Chapter 16

Interplay between Hypoxia, Inflammation and Adipocyte Remodeling in the Metabolic Syndrome

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Additional information is available at the end of the chapter

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Abstract

Obesity, a major social and health problem in many countries, is due to the accumulation of white adipose tissue in subcutaneous and visceral depots. The discovery of adipocytes capacity of synthesis of numerous adipocytokines and growth factors and the cross talk between adipocytes and cells of the adipose stromo-vascular fraction had highlighted the role of adipose tissue dysfunction in obesity. In visceral obesity the unbalanced synthesis of pro- and anti-inflammatory adipocytokines contributes to the development of the metabolic syndrome which cumulates the factors that increase the risk for ischemic heart disease and cerebral stroke. Adipose tissue accumulation is associated with a state of chronic inflammation, and local hypoxia is considered its underlying cause due to the hypertrophic or/and the hyperplasic growth of the fat pad. Adipose tissue hypoxia is one of the first pathophysiological changes and was placed as a missing link between obesity and low-grade inflammation present in the metabolic syndrome. Hypoxia is a major trigger for adipose tissue remodeling including adipocyte death, inflammation, tissue fibrosis, and angiogenesis. Recently, the role of hypoxia in brown adipose tissue dysfunction, a tissue presumed as the biologic counterbalance of the metabolic disturbances in human obesity, is discussed.

Keywords: adipose tissue, hypoxia, inflammation, metabolic syndrome, fibrosis, angiogenesis

1. Introduction

Until two decades ago, the adipose tissue has been considered one of the least dynamic structures of the mammalian organism involved exclusively in fat storage. Some key events have changed this mechanistic point of view, and now the whole fat of an organism is viewed as a complex organ composed of at least two main varieties of adipose tissues: the white adipose tissue (WAT) containing unilocular adipocytes and the brown adipose tissue (BAT) formed by multilocular adipocytes. Besides this different type of adipocytes, both tissues contain a non-adipocitary stromo-vascular fraction that includes undifferentiated cells, preadipocytes, fibroblasts, inflammatory cells, and various amounts of vessels and nerves. The adult adipose organ is divided into two types of depots: subcutaneous/peripheral and visceral/central constituted of lobules of unilocular adipocytes sustained by the stromo-vascular fraction well vascularized and innervated [1–5].

A significant development in the knowledge of adipose tissue is related to its function as an endocrine organ, both types of tissues being able to elaborate adipocytokines, humoral factors with various metabolic, vascular, pro-inflammatory, and anti-inflammatory roles [2, 4].

The accumulation of WAT in physiological depots leads to obesity characterized by an increase of the body mass index (BMI) over 30 kg/m^2 [6]. Obesity became a major social and health problem in many countries (between one quarter and one third of the population), a recent report of the World Health Organization (WHO) mentioning more than 1.9 billion of adult overweight subjects worldwide, more than 600 millions being obese [7]. From a pathogenically point of view, the quality and the distribution of adipose tissue seem to be more important in triggering the metabolic syndrome than the quantity of the fat per se. A direct relationship is accepted between abdominal/visceral fat accumulation—apple-shaped obesity—and the emergence and development of the metabolic syndrome or abdominal and pelvic cancers. Unbalanced synthesis of pro- and anti-inflammatory adipocytokines in visceral obesity contributes to the development of many features of the metabolic syndrome which cumulates the factors that increase the risk for ischemic heart disease and cerebral stroke: apple-shaped fat deposition, impaired glucose metabolism, dyslipidemia, and high blood pressure [8–10]. Pear-shaped obesity—i.e., subcutaneous fat accumulation—has a minimal risk for the development of such pathologies even at the same BMI greater than 30 kg/m^2 [11–13].

Hypoxia is one of the mechanisms responsible for the development of the metabolic changes and the pro-inflammatory milieu of white adipose tissue in obesity [3].

Tissue partial O_2 pressure (pO_2) reflects the balance between O_2 delivery and consumption, and continuous, chronic low O_2 tension occurs as a tissue inability to provide adequate compensatory vascular supply [3, 14, 15]. Obviously, adipose tissue hypoxia has a polymorphic feature since it depends on the adipose tissue blood flow regulation, different between the adipose phenotype WAT or BAT, the adipose fat pad localization, subcutaneous or visceral, and the delay of time between the onset of hypoxia and its quantification.

In healthy lean young adult, the pO_2 in adipose tissue is considered 55–60 mm Hg [14, 16] similar to the general tissue oxygenation [4], but important differences were reported in obese

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subjects. Oxygen supply was found markedly lower (44.7 mm Hg) in obese subjects in fasting and postprandial status than in lean subjects (55.4 mmHg) [14, 17], but Goossens et al. [18] found that in WAT of obese subjects the pO_2 was even higher (67.4 mmHg) than lean (46.8 mmHg). Of note, the pO_2 is not in a direct relation with the surface of the vascular network in the adipose pad. Capillary density for both subcutaneous and visceral depots is lower in obese human than in lean, but in lean subjects, the density is greater in visceral location [14, 19]. Even if BAT adipose tissue is more vascularized than WAT, it was indicated that obesity also causes BAT hypoxia, the same response being noted in multilocular adipocytes that became larger in obese animals [20]. Interestingly, BAT hypoxia seems to be temperature-dependent. Xue et al. proved that there is no hypoxia in mice housed at 30°C, but it appears in animals living at 4°C [21].

Adipose tissue is one of the most plastic organs in adults gifted with the ability of a continuous remodeling—extends or regresses depending on nutrient intake. The plasticity of any tissue is due to its capacity of extending vasculature which requires the cross talk between adipocytes and stromal and endothelial cells in the case of adipose tissue.

There are several arguments in favor of this "hypoxia concept." Normally, each adipocyte is surrounded by capillaries, and it is widely accepted that WAT is poorly oxygenated in obese individuals because adipocytes may be up to 200 μm, so larger than the normal diffusion distance of oxygen within tissues. As adipose tissue mass rapidly increases, clusters of unilocular adipocyte distance from the vessels and pockets of hypoxia are generated [22]. Another cause presumed for adipose tissue hypoxia is the loss of endothelial cells usually associated with the damage of parenchymal cells in other tissues. Recently, the interest in brown adipose tissue (BAT) increased, and studies have indicated that obesity determines also BAT hypoxia and the loss of its thermogenic capacity [20].

Chronic adipose tissue hypoxia has been suggested to be part of the pathogenesis of adipocyte dysfunction [14, 23, 24]. Local hypoxia triggers the generation of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress [25] and initiates the inflammatory response able to regulate the balance between angiogenic factors and inhibitors in order to stimulate angiogenesis and increase blood flow. The paucity of endothelial barrier is associated with the release of profibrogenic and pro-inflammatory cytokines and an augmented influx of inflammatory cells [26]. There is considerable evidence that obese adipose tissue is markedly infiltrated with macrophages which participate in the inflammatory pathways and are very important in adipose tissue remodeling, macrophage infiltration being signalized by lipid-overloaded adipocytes necrosis. Numerous reports emphasized that visceral adipose tissue in obese individuals is more fibrotic than that of lean subjects [27–29].

Normally, BAT and WAT produce various pro-angiogenic factors and cytokines able to induce remodeling of the vasculature, and as a response to hypoxia, an unbalanced production of these multiple bioactive pro-angiogenic and antiapoptotic growth factors synthesized by the adipose stromal cells may occur. Local hypoxia in obese is the underlying cause of an increase of macrophage cell number accompanied by the state of chronic inflammation and impaired adipokine secretion. Hypoxia promotes the delivery of many adipocytokines related to inflammation and tissue remodeling needed for angiogenesis to the ischemic tissue, such as

macrophage migration inhibitory factor (MIF), granulocyte-macrophage colony-stimulating factor (GM-CSF), matrix metalloproteinases MMP-2 and MMP-9, transforming growth factor (TGF)-β, vascular endothelial growth factor (VEGF), interleukins (IL-1, IL-6, IL-10), tumor necrosis factor (TNF)-α, angiopoietin-like (Angptl)-4, and leptin [4, 15, 22, 30–32].

This chapter summarizes the potential links between hypoxia, inflammation, adipocyte hypertrophy, and macrophage infiltration of adipose tissue and the effects of inflammatory mediators on its remodeling.

2. Essentials of adipose organ structure and functions

The adult adipose organ is composed by two types of adipose depots divided into adipose lobules of (i) unilocular adipose tissue (WAT—white adipose tissue) composed of unilocular cells and (ii) brown adipose tissue (BAT), formed by multilocular adipocytes (**Figure 1a** and **b**).

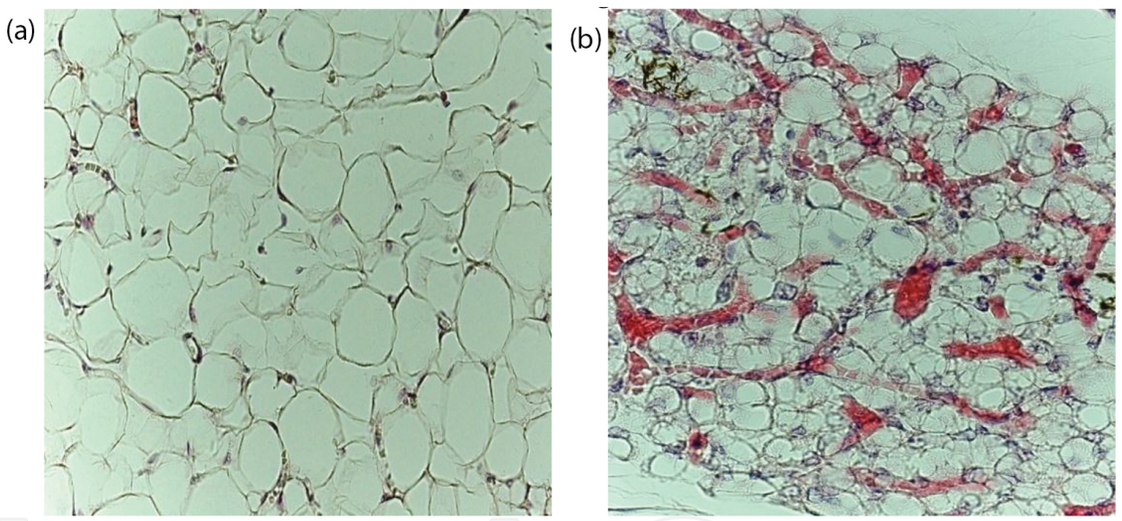

Figure 1. (a) Human adult subcutaneous WAT (hematoxylin and eosin staining, ob. ×40) and (b) human newborn visceral adipose depot with unilocular WAT and multilocular BAT adipocytes (hematoxylin and eosin staining, ob. ×20).

Both types of cells organized into adipose lobules are sustained by the stromo-vascular fraction well vascularized and innervated [11, 33]. Anatomically, WAT depots are located primarily in two major areas—subcutaneous/peripheral and visceral/central, which differ in the composition of the stromo-vascular fraction [34, 35]. Although at a first view the adipose tissue looks quite simple, a deeper molecular analysis revealed a high heterogeneity of cells. With respect to adipose cells, recent research identified both in rodent and men, the third type of adipocytes with common features of WAT and BAT adipocytes named "brite" or "beige" cells.

BAT, so named because of its yellow-brown color in vivo due to a very rich vascularization, is distinguishable morphologically from WAT by its cytoplasmic multiple droplets of stored triglycerides, while WAT contains a single large droplet. The multilocular cells are rich in

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mitochondria containing the uncoupling protein (UCP)1 which is uniquely present in BAT and therefore considered a marker for it (**Figure 2**).

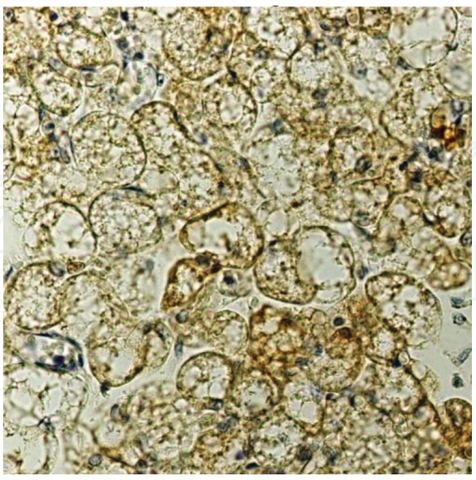

Figure 2. Newborn human brown adipocytes labeled with UCP1 (IHC, ob. ×40).

In humans, two types of BAT are present: (i) the classical (or constitutive BAT—cBAT) that is fully developed at birth and then reduced to remain in human adult only in a symmetrical cervical position and around the clavicles as very recently localized by PET/CT scanning, and the second type of brown adipocytes named "beige" or "brite" (brown in white), inducible or recruitable BAT (rBAT). This is composed of isolated brown multilocular cells resident between white cells mainly in subcutaneous depots [36, 37]. WAT is recognized as the site of fat storage, while BAT acts, as in rodents, as a heat-generating tissue through uncoupled oxidative phosphorylation which involves the action of UCP1 [38].

The functional complexity of adipose tissue is also due to the heterogeneity of cell phenotypes located in the non-adipocitary stromo-vascular fraction that includes undifferentiated or mesenchymal cells, preadipocytes, fibroblasts, and inflammatory cells (macrophages, lymphocytes, and mast cells). These cells are surrounded by a very complex network of vessels and nerves. The vascular network is more developed and branched in BAT than in WAT [39]. Normal metabolic functions and their imbalance involve a cross talk between adipocytes and the cells from the stromo-vascular fraction mediated by the components of adipose tissue extracellular matrix (ECM).

WAT secretoma. The discovery of leptin by Friedman in 1994 initiated the recognition of white adipocytes as major endocrine cells that secrete numerous bioactive molecules: lipids (such as free fatty acids mobilized in lipolysis, prostaglandins, and endocannabinoids) and proteins (termed "adipokines" or "adipocytokines" with metabolic and pro-/anti-inflammatory functions) [40]. Several adipocytokines are listed in **Table 1**. Impaired production of adipokines is associated with the pathogenesis of obesity-related disorders—type 2 diabetes mellitus, metabolic syndrome, cardiovascular diseases, and certain types of cancer [2, 41–44]. Generally, blood adipocytokine levels rise with the increase of fat mass except for adiponectin and omentin levels which are reported to be lower in obese and overweight subjects [31, 45, 46].

Adipocytokine	Function
Leptin	Feeding behavior, fat mass, pro-angiogenic
Adiponectin	Insulin sensitivity, anti-inflammatory, pro-angiogenic
Resistin	Insulin resistance, pro-inflammatory, antiangiogenic
Visfatin (pre-B-cell colony-enhancing factor, PBEF)	Insulin resistance, pro-inflammatory
Vaspin (visceral adipose tissue-derived serpin)	Insulin resistance
Omentin	Insulin resistance
Retinol-binding protein (RBP)-4	Insulin resistance
Serum amyloid A	Insulin resistance, pro-inflammatory
Cholesteryl ester transfer protein (CETP)	Lipid metabolism
Lipoprotein lipase (LPL)	Lipid metabolism
Adipocyte fatty acid-binding protein (A-FABP)-4	Lipid metabolism
Perilipin	Lipid metabolism
Apelin	Vasodilatation, pro-angiogenic
Angiotensinogen	Regulation of blood pressure
Angiotensin II	Regulation of blood pressure
Adipsin (adipocyte trypsin/complement factor D)	Lipid and glucose metabolism, inflammation
Tumor necrosis factor (TNF)-α	Pro-inflammatory
Interleukin 6 (IL-6)	Pro-inflammatory
C-reactive protein (CRP)	Pro-inflammatory
Plasminogen activator inhibitor (PAI)-1	Fibrinolysis, pro-angiogenic
Monocyte chemoattractant protein (MCP)-1	Macrophage activation
Intercellular adhesion molecule (ICAM)-1	Macrophage activation
Fibroblast growth factor (FGF)-2	Pro-angiogenic
Hepatocyte growth factor (HGF)	Pro-angiogenic
Platelet-derived growth factor (PDGF)	Pro-angiogenic
Vascular endothelial growth factor (VEGF)	Pro-angiogenic
Transforming growth factor (TGF)-β	Inflammation, fibrosis
Matrix metalloproteinases (MMPs)	Pro- and antiangiogenic, ECM remodeling
Tissue inhibitor of metalloproteinases (TIMPs)	Antiangiogenic, ECM remosdeling

Table 1. Adipocytokines and their main biological effects (adapted with permission from [31]).

Leptin and adiponectin are the most important hormones secreted by white adipocytes with multiple metabolic roles (regulating appetite and energy balance, insulin sensitivity) but also encompass angiogenic and anti-inflammatory actions [2, 4]. Leptin increases the vascular permeability in adipose tissue and influences microvessels density [47].

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Adiponectin is regarded as a link between obesity and related metabolic disorders because it improves glucose and lipid metabolism and prevents inflammation [15]. There are many other members of the "adipokinome" involved in the inflammatory response: tumor necrosis factor (TNF)-α, interleukins (IL-6, IL-8, IL-10), monocyte chemoattractant protein (MCP)-1, and macrophage migration inhibitory factor (MIF) [4, 15, 22]. Besides the adipocytes, many other cells from the stromo-vascular fraction secrete inflammatory cytokines and chemokines in response to adipocyte hypertrophy or hypoxic conditions. Other adipokines related to inflammation include several crucial angiogenic factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF)-1, angiopoetin-2, nerve growth factor (NGF), plasminogen activator inhibitor (PAI)-1, apelin, and adipsin [4, 30–32]. The release of numerous inflammatory adipocytokines is markedly increased in obesity-related diseases. Subcutaneous and visceral adipose tissue display differences in their adipokinome. Even if the results of in vitro and in vivo studies are controversial, it can be assumed, for example, that leptin and adiponectin are mainly produced in vivo by the subcutaneous adipocytes, while others (angiotensinogen, A-fatty acid-binding protein (FABP)-4, IL-6) are secreted at higher levels in visceral adipose tissue [48–51].

3. Adipose tissue dysfunction and hypoxia

Adipocyte capacity of synthesis corroborated with the clinical observation that a proportion of obese individuals seem to be protected against metabolic syndrome [52] had highlighted the role of adipose tissue dysfunction in obesity. Obesity is so long considered a genetic predisposition that promotes the excess of energy intake or the scarce energy expenditure.

In humans, the adipose tissue from the two main locations (subcutaneous and visceral) shows anatomical and functional differences (in contrast to subcutaneous adipose tissue, abdominal depots drain directly onto the portal circulation [31]) and different gene expressions.

Oxygen is a main nutritional factor without which oxidation of nutrients in aerobic tissues cannot take place. The decrease of oxygen level in various tissues can occur even if the total amount provided to the organism is not reduced [20]. Evinced hypoxia that follows low oxygen tension has numerous implications for cellular metabolism and transcriptional program [27]. Recent research suggests that adipose tissue hypoxia occurs in obese mice and even in human subjects. In obese rodents the existence of hypoxia was demonstrated by qualitative reaction (using hypoxic cell markers, such as pimonidazole—PIMO) or quantitative technique using needle-type oxygen sensors [3, 15, 22, 23]. In human obese subjects, the results are more controversial since normoxia and even hyperoxia have been reported in various experiments [18, 20, 53].

Chronic hypoxia has been suggested to be part of the pathogenic pathways leading to adipose tissue dysfunction [14, 23, 24].

Local hypoxia triggers the main alterations defining the adipose tissue dysfunction: generation of ROS and oxidative stress [54], ER stress [25], adipocyte death [55], inhibition of adiponectin

expression [55, 56], and leptin hyperproduction [57] and initiates the inflammatory response able to regulate the balance between angiogenic and inhibitor factors in order to stimulate angiogenesis and increase blood flow.

More causes of adipose tissue hypoxia are discussed, this concept being related to the histological changes of the adipose obese tissue—hyperplasia and adipocyte hypertrophy. Reduction of blood supply in adipose pads is a common mechanism of tissue hypoxia. Reduced adipose tissue blood flow in obese rats and humans was reported many years ago (Larsen et al, 1966, West et al., 1987 cited by [26]), being associated with insulin resistance in obese individuals [17, 18]. Adipose tissue angiogenesis is insufficient to maintain normoxia in the growing number of fat-storing cells in adipose depots as they are in obesity. Histological analysis has demonstrated a scarce capillary network in abdominal subcutaneous depots in obese subjects compared to the leans [14, 18].

A second cause is related to the increased size of adipocytes—hypertrophy—reaching in obese subjects a diameter larger than 150–200 μm [45]. This exceeds the normal capacity of oxygen diffusion through the tissue (100–200 μm), and oxygenation of adipose tissue will be compromised [58].

Hypoxia-inducible factor (HIF)-1 is the key transcriptional factor involved in response to hypoxia, which moves into the nucleus and binds to hypoxia-response elements from a myriad of target genes to initiate their transcription [3]. Both murine and human adipocytes exhibit extensive functional changes in culture in response to HIF-1, which alters the expression of up to 1300 genes [59]. These include genes encoding key adipokines, such as leptin, apelin, visfatin, TNF-α, IL-1, IL-6, VEGF, angiopoietin-like protein (Angptl)-4, MIF, PAI-1, and matrix metalloproteinases 2 and 9 (MMP-2, MMP-9), which are upregulated, and adiponectin, peroxisome proliferator-activated receptor (PPAR)-γ which is downregulated [3, 20, 55, 60, 61].

Hypoxia alters genes encoding key proteins for metabolic processes: glucose uptake, glycolysis, oxidative metabolism, lipolysis, and lipogenesis. Glucose uptake into adipocytes is stimulated by hypoxia because the expression of GLUT transporters is upregulated [20, 55, 62]. A switch from aerobic to anaerobic metabolism in hypoxic adipocytes is sustained by the increased activity of some glycolytic enzymes (e.g., phosphofructokinase [63]) and a net lactate release. Many studies focused on hypoxia-induced derangements of lipid metabolism reporting an increased lipolysis rather than unchanged but reduced lipogenesis in hypoxic adipocytes [64–66].

It seems that various degrees of adipose tissue hypoxia have different metabolic effects, and it is supposed also that subcutaneous and visceral adipocytes respond differently to factors that mediate tissue hypoxia. A recent study demonstrated that hypercaloric diet induces more severe hypoxia in mesenteric adipose tissue of mice than in the subcutaneous one [67].

Another direct effect of hypoxia is induction of insulin resistance via the upregulation of certain adipokines, the impairment of insulin-signaling pathway being a key change for white adipocyte dysfunction in obese subjects [3, 4].

Adipose tissue is one of the most plastic entities of an organism in terms of growth in the childhood and even in the adulthood in normal and pathological conditions, responding

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rapidly and dynamically to nutrient excess or starvation. Nor normal or pathological tissue, therefore nor the adipose tissue, is able to grow, develop, and function in the absence of an appropriate vascular network. Therefore, the hypoxia-induced expression of VEGF, the main angiogenic factor, and of certain adipokines, such as angiopoietin-2, Angptl-4, and leptin, sustains the stimulation of angiogenesis in obese adipose tissue [68–70]. Experimental data emphasize the induction of a pro-fibrotic switch of the transcriptional program in hypoxic adipocytes, fibrosis being another feature of adipose tissue dysfunction in obesity [29, 71]. Preadipocytes, pro-inflammatory cells, and fibroblasts from WAT as well as adipocytes respond to hypoxic conditions, favoring cellular events that lead to inflammation and fibrosis. Biostatistical analysis of WAT transcriptome had demonstrated a positive correlation between fat mass, degree of inflammation, and synthesis of ECM in obesity complications [72].

4. WAT hypoxia: a link between obesity and inflammation

The necessary link between abdominal (visceral or central) obesity and the development of type 2 diabetes and metabolic syndrome (which includes atherosclerosis, hypertension, and hyperlipidemia) due to the expanding fat mass and adipose tissue dysfunction was first demonstrated by Spiegelman's group [73, 74]. The mild inflammation status of the adipose tissue in obese subjects is induced by the peculiar role occupied by TNF-α, a 26 kDa transmembrane protein secreted as a cytokine and acting as an endotoxin-induced factor causing necrosis of tumors in vitro and cachexia in vivo, so naturally linked to the energy homeostasis [1]. They discovered that TNF-α is an active biofactor secreted by adipocytes and stromovascular cells positively correlated with obesity and insulin resistance.

Many signaling pathways have been proposed to be involved in the pathogenesis of obesity-associated inflammation called also "metaflammation" [75] such as (i) activation of toll-like receptor 4 (TLR4) by free fatty acids released after lipolysis [76], (ii) activation of protein kinase C (PKC) by diacylglicerol and ceramide [77]), (iii) induction of ER stress [25, 78] and oxidative stress [79], and (iv) adipocyte death [39]. Recent research data suggest that adipose tissue hypoxia is one of the first pathophysiological changes and was placed as a missing link between obesity and low-grade inflammation [61, 80].

Clinical and physiological data argue that in the whole organism the oxygen level is not the same in all the tissues nor constant for the same tissue and an isolate organ or tissue may lack oxygen even if the total supply is not compromised. This seems to be the case of the hypoxia inside the WAT human depots, the expanding adipose lobules or hypertrophic adipocytes resting isolated in pockets of tissue that lack the vascular supply, while other areas could be in normoxia or even hyperoxia [20]. The lack of oxygen perfusion for the hypertrophic adipocytes made them necrotic and finally they died. Dead adipocytes and free lipid droplets liberatedly act as recruitment factors for macrophages [39]. Besides adipocytes, preadipocytes and macrophages (the main players in WAT inflammatory response stimulating the inflammatory state in adipose tissue by the release of pro-inflammatory cytokines, such as TNF-α and interleukins) also respond to hypoxia. For such controversial results regarding the hypoxia

in human adipose tissue, one must consider the technique accuracy and the methodological issues, minding that the same depot could be polarized toward hypoxic areas or inflamed and hypervascularized nests. Such a clustered differentiation is not unique in the adipose tissue since data demonstrated that in obese adipose tissue the switch from M2a macrophages discriminative for lean mice to M1 inflammatory phenotype takes place in well-defined spatiotemporal areas inside the same adipose depot [81].

In order to assess the involvement of TLR signaling in inflammation in obesity-related diseases, we analyzed the expression of TLR-2, TLR-4, TNF-α, and CD-68 in subcutaneous and visceral adipose depots from lean, obese, and obese diabetic subjects. We observed that both types of depots showed an increased number of small- and medium-dilated vessels with many CD68-positive cells [82]. In the peritoneal depots, we observed leukocyte margination with CD68-positive cells, but we didn't notice the presence of macrophages crowns in none of the samples analyzed, as Cinti and coworkers found in adipose tissue with hypertrophic cells [39]. Data obtained proved that same cells from the visceral adipose depots of obese and obese-diabetic patients, mainly macrophages, intravascular leukocytes, and endothelial cells, showed a positive reaction for both TLR-4 and TNF-α [82], proving that TLR4 activation contributes to the inflammatory process in obesity and the onset of the metabolic syndrome (**Figure 3**).

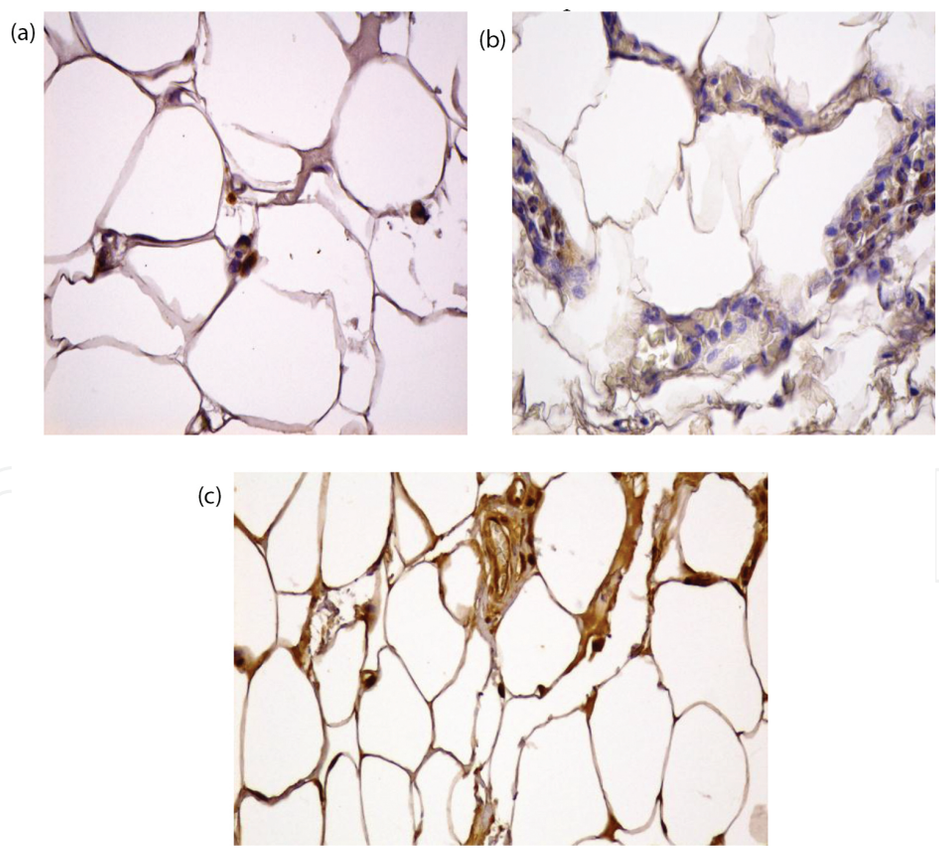

Figure 3. Immunostaining for CD68, TLR-4, and TNF-α of visceral obese adipose depots. (a) CD68-positive leukocytes between adipocytes (ob. ×40) in adipose peritoneal depots, (b) TLR-4-positive leukocytes and endothelial cells (ob. ×40), (c) intense-positive TNF-α reaction in visceral depots (ob. ×40).

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Summarizing the data linking the cellular and molecular alterations of the adipose tissue in obesity to the adipose tissue dysfunction, among the three events highlighted—oxidative stress, ER stress, and local hypoxia—hypoxia might be the first in a logical chronologically order, since it promotes oxidative and ER stress. In obesity, quick changes from normoxia/hyperoxia to hypoxia would be needed in order to induce oxidative stress [16]. Adipose tissue hypoxia induces inflammation through activation of two main transcription factors, HIF-1α and nuclear factor (NF)-KB, each of them activating transcription of a variety of genes encoding angiogenic and/or pro-inflammatory adipocytokines [26, 83]. Available data demonstrate that in rodent, HIF-1α upregulation starts in the first 1–3 days after the administration of a high-fat diet, before inflammation and insulin resistance develop [15, 19, 84].

5. Hypoxia: a major trigger for adipose tissue remodeling

Adipose tissue hypoxia is a concept that can practically explain the main alterations defining the adipose tissue dysfunction due to obesity: chronic inflammation, leptin expression, adiponectin reduction, adipocyte death followed by the invasion of monocytes and activation of macrophages, elevated lipolysis and adipocyte insulin resistance, and increased activity of ROS [3, 15, 55]. This entire cellular and molecular imbalance is followed by a compulsory adipose depot remodeling. The concept of remodeling of adipose tissue refers, as in all other entities, to the turnover of the cells and of the ECM in response to the requirement for growth and expansion of the adipose depots [85]. The molecules (cytokines, adipokines, growth factors, and proteases) involved in adipose tissue remodeling are synthesized and act as a permanent result of the cross talk between adipocytes and stromal cells.

5.1. Adipocyte death and inflammation

Adipocyte death is accepted as the main trigger for the adipose tissue remodeling [84], but the cause of this event is not consensual: the adipocyte size or the hypoxic milieu. In mice a positive robust correlation exists between adipocyte size and adipocyte death [39]. Consecutively macrophages are accumulating in crown-like structures being a source of numerous pro-inflammatory cytokines. A difference in the incidence of dead adipocytes was noted, the intra-abdominal cells being more susceptible than those of the inguinal depots. The clearance of the cellular detritus by the macrophages is the trigger for a homeostatic remodeling program that will allow the further expansion of the adipose depots that include matrix remodeling and vasculogenesis. Foci of adipocyte death are therefore areas where macrophages promote obesity-associated inflammation [39]. Interestingly, adipocyte loss is associated with phenotypic changes in stromal monocytic-macrophage cells. In a chronologically sequence, after the scavenging, the place occupied by the huge dead adipocytes was taken by small-size adipocytes, and the former hypertrophic adipose tissue became hyperplasic (Faust et al., 1984 cited by [84]). As a new study demonstrated that the macrophages are crowded in foci of hypoxic tissue, a second theory emphasizes that adipocyte death is caused by the hypoxia and the macrophages are trapped into the hypoxic areas by MIF [65, 86].

5.2. Hypoxia underpins adipose tissue fibrosis

There are several recent studies involving fibrosis of adipose depots in installing hypoxia and insulin resistance [27, 28, 87].

Scherer's research group proposed that in adipose obese tissues hypoxia is the most important driving force downstreaming the events associated with inflammation and fibrosis [27]. They found that in adipose tissue from the transgenic mice HIF-1α-ΔODD, in which a dominant-active deletion mutation of HIF-1α is overexpressed, fed with a hypercaloric diet, the transcription factor HIF-1α failed to promote the pro-angiogenic program by targeting genes, such as VEGF-A. Moreover, in these mice HIF-1α induces the fibrotic program by an increased synthesis of fibrotic proteins, such as lysyl oxidase (LOX), type I and type III collagens, tissue inhibitor of matrix metalloproteinases (TIMP)-1, and connective tissue growth factor (CTGF). Histology performed with trichromic staining revealed thick fibrotic streaks composed of type I collagen fibers, similar results being reported also for the adipose pads from obese human subjects [88].

LOX is a known target gene of HIF-1α, and in adipose tissue of ob/ob transgenic mouse, LOX is found in increased level compared to wild type [27]. LOX cross-links elastin and collagens in ECM and creates ECM-resistant bands of fibrosis. In adipose tissue of ob/ob mouse, these collagen bundle "streaks" are found outside the "crown-like" structures previously described [27, 39, 84]. The conclusion derived was that collagen synthesis and deposition could be anterior to the accumulation of macrophages surrounding the adipose cells because hypoxia-induced fibrotic program develops shortly after the high-fat diet is established [27]. So adipose tissue fibrosis is not necessarily induced by inflammation but could be rather an upstream phenomenon through the synthesis of HIF-1α and LOX.

From a different point of view, like in other tissues, adipose tissue fibrosis develops as a result of a persistent inflammation and a failure of the normal tissue repair with *restitutio ad integrum*.

Interestingly, fibrosis of adipose depots in obesity seems to display an otherwise intensity as the inflammation and hypoxia, those visceral seeming to be not only less fibrotic than those peripheral but also with a different distribution of collagen fibers, especially pericellular or intraparenchymatous [28]. As the visceral depots are more inflamed than the subcutaneous depots as we showed [88], this observation contradicts the accepted biological sequence that fibrosis develops as a result of an excessive and altered ECM synthesis and storage by resident cells activated in an inflammatory environment. This abnormal amount of fibrotic matrix in the subcutaneous adipose tissue could be explained if we keep in mind the histology of the host tissue where the adipose depots expand (the subcutaneous adipose tissue develops toward a much more dense tissue than the visceral one). In obese subjects fibrosis accumulates in pericellular areas—lining each adipose cell or a group of cells (interstitial fibrosis) and around the vessels (**Figure 4**).

Collagen phenotypes are also different, types I, III, and VI being present in pericellular position but only I and III form thick bundles appearing as interlobular septa surrounding more cells [28]. In visceral (omental) depots, the accumulation of fibers in pericellular position is associated with small adipocyte size and a lowest quantity of circulating triglycerides, proving that

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the subjects with smaller adipocytes have a less adverse metabolic profile [27, 89], so fibrosis may act as a protective reaction. In the adipose depots, the significance of type VI collagen seems to be peculiar, since its appearance changes dramatically through adipogenesis [90]. Transgenic mouse col 6KO ob/ob shows reduced necrotic cell death and consumes only a half of the amount of food that ob/ob strain [91] and type VI collagen levels correlate with hyperglycemia and insulin resistance [87, 92]. Obese humans expressed higher levels of type VI collagen and macrophage markers [92].

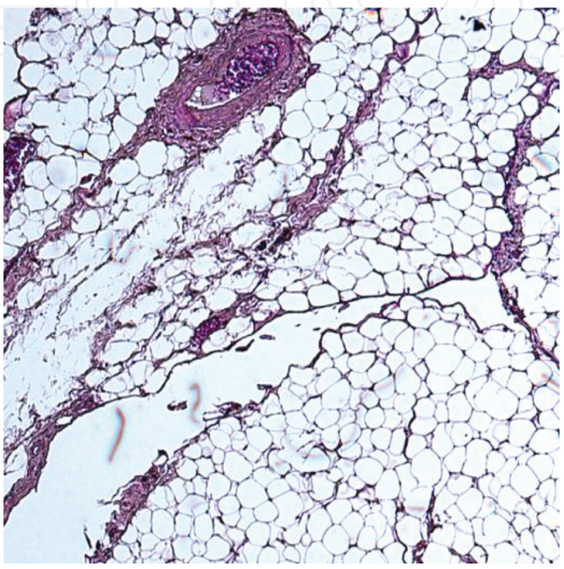

Figure 4. Pericellular and interstitial fibrosis in a visceral adipose depot from an adult obese subject (ob. ×20).

5.3. Hypoxia-induced angiogenesis in white adipose tissue

Being a compulsory condition for the expansion of any tissue, angiogenesis is a very limited process in normal adulthood (in endometrial cyclic physiology or wound healing), and the endothelial cells of the adult capillary network are in a relatively quiescent state.

In adipose tissue, angiogenesis is a very complex phenomenon regulated by a lot of molecules (hormones, cytokines, and growth factors) secreted by the stromo-vascular cells, including endothelial cells, and also by the adipocytes and preadipocytes [93, 94].

During adipose depot development, adipogenesis and vasculogenesis are temporally and spatially dependent, and in an adipose depot, the vascular network seems to act as a self-stop for the adipose expansion, since the inhibition of angiogenesis reduces the adipose tissue mass [95, 96].

In hypoxia induced by a high caloric intake, the vascular network does not progress uniformly between depots, due to the differences between the initial degree of vascularization and the rate/capacity of neovascularization during adipose tissue expansion. Hypoxia is more severe in mesenteric visceral depots than in subcutaneous [67], and at the same time, human visceral depots reveal a greater capillary density and angiogenic capacity than the subcutaneous

adipose tissue [97, 98]. In their study using CD31+/CD34+ immunolabeling, Villaret and coworkers revealed that in obesity the capillary network is more developed and endothelial cell number is greater in visceral than in subcutaneous adipose depots, so increased hypoxia of visceral adipose tissue is not necessarily a consequence of capillary rarefaction [98]. The pro-angiogenic and pro-inflammatory phenotype of visceral adipose tissue could be related to endothelial cell senescence proved by an altered expression of some senescence markers such as IGFBP3, γH2AX, and SIRT1. They postulated at least two main causes for endothelial cell senescence in visceral adipose tissue: increased cell replication and oxidative stress [98]. Hyperplasia of obese visceral adipose tissue is responsible for an increased secretion of VEGF-A2 that stimulates endothelial cell proliferation.

The compensatory angiogenesis could prevent the metabolic disturbances induced by the hypoxia. It seems that not in all conditions the expansion of adipose tissue is associated with inflammation if an appropriate capillary bed is developed. If this condition is satisfied, the obese subjects are termed "metabolically healthy obese" because they may expand their adipocyte depots without inflammation consequences. This kind of expansion is associated with an enlargement of a given fat pad through recruitment of new adipocytes along with an adequate development of the vasculature, minimal associated fibrosis, and the lack of hypoxia and inflammation [83, 99].

The effects of hypoxia for obese humans have been recently disputed, the reactions triggered by the oxygen deprivation being a matter of severity, duration, and environment, since results between in vitro experiments, cell cultures under acute hypoxia, animal models, and human obese subjects are different. In recent studies, opposite data are reported by Goossens's research group. Their experiments revealed an increased pO_2 in obese insulin-resistant subjects and a positive correlation between pO_2 and gene expression for pro-inflammatory markers and an inverse association between pO_2 and peripheral insulin sensitivity [18]. In another experiment, after exposing mice at normoxia and hypoxia for the same duration of time, the authors reported a decrease in adipocyte size, macrophages infiltration, and inflammatory cell genes in adipose tissue from hypoxic animals [100]. The same results were reported for obese men exposed for 10 nights to hypoxia consecutively followed by increased insulin sensitivity [101]. Moreover, it was presumed that the obstructive sleep apnea could be a protective mechanism to maintain energy homeostasis in obese subjects [102, 103]. Angiogenesis in hypoxic tissues is controlled by HIF-1α, so-called the master regulatory of cellular and tissue response to hypoxic stress. In adipose hypoxic tissue, HIF-1 induces angiogenesis by upregulating VEGF gene. VEGF-A is the only endothelial growth factor that stimulates ECM degradation, proliferation, migration, and tube formation of endothelial cells [104]. VEGF secretion is regulated also by insulin stimulation, growth factor, and cytokines, such as PDGF, EGF, TNF-α, TGF-α, and IL-1β [99, 104].

As we showed in a previous study, VEGF immunohistochemical expression was higher in the adipose tissue of obese and obese-diabetic patients, especially in peritoneal depots. In normal weight subjects, both peripheral and central depots were VEGF negative [88].

It was demonstrated that an overexpression of VEGF in transgenic animals increased the vascularization and reversed the metabolic dysfunctions induced by a hypercaloric diet [80].

Recently, it had been claimed that the mechanism of VEGF promoting angiogenesis in adipose tissue is controlled by HIF-1β, while HIF-1α seems to regulate vascularization in BAT but not in WAT and additionally to promote WAT inflammation [105].

Adipokines, such as leptin and adiponectin, have also angiogenic properties that are stimulated in metabolically challenging conditions: leptin stimulates the angiogenic program upregulating VEGF expression, increases the vascular permeability by the formation of fenestrations in endothelial cells, and influences microvessel density [106]. For adiponectin, the results are conflicting: one supposed to be antiangiogenic because it inhibits endothelial cell migration and proliferation in vitro and neoangiogenesis in vivo [107] and others pro-angiogenic [99, 108].

6. Hypoxia: a trigger for BAT whitening and WAT browning

BAT presence has been reported once for small rodents and newborns, but recently, evidence for metabolically active BAT in adult humans has been reported [109]. BAT activation under β-adrenergic signaling is important for heat generation through uncoupled oxidative phosphorylation as a result of activation of non-shivering thermogenesis [110]. For this function, an important blood supply is required to provide the amount of oxygen and nutrients, and therefore BAT is much more vascularized than WAT. In relation to energy balance, an inverse relationship is accepted between BMI and age, BAT being less active in older subjects and in obese [111, 112]. Recent experiments highlighted the importance of hypoxia in BAT dysfunction too, the lack of an adequate BAT vascularization being involved in the overall dysfunction of the adipose organ in obesity [20]. BAT activity reduces the development of metabolic syndrome, and its activation increases insulin sensitivity and contributes to glucose homeostasis [113]. Having a high oxidative capacity, it is presumed that BAT is a contributor to systemic metabolic homeostasis, and this function was impaired in obesity, as demonstrated in the experiment performed by Shimizu and coworkers [114]. They proved in mice that obesity affects the density of the capillary network in BAT much more than in WAT and induced hypoxia in this organ. This elegant experiment shows in a very credible manner that the transition of phenotype from brown to white adipocytes is induced by the diminution of vascularization, a reverse mechanism of BAT differentiation observed in fetal development when the appearance of multilocularity is anticipated by the branching of the capillary loops (personal unpublished data). This vascular dysfunction is followed by the "whitening of the brown fat" (diminished β-adrenergic signaling, the appearance of enlarged lipid droplets in the cells and loss of mitochondria) and can impact obesity and obesity-related diseases [115]. HIF-1α increased level and suppression of UCP1 gene were observed in hypoxic BAT [114]. The same influences that hypoxia exerts on gene expression in WAT have been reported also for BAT, such as increased expression of leptin, VEGF, IL-6, and GLUT1. Due to loss of mitochondria, high glucose uptake will be accompanied by the same switch to anaerobic glycolysis as in WAT. Besides triggering inflammation in macrophages, lactate is supposed to be involved also in "browning of the white fat," recent experimental data proving that in vitro lactate induces the expression of genes encoding UCP1 and proteins involved in mitochondrial

oxidation in mice and human white adipocytes [116]. Same authors demonstrated that lactate also controls the browning process in vivo because it regulates Ucp1 expression in a PPAR-δ-dependent manner, the combination of lactate and PPAR-δ ligand rosiglitazone constituting a strong inducer of an increased expression of some mitochondrial oxidation markers in mice white adipose depots [116]. Based on this observation, one can assume that lactate could be responsible for the recruitment of "brite" cells. In light of these results, the recruitment and activation of BAT are regarded as a potential new target for strategies to counteract obesity-induced changes.

In conclusion, hypoxia could be regarded as the leading cause of adipose tissue remodeling rather than as a consequence of the functional changes in the adipose organ. Due to the interplay between hypoxia, inflammation, and angiogenesis, targeting hypoxia pathways could be a valuable therapeutic approach to reduce the clinical consequences of the metabolic syndrome.

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