding studies in humans have provided evidence for dramatic interindividual differences in the energy cost of feeding (115-117).

The last several years have seen an explosion of information related to the transcriptional control of brown fat cell development, differentiation, and function. The Zn-finger transcriptional regulator PR domain containing 16 (PRDM16) has recently emerged as a dominant driver of brown fat cell fate (118,119). Bone morphogenetic protein 7 (BMP7) was also recently shown to specifically direct brown adipocyte differentiation, including induction of Prdm16 and Ucp1 gene expression (120). Synthetic chemicals or endogenous factors (e.g., BMP7 itself) that activate PRDM16 function or mimic its action in brown adipocyte development may be viable antiobesity drugs. Alternatively, it may be possible to engineer synthetic brown adipocytes ex vivo for autologous transplantation. Of course, it will first be important to establish that these developmental pathways are conserved in human BAT (121).

Nevertheless, the new human data have invigorated interest and excitement in the function and physiological relevance of BAT. Hopefully, these findings can be translated translated into 1) a better understanding of the mechanisms that work together to regulate body weight and 2) novel therapeutic interventions to reduce the burden of obesity in our society (121).

Adipocyte Differentiation

The differentiation of adipocytes represents a complex process dependent on the strict temporal regulation of multiple inhibitory and stimulatory signaling events, the net sum of which ultimately leads to the expression of several hundred differentiation-dependent downstream adipocyte-specific and adipocyte-associated genes as well as an increased capacity of the cell for lipid-filling, or lipogenesis (34,38,122). Following initiation of differentiation by a variety of requisite factors, adipocyte precursor cells express a cascade of transcription factors, transcriptional coactivators, and cell-cycle proteins that each contribute to the regulation of subsequent steps in the differentiation program. Once triggered, transcription factors act cooperatively and sequentially to promote the necessary stages that drive the adipogenic program. The most prominent of these adipogenic transcription factors, the peroxisome proliferator-activated receptors (PPARs) and CCAAT/enhancer-binding proteins (C/EBPs) (18).

Characterization of regulatory regions of adipose-specific genes has led to the identification of the transcription factors peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer binding protein (C/EBP), which play a key role in the complex transcriptional cascade during adipocyte differentiation. Growth and differentiation of preadipocytes is controlled by communication between individual cells or between cells and the extracellular environment. Various hormones and growth factors that affect adipocyte differentiation in a positive or negative manner have been identified. In addition, components involved in cell-cell or cell-matrix interactions such as preadipocyte factor-1 and extracellular matrix proteins are also pivotal in regulating the differentiation process. Identification of these molecules has yielded clues to the biochemical pathways that ultimately result in transcriptional activation via PPAR-γ and C/EBP. Studies on the regulation of the these transcription factors and the mode of action of various agents that influence adipocyte differentiation will reveal the physiological and pathophysiological mechanisms underlying adipose tissue development (39).

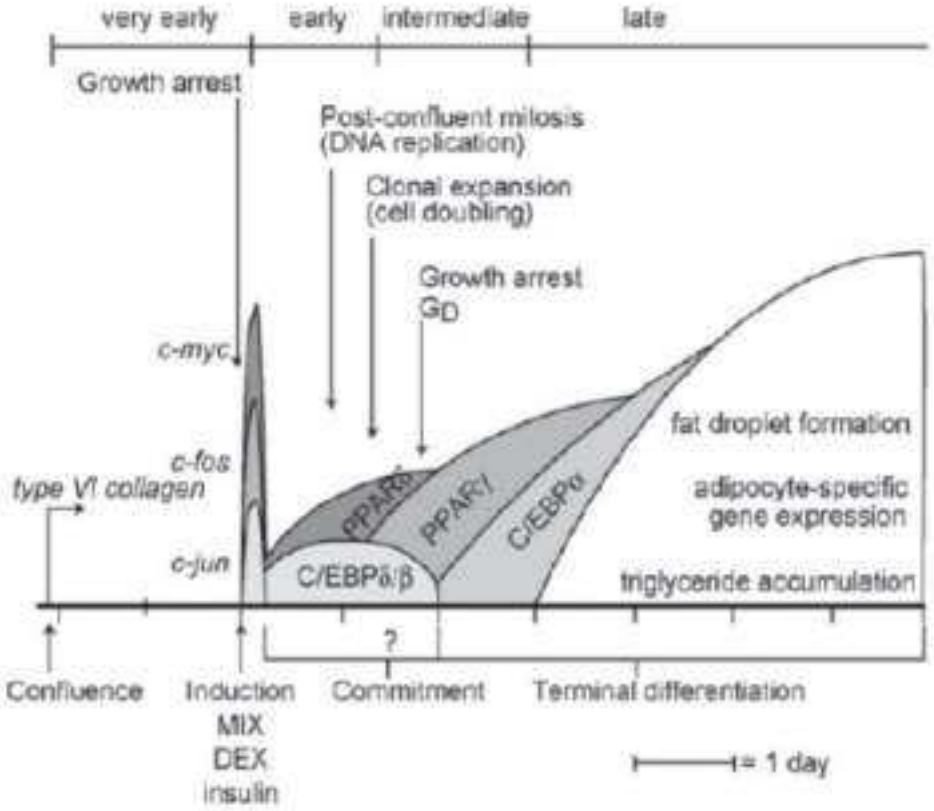

Fig 3. Progression of 3T3-L1 preadipocyte differentiation (Adapted with permission from Avram MM et al J Am Acad Dermatol 2007)

The effects of TGF-β on the differentiation of various tissues of mesenchymal and epithelial origin include both the prevention and induction of expression of specific phenotypes. It has also been observed that TGF-β inhibits adipocytogenesis of the murine 3T3-F442A cell line by signaling through Smad3 (123). Moreover, TGF-β/ Smad3 has been shown to inhibit the induction of PPAR-γ in murine 3T3-F442A and NIH3T3 cells (123,124). Despite its ability to inhibit adipocyte differentiation, TGF-β is expressed in cultured adipocytes and adipose tissue (125,126). Therefore, there may be endogenous mechanisms that affect TGF-β signaling in adipocyte differentiation.

Horie et al demonstrated that insulin specifically antagonizes TGF-β signaling in preadipocytes by stabilizing the putative Smad transcriptional corepressor Triglyceride – interacting factor (TGIF) and regulates adipocyte differentiation (127).

Insulin is a major adipogen. It stimulates the uptake of glucose, which is then converted into triglycerides and stored in fat droplets leading to adipocyte maturation; insulin also promotes the differentiation of preadipocytes in the stroma of the adipose tissue into adipocytes (128).

Insulin is also known to acutely increase the reactive oxygen species (ROS) production in adipocytes (129), but the enzymes responsible for ROS formation in these cells have not been extensively characterized. Moreover, it is unknown whether ROS are required for insulin-induced differentiation. Besides mitochondria, the Nox family of NADPH oxidases is considered the most important source of ROS in the body (130). It is accepted that Nox1- and Nox2-dependent ROS formation requires activation of the proteins by cytosolic activators, whereas Nox4 is constitutively active and independent of activator proteins (130,131). It is therefore assumed that Nox1 and -2 mediate short-term effects, whereas Nox4 is responsible for long-lasting events such as controlling cell cycle progression and proliferation (132).

Nox4 acts as a switch from insulin-induced proliferation to differentiation by controlling MKP-1 expression, which limits ERK1/2 signaling (133).

The combination of expression data and functional assay results identified a role for miR-143 in adipocyte differentiation. miR-143 levels increased in differentiating adipocytes, and inhibition of miR-143 effectively inhibited adipocyte differentiation. In addition, protein levels of the proposed miR-143 target ERK5 (4) were higher in ASO (Antisense Oligonucleotide) – treated adipocytes. These results demonstrate that miR-143 is involved in adipocyte differentiation and may act through target gene ERK5 (134).

The zinc finger transcription factor GATA-2 and GATA-3 are expressed in adipocyte precursors and control the preadipocyte-to-adipocyte transition. Constitutive expression of both GATA-2 and GATA-3 suppressed adipocyte differentiation, partially through direct binding to the PPARγ promoter and suppression of its basal activity (68).

Both GATA-2 and GATA-3 form protein complexes with CCAAT/enhancer binding protein alpha (C/EBPα) and C/EBPβ, members of a family of transcription factors that are integral to adipogenesis. This interaction to the basic leucine zipper domain of C/EBPα and a region adjacent to the carboxyl zinc finger of GATA-2. The interaction between GATA and C/EBP factors is critical for the ability of GATA to suppress adipocyte differentiation. Thus in addition to its previously recognized function in suppressing PPARγ transcriptional activity, interaction of GATA factors with C/EBP is necessary for their ability to negatively regulate adipogenesis (68).

The process of adipocyte differentiation therefore requires not only cell cycle withdrawal and expression of specic transcription factors, but also a proper extracellular environment which transduces external signals to the nucleus via a cascade of intracellular signaling. One such molecule, preadipocyte factor-1 (pref-1), that originally cloned by differential screening in an attempt to identify regulatory molecules for adipogenesis (135-138).

Pref-1 is a transmembrane protein having epidermal growth factor (EGF)-like repeats in the extracellular domain, a juxtamembrane region, a single transmembrane domain, and a short cytoplasmic tail. Pref-1 is found in 3T3-L1 preadipocytes but disappears after their conversion into adipocytes (136,139). Pref-1, therefore, is used as a marker for preadipocytes (140-142). Pref-1 directly activates the MEK/ERK pathway and that ERK phosphorylation peaking at day 2 is responsible for Pref-1 inhibition of adipogenesis (143).

α-Lipoic acid (LA) has been demonstrated to activate the insulin signaling pathway and to exert insulin-like actions in adipose and muscle cells. LA inhibits insulin or the hormonal mixture-induced differentiation of 3T3-L1 pre-adipocytes by modulating activity and/or expression of pro- or anti-adipogenic transcription factors mainly through activating the MAPK pathways (144).

The apparent number of preadipocytes in the abdominal subcutaneous tissue that can undergo differentiation is reduced in obesity with enlarged fat cells, possibly because of increased MAP4K4 (Mitogen Activated Protein 4 Kinase) levels. TNF-α promoted a macrophage-like phenotype of the preadipocytes, including several macrophage markers. These results document the plasticity of human preadipocytes and the inverse relationship between lipid storage and proinflammatory capacity (145).

Adipose Tissue Angiogenesis

Adipose tissue is highly vascularized, and each adipocyte is nourished by an extensive capillary network (147-149). In vitro studies indicated that depot-dependent vascular traits may be attributable to intrinsic growth characteristics of adipose tissue endothelial cells. These studies indicate that adipogenesis may be regulated by factors that drive angiogenesis. Fundamental aspects of angiogenesis, including basement membrane breakdown, vasculogenesis, angiogenic remodeling, vessel stabilization, and vascular permeability. Critical angiogenic factors include vascular endothelial growth factor (VEGF), VEGF receptors, angiopoietins (Ang), ephrins, matrix metalloproteinases, and the plasminogen enzymatic system. Vascular endothelial growth factor is the most critical factor because it initiates the formation of immature vessels and disruption of a single VEGF allele leads to embryonic lethality in mice. Expression of VEGF is influenced by hypoxia, insulin, growth factors, and several cytokines (149).

Vascular endothelial growth factor expression and secretion by adipocytes is regulated by insulin and hypoxia, and is associated with adipose tissue accretion. Vascular endothelial growth factor accounts for most of the angiogenic activity of adipose tissue (149).

Growing adipocytes produce a dozen angiogenic factors including leptin, VEGF, FGF-2, HGF, IGF, TNF-α, TGF-β, placental growth factor (PlGF), VEGF-C, resistin, tissue factor (TF), neuropeptide Y (NPY), heparin-binding epidermal growth factor, and Angs (150-158). Preadipocytes and adipocytes also produce non-protein small lipid molecules such as monobutyrin that stimulate angiogenesis in the adipose tissue (159,160). ASCs secrete high levels of a number of angiogenic factors including VEGF, HGF, GM-CSF, FGF-2, and TGF-β (161). Recruitment of inflammatory cells also significantly contributes to adipose neovascularization. For example, activated macrophages produce potent angiogenic factors such as TNF-α, VEGF, FGF-2, IL-1b, IL-6, and IL-8 (87,162).

Taken together, these findings support the notion that adipose tissue development requires constant vascular remodeling and that multiple angiogenic molecules produced in adipose tissue may contribute to the complex regulation of adipogenesis (163,164).

Lipid Droplet Biogenesis

Most organisms transport or store neutral lipids as lipid bodies – lipid droplets that usually are bounded by specific proteins and (phospho)lipids (165). The early phases of lipid-body biogenesis probably proceed by a similar mechanism in white- and brown adipose-tissue adipocytes. Early in adipocyte differentiation, nascent lipid bodies appear to arise from, and sometimes appear to be enfolded by, ER membranes. Such lipid bodies contain an ~50-kDa surface-bound acylated protein – adipose differentiation – related protein (ADRP) – that originally was isolated as a strongly induced marker of adipocyte differentiation (166) During the further differentiation of adipocytes, perilipins (a class of 42–56-kDa polypeptides) replace ADRP as the predominant protein on the surfaces of lipid bodies. The N-terminal 105 residues of the perilipins are similar to ADRP, although perilipins are found only in adipocytes and steroidogenic cells. As in the case of ADRP, perilipin expression is concurrent with lipid-body formation (165).

Adipocyte differentiation in white adipose tissue involves the coalescence of small nascent lipid bodies to form the one or more large TAG droplets found in mature cells. By contrast, most other cell types contain numerous smaller lipid bodies (165).

An endoplasmic reticulum localization of the lipid droplet assembly is consistent with the observation that microsomal membrane proteins are involved in the assembly of lipid droplets. These include phospholipase D1 (PLD1), which is essential for the assembly of lipid droplets (167,168), and enzymes involved in triglyceride biosynthesis – glycerol-3-phosphate acyltransferase (GPAT) (169,170), phosphatidic acid phosphohydrolase (PAP) (171,172) and diacylglycerol acyltransferase (DGAT) (172,173).

The lipid droplets that are formed at isolated microsomal membranes are 0.1–0.4μm in diameter, which is the size of the smallest droplets observed in cells by electron microscopy, and their assembly is totally dependent on the rate of triglyceride biosynthesis (167).

Once formed, lipid droplets are transported on microtubules (174,175). The motor protein dynein has

been shown to be present on the droplets (175) and sorted to the droplets following phosphorylation by the cytosolic protein extracellular-regulated kinase 2 (ERK2) (176). Both dynein and microtubules are essential for lipid droplet fusion (168,175).

Lipid droplets in the cytosol are several folds larger than the ones formed from the microsomal membranes. the droplets can increase in size by a fusion process that is independent of triglyceride biosynthesis (175). The involvement of α-soluble N-ethylmaleimi de-sensitive factor adaptor protein receptors (SNAREs) in the fusion between transport vesicles and target membranes has been described in detail (176,177). A SNARE on the transport vesicle (v-SNARE or R-SNARE) interacts with SNAREs present on the target membrane (t-SNARE or Q-SNAREs) to form a SNARE complex.

After fusion, the SNARE complex is unwound by the ATPase N-ethylmaleimide-sensitive factor (NSF) and its adaptor α-soluble NSF adaptor protein (α-SNAP). These SNAREs and α-SNAP are essential for the fusion between the droplets. Thus lipid droplets grow in size by a fusion process catalyzed by the SNARE system (179).

Insulin resistance is highly associated with increased accumulation of lipids in skeletal muscle (180-187), and studies have identified SNAP23 as a potential link between insulin resistance and inflow of fatty acids to the cell (179).

Furthermore, it is not the lipid droplets per se that promote the development of insulin resistance but the influence of the fatty acids on SNAP23. This could either be an indirect influence through the insulin signal or a direct influence of the fatty acids on SNAP23 structure or sorting. The effect of fatty acids on the insulin signal is well established (188) and is proposed to be mediated by fatty acid metabolites such as diglycerides (189,190), ceramides (191) and partially oxidized fatty acids (192). The exact mechanism, however, is not known.

Recent evidence suggests that lipid droplets can form tight interactions with other organelles such as peroxisomes, the endoplasmic reticulum, endosomes and mitochondria (192-196). Lipid droplets are proposed to interact with the outer leaflet of the peroxisome membrane (197) that is similar to our proposed model for the fusion between lipid droplets. It would thus be of interest to determine the role of the SNARE system in the interaction between lipid droplets and peroxisomes. The interaction between lipid droplets and the endoplasmic reticulum involves a tethering mechanism rather than a fusion process, and seems to be mediated by the GTPase Rab18 (192,193). Little is known about the interaction with endosomes (which is dependent on Rab5 (195) or with mitochondria (196).

Fat-specific protein of 27 kDa (FSP27) also known as Cidec is a highly expressed adipocyte protein that promotes triglyceride accumulation within lipid droplets. In this issue of the JCI, Nishino et al. show that FSP27 also helps to maintain the characteristically large unilocular lipid droplet structure within each white adipocyte. Fragmentation of lipid droplets in white adipocytes from FSP27-KO mice caused both increased lipolysis and upregulation of genes enhancing mitochondrial oxidative metabolism. This increased energy expenditure in turn protected the mice from diet-induced obesity and insulin resistance. These new results highlight powerful mechanisms that tightly coordinate rates of triglyceride storage in lipid droplets with mitochondrial fatty acid oxidation in white adipocytes (197).

Lipid droplets are dynamic and heterogeneous in size, location, and protein content. The proteins that coat lipid droplets change during lipid droplet biogenesis and are dependent upon multiple factors, including tissue-specific expression and metabolic state (basal vs. lipogenic vs. lipolytic). New data suggest that proteins previously implicated in vesicle trafficking, including Rabs, SNAREs, and motor and cytoskeletal proteins, likely orchestrate the movement and fusion of lipid droplets. Thus, rather than inert cytoplasmic inclusions, lipid droplets are now appreciated as dynamic organelles that are critical for management of cellular lipid stores. (199).

PAT Protein

In addition to the proteins discussed above, a number of other proteins have been identified on lipid droplets. The most well known of these are the PAT proteins, named after the first identified species perilipin, ADRP and tail-interacting protein 47 (TIP47) (200-202). These proteins contain one PAT domain in the N-terminus and a second in the C-terminus.

Adipophilin also called adipose differentiation-related protein, TIP47 (for Tail-Interacting Protein of 47 kDa), S3-12, OXPAT (also called Myocardial Lipid Droplet Protein or MLDP and Lipid Storage Droplet Protein 5 or LSDP5), and LSD1 and LSD2 (for Lipid Storage Droplet proteins 1 and 2). And perilipin members of this family share varying levels of sequence similarity, lipid droplet association, and functions in stabilizing lipid droplets (203).

The most recently described member of the perilipin family has been given several names, including OXPAT (for a PAT family protein expressed in oxidative tissues) (203),

MLDP (204), and LSDP5 (201). OXPAT/MLDP is most highly expressed in heart and slow-twitch muscle, with lower levels in fast-twitch muscle, liver, white and brown adipose tissue, testis, and adrenal gland (201,203,204). OXPAT/MLDP is related to TIP47 (30% identity) and adipophilin (26% identity) throughout the amino acid sequence (203).

Furthermore, like TIP47, OXPAT/MLDP is stable in the cytoplasm but is recruited to lipid droplets under conditions that promote lipid droplet formation (203). The two remaining members of the protein family, perilipin and S3-12, have divergent amino acid sequences relative to TIP47, adipophilin, OXPAT/MLDP, and each other.

Three protein isoforms of perilipin have been described that arise from the translation of alternatively spliced mRNA (205,208). Perilipin A is the largest protein (517 amino acids in mice) and the most abundant protein on adipocyte lipid droplets (206,207); perilipin B, a less abundant protein, shares 405 amino acids with perilipin A followed by 17 unique amino acids at the C terminus (in mice) (206,207); perilipin C is an even shorter isoform that is expressed only in steroidogenic cells (207,208).

Functional studies in cell culture and animal models have demonstrated that perilipin and the related proteins in the perilipin family regulate the lipolysis of stored neutral lipids.

Adipophilin is the major surface protein on the lipid droplets of fibroblasts, but ectopic expression of perilipin A replaces the adipophilin content of the droplets in favor of a perilipin coat (209,210). These data suggest that perilipin A competes with adipophilin for binding to lipid droplets and is more effective at attenuating lipolysis than adipophilin. Loss of adipocyte perilipin content, with the concomitant loss of the protection of stored triacylglycerol from cytosolic lipases, is part of the mechanism by which TNF-a increases lipolysis; the resulting increased flux of fatty acids may contribute to local effects on gene expression within adipocytes as well as to distal effects of fatty acids on insulin sensitivity in other tissues (202).

Under basal conditions, perilipin may bind proteins that facilitate triacylglycerol storage while allowing a low level of lipolysis. When PKA is activated, phosphorylated perilipin disperses the basal coat proteins to make way for powerful lipolytic machinery, including lipases and trafficking molecules, some of which may facilitate the budding off of microlipid droplets (202).

Two examples of perilipin binding proteins have been reported. Hormone-sensitive lipase requires perilipin A to dock on lipid droplets and gain access to lipid substrates after hormonal stimulation of adipocytes, as discussed. An example of a protein that binds to perilipin A under basal conditions is CGI-58, also called ABHD5 (for α/β hydrolase fold domain 5) (211,212). C More recent studies suggest that CGI-58 serves as a coactivator of ATGL, stimulating triacylglycerol hydrolysis by as much as 20-fold (213,214).

Gel mobility shift and chromatin immunoprecipitation assays showed that endogenous PPARγ protein binds to the perilipin promoter. PPARγ2, an isoform exclusively expressed in adipocytes, was found to be the most potent regulator from among the PPAR family members including PPARα and PPARγ1. These results make evident the fact that perilipin gene expression in differentiating adipocytes is crucially regulated by PPARγ2, providing new insights into the adipogenic action of PPARγ2 and adipose-specific gene expression, as well as potential anti-obesity pharmaceutical agents targeted to a reduction of the perilipin gene product (215).

Polymorphisms at the perilipin locus are associated with anthropometric measures and the risk of obesity in a gender specific fashion, in several ethnic groups from different studies including Whites (two studies) (216,217), Malays (218) and Asian Indians (216). The nature of the associations, however depends on the intragenic linkage disequilibrium structure of the perilipin locus in the various populations (220).

Dietary fat may interact with polymorphisms at the perilipin locus to modulate diabetes related traits, data from the Singapore population, taking into consideration dietary macronutrient intake (219). Tai ES et al found evidence of an interaction between dietary fat (specifically saturated fat) intake, polymorphisms at the perilipin locus (11482G>A and 114995A>T) and insulin resistance (220).

In response to cold, norepinephrine (NE)-induced triacylglycerol hydrolysis (lipolysis) in adipocytes of brown adipose tissue (BAT) provides fatty acid substrates to mitochondria for heat generation (adaptive thermogenesis). NE-induced lipolysis is mediated by protein kinase A (PKA)-dependent phosphorylation of perilipin (221).

Perilipin seems important for the regulation of lipolysis in human fat cells. Obesity and a polymorphism in the perilipin gene associate with decreased protein content of perilipin and increased basal (unstrained) and noradrenaline-induced lipolysis. Low perilipin content also associate with high in vivo lipolytic activity. Perilipin could be a factor behind impaired lipolysis in insulin-resistant conditions (222).

Adipose Tissue Lipolysis

One of the central reactions in bodily energy metabolism is lipolysis in adipocytes, the reaction that governs the release of stored fatty acids from the adipocyte triacylglycerol pool, which constitutes the major energy reserve in animals. These fatty acids are then transported by serum albumin to various tissues to supply their energy requirements. This reaction was previously thought to result from phosphorylation and activation of hormone-sensitive lipase (HSL) by protein kinase A (PKA) but is now known to be governed by a translocation of the lipase from the cytosol to the surface of the intracellular lipid droplet that houses the reservoir of TAG. This droplet is coated with perilipin A, which is also phosphorylated by PKA in response to lipolytic stimuli, and phosphorylation of perilipin A is essential for HSL translocation and stimulated lipolysis (223).

Release of fatty acids (FAs) from adipose tissue through lipolysis in fat cells is a key event in many processes. FAs are not only energy substrates but also signalling molecules and substrates for lipoprotein production by the liver. Fat cells consist of>95% triglycerides that are hydrolysed during lipolysis to glycerol and FAs (224).

In contrast to these "beneficial" characteristics, unesterified FAs can become deleterious for cells when present even at relatively low concentrations. The chronic exposure of nonadipose cells and tissues to elevated concentrations of FAs triggers adverse effects subsumed under the term of "lipotoxicity" (225,226).

The mobilization of FAs from all fat depots depends on the activity of TG hydrolases. Currently, three enzymes are known to hydrolyze TG, the well-studied hormone-sensitive lipase (HSL) and monoglyceride lipase (MGL), discovered more than 40 years ago, as well as the relatively recently identified adipose triglyceride lipase (ATGL). The phenotype of HSL- and ATGL-deficient mice, as well as the disease pattern of patients with defective ATGL activity (due to mutation in ATGL or in the enzyme's activator, CGI-58), suggest that the consecutive action of ATGL, HSL, and MGL is responsible for the complete hydrolysis of a TG molecule. The complex regulation of these enzymes by numerous, partially uncharacterized effectors creates the "lipolysome," a complex metabolic network that contributes to the control of lipid and energy homeostasis (227).

Numerous lipolytic and antilipolytic effectors control the catabolism of stored fat in various tissues (228,229). These include hormones, cytokines, and adipokines. In adipose tissue, the most potent stimulatory signals are catecholamines acting on β-adrenergic receptors (230). In human adipose tissue, only β1 and β2 receptors induce lipolysis. When catecholamines bind to these receptors, stimulatory Gs proteins activate adenylate cyclase, causing a rise in cAMP levels and elevated activity of cAMP-dependent protein kinase-A (PKA) (229,231,232). PKA-mediated phosphorlylation of target proteins, including lipolytic enzymes and lipid droplet associated proteins, induces an increased release of FAs and glycerol from adipose tissue up to 100-fold. Other hormones that stimulate PKA via Gs protein-coupled receptors include glucagon, parathyroid hormone, thyrotropin, α-melanocyte-stimulating hormone, and adrenocorticotropin. Several antilipolytic factors have been shown to act through inhibitory Gi protein-coupled receptors (229). These factors include catecholamines acting through α2-adrenergic receptors (230), adenosine (A1-adenosine receptor) (14), prostaglandin (E2 receptor) (234), NPY (NPY-1 receptor) (235), and nicotinic acid (GPR109A receptor) (236). The relative distribution of α- and β-adrenergic receptors therefore determines the lipolytic activity in a tissue- and cell type-specific manner. Insulin and insulin-like growth factor represent the most potent inhibitory hormones in lipolysis (228,237). Their effects are primarily communicated through the insulin receptor (IR), polyphosphorylation of insulin receptor substrates 1–4 (IRS1–4), activation of phosphatidylinositol-3 kinase (PI3K), and the induction of the protein kinase B/AKT (PKB/AKT). Complexity in this essentially linear pathway is added by the divergence at so-called critical nodes that interact with other signaling cascades (238). Critical nodes in the IR pathway include the IR and IRS interacting with cytokine and extracellular signal-regulated kinase (ERK) signaling and PI3K activating both 3-phosphoinositidedependent protein kinases (PDK1 and 2) as well as atypical protein kinases C (PKCl and z). At this point, a signaling network is established that regulates innumerable biological processes (possibly more than 1,000). Lipolysis is affected in multiple steps, including the phosphorylation of phosphodiesterase 3B, causing the degradation of cAMP and loss of PKA activation (237).

The mechanisms through which other effectors regulate lipolysis are less well characterized. These include tumor necrosis factor-α (TNFα), growth hormone, the Cide domain-containing proteins (CideN) family of proteins (CIDEA, -B, and -C), and the CopI-ARF vesicle transport machinery described below.

HSL exhibits broad substrate specificity capable of hydolyzing TG, diacylglycerol (DG), monoacylglycerol (MG), cholesteryl esters (CEs), retinyl esters (REs), and other ester substrates such as p-nitrophenyl butyrate (239). The relative maximal hydrolysis rates are in the range of

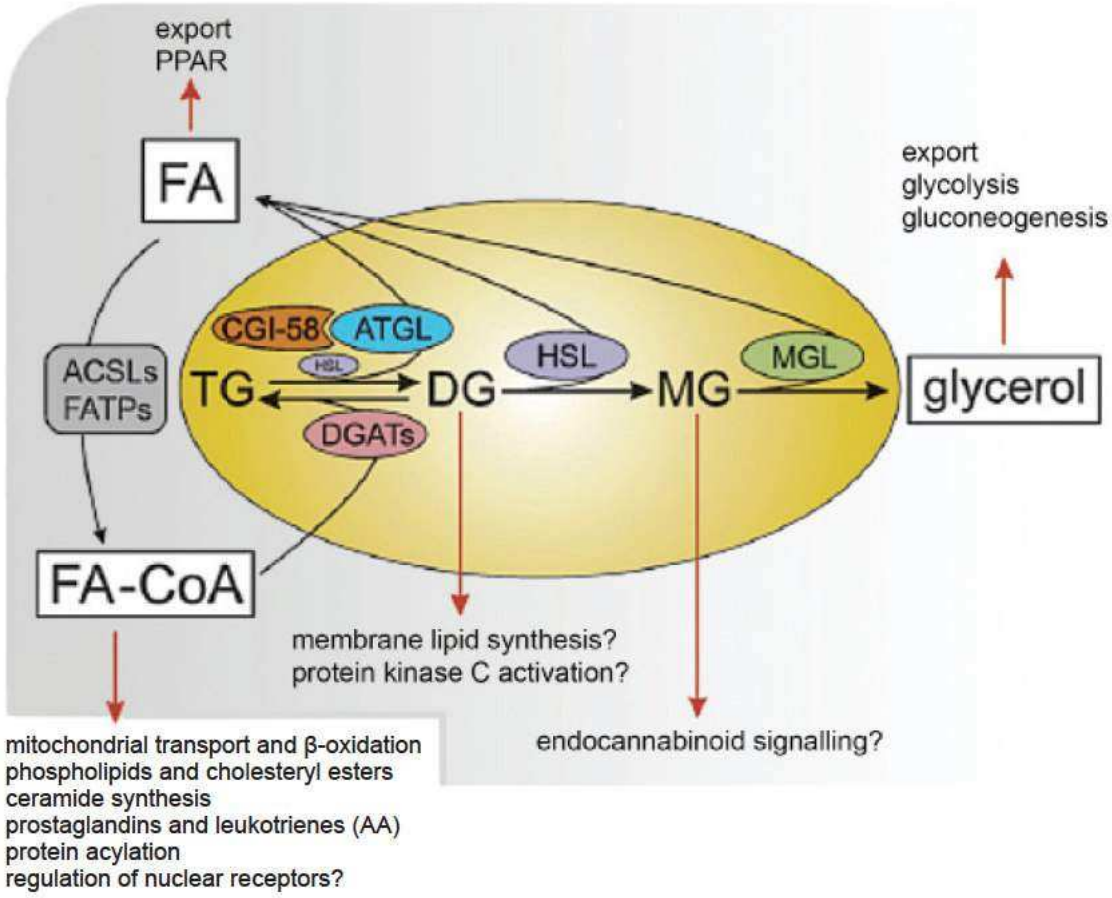

Fig 4. Simplified summary of the lipolytic process and the involved metabolic intermediates (Adapted with permission from Zechner R et al, J Lipid Res 2009).

1: 10: 1: 4: 2 for TG: DG: MG:CE: RE. Thus, TGs are actually the worst substrate for HSL among all these natural lipid esters, whereas DGs are the best. In 2004, three groups independently published the discovery of an enzyme able to hydrolyze TG and named it ATGL (240), desnutrin (241), or calcium-independent phospholipase A2z (iPLA2z) (242).

ATGL exhibits 10-fold higher substrate specificity for TG than for DG and selectively performs the first step in TG hydrolysis, resulting in the formation of DG and FA (241).

ATGL is most closely related to a group of five genes and proteins named patatinlike phospholipase domain-containing 1 to 5 (PNPLA1-5) (243,244). Members of this protein family in addition to ATGL (PNPLA2) are PNPLA1, adiponutrin (PNPLA3), GS2 (PNPLA4), and GS2-like (PNPLA5). More distantly related members of ATGL include neuropathy target esterase (NTE, PNPLA6), NTE-related esterase (NRE, PNPLA7), calcium-independent phospholipase A2g (iPLA2g, PNPLA8), and phospholipase A2 group VI (PLA2G6, PNPLA9). Like ATGL, adiponutrin, GS2, and GS2-like also exhibit hydrolase and transacylase activity in in vitro assays (242). Low specific phospholipase activity was reported for ATGL, adiponutrin, and GS2-like (242,245).

A lipid droplet protein, CGI-58 or ABHD5, was found to activate ATGL (213). In the presence of CGI-58, the TG hydrolase activity of mouse ATGL is induced approximately 20-fold. Human ATGL is also activated by CGI-58, although to a lesser degree (approximately 5-fold ATGL induction). Importantly, these findings provided a biochemical explanation for a human disorder. In 2001, Lefevre et al. (125) discovered that mutations in the gene for CGI-58 are causative for a lipid storage disorder designated "neutral lipid storage disease" or Chanarin Dorfman Syndrome. The crucial role of perilipin in the ATGL/CGI-58-mediated hydrolysis of TG became evident in an elegant study by Miyoshi et al. (247) showing that hormone stimulated lipolysis depended on perilipin and ATGL. The authors demonstrated that perilipin

phosphorylation of residue serine-517 is essential for ATGL-mediated lipolysis and represents a prerequisite for the function of subsequent lipase activity of HSL.

In addition to PAT proteins, other proteins found on lipid droplets are also involved in the regulation of lipolysis. Surprisingly, searching for receptors and binding proteins for pigment epithelium derived factor (PEDF), Notari et al. (245) identified ATGL as a PEDF binding protein and proposed to name the enzyme PEDF-receptor. Apparently, ATGL is highly expressed in the pigment epithelium and can be found on the plasma membrane, where it binds to PEDF and exhibits phospholipase activity. Another group of lipid droplet binding proteins that regulate lipolysis belongs to the CideN family. CideN proteins were originally discovered because of their structural similarity to DNA fragmentation factors and were believed to regulate cell death activation (135). Recently, members of the CideN family were shown to affect lipid droplet morphology and turnover. CideA and CideC/Fsp27 bind to lipid droplets and colocalize with perilipin (248,249). Overexpression of these factors inhibits fat catabolism and induces cellular lipid accumulation (249). Consistent with these findings, mice that lack CideC/FSP27 have smaller, multilocular lipid droplets, decreased fat mass, lower levels of plasma FAs, and increased insulin sensitivity (198,251).

Taken together, these results suggest that lipases are embedded in a complex "lipolysome" consisting of the actual lipolytic enzymes and numerous modulators of enzyme activity (227).

Additionally, better understanding of the "lipolysome" might lead to pharmacological treatment controlling the release of FA and other lipolytic products involved in the development of insulin resistance and type 2 diabetes (227).

Adipose Tissue as an Endocrine Organ

As the master regulator of systemic lipid storage and through secretion of a number of these adipokines, adipose tissue has an influence on many processes, including energy metabolism, inflammation, and pathophysiological changes such as cancer and infectious disease (252). At the interface of energy metabolism and inflammation, adipose tissue also plays a key role in the development of the metabolic syndrome. As such, our views of adipose tissue have changed significantly over the past 20 years. Initially considered an inert storage compartment for triglycerides, pioneering work from the Spiegelman and Flier (253) laboratories in the mid-1980s highlighted for the first time that adipocytes are an abundant source of a specific secretory protein, called adipsin or complement factor D. In 1995, Jeffrey Friedman's (254) group identified leptin as a fat cell–specific secretory factor deficient in the ob/ob mouse that mediates the hormonal axis between fat and the brain.

Around the same time, we and others described a protein that initially termed Acrp30, which later became known as adiponectin (255-258). Additional proteins have joined this exclusive club of adipocyte-specific secretory proteins since then, including adipokines such as resistin (259,260) and acylation-stimulating protein (261), as well as the recently described visfatin (262,263) and retinolbinding protein-4 (264). Enzymes such as lipoprotein lipase are also abundantly produced and released from adipocytes. Finally, many proinflammatory cytokines and acute phase reactants originate in the adipocyte. These include α1 acid glycoprotein, serum amyloid A, the C-reactive protein homolog pentraxin-3, the lipocalin 24p3, and a host of cytokines (265).

Using adipokines as one of the major communication tools, adipocytes affect a large number of other tissues, such as the liver, muscle, the brain, the reproductive system, pancreatic β-cells, and, as mentioned above, the vasculature (266).

It is now clear that adipose tissue is a complex and highly active metabolic and endocrine organ (267,268). Besides adipocytes, adipose tissue contains connective tissue matrix, nerve tissue, stromovascular cells, and immune cells (14). Although adipocytes express and secrete several endocrine hormones such as leptin and adiponectin, many secreted proteins are derived from the nonadipocyte fraction of adipose tissue (269). Regardless, these components function as an integrated unit, making adipose tissue a true endocrine organ (14).

As a further level of complexity, there is considerable heterogeneity among the various adipose tissue depots. The sc and visceral adipose tissue depots have been the best characterized, particularly with respects to contribution to disease. Visceral adipose tissue is associated with increased risk for multiple medical morbidities including the metabolic syndrome. This observed difference in disease risk may be due to differences in endocrine function among adipose tissue depots. The anatomic location of

each adipose tissue depot itself affects endocrine function. Endocrine hormones derived from visceral adipose tissue are secreted into the portal system and have direct access to the liver, whereas those derived from sc adipose tissue are secreted into the systemic circulation. Hence, the former have a relatively greater effect on hepatic metabolic function (270).

So far, many adipokines have been identified (Table 1). They all integrate in a communications network with other tissues and organs such as the skeletal muscle, adrenal cortex, brain and sympathetic nervous system and participate in appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and haemostasis (271).

Table 1. Adipokines and their main effects (271).

Parameter	Effects on
LPL	Lipid metabolism
HSL	Lipid metabolism
Perilipin	Lipid metabolism
aP2	Lipid metabolism
CETP	Lipid metabolism
RBP4	Lipid metabolism, insulin resistance
IL-6	Inflammation, atherosclerosis, insulin resistance
TNF-α	Inflammation, atherosclerosis, insulin resistance
Adipsin/ASP	Immune – stress response
Metallothionein	Immune – stress response
Angiotensinogen	Vascular homeostasis
PAI – I	Vascular homeostasis
Adiponectin	Inflammation, atherosclerosis, insulin resistance
PPAR-γ	Lipid metabolism, inflammation, Vascular homeostasis
CRP	Inflammation, atherosclerosis, insulin resistance
IGF-1	Lipid metabolism, insulin resistance
TGF-β	Cell adhesion and migration, growth and differentiation
Monobutyrin	vasodilation of the microvessel
Uncoupling proteins	Energy balance and thermoregulation
Steroid hormones	Lipid metabolism, insulin resistance
Leptin	Food regulation, reproduction, angiogenesis, immunity
Resistin	Inflammation, insulin resistance
P450 arom	Lipid metabolism
Apelin	Insulin resistance
Visfatin	Insulin resistance
Vaspin	Insulin resistance
ZAG	Lipid metabolism, cancer cachexia.

Abbreviations: LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; aP2, adipocyte lipid-binding protein; RBP, retinol-binding protein; IGF-1, insulin-like growth factor-1; TGF-β, transforming growth factor-β; PPAR-γ, peroxisome proliferator-activated receptor γ; ZAG, zinc-a2-glycoprotein.

Over 90% of adipokine release by adipose tissue, except for adiponectin and leptin, was due to nonfat cells. Although PAI-1 was released to the medium by adipocytes in amounts 30% of that by the tissue matrix the release of all other adipokines by adipocytes was less than 15% of that by the tissue matrix. Furthermore, the greater release of VEGF, IL-6, and PAI-1 by visceral adipose tissue as opposed to abdominal sc adipose tissue was due to the nonfat cells of the tissue (269).

The concentration in blood of many adipokines, hormones, and acute-phase proteins is altered in human obesity. Leptin is elevated, whereas plasma adiponectin is reduced in obese humans (14,267,272-275)). C-reactive protein (CRP) (276-282) is an example of an acute-phase protein whose circulating level is higher in obese than in nonobese individuals. Blood levels of IL-10 (282), IL-6 (278-286), IL-8 (285,287), plasminogen activator inhibitor 1 (PAI-1) (284,288-291), TNF-α (278,292), and hepatocyte growth factor (HGF) (293) have all been reported to be elevated in obesity.

Adipocyte size is an important determinant of adipokine secretion. There seems to be a differential expression of pro- and antiinflammatory factors with increasing adipocyte size resulting in a shift toward dominance of proinflammatory adipokines largely as a result of a dysregulation of hypertrophic, very large cells (294).

The existence of a network of adipose tissue signaling pathways, arranged in a hierarchical fashion, constitutes a metabolic repertoire that enables the organism to adapt to a wide range of different metabolic challenges, such as starvation, stress, infection, and short periods of gross energy excess (268). Unraveling the diverse hormonal and neuroendocrine systems that regulate energy balance and body fat has been a long-standing challenge in biology, with obesity as an increasingly important public health focus (295).

Adipocyte Dysfunction

Abdominal obesity and adipose tissue dysfunction are major risk factors for chronic diseases, such as insulin resistance, type 2 diabetes, and cardiovascular diseases. Insulin resistance is associated with alterations in glucose and lipid homeostasis. During the genesis of obesity, adipose tissue is one of the first tissues affected by insulin resistance. This phenomenon is closely associated with the development of a proinflammatory state within the adipose tissue. In addition to this proinflammatory state, obesity is associated with the formation of hypoxic areas within the tissue (296).

Obesity-associated inflammation leads to highly dysregulated adipose tissue with an altered pattern of secreted adipokines and increased lipolysis (297,298). Secretion of cytokines like tumor necrosis factor (TNF)-α in the adipose tissue impairs the differentiation of preadipocytes, reduces adiponectin secretion, and promotes a proinflammatory state, which in turn further promotes the local secretion of cytokines and chemokines (299,300). In addition, both interleukin (IL)-6 and TNF-α induce insulin resistance in the adipose cells at the level of insulin signaling and action because of reduced expression of insulin receptor substrate-1 and GLUT4 (299,301).

Obesity, both in animal models and in humans, is associated with an increase in different markers of inflammatory cells, such as CD68, macrophage inflammatory protein (MIP)-1α, EMR (epidermal growth factor–like module containing mucin-like hormone receptor), and ADAM-8 (a disintegrin and metalloproteinase domain-8) in the adipose tissue (297,300,302). In fact, using such markers, Weisberg et al. (80) reported that up to 50% of the cells in the adipose tissue were positive for CD68 and thus could be classified as macrophages. They also reported that the CD68-positive cells were the major producers of the different cytokines studied (80,145).

Obesity as excess of adipose tissue is attributed to hypertrophy and hyperplasia of adipocytes. Adipocytes become hypertrophic during the development of obesity, and their size increases up to 140–180 μm in diameter (303). Adipocytes have a limited capacity for hypertrophy; one reason for this is considered the diffusion limit of oxygen, which is at most 100 μm (304). Therefore, it is possible that hypertrophic adipocytes might endure less than adequate oxygen supply. Hypoxia occurs when oxygen availability does not match the demand of the surrounding tissue, resulting in decreased oxygen tension (26).

An important and well-characterized key regulator of the adaptive response to alterations in oxygen tension is hypoxia-inducible factor-1 (HIF1), a transcription factor that accumulates during hypoxia and increases the mRNA expression of a wide variety of genes that stimulate erythropoiesis, angiogenesis, and glycolysis (305). On the other hand, hypoxic cells also provoke HIF1-independent adaptive responses. Previous reports have shown that the unfolded protein response (UPR), an HIF1-independent signaling pathway, is activated in the presence of hypoxia and contributes to cellular adaptation of this stress (26).

Many disturbances including hypoxia cause accumulation of unfolded proteins in the ER, resulting in

ER stress. To cope with the ER stress, cells trigger a set of pathways known as UPR, which is mediated by three types of ER-transmembrane proteins, inositol-requiring protein-1 (IRE1), RNA-dependent protein kinase–like ER eukaryotic translation initiation factor 2α kinase (PERK), and activating transcription factor 6 (ATF6) (306).

Exposure of adipocytes to hypoxia elicits dysregulated production of adipocytokines and that hypoxia-induced downregulation of adiponectin mRNA is mediated by ER stress–dependent transcriptional and –independent posttranscriptional mechanisms (26). Dysregulated production of adipocytokines is associated with the pathophysiology of obesity-related metabolic diseases (307-311).

It is imperative to understand the alterations that occur from the life of a healthy adipocyte to a hypertrophic insulin-resistant adipocyte to perhaps even a dead adipocyte. A deeper knowledge of adipose tissue expansion and the dysfunction that follows will be critical to our thinking and to therapy in the coming years (312).

Obesity, Inflammation and Insulin Resistance

Obesity-associated insulin resistance is a major risk factor for type 2 diabetes and cardiovascular disease. In the past decade, a large number of endocrine, inflammatory, neural, and cell-intrinsic pathways have been shown to be dysregulated in obesity. Although it is possible that one of these factors plays a dominant role, many of these factors are interdependent, and it is likely that their dynamic interplay underlies the pathophysiology of insulin resistance. Understanding the biology of these systems will inform the search for interventions that specifically prevent or treat insulin resistance and its associated pathologies (313).

The complex processes linking obesity to its deleterious health consequences are finally being unraveled. Inflammation is receiving increased attention for its potential role in the pathogenesis of disorders ranging from insulin resistance and type 2 diabetes to fatty liver and cardiovascular disease (314).

Hotamisligil et al. (307) were the first to describe a molecular connection between inflammation and obesity, when TNF-α (tumor necrosis factor-α), an inflammatory cytokine, was found to be expressed in adipose tissue in obesity animal models.

This inflammatory state is characterized by an increase in macrophage numbers and expression of macrophage markers, such as CD68 (80,81). Expression of insulin-resistance genes and the local production of their protein products by macrophages in adipose tissue are also increased in obese compared with non-obese subjects (6). These and other changes may contribute to increased lipolysis and adiponectin deficiency which characterize adipose tissue of insulin-resistant subjects and may increase liver fat content (315).

Starting with the discovery of leptin in 1994 and subsequently of many other adipokines, adipose tissue has been identified as an endocrine organ, leading to the first revolution in adipose tissue biology (270). The second revolution in adipose tissue biology was the identification of adipose tissue as an organ at the interface of inflammation, insulin resistance, and cardiovascular disease. Moreover, in the past 5 years obesity has been shown to be associated with a low-grade state of inflammation, resulting from increased adipocyte activity and increased immune cell infiltration of adipose tissue (316,317), which may induce insulin resistance and other manifestations of metabolic syndrome as cardiovascular disease (318-320).

Activation of macrophages infiltrating adipose tissue leads to the release of a variety of chemokines and proinflammatory cytokines that initiate a paracrine process with the activation of proinflammatory pathways, contributing to the propagation of additional macrophage recruitment (332). An interesting hypothesis regarding the causes of macrophage infiltration of adipose tissue is that macrophages are recruited to phagocytose dead or dying adipocytes present in the expanding adipose tissue depot. Cinti et al. (12) have recently shown that more than 90%of macrophages infiltrating the adipose tissue of obese humans and animals are present around dead adipocytes, forming characteristic elements called crown-like structures (CLS). Recently, the same authors described a significantly higher proportion of CLS in the visceral as opposed to subcutaneous depots of genetically obese mice (321).

Clearance of dead adipocytes by ATMΦs is an initial remodeling event required for AT repair and differentiation of new adipocytes at sites of adipocyte loss. MΦ-mediated cell killing is a feature of various forms of tissue remodeling (322), rendering it plausible that ATMΦs actively participate in adipocyte execution (12). The clearance of dead adipocytes is likely to promote proinflammatory ATMΦ

activation, reflecting both the necrotic-like morphology of adipocyte death and ATMΦ fusion (22). MΦ fusion, which synergistically increases MΦ absorptive capacity, requires TNF-α autocrine/paracrine signaling (323), suggesting that CLS and multinucleate giant cells (MGCs) may be chronic sources of TNF-α. Moreover, because each dead adipocyte "recruits" dozens of ATMΦs, a low frequency of adipocyte death may be sufficient to cause AT inflammation and promote insulin resistance. Excess nutrient intake is the main cause for obesity, and several recent studies have implicated ER stress as an early consequence of nutrient excess and a cause for the development of insulin resistance and inflammation (325).

The adipose tissue has to take up and store excess calories as fat and, hence, needs to synthesize many proteins to meet this challenge. In addition, the massive expansion of adipose tissue requires synthesis of structural proteins. Fatty acids, which are an important part of overnutrition and which have been shown to induce ER stress in cultured hepatocytes and pancreatic β-cells (326), are likely to be among the agents causing ER stress. ER stress has been demonstrated to trigger activation of several serine/threonine kinases, including c-jun NH2-terminal kinase (JNK) and IκB-α kinase (IKK). For instance, ER stress leads to formation of the IRE-1α-TRAF 2 complex, which results in phosphorylation and activation of IKK. IKK phosphorylates and inactivates IκB-α resulting in activation and nuclear translocation of nuclear factor κ B, which is a key promoter of inflammation (327). The IRE-1α-TRAF 2 complex also phosphorylates and activates JNK, which induces the expression of proinflammatory cytokines and induces insulin resistance via serine phosphorylation of insulin receptor substrates 1 and 2 (328). ER stress is also a major source for the production of reactive oxygen species (ROS). This can occur via activation of protein disulfide isomerase (PDI), an enzyme which catalyzes disulfide bridge formation and in the process generates ROS. ROS are known to promote insulin resistance and inflammation (327,329). Thus, the ER may be a proximal site that senses nutritional excess and translates it into metabolic and inflammatory responses. Adipogenesis is associated with changes in amount and subunit composition of the NF-κB complexes. NF-κB subunits p65 (RelA), p68 (RelB), and IéB are upregulated during fat cell differentiation (330).

The discovery that obesity also activates intracellular pathways, including the IKK-β– NF-κB and JNK pathways (328,331) added fuel to the inflammatory hypothesis. Upregulation of the IKK-β–NF-κB axis leads to excess production of multiple potential mediators of inflammation, whereas JNK activation impinges upon insulin signaling through phosphorylation of serine residues of insulin receptor substrate-1. Stimuli potentially activating both IKK-β–NF-κB and JNK in obesity can be separated into extracellular ligands, such as the proinflammatory cytokines TNF-α, IL-1β and IL-6 or fatty acids binding to Toll-like receptors, and intracellular stimuli such as endoplasmic reticulum or oxidative stress, and ceramides (318). Determining which processes initiate obesity induced inflammation is an active area of investigation (314).

In conclusion, chronic tissue inflammatory responses may be part of a physiologic purpose, as adaptive restoration of tissue homeostasis in response to cellular stress. However, this response often becomes chronic in obesity, where the pathophysiological consequences of chronic adipose tissue inflammation result in insulin resistance (332).

Prediapocyte Plasticity

Preadipocytes are present throughout adult life in adipose tissues and can proliferate and differentiate into mature adipocytes according to the energy balance. An increasing number of reports demonstrate that cells from adipose lineages (preadipocytes and adipocytes) and macrophages share numerous functional or antigenic properties (333).

Recent studies suggest that preadipocyte and macrophage phenotypes are very similar and that preadipocytes have the potential to be very efficiently and rapidly converted into macrophages. This work emphasizes the great cellular plasticity of adipose precursors and reinforces the link between adipose tissue and innate immunity processes (333).

The emerging field of regenerative medicine will require a reliable source of stem cells in addition to biomaterial scaffolds and cytokine growth factors. Adipose tissue represents an abundant and accessible source of adult stem cells with the ability to differentiate along multiple lineage pathways (334).

Adipose tissue is now also regarded as a promising source of adult stem cells, as adipose tissue has plenty of progenitor cells, some of which can differentiate into diverse lineages (84). A component of fibroblast – like stromal cells obtained from liposuction aspirates can differentiate into various cell lineages (84), including adipogenic, osteogenic (334), chondrogenic (335), myogenic (337), cardiomyogenic (337), and neurogenic (338), thus, adipose tissue – derived stromal cells are now called adipose – derived stem/stromal/progenitor cells (ASCs) and are expected to become a valuable tools for a wide range of cell – based therapies (339).

ASCs are currently being used in some clinical trials, including treatments for bone defects (autologous fresh ASCs) (340), rectrovaginal fistula (atulogous cultured ASCs) (341), graft – versus – host disease (nonautologous ASCs) (342), and soft tissue grafting (autologous fresh ASCs) (343-346). ASCs have been found to have potential similarities to bone marrow – derived mesenchymal stem cells and are now of great interest as a tool for cell therapies (347).

Conclusions

During the last decade, understanding the biology of adipose tissue and, in particular, its secretory functions have dramatically improved, and this has completely modified our understanding of the pathophysiological link between the increase of fat mass, namely obesity, insulin resistance and cardiovascular complications.

As our understanding of the integrative biology of adipose tissue increases, we hope that these, and many other aspects of adipose tissue function, will be clarified.

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