Revisiting the adipocyte: a model for integration of cytokine signaling in the regulation of energy metabolism

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Adipose tissue constitutes an extremely active endocrine organ with a network of signaling pathways enabling the organism to adapt to a wide range of different metabolic challenges, such as starvation, stress, infection, and short periods of gross energy excess. The functional pleiotropism of adipose tissue relies on its ability to synthesize and release a huge variety of hormones, cytokines, complement and growth factors, extracellular matrix proteins, and vasoactive factors, collectively termed adipokines. Obesity is associated with adipose tissue dysfunction leading to the onset of several pathologies including type 2 diabetes, dyslipidemia, nonalcoholic fatty liver, or hypertension, among others. The mechanisms underlying the development of obesity and its associated comorbidities include the hypertrophy and/or hyperplasia of adipocytes, adipose tissue inflammation, impaired extracellular matrix remodeling, and fibrosis together with an altered secretion of adipokines. Recently, the potential role of brown and beige adipose tissue in the protection against obesity has been also recognized. In contrast to white adipocytes, which store energy in the form of fat, brown and beige fat cells display energy-dissipating capacity through the promotion of triacylglycerol clearance, glucose disposal, and generation of heat for thermogenesis. Identification of the morphological and molecular changes in white, beige, and brown adipose tissue during weight gain is of utmost relevance for the identification of pharmacological targets for the treatment of obesity and its associated metabolic diseases.
Table 1. Adipokines secreted by adipose tissue

<table>
<thead>
<tr>
<th>Adipokine</th>
<th>Main Metabolic Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>Hormone with antidiabetic, antiatherogenic, and anti-inflammatory properties</td>
<td>(195, 261, 284)</td>
</tr>
<tr>
<td>Adipin</td>
<td>Protein involved in activation of the alternative complement pathway</td>
<td>(202)</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>Precursor molecule of angiotensin II that causes vasoconstriction, increased BP, and release of aldosterone from the adrenal cortex</td>
<td>(151)</td>
</tr>
<tr>
<td>Apelin</td>
<td>Peptide implicated in control of BP and one of the most potent stimulators of cardiac contractility; inhibits insulin secretion; involved in lipolysis</td>
<td>(310)</td>
</tr>
<tr>
<td>ASP</td>
<td>Hormone produced by the complement pathway that regulates whole body glucose and lipid metabolism</td>
<td>(46)</td>
</tr>
<tr>
<td>Calprotectin</td>
<td>Proinflammatory factor involved in cell adhesion, chemotaxis, and antimicrobial activity</td>
<td>(329)</td>
</tr>
<tr>
<td>Cardiotrophin-1</td>
<td>Cytokine involved in hypertrophy of cardiomyocytes</td>
<td>(215)</td>
</tr>
<tr>
<td>Cathepsins S, L, K</td>
<td>Cysteine proteases that promote adipogenesis and extracellular matrix remodeling</td>
<td>(214, 307, 308)</td>
</tr>
<tr>
<td>Chemerin</td>
<td>Chemoattractant protein involved in adaptive and innate immunity and in adipogenesis</td>
<td>(105)</td>
</tr>
<tr>
<td>CCL2, 3, 5, 7, 8, 11</td>
<td>Chemokines involved in monocyte chemotaxis</td>
<td>(138)</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Lipoprotein that promotes tumor progression and angiogenesis and is involved in metabolic and cardiovascular diseases</td>
<td>(238, 245)</td>
</tr>
<tr>
<td>CRP</td>
<td>Acute-phase reactant involved in inflammatory processes</td>
<td>(199)</td>
</tr>
<tr>
<td>Fetuin A</td>
<td>Protein that reflects liver fat content; associated with lipid-induced inflammation and insulin resistance; promotes cancer progression</td>
<td>(126, 255)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Proinflammatory cytokine implicated in acute-phase responses</td>
<td>(158)</td>
</tr>
<tr>
<td>FIAF/ANGPTL4</td>
<td>Protein induced by fasting and hypoxia</td>
<td>(348)</td>
</tr>
<tr>
<td>FNDC5/Virgin</td>
<td>Myokine/adipokine involved in promotion of myogenesis and fat browning</td>
<td>(206, 259, 260)</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>Orexigenic and adipogenic hormone that exerts a depressor effect on BP and acts as a cardioprotective agent</td>
<td>(267, 268)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Acute-phase reactant with angiogenic and chemotactic properties</td>
<td>(76)</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>Proinflammatory cytokine that activates MMP-9</td>
<td>(17)</td>
</tr>
<tr>
<td>HGF</td>
<td>Factor that stimulates proliferation and development in adipocytes and antiinflammatory effects</td>
<td>(18, 174)</td>
</tr>
<tr>
<td>HMGB1</td>
<td>Alarmin involved in DNA repair and secretion of insulin in pancreatic β-cells</td>
<td>(113)</td>
</tr>
<tr>
<td>Hsp72</td>
<td>Damage-activated protein that induces neutrophil and natural killer cell recruitment</td>
<td>(271)</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Factor that stimulates proliferation and differentiation in adipocytes</td>
<td>(44, 285)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Proinflammatory cytokine involved in a paracrine inflammatory pathway in adipose tissue</td>
<td>(179)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Proinflammatory cytokine implicated in acute-phase responses</td>
<td>(203)</td>
</tr>
<tr>
<td>IL-5/CXCL8</td>
<td>Chemokine involved in pathogenesis of atherosclerosis and cardiovascular diseases</td>
<td>(29)</td>
</tr>
<tr>
<td>IP-10/CXCL10</td>
<td>Chemokine produced by T cells</td>
<td>(127)</td>
</tr>
<tr>
<td>Leptin</td>
<td>Anorexigenic hormone with lipolytic and vasoactive effects in addition to other pleiotropic activities</td>
<td>(83, 97, 265, 359)</td>
</tr>
<tr>
<td>Lipocalin-2</td>
<td>Adipokine showing anti-inflammatory properties</td>
<td>(38)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Chemoattractant protein that promotes inflammation and macrophage infiltration in adipose tissue</td>
<td>(280)</td>
</tr>
<tr>
<td>MIF</td>
<td>Factor involved in proinflammatory processes and immunoregulation</td>
<td>(278)</td>
</tr>
<tr>
<td>MMP-1, 2, and 9</td>
<td>Proteins implicated in adipogenesis</td>
<td>(185)</td>
</tr>
<tr>
<td>NUCB2/Nesfatin-1</td>
<td>Anorexigenic peptide also involved in the inflammatory response</td>
<td>(249, 289)</td>
</tr>
<tr>
<td>NGF</td>
<td>Neurotropin involved in development and survival of sympathetic neurons</td>
<td>(299)</td>
</tr>
<tr>
<td>Omentin</td>
<td>Novel adipokine that modulates insulin sensitivity and exerts anti-inflammatory properties</td>
<td>(345)</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Proinflammatory factor involved in vascular and myocardial remodeling; cytokine implicated in insulin resistance and cancer</td>
<td>(99)</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Potent inhibitor of fibrinolysis implicated in development of atherosclerotic plaques</td>
<td>(157)</td>
</tr>
<tr>
<td>PEDF</td>
<td>Secreted glycoprotein that belongs to the noninhibitory serpin group with antiangiogenic, antioxidant, antiinflammatory, and lipolytic effects</td>
<td>(50, 276)</td>
</tr>
<tr>
<td>PGE2 and PGF2α</td>
<td>Factors implicated in regulatory functions such as inflammation, blood clotting, ovulation, menstruation, and acid secretion</td>
<td>(204)</td>
</tr>
<tr>
<td>Programin</td>
<td>Chemoattractant protein involved in adipose tissue inflammation and neurodegenerative diseases</td>
<td>(350)</td>
</tr>
<tr>
<td>RBP4</td>
<td>Factor involved in development of insulin resistance, visceral fat distribution, and dyslipidemia</td>
<td>(248)</td>
</tr>
<tr>
<td>Resistin</td>
<td>Hormone involved in development of insulin resistance, which participates in the proinflammatory response and polarization toward the M1 phenotype</td>
<td>(334)</td>
</tr>
<tr>
<td>SAA</td>
<td>Acute-phase reactant produced in response to injury, infection, or inflammation</td>
<td>(101)</td>
</tr>
<tr>
<td>SRFP5</td>
<td>Inhibitor of WNT5α signaling with anti-inflammatory properties</td>
<td>(233)</td>
</tr>
<tr>
<td>STAMP2</td>
<td>Metalloeductase that plays a role in cellular import of copper and iron and in reduction of macrophage recruitment and polarization toward the M1 phenotype</td>
<td>(321)</td>
</tr>
<tr>
<td>Tenasin C</td>
<td>Damage-activated protein that induces immune responses and extracellular matrix remodeling</td>
<td>(152)</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Regulatory factor of preadipocyte proliferation, differentiation, and apoptosis</td>
<td>(279)</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Major cellular initiator of the coagulation cascade</td>
<td>(279)</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Inhibitor that decreases adipogenesis and impairs glucose tolerance</td>
<td>(150)</td>
</tr>
<tr>
<td>TNFα</td>
<td>Proinflammatory cytokine involved in systemic inflammation and development of insulin resistance in obesity</td>
<td>(135)</td>
</tr>
<tr>
<td>TWEAK</td>
<td>Proinflammatory cytokine of the TNF superfamily that inhibits adipocyte tissue growth</td>
<td>(42)</td>
</tr>
<tr>
<td>Vaspin</td>
<td>Adipokine of the serine protease inhibitor family showing insulin-sensitising effects</td>
<td>(130)</td>
</tr>
<tr>
<td>VEGF</td>
<td>Factor involved in angiogenesis stimulation in adipose tissue</td>
<td>(98, 100)</td>
</tr>
<tr>
<td>Visfatin/NAMPT</td>
<td>NAD+ biosynthetic enzyme involved in regulation of β-pancreatic cells</td>
<td>(257)</td>
</tr>
<tr>
<td>WNT5a</td>
<td>Secreted glycoprotein of the WNT family with anti-angiogenic and proinflammatory actions</td>
<td>(21, 32, 243)</td>
</tr>
<tr>
<td>WISP1</td>
<td>Secreted matricellular protein that regulates adipogenesis and adipose tissue inflammation</td>
<td>(238, 245)</td>
</tr>
<tr>
<td>YKL-40</td>
<td>Proinflammatory factor that stimulates innate immune system, extracellular matrix remodeling, and angiogenesis</td>
<td>(34, 254)</td>
</tr>
<tr>
<td>Zinc α2-glycoprotein</td>
<td>Soluble glycoprotein with lipolytic effects</td>
<td>(22)</td>
</tr>
</tbody>
</table>

**ANGPTL4, angiopeptin-like 4; ASP, acylation-stimulating protein; BP, blood pressure; CCL, chemokine (CC motif) ligand; CRP, C-reactive protein; FGF-21, fibroblast growth factor-21; FIAF, fasting induced adipose factor; FNDC5, fibronectin type III domain containing 5; HMGB1, high-mobility group B1; IL, interleukin; MCP-1, monocyte chemotactant protein 1; MIF, macrophage migration inhibitory factor; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; PEDF, pigment epithelium-derived factor; PG1 and PGF2α, prostaglandin I2 and IIα; RBP4, retinol binding protein-4; SAA, serum amyloid A; STAMP2, six-transmembrane protein of prostate 2; TGFβ, transforming growth factor-β; TIMP-1, tissue inhibitor of matrix metalloproteinase-1; TNFα, tumor necrosis factor-α; TWEAK, TNF-like weak inducer of apoptosis; VEGF, vascular endothelial growth factor; WISP1, Wnt1-inducible signaling pathway protein-1; YKL-40, chitinase-like 1 protein.**

Expand in size during periods of positive energy balance, such as overfeeding or sedentary lifestyle, becoming hypertrophic. The pathological expansion of the adipose tissue during weight gain is accompanied by adipose tissue inflammation, fibrosis, and altered adipokine profile that ultimately promote obesity-associated metabolic alterations. The present review focuses on the advances in the morphological and functional changes in white adipose tissue during the onset of obesity, with special emphasis on its endocrine role in inflammatory processes and insulin sensitivity as well as the potential involvement of...
brown and beige fat depots as thermogenic, energy-dissipating tissues.

Ontogeny and Function of White, Brown, and Beige Adipose Tissue

According to their morphology, function, and location, adipose tissue can be broadly classified into two main types: white and brown adipose tissue (80, 96). White adipocytes are usually large, round cells with a diameter that varies from 25 to 200 μm, containing a large unilocular lipid droplet surrounded by a thin layer of cytoplasm with few mitochondria, and a flattened nucleus located in the periphery (79). The main functions of white adipocytes are storage of energy in the form of fat, lipolysis, and secretion of adipokines. White fat cells are part of the subcutaneous abdominal or visceral, retroperitoneal, inguinal, and gonadal fat depots (47, 262, 312). Brown adipocytes are cells with polygonal shape, normal cytoplasm with multilocular lipid droplets, and containing a central and round nucleus, together with a large quantity of mitochondria with laminar cristae (95). The main biological function of brown adipocytes is thermogenesis, and they also can store energy in the form of fat and secrete adipokines but to a lesser extent than that of white adipocytes (80). The thermogenic ability of brown adipocytes derives from the presence of uncoupling protein-1 (UCP1), a mitochondrial protein that induces heat production by uncoupling respiration from ATP synthesis. In new-borns, brown fat is abundant in the neck and interscapular region to prevent hypothermia (218). It was believed that only vestigial amounts were present in adults as a result of involution during infancy. However, positron electron tomography studies have revealed that adults show active brown fat in the neck, supraclavicular fat, mediastinum, and paravertebral and suprarenal fat (53, 324, 327).

White and brown adipocytes derive from the same mesenchymal stem cells (65). Nevertheless, during gastrulation, the mesenchymal stem cells of the paraxial mesoderm express the transcription factor Myf5, and the mesenchymal stem cells of the lateral mesoderm do not express this transcription factor. Myf5-expressing cells will differentiate into brown adipocytes or myocytes, whereas cells without Myf5 will become white adipocytes or blood vessel-associated pericytes.

Recent studies have revealed the presence of brown-like cells within adipose tissue, termed beige or brite (brown-in-white) adipocytes (340). Beige adipocytes are Myf5-negative cells that seem to originate from endothelial and perivascular cells within white adipose tissue with a unique gene signature (54, 184, 340) that is different from that of white and brown adipocytes (16, 98, 184). Under basal conditions, beige adipocytes express low levels of UCP1, but upon β-adrenergic stimulation induced by cold exposure or exercise, beige adipocytes produce high amounts of UCP1 and show thermogenic properties (340).

White adipogenesis. Adipogenesis is a highly controlled process whereby mesenchymal stem cells are differentiated into mature adipocytes (80, 273). Two main steps are involved in adipogenesis: 1) determination, i.e., commitment of a pluripotent stem cell to a unipotent preadipocyte that is morphologically identical to the precursor stem cell but has lost the potential to differentiate into other cell types; and 2) terminal differentiation, i.e., the process whereby preadipocytes acquire the phenotype and functional characteristics of mature adipocytes.

The biological process of adipogenesis is complex and regulated by multiple factors. Bone morphogenetic proteins (BMP) are potent growth factors of the tumor growth factor-β (TGFβ) superfamily that are involved in the determination or commitment of pluripotent stem cells to the adipocyte, chondrocyte, or osteoblast lineage (2, 12). BMP interacts with specific receptors on the cell surface, BMP receptor types 1 and 2 (BMPR1 and BMPR2). Binding of BMPs with the complex BMPR1 and BMPR2 induces the phosphorylation of SMAD1, -5, and -8, which form a complex with SMAD4 that translocates to the nucleus and regulates gene expression. The activation of the complex BMPR1:BMPR2 also stimulates the MKK3/p38 MAPK and TAK1 pathways, activating the expression of BMP target genes. The determination of white adipocytes is positively regulated by BMP2 and BMP4 (Fig. 1) (137, 309). Nonetheless, many transcription factors repress adipogenesis, including several members of the GATA-binding and forkhead families, Notch and Wnt signaling, as well as Kruppel-like factor 2 and 7 (KLF2 and KLF7) and DNA damage-inducible transcript 3/CHOP proteins (273). Committed preadipocytes also secrete the protein gremlin-1 (GREM1), a potent inhibitor of BMP4 with a considerably higher expression in hypertrophic obesity (111).

The transcription factors peroxisome proliferator-activated receptor-γ (PPARγ), C/EBP, and sterol regulatory element-binding protein-1 (SREBP1) are key for the terminal differentiation of adipocytes (Fig. 1) (161). Low or null levels of these transcription factors are usually found in preadipocytes, but their expression is increased during adipocyte differentiation (161). The binding of these transcription factors to the promoters of adipose-specific genes results in an increased expression of markers of adipocyte differentiation (19, 161). PPARγ is a member of the nuclear receptor superfamily and is considered the master regulator of adipogenesis, because all adipogenic factors require the presence of PPARγ to promote adipogenesis. The alternative splicing of PPARG originates in two isoforms: PPARγ1, the predominant isoform in fat cells, and PPARγ2, which is 30 amino acids longer than PPARγ1. PPARγ heterodimerizes with another nuclear receptor, the retinoid X receptor-α (RXRα) to bind DNA and promote the transcription of adipocyte-specific genes such as leptin, adiponectin, fatty acid-binding protein-4 (FABP4), or perilipin, among others (274). C/EBP belongs to the basic-leucine zipper class of transcription factors and presents six isoforms (C/EBPα, β, δ, ε, γ, and ζ) that are involved in the early induction of adipocyte differentiation (274). The increase in intracellular cyclic AMP leads to the activation of a signaling cascade that triggers the translocation of C/EBP to the nucleus and promotes the transcription of several adipocyte-specific genes (270). After hormonal induction, a rapid and transient increase in transcription and expression of C/EBPβ and C/EBPδ is observed in preadipocytes that activates the expression of PPARγ and C/EBPα (56). The activation of C/EBPα is followed by transcriptional activation of many genes encoding proteins involved in creating the adipocyte phenotype (GLUT4, SCD1, LEP, FABP4) (110). C/EBPζ, a dominant inhibitor of C/EBPα and -β, is induced in late adipocyte differentiation and has been proposed as an inhibitor of adipogenesis. Finally, the third key transcrip-
tion factor for adipogenesis, SREBP1 (formerly known as adipocyte determination and differentiation factor 1, ADD1) belongs to the basic helix-loop-helix family of transcription factors (161, 274). During adipocyte differentiation, SREBP1 is activated and translocated into the nucleus, where it recognizes sterol response elements (SRE) and induces the expressions of lipogenic enzymes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lipoprotein lipase (LPL), and stearoyl-CoA desaturase-1 (SCD1), as well as of an unknown activator of PPARα (5, 161, 162, 246).

Brown adipogenesis. BMP7 and PR domain containing 16 (PRDM16) play a key role in the determination of brown adipocytes (Fig. 1) (319). Stimulation of mesenchymal progenitor stem cells with BMP7 stimulates the commitment of brown adipocytes through the following mechanisms: 1) inhibition of adipogenic inhibitors, such as Pref-1, WNT10a, or nectin; 2) induction of adipogenic markers that are common to brown and white adipocytes, such as PPAR or C/EBP; 3) upregulation of brown fat-specific markers, such as PRDM16, PPAR coactivator 1α/β (PGC-1α/β), and UCP1; and 4) induction of mitochondrial biogenesis by increasing mitochondrial components such as mitochondrial transcription factor A (TFAM), cytochrome c, or nuclear respiratory factor 1 (NRF1). PRDM16 is a zinc finger transcription factor that controls the bidirectional...
switch of Myf5+ cells into brown adipocytes rather than myoblasts (14, 15, 86, 286). PRDM16 stimulates brown adipogenesis by binding to PPARγ and PGC-1α, activating their transcriptional function (286). On the one hand, PRDM16 represses the expression of white adipocyte and myogenic-selective genes via binding with corepressors COOH-terminal binding protein-1/2 (CBP1/2) and euchromatic histone-lysine N-methyltransferase-1 (EHMT1) (118, 229, 326). On the other hand, PRDM16 binds PPARγ and PGC-1α/β through its zinc fingers and stimulates the transcription of brown-selective genes. Recently, it has been shown that PRDM16 enhances nuclear receptor-dependent transcription of UCP1 through interactions with the Mediator subunit MED1 (14, 16, 140).

PGC-1 is a transcriptional coactivator extremely important for the induction of gene expression of specific markers of brown adipocytes (UCP1, UCP3) as well as for the promotion of mitochondrial biogenesis (15, 72). Three members of the PGC-1 family have been described: PGC-1α, PGC-1β, and PGC-related coactivator (72). The stimulation of β-adrenergic receptors by cold, exercise, or diet in brown adipose tissue activates PGC-1α (72). Consequently, PGC-1α coactivates the PPARγ-RXRα heterodimer, promoting the expression of the thermogenic genes UCP1 and UCP3, binding also to the homodimer of NRF-1 and NRF-2 that are involved in mitochondrial DNA replication (320). One of the best molecular markers for classical brown adipocytes in mice and humans is the Zic family member 1 (ZIC1), whose expression is very low in beige/brite adipocytes (144, 184, 330, 340). Analysis of the gene signature of human BAT in the supraclavicular region revealed an upregulation of other BAT-selective markers, including LIM homeobox 8 (LHX8), microRNA 206 (mir-206), and 133b (miR-133b) (144).

**Fat browning.** The mechanisms by which white adipocytes acquire brown-like properties are generally referred as “fat browning” (218). The capacity of adult humans to develop brown adipocytes has long been recognized, as brown adipocytes arise in adipose tissue of patients with pheochromocytoma due to the tumor-mediated release of catecholamines, which are known BAT-inducing agents (41, 77). These data suggest that fat browning also occurs in humans and might be harnessed for therapeutic purposes against diet-induced obesity and metabolic diseases (95).

To date, the cellular origin of beige adipocytes remains enigmatic (Fig. 1), since it has been proposed that beige fat cells originate from smooth-muscle-like cells residing in white adipose tissue and existing adipocytes (187, 286). After induction, beige adipocytes acquire a differential gene expression profile that can be used to distinguish them from classical brown adipocytes despite the common expression of genes involved in thermogenesis, such as UCP1, PGC-1α (PPARGC1A), deiodinase type II (DIO2), and β3-adrenergic receptor (ADRB3) (184, 340). The genetic markers of the beige cell identity include TNF receptor superfamily member 9 (CD137), transmembrane protein-26 (TMEM26), T box-associated transcription factor (TBX1), or short-stature homeobox-2 (SHOX2) (54, 144, 184, 293, 340).

Cold exposure and β-adrenergic stimulation are the main inducers of fat browning (41, 217, 218). However, several additional factors able to regulate fat browning have been discovered in recent years, such as irisin, fibroblast growth factor 21 (FGF21), follistatin, β-aminoisobutyric acid (BAIBA), meteorin-like, PPARγ agonists, BMP8B, myostatin, and leptin, among others (25, 28, 230, 252, 258, 260, 291, 336). Special attention has been paid in obesity research to the ability of irisin to promote fat browning. Bööst et al. identified fibronectin type III containing five (FNDC5), a myokine induced in response to exercise and/or PGC-1α activation (25). The cleavage of FNDC5 releases a soluble protein, termed irisin, which is released into the bloodstream and promotes fat browning. Myostatin and leptin have been shown to negatively regulate irisin-induced fat browning (260, 291), confirming the presence of negative feedback mechanisms to prevent excessive energy dissipation by fat browning. Considerable controversy exists on the role of irisin in human metabolism, with several studies showing exercise and high-intensity training protocols being particularly effective in raising circulating irisin in humans (25, 139, 143, 225), whereas others were not able to find any association (122, 132, 173, 316). Moreover, recent reports even argued against the existence of circulating irisin, since the human FNDC5 gene starts with a noncanonical ATA codon that might represent a null mutation preventing irisin transcription (3, 66). Most of the studies used for circulating irisin detection relied on commercial antibodies and ELISA assays. In this regard, the detection and quantitation of circulating irisin by quantitative mass spectrometry with control peptides marked with heavy stable isotopes as standards has so far settled the existence of human irisin in plasma and its regulation by exercise (143).

**Biological and Morphological Changes of White Adipose Tissue in Obesity**

The World Health Organization (WHO) recognized obesity as a disease in 1948 (87, 141). Obesity is defined as an excess of adiposity, with this excess of body fat being associated with the onset of other pathologies, such as type 2 diabetes, cardiovascular diseases, sleep apnea, arthritis, and some types of cancer, among others (150, 165). The prevalence of obesity has reached pandemic proportions, with more than two billion adults being overweight (BMI ≥25 kg/m²) and with 671 million individuals being obese (BMI ≥30 kg/m²) worldwide in 2013 (88, 219). BMI is the most widely used method to diagnose obesity, but it is only a surrogate for body fatness and does not provide an accurate measurement of body composition (23, 247). In this regard, the development of tools to more precisely measure body fat, including DEXA, air displacement plethysmography, or bioimpedance, has enabled the classification of individuals according to the degree of their true adiposity (23). The cut-off points, according to the percentage of body fat (%BF), are 20.1 and 24.9% for men and 30.1 and 34.9% for women for obesity (91). Noteworthy, individuals classified as normal-weight by BMI with excess adiposity exhibit higher waist circumference, worse lipid profile, higher glycemia, and cardiovascular risk factors than subjects with normal BMI and %BF (102, 103). In addition to the amount of body fatness, fat distribution is also important for the development of obesity-associated pathologies (262). Visceral obesity, as determined by an increased waist circumference or waist-to-hip ratio or an elevated intra-abdominal fat area by image analysis at the lumbosacral level, is associated with an increase in metabolic...
alterations, elevated cardiovascular risk, and premature death (172, 351).

In the present section, the biological and morphological changes that take place during the pathological expansion of white adipose tissue in obesity, ultimately leading to obesity-associated pathologies, are reviewed.

Adipose tissue expansion: hyperplasia and hypertrophy. Adipose tissue growth is a tightly regulated biological process, since both the excess (overweight and obesity) and the partial or total absence (lipodystrophies) of adipose tissue are associated with metabolic disturbances. Adipose tissue mass is determined by two mechanisms: hypertrophy (increase in cell size) and hyperplasia (increase in cell number) (Fig. 2) (8, 10).

Fat cell progenitors are set during the prenatal period by the mechanisms described above in the first section. Adipocyte number increases after birth and during adolescence, which represent critical periods for subsequent obesity development but varies little during adulthood, where the adipose tissue grows mainly by adipocyte hypertrophy (9, 300). It can be questioned whether obesity is the result of hypertrophy or hyperplasia. Hypertrophy is the main contributor to adipose tissue enlargement in order to meet the need for fat accumulation in the progression of obesity, whereas hyperplasia contributes less to this increase because it occurs in small cells with low fat storage capacity (123, 146). Nonetheless, hyperplasia can be correlated with beneficial metabolic properties, whereas hypertrophy is associated with the development of metabolic derangements and elevated cardiovascular risk in obesity (8, 9, 123, 131).

Adipocyte hyperplasia. Preadipocytes differentiate into adipocytes in response to insulin-like growth factor I (IGF-I), lipids, glucocorticoids, ghrelin, and other signals (267, 274). These factors stimulate a cascade involving C/EBP, PPARγ, SREBP1, and other transcription factors orchestrating the expression of adipocyte-specific genes during adipogenesis. Human preadipocyte capacity for adipogenesis shows sex and fat depot differences (191, 313). Women present a higher proportion of early differentiated adipocytes than men, with abdominal subcutaneous preadipocytes having a greater capacity for adipogenesis than omental cells. Although the adipocyte number tends to be stable during adult life, visceral and subcutaneous fat depots can enlarge via hyperplasia and/or hypertrophy during weight gain (313, 314). Obesity and obesity-associated type 2 diabetes are usually associated with adipose tissue hypertrophy and hypoplasia due to a lower potential of adipogenesis of preadipocytes (216, 244, 325). However, some individuals can respond to overfeeding with hyperplasia, resulting in a healthier obese phenotype (123, 146). In addition,
hyperplasia is also markedly correlated with obesity severity, becoming more evident in morbidly obese individuals (10). Hyperplasia in both visceral and subcutaneous adipose regions appears to be protective against lipid as well as glucose/insulin abnormalities in obesity (131).

**Adipocyte hypertrophy.** Adipocyte volume increases by excess accumulation of TG as a result of the esterification of three free fatty acids (FFA) with one glycerol 3-phosphate in circumstances of positive energy balance, such as overfeeding or sedentary lifestyle (85). FFA transmembrane transport is produced in cholesterol- and sphingolipid-rich lipid rafts of the plasma membrane of adipocytes, which are enriched in caveolae containing FABP4, fatty acid translocase (FAT, CD36) or fatty acid transporter protein-1 (FATP1). Caveolin-1, the most abundant protein coating caveolae, is necessary to target CD36 to the plasma membrane and, hence, regulate the surface availability of CD36 (84). Moreover, LPL, located on the surface of adipocytes, removes FFA from both chylomicrons and very low-density lipoproteins (VLDL) (263). On the other hand, glycerol 3-phosphate, the second metabolite required for TAG biosynthesis, derives from three different metabolic sources: 1) glucose, since glycerol 3-phosphate is an intermediate metabolite of glycolysis; 2) lipolysis-derived glycerol, which is phosphorylated by the glycerol kinase (GK) enzyme; and 3) aquaglyceroporin (AQP)-mediated glycerol uptake (263). AQP7 is considered the main glycerol channel facilitating glycerol release/uptake in adipocytes (78, 117, 129, 199), but other glycerol channels such as AQP3, AQP5, AQP9, AQP10, and AQP11 also contribute to glycerol transport in fat cells (175, 192, 193, 264). Insulin constitutes an important regulator of adipocyte hypertrophy, but not of hyperplasia, since low levels of insulin receptor are found in preadipocytes (112). Several mechanisms contribute to insulin-induced TG accumulation, including the increase in glycerol uptake through the translocation of glucose transporter 4 (GLUT4) from the cytoplasm to the plasma membrane (116), the activation of LPL (75) and the stimulation of glycerol uptake through the upregulation of AQP3, AQP7, and AQP9 (264) in human fat cells (Fig. 2).

Increased adipocyte volume in obese patients is associated with an impaired mitochondrial function and changes in membrane proteins as well as higher cell death and inflammation, which contribute to the development of obesity-associated metabolic disturbances (123). These metabolic alterations associated with adipocyte hypertrophy show fat depot-specific differences, with enlarged visceral adipocytes being linked to dyslipidemia while large subcutaneous fat cells being associated with impaired glucose metabolism and insulin resistance (131).

**Increased adipocyte cell death: apoptosis and autophagy.** Human adipose tissue is very dynamic, with 10% of adipocytes being renewed each year after cell death (300). However, the generation of hypoxic areas together with the proinflammatory milieu of the adipose tissue in obesity is associated with an increase in adipocyte cell death that is further aggravated by insulin resistance (48, 268). Two forms of programmed cell death, apoptosis and autophagy, are increased in the adipose tissue of obese subjects (48, 106, 167, 268). Apoptosis, or type I cell death, is characterized by morphological and biochemical hallmarks, including cell shrinkage, chromatin condensation (pyknosis), membrane blebbing, nuclear DNA fragmentation (karyorrhexis), and apoptotic body formation (48, 89, 94). The caspases constitute a family of aspartate-specific cysteine proteases that play a key role in apoptosis as effector molecules in the process of cell death. Autophagy, or type II cell death, is characterized by sequestering cytosolic organelles and proteins in double-membrane vesicles, termed autophagosomes, that translocate to lysosomes for fusion and content degradation (296). The proteins encoded by autophagy-related genes (ATG) are required for the formation of autophagosomes.

**Adipocyte apoptosis.** Apoptotic adipocytes can be identified in histological sections of adipose tissue surrounded by macrophages forming the characteristic “crown-like structures” and lack perilipin, a protein coating lipid droplets (48). The apoptotic process can be activated via two main sites: 1) at the plasma membrane upon ligation of death receptors (extrinsic pathway) and 2) at the mitochondria, inducing the release of cytochrome c and other mitochondrial proteins (intrinsic pathway) (Fig. 3). The extrinsic pathway of apoptosis is initiated by the stimulation of death receptors of the TNF receptor superfamily, such as TNF receptor 1 (TNFR1), CD95 (APO-1/Fas), or death receptor 3–6 (DR3-6), during the pathological expansion of adipose tissue in obesity (128, 156, 213, 223). The presence of their ligands, namely TNFα, Fas ligand (FAS-L or CD95-L), or TNF-related apoptosis-induced ligand (TRAIL), in adipose tissue is due to their synthesis by adipocytes and adipose tissue-embedded macrophages. TNFα is overexpressed in adipose tissue of obese individuals and constitutes a well-known regulator of apoptosis in white and brown adipocytes (134, 221, 223). Upon binding to TNFR1, TNFα triggers caspase-8 cleavage and activation, which further activates caspase-3, leading to adipocyte cell death (268). Other death receptor ligands, such as FAS-L or TRAIL, are also associated with adiposity and are related to adipocyte apoptosis (4, 90, 156).

The intrinsic or mitochondrial pathway is triggered by the release of several mitochondrial proteins, such as cytochrome c, Smac (second mitochondria-derived activator of caspase)/Diablo [direct inhibitor of apoptosis protein (IAP)-binding protein with low PI], Omi/HtrA2, or endonuclease G, from the mitochondrial intermembrane space to the cytoplasm (89, 331). The released cytochrome c binds and oligomerizes cytosolic protein Apaf-1 to facilitate the formation of the apoptosome (89). The apoptosome recruits and activates procaspase-9 via Apaf-1, and in turn, active caspase-9 cleaves and activates the effectors caspase-3 and caspase-7. On the other hand, Smac/Diablo and Omi/HtrA2 promote caspase activation through the neutralization of inhibitor of apoptosis proteins (IAPs) (89, 331). Serum deprivation, medium-chain fatty acids octanoate and decanoate, resveratrol, or thiazolidinediones are initiators of apoptosis through the mitochondrial pathway in adipocytes (194, 221, 341, 344).

Obesity is associated with the activation of both the extrinsic and intrinsic pathways of adipocyte apoptosis (4, 48, 268). Interestingly, transgenic mice lacking the Bid gene, a key proapoptotic molecule that serves as a link between these two apoptotic pathways, exhibit reduced caspase activation, adipocyte apoptosis, and adipose tissue macrophage recruitment and are protected against the development of systemic insulin resistance and hepatic steatosis (4). These data strongly suggest that adipocyte apoptosis constitutes a key initial event that
contributes to obesity-associated adipose tissue inflammation, insulin resistance, and fatty-liver.

**Autophagy in adipocytes.** Under physiological conditions, when nutrients are abundant, autophagy is maintained in a basal state mainly for housekeeping purposes such as degradation of bulk cytoplasmic contents, abnormal protein aggregates, and damaged organelles (297). Autophagy is induced in response to nutrient starvation or stress. Depletion of nutrients activates an important energy sensor, AMP-activated protein kinase (AMPK), which, in turn, inhibits mammalian target of rapamycin (mTOR; the best characterized negative regulator of autophagy) and activates the phosphorylation of unc51-like kinase-1 (ULK1) (232, 297). Active ULK1 initiates the formation of the phagophore by the phosphorylation of beclin-1, a protein that recruits regulatory proteins to the VPS34 complex (class III PI3K), which is essential for the activity of the phagophore (275). During the elongation of the phagophore, ATG7 induces the conjugation of ATG12 to ATG5 as well as the conjugation of cytosolic light chain 3 (LC3)-I to phosphatidylethanolamine to generate LC3-II, one of the best characterized components of the autophagosomes (269). The hydrolysis of engulfed cargo in the autolysosomes generates amino acids and FFA that are released into the cytoplasm for essential biosynthetic functions (297).

Autophagy actively participates in the regulation of adipocyte differentiation, fat storage, and inflammation (142, 297, 298). Autophagy is involved in adipogenic differentiation by repressing proteasome-dependent and PPARγ degradation (353). In line with these observations, Atg5 and Atg7 knockout mice show a reduction in adipose mass, suggesting that autophagy is essential for normal adipogenesis (298, 357). Analogously, Atg5 and Atg7 knockdown 3T3-L1 adipocytes decrease the intracellular content of TG and reduce the expression of the key adipogenic transcription factors C/EBPα, C/EBPβ, and PPARγ (298). Autophagy inhibition with 3-methyladenine reduces the expression of inflammatory-related factors, such as MCP-1, IL-1β, IL-6, and IL-8, caused by endoplasmic reticulum (ER) stress, a fact that reveals the important role of autophagy as regulator of adipocyte inflammation (142, 349).

Obesity is associated with increased accumulation of autophagosomes, an attenuated mTOR signaling, as well as

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**Fig. 3. Adipocyte apoptosis.** Activation of caspases, the effectors of apoptosis, in adipocytes can be stimulated by 1) the extrinsic pathway, which is triggered by stimulation of death receptors of the TNF receptor superfamily, such as TNF receptor 1 (TNFR1), CD95 (APO-1/Fas), or death receptor 3–6 (DR3-6), and 2) the intrinsic pathway, which involves mitochondrial release of apoptogenic factors, including cytochrome c and Smac/Diablo proteins. Obesity is associated with increased activation of death receptors and mitochondrial pathways, leading to the stimulation of effector caspases and adipocyte apoptosis. FADD, Fas-associated death domain; FAS-L, Fas ligand; IAPs, inhibitor of apoptosis proteins; Smac, second mitochondria-derived activator of caspase; TNFα, tumor necrosis factor-α; TRAIL, TNF-related apoptosis-induced ligand; TRAIL-R2, TRAIL receptor 2.
enhanced expression of key autophagy-related proteins beclin-1, ATG5, ATG7, ATG12, and LC3B (166, 167, 201, 268). Markers of autophagy are correlated with whole body adiposity, visceral fat distribution, and adipocyte hypertrophy (167, 268). However, the altered expression of autophagy in human obesity appears to be related to the degree of insulin resistance rather than to excess adiposity (268). Several plausible mechanisms are involved in the altered autophagy in obesity: 1) insulin represents a major inhibitor of autophagy, with insulin resistance being a potential activator of this process, since patients with type 2 diabetes show elevated formation of autophagosomes in the adipose tissue (166, 232); 2) the orexigenic hormone ghrelin also acts as a negative regulator of autophagy in human adipocytes through the downregulation of ATG5 and ATG7, but its circulating levels are decreased in obesity (268, 318); and 3) the inflammatory response induced by TNFα and JNK-dependent ER stress inducers of adipocyte autophagy, with both processes being upregulated in obesity (268, 347). Taken together, the increased autophagy found in the adipose tissue of obese patients might constitute an attempt to limit the excessive adipocyte hypertrophy and inflammation in order to prevent adipose tissue dysfunction.

Adipose tissue inflammation: cross talk between immune cells and adipocytes. The finding that TNFα is overexpressed in adipose tissue of obese individuals revolutionized the field of obesity research in the late 1990s (134). Six years later, increased infiltration of macrophages in the adipose tissue of obese mice and humans that is directly proportional to measures of adiposity was reported (333). These initial observations provided a new context for understanding obesity-related inflammation. Nowadays, it is well known that adipose tissue macrophages can be resident or recruited and that their phenotype and function predict its metabolic role. In this sense, macrophages are classified according to their cell surface markers and secretory profile as M1, or “classical”, and M2, or “alternative” (189, 190). M1 macrophages play a key role against bacterial and viral infections, being activated by interferon-γ (IFNγ) and lipopolysaccharide (LPS), and secrete proinflammatory cytokines, including TNFα, IL-1β, and IL-6 as well as monocyte-chemoattractant protein-1 (MCP-1), and large amounts of nitric oxide (NO) through the stimulation of inducible NO synthase (iNOS). By contrast, M2 macrophages are associated with anti-inflammatory responses, helminth infection, tissue remodeling, fibrosis, and tumor progression, are induced by exposure to IL-14 and IL-13, and produce immunosuppressor factors such as IL-10 or IL-1 receptor agonist (IL-1RA). Additionally, arginase-1, an enzyme that blocks iNOS activity by competing for the arginine substrate, is increased in M2 macrophages. Obesity is associated with a phenotypic switch from an M2-anti-inflammatory state toward an M1-proinflammatory state (189). An alternative hypothesis for adipose tissue macrophage activation based on metabolic dysfunction has been recently proposed by several authors (30, 170). The exposure of macrophages to a mixture of glucose, insulin, and palmitate (i.e., “metabolic activation”) drives the activation of macrophages through distinct pathways than classically activated M1 macrophages (Fig. 4). “Metabolically” activated macrophages secrete proinflammatory cytokines and specifically express cell surface proteins ABCA1, CD36, and PLIN2 but fail to express cell surface markers of M1 (CD38, CD319, or CD274) or M2 (CD163, CD206, TFRC, or TGFβ1) macrophages (170). An increase in metabolically activated macrophages has been found in the adipose tissue of obese humans and are positively correlated with adiposity. Together, the metabolic dysfunction drives to a proinflammatory phenotype that is mechanistically different from classical M1 activation.

Growing evidence has revealed that obesity also induces an immune response involving other immune cells of the adaptive (B and T lymphocytes) and innate (dendritic cells, mast cells or eosinophils) system (20, 63, 186, 292, 338, 339) that further aggravates systemic inflammation and contributes to the onset of obesity-associated comorbidities (Fig. 4). However, the metabolic regulation of inflammatory responses in the adipose tissue is related not only to obesity but also to fasting, cold challenge, weight loss, and acute lipolysis (70). These complex interactions have given rise to the study of “immunometabolism”, a field of research that focuses on the integration of metabolic and immune pathways that is crucial for systemic homeostasis.

Resident immune cells in adipose tissue: “healthy” immunometabolism. In contrast to the belief that adipose tissue inflammation exerts a fundamentally negative impact on metabolism, Wernstedt Austerholm and colleagues (335) postulated the concept of “healthy inflammation” that scavenges the debris of apoptotic adipocytes and enables adipogenesis as well as appropriate extracellular matrix remodeling during the adipose tissue expansion. The healthy expansion of the adipose tissue in circumstances of positive energy balance, such as overnutrition, requires an acute local inflammation in order to prevent lipotoxicity and ectopic lipid accumulation (104, 335). In this regard, a recent report showing the analysis of three animal models with constitutive or inducible expression of anti-inflammatory factors revealed their inability to expand the adipose tissue, leading to ectopic lipid deposition and deteriorated metabolic profile (335).

Macrophages constitute an important fraction of non-adipocyte cells within the adipose tissue, accounting for almost 10% of the total cell number in the lean “healthy” adipose tissue (168). The main population of resident macrophages in the lean healthy state corresponds to the M2 phenotype, and their main function includes tissue surveillance and extracellular matrix remodeling as well as maintaining insulin sensitivity in the adipose tissue through PPARγ-dependent mechanisms (227).

T lymphocytes and eosinophils are involved in the activation state of adipose tissue M2 macrophages (Fig. 4). In lean mice, ~10% of the stroma-vascular fraction is composed of CD3+ T lymphocytes with 1:3 being CD8+ or CD4+ T cells. The population of CD4+ T cells can be further subclassified into proinflammatory T helper types 1 and 17 (Th1 and Th17) and anti-inflammatory Th2 and T-regulatory cells (Treg) that express forkhead box protein-3 (FOXP3) (71, 209, 304). The population of Th2 and Treg is usually constant and regulates the expansion of proinflammatory Th1 cells. On the one hand, lean, but not obese, adipose tissue is enriched in Th2 and Treg, which secrete the anti-inflammatory IL-10 and also induce IL-10 synthesis in M2 macrophages (71). Moreover, M2 macrophages might promote insulin sensitivity through IL-10 by antagonizing TNFα-induced insulin resistance. On the other hand, eosinophils migrate into adipose tissue through α4- and αL-integrin-dependent mechanisms and sustain alternatively
activated M2 macrophages through an IL-4/IL-13-dependent mechanism (338). The pseudokinase TRIB1 is also essential for the differentiation of tissue-resident M2 macrophages (281). Mice lacking Trib1 exhibit a significant reduction of adipose tissue M2 macrophages and eosinophils, suggesting that the defect in M2 polarization could be, in part, secondary to diminished levels of eosinophil-derived IL-4 under homeostatic conditions. Eosinophils also play an important role in metabolic homeostasis, since eosinophil-deficient mice develop more severe obesity and insulin resistance than eosinophil-deficient mice, whereas helminth-induced adipose tissue eosinophilia enhances glucose tolerance (338).

Recruitment of proinflammatory immune cells in obesity perpetuates chronic adipose tissue and systemic inflammation. Obesity is associated with an increased infiltration of macrophages, neutrophils, foam cells, T and B cells, mastocytes, and dendritic cells (63, 186, 292, 333, 338). Adipose tissue hypertrophy and/or hyperplasia is associated with an increased cell death, which enhances the recruitment of macrophages that form the characteristic crown-like structures around fat cells (48, 305). Weight gain induces chemokine production, such as MCP-1, IL-8, and chemokine (C-C motif) ligands 2 and 5 (CCL2 and -5), to promote recruitment of circulating proinflammatory monocytes, which differentiate into an M1 macrophage phenotype (52, 59, 154, 189, 190). Moreover, it has been recently reported that in situ proliferation of local macrophages also contributes to increased adipose tissue inflammation (6).

The transcription factor interferon regulatory factor 5 (IRF5) contributes to the polarization of local or recruited macrophages toward an inflammatory M1 phenotype (Fig. 4) (55, 171). The increased cytokine production from M1 macrophages (TNFα, IL-6, and IL-1β), together with the reduced anti-inflammatory signals from the M2 macrophages (IL-10 and IL-1Ra), promotes adipose tissue dysfunction and impairs insulin sensitivity (239). It has been recently demonstrated that netrin-1, a factor secreted by macrophages that promotes adipose tissue inflammation. In the healthy state, adipose tissue inflammation is suppressed by IL-4, IL-10, and IL-13, secreted by eosinophils, and Th2 and Treg cells that contribute to the activation of M2 macrophages. The pathological expansion of adipose tissue in obesity is associated with increased infiltration of macrophages, neutrophils, foam cells, T and B cells, mastocytes, and dendritic cells. Obesity is characterized by polarization of macrophages toward a proinflammatory M1 phenotype as well as with the shift from anti-inflammatory Th2 and Treg CD4+ cells toward the proinflammatory Th1 and Th17 CD4+ cells, particularly in the visceral depot. Another hallmark of adipose tissue inflammation in obesity is increased adipocyte cell death; apoptotic adipocytes are usually surrounded by macrophages forming the characteristic “crown-like structures”. In addition, adipocytes further contribute to the inflammatory milieu by the secretion of proinflammatory cytokines, chemokines, and damage-associated molecular pattern (DAMP) molecules. Recently, a phenotype of proinflammatory macrophages different from classically activated M1 macrophages has been identified, which are “metabolically” activated by glucose, insulin, and palmitate and with the ability to secrete proinflammatory cytokines.
pose tissue macrophage retention, is increased in obesity, thereby perpetuating adipose tissue inflammation and insulin resistance (250). Regarding the metabolic activation of macrophages, it can be induced at least by two independent mechanisms: 1) binding of palmitate, a saturated FFA, to cell surface Toll-like receptors 2 and 4 (TLR2 and -4), which drives to cytokine production through NF-κB-dependent mechanisms; and 2) activation of p62/SQSTM1 and PPARY induced by palmitate internalization, which triggers lipid metabolism and limits inflammation (170). The balance of these two pathways determines the pro- or anti-inflammatory response of macrophages to metabolic dysfunction, leading to a complex spectrum of macrophage types ranging from “M1-like” to “M2-like” phenotypes.

Adipose tissue-infiltrated CD4+ and CD8+ T cells also produce proinflammatory cytokines, leading to chronic inflammation and inhibiting insulin signaling (196, 222). The shift from anti-inflammatory Th2 and Treg CD4+ cells toward the proinflammatory Th1 and Th17 CD4+ cells, particularly in the visceral depot, contributes to adipose tissue inflammation associated with insulin resistance (64, 163) (Fig. 4). Th1 and Th17 secrete IFNγ and IL-17, respectively, stimulating the release of proinflammatory TNFα and IL-6 from M1 macrophages (287). Adipocytes themselves exhibit immune cell-like functions that lead to CD4+ T cell activation, triggering inflammation independently of macrophage infiltration (197). CD8+ T cells are involved in the initiation and propagation of adipose tissue inflammation, since they increase and promote the recruitment and activation of adipose tissue macrophages (222). The clonal expansion and differentiation into effector CD8+ T cells seems to be antigen driven (7, 222). After pathogen clearance, the majority of the effector CD8+ T cells die and a small population of CD8+ T cells survives as memory T cells (51, 283). Upon antigen resensitization, resident memory CD8+ T cells secrete cytokines that trigger rapid adaptive and innate immune responses. The cell survival and self-renewal of memory CD8+ T cells is dependent on cytokines such as IL-7 and IL-15 (51). IL-7 induces the expression of the glycerol channel AQP9 and the subsequent glycerol transport in memory CD8+ T cells, which is necessary for TG synthesis and the maintenance of cellular bioenergetics in order to ensure the longevity of these immune cells.

Adipose tissue fibrosis. Extracellular matrix remodeling requires a balance between the synthesis of the components of the adipose tissue, such as collagens, and their degradation by the fibrinolytic system and metalloproteases (60, 306). Adipocytes are embedded in a unique extracellular matrix (all types of collagens, laminins, fibronectin, and proteoglycans) that provides mechanical support in addition to participating in several signaling events (207, 241). The maintenance of high flexibility of the extracellular matrix allows a healthy adipose tissue expansion (107). By contrast, fibrosis results from an exacerbated synthesis of fibrillar components, such as collagens I, III, and VI, ultimately leading to adipose tissue dysfunction and metabolic alterations (306). Genetic deletion of collagens in transgenic mice is related to profound changes in adipocyte size and metabolic derangements. In this regard, obese ob/ob mice lacking collagen VI develop adipose tissue hypertrophy in association with metabolic improvements (157). In addition, removal of the extracellular matrix by proteases such as matrix metalloproteases is necessary to allow adipocyte hypertrophy (38, 43). Accordingly, genetic ablation of MT1-MMP, a metalloprotease involved in collagen I degradation, leads to fibrosis around small adipocytes together with the development of severe metabolic disorders (45).

**Metabolic consequences of adipose tissue fibrosis.** Obesity is associated with an abnormal extracellular matrix remodeling that may decrease its flexibility, leading to adipocyte dysfunction (125, 157, 212). One of the mechanisms that trigger the accumulation of extracellular matrix components is adipose tissue hypoxia, which ultimately leads to fibrosis (115). Hypoxia in adipose tissue results in the stabilization of hypoxia-inducible factor 1α (HIF-1α), which in turn induces the expression of fibrotic proteins such as lysyl oxidase (LOX), elastin, collagen I and III, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and connective tissue growth factor (CTGF) (115, 317). In this sense, obese subjects exhibit reduced elastin and increased collagen I, III, V, and VI, and CTGF in adipose tissue, with visceral fat showing more fibrotic strokes than subcutaneous adipose tissue (61, 301, 302). Preadipocytes constitute the major source of collagen I, fibronectin, and tenasin C, which are involved in the formation of the collagen network (61, 125, 153). Contact with factors released by activated macrophages leads to profound modifications of the human preadipocyte phenotype, producing fibrotic components with increased migration and proliferation (153). Adipocytes surrounded by fibrotic depots become less metabolically active because of the increased mechanic tension (241).

The ability of adipose tissue to expand during surplus energy is limited, and when it is exceeded, fat is stored in other metabolically more harmful lipid depots, such as the liver, pancreas, myocardium, skeletal muscle, and blood vessels (107, 328). Abnormal fibrosis in the adipose tissue limits its expandability, resulting in ectopic lipid accumulation in peripheral tissues, such as liver, skeletal muscle, or pancreas. In the postprandial state, a reduction in the antilipolytic action of insulin, leading to elevated serum FFA and glycerol, takes place (85). In humans, venous drainage of visceral adipose tissue is via the portal vein, which provides FFA and glycerol as substrates for hepatic lipid and glucose metabolism (266, 290, 355), while the increased lipolysis in the retroperitoneal and epididymal fat depots constitutes the main source for these metabolites for hepatic metabolism in rodents (92, 311). The elevated concentrations of FFA reduce the degradation of apolipoprotein B and insulin, which may contribute to the development of the dyslipidemia, hyperinsulinemia, and insulin resistance observed in visceral obesity (178, 262, 328). Interestingly, both adipose tissue stiffness and elevated serum FFA are associated with markers of hepatocellular damage as well as with liver steatosis and fibrosis (1). On the other hand, obese patients show a decrease in glycerol permeability as well as lower expression of hepatic AQP9, the glycerol channel that mediates glycerol influx into hepatocytes, which suggests an attempt to prevent a further accumulation of TG within the liver parenchyma (266).**

**Altered secretion of adipokines.** Adipin (also known as complement factor D) was originally identified in 1987 as a highly differentiation-dependent gene in 3T3-L1 adipocytes (49). In 1993, increased expression of TNFα in the adipose tissue of different rodent models of obesity was described, providing evidence for a functional link between obesity and inflammation (133). One year later, leptin, the product of the
discuss their metabolic regulatory properties.

Novel proinflammatory and anti-inflammatory adipokines and function, that promote inflammatory responses and metabolic adiponectin, caused by excess adiposity and adipocyte dys-

Obesity is associated with an altered secretion of adipokines represented by acute-phase reactants (C-reactive protein, serum amyloid A, plasminogen activator inhibitor 1, haptoglobin), cytokines (TNF-α, IL-6, IL-10, IL-R1a, TGFβ), chemokines [MCP-1, macrophage inflammatory protein-2 (MIP-2), CCL2, CCL5, IL-8/CXCL8, IFNγ-inducible protein 10/ CXCL10], damage-associated molecular pattern molecules (DAMPs; tenasin C, calprotectin, heat shock protein 72, and HMGB1), and proinflammatory (leptin, resistin, osteopontin, chemerin, WNT5A, among others) and anti-inflammatory (adiponectin, SRFP5, omentin, ghrelin, and lipocalin-2) factors. Obesity is associated with an altered secretion of adipokines with a special upregulation in the secretion of proinflammatory adipokines and a downregulation of the anti-inflammatory adiponectin, caused by excess adiposity and adipocyte dys-

function, that promote inflammatory responses and metabolic dysfunction (235). Below, we briefly describe a selection of novel proinflammatory and anti-inflammatory adipokines and discuss their metabolic regulatory properties.

OSTEOPONTIN. Osteopontin (OPN), also known as early T lymphocyte activation (Eta-1), bone sialoprotein-1, and secreted phosphoprotein-1 (SPP1) among others, is a 40- to 80-kDa secreted matrix glycoprotein and proinflammatory cytokine that has been characterized as a major component of cell-mediated immunity (11). Alternative splicing of OPN mRNA generates two isoforms, a secreted and an intracellular form of OPN (294). In adipose tissue, OPN is mainly expressed by different cellular types of the stromal-vascular fraction such as lymphocytes, endothelial cells, macrophages, vascular smooth muscle cells, and mesenchymal stem cells (282), but also by adipocytes (99). Its ability to interact with integrin surface receptors through an Arg-Gly-Asp (RGD) sequence and with the CD44 receptor has established OPN as an important attachment and signaling molecule (58). OPN expression in adipose tissue-embedded macrophages is strongly upregulated in obesity (99, 160). The secretion of OPN from adipocytes and macrophages mediates monocyte adhesion, migration, differentiation, and phagocytosis (93, 211, 226) as well as the generation of the proinflammatory Th1 and Th17 cells (295). Plasma OPN and its expression in visceral adipose tissue are increased in obesity and obesity-associated type 2 diabetes promoting macrophage recruitment and adipose tissue inflammation in these pathologies (99, 224). In this regard, OPN expression can be induced by proinflammatory cytokines such as TNFα and TGFβ, hypoxia and hyperglycemia, all well-

known factors of obesity-related inflammation (282). Moreover, OPN deletion prevents the development of obesity and hepatic steatosis via impaired adipose tissue matrix remodeling and reduced inflammation and fibrosis in adipose tissue and liver in mice (159, 177). Interestingly, diet-induced weight loss is associated with a reduction in plasmatic and gene expression levels of OPN in visceral fat in obese animals and humans (99, 176, 177, 224), which may reflect an improvement in systemic and local inflammation.

CHEMERIN. Chemerin, also known as retinoic acid receptor responder 2 (RARRES2) and tazarotene-induced gene 2 (TIG2) is a 16-kDa secreted protein that plays an important role in adaptive and innate immunity as well as in adipogenesis, metabolic function, and glucose metabolism (337). Chemerin promotes the chemotaxis of multiple immune cells, including leukocytes, macrophages, immature myeloid cells, dendritic cells, microglial, and natural killer T cells (337). Moreover, chemerin stimulates macrophage adhesion to the extracellular matrix protein fibronectin as well as to the adhesion molecule VCAM-1 (119). Human adipocytes express chemerin and its receptor chemokine-like receptor 1 CMKLR1 (ChemR23 or DEZ) (27, 35). Chemerin acts locally on adipocytes, inducing adipogenesis and lipolysis, but it is also secreted to the bloodstream, promoting insulin resistance in skeletal muscle (105, 272, 288). Obesity and metabolic syn-

drome are associated with increased circulating levels of chemerin, which are significantly decreased after weight loss induced by bariatric surgery (27, 35, 69, 315). A higher gene expression of chemerin and its receptor CMKLR1 has been also found in the visceral adipose tissue of obese patients (27, 35). Chemerin expression is upregulated by TNFα and IL-1β in murine and human fat cells (35, 169). Taken together, the increased levels of chemerin in obesity and its positive association with inflammatory markers suggest a role for this chemoattractant protein in the establishment of obesity-associated low-grade chronic inflammation.

LIPOCALIN-2. Lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), neu-related lipocalin, oncogene 24p3, siderocalin, and urocalcin, is a secretory glycoprotein member of 25 kDa of the highly heterogeneous family of lipocalins that was originally isolated from secondary granules of human neutrophils (164). LCN2 is a component of the innate immune system that acts as an iron carrier protein and plays a key role in acute-phase response to infection (73) and in regulation of macrophage polarization (108). LCN2 has been also identified as an adipokine secreted from murine and human adipose tissue involved in the regulation of energy metabolism and glucose and lipid homeostasis, as well as in insulin resistance (38, 332, 354). LCN2 increases during adi-

ogenesis, and its expression is sex, age, and depot dependent as well as inducible by LPS, proinflammatory cytokines TNFα, IL-1β, and IL-6 and saturated fat (109, 205, 354, 356). Lcn2 knockout mice are cold-intolerant and develop increased body fat mass, show exacerbated adipocyte hypertrophy, dyslipidemia, fatty liver, and insulin resistance on high-fat diet feeding (109, 145, 358). Obesity and obesity-associated insulin resistance are related to an upregulation of LCN2 that is linked to iron status and inflammation (37, 38, 205, 343). Since LCN2 antagonizes the inflammatory effects of TNFα on adipocytes and macrophages in adipose tissue (354), its increased levels in obesity might constitute a compensatory mechanism to reduce adipose tissue inflammation through mechanisms involving iron homeostasis.

CHITINASE-3-LIKE 1, OR YKL-40. YKL-40, also known as carti-
lage glycoprotein-39 (gp39) or chitinase-3-like 1 (CHI3L1), is a 40-kDa heparin- and chitin-binding lectin protein that belongs to the family of glycosyl hydrolases (114). The main function of YKL-40 is the regulation of tissue inflammation
and extracellular matrix remodeling, with its concentrations being increased in various inflammatory conditions. YKL-40 constitutes a well-known autoantigen in rheumatoid arthritis by inducing T cell-mediated autoimmune response (242, 322). YKL-40 is also involved in the progression of atherosclerosis by promoting angiogenesis, chemotaxis, cell attachment, and migration of smooth muscle cells as well as the maturation of monocytes to macrophages (254, 256). The role of YKL-40 in cancer is supported by its ability to induce tumor cell proliferation and invasion (39, 148). Moreover, circulating concentrations of YKL-40 are reportedly increased in chronic liver disease and related to the amount of liver fibrosis (147).

Several cell types of adipose tissue, including adipocytes and endothelial, smooth muscle, and immune cells express and secrete YKL-40 (24, 220, 254). In line with the above-mentioned increase of YKL-40 in pathological conditions related to inflammation, upregulation of this protein has been reported in adipose tissue obtained from patients with obesity (34, 40, 124) and type 1 and type 2 diabetes (220, 253). Both circulating and visceral adipose tissue expression of YKL-40 are associated with variables of impaired glucose metabolism and inflammation in obese patients (34, 220), suggesting a relevant role in the onset of low-grade inflammation associated with insulin resistance.

Tenascin C. Tenascin C is an extracellular matrix protein that belongs to the tenasin family (149). The protein is assembled into a hexamer with each monomer comprising an assembly domain, epidermal growth factor-like (EGFL) repeats, fibronectin type III repeats (FN III), and a fibronectin globe. The expression pattern of TNC is dynamic, with low or null levels found in most healthy tissues and being specifically induced and tightly controlled during acute inflammation and persistently expressed in chronic inflammation (149, 200). In this regard, tenasin C has been identified as the endogenous activator of TLR4, whose activation induces the release of proinflammatory cytokines as well as the expression of pro-collagen type 1 and integrin-1 (121, 188, 200). Tenasin C orchestrates the inflammatory response induced by the activation of the TLR4 in response to bacterial LPS. Tenasin C enables the expression and release of critical proinflammatory cytokines upon LPS activation of TLR4 and suppresses the synthesis of anti-inflammatory cytokines via the induction of microRNA miR-155 (200). Interestingly, increased tenasin C and TLR4 levels have been reported in visceral adipose tissue in the context of obesity and obesity-associated type 2 diabetes and nonalcoholic fatty liver disease (36). The gene expression of tenasin C is upregulated in human adipocytes by the inflammatory factors TNFα and LPS, while tenasin C, in turn, upregulates MMP9 expression, which provides evidence for the link of this DAMP to inflammation and extracellular matrix remodeling in obesity (36).

Calprotectin. Calprotectin is a 24-kDa heterodimer consisting of light S100A8 (calgranulin A, MRP8) and heavy S100A9 (calgranulin B, MRP14) subunits, which are members of the low-molecular-weight members of a subfamily of S100 calcium-binding proteins called calgranulins (228). There are two major forms of calprotectin, the secreted soluble form and the plasmatic membrane integrated form (231, 352). The soluble form of calprotectin can be found in various body fluids and excreta, i.e., in plasma, urine, saliva, intestinal fluid, feces, cerebrospinal fluid, and synovial fluid. The heterocomplex S100A8/A9 is mainly expressed in cells of the myeloid lineage, including neutrophils and monocytes, and early differentiation states of macrophages (251). Calprotectin expression and secretion are stimulated by various inflammatory factors such as IFNγ or TNFα, (251, 342). S100A8 and S100A9 are released by phagocytes and amplify the inflammatory response induced by phagocyte activation during sepsis (329). In line with this function, calprotectin is considered a DAMP, with its circulating levels increasing more than 100-fold during inflammatory conditions, including rheumatoid arthritis, allograft rejections, inflammatory bowel disease, cancer, and cardiovascular diseases (62, 74, 180, 240). Calprotectin also constitutes an adipokine, with its circulating and visceral adipose tissue expression being increased in obesity as well as in type 1 and type 2 diabetes (26, 33, 208, 231). The positive association of adipose tissue expression of calprotectin with inflammatory markers suggests a role for this DAMP as a chemotactic factor for macrophages to visceral fat, increasing inflammation and the development of obesity-associated pathologies.

Brown and Beige Adipose Tissues: New Players in the Onset of Obesity

The presence of brown adipose tissue (BAT) has been traditionally recognized in human newborns, wherein the UCP1 content increases in the final trimester of gestation (136). In 2009, three independent studies based on fluorodeoxy-[18F]glucose-based positron emission tomography scans showed the presence of metabolically active BAT in adult humans (53, 324, 327). Although BAT represents a small fraction of the body cell mass (~0.1%), it has a remarkable energy-dissipating capacity through the promotion of TG clearance, glucose disposal, and generation of heat for thermogenesis (80, 181, 217). Therefore, the amount of BAT activity impacts whole body energy balance and may be key for the onset of obesity and its associated metabolic derangements. Cold exposure activates BAT to produce nonshivering thermogenesis (323, 324). In this regard, a recent report showed that human subcutaneous fat increases acutely the expression of UCP1 and beige-selective genes TMEM26 and TBX1 during winter compared with summer, with this response to the cold stimulus being inhibited by obesity and inflammation (155). BAT activity is higher in women than in men and progressively decreases with age (13). Interestingly, an inverse association between active BAT and fat mass, BMI, and fasting glycemia has been reported (53, 237, 277, 346), confirming the potential of BAT as a relevant target for pharmacological and gene expression manipulation to combat human obesity.

Some authors raised the question whether human BAT is composed of classical brown adipocytes or beige/brite fat cells (31, 184, 340). Post mortem magnetic resonance imaging and histological and biochemical analyses in eighth human infants provided evidence for two types of BAT in humans (184). The supraclavicular, interescapular, and perirenal regions show similar expression of BAT-characteristic genes, namely UCP1, PPARGC1A, PRDM16, DIO2 (deiodinase type II), and ADRB3 (β3-adrenergic receptor). However, the expression of the classical brown adipocyte marker ZIC1w was highest in the BAT from the interscapular region, whereas the supraclavicular one showed higher expression of the beige adipocyte marker TBX1. Thus, the presence of different populations of brown and beige

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adipocytes might be differentially affected by external stimuli, representing distinct potential targets for therapeutic interventions.

To date, the evidence supporting the metabolic significance of beige adipose tissue in the human supraclavicular area is largely associative (144, 182, 236). Positron emission tomography studies showing beige activity in the human neck used fluorodeoxy-[18F]glucose as a tracer, when FFA rather than glucose, constitutes the main substrate for UCP1 activation in active beige adipocytes (183). However, a recent study has revealed the capacity of FGF21 to differentiate primary human preadipocytes harvested from the neck into functional beige adipocytes exhibiting high oxygen consumptive, fatty acid oxidative, and thermogenic capacities (182). Further studies are needed to analyze the hormonal regulation of beige adipocyte differentiation and/or activation as well as its functional relevance in the etiopathology of human obesity.

Conclusions

Adipose tissue plays a key role in the regulation of energy metabolism through its endocrine effects (adipokines), its ability to synthesize/hydrolyze TG in response to energy demands, as well as its participation in thermal regulation through brown and beige adipocytes. The pathological expansion of adipose tissue during weight gain leads to an inflamed, fibrotic, and dysfunctional adipose tissue that favors ectopic fat accumulation and insulin resistance (Fig. 5). The discovery of brown and beige adipose tissue in human adults during recent years has provided a new potential therapeutic target against obesity due to their energy-dissipating thermogenic properties. Thus, the reduced mass and/or activity of brown and beige fat depots may also contribute to the pathogenesis of the detrimental complications of obesity. In summary, adipose tissue is an extraordinarily plastic and dynamic metabolic organ, with the identification of the morphological and molecular changes involved in its pathological expansion being of utmost relevance for the identification of pharmacological targets for the treatment of obesity and its associated metabolic diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


Fig. 5. Alterations of white, brown, and beige adipose tissue involved in the onset of obesity and its associated comorbidities.
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