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Review

Adipose tissue and adipocyte dysregulation

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Abstract

Obesity-associated insulin resistance is a complex disorder involving a number of candidate molecules, pathways and transduction systems possessing potential causal actions. Inflammation in adipose tissue (AT) is one mechanism proposed to explain the development of insulin resistance, while identification of factors that lead to or cause AT dysfunction when it reaches its limit of expansion represents an important challenge. Pathological expansion of AT is characterized by changes in its blood flow, and the presence of enlarged and dysfunctional adipocytes that begin an inflammatory campaign of altered adipokine and cytokine secretions. Adipocyte senescence, necrosis and death are associated with increased immune cell and macrophage infiltration of AT in obesity. This can boost inflammation and reinforce fat cell dysfunction and death. In addition, pathological fat mass expansion is also related to limited recruitment of fat cell progenitors able to proliferate and differentiate into healthy small fat cells to compensate for cell death and preserve adipocyte numbers. Limiting vascular development and enhancing fibrotic processes worsen inflammation towards chronic irreversibility. The AT expandability hypothesis states that failure of AT expansion is one of the key factors linking positive energy balance and cardiometabolic risks, not obesity *per se*. Besides the usual treatment of obesity based on behavioral approaches (specific dietary/nutritional approaches together with increased physical activity), a number of questions remain concerning the possible recovery of metabolic health after inflammation-preventing interventions.

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Keywords: Fat cells; Blood flow; Inflammation; Lipogenesis; Lipolysis; Adipogenesis; Macrophages; Insulin resistance; Adipocyte hypertrophy

Résumé

Dysfonctions du tissu adipeux et des adipocytes.

L'insulino résistance associée à l'obésité est un désordre aux origines complexes qui implique de nombreuses molécules, dispositifs de signalisation cellulaire et voies métaboliques susceptibles de jouer un rôle causal. L'apparition d'une inflammation au sein du tissu adipeux est un des déterminants des mécanismes proposés. L'identification des facteurs qui sont à l'origine des dysfonctions du tissu adipeux lorsqu'il atteint ses limites d'expansion reste un objectif de recherche important. Les altérations pathologiques du tissu adipeux sont caractérisées par des altérations du flux sanguin local et l'apparition d'adipocytes hypertrophiés aux fonctions perturbées qui s'engagent dans un programme inflammatoire avec des modifications notables de la production d'adipokines et de cytokines. La sénescence, nécrose et mort des adipocytes est associée à une infiltration accrue de cellules immunitaires et de macrophages dans le tissu adipeux de l'obèse. Ce processus peut renforcer l'inflammation et aggraver les dysfonctions cellulaires et accélérer les processus de nécrose et d'apoptose. L'accroissement pathologique de la masse grasse est associé à une diminution du recrutement des progéniteurs adipocytaires capables de proliférer et de se différencier en adipocytes sains de petite taille, capables de compenser la mort des adipocytes et assurer la constance de leur nombre. La limite d'expansion de la vascularisation du tissu adipeux et l'aggravation de processus fibrotiques vont orienter un état inflammatoire réversible initial vers un état chronique irréversible. L'hypothèse sur l'expansibilité du tissu adipeux propose que la limite d'expansion que peut atteindre la masse grasse représente un des facteurs essentiels liant une situation de balance énergétique positive excédentaire et l'apparition de risques cardiométaboliques. En dehors des traitements usuels de l'obésité basés sur des approches comportementales (*i.e.*, approches nutritionnelles associées à une augmentation de l'activité physique), de nombreuses questions subsistent sur la possibilité d'améliorer la santé cardiométabolique à l'aide d'interventions prévenant l'apparition de processus inflammatoires.

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Mots clés : Cellules adipeuses ; Flux sanguin ; Inflammation ; Lipogénèse ; Lipolyse ; Adipogénèse ; Macrophages ; Insulino résistance ; Hypertrophie adipocyte

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1. Introduction

Numerous studies have revealed various aspects of adipose tissue (AT) and adipocyte dysfunction in the pathophysiology of insulin resistance and other obesity-related diseases. It is currently accepted that excess body fat is associated with dysfunctional metabolism, systemic inflammation characterized by elevated circulating concentrations of proinflammatory markers, and increased risks of type 2 diabetes (T2D) and cardiovascular problems. The inflammatory state, which interferes with normal metabolism and disrupts insulin signaling, does not accord with the classical inflammatory paradigm, as it is associated with a reduced metabolic rate, as discussed in a recent review [1] (Fig. 1). AT is a heterogeneous tissue composed of adipocytes, and various microvascular and immune cells in its stromal vascular fraction (SVF). When considering the impact of AT on metabolism, its anatomical distribution plays an important role. Basically, AT is divided into two compartments: central (the subcutaneous upper abdominal and visceral fat masses); and peripheral (hip and gluteal–femoral fat). Excess central fat contributes to dyslipidaemia, hypertension and insulin resistance [2], whereas excess femoral fat has protective metabolic effects [3] and is associated with a reduced cardiovascular risk [4]. Considerable research has been devoted to unravelling the functional specificity of the various fat deposits in normal-weight, overweight and obese patients (see reviews [2,5]). Neural mechanisms are crucial for circadian rhythms and are also implicated in an important dialogue with AT in the control of homeostasis of energy stores and fuel metabolism. Circulating hormones such as insulin and other signals originating in AT, including metabolites such as fatty acids (FAs) and adipokines such as leptin, adiponectin, retinol-binding protein 4 (RBP4) and apelin, circulate in proportion to body fat extent and send messages to the brain which, in response, sends signals to regulate food intake and to fuel metabolism (Figs. 1–3).

AT is now recognized as the predominant contributor to systemic inflammation, which characterizes the obese state. It is currently accepted that obesity is associated with macrophage and other immune cell infiltration in both human and rodent AT. A larger number of macrophages is observed in omental AT compared with subcutaneous AT, and the number of macrophage infiltrates is positively correlated to body mass index (BMI), while levels of C-reactive protein (CRP) and toll-like receptor 4 (TLR4) expression correlate with the size of adipocytes [6]. Macrophage abundance is thought to contribute to regional cytokine production, although cytokine and chemokine synthesis by human primary adipocytes may also play a role [7,8]. The AT becomes dysfunctional in obesity, with a tendency towards overproduction of proinflammatory adipokines, and reduced production of anti-inflammatory and insulin-sensitizing adipokines such as adiponectin [9]. Moreover, enhanced fibrotic processes can worsen inflammation towards chronic irreversibility.

Another important element of AT dysfunction concerns vascular-related problems and dysregulation of adipose tissue blood flow (ATBF). Altered AT perfusion can also perturb hormone signaling and the flux of various metabolic substrates,

while AT and adipocyte production can affect vascular tone and modify reactivity in the AT vascular bed. Impaired ATBF has been reported in both obesity and T2D [10]. Hypoxia is another possible cause of AT inflammation in obesity, and has been proposed as an AT response that may be contributing to chronic inflammation [11].

On considering AT and adipocyte dysfunction during fat accumulation in states of positive energy balance, according to the AT expandability hypothesis—based a number of studies in rodents—AT dysfunction can lead to limitation of AT expansion [12]. When AT ceases to store lipids and energy efficiently, the lipid flux is directed towards non-adipose organs. This ectopic accumulation of lipids in cells other than adipocytes promotes a number of lipotoxic insults in those cells, thereby leading to insulin resistance, apoptosis and inflammation [13]. Although the AT expandability concept is mostly based on experimental data from rodent models, some clinical studies also support the hypothesis [14].

The objective of the present review is to describe, mainly in humans, the AT, ATBF and adipocyte dysfunction that arises during expansion of fat deposits, and to review and discuss the relationships between AT perturbations and the risk of developing obesity-related diseases such as insulin resistance and T2D, and the increased cardiovascular burden often seen in the obese. Various factors that might interfere with AT expandability (such as disturbed adipogenesis and angiogenesis, altered lipogenesis/lipolysis balance, mechanical limitations of AT expansion and immune cell infiltration) are also considered.

2. Changes in adipose tissue blood flow

Blood vessels play an important role in AT function and pathology, and its microcirculation is affected by AT expansion. Thus, any exploration of AT dysregulation should include the macro- and microvasculature modifications and ATBF changes that arise during fat mass expansion.

AT possesses a dense network of capillaries that varies between depots, while reduced capillary density (rarefaction) in AT has been reported in the obese [15,16]. As angiogenesis (new blood vessel formation) is required for AT expansion, inhibitors of angiogenesis also inhibit AT expansion, and can reverse obesity in genetic and dietary rodent models of obesity. Fat pads that are actively expanding contain a high-density of vessels compared with other fat pads, at least in rodents. Also, AT function is intimately linked with microvascular function. In addition to a role in hypoxia prevention, microvascular modifications and endothelial cell dysfunction can also alter expression of adhesion molecules and chemotactic factors affecting endothelial permeability, transendothelial transport and immune cell mobility, and may be the source of fat cell progenitors [17]. Indeed, all endothelial cell and AT vasculature disturbances could be either the source or accelerator of the inflammatory process. Lymphatic circulation status in AT has also been neglected [18]. Efferent and afferent lymphatic vessels of superficial lymph nodes are encapsulated in AT, and may be considered an energy reservoir for immune responses [19]. Inflammation, radiotherapy and some surgical procedures

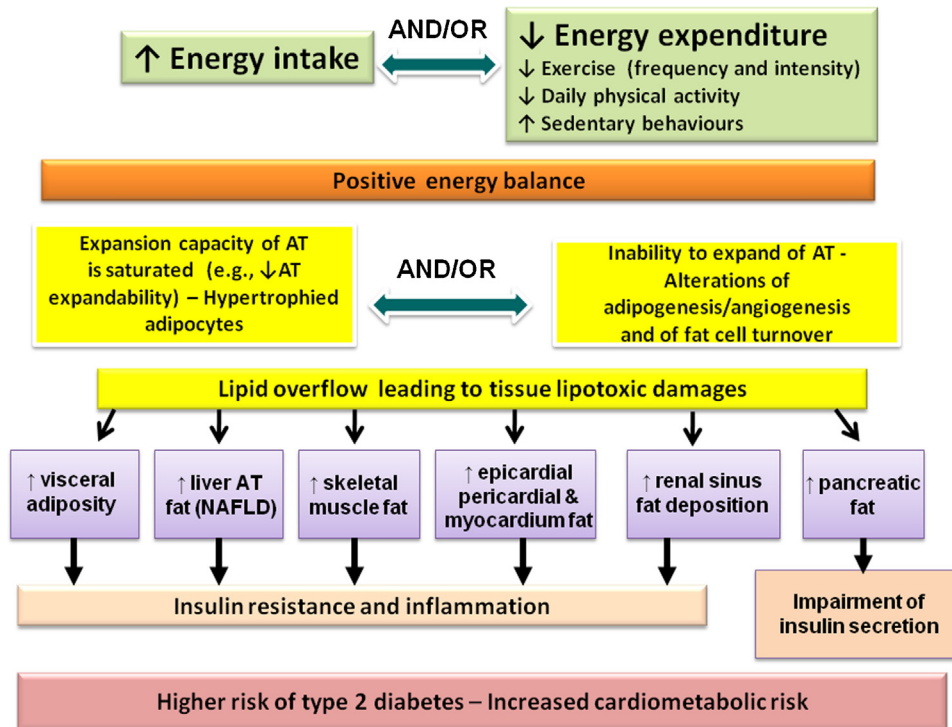


Fig. 1. Consequences of chronic positive energy balance leading to obesity and adipose tissue (AT) dysfunction. Deposition of non-esterified fatty acids (NEFAs) as triacylglycerols in the liver, skeletal muscle, epi-/pericardium and pancreas create a lipotoxic setting (with ectopic fat deposition and lipid-driven toxicity). In addition to NEFA release, adipocytes produce pro- and anti-inflammatory molecules at levels that deteriorate in obesity. Adapted from Tchernof and Despres [2].

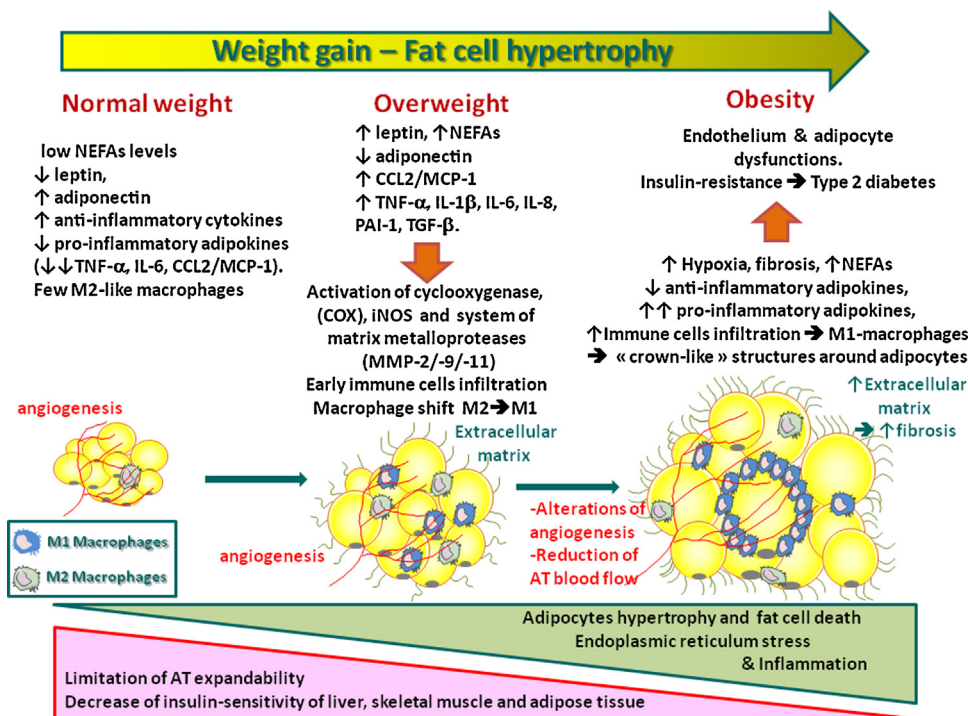


Fig. 2. Pathological changes over time in adipose tissue (AT) from expansion to obesity. Changes in extracellular matrix composition and angiogenesis aggravate the deleterious conditions caused by AT expansion. The vicious circle progresses towards aggravated insulin resistance, and increases the risk of type 2 diabetes and cardiometabolic disorders. NEFAs: non-esterified fatty acids; CCL2/MCP-1: C-C motif chemokine ligand 2/monocyte chemoattractant protein-1; TNF: tumour necrosis factor; IL: interleukin; PAI-1: plasminogen activator inhibitor 1; TGF: transforming growth factor; COX: cyclooxygenase; iNOS: inducible nitric oxide synthase; MMP: matrix metalloproteinase.

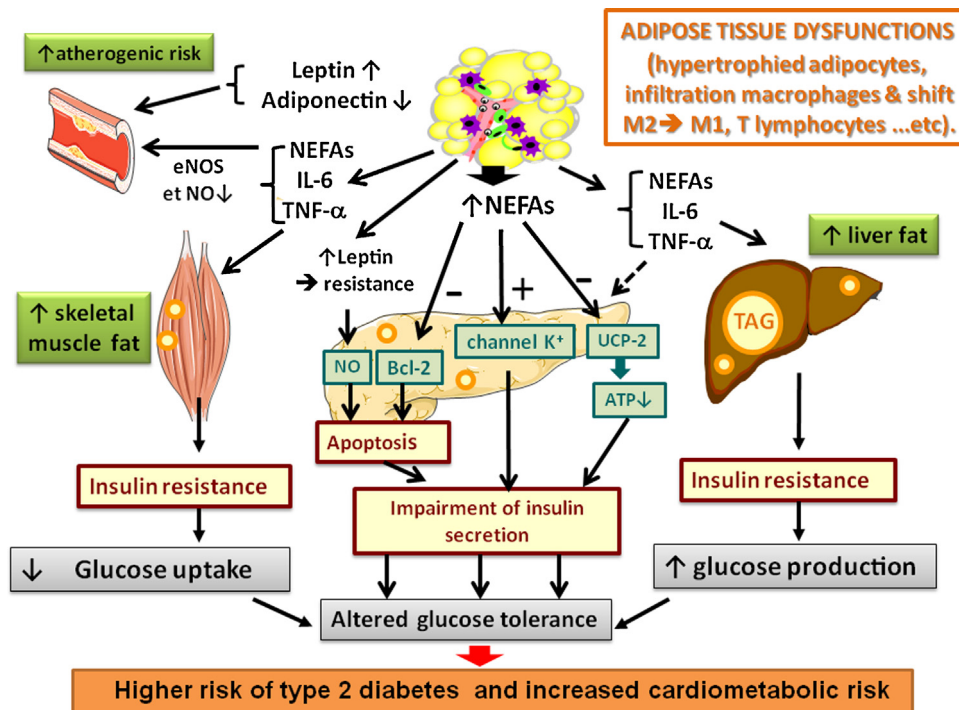


Fig. 3. Adipose tissue (AT) dysfunction (changes in secretory capacity of adipocytes and cells in the stromal vascular fraction) has multiple effects on liver, skeletal muscle, pancreas and blood vessels, ultimately increasing risks of insulin resistance, type 2 diabetes and cardiometabolic disorders. Studies *in vitro* show that long-chain acyl-CoA and non-esterified fatty acids (NEFAs) promote K⁺ channels in β cells and decrease their insulin secretion. Leptin antiapoptotic effects are mediated by inhibition of nitric oxide (NO); NO induces β-cell apoptosis *via* DNA lesions, depletion of calcium stores and endoplasmic reticulum stress. Increased circulating levels of NEFAs, IL-6, TNF-α and leptin in insulin resistance also reduce endothelial NO synthase (eNOS), while decreased NO production leads to endothelial dysfunction, affecting vascular reactivity. Fat cell production of adiponectin, a potent anti-inflammatory, insulin-sensitizing and antiatherogenic hormone, is also defective in dysfunctional AT. UCP-2: uncoupling protein-2; ATP: adenosine triphosphate; TNF: tumour necrosis factor; IL: interleukin.

are known to alter lymphatic function and transport activity in humans, and lymphatic transport deficiency leading to lymphoedema has been associated with AT expansion in the affected limb, while malformed lymphatic vessels have been observed in lipidaemia (pathological regionalized lipid accumulation and AT expansion). Consistent with such clinical observations, AT accumulation has also been reported in various mouse models of experimentally induced lymphatic dysfunction.

The standard reference human ATBF value has been reported to be around 28 mL/min/kg, based on xenon-133 washout measurements in subcutaneous AT. However, human ATBF is notably heterogeneous, varying by at least fivefold according to the method of measurement used. Various factors influencing ATBF (such as insulin, sympathetic and parasympathetic nervous system factors, nitric oxide, the renin–angiotensin system, endothelin 1, gastrointestinal hormones, natriuretic peptides and leptin) have recently been reviewed [10]. An enhanced ATBF facilitates plasma triglyceride extraction, enables hormone access to fat cells, and facilitates signaling between AT secretions and other tissues involved in the regulation of metabolism. ATBF also plays an integral role in oxygen supply. Sufficient increases in ATBF after meals are known to facilitate energy storage in fat, and ATBF is higher in abdominal than in femoral fat [20]. ATBF also exhibits a greater degree of modulation during physical exercise (which promotes strong sympathetic nervous system activation and epinephrine release by the adrenal medulla) and increases during moderate-intensity

exercise. In healthy individuals after a standard meal, ATBF rises following an oral glucose load or ingestion of a mixed meal and peaks earlier than plasma triglyceride levels [21]. Also, subjects with high ATBF responses have significantly greater increases in plasma noradrenaline, indicating a link between postprandial insulinaemia, sympathetic activation and the ATBF response [22].

2.1. Dysfunctional ATBF in obesity and insulin resistance

Insulin resistance is a known determinant of reduced ATBF responsiveness, more so than even obesity, as glucose-induced ATBF increases after a meal are suppressed by any stage of glucose intolerance, by overt T2D and even in first-degree relatives of people with T2D [22,23]. Microcirculation dynamics in subcutaneous AT are impaired in the postprandial state in T2D [24], and the reduced ATBF in obese and insulin-resistant individuals with impaired postprandial vasodilatation could contribute to changes in lipid homeostasis in insulin-resistant subjects and predispose to cardiovascular disease. However, the mechanisms behind the lack of postprandial ATBF responsiveness in insulin resistance remain largely unexplained.

AT expansion due to the enlargement of adipocytes can lead to relative AT hypoperfusion. In obesity, expansion of AT mass and adipocyte size with the concomitant increase in capillary density can lead to changes in the diffusion distance between capillaries and adipocytes [25] and in blood flow per cell that,

in turn, could be responsible for the reduction in basal and stimulated ATBF. Lower capillary density has been reported in both visceral and subcutaneous AT depots in obese compared with lean individuals [15]. Thus, it is suspected that the supply of oxygen in AT may be restricted in obesity. Tissue oxygen partial pressure (pO_2) reflects the balance between O_2 delivery and consumption. AT is not a major consumer of O_2 (making up only 5% of whole-body O_2 consumption in normal-weight individuals) compared with skeletal muscle. Yet, hypoxia has been described in the AT of obese mice, with hypoxic areas identified using pimonidazole as a marker [16], although the effects of adiposity on arterial O_2 content and delivery of O_2 to AT in humans are still unclear. Metabolic signatures of AT hypoxia have also been questioned: although O_2 delivery to AT in obesity is reduced, O_2 consumption is also low, and the metabolic signatures of human AT do not support the notion of a state of hypoxia in the obese [26]. In general and based on studies in experimental animals, hypoxia is seen in AT depots (at least in rodents), in cases of genetic obesity and obesity-induced by a high-fat diet [20,27]. In human AT, future studies devoted to improved pO_2 determinations are needed, with special attention focused on the metabolic responses induced by pO_2 changes within human physiological ranges (3–11% O_2) [28].

3. Adipose tissue dysfunction and obesity

Obesity leads to features of metabolic dysfunction in the AT that could contribute to later impairment of insulin action. For this reason, the following should be considered when characterizing AT dysfunction:

- changes in lipid-storing capacity and lipid mobilization processes in mature adipocytes represent important elements of AT dysfunction, as both play a major role in buffering non-esterified fatty acid (NEFA) flux in the postprandial period or when fasting [29];
- adipokine/cytokine/chemokine production may be impaired in hypertrophied adipocytes, immune cells and/or fat cell progenitors of the SVF;
- macrophage and immune cell recruitment and infiltration into AT may constitute the origins of proinflammatory events and remodelling processes;
- extracellular matrix remodelling of adipogenic processes as well as angiogenesis may be impaired.

3.1. Altered lipid-storing capacity and lipid mobilization processes

Storage of FAs by AT in the form of triacylglycerols (TAG) is essential for limiting their deleterious systemic impact when plasma concentrations are increased [29]. FAs are structural elements in plasma membrane lipids and effective energy substrates. They also serve as potent second messengers, are implicated in hepatic and skeletal muscle FA-induced insulin resistance [30], signal nutrient mediation to the central nervous system (CNS) and have central effects on insulin action. In

addition, FAs can induce the endoplasmic reticulum stress response and numerous adipocyte disturbances [1].

The idea that AT may have a limited storage capacity that affects metabolic health is not new, and it has been proposed that insulin resistance might represent an adaptation for weight maintenance [31], although evidence was lacking. Now, however, it is well recognized that AT storage capacity for FAs prevents the deleterious effects of high FA concentrations in target cells as well as their storage and deleterious actions in other tissues (lipotoxicity) [13]. However, AT buffering of lipid fluxes is impaired in obesity through defects in the ability of AT to respond rapidly to the dynamic situation that arises after meals and also in lipodystrophy, where there is insufficient AT to provide the necessary buffering [29]. Mobilization of FAs after TAG lipolysis represents the other component of FA homeostasis, and perturbed lipolysis is known to impinge on FA bioavailability.

As the biochemistry and pathophysiology of intravascular and intracellular lipolysis have already been summarized in recent reviews [32–34], the discussion here is limited to AT-dependent events. Intravascular lipolysis, which controls the release of FAs from TAG-rich lipoproteins, is a significant source of both portal and systemic FAs, and can lead to spillover of FAs beyond AT. Intracellular lipolysis, the hydrolysis of TAG stored in adipocytes, involves three different lipases, and is subject to a sophisticated mix of hormonal and metabolic agents in its regulation [32]. Re-esterification—esterification of non-esterified fatty acids (NEFAs) on existing glyceride molecules—takes place concurrently with lipolysis. In humans, the re-esterification rate is estimated to be 50–75%, but can decrease to 20–35% with fasting and exercise.

3.1.1. Fat storage defects

The development of obesity and its complications is related to modifications in lipid turnover in AT. During the lifespan of an adipocyte (9.5 years, on average, in human subcutaneous AT) [35], the mechanism of lipid turnover is such that TAG is replaced six times faster on average [36]. Lipoprotein lipase (LPL) is the gatekeeper of fat storage in AT, but the action of LPL on chylomicrons may also be a source of plasma NEFAs released into the circulation by an escape (or spillover) mechanism. Briefly, intravascular lipolysis involves the transfer of LPL from adipocytes, where it is synthesized, to the luminal face of capillary endothelial cells by a protein responsible for its transcytosis—glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) [37]. After its transfer to capillaries, LPL is anchored to endothelial cells by electrostatic interactions between its heparin-binding motifs and negatively charged heparan sulphate proteoglycans (HSPGs) [33]. LPL-mediated FA uptake is a process of variable efficacy, and the loss of LPL activity leads to familial chylomicronaemia syndrome, characterized by strongly elevated plasma TAG levels. Dozens of LPL gene mutations have been described as well as mutations of various regulators of LPL activity that also lead to LPL deficiency [apolipoprotein C-II (apoC-II), GPIHBP1 and lipase maturation factor 1 (LMF1), important for LPL dimerization/activation]. Some mutations of the LPL gene abolish its

transcription or lead to the production of unstable transcripts, while others prevent LPL secretion and its transcytosis *via* GPI-HBP1 and, as a consequence, normal activation of the enzyme [34]. When released, FAs have to cross the endothelial barrier before being taken up by adipocytes. Various FA-binding and lipid droplet-associated proteins (which package TAG into lipid droplets) have been identified in adipocytes [38], and are implicated in the ability of fat cells to sequester FAs and TAG *via* modulation of lipid droplet size, and to control lipolytic processes [39].

As long as there is efficient LPL activity, and normal TAG synthesis pathways and capacity to store fat in AT, there is no ectopic lipid deposition. Thus, the storage of lipids in the harmless compartment constituted by AT protects against ectopic fat deposition. However, impaired AT storage (reduced FA uptake or increased spillover leading to excess FA ‘escape’ from AT) of ingested fat drives fat deposition into non-adipose tissues. Also, the idea that the limited storage capacity of AT affects metabolic health [31] has been strengthened over the past few decades. When the storage capacity of AT is overcome [40], ectopic deposition of lipids is seen in the liver and skeletal muscle [13]. Obesity-associated insulin resistance is thought to depend on two major mechanisms:

- dysregulation of AT expandability;
- abnormal production of adipokines [12,41].

A genetic predisposition for T2D, but not obesity, is associated with an impaired ability to recruit new adipose cells to store excess lipids in subcutaneous AT, thereby also promoting ectopic lipid deposition [42,43]. Another putative mechanism to explain AT storage defects is *de novo* lipogenesis (DNL). Although there is no evidence of increased AT lipogenic capacity in obese people [44], DNL is downregulated as adipocytes expand [45].

3.1.2. Defects of fat mobilization

In the fasting state, plasma NEFAs arise almost entirely from hydrolysis of TAG within adipocytes. In the obese state, the limited increase in plasma NEFAs is partly explained by decreased NEFA production in subcutaneous AT, from where most NEFAs originate. In addition, it has long been recognized that plasma NEFA concentrations are commonly raised in obesity, leading to the argument that increased NEFA release is associated with fat mass expansion. However, a point of view different from the previous conventional ones has recently been proposed—that fasting plasma NEFA concentration is largely unrelated to body fat mass [46]. An increased basal fasting lipolytic rate and impaired catecholamine-induced lipolysis in subcutaneous AT is a common feature in obese subjects, as are raised levels of cyclic adenosine monophosphate (cAMP) in larger adipocytes, known to be less insulin-sensitive, and impaired insulin antilipolytic action, which enhances lipolytic rate. Large adipocytes have an increased lipolytic capacity probably due to enrichment of distal regulatory proteins in the lipolytic cascade such as hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL) and perilipin [47]. Various alterations at multiple levels

in the lipolytic cascade are also related to resistance to lipolytic hormones, and defects in the expression of HSL, ATGL (and its regulator CGI-58), $\beta_{1/2}$ -adrenergic receptors, perilipin and the regulatory subunit of protein kinase A and aquaporin-7 (involved in glycerol release by fat cells) have been reported in obese subjects [32]. In adipocytes, altering lipase content quantitatively changes lipolysis and re-esterification fluxes [48]. Also, lipolytic resistance to catecholamines may involve increased antilipolytic responsiveness of α_2 -adrenergic receptors, which are highly expressed in hypertrophied adipocytes. Fat cell α_2 -adrenergic receptor expression is positively correlated to fat cell size—the larger the fat cell, the higher the receptor number and weaker the lipolytic responsiveness to catecholamines [49]. Of the factors that contribute to lipolysis disturbances in obesity, tumour necrosis factor (TNF)- α , secreted by adipocytes and AT macrophages, chronically stimulates lipolysis and attenuates the antilipolytic action of insulin [50].

3.2. Fat cell hypertrophy-related events

Obese patients with smaller-size adipocytes exhibit better lipid profiles [51], while those with adipocyte hypertrophy have more adverse metabolic profiles than those with cellular hyperplasia (increased numbers of small adipocytes) at similar BMI levels [52]. What about fat cell hypertrophy-related metabolic disturbances (Fig. 2)? Obese people with larger adipocytes in their subcutaneous AT are more hyperinsulinaemic and glucose intolerant than lean people with smaller adipocytes, so the presence of larger subcutaneous adipocytes may be predictive of T2D development. It has long been recognized that larger adipocytes are more insulin-resistant *in vitro*: the antilipolytic action of insulin is reduced. There is also a strong association between small adipocytes and insulin sensitivity independent of BMI [45], a finding in line with a previous prospective study of Pima Indians [53]. Moreover, a downregulation of DNL and lipogenic gene expression has been observed in larger adipocytes [45]. Defects of subcutaneous fat storage may underlie obesity-associated insulin resistance and, as the severity of insulin resistance varies widely among obese people, this may explain the heterogeneity found in the study’s results [54]. In a different study using periumbilical subcutaneous AT samples and in contrast to previous findings, insulin resistance was associated with a higher ratio of small-to-large cells. It was also shown [based on real-time polymerase chain reaction (PCR) assay] that a two- to threefold lower expression of genes encoding markers of adipose cell differentiation, such as peroxisome proliferator-activated receptors- γ_1/γ_2 (PPAR- γ_1/γ_2), glucose transporter type 4 (GLUT4), adiponectin and sterol response element-binding protein-1c (SREBP-1c), was described in insulin-resistant compared with insulin-sensitive individuals [55]. The fact that insulin resistance was associated with an expanded population of small adipose cells with concomitantly decreased expression of differentiation markers suggests that impaired adipose cell differentiation, reflecting defects in fat storage, may be involved in obesity-related insulin resistance [55].

Adipocyte size varies not only between anatomical AT locations, but also within each depot [56]. Adipocyte size in both subcutaneous and omental fat is increased as body fat mass increases. In terms of highest body fat mass values, fat accumulation is progressively higher in the subcutaneous rather than visceral fat compartment, suggesting that fat cell hyperplasia can occur in subcutaneous fat [57]. Most studies of cell size have involved pools of fat cells of variable size taken from patients or rodents of different ages and weights. Under such conditions, it is difficult to determine the influence of cell size *per se* from size changes due to ageing. Thus, an important challenge in the investigation of cell-size-related events is to separate fat cells according to size from the same biopsy sample. The reason for adipocyte size heterogeneity within a given depot is unknown, and the mechanisms that govern adipocyte size are still a matter of debate, although enlarged adipocytes are considered to arise from increased fat deposition (lipogenesis) due to FAs and glucose from plasma or DNL.

Flotation or filtration methods have been used to separate fat cell populations. In one study of Zucker rats, large fat cells were compared with small fat cells from the same fat depot. Compared with small fat cells, FA synthase and LPL activities were increased two- and sevenfold, respectively, GLUT4 protein concentration was increased threefold, leptin expression was increased and lipolytic capacity was increased fourfold in large fat cells. In addition, the β 1-integrin/extracellular signal-regulated kinase (ERK) signaling pathway present in mature adipocytes was also affected by fat cell size. In large vs. small cells, cytoplasmic concentrations of ERK1/2 were increased twofold, whereas their activities were increased 10-fold. Increases in cell size could therefore modify the relationship between fat cells and extracellular matrix. As ERK modulates a number of transcription factors and, subsequently, the expression of various genes, this pathway has been proposed to play an important role in the adaptation of AT function to cell size [58].

FA uptake in human AT explants has also been measured, using an original single-cell analytical method. Subcutaneous fat cells are heterogeneous in size and intrinsically insulin-sensitive. Smaller adipocytes respond to insulin by increasing lipid uptake, while larger fat cells are less sensitive and insulin-resistant at the same anatomical location [59]. However, the fact that FA uptake and insulin antilipolytic effects are negatively regulated by fat cell size questions the physiological relevance of such adaptations. Is it a case of feedback inhibition of insulin-dependent events aimed at physiological prevention of lipid overload in the expanding adipocyte? These results fit with the downregulation of lipogenic genes, as previously mentioned, which is thought to limit expansion of TAG stores [45]. Limiting the excess fat cell enlargement that leads to detrimental morphology could prevent some of the functional changes reported in AT (such as hypoxia, inflammation, decreased secretion of anti-inflammatory cytokines and increased basal lipolysis).

Recent gene expression studies and proteomic analyses in fat-specific insulin receptor knockout (FIRKO) mice have revealed numerous classes of molecules that are differentially expressed in small and large fat cells. A total of 27 alterations in

protein expression at key stages of lipid and energy metabolism, coordinated and regulated by adipocyte cell size and/or able to impair insulin signaling, has been defined [60]. However, it is not yet known whether changes in fat deposition induce adipocyte protein expression or whether protein expression governs fat deposition and, thus, adipocyte size.

Caveolae, small invaginations in the cell surface membrane that are highly enriched with cholesterol, sphingolipids, and the specific proteins cavin and caveolin, represent 30–50% of adipocyte surface area and may be involved in sensing membrane tension during cellular swelling. These structures are important for adjusting the size of growing adipocytes and are also critical for insulin signaling [61]. The question is: how does the expression of plasma membrane lipids and functional proteins contribute to fat cell size determination and putative dysfunction? A decrease in membrane cholesterol with increasing fat cell size has been reported, as has an association between membrane cholesterol/caveolae density and cell size. Also, altering cholesterol balance in a way resembling that of hypertrophied fat cells from obese rodents or humans can profoundly modify adipocyte metabolism [62]. The average density of caveolar proteins in the fat cell plasma membrane and cytoplasmic rim decreases as the mean adipocyte size increases [63]. Does this have an impact of other organelle dysfunction? In fact, a key regulator of the inflammatory response is organelle dysfunction in metabolic cells. It is known that the liver and AT in obesity exhibit increased levels of endoplasmic reticulum stress, as measured by activation of the unfolded protein response [1,64]. Nevertheless, the mechanisms involved in the control of human fat cell size have yet to be properly elucidated. As their links to fat cell size increase, organelle dysfunction and metabolic dysregulation needs to be studied in greater depth in human adipocytes.

3.3. Endocrine mechanisms: impaired adipokine production by hypertrophied adipocytes

A wide range of secreted factors from AT has been discovered. Some are known cytokines and chemokines such as TNF- α and interleukin (IL)-6, while others are novel secreted factors such as adiponectin, apelin, RBP4 and angiopoietin-like protein 2 (Angptl2) and among the most commonly discussed. Fat cell secretions have been the subject of several recent reviews and so are not detailed here [65,66]. The discovery of adipokines is still ongoing and new candidates are regularly emerging. Among these numerous AT-derived products, however, it is essential to make a distinction between those essentially produced by adipocytes and those secreted by cells of the SVF, particularly immune cells (see below). Release of interleukins and other inflammatory cytokines is enhanced in human AT by obesity. However, this release is mainly due to non-fat cells in the AT [67]. In addition, as several adipokines have been associated with inflammation, they have also been the subject of clinical studies [65,66].

Is there perturbed adipokine secretion in the dysfunctional hypertrophied adipocyte? During periods of prolonged caloric overload, the resultant inflammatory response leads to adipocyte

dysfunction. Hypertrophied adipocytes are known to secrete large amounts of monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2) and TNF- α , which act to modulate the AT inflammatory response. MCP-1 also enhances macrophage infiltration of AT in the obese (see below) and, at elevated levels, can promote adipocyte dedifferentiation and pathologies associated with hyperinsulinaemia and obesity [68].

The inhibitory effects of insulin promoted by AT-secreted chemokines/cytokines work *via* activation of several inflammatory kinases, such as c-Jun N-terminal kinase (JNK), inhibitor κ B kinase (IKK) and protein kinase R (PKR), identified in metabolic tissues that target insulin signaling pathways and especially insulin receptor substrate 1 (IRS-1) [1]. In addition to these kinases, other networks may be involved in the inhibition of insulin signaling, including kinases such as protein kinase C θ (PKC θ), ERK and the mammalian target of rapamycin (mTOR), which may also be involved in the inhibitory phosphorylation of IRS-1 [69]. Obesity is associated with increased activation of the c-Jun NH2-terminal kinase (JNK) in AT [70], while specific suppression of JNK in AT has recently been shown to counteract the metabolic dysregulation and systemic inflammation induced by a high-fat diet in rodents [71]. Regional human AT differences and adipocyte dysfunction leading to various degrees of insulin resistance merit deeper attention [54].

Separation of human adipocytes in AT samples into populations of small and large cells has identified some genes with higher expression [including leptin, serum amyloid A (SAA) and transmembrane 4 L six family member 1 (TM4SF1)] in large fat cells than in small cells [72]. Indeed, leptin production is markedly increased in large adipocytes, and a close positive correlation between leptin mRNA levels and adipocyte volume is found in small to hypertrophic adipocytes in mice [73]. Among its pleiotropic effects, leptin can modulate T-cell immune responses and increase the production of proinflammatory cytokines by immune cells recruited in AT. In contrast, hypertrophic adipocytes release less adiponectin and apelin, two adipokines with beneficial effects on insulin sensitivity and vascular function. In summary, hypertrophied adipocytes are in a state of hypersecretion of proinflammatory and pro-diabetic adipokines concomitantly with decreased production of adipokines affording protection against inflammation and diabetes. Thus, in AT in the obese, besides disturbances in secretory activity, adipocyte hypertrophy is also associated with altered adipocytes (probably dead adipocytes) surrounded by macrophages forming ‘crown-like structures’ that are probably scavenging adipocyte debris [74]. However, the adipocyte events triggering adipocyte death are still poorly understood.

4. Deleterious effects of macrophage and other immune cell infiltration of AT

In 2003, the infiltration of macrophages into the AT of genetically obese (leptin-deficient or leptin-receptor-deficient) and diet-induced obese (DIO) mouse models was reported, and later followed by reports of infiltration involving other immune cells of T-lymphocyte lineage (such as CD4⁺ and CD8⁺ T cells,

T_{reg} and mast cells). In fact, CD8⁺ T-cell infiltration precedes macrophage accumulation in rodent AT [75,76], while CD4(+) T-lymphocytes, which reside in visceral AT, have been shown to control insulin resistance in DIO mice [77]. Other reports have followed these preliminary observations and confirmed the T-lymphocyte/macrophage-driven inflammatory responses in AT in obesity. In addition, obesity-induced insulin resistance has been observed along with macrophage and immune cell infiltration. Macrophages may be organized into crown-like structures surrounding necrotic adipocytes [74]. In fact, based on studies of macrophage dynamics in the AT of murine models, it has been proposed that obesity is accompanied by transformation of AT macrophages [78] from an anti-inflammatory “alternatively activated” (M2) state seen in lean mice to the more inflammatory “classically activated” (M1) state [79,80]. However, as several studies have already investigated the changes over time in the balance of such polarized macrophage populations, this aspect is not covered here in detail (see review [81]).

As in mouse studies, the AT in obese subjects is known to contain an increased number of macrophages [82,83]. Such increases are seen in visceral depots and are considered to correlate with insulin resistance risk in patients with visceral obesity [84–86]. Also, in human AT, the macrophages that accumulate during fat mass development exhibit a particular mixture of M1 and M2 remodelling phenotypes [87]. An improved inflammatory profile has been described after weight loss due to bypass surgery and was related to a reduced number of macrophages in subcutaneous AT [88]. This result throws light on an important point: the possible reversibility of macrophage infiltration.

Based on numerous studies in mouse models, several mechanisms of macrophage recruitment during fat pad expansion have been proposed and reviewed by various authors [81,89,90]. Hypoxia (see above) is thought to be involved in the induction of hypoxia-induced fibrosis and stimulation of local inflammatory responses in mouse AT [91]. Activation of hypoxia-inducible factor (HIF)-1 α , involved in transcription, in visceral white adipocytes in mice is critical for maintaining diet-related obesity and associated pathologies such as glucose intolerance, insulin resistance and cardiomyopathy [92]. Likewise, many AT secretions related to inflammation and thought to be implicated in insulin resistance are also hypoxia-induced, including macrophage migration inhibitory factor (MIF), matrix metalloproteinase (MMP)-2 and MMP-9, IL-6, TNF- α , MCP-1, plasminogen activator inhibitor (PAI)-1, vascular endothelial growth factor (VEGF), leptin and Angptl4. In support of this idea, selectively treating mice by HIF-1 α inhibitor (PX-478) or AT-specific genetic inhibition of endogenous HIF-1 α activity ameliorated AT metabolic dysfunction and reduced the macrophage crown-like structures surrounding adipocytes [81,93]. Thus, inhibition of HIF-1 α in AT can improve obesity and insulin resistance.

The link between fat cell death and macrophage infiltration has been confirmed in a transgenic model of lipoatrophy in which rapid accumulation of macrophages and marked remodelling of fat pads were observed [94]. Caspase activation and adipocyte apoptosis were both markedly increased in AT in both obese humans and DIO mice, and both the extrinsic

death-receptor-mediated and intrinsic mitochondrial-mediated pathways of apoptosis were activated. Inactivation of BID (BH3-interacting domain death agonist), a key proapoptotic molecule, reduced caspase activation and adipocyte apoptosis, prevented AT macrophage infiltration and protected against the development of systemic insulin resistance [95]. Adipocyte apoptosis has been proposed as the key initial event contributing to macrophage infiltration of AT.

However, another theory suggests that adipocyte-derived chemotactic factors are involved in activation of the MCP-1/C-C chemokine receptor 2 (CCR2) pathways [96,97]. Increased levels of proinflammatory factors [MCP-1/-2/-3, chemokine (C-X-C motif) ligand 14 (CXCL14), MIP-1 α , and regulated on activation, normal T-cell-expressed and -secreted (RANTES) chemokines] have been described in the AT of obese mice [83,98] and may be involved in macrophage recruitment. At the same time, there is a decreased production of anti-inflammatory factors such as adiponectin, secreted frizzled-related protein 5 (SFRP5) and apelin. The impact of such deregulated production/secretion of adipokines in the initiation/enhancement of inflammation and metabolic diseases has been extensively reviewed elsewhere [9] and requires further investigation in humans.

Some authors have focused on the putative role of extracellular NEFAs in AT in the initiation of macrophage recruitment, and macrophages may also be activated by various lipid metabolites secreted by adipocytes. Saturated FAs stimulate intracellular proinflammatory pathways in a TLR4-dependent manner involving nuclear factor (NF)- κ B pathways. However, how NEFAs activate TLR4 signalling remains unclear. Also, direct interactions of saturated FAs and TLR4 have been questioned, as the hepatocyte-secreted fetuin-A acts as an adaptor between NEFAs and TLR4 signalling in lipid-induced inflammation [99]. In humans, interactions between NEFAs and fetuin-A concentrations can induce insulin resistance [100]. Acute weight loss [101] or treatment with a lipolytic agent [102] can also promote rapid and transient recruitment of macrophages. The effect is clearly driven by enhanced lipolysis and is dependent on NEFA release. However, the number of studies so far is limited in both rodents and humans, and the validity of animal models in translational research requires deeper investigation.

5. Extracellular matrix remodelling and impaired adipogenesis/angiogenesis

In addition to fat cell enlargement, special attention needs to be paid to extracellular matrix (ECM) remodelling, as adipocytes and other SVF cells are embedded within the ECM and could exert mechanical constraints on AT expansion [12]. Moreover, the regulation of both adipogenic and angiogenic processes plays a major role in fat mass expansion and AT turnover and expandability. Fibrosis is now considered a hallmark of altered human AT in obesity. The ECM provides both mechanical and structural support to the microenvironment of AT, and ECM-derived proteolytic fragments may be involved in the activation of various signaling pathways and could influence events in neighbouring cells expressing ECM receptors such as integrins.

ECM remodelling is known to play a major role in adipogenesis and AT architecture, and to contribute to accommodation of obesity-induced cellular alterations (Fig. 2). Excess synthesis of ECM components may also take place in chronic inflammation. Human preadipocytes are able to secrete ECM components and fibrosis factors when subjected to inflammatory conditions (challenged by macrophage-secreted products) [103,104], which indicates that macrophage–preadipocyte interactions play an important role in the development of AT fibrosis. Indeed, the kinetics of fibrotic depot accumulation in human AT merit further investigation. In humans, immunohistochemistry has revealed that collagen type VI (COL6; encoded by *Col6a1*, *Col6a2* and *Col6a3*) is present in AT ECM. COL6 α 3-subunit mRNA correlates with BMI [105], and increased COL6 deposition has been reported in human AT concomitantly with increased expression of inflammatory genes [103]. COL6 is highly expressed in AT and upregulated in the obese state [106], and a cleavage product of the COL6 α 3 chain—endotrophin (ETP)—is involved in augmented fibrosis, angiogenesis and inflammation through recruitment of macrophages and endothelial cells [107].

Fibrotic elements (on picrosirius red and COL6 staining of human AT sections) appear to be distributed around each adipocyte (pericellular fibrosis) in human AT [51]. Some adipocytes surrounded by fibrotic elements are reminiscent of the perilipin-negative adipocytes described in the macrophage crown-like structures surrounding adipocytes [74]. Diminished fat mass loss is reported in patients with high levels of fibrosis [51]. Is the fibrotic pattern a potential predictor of resistance to weight loss in obese subjects?

During weight gain, fat depots enlarge due to hyperplasia, hypertrophy or both, and new adipocytes appear more rapidly in some depots than in others [108]. Striking differences have been reported in fat cell progenitor replication, differentiation and susceptibility to apoptosis or senescence, although the abundance of fat cell progenitors and preadipocytes is poorly understood in human fat depots. These cells, which are partly related to macrophages (by cytokine secretion, but not phagocytosis), could dedifferentiate in old age, switching to a proinflammatory, tissue-remodelling, senescent-like state [5]. The ability of stromal vascular cells in human AT to undergo adipogenic differentiation is reduced in hypertrophic obesity, and the degree of impairment correlates positively with the size of mature donor adipocytes. This suggests it is primarily due to impaired preadipocyte differentiation rather than a lack of early precursor cells [109]. Recently, it has been shown that TNF- α , but not MCP-1, resistin or IL-6, can completely prevent normal adipogenesis in 3T3-L1 preadipocytes as well as in human fat cell progenitors. Terminal differentiation to adipose cells was completely prevented [109]. Briefly, TNF- α prevented the normal development of preadipocytes into fully differentiated adipose cells *via* activation of the WNT/ β -catenin pathway, known to be highly active in precursor cells and able to control the fate of mesenchymal stem cells (MSCs) towards adipogenic, osteogenic or myogenic differentiation [110]. When the WNT signalling pathway is activated in human fat cell progenitors, they become orientated towards a macrophage-like phenotype

[109]. These findings indicate that the AT dysfunction related to fat cell enlargement *via* local production of TNF- α controls the fate of progenitors. Many stromal cells in human AT appear unable to undergo final differentiation into mature adipocytes, as they are under the control of paracrine regulators of the pathways controlling final maturation of fat cells. Such prevention of final progenitor differentiation offers an explanation for why adipocytes in obesity expand in size rather than recruit new preadipocytes in the AT to cope with the increased lipid accumulation.

AT growth is an angiogenesis-dependent event. Rodent studies have clearly shown that modulators of angiogenesis affect fat mass expansion and metabolism by regulating the growth and remodelling of AT vasculature [111]. The AT (adipocytes and SVF cells) is an important site for the production of proangiogenic factors such as VEGF, placental growth factor, leptin and apelin, as well as antiangiogenic factors such as thrombospondin and endostatin. However, the heterogeneity and scarcity of results in humans limit straightforward conclusions. Increased serum concentrations of vascular growth factors and the angiogenesis inhibitor endostatin are seen in overweight and obese subjects. Angiogenesis promoted by human subcutaneous AT and by visceral AT explants from the same obese subjects was assessed in an *in vivo* model of chick chorioallantoic membrane (CAM), and showed that angiogenic potency was not related to either AT localization, adipocyte size or infiltration of inflammatory cells [112]. In another study, AT angiogenic capacity was determined by assessing capillary density using AT explants embedded in BD MatrigelTM. Subcutaneous capillary density and angiogenic capacity were both decreased in the morbidly obese, and angiogenic capacity correlated negatively with insulin sensitivity.

Gene array analyses have also revealed differences in the expression of angiogenic genes between depots, including increased subcutaneous expression of Angptl4, which is proangiogenic in AT [113]. However, quantification of capillary vessel density in humans gives divergent results. In one group, overweight/obese subjects had lower capillary density and lower VEGF, suggesting AT rarefaction (capillary dropout) compared with lean subjects [15]. Others reported increased AT vascular density in obese subjects after weight loss [114]. More recently, vascular density in subcutaneous AT was found to correlate positively with BMI, while vascular density in omental AT correlated with waist circumference. The number of vessels per adipocyte and expression of VEGF receptor 2 correlated with adipocyte area in both fat depots, a result suggesting that adipocyte hypertrophy stimulates angiogenesis [115]. In that study, no correlation was found between metabolic disorder and vascular density or expression of angiogenic factors in either omental or subcutaneous AT.

In another study, the proportion of endothelial cells in subcutaneous AT remained constant while BMI increased, a result that argues for a parallel increase in adipocyte number and vascular density [116]. Studies of AT endothelial cell properties have revealed that vascular density and endothelial cell abundance are higher in visceral AT. Moreover, visceral endothelial cells exhibit a more marked angiogenic and inflammatory status (with

decreased expression of metabolism-related genes) than subcutaneous cells. The phenotype of endothelial cells in visceral AT may be related to premature endothelial cell senescence, and these cells may also be contributing to hypoxia and inflammation [116].

In addition, the remodelling capability of AT can influence weight loss. A study of obese patients who lost weight after gastric bypass found that vascular density was negatively correlated with weight loss whereas VEGF expression correlated positively. High VEGF expression was related to high remodelling. In summary, weight loss after bariatric surgery correlated negatively with adipocyte hypertrophy and vascular density, and positively with inflammation and angiogenesis in AT [115].

6. Conclusion and future trends

AT presents with a number of dysfunctions that show up over the course of its expansion. This includes several steps that alter the lipid-storage capacity of fat cells, and lead to failure of adipogenic/angiogenic pathways and changes in the local blood flow. These events could be the origin of uncontrolled circulating levels of NEFAs and/or their ectopic deposition in the form of TAG in the liver, skeletal muscle and pancreas, which then become involved in a lipotoxic situation [13]. In addition, there are interdepot differences in vascular bed structure, infiltration of inflammatory cells, and secretion of adipokines and cytokines involved in the initiation of the chronic low-grade inflammatory response observed in obesity. The inhibitory effects of insulin promoted by a number of AT-secreted chemokines/cytokines operate through several inflammatory kinases, such as JNK, IKK and PKR, which decrease the efficiency of insulin signalling in metabolic tissues.

Furthermore, a large proportion of individuals are insulin-resistant, and the questions already raised long ago [31] remain: Are there any beneficial effects with low-grade insulin resistance? What about the possible management of low-grade insulin resistance in the long term? The curtailed insulin effects observed in insulin-resistant patients could be considered an adaptive physiological process against abundance to prevent further weight gain. In fact, the reduced insulin action might even prevent additional weight gain in obese people who have problems controlling their food intakes and maintaining reasonable levels of physical activity. Unfortunately, however, insulin resistance leads to T2D in patients with such a genetic susceptibility. Some of the AT disturbances arising during AT expansion in humans could tie in with the AT expandability concept proposed by Virtue and Vidal-Puig [12], which was mainly developed from experimental data in rodent models, but is now supported by some studies in humans, although there are major differences in the rate and extent of AT dysfunction and obesity-related problems according to anatomical AT distribution [4] and in different ethnic groups [14]. Nevertheless, as long as energy surplus persists and the AT storage capacity is able to accommodate the continuing energy and lipid excess, metabolic and cardiovascular health can be preserved in spite of obesity. Moreover, as long as FAs are sequestered as triglycerides in expanding adipocytes, lipotoxic insults will

not arise, and those able to undergo unlimited AT expansion will remain protected against lipotoxicity; women with large hip and thigh circumferences belong in this category. Given such discrepancies, some authors claim that a new definition of obesity based on lipotoxicity effects may be useful [14].

In any case, the mechanisms responsible for the regional development of fat deposition and AT remodelling should now be explored in greater depth to elucidate why metabolic risks are increased with central obesity, while expansion of peripheral fat depots affords some protection against metabolic problems. Also, aside from the usual treatments for obesity based on behavioural approaches (specific dietary/nutritional approaches along with increased physical activity), there are question marks over the possible recovery of metabolic health after interventions that prevent inflammation. Is there any value in using pharmacological interventions to inhibit inflammatory pathways activated by AT dysfunction? Restoration of the production/secretion of endogenous anti-inflammatory molecules such as adiponectin and apelin may constitute another beneficial strategy. As recently discussed [1], a number of agents showing beneficial effects on inflammation and insulin sensitivity in rodents should also be investigated in humans even though the validity of animal models in translational research has recently been questioned, given that genomic responses in mouse models are poor mimics of human inflammatory disease [117].

Disclosure of interest

The author declares that he has no conflicts of interest concerning this article.

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