


Focus: Metabolism

Adipose tissue: between the extremes

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Abstract

Adipose tissue represents a critical component in healthy energy homeostasis. It fulfills important roles in whole-body lipid handling, serves as the body's major energy storage compartment and insulation barrier, and secretes numerous endocrine mediators such as adipokines or lipokines. As a consequence, dysfunction of these processes in adipose tissue compartments is tightly linked to severe metabolic disorders, including obesity, metabolic syndrome, lipodystrophy, and cachexia. While numerous studies have addressed causes and consequences of obesity-related adipose tissue hypertrophy and hyperplasia for health, critical pathways and mechanisms in (involuntary) adipose tissue loss as well as its systemic metabolic consequences are far less understood. In this review, we discuss the current understanding of conditions of adipose tissue wasting and review microenvironmental determinants of adipocyte (dys)function in related pathophysiologies.

Keywords adipose tissue dysfunction; cachexia; lipodystrophy; metabolic disorders; obesity

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Functional adipose tissue is essential for health

The adipose organ can be divided into two distinct types of adipose tissues, white and brown. Brown adipose tissue (BAT) dissipates energy by producing heat (thermogenesis) via the uncoupling of the activity of the mitochondrial electron transport chain through the specific expression of uncoupling protein 1 (UCP1). BAT is composed of brown adipocytes characterized by a high mitochondrial content and endowed with a high capacity of lipid oxidation (Berriel Diaz *et al.*, 2014). In addition to the thermogenic brown adipocytes located in BAT, white adipose tissue (WAT) contains thermogenic fat cells, called “brown-in-white” (“brite”) or “beige” adipocytes which are able to burn fat and carbohydrates via non-shivering thermogenesis (Petrovic *et al.*, 2010; Vegiopoulos *et al.*, 2010).

White adipose tissue is specialized in the storage and release of fat, the balance of which is critical to maintain healthy energy homeostasis (Rohm *et al.*, 2013). Indeed, in humans, a combination of excessive lipid storage and decreased removal

leads to obesity and associated comorbidities, including insulin resistance and type 2 diabetes (Langin, 2011), now affecting more than 500 million overweight and/or obese people worldwide (Finucane *et al.*, 2011).

In addition to its lipid-storing capacity, WAT has been described as an important endocrine organ controlling the systemic handling of energy substrates (Galic *et al.*, 2009). Also, intracellular WAT lipid homeostasis is a key determinant of body weight and insulin sensitivity in both mice and humans (Yu & Ginsberg, 2005). In this respect, excessive lipid load causes adipocyte stress, which in turn accounts for many adverse effects of obesity, particularly alterations in adipocytokine release and a low-grade inflammatory response, ultimately leading to the development of metabolic dysfunction such as worsened insulin sensitivity and glucose intolerance (Hotamisligil, 2006; Rasouli & Kern, 2008). Due to their critical role in the maintenance of proper adipocyte function, both storage and release of lipids in WAT are physiologically under tight hormonal control: Whereas insulin promotes triglyceride (TG) storage during the postprandial phase, catecholamines trigger the breakdown of TG into glycerol and fatty acids (“lipolysis”) to provide energy substrates for other organs, including liver and skeletal muscle, during fasting (Yu & Ginsberg, 2005). An intriguing but still largely unexplored question is how the local microenvironment determines adipose tissue function and its impact on systemic metabolism.

Furthermore, whereas numerous studies have highlighted mechanisms in obesity predisposition, development, and manifestation, also conditions of absence or scarcity of WAT such as lipodystrophy and cachexia are associated with severe metabolic complications (Shimomura *et al.*, 1998; Petruzzelli & Wagner, 2016). Intriguingly, many of these metabolic conditions, including insulin resistance, glucose intolerance, and inflammation, are commonly shared between both opposing states of WAT mass, that is, scarcity of WAT and obesity, suggesting similar or even identical molecular mechanisms linking these conditions. However, how and whether obesity and adipose tissue deficiency couple at the molecular level, and whether scientists and clinicians can learn from either side to therapeutically counteract the opposite phenotype remain to be solved.

Given the high number of excellent reviews summarizing pathomechanisms in obesity development, this review aimed to highlight our current understanding of both mechanisms of WAT energy handling and also local determinants of WAT functionality as particularly related to lipodystrophy, cancer cachexia, and other states of WAT deficiencies.

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Handling of energy substrates determines the adipocyte impact on systemic energy balance

Organismal survival depends on the ability of adipocytes to temporarily store excess substrates and mobilize them upon decreased external nutrient supply. Substrates are stored mainly as triacylglycerols in lipid droplets and are released as fatty acids and glycerol by lipolysis. In the postprandial state, adipocytes take up fatty acids delivered by gut-derived chylomicrons or lipoprotein particles synthesized in the gut and the liver. Intracellularly, fatty acids are sequentially esterified to glycerol to form triacylglycerols and thus be incorporated as neutral lipids into the lipid droplet. Although the liver has been shown to be the major site of conversion of carbohydrates to lipid, adipocytes are also able to synthesize fatty acids from excess circulating glucose (*de novo* lipogenesis) (Abel *et al*, 2001; Mao *et al*, 2009).

During prolonged fasting or physical activity, the acyl moieties are released from triglycerides through the sequential action of lipases, namely adipose triglyceride lipase (ATGL/*Pnpla2*), hormone-sensitive lipase (HSL/*Lipe*) and monoglyceride lipase (MGL/*Mgl1*). Following transport mainly through the lymph, these fatty acids become systemically available for oxidation and ATP synthesis. In addition, released glycerol can serve as a substrate for gluconeogenesis in the liver.

The regulation of uptake of circulating substrates, triglyceride biosynthesis as well as lipolysis depends on the balance of systemic anabolic and catabolic signals. A complex interplay between multiple endocrine mediators and the sympathetic nervous system has been shown to govern adipocyte metabolism. Among these signals, the pancreatic hormone insulin seems to play a central role as a gauge of short-term systemic fuel availability. In the postprandial state, insulin promotes glucose and fatty acid uptake as well as lipogenesis and suppresses triglyceride lipolysis (Samuel & Shulman, 2012). The key role of insulin is highlighted by the severe lipodystrophy observed in adipocyte-specific insulin receptor knockout mice (Lee *et al*, 2013a; Qiang *et al*, 2016). However, as discussed below, local signals in the adipose tissue microenvironment can affect adipocyte metabolism with impact on systemic homeostasis.

The chronic availability of excess nutrients leads to enlargement of lipid droplets and adipocyte hypertrophy, culminating in larger fat depots and body weight gain. In fact, the gradual development of overweight as observed in the majority of the Western world population has been estimated to result from an imbalance of 50–100 kcal per day over several years (Mozaffarian *et al*, 2011). Adipocyte hypertrophy is not only characteristic for adipose tissue in obesity but is also associated with the development of obesity-associated pathology [(Blüher, 2013; Spalding *et al*, 2008) and refs therein]. In particular, hypertrophy correlated with dyslipidemia, inflammation, and impaired glucose homeostasis in humans (Hoffstedt *et al*, 2010; Klötting *et al*, 2010). Conversely, adipocyte size was shown to be smaller in obese individuals without metabolic disease (“metabolically healthy obese”) compared to obese patients with metabolic complications (Klötting *et al*, 2010). These clinical findings could be interpreted in two ways: (i) large adipocytes are pathogenic *per se* and/or (ii) the inability of adipocytes to further expand limits the capacity for excess fatty acid storage with the consequence of systemically elevated lipid levels. A recent report demonstrated that hypertrophic adipocytes had reduced

ability for insulin-stimulated glucose uptake in a cell-autonomous manner (Kim *et al*, 2015). However, increased adipocyte size has been shown to correlate with improved systemic glucose homeostasis in certain transgenic mouse models, arguing against a general pathogenic effect of hypertrophic adipocytes (Khan *et al*, 2009; Abreu-Vieira *et al*, 2015). Furthermore, the inability to efficiently store lipid in adipocytes of lipodystrophic patients and mouse models is associated with metabolic dysfunction as discussed below. Thus, it appears more likely that the limited storage capacity and the concomitant increase in ectopic lipids are major contributors to the pathogenesis of metabolic disease, as proposed in the “expandability hypothesis” (Unger & Scherer, 2010; Virtue & Vidal-Puig, 2010). Specific intermediates of fatty acid metabolism which accumulate under conditions of impaired triglyceride storage seem to play a key role in this process by causing cellular stress in adipocytes and systemically lipotoxicity (Fig 1, Box 1).

Which are the consequences of exceeded adipocyte storage capacity for the function of remote organs? As discussed in Box 1, excess circulating lipid and the concomitant increase in “reactive” lipid metabolites in remote tissues directly impact on insulin sensitivity in liver and muscle cells, resulting in increased glucose output in liver and reduced uptake in muscle, which are important contributors to impaired glucose tolerance and type 2 diabetes (Samuel & Shulman, 2012). In parallel, key nodes of insulin signaling can be inhibited indirectly by several central inflammatory pathways. Indeed, overloaded adipose tissue is associated with the activation of inflammatory and immune cells along with a network of inflammatory mediators locally as well as systemically. Chronic low-level inflammation results in insulin resistance, as demonstrated in numerous models with genetic inactivation of key signal transducers including, for example, tumor necrosis factor- α (TNF- α), I κ B kinase (IKK β) and c-Jun N-terminal kinase (JNK) [reviewed by Lackey and Olefsky (2016)]. Most recent advances in this direction revealed the role of the lipid mediator leukotriene B4 and the macrophage-derived Galectin-3 in the induction of obesity-associated systemic insulin resistance (Li *et al*, 2015, 2016). In addition to inflammation-related cytokines, alterations in secreted adipocyte-derived polypeptides (adipokines) mediate adipose tissue dysfunction to remote organs, including liver, muscle, and brain, thereby substantially contributing to systemic metabolic deterioration (Box 2). Furthermore, although the local signaling function of lipid mediators is well established, recent studies have revealed products of adipocyte lipid metabolism as beneficial endocrine regulators (“lipokines”) (Yilmaz *et al*, 2016). In particular, both palmitoleate (C16:1) and palmitic acid-hydroxy-stearic acid, deriving from adipocyte *de novo* lipogenesis, are able to improve systemic glucose metabolism and a reduction in their systemic levels may contribute to metabolic disease in humans (Cao *et al*, 2008; Yore *et al*, 2014).

Whereas the effects of overloaded adipose tissue on insulin sensitivity in liver and muscle are central in our understanding of lipid-induced systemic metabolic dysfunction, a simpler metabolic component needs to be considered. Impaired uptake of excess fatty acids or increased lipolysis rates in adipocytes result in higher fatty acid flux to the liver. This is a major contributor to the accumulation of lipids in the liver leading to non-alcoholic fatty liver disease, which can progress to non-alcoholic steatohepatitis involving inflammation and fibrosis (Donnelly *et al*, 2005). Importantly, these conditions are strongly associated with cardiometabolic disease and

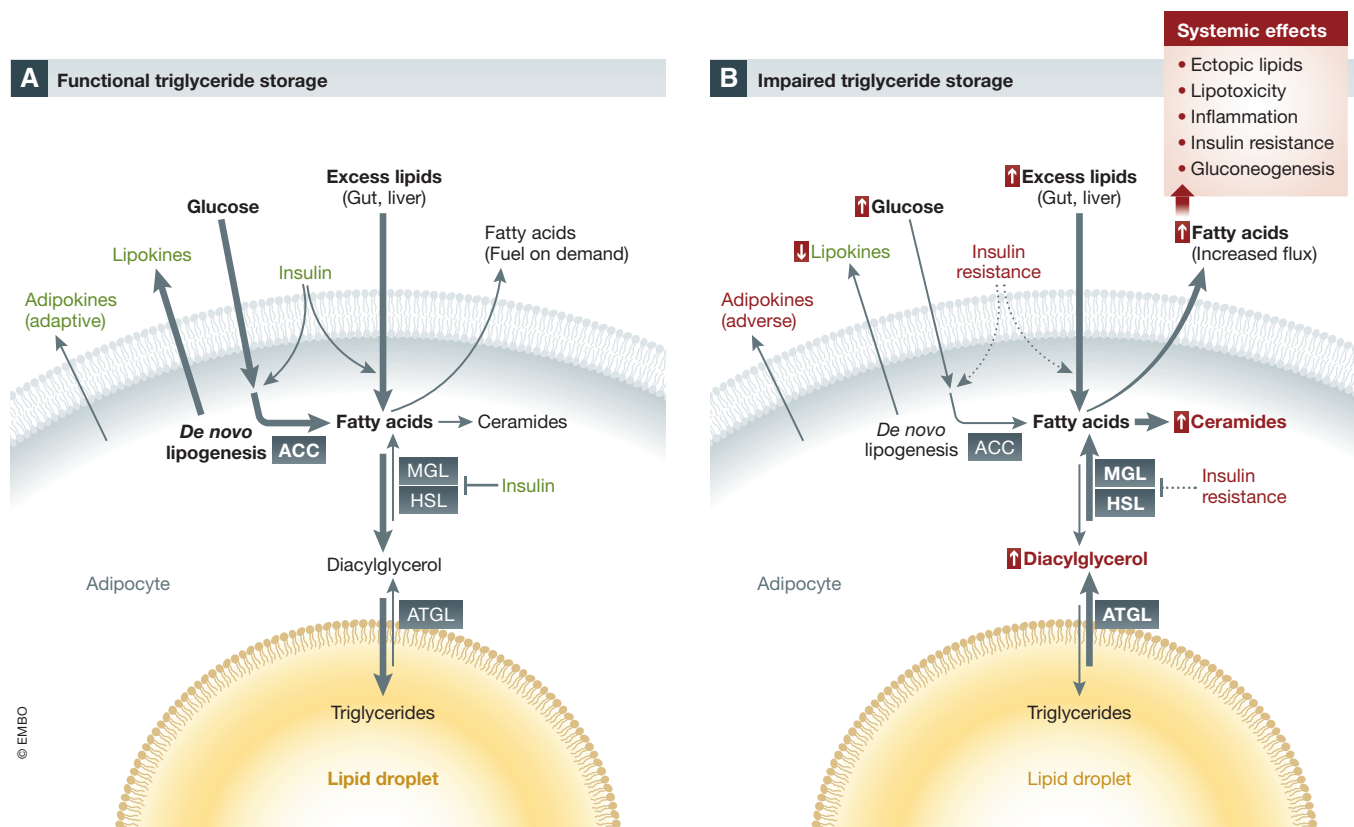


Figure 1. Lipid storage and metabolism in healthy and dysfunctional adipocytes.

(A) Functional adipocytes metabolize and store excess circulating lipids and glucose in the inert form of triglycerides in the lipid droplet. Fatty acids can be mobilized by lipolysis on demand. Pancreatic insulin promotes *de novo* lipogenesis as well as the uptake and storage of circulating lipids. The secretion of adipokines and lipokines by functional adipocytes contributes to systemic metabolic regulation. (B) Impairment of triglyceride storage in overloaded or dysfunctional adipocytes is associated with constitutive fatty acid mobilization, reduced glucose uptake and *de novo* lipogenesis as well as the generation of lipotoxic diacylglycerol and ceramides (these intermediates also accumulate in remote tissues). These metabolic changes are partly due to insulin resistance (see also Fig 4). Dysfunctional adipocytes are characterized by reduced production of certain lipokines as well as an adverse adipokine profile. Overall, the increased flux of fatty acids away from adipocytes promotes systemic metabolic dysfunction. Adaptive and pathogenic effects are shown in green and red, respectively. MGL, monoglyceride lipase; HSL, hormone-sensitive lipase; ATGL, adipose triglyceride lipase; ACC, acetyl-CoA carboxylase.

are prominent risk factors for hepatocellular carcinoma (Cornier *et al*, 2008; Yki-Jarvinen, 2014). In addition, liver glucose output and thereby blood glucose regulation have been shown to be driven by the control of adipose tissue lipolysis rather than liver insulin action (Rebrin *et al*, 1996; Mittelman & Bergman, 2000; Titchenell *et al*, 2016). Using sophisticated *in vivo* flux analysis, the Shulman

laboratory could show that the availability of adipose-derived fatty acids determined acetyl-CoA levels in the liver and thereby glucose output and blood glucose regulation, independently of liver insulin sensitivity (Perry *et al*, 2015). This depended on increased gluconeogenic flux through the allosteric activation of pyruvate carboxylase by acetyl-CoA.

Box 1: Impaired triglyceride storage and lipotoxicity

The biochemical form in which excess fatty acids exist in cells and tissues is critical for their pathogenicity. Thus, triglycerides stored in lipid droplets represent an inert non-pathogenic form as demonstrated in mouse models with genetic modifications influencing the final step of triglyceride biosynthesis (Samuel & Shulman, 2012). In line with this, mutations in genes involved in lipid droplet formation cause lipodystrophy and metabolic syndrome (as discussed below). In contrast, various intermediates of fatty acid metabolism have been shown to cause cellular stress and toxicity (lipotoxicity) in adipocytes and other relevant cell types, including myocytes, hepatocytes, and immune cells (Fig 1). A well-established case is represented by diacylglycerol, an intermediate of lipolysis and fatty acid esterification which can activate members of the protein kinase C (PKC) family to inhibit insulin signaling in mice and humans [reviewed in Shulman (2014)]. Saturated fatty acids have been shown to act as ligands for the Toll-like receptor 4, thereby promoting inflammation and insulin resistance (Shi *et al*, 2006; Lackey & Olefsky, 2016). Excess availability of saturated fatty acids is also associated with increased generation of the sphingolipid ceramide. Ceramides can interfere with insulin signaling and mitochondrial oxidation and promote endoplasmic reticulum stress and apoptosis (Chaurasia & Summers, 2015). Importantly, inhibition of ceramide synthesis was protective against insulin resistance in mice (Holland *et al*, 2007), and others reviewed by Chaurasia and Summers (2015).

Box 2: Adipokines and the endocrine function of fat

Beyond the systemic impact of local lipid metabolism, adipocytes substantially affect metabolic regulation of remote tissues through the endocrine action of secreted polypeptides termed adipokines. The plethora of adipokines, identified in the last two decades, essentially influence every organ system mediating physiological effects on metabolism, immunity, behavior, cardiovascular function, and reproduction, often with disease relevance (Fasshauer & Blüher, 2015). A prime example for how aberrant expression of adipokines contributes to systemic metabolic pathogenesis upon adipocyte dysfunction is given by adiponectin. Adiponectin promotes systemic insulin sensitivity and improves glucose and lipid metabolism in liver and muscle as well as pancreatic endocrine function (Stern *et al*, 2016). Most of its actions appear to be mediated by activation of the adenosine monophosphate-activated protein kinase (AMPK) and the ceramidase-mediated reduction in ceramide levels downstream of the AdipoR1 and R2 transmembrane adiponectin receptors. Adiponectin levels are reduced in obesity and show a remarkable inverse correlation with insulin resistance and type 2 diabetes in humans (Spranger *et al*, 2003; Klötting *et al*, 2010; Ye & Scherer, 2013). Thus, the loss of protective effects of adiponectin is likely to be a major pathogenic factor in metabolic disease. The prototype of adipokines is probably best represented by leptin (Friedman, 2016). The overtly obese phenotypes of humans and mice with inactivating mutations in the leptin/leptin receptor system illustrates the physiological importance of this pathway (Farooqi *et al*, 1999; Friedman, 2016). Although the most potent effects of leptin are mediated through the regulation of food intake and energy expenditure in the central nervous system, leptin modulates lipid metabolism and insulin sensitivity in several tissues, either directly or through the central nervous system (Stern *et al*, 2016). In contrast to adiponectin, leptin levels are increased in obesity but the protective effects of leptin are hindered by resistance to leptin signaling in the central nervous system (leptin resistance). Nevertheless, the potency of the leptin system is highlighted by the exploitation of leptin administration for the treatment of metabolic disorders in the absence of obesity, in particular lipodystrophy (see Box 3), non-alcoholic fatty liver disease and potentially type 1 diabetes (Wang *et al*, 2010; Stern *et al*, 2016).

A newer development relates to the role of secreted fatty acid binding protein 4 (FABP4). Proteins in the FABP family are able to bind lipophilic molecules intracellularly and influence lipid transport and metabolism (Hotamisligil & Bernlohr, 2015). Recently, circulating FABP4, secretion of which seems to be a regulated process, was shown to enhance hepatic glucose production (Cao *et al*, 2013). Along with accumulating evidence supporting a correlation of circulating FABP4 with obesity and metabolic disease, these findings define FABP4 as a novel disease-relevant adipokine (Hotamisligil & Bernlohr, 2015).

Beyond the regulation of glucose levels by liver and muscle, newer concepts of how systemic excess lipids affect metabolic homeostasis and promote cardiometabolic disease have emerged. These include hypothalamic and pancreatic lipotoxicity and inflammation, in addition to the well-established links to atherosclerosis and heart disease (Cornier *et al*, 2008; Janikiewicz *et al*, 2015; Kälén *et al*, 2015). Local toxic and inflammatory effects of excess lipids in the hypothalamus can impair the ability of this key central nervous system site to regulate food intake, systemic energy expenditure, and peripheral metabolism (Kälén *et al*, 2015). Similarly, ectopic fat and inflammation in the pancreas have been suggested to compromise the endocrine function, in particular insulin secretion (Janikiewicz *et al*, 2015).

Lipodystrophy

Lipodystrophies (LD) are defined by the partial or complete absence of metabolically active adipose tissue and can be acquired or originated in a genetic defect (Garg & Agarwal, 2009). In line with the idea of a critical functional adipose tissue mass, insulin resistance, dyslipidemia, hypertension, and diabetes often accompany lipodystrophy, and the degree of fat loss determines the degree of metabolic disease, arguing for the “expandability hypothesis” as outlined above (Fig 2) (Unger & Scherer, 2010; Virtue & Vidal-Puig, 2010). While circulating triglyceride levels are elevated, free fatty acid levels are mostly unaffected in lipodystrophy (Patni & Garg, 2015). Adipose tissue expansion has been shown to have beneficial effects in both LD and obesity: Adipose tissue transplantation into dyslipidemic, insulin-resistant A-ZIP/F-1 “fatless” mice reversed both ectopic fat accumulation and insulin resistance (Kim *et al*, 2000). Adipocyte progenitor injection had the same effect (Rodeheffer *et al*, 2008). In addition, adipose tissue expansion by increased adiponectin levels improved metabolic health in leptin-deficient obese ob/ob mice (Kim *et al*, 2007). Thus, adipose tissue plays a metaboloprotective role in the face of over-nutrition by storing excessive supplies. The major factors believed to cause metabolic syndrome in lipodystrophy are ectopic fat or “reactive” lipid species, and alterations in the adipokine profile (see Box 3). Adipose tissue inflammation as typically

seen in obesity is not commonly observed in LD, although present in HIV-related and some types of partial LD (Jan *et al*, 2004; Gandotra *et al*, 2011), and a recent study in Fsp27-deficient lipodystrophic mice shows that hepatic steatosis and insulin resistance are independent of adipose tissue inflammation (Zhou *et al*, 2003).

Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder caused by mutations in genes involved in triglyceride synthesis and lipid droplet formation, namely AGPAT2 (lysophosphatidic acid acyltransferase- β), BSCL2 (Seipin), CAV1 (Caveolin 1), and PTRF (Cavin-1) (Magre *et al*, 2001; Agarwal *et al*, 2002; Kim *et al*, 2008; Hayashi *et al*, 2009). AGPAT2 directly affects adipocyte differentiation via AKT and PPAR γ pathways (Patni & Garg, 2015). It catalyzes the second step in the *de novo* phospholipid synthesis pathway and plays a key role in triglyceride and glycerophospholipid formation from glycerol-3-phosphate (Agarwal *et al*, 2002). Thus, mutations in AGPAT2 lead to dysfunctional triglyceride storage, and in addition cause the accumulation of pathway intermediates such as lysophosphatidic acid (LPA). Increased LPA levels may further decrease adipose tissue mass and functionality by inhibiting adipogenesis and adipose tissue expansion (Rancoule *et al*, 2014a). Indeed, mice treated with the LPA-receptor antagonist Ki16425 showed enhanced adipose tissue expansion during high-fat diet feeding. Secreted LPA has also been implicated in glucose sensitivity (Rancoule *et al*, 2014a). Interestingly, a recent report also links LPA to increased adipose tissue fibrosis in obesity (Rancoule *et al*, 2014b), and LPA may thus be involved in lipodystrophy-induced fibrosis as discussed below. BSCL2, CAV1, and PTRF, which are mutated in other forms of CGL, are mainly involved in vesicle trafficking and affect the formation or maturation of lipid droplets (Patni & Garg, 2015).

Congenital generalized lipodystrophy is characterized by near-complete lack of body fat present at birth or from a very young age (Patni & Garg, 2015). Interestingly, while these patients have almost no metabolically active storage fat, mechanical fat (i.e., in joints, orbits, palms, and soles) is still present (Garg *et al*, 1992). Patients develop signs of metabolic syndrome during childhood, including dyslipidemia and ectopic triglyceride accumulation (Berardinelli,

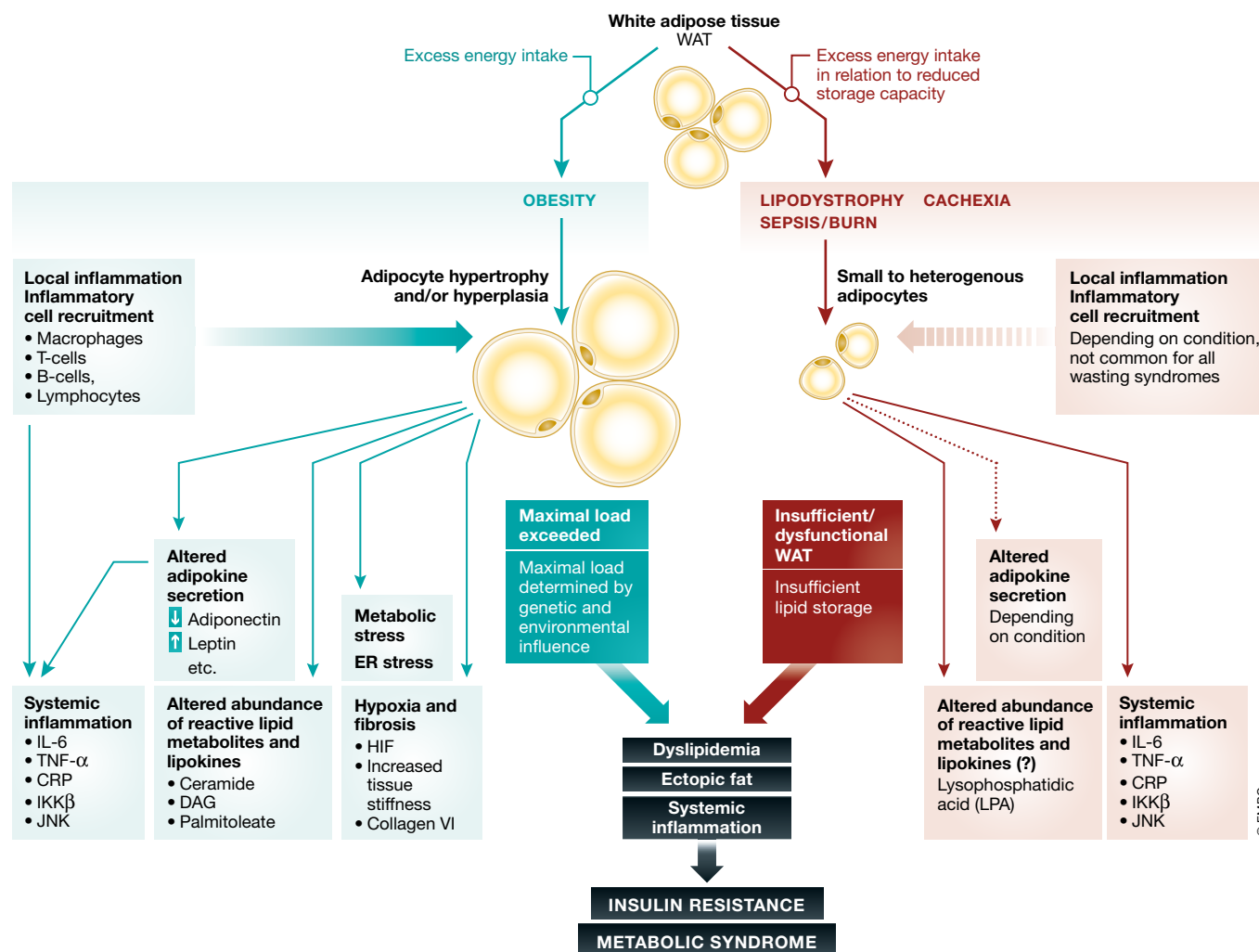


Figure 2. Obesity and wasting diseases in light of the adipose tissue expandability hypothesis.

Both obesity and wasting diseases cause adipose tissue dysfunction due to insufficient storage capacity, resulting in dyslipidemia, systemic inflammation and altered adipokine profiles. Combination of these factors favors the development of insulin resistance and the metabolic syndrome, which further impairs adipose tissue function, creating a futile cycle. IKK β , I κ B kinase; HIF, hypoxia-inducible factor; IL-6, interleukin-6; JNK, c-Jun N-terminal kinase; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein; DAG, diacylglycerol.

1954) and hyperinsulinemia or type 2 diabetes (Van Maldergem *et al*, 2002).

Depending on the degree of adipose tissue loss, the same symptoms are observed in partial lipodystrophies, both familial forms [familial partial lipodystrophy, FPLD (Garg, 2000)] and acquired forms, most commonly occurring in patients with human immunodeficiency virus (HIV) receiving highly active antiretroviral therapy (HAART) with protease inhibitors (Blumer *et al*, 2008). Indeed, it has been reported that up to 79% of lipodystrophic patients on HAART have insulin resistance or type 2 diabetes (Vigouroux *et al*, 1999). Protease inhibitor treatment leads to reduced levels of key transcription factors involved in regulating adipocyte differentiation and function, such as PPAR γ , C/EBP α , ATF-4, CHOP, and XBP-1, and increase levels of reactive oxygen species, causing inflammation. This leads to permanent alteration of adipose tissue functionality, so that lipodystrophy persists even after the protease treatment is

discontinued (Nolis, 2014). Impaired PPAR γ activity has also been reported in patients with FPLD3, where PPAR γ is mutated (Agarwal & Garg, 2002). Likewise, mutations in AKT2 and PLIN1 impair adipocyte function and cause FPLD, and a mutation in Lamin A/C (LMNA) causes cell death by weakening the nuclear envelope (Nolis, 2014). Unless CGL, FPLD results in partial AT loss mainly from the subcutaneous depots in the extremities and upper trunk region. Other adipose depots, such as those in the facial or vulvar areas, are often enlarged. Why some AT depots are affected by the lipodystrophy while others are preserved remains to be clarified, but likely traces back to alterations in microenvironment, extracellular matrix (ECM) composition, or different progenitor populations.

Cachexia

Adipose tissue insufficiency and dysfunction also occurs in cachexia, a pathological wasting syndrome defined by muscle and

Box 3: Lipodystrophy and metabolic disease

Lipodystrophies can be acquired or genetic diseases and are defined by various degrees of adipose tissue deficiency. They are often accompanied by metabolic complications such as insulin resistance, dyslipidemia, or cardiovascular disease. The severity of metabolic disease in LD depends on the degree of fat loss. The two major factors causing metabolic dysfunction associated with LD are believed to be dyslipidemia and alterations in the adipokine profile. Firstly, a deficit in adipose tissue leads to triglyceride redistribution toward other organs, like skeletal muscle or liver (Garg, 2004), leading to organ damage and reduced whole-body insulin sensitivity. This is underlined by the observation that over-expression of Agpat2 in livers of Agpat2^{-/-} mice did not reverse lipodystrophy-induced fatty liver disease (Agarwal et al, 2011). Hepatic steatosis is a characteristic of CGL (Berardinelli, 1954). Defective adipose tissue storage also leads to elevated circulating levels of "reactive" lipid metabolites, cholesterol, and triglycerides, causing atherosclerosis and cardiovascular disease, as shown in adipose tissue-deficient Seipin^{-/-} mice (Wang et al, 2015) and patients with CGL (Sanon et al, 2016).

In addition to dyslipidemia, adipokine and cytokine profiles are frequently altered in patients with LD. Both adiponectin and leptin levels are very low in LD, particularly in generalized LD (Haque et al, 2002). Whereas the reduction in adiponectin levels affects lipid metabolism, inflammation and insulin sensitivity in remote organs, low leptin levels increase the systemic lipid load by acting mainly centrally to increase food intake. A mouse study investigating the effect of leptin on liver in Agpat2^{-/-} mice suggests that the major function of leptin is this setting lies in regulation of food intake, as leptin protects Agpat2^{-/-} mice from hepatic steatosis and insulin resistance despite the deletion of the leptin receptor in hepatocytes (Cortes et al, 2014). Elevated energy expenditure has been measured in patients with LD, which is directly proportional to their energy intake. A calorie-restricted diet not only reversed energy expenditure, but also reversed the adverse metabolic effects of LD (Robbins et al, 1979), further underlining the importance of (leptin-regulated) food intake. In a mouse model of generalized LD, a combination therapy of adiponectin and leptin fully reversed insulin resistance (Yamauchi et al, 2001). Other studies performed in a range of murine LD models ranging from HAART to CGL have shown improved lipid profiles and insulin sensitivity by adiponectin and leptin therapy (Shimomura et al, 1999; Ebihara et al, 2001; Duntas et al, 2004; Xu et al, 2004). In patients with LD, the synthetic leptin analogue metreleptin (human recombinant methionyl leptin) has first been described to have beneficial effects in patients with LD over a decade ago (Oral et al, 2002) and is now successfully used to treat dyslipidemia and insulin resistance due to CGL (Javor et al, 2005; Ebihara et al, 2007), partial LD (Chong et al, 2010; Diker-Cohen et al, 2015; Ajluni et al, 2016), and antiretroviral therapy-induced LD (Lee et al, 2006; Mulligan et al, 2009).

adipose tissue loss that cannot be reversed by nutrition (Fearon et al, 2011). The wasting can be caused by a range of chronic diseases including chronic obstructive pulmonary disease, congestive heart failure, rheumatoid arthritis, chronic kidney disease, and HIV infection (Morley et al, 2006). It also occurs in up to 30% of patients with cancer (Morley et al, 2006) and is associated with a significantly increased mortality risk (Utech et al, 2012). Despite the clear clinical relevance, the mechanisms by which cachexia develops are still not well understood. Initially, wasting in cancer cachexia was thought to be caused exclusively by enhanced energy requirements of the tumor (Theologides, 1979). Indeed, tumors require substantial amounts of energy. A study by Liefers et al estimated that a metastatic colon cancer required up to 17,700 kcal in 3 months (Liefers et al, 2009). However, tumor size and degree of cachexia do not correlate, and tumors of the same type and degree can cause cachexia in some patients but not in others, just like outcomes in sepsis can be influenced by genetic variations in immunity (Fearon et al, 2012). Thus, the original idea was expanded to the concept of a cachectic metabolism in which tumors secrete factors that alter systemic metabolism, overall favoring catabolism (Fearon et al, 2012). A number of tumor-secreted or tumor-induced/host-secreted factors involved in cachexia development have already been described, including the inflammatory cytokines interleukin 1 and interleukin 6 (IL-1, IL-6) (Uehara et al, 1989; Strassmann et al, 1992; Baltgalvis et al, 2008), tumor necrosis factor-alpha (TNF- α) (Oliff et al, 1987), and interferon gamma (IFN- γ) (Acharyya et al, 2004), but also other factors such as zinc-alpha2-glycoprotein (ZAG) (Mracek et al, 2011), myostatin (Zimmers et al, 2002), ataxin 10 (Schäfer et al, 2016) and parathyroid hormone-related protein (PTHrP) (Kir et al, 2014). Despite the advance in identifying cachexia-inducing agents, treatment success has been limited so far, which can be attributed to a complex interplay between several tumor- and/or host-derived factors responsible for inducing wasting (Schäfer et al, 2016).

Cachexia has long been regarded as a muscle wasting disorder, but recently, adipose tissue wasting has started to gain attention in

regard to cachexia (Fearon et al, 2011), especially after noting that fat mass was lost before lean mass during the progression of cancer cachexia (Fouladi et al, 2005). Increased adipose tissue lipolysis is a key element in the development of cachexia (Zuijgeest-van Leeuwen et al, 2000). Indeed, increased levels of free fatty acids and glycerol are often observed in patients with cachexia (Tisdale, 2009), and many of the above-mentioned cachexokines induce lipolysis (Das et al, 2011). Microarray analysis of adipose tissue from patients with cancer cachexia revealed significant induction of genes involved in energy turnover and fatty acid degradation (Dahlman et al, 2010). The key enzymes of lipolysis, ATGL and HSL, play major roles in disease progression. ATGL and HSL activities are increased in patients with cancer cachexia (Das et al, 2011). Knockout of either of these lipases protected against cancer-induced adipose tissue loss and partly inhibited lean mass wasting. In line with a central role of lipolysis for disease progression, other cachexokines such as IL-6, TNF- α , or ZAG were unchanged in these models (Das et al, 2011), and *in vitro* studies confirmed that tumor-derived factor(s) rather than secondary effects were responsible for the induction of lipolysis (Rohm et al, 2016). Thus, disruption of fat catabolism has the potential to cause whole-body wasting, and therapies targeting adipocyte lipolysis are already under investigation for cachexia treatment (Mayer et al, 2013).

While enhanced adipose tissue lipolysis in cachexia is well established, the influence of lipogenesis on fat wasting is less clear. With dwindling adipose tissue supplies, one would expect reduced lipid synthesis in cachexia, and indeed, early studies in mice suggested that *de novo* lipogenesis was inhibited in tumor-bearing animals (Trew & Begg, 1959; Lanza-Jacoby et al, 1984). However, newer evidence suggests that in addition to lipolysis, lipogenesis is also increased in cachexia (Mulligan & Tisdale, 1991; Rohm et al, 2016). In the latter study, adipocytes showed both markedly enhanced lipolysis and triglyceride synthesis in response to tumor-derived stimuli, suggesting enhanced substrate cycling. Increased production of triacylglycerol-bound glycerol and *de novo* lipid synthesis

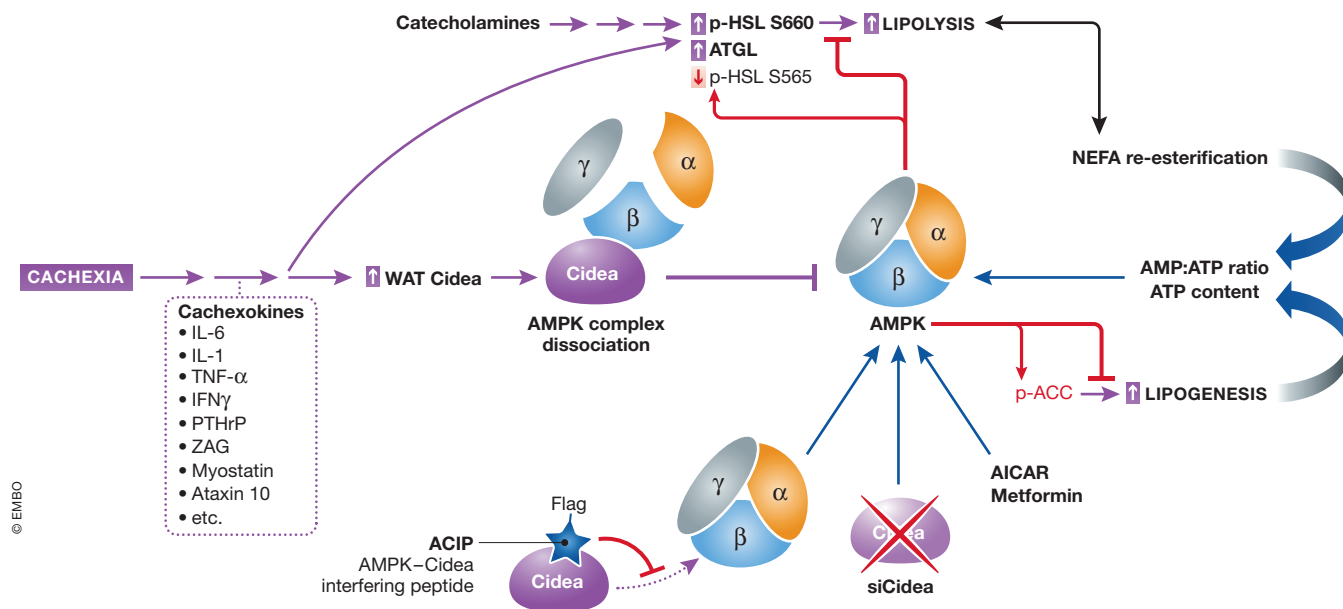


Figure 3. Activation of energy wasting pathways in cancer cachexia.

AMPK (adenosine monophosphate-activated protein kinase) in adipose tissue phosphorylates HSL (hormone-sensitive lipase) and ACC (acetyl-CoA carboxylase) at inhibitory sites, blocking lipolysis and lipogenesis. Cachexia-inducing tumors activate adipocyte lipolysis and lipogenesis by (i) upregulating lipase levels (ATGL, HSL), and (ii) reducing the inhibitory AMPK activity. Cachexia causes reduced AMPK activity due to increased levels and binding to CIDEA (cell death-inducing DNA fragmentation factor- α -like effector A) resulting in AMPK complex dissociation. Treatments targeting adipose tissue AMPK function (including ACIP, AMPK-CIDEA interfering peptide) have proven beneficial in counteracting cachexia. ZAG, zinc- α 2-glycoprotein; PTHrP, parathyroid hormone-related protein; NEFA, non-esterified fatty acid.

has also been described in a different model of cancer cachexia (Beck & Tisdale, 2004). Since substrate cycling is an energy costly process, the parallel induction of lipolysis and lipogenesis in the adipocyte leads to enhanced energy demand. In the face of energy undersupply due to cachectic metabolism and anorexia, this contributes to adipose tissue loss in cachexia. In line with this, ATP levels in cachectic adipose tissue are reduced by more than 50% (Rohm *et al*, 2016).

Energy deprivation usually activates the cellular energy sensor adenosine monophosphate-activated protein kinase (AMPK) by a conformational change in its gamma subunit and subsequent activating phosphorylation of its alpha subunit (Hardie *et al*, 2012). Generally speaking, active AMPK promotes energy conservation by activating ATP-producing catabolic pathways and inhibiting ATP-consuming anabolic pathways (Hardie *et al*, 2012). In adipocytes, AMPK activation leads to inhibition of fatty acid synthesis and lipolysis by inhibitory phosphorylation of the key enzymes HSL (Garton & Yeaman, 1990) and acetyl-CoA carboxylase (ACC) (Sullivan *et al*, 1994). In cachexia, however, this regulation is dysfunctional: AMPK levels and protein activity in cachectic adipose tissue are low despite energy deprivation (Rohm *et al*, 2016). Thus, an essential brake to energy wasting is missing. In line with a central role of adipocyte AMPK activity in fat wasting, a previous study on AMPK knockout mice (Prkab1^{-/-}, knock out of AMPK β 1 subunit) described reduced body fat content, slightly elevated circulating fatty acid levels, and enhanced adipocyte lipogenesis (Dzamko *et al*, 2010). Interestingly, adipocyte lipolysis was not altered in this study, arguing for additional regulatory factors. Chronic AMPK activation by the AMP analogue AICAR (5-aminoimidazole-4-carboxamide riboside) has been shown to

inhibit lipolysis in both mice and men (Boon *et al*, 2008; Anthony *et al*, 2009). Thus, under certain conditions, AMPK inhibition can promote lipid mobilization.

How is AMPK dysregulated in cancer cachexia? We have recently shown that the AMPK dysfunction in cachexia roots in AMPK complex disintegration and subsequent protein degradation caused—at least in part—by interaction with CIDEA (Fig 3) (Rohm *et al*, 2016). Cell death-inducing DNA fragmentation factor- α -like effector A (CIDEA) has previously been reported to interact with the AMPK β 1 subunit (Qi *et al*, 2008), thereby preventing AMPK complex formation and destabilizing the protein. Cidea^{-/-} mice displayed lower concentrations of plasma free fatty acids and dampened fatty acid release from BAT (Zhou *et al*, 2003). We and others have shown that CIDEA is upregulated in cachectic adipose tissue in both patients and rodents (Laurencikiene *et al*, 2008; Rohm *et al*, 2016). In cachexia, CIDEA binds the AMPK β 1 subunit, the AMPK complex dissociates, and major brakes in lipolysis and lipogenesis are disabled (Rohm *et al*, 2016). Under normal conditions, CIDEA is hardly expressed in WAT, particularly in rodents, and is considered to be a brown fat marker (Zhou *et al*, 2003; Laurencikiene *et al*, 2008). However, CIDEA expression is induced during browning of WAT. Brite adipose tissue has the potential to create increased energy expenditure and body weight as well as adipose tissue loss (Sidossis & Kajimura, 2015). It has recently been proposed that WAT browning may play a role in the progression of wasting during cancer- (Kir *et al*, 2014; Petruzzelli *et al*, 2014) and nephrectomy-induced (Kir *et al*, 2016) cachexia. These studies showed elevated brown fat markers, including UCP1 and CIDEA, in cachectic WAT and proposed that browning-induced elevated energy expenditure contributed to wasting. However, elevated resting energy

expenditure is often not present in cachexia, both in patients and mouse models (Blum *et al*, 2011; Rohm *et al*, 2016), arguing for a different role of CIDEA in cachexia, such as in AMPK regulation. In line with this, cachexia has been shown to develop both under thermoneutral conditions, when the thermogenic program is largely inactive (Cui *et al*, 2016), and under blockage of beta-adrenergic signaling (Rohm *et al*, 2016). Also, UCP1^{-/-} mice injected with cachexia-inducing Lewis lung cancer (LLC) cells showed no difference in lipolysis, energy expenditure, or loss of body weight, lean mass and adipose tissue mass compared to their LLC-injected wild-type littermates (Rohm *et al*, 2016).

Thus, there are still many things to be learned about the role of adipose tissue in cachexia. It certainly plays a prominent role in disease progression, as a number of studies over the last few years show (Das *et al*, 2011; Kir *et al*, 2014; Petruzzelli *et al*, 2014; Rohm *et al*, 2016). It may also be a promising therapeutic target, especially since treatment options for cachexia are limited and altering major clinical outcomes is not possible so far (Fearon *et al*, 2013). Current lines of treatment involve nutritional supplementation, resistance exercise, steroid hormones, and inhibition of inflammatory mediators, for example using thalidomide or cyclooxygenase inhibitors (such as indomethacin and ibuprofen) (Fearon *et al*, 2013). Newer approaches include the use of the hunger hormone ghrelin or anti-myostatin antibodies (Fearon *et al*, 2013). Cachexia is a multifactorial disease and as such is unlikely to be cured by a single agent. Thus, including modulators of adipose tissue metabolism is a promising future approach to treat the disease. Kir *et al* (2014) have shown that blocking PTHrP action on adipose tissue prevented cachexia in LLC-injected mice. Petruzzelli *et al* (2014) have shown that blocking IL-6 could maintain adipose tissue integrity and prevented wasting in a cachexia model. Other adipocentric therapies, for example, Atglistatin, which targets lipolysis by inhibiting ATGL, are currently developed (Mayer *et al*, 2013). We have recently shown that maintaining adipose tissue AMPK functionality by introducing a peptide blocking the CIDEA-AMPK interaction (termed AMPK-CIDEA interfering peptide, ACIP) prevented excessive lipolysis and prolonged life of cachectic animals (Rohm *et al*, 2016). In line with this, activating AMPK in human adipocytes using biguanides and thiazolidinediones (TZD) has been shown to inhibit lipolysis (Bourron *et al*, 2010), and metformin treatment of cachectic rats has proven effective in preventing wasting (Oliveira & Gomes-Marcondes, 2016).

Burn injury

Novel treatment approaches gained from cachexia research have the potential to improve adipose tissue wasting in other diseases, too. Burn injury, for example, has a strong impact on adipose tissue metabolism. Severe burn injuries, which cover 30% or more of the total body, induce lasting metabolic changes in part by altering adipose tissue function (Rojas *et al*, 2012). The trauma induces a biphasic metabolic response, with an initial phase of reduced energy expenditure, followed by a prolonged phase of increased resting energy expenditure and hypermetabolism (Breznock, 1980). This second phase can last for years after the actual burn injury and is associated with lean mass and adipose tissue loss, increased cardiac output and metabolic rate as well as poor immune function. This is often accompanied by insulin resistance (Rojas *et al*, 2012).

A major factor in the development of hypermetabolism due to burn injuries are catecholamines (Wilmore *et al*, 1974; Kulp *et al*, 2010). Catecholamine levels can remain elevated for years after the actual injury (Rojas *et al*, 2012), and a high catecholamine concentration is associated with a more severe form of hypermetabolism (Williams *et al*, 2009). Blocking catecholamine signaling, for example, by the β -adrenergic receptor antagonist propranolol, reduces burn-induced hypermetabolism (Pereira *et al*, 2005). Catecholamines are also major drivers of adipocyte lipolysis. Lipolysis and fatty acid release into the circulation have been shown to be elevated upon burn injury (Williams *et al*, 2009). Interestingly, a recent study showed that adipose tissue loss due to septic burn injury could be traced back to a loss in AMPK-dependent inhibition of lipolysis in the adipose tissue (Diao *et al*, 2015). Similar to cachexia, burn injury often presents with elevated levels of inflammatory cytokines such as IL-6 (Abdullahi *et al*, 2017). Studies in mice have shown that IL-6 [which in case of burn injury is released from the bone marrow (Abdullahi *et al*, 2017)] has the potential to induce hypermetabolism, and IL-6 blockage can prevent adipose tissue defects in burn injury (Abdullahi *et al*, 2017). Interestingly, burn injury has recently been linked to increased adipose tissue browning (Patsouris *et al*, 2015; Abdullahi *et al*, 2017), and multilocular, UCP1-positive adipocytes have been found in WAT from burn patients (Sidossis *et al*, 2015). In the latter study, UCP1 mRNA levels, mitochondrial density, and leak respiratory capacity were all increased in adipocytes after burn injury (Sidossis *et al*, 2015).

Local determinants of adipose tissue functionality

Adipose tissue cellularity (number and size of adipocytes)

The capacity of adipose tissue to store excess nutrients safely and without metabolic overloading of adipocytes depends on the number of adipocytes per depot. The fact that metabolically healthy obese are predicted to have higher adipocyte numbers compared to weight-matched insulin-resistant individuals suggest that adipose tissue hyperplasia is beneficial (Klötting *et al*, 2010). In accordance, insulin sensitization through treatment with the anti-diabetic TZD is associated with increased numbers of smaller adipocytes (Okuno *et al*, 1998; Tang *et al*, 2011). The number of adipocytes depends on the balance between cell death and adipogenesis, that is, the formation of new adipocytes from progenitor cells. Adipocyte progenitors have been recently identified *in vivo* as immature mesenchymal stromal cells largely associated with the vasculature (Rodeheffer *et al*, 2008; Tang *et al*, 2008). The turnover rate in adult humans has been estimated at 10% per year, rather low levels compared to other tissues (Spalding *et al*, 2008). In any case, the size of fat depots correlates with the number of adipocytes, as shown for some depots in humans (Arner *et al*, 2013). Importantly, obese individuals have higher total adipocyte numbers, and this becomes evident already in childhood obesity (Spalding *et al*, 2008). Since the genetic determination of obesity has been demonstrated by GWAS as well as monogenic phenotypes, it is likely that the regulation of adipocyte numbers will have a genetic component (Dahlman *et al*, 2016). However, this remains to be proven by human studies focusing on adipose tissue cellularity.

How does the nutritional environment influence adipocyte numbers? In a unique study with healthy adults, Tchoukalova *et al*

(2010) could show that 8 weeks of overfeeding increased the number of adipocytes in association with the enlargement of the fat depot by 1.6 kg. In rodents, adipose tissue hyperplasia has been demonstrated in hyperphagic dietary and genetic models of obesity (Johnson & Hirsch, 1972; Klyde & Hirsch, 1979). More recent genetic fate mapping experiments could prove that this occurs through the formation of new adipocytes through differentiation of progenitor cells (Lee *et al*, 2012; Wang *et al*, 2013; Vishvanath *et al*, 2016). Intriguingly, work from the Rodeheffer laboratory revealed that a hypercaloric diet with high fat content (HFD) stimulated a proliferation wave of adipocyte progenitors, which subsequently gave rise to mature adipocytes, thereby contributing substantially to adipose tissue expansion (Jeffery *et al*, 2015). To which extent progenitor differentiation without preceding cell proliferation contributes to fat hyperplasia remains to be determined.

A common clue from the above studies was the marked fat depot-specificity of the hyperplastic response to the diet. Thus, increased adipocyte numbers could be detected in the femoral subcutaneous fat depot in humans but not in the upper abdominal subcutaneous depot (Tchoukalova *et al*, 2010). In male mice, higher rates of proliferation/adipogenesis were shown in the intra-abdominal gonadal fat depot but not in the posterior subcutaneous depot (Wang *et al*, 2013; Jeffery *et al*, 2015). These differences can have substantial impact on the systemic adaptation of metabolism given the pathophysiological relevance of the individual depots. Accumulation of fat in the intra-abdominal/visceral depots is a central feature of the metabolic syndrome and is associated with insulin resistance and increased risk for diabetes and cardiovascular disease (Cornier *et al*, 2008; Lee *et al*, 2013b). In contrast, higher subcutaneous fat mass is associated with improved metabolic parameters including insulin sensitivity and blood lipid profiles. Interestingly, female mice increased progenitor proliferation and adipogenesis in both the intra-abdominal and the subcutaneous depots in response to HFD (Jeffery *et al*, 2016). This is in line with the fact that weight gain in premenopausal women generally comes along with preferential subcutaneous fat accumulation, whereas men show increased visceral fat deposition. This differential fat distribution has been suggested to contribute to the relative protection from cardiometabolic disease in women (Karastergiou *et al*, 2012). Taken together, location-specific hyperplastic expansion of adipose tissue can affect body fat distribution. Although direct proof is pending, the adaptive increase in adipocyte numbers is likely to contribute to systemic metabolic homeostasis and influence pathogenesis. This notion is supported by a study using stable isotope tracing to model proliferation-dependent adipocyte turnover in mice (Kim *et al*, 2014). The authors concluded that HFD-induced adipogenesis occurred predominantly in juvenile mice and that the reduction in hyperplastic potential in adults could be responsible for the development of insulin resistance. Furthermore, Lotta *et al* could identify a genetic association of 53 loci with traits related to insulin resistance as well with type 2 diabetes and coronary heart disease (Lotta *et al*, 2017). Remarkably, genetic predisposition for insulin resistance predicted by these loci was associated with reduced fat deposition in peripheral “metabolically safe” depots. Moreover, the study revealed common links to lipodystrophy-related insulin resistance, highlighting the importance of adipose tissue function for systemic homeostasis.

The molecular regulation of adipogenesis has been investigated over several decades, and a comprehensive network of signaling, transcriptional, and epigenetic factors has been described (Cristancho & Lazar, 2011). However, with limited exceptions, the links to the regulation of adipocyte number *in vivo* including the genetic determination of adipose tissue growth in humans have not been established. In particular, the mechanisms by which adipocyte progenitor proliferation and differentiation are controlled by systemic factors and the microenvironment in response to excess nutrients are poorly understood. In a recent report, activation of the Akt2 signal transducer in adipocyte progenitors was shown to be crucial for their HFD-induced proliferation and adipogenesis (Jeffery *et al*, 2015). This is consistent with the pro-adipogenic effect of insulin and insulin-like growth factor 1 (IGF1) as upstream Akt activators in cell models of adipogenesis (Garten *et al*, 2012). It remains to be determined to which extent insulin/IGF1 mediates the systemic anabolic state to adipocyte progenitors upon over-nutrition *in vivo*.

The role of the microenvironment as the adipocyte progenitor niche in the regulation of tissue plasticity is increasingly attracting attention. Obesity is associated with changes in the extracellular matrix (ECM, discussed below) and manipulation of the ECM has been shown to alter diet-induced adipose tissue growth (Christiaens *et al*, 2008). Given the established function of the ECM in the control of cell fate in multiple stem cell systems, it is likely that future studies will reveal similar findings in relation to adipose tissue (Guilak *et al*, 2009; Cristancho & Lazar, 2011). A further emerging concept is the involvement of transient local inflammation as a trigger for adipose tissue remodeling, both in the context of hyperplastic expansion and browning of white fat depots. Thus, genetic inhibition of central inflammatory pathways in fat resulted in reduced tissue growth (Wernstedt Asterholm *et al*, 2014). Lee *et al* (2013c) described the induction of an adipogenic niche by HFD and other triggers with macrophages playing a central role. Generally, according to the current consensus, adipocyte progenitor responses are driven by M2-type macrophages and type 2 innate immune cells during adaptive tissue remodeling (Brestoff & Artis, 2015; Odegaard & Chawla, 2015).

Inflammation

In contrast to transient adaptive inflammatory responses, chronic inflammation due to adipocyte overloading and lipotoxicity has a major impact on adipocyte metabolism and adipose tissue function. Thus, macrophage infiltration of adipose tissue was a strong predictor of insulin resistance in obese subjects after matching for BMI and other factors (Klötting *et al*, 2010). Similarly, one of the effects of bariatric surgery is the reduction of macrophage infiltration in conjunction with insulin sensitization (Frikke-Schmidt *et al*, 2016). The causal relationship between obesity-induced chronic inflammation and metabolic dysfunction has been established in numerous mouse models with genetic manipulation of key inflammatory pathways [reviewed in (Lackey & Olefsky, 2016)]. Inflammatory mediators can affect adipocyte metabolism in several ways. The inhibition of insulin signaling compromises the clearance of glucose and fatty acids in the postprandial state. In addition, the stimulation of lipolysis further contributes to fatty acid flux to the liver and other organs, leading to systemic lipotoxicity and insulin resistance as discussed above (Perry *et al*, 2015).

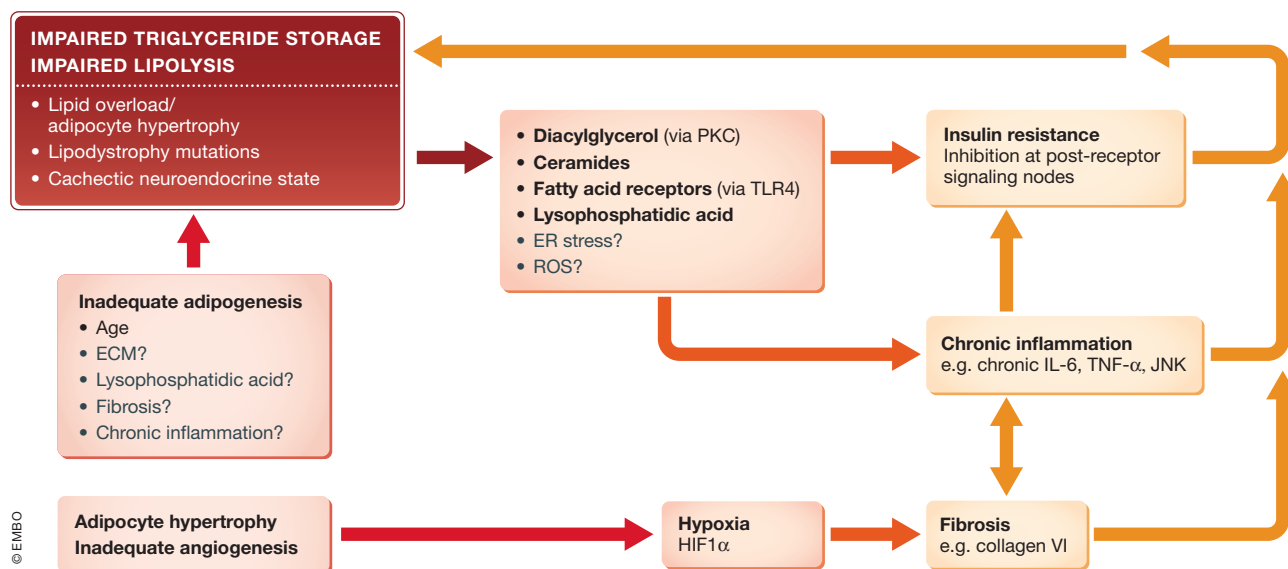


Figure 4. Futile cycles driving impaired triglyceride storage and lipolysis.

Adipocyte overloading in the absence of compensatory adipocyte formation and angiogenesis or lipodystrophy-associated impaired triglyceride storage can increase “reactive” lipid intermediates, other cell stressors, and hypoxia and thereby promote inflammation, fibrosis and insulin resistance. These factors interact and synergistically feed back to deteriorate adipocyte metabolism and safe lipid storage. ECM, extracellular matrix; ER, endoplasmic reticulum; HIF1 α , hypoxia-inducible factor 1 α ; PKC, protein kinase C; ROS, reactive oxygen species; TLR4, Toll-like receptor 4.

Furthermore, certain cytokines have been shown to inhibit the formation of new adipocytes, thereby possibly limiting the storage capacity of the tissue (Gustafson *et al*, 2009). It becomes apparent that a futile cycle between impaired lipid storage, inflammation and insulin resistance drives the metabolic syndrome and progression to disease (Fig 4).

As discussed below, inflammation can also promote fibrosis as a further pathogenic feature in adipose tissue. Finally, beyond inflammation, vascularization and angiogenesis as well as sympathetic innervation have been suggested to be important determinants of adipocyte metabolism and adipose tissue expansion (Bartness & Song, 2007; Cao, 2013).

Extracellular matrix and fibrosis

The ECM plays an important role in regulating tissue integrity and function. In recent years, adipose tissue ECM has emerged as a new regulator of adipose tissue differentiation, metabolism, and inflammation, and as such has important implications for metabolic disease. The ECM consists of a fibrillary network of structural and adhesive proteins, mainly fibronectin and collagens, which provide both mechanical support and create micro-domains as basis for cellular signaling. Other components include laminin, elastin, proteoglycans, and other polysaccharides, and ECM components are also directly linked to membrane receptors such as integrins (Mariman & Wang, 2010; Sun *et al*, 2013). Matrix metalloproteinases (MMPs) dynamically degrade and remodel the ECM (Sun *et al*, 2013). In adipose tissue, ECM components are produced by adipocytes, pre-adipocytes and inflammatory cells (Buechler *et al*, 2015). They regulate mechanical properties, adipogenesis, cellular expansion and lipid droplet growth (Nakajima *et al*, 2002; Alkhouli *et al*, 2013). For example, studies using different ECM components like fibronectin or laminin for pre-adipocyte culture demonstrate that

ECM remodeling from a fibrillar to a laminar structure is required for adipocyte differentiation (Spiegelman & Ginty, 1983; O’Connor *et al*, 2003).

Excess accumulation of ECM is termed fibrosis. Fibrosis is a ubiquitous response to chronic inflammation (Wynn, 2007), and increased fibrosis is commonly observed in adipose tissue of obese mice and patients (Strissel *et al*, 2007; Henegar *et al*, 2008; Divoux *et al*, 2010; Sun *et al*, 2013). Fibrotic areas and collagen accumulation in the interstitial and pericellular space are up to 4 times higher in obese than in lean patients (Divoux *et al*, 2010). Collagen VI expression in human adipose tissue is increased already after 8 weeks of overfeeding (Pasarica *et al*, 2009), and Col6 knockout in obese ob/ob or high-fat diet fed mice reduces weight gain and adiposity (Khan *et al*, 2009). Interestingly, weight loss after bariatric surgery does not resolve fibrosis in obese adipose tissue, even 2 years after surgery (Canello *et al*, 2013). Hypoxia is thought to be one of the main drivers of fibrosis in obesity. Obesity induces reduced capillary density (Spencer *et al*, 2011) and local hypoxia at the site of hypertrophic adipocytes, which leads to the activation of hypoxia-inducible factor 1 alpha (HIF1 α). HIF1 α stimulates ECM synthesis (Halberg *et al*, 2009) and cross-linking (Mariman & Wang, 2010), but also inflammatory gene expression, leading to macrophage recruitment. HIF1 α overexpression in adipocytes is sufficient to stimulate fibrosis and local inflammation (Halberg *et al*, 2009). In addition to adipocytes, macrophages and mast cells are also profibrotic (Divoux *et al*, 2012; Hirai *et al*, 2014; Jang *et al*, 2016), so inflammatory cell recruitment contributes to fibrosis development in obesity. Increased ECM, particularly surrounding adipocytes (Divoux *et al*, 2010), can further lead to adipocyte necrosis and additional inflammatory cell recruitment. Inflammation also influences adipocyte differentiation and lipid metabolism (Virtue & Vidal-Puig, 2010). Adipose stiffness due to increased ECM limits

adipocyte growth (Abdenmour *et al*, 2014), which in line with the “expandability hypothesis” (Virtue & Vidal-Puig, 2010) leads to dyslipidemia and metabolic disease. In line with this, mice lacking Col6 show extreme adipocyte hypertrophy without metabolic disease (Khan *et al*, 2009). Fibrosis and inflammation are tightly linked, but it is unclear so far which comes first in adipose tissue. Fibrosis is a universal response to inflammation, an attempt of wound healing that occurs in multiple tissues upon chronic inflammation. A microarray time course of diet-induced obesity suggests that inflammation is the primary driver of fibrosis, since in this study, fibrosis is a late event in obesity development (McGregor *et al*, 2013). On the other hand, fibrosis also causes inflammation: Hypertrophic adipocytes induce hypoxia, thereby activating Hif1 α and promoting fibrosis, which then causes adipose tissue stiffness, cell death, and inflammatory cell recruitment. Hypoxia in adipose tissue is one of the first pathological changes that occurs during obesity development (Halberg *et al*, 2009). In line with this, Halberg *et al* (2009) showed that fibrosis markers appeared before inflammatory markers upon high-fat diet feeding. Increased adipocyte necrosis due to tissue restriction, increased deposition of collagens, and integrin signaling attract pro-inflammatory cells, thereby promoting further fibrosis. In line with this, a recent study has shown that deletion of the integrin CD11b inhibits alternative activation and proliferation of adipose tissue macrophages (McGregor *et al*, 2013; Zheng *et al*, 2015). Other factors, such as LPA or the adipokines adiponectin and leptin, have been described to influence fibrosis independent of inflammation (Rancoule *et al*, 2014b; Saxena & Anania, 2015). In lipodystrophy (discussed below), adipose tissue inflammation is not always present, while fibrosis occurs frequently. Thus, more work is still needed to fully understand the mechanistic links between the two conditions.

Likewise, it is unclear so far whether the increased adipose tissue fibrosis in obesity is linked to metabolic disease, that is, whether it is beneficial or detrimental. The strong correlation with both local inflammation and systemic inflammation (Sun *et al*, 2013) would suggest that high adipose tissue fibrosis is metabolically unfavorable. Indeed, some studies described a positive correlation between fibrosis and markers of metabolic disease or insulin resistance (Pasarica *et al*, 2009; Spencer *et al*, 2011). Tissue stiffness as measured by shear-wave velocity was also increased in adipose tissue of obese diabetic compared to non-diabetic patients (Abdenmour *et al*, 2014). However, other studies detected no correlation between total adipose tissue fibrosis and diabetes (Divoux *et al*, 2010), or provided evidence for reduced fibrosis in obese diabetic patients when compared to metabolically healthy obese (Lackey *et al*, 2014; Muir *et al*, 2016). Thus, the relationship between fibrosis and diabetes is not fully understood yet, and variability likely arises through differences in methodology, that is, different fat depots studied, different ways to measure fibrosis, etc., as well as a high variability between individuals, as noted particularly in the obese state by Pasarica *et al* (2009).

Fibrosis and adipose tissue stiffness in wasting

Transgenic mice with overexpression of a constitutively active TGF- β develop a phenotype resembling lipodystrophy, with extremely reduced adipose tissue mass, which is mainly attributed to inhibited adipogenesis upon TGF- β overexpression. They also display fibrosis of the liver, kidney, and adipose tissue, as measured by trichrome

staining (Clouthier *et al*, 1997). Interestingly, this mouse also displayed an altered BAT with white appearance, fibrosis and mostly unilocular adipocytes (Clouthier *et al*, 1997). In patients with HIV-induced lipodystrophy, adipose tissue fibrosis presents in combination with inflammation, apoptosis, and decreased adipogenesis (Jan *et al*, 2004). A different study on patients with partial lipodystrophy (either by LMNA mutation or HIV protease inhibitor treatment-induced) describes increased fibrosis and a larger number of small adipocytes in the remaining adipose tissues (Berezat *et al*, 2011). This was without effects on inflammation or angiogenesis. However, these patients displayed increased UCP1 expression and mitochondrial function in their remaining WAT (Berezat *et al*, 2011). Patients with partial lipodystrophy caused by PLIN1 mutations display smaller adipocytes accompanied by macrophage infiltration and marked fibrosis (Gandotra *et al*, 2011).

In a microarray from cachectic adipose tissue of patients, ECM, actin cytoskeleton and focal adhesion were the most strongly down-regulated pathways (Dahlman *et al*, 2010). The observed gene expression changes were largely reciprocal to those previously associated with obesity (Dahlman *et al*, 2005), arguing for an important role of ECM plasticity and cytoskeleton for adipose tissue wasting. ECM genes are also strongly downregulated in human adipose tissue after long-term weight reduction (Kolehmainen *et al*, 2008). This may represent adaptations of the adipose tissue to reduced lipid droplet volume and nutrient or oxygen availability.

Of note, downregulated expression of ECM-related proteins does not necessarily equal reduced ECM accumulation, since negative regulators such as MMPs or signaling molecules are also included in this list (Dahlman *et al*, 2010). The observation that AMPK activation using metformin inhibits fibrotic gene expression and collagen deposition in genetic or diet-induced obese mice, and interstitial fibrosis is associated with AMPK inactivation in adipose tissue of obese humans (Luo *et al*, 2016), opens the question whether fibrosis is also involved in adipose tissue wasting during cachexia development, where AMPK function is impaired (Rohm *et al*, 2016). Studies in this field are rare to date, but initial reports describe increased interstitial space and collagen staining in cachectic adipose tissue from patients (Mracek *et al*, 2011). Picro Sirius Red staining and expression of collagens Col3a1 and Col6a1 (Alves *et al*, 2015) as well as tissue remodeling and accumulation of inflammatory cells in fibrotic areas (Batista *et al*, 2016) was observed in adipose tissue of patients with cancer cachexia. These changes in fibrosis were associated with reduced adipocyte size and adipose tissue atrophy (Batista *et al*, 2016).

MMP-2, MMP-3, and MMP-14, which degrade components of the ECM and are associated with pathology in models of heart failure and muscular dystrophy, have been shown to be induced in cardiac and skeletal muscle in the Colon26 mouse model of cancer cachexia (Devine *et al*, 2015). Cardiac fibrosis is also noted. Whether altered MMP expression in cachectic adipose tissue also plays a role in disease progression is not known so far. There certainly is evidence for increased fibrosis in cachectic adipose tissue, both in mice and men (Mracek *et al*, 2011; Batista *et al*, 2016; Luo *et al*, 2016). However, whether this is cause or consequence of the tissue wasting in cachexia remains to be investigated. Of note, patients with burn injury also display a higher collagen content in their adipose tissue, arguing for a role of adipose tissue remodeling in burn injuries, too (Saraf *et al*, 2016).

Table 1. Comparison of adipose tissue-specific and systemic effects in obesity, metabolically healthy obesity, lipodystrophy, and wasting disease.

	Obesity (insulin resistant)	Obesity (insulin sensitive, metabolically healthy)	Lipodystrophy	Cachexia	References
Adipose tissue					
Storage capacity	Exceeded	Sufficient	Insufficient	Insufficient	Garg & Agarwal (2009); Lotta et al (2017); Unger & Scherer (2010); Virtue & Vidal-Puig (2010)
Relative adipocyte number (Adipogenesis)	Low	High (in particular in subcutaneous depots)	Low	Unchanged	Berezat et al (2011); Dahlman et al (2010); Hoffstedt et al (2010); Jan et al (2004); Klötting et al (2010); Lotta et al (2017); Okuno et al (1998); Rodeheffer et al (2008); Tang et al (2011);
Adipocyte size	Large	Small	Heterogenous	Small	Dahlman et al (2010); Klötting et al (2010); Okuno et al (1998); Spalding et al (2008); Tang et al (2011)
Chronic immune cell infiltration and inflammation (type 1)	High	Low	Normal to high (depending on type of LD)	High	Blüher (2013); Gandotra et al (2011); Jan et al (2004); Klötting et al (2010); Lackey & Olefsky (2016)
Fibrosis	High	Low	High	High (?)	Abdenmour et al (2014); Batista et al (2016); Berezat et al (2011); Gandotra et al (2011); Jan et al (2004); Khan et al (2009); Lackey et al (2014); Muir et al (2016)
Systemic					
Energy balance (intake – expenditure)	Positive	Positive	Balanced	Negative	Blum et al (2011); Kir et al (2014); Langin (2011); Petruzzelli et al (2014); Rohm et al (2016)
Ectopic fat and “reactive” lipids	High	Low	High	High (?)	Unger & Scherer (2010); Samuel & Shulman (2012); Van Maldergem et al (2002); Garg (2004)
Inflammation	High	Low	High	High	Batista et al (2016); Fouladiun et al (2005); Gandotra et al (2011); Jan et al (2004); Lackey & Olefsky (2016)
Adipokine profiles	Adverse	Normal	Adverse	Adverse	Fasshauer & Blüher (2015); Fouladiun et al (2005);
Adiponectin	Low	High	Low	High?	Haque et al (2002); Stern et al (2016)
Leptin	High	High	Low	Low?	
Insulin resistance	Present	Normal	Present	Present	Blüher (2013); Garg & Agarwal (2009); Holroyde (1984); Klötting et al (2010)

Lessons learned

Given its localization across different organ compartments, adipose tissue depots represent key checkpoints in systemic energy homeostasis. At the cellular level, energy handling within the adipocyte largely determines overall adipose tissue functionality and recent

studies have highlighted the importance of local, intra-adipose “niches” for adipocyte integrity. As described above, not only obesity-related energy excess but also—still “under-investigated”—conditions of adipose tissue scarcity are characterized by adipocyte and niche dysfunctions which translate into disturbances of systemic energy balance and organismal wasting conditions (Table 1). In this

respect, key questions for future research include the role of ECM remodeling and fibrosis for adipose tissue wasting conditions, for example in cancer cachexia, and to which degree the protection of adipose tissue integrity may contribute to the prevention and/or therapy of human wasting disorders. Indeed, high levels of fibrosis have been shown to correlate with little weight loss after bariatric surgery (Divoux *et al*, 2010), that is, low fibrosis is associated with enhanced weight loss, opening the seemingly paradoxical question whether a high fibrotic burden might even serve a protective role against cachectic conditions in the tumor-bearing state. In light of the clinical relevance of wasting disorders and the current lack of effective predictive and therapeutic options in humans, a certain re-focusing of clinical and research efforts onto the maintenance of healthy adipocytes and their niches under these conditions seems to be justified and mandatory in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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