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Mechanisms of obesity-induced metabolic and vascular dysfunctions

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1. ABSTRACT

Obesity has reached epidemic proportions and its prevalence is climbing. Obesity is characterized by hypertrophied adipocytes with a dysregulated adipokine secretion profile, increased recruitment of inflammatory cells, and impaired metabolic homeostasis that eventually results in the development of systemic insulin resistance, a phenotype of type 2 diabetes. Nitric oxide synthase (NOS) is an enzyme that converts L-arginine to nitric oxide (NO), which functions to maintain vascular and adipocyte homeostasis. Arginase is a ureohydrolase enzyme that competes with NOS for L-arginine. Arginase

activity/expression is upregulated in obesity, which results in diminished bioavailability of NO, impairing both adipocyte and vascular endothelial cell function. Given the emerging role of NO in the regulation of adipocyte physiology and metabolic capacity, this review explores the interplay between arginase and NO, and their effect on the development of metabolic disorders, cardiovascular diseases, and mitochondrial dysfunction in obesity. A comprehensive understanding of the mechanisms involved in the development of obesity-induced metabolic and vascular dysfunction is necessary for the identification of more effective and tailored therapeutic avenues for their prevention and treatment.

2. INTRODUCTION

Obesity, a condition characterized by the excessive accumulation and storage of fat in the body, is generally defined as a body mass index (BMI: weight-lbs/(height-inches)^2 x 703) of 30 or greater. Obesity is considered the core of metabolic disorders and an independent risk factor for all-cause mortality in the general population, particularly from cardiovascular disease (1). The vast majority of patients with type 2 diabetes (T2D) exhibit obesity and insulin resistance (2, 3). According to the World Health Organization, obesity is now considered a serious health problem worldwide, with its prevalence nearly tripling over the past 40 years due to overnutrition and reduced physical activity (4). A key function of adipose, or fat, tissue is energy homeostasis. Adipose tissue stores excess nutrients (ie: glucose and fatty acids) through the process of lipogenesis. In conditions of nutrient deficiency, it ensures a stable supply of energy to all organs and tissues through lipolysis (5).

Adipose tissue is the largest endocrine organ in the body, consisting mainly of adipocytes which are capable of secreting a variety of cell signaling cytokines, known as adipokines (6). These adipokines, particularly those in visceral adipose tissue (VAT), can regulate local and systemic inflammation as well as energy homeostasis (7). Healthy adipocytes are insulin sensitive, a trait essential for adipocyte glucose uptake and for the prevention of hepatic gluconeogenesis, which allows for the maintenance of normal blood glucose levels (8). Insulin resistance is an important feature of metabolically unhealthy obesity, a condition which differs from healthy obesity in terms of fat distribution. Metabolically healthy obese individuals exhibit increased subcutaneous adipose tissue (SAT) mass with less inflammation, less VAT and ectopic (liver and skeletal muscle) fat accumulation, and a normal adipokine secretion profile compared to metabolically unhealthy obese individuals (9). Studies from many groups have led to our current understanding that vascular pathology and dysfunction of obesity-related metabolic dysfunction develops through a chronic and progressive inflammatory process (10-12).

The pathogenesis of obesity is far more complex than just lipid accumulation and involves interactions among many cell types (Figure 1). With expansion of the VAT, hypertrophy of adipocytes, and inadequate vascularity (impaired angiogenesis), hypoxia occurs, causing the release of inflammatory cytokines and chemokines. These factors 'activate' endothelial cells by enhancing leukocyte and monocyte adhesion to the endothelium and inducing tissue infiltration by pro-inflammatory macrophages. This further elevates levels of inflammatory factors, triggering a vicious cycle of inflammation (13, 14). Nitric oxide (NO) has been recognized as a key regulator of body composition, energy metabolism, and vascular function. NO is produced from L-arginine by three NO synthase (NOS) isoforms: endothelial NOS (eNOS/NOS3), inducible (iNOS/NOS2), and neuronal NOS (nNOS, NOS1) (15, 16). NO produced by eNOS (nanomolar range) relaxes vascular smooth muscle cells and prevents their excessive proliferation, increases blood flow, and suppresses platelet aggregation (17-19). This eNOS-produced NO also prevents 'activation' of endothelial cells by suppressing the release of factors that trigger migration and adhesion of leukocytes

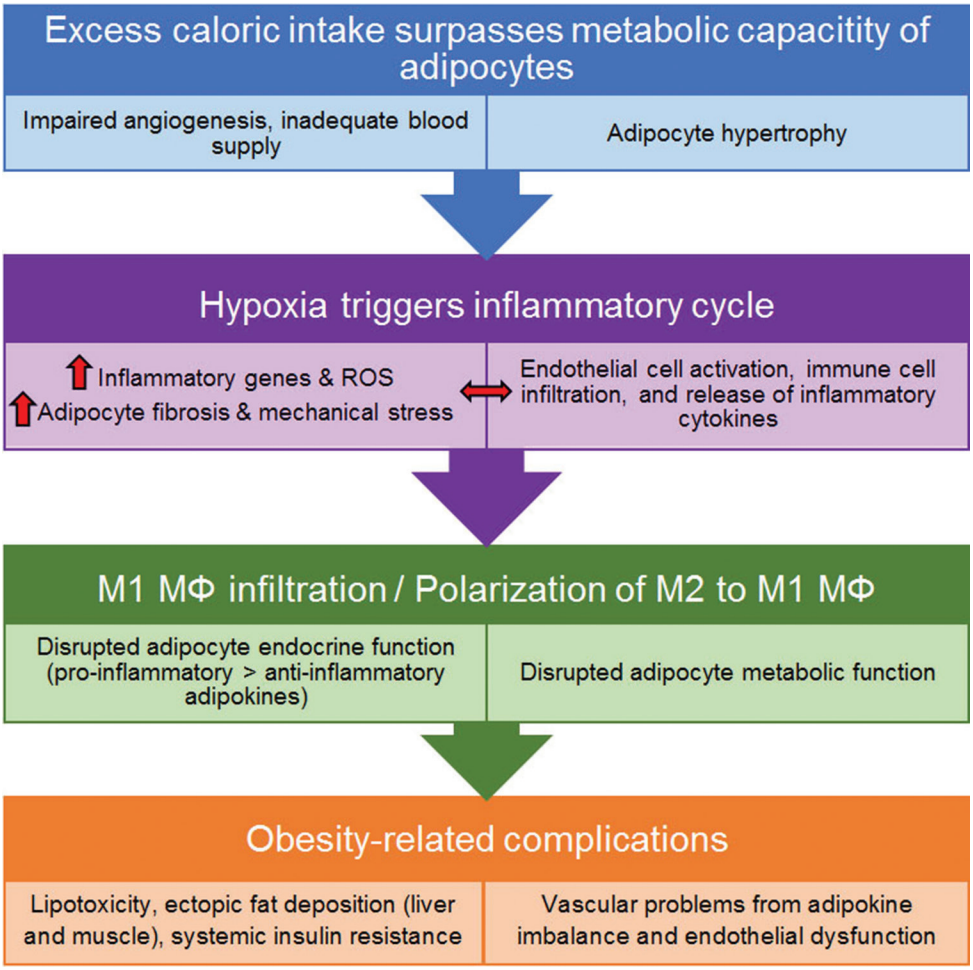

Figure 1. Schematic illustrating the development of metabolic and cardiovascular dysfunctions in obesity.

and monocytes to the endothelium, preventing infiltration of inflammatory macrophages. Endothelial NO concentration and production are suppressed in obesity (20, 21). Inducible NOS (iNOS), in contrast, produces much higher and toxic levels of NO (micromolar range) and is found in adipocytes and pro-inflammatory macrophages. NO production by iNOS is elevated in obesity (22).

With the exception of nNOS, the genes related to the NO system (eNOS, iNOS, subunits of the soluble guanylate cyclase (sGC), and both genes encoding cGMP-dependent protein kinases) are expressed in subcutaneous human adipose tissue and isolated adipocytes. Under physiological conditions, eNOS appears to be the predominant NOS isoform in human adipocytes (23). Expression of eNOS has been reported in human, rat, and mouse adipose tissue (24). eNOS synthesizes NO through the oxidation of the semi-essential amino acid, L-arginine (25, 26). NO signaling mechanisms involve either the activation of sGC, which increases the levels of the secondary messenger cGMP, or the posttranslational modification of the cysteine thiol group (S-nitrosylation) of various proteins to form nitrosothiols (SNO), directly affecting signal transduction (27, 28).

Reactive oxygen species (ROS) are often greatly elevated in obesity and hyperglycemia and can have serious pathological effects. ROS include hydrogen peroxide (H_2O_2), superoxide ($O2^-$), hydroxyl radical (OH), high levels of nitric oxide (NO), and peroxynitrite ($ONOO^-$). These ROS are products of numerous enzymatic reactions that occur within various subcellular compartments. Chronic hypernutrition induces the production of superoxide from NADPH oxidases, mitochondrial oxidative phosphorylation, and endothelial dysfunction/eNOS uncoupling (29-32). Chronic inflammation in adipose tissue can further perpetuate the vicious cycle of inflammation by promoting the infiltration of pro-inflammatory, ROS-producing macrophages (33, 34). Obesity also is associated with the depletion or decreased activity of antioxidant defense enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (33, 35). Acute changes in ROS concentration are important for cellular homeostasis and normal physiological processes where the ROS contribute to protective immune responses and act as intracellular signaling molecules that can induce insulin secretion and insulin sensitivity (36, 37). However, if not properly managed, ROS accumulation that exceeds the cellular antioxidant capacity may lead to maladaptive responses that result in metabolic dysfunction and inflammation (38, 39).

3. OBESITY-INDUCED ADIPOSE TISSUE DYSFUNCTION AND METABOLIC DYSREGULATION

3.1. Impaired adipogenesis

Adipose tissue expansion occurs through enlargement of existing adipocytes (hypertrophy) and/or through increased number of adipocytes (hyperplasia/adipogenesis). Adipogenesis occurs in two consecutive phases: first, mesenchymal stem cells commit to the formation of preadipocytes, which is then followed by terminal differentiation (40). The signaling mechanisms driving adipogenesis are not clearly understood. What is known is that the commitment step involves repression of zinc-finger protein 521 (ZNF521) and bone morphogenetic protein 4 (BMP4), which ultimately leads to the activation of ZNF423 and its downstream target PPAR gamma. The process of adipogenesis also involves the sequential activation of several C/EBP transcription factors, C/EBP beta, sigma, and alpha. Activated PPAR gamma and C/EBP alpha then drive the terminal differentiation of preadipocytes (41-43).

It has been suggested that a causal relationship exists between adipocyte size and the formation of new adipocytes. Individuals with large subcutaneous adipocytes have poor differentiation capacity, either due to elevated dedifferentiation signals or downregulation of differentiation factors, both of which are associated with a high risk of T2D (44-46). Hypertrophied adipocytes showed reduced potential to recruit mesenchymal stem cells and promote their terminal differentiation into new adipocytes, secondary to impaired PPAR gamma activation and adipocyte differentiation (46, 47). Mature, healthy adipocytes secrete BMP4 during adipogenesis, causing mesenchymal stem cell commitment to an adipogenic phenotype. In an attempt to avoid dysregulation, hypertrophied adipocytes secrete higher levels of BMP4 to recruit preadipocytes. However, this process becomes futile due to increased secretion of endogenous BMP4 antagonist, Gremlin1. Inhibition of Gremlin1 has been shown to enhance the process of adipogenesis, restoring some of the buffering functionality of these adipocytes (48).

3.2. Enhanced inflammatory response and disrupted adipokine profile

Adipokines are cytokines secreted from adipose tissue that play an important role in maintaining energy homeostasis (49). Additionally, their immunomodulatory activities contribute to the chronic low-grade inflammation associated with obesity (50). During obesity, there is increased secretion of several pro-inflammatory adipokines that occurs in tandem

with downregulation of anti-inflammatory adipokines (51). This adipokine imbalance is pivotal in the development of metabolic disorders and cardiovascular disease (52). Obesity disrupts adipose tissue homeostasis through deregulation of adipogenesis, reduced angiogenesis, and localized hypoxia, creating an environment of high cellular stress (53). Adipose tissue of obese subjects, primarily their VAT and to a lesser extent, their SAT, has been shown to sustain a state of chronic low-grade inflammation, which has been linked to the development of insulin resistance (54). The physiological response to this elevated adipocyte stress is the release of inflammatory cytokines and chemokines, chiefly, monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-alpha), which recruit more inflammatory cells, thus further perpetuating the cycle of cellular stress, inflammation, and impaired macrophage emigration (55, 56). On the molecular level, hypertrophied adipocytes secrete saturated fatty acids which activate the toll-like receptor (TLR)-4 on macrophages. This TLR-4 activation results in increased activity of the transcription factor, nuclear factor kappa -light-chain-enhancer of activated B cells (NF-kappaB), which upregulates expression of TNF-alpha (57, 58). TNF-alpha increases adipocyte lipolysis, producing more free fatty acids, and also promotes adipocyte expression of intracellular adhesion molecule-1 (ICAM-1) and MCP-1. These proteins recruit circulating inflammatory monocytes and promote their differentiation into macrophages, exacerbating inflammation. Hypertrophied adipocytes also express lower levels of adiponectin, an anti-inflammatory adipokine that inhibits TLR-activated NF-kappaB, allowing the expression of TNF-alpha to proceed relatively unhindered (59-61).

Adipose tissue macrophages represent about 40% of all adipose tissue cells during metabolic stress (62, 63). In healthy adipose tissue, the resident macrophages are primarily polarized towards the anti-inflammatory, reparative M2 phenotype. These cells secrete anti-inflammatory cytokines, like IL-10, and perform immune surveillance and lipid buffering functions to maintain a state of insulin sensitivity (64, 65). However, under obese conditions, hypoxic adipocytes secrete chemotactic molecules to recruit inflammatory monocytes, activated T cells, and B cells. The presence of activated T cells coupled with the reduced number of regulatory T cells (Tregs), the immunosuppressive subpopulation of T cells that downregulate the activation and proliferation of effector T cells, leads to a phenotypic shift in the macrophage population towards the pro-inflammatory, M1 phenotype (66). The M1 macrophages secrete mainly pro-inflammatory cytokines such as TNF-alpha and IL-6 (67). The degree of insulin resistance positively correlates with the amount of infiltrating M1-like macrophages in adipose tissue (68). It has been proposed that the key link between inflammatory stimuli and insulin resistance is the intracellular activation and nuclear translocation of NF-kappaB in response to increased pro-inflammatory stimuli (69). Anti-inflammatory drugs, such as salicylates, have been shown to inhibit NF-kappaB and improve insulin sensitivity in obese rodents and diabetic patients (70). In adipose tissue of obese mice, the c-Jun N-terminal kinase (JNK)–activator protein-1 (AP-1) pathway is activated. Activation of the JNK/AP-1 pathway results in phosphorylation of insulin receptor substrate-1 (IRS-1) at its negative regulatory site, preventing interaction with the insulin receptor and inhibiting the insulin signaling pathway (71). Our recent studies showed that systemic administration of an arginase inhibitor or deletion of endothelial arginase 1, an isoform of arginase that competes with eNOS for available L-arginine, protected mice against obesity-induced inflammatory responses, indicating protective functions of NO (72, 73). Another study showed that arginase 2 activity promoted a pro-inflammatory macrophage response through increased generation of mitochondrial oxidative stress. The formation of excess mitochondrial ROS contributed to the development of insulin resistance and atherosclerosis (74).

3.3 Adipokines

Adipokines are a group of proteins composed of cytokines, chemokines, and hormones that are secreted from adipose tissue. They play important roles in the maintenance of energy homeostasis, appetite, glucose and lipid metabolism, insulin sensitivity, angiogenesis, immunity and inflammation, hemostasis, and blood pressure (75). Adipokines are classified as either pro-inflammatory or anti-inflammatory. The former has been shown to be elevated at the expense of the latter in obesity. This adipokine imbalance is believed to be the link between obesity, metabolic disorders, and cardiovascular diseases. Pro-inflammatory adipokines include leptin, resistin, TNF-alpha, retinol binding protein 4, lipocalin 2, angiopoietin-like protein 2, and visfatin. Anti-inflammatory adipokines include adiponectin, omentin, and adipolin (51, 76).

3.3.1. Leptin

Leptin is considered a pro-inflammatory adipokine since it induces production of ROS, TNF-alpha, and IL-6 by macrophages and monocytes, which in turn initiate the production of more leptin (77). However, leptin has many beneficial roles outside of its inflammatory effects. Leptin is a 16 kDa adipokine produced primarily in adipocytes from the LEP gene, the human homologue of the murine obese (ob) gene (78). Leptin receptors are produced from the diabetes (db) gene (79). Leptin regulates appetite and food intake by communicating energy status to the central nervous system (80). Leptin enhances glucose utilization and insulin sensitivity under normal conditions and ameliorates hyperlipidemia as shown in both experimental and clinical studies (81, 82). In mouse models, severe obesity can be induced by mutations in either the ob or db genes (78, 83). However, hyperleptinemia is common in clinical settings and administration of exogenous leptin does not result in weight loss, indicating that leptin resistance could be due to downregulation of its receptor or impairment of signal transduction (84, 85). The form of leptin resistance seen primarily in obesity occurs through inhibition of JAK2/STAT3 signaling, which is normally activated once leptin binds its receptor (86). Increased activity of the protein suppressor of cytokine signaling 3 (SOCS3) inhibits activation of the JAK/STAT3 pathway, reducing leptin signal transduction (87-89).

The cardiovascular effects of leptin are controversial. Elevated leptin levels are associated with hypertension caused by chronic activation of the sympathetic nervous system (90, 91). Additionally, studies that investigated the metabolic effects of insulin showed that prolonged exposure of rat adipocytes to high leptin concentrations (>1 nM), resulted in dose-dependent inhibition of insulin-stimulated glucose uptake, which was paralleled by decreased lipogenesis (92). Inhibition of insulin-stimulated glucose uptake and downregulation of lipogenesis are key events that can lead to the development of insulin resistance and cardiovascular dysfunction. Increased serum levels of leptin and arginase 1 have been found in obese patients (93). In contrast, leptin-mediated vasodilatory effects from increased NO production are impaired under pathological conditions, including obesity and metabolic syndrome (94). Leptin-deficient mice showed significant elevation in arginase activity in wounded skin, which correlated with impaired skin repair, likely due to decreases in endothelial cell-derived NO needed for angiogenic repair and unchecked inflammatory responses (95). The impaired tissue repair in leptin-deficient mice was abolished with administration of exogenous leptin (96).

3.3.2. Resistin

Resistin is a pro-inflammatory adipokine produced primarily from adipocytes in rodents, and monocytes and macrophages in humans (97). Elevated serum levels of resistin are associated with metabolic disorders and diabetic microvascular complications mediated by endothelial dysfunction (98). Interestingly, obesity is still seen in resistin-deficient mice, despite improved glucose tolerance and insulin sensitivity (99). Pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha induce

transcription of the resistin gene (RETN) in human mononuclear cells, which leads to the expression of more pro-inflammatory cytokines, resulting in the precipitation of inflammation (100). Resistin activates SOCS3, an inhibitor of the insulin signaling pathway, thereby inducing insulin resistance (101). *In vivo* supplementation of eNOS substrate, L-arginine, to mice fed a high-fat diet (HFD), enhanced insulin sensitivity without affecting resistin levels (102). Previous reports have shown that inflammatory stimuli produce high levels of iNOS-generated NO which promotes resistin expression, while iNOS inhibition reduces resistin expression, confirming the deleterious effect of high NO levels (103, 104).

3.3.3. Tumor necrosis factor-alpha

Tumor Necrosis Factor-alpha (TNF-alpha) is a pro-inflammatory cytokine, which in obesity, is heavily produced by monocytes and macrophages present in the stromal vascular fraction of adipose tissue. TNF-alpha levels have been found to positively correlate with obesity and T2D (54). TNF-alpha plays a central role in the development of insulin resistance and inflammation by inducing a repressive form of insulin receptor substrate-1 (IRS-1), effectively halting the insulin signaling pathway (105). Interestingly, short-term treatment (~4 weeks) with TNF-alpha blockers in obese diabetic patients and patients with metabolic syndrome reduced inflammatory responses, but did not improve insulin signaling suppression (54, 106). However, patients with metabolic syndrome that were treated with TNF-alpha blockers for a prolonged period (~6 months), showed lower fasting glucose levels, indicating improvement in insulin resistance and glucose uptake (107). In addition to its non-vascular effects, TNF-alpha has been shown to induce impairment of NO-mediated vasodilation in the small arteries found in the visceral fat of obese patients (108). It also has been shown that TNF-alpha activity impairs NO-induced vascular endothelial vasorelaxation through upregulation of arginase 1 expression/activity in ischemia-reperfusion injuries (109). In addition, TNF- alpha functions to reduce the levels of the anti-inflammatory adipokine, adiponectin, and increase the level of the pro-inflammatory adipokine, visfatin/NAMPT (110).

3.3.4. Retinol binding protein 4 (RBP4)

Retinol binding protein 4 (RBP4) is a blood transporter for retinol (vitamin A) secreted by the liver, adipose tissue, and macrophages (111). RBP4 serum level positively correlates with metabolic disorders, obesity, insulin resistance, and pro-atherogenic conditions (112). RBP4 induces insulin resistance by preventing insulin-initiated phosphorylation of insulin receptor substrate 1 (IRS-1) (113). RBP4 levels can be used to determine the predisposition of patients to atherosclerosis due to its positive correlation with obesity and pro-atherogenic markers (112). Mice lacking RBP4 exhibit reduced systolic blood pressure through enhanced eNOS phosphorylation and NO-mediated vasodilation (114).

3.3.5. Lipocalin 2

Lipocalin 2 is a carrier of retinoids, arachidonic acid, steroids, leukotriene B4, and platelet activating factor. Lipocalin 2 is produced primarily by adipocytes and macrophages upon activation of NF-kappaB. Elevated serum levels of lipocalin 2 positively correlate with metabolic disorders and inflammation (115, 116). Lipocalin-2 has been shown to cause M1 macrophage polarization while suppressing formation of the M2 macrophage phenotype, thereby increasing expression of iNOS and decreasing arginase 1 activity in macrophages (117). Inhibition of iNOS, pharmacologically or via gene silencing, prevents IL-1beta and IFN-gamma-induced lipocalin 2 expression (118). Paradoxically, lipocalin 2 knockout mice showed increased body weight, adipose tissue weight, and insulin resistance compared to wild type mice (119). Also at odds with its association with metabolic disorders, lipocalin 2 was recently reported to interact synergistically with insulin and retinoic acid in the activation of beige adipocytes with a resultant thermogenesis (120). The mechanisms behind these apparent contrasting effects of lipocalin 2 have yet to be elucidated.

3.3.6. Angiopoietin-like protein 2 (ANGPTL2)

Angiopoietin-like protein 2 (ANGPTL2) is an adipokine produced mainly from adipocytes, macrophages, and endothelial cells and is involved in the development of insulin resistance and inflammation (121). Serum and adipose tissue levels of ANGPTL2 positively correlate with metabolic disorders and inflammation (122). ANGPTL2 transgenic mice have been shown to have reduced eNOS expression, which is indicative of impaired NO-mediated vasorelaxation (123). This is in contrast to another study which showed that ANGPTL2 improves insulin sensitivity and lipid profile in genetically diabetic mice (124). The reasons for these differing effects of ANGPTL2 activity have not been resolved.

3.3.7. Visfatin

Visfatin, also known as cytokine pre-B cell colony enhancing factor (PBEF), or nicotinamide phosphoribosyltransferase (NAMPT), is produced primarily in adipocytes and macrophages (125, 126). This adipokine was initially thought to have insulin-mimetic effects, but this response has not been observed in humans (125, 127). However, administration of visfatin has been shown to ameliorate glucose intolerance and improve hepatic insulin sensitivity (128). The controversy over visfatin function was highly debated but more recent data indicate that serum levels of visfatin are higher in obese and T2D patients. This study suggested that visfatin-induced the release of pro-inflammatory cytokines, like TNF-alpha, which contributed to the onset of insulin resistance (129, 130). This pro-inflammatory role is reported to involve activation of p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK) pathways (131). It also has been reported that circulating levels of visfatin are markedly elevated during atherosclerosis and that this increase was closely associated with decreased levels of L-arginine and NO, and increased levels of an endogenous inhibitor of NOS, asymmetric dimethylarginine (ADMA) (132).

The contrasting characterizations of visfatin as pro-inflammatory versus anti-inflammatory may be due to the differences between the extracellular and intracellular actions of visfatin, and whether its function is mediated by enzyme activity or by activation of the unknown visfatin receptor (126). Intracellular visfatin/NAMPT produces NAD^+. NAD^+ is essential for the activity of sirtuin1 (SIRT1), a protein and histone deacetylase, which exerts many beneficial effects on cellular metabolism and vascular function (133). SIRT1 induces eNOS activity and NO production and thus improves cardiovascular function (134).

3.3.8. Adiponectin

Adiponectin is an anti-inflammatory adipokine synthesized only by adipocytes. Compared to most other adipokines, the healthy plasma concentration of adiponectin is high (~3-30 µg/mL) (135-137). Adiponectin enhances insulin sensitivity by increasing glucose and fatty acid metabolism through the activation of AMP Kinase (AMPK) and PPAR alpha (138-140). Conditions that adversely affect adiponectin concentrations are hypoxia, pro-inflammatory cytokines, and oxidative stress (141). The plasma adiponectin levels in obese subjects negatively correlate with plasma lipid peroxidation, a marker of oxidative stress (33). Overexpression of adiponectin in ob/ob mice results in healthy adipogenesis with expansion of the subcutaneous adipose tissue and insulin sensitivity similar to that of lean mice (142).

The effects of adiponectin on cellular metabolism and insulin sensitivity are important for the maintenance of good health (52). Adiponectin exerts an anti-inflammatory effect by repressing TNF-alpha production and promoting eNOS activity (143). Moreover, adiponectin inhibits toll-like receptor-induced activation of NF-kappaB and limits macrophage polarization to pro-inflammatory M1 macrophages, while simultaneously increasing the number of anti-inflammatory M2 macrophages (144).

Adequate adiponectin levels are associated with proper eNOS function. eNOS-deficient mice showed reduced adiponectin levels while mice overexpressing dimethylarginine dimethylaminohydrolase (DDAH), the enzyme responsible for the degradation of endogenous eNOS inhibitor, ADMA, showed higher adiponectin levels (145, 146). In turn, adiponectin can enhance NO levels by increasing eNOS mRNA stability and eNOS phosphorylation (147, 148). Adiponectin has also been shown to impede NO degradation through suppression of superoxide anion formation (149). Additionally, global deletion of the mitochondrial arginase isoform, arginase 2, induced a significant increase in adiponectin expression in epididymal adipose tissue with no significant effect on circulating adiponectin or hepatic levels, suggestive of a local autocrine effect (150).

3.3.9. Omentin

Omentin is an anti-inflammatory adipokine produced in adipose tissue that exhibits insulin-sensitizing properties through activation of the Akt signaling pathway (151). It has been shown that circulating levels of omentin are decreased in obese patients with insulin resistance (152). Omentin expression in both visceral and subcutaneous adipose tissue was found to correlate positively with the expression of neuropeptide Y (NPY), the most potent appetite stimulating peptide, suggesting that omentin may play a role in appetite modulation (153, 154).

In addition, omentin has been associated with reduced inflammation, improved lipid metabolism and vasodilation, and a reduction in the development of obesity-related cardiovascular disease and atherosclerosis. Omentin induces adiponectin expression, resulting in improved fatty acid breakdown and increased insulin-mediated glucose uptake (155). Omentin also stimulates endothelial-derived NO production, resulting in vasorelaxation, maintained endothelial barrier function, and reduced inflammation (156, 157). In addition to its positive regulatory roles, omentin has been shown to suppress TNF-alpha production (158). These various functions of omentin protect against atherosclerosis and obesity-related cardiovascular disorders.

3.3.10. Adipolin

Adipolin is an anti-inflammatory, insulin-sensitizing adipokine, primarily secreted from adipose tissue. Adipolin levels are reduced in obese mice and are negatively correlated with insulin resistance (159). Adipolin reduces inflammation through the inhibition of macrophage recruitment and secretion of pro-inflammatory cytokines (160).

3.4. Premature cellular senescence in adipose tissue

Cellular senescence is a state of irreversible replicative arrest that is associated with aging. It is initiated by a variety of factors including progressive telomere shortening through many cell cycles, buildup of reactive oxygen species (ROS), DNA damage, growth factors, and other metabolic and mitogenic stressors (161). This process is not only a stress response to severe cellular damage designed to protect against the proliferation of aberrant cells, but is also involved in development (162). The accumulation of these senescence-inducing factors triggers the upregulation of cyclin-dependent kinase inhibitors, $p16^{INK4a}$ and p53/p21, which arrest the cell cycle (163). However, premature senescence has been observed in the preadipocytes and adipocytes of the visceral adipose tissue (VAT) of young obese humans and animals (164). This phenomenon is promoted by nutrient excess, which contributes to oxidative stress, adipose tissue metabolic dysregulation, and inflammation (33, 61, 65, 165).

The marked accumulation of senescent cells in the visceral adipose tissue of obese humans and animals, compared to their age-matched counterparts, is correlated with compromised adipose tissue and mitochondrial function (166). The effect of obesity-induced senescence is particularly evident in preadipocytes, where it contributes to the

reduction of adipogenesis and lipogenesis, leading to lipotoxicity and inflammation (167). Although senescent cells cannot divide, they remain metabolically active. Especially in the VAT, senescent cells readily produce and release pro-inflammatory cytokines, chemokines, and growth factors. This process, which has been termed the senescence-associated secretory phenotype (SASP), enhances inflammation and adipose tissue dysfunction (166). The SASP release of MCP-1 further exacerbates the inflammatory state by promoting pro-inflammatory macrophage infiltration (168, 169). Additionally, the accumulation of senescent cells in the VAT can induce senescence in neighboring cells in a feed-forward mechanism (161).

In addition to the deleterious effects of obesity-induced preadipocyte and adipocyte senescence, senescence in VAT endothelial cells (EC) also plays a key role in VAT dysfunction (170). The process of transporting fatty acids (FAs) into adipocytes requires the microvascular endothelium. Senescence in these VAT EC has been reported to block fatty acid transport into adipocytes by a mechanism involving reduced PPAR gamma expression and activity (171, 172). The failure of adipocytes to take up free FAs results in their inability to store FAs in the VAT, leading to ectopic fat deposition and toxicity in the skeletal muscle and liver. Endothelial cells from the VAT of obese subjects also have been shown to exhibit the SASP, possessing inflammatory and angiogenic secretory profiles (170).

Another cell type found in VAT that can be forced into senescence by overnutrition are T cells. T cell senescence has also been noted in the VAT of diet-induced obese mice. These senescent $CD4^+$-associated T cells appear to enhance the inflammatory environment in obese VAT by releasing large amounts of osteopontin (173). Osteopontin, an inflammatory cytokine, has been reported to be elevated in the blood of obese diabetic and insulin-resistant patients. This is correlated with the severity of coronary artery disease, and plays a causative role in VAT inflammation, macrophage infiltration, and insulin resistance (174, 175).

4. IMPAIRED GLUCOSE METABOLISM AND INSULIN SENSITIVITY

The metabolic effects of insulin are mediated through a signal cascade initiated by the binding of insulin to its receptor (INSR) (Figure 2). Insulin binding triggers a conformational change in the receptor, leading to activation of the tyrosine kinase domain through autophosphorylation (176). The activated receptor phosphorylates insulin receptor substrates (IRSs), which in turn, bind phosphoinositide 3' kinase (PI3K). PI3K-IRS-1 phosphorylates its plasma membrane-bound substrate, phosphatidylinositol (3,4)-bisphosphate (PIP_2) forming secondary messenger, phosphatidylinositol (3,4,5)-triphosphate (PIP_3). PIP_3 is required for the activation of protein kinase B (PBK or Akt), which once activated, mediates the translocation of glucose transporter 4 (GLUT4) to the plasma membrane (176). In addition to promoting cellular glucose uptake, insulin also increases the expression of transcription factor adipocyte determination and differentiation-dependent factor 1 (ADD1). ADD1 upregulates expression of two genes: fatty acid synthase (FAS), an enzyme critical in lipogenesis, and leptin, which is responsible for appetite suppression (177).

Obesity is associated with a decrease in insulin-dependent GLUT4 expression and membrane translocation, which results in reduced glucose uptake and the subsequent development of hyperglycemia (178-180). The mechanism for the development of insulin resistance is not fully understood, but it is postulated that insulin resistance manifests as a defensive response from hypertrophied adipocytes (181). The onset of insulin resistance is an initial step in the development of T2D (181). Cell starvation from the lack of intracellular glucose results in an increase in pancreatic insulin secretion, triggered by

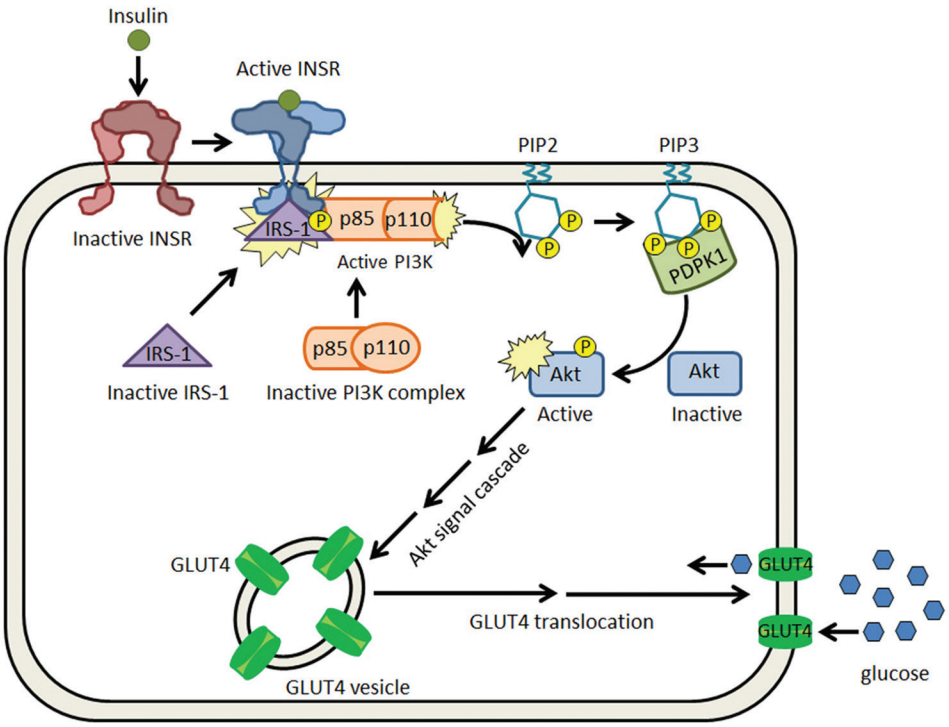

Figure 2. Insulin signaling pathway. Insulin binds to the inactive insulin receptor (INSR) and elicits a conformational change. This allows IRS-1 to bind to the intracellular domain of the INSR, where it is phosphorylated and subsequently able to bind to p85, the regulatory domain of PI3K. The binding of the p85 domain in PI3K activates its kinase domain, p110, which phosphorylates PIP2, producing PIP3. PIP3 is bound by phosphoinositide-dependent kinase-1 (PDPK1), which activates Akt through phosphorylation. Activated Akt acts through a signaling cascade to promote GLUT4 translocation to the plasma membrane to facilitate glucose uptake.

increased fat metabolism and the production of ketones (182, 183). This increase in insulin results in hyperinsulinemia because the insulin receptors no longer respond properly to insulin levels. Sustained, elevated insulin release leads to dysfunction in its production and pancreatic beta-cell failure, with ensuing hypoinsulinemia and hyperglycemia (184).

Many factors are associated with the development of insulin resistance in obesity including adipocyte dysfunction, elevated oxidative stress, and high levels of NO produced by iNOS. Adipocyte hypertrophy occurs as a buffering response to chronic overnutrition that protects other tissues from lipotoxicity (185). The development of adipocyte hypertrophy has systemic deleterious effects, in addition to promoting adipocyte dysfunction. In fact, genetic deletion of GLUT4 from adipose tissue resulted in systemic insulin resistance similar to that seen with the same deletion in skeletal muscle, tissue crucial for glucose uptake. In obese women, adipose tissue expression of GLUT4 and IRS-1 were found to be significantly reduced, indicating that obesity-induced adipocyte hypertrophy is linked to development of insulin resistance (186). This decrease in GLUT4 expression in subcutaneous adipocytes was also seen in patients who developed T2D (187). Obesity has also been shown to induce systemic and local oxidative stress, which is suggested to be critical in the pathogenesis of metabolic syndrome. Reactive oxygen species (ROS) are produced from many sources in response to the increase in fatty acids present in visceral adipose tissue under obese conditions (33, 188). A transient

increase of intracellular ROS is essential for insulin signaling and glucose uptake. However, chronic elevation of intracellular ROS induces insulin resistance through suppression of the insulin signaling pathway (33, 178-180, 189). Another mechanism that contributes to the pathogenesis of insulin resistance is abnormal eNOS and iNOS activity. Insulin-stimulated glucose uptake in adipose tissue and skeletal muscle is NO-dependent, and these physiological levels of NO are produced by constitutively active eNOS (190, 191). In obesity, proper eNOS function is compromised (192). Mice fed a high-fat diet (HFD) become obese, but supplementation with L-arginine, the substrate for eNOS, increased NO production and improved their insulin sensitivity (102). Inducible NOS (iNOS) has a pro-inflammatory role in the immune response and is upregulated in obesity, diminishing insulin sensitivity (193). Interestingly, inhibition of all NOS isoforms restored adipocyte insulin sensitivity, suggesting that inhibition of the detrimental iNOS activity is more important than the beneficial, constitutive activity of eNOS in regards to the maintenance of the homeostatic insulin response (103, 194, 195). These compounding effects promoted in obesity contribute to the type 2 diabetic state consisting of impaired glucose metabolism and insulin resistance.

5. IMPAIRED PROTEIN AND MUSCLE METABOLISM

In contrast to the increased adiposity seen in obesity and T2D, muscle mass in these conditions decreases due to impaired protein synthesis and increased muscle degradation in a process known as sarcopenia. The process of sarcopenia is accelerated with aging. In healthy subjects, insulin stimulates protein anabolism by simultaneously promoting protein synthesis and reducing protein catabolism (196). A large survey reported an inverse relationship between the skeletal muscle (SM) index (ratio of SM mass to body weight) and insulin resistance, indicating that patients with insulin resistance were likely to have increased muscle atrophy (197). Furthermore, muscle from obese and/or T2D patients has been found to exhibit increased levels of myostatin, a hormone known to reduce skeletal muscle mass (198, 199). Additionally, obese women have been shown to display more resistance to insulin-stimulated protein anabolism than lean women (200). Ectopic fat deposition in the skeletal muscle, or myosteatosis, has been shown to contribute to impaired protein anabolism and muscle function (201, 202). Diets high in protein and essential branched-chain amino acids (BCAA - leucine, isoleucine and valine) are very important for promotion of protein anabolism and the maintenance of muscle mass (203). Activation of mammalian target of rapamycin complex 1 (mTORC1) is believed to be centrally involved in this process.

Adipocytes also catabolize BCAAs to produce precursors required for fatty acid and sterol biosynthesis. This catabolic process increases dramatically with adipogenesis indicating that homeostasis of protein metabolism is distorted in obesity (204). Mice deficient in the enzyme responsible for BCAA catabolism showed decreased adiposity despite higher food intake and enhanced energy expenditure (205). Mice fed a leucine-deficient diet showed reduced adipose tissue weight and lipogenesis, with enhanced lipolysis and energy expenditure (206).

6. IMPAIRED LIPID METABOLISM

One of the primary functions of adipocytes is the storage of lipids in the form of triacylglycerol (TAG), which constitutes about 90% of adipocyte volume. The effect of insulin on lipid metabolism is highly coordinated. It simultaneously stimulates fatty acid anabolism (lipogenesis) through upregulation of lipogenic enzymes like acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), while preventing lipid catabolism (lipolysis) by inhibiting the phosphorylation and activation of hormone-sensitive lipase (HSL) (207-209). In obesity, adipocytes are abnormally enlarged and the usually, tightly regulated effects of

insulin on lipid metabolism are lost. Basal lipolysis is increased in obesity, resulting in hyperlipidemia due to increased secretion of lipolytic adipokines, serum amyloid A (SAA), IL-6, and TNF-alpha, from the hypertrophied adipocytes (210, 211). Concurrently, these dysfunctional adipocytes exhibit lower rates of lipogenesis as the rates of lipolysis increase, a phenotype commonly seen in insulin resistance (212). During obesity, the increased levels of free fatty acids and cholesterol result in myosteatosis and hepatosteatosis, or the build of ectopic lipid deposits in the skeletal muscle and liver (213-215).

Lipoprotein lipase (LPL) is an enzyme critical for the hydrolysis of TAG in circulating chylomicrons and very low-density lipoproteins (VLDL). LPL hydrolyzes TAG into two free fatty acids and one monoacylglycerol (216). LPL is expressed in adipocytes and subsequently transported to the capillary endothelium (217, 218). In adipose tissue, LPL influences fatty acid (FA) uptake for lipid storage and in skeletal and cardiac muscle, LPL induces FA uptake to provide these energetically-active cells with fuel (219, 220). Previous studies in mice on a high-fat diet (HFD) have shown that the skeletal muscle-specific deletion of LPL reduced lipid deposition and increased insulin sensitivity in the muscles (221, 222). This result indicates that LPL-induced uptake of fatty acids in skeletal muscle is detrimental in obesity due to the extreme excess of circulating lipids. When transgenic mice, overexpressing LPL in their adipose tissue, were challenged with a HFD, they exhibited elevated adiponectin levels, improved glucose and insulin tolerance, and increased energy expenditure when compared to control mice on the same diet (223). The positive, anti-inflammatory effect of LPL seen in adipocytes was suppressed in cultured adipocytes treated with TNF-alpha due to the activation of inducible nitric oxide synthase (iNOS), which produced toxic levels of nitric oxide (224). Other *in vitro* studies demonstrated that both a NO-releasing compound and the NOS substrate (hydroxylamine and L-arginine, respectively) increased LPL activity as seen by adipocyte differentiation and lipid accumulation (225). These data indicate that the toxic levels of NO from iNOS have a deleterious effect on LPL function, while moderate levels of NO promote LPL activity.

In addition to LPL dysfunction, mitochondrial lipid metabolism also is adversely affected by obesity. In lean conditions, fatty acids are internalized and transported into mitochondria, where they undergo beta-oxidation to produce acetyl-CoA. Acetyl-CoA is subsequently shuttled into the citric acid cycle to produce energy for the cell. In obesity, the mitochondria in white adipose tissue are inundated with excess lipids and incomplete beta-oxidation occurs. Incomplete beta-oxidation coupled with lipid overloading results in accumulation of toxic lipid intermediates and ROS, which promote insulin resistance (226, 227).

7. ROLE OF MITOCHONDRIAL DYSFUNCTION AND ER STRESS IN OBESITY

Mitochondria are dynamic organelles, critical for the maintenance of energy homeostasis. The mitochondria produce ATP, a form of cellular energy currency, through a sequence of processes that terminate with the electron transport chain (ETC): the citric acid cycle (CAC), pyruvate decarboxylation, fatty acid beta-oxidation, branched chain amino acid degradation, and oxidative phosphorylation (228). Mitochondria also internalize Ca^{2+}, an important physiological process that influences mitochondria metabolism, intracellular Ca^{2+} signaling, and under conditions of oxidative stress, this uptake leads to initiation of apoptosis, or programmed cell death (229).

7.1. Mitochondrial dysfunction

In response to increased energy expenditure, healthy cells undergo mitochondrial biogenesis, a process where cells increase their mitochondrial mass in order to increase their individual ATP production.

Mitochondrial biogenesis is closely associated with the process of adipogenesis, indicating the importance of mitochondria to healthy adipocyte physiology and function (230-234). In adipose tissue, eNOS-produced NO plays an important role in mitochondrial biogenesis by increasing oxygen consumption and energy expenditure, inducing gene expression, and promoting protein kinase G (PKG)-dependent phosphorylation of AMP-activated protein kinase (AMPK) (20, 235). Activation of AMPK promotes expression of PPAR gamma coactivator 1 alpha (PGC-1 alpha), leading to increased expression of PPAR gamma, a protein that upregulates adipogenesis (236, 237).

Mitochondrial dysfunction, a process defined by poor ATP production, often occurs in obesity. There is still some debate on whether mitochondrial dysfunction is a cause or consequence of obesity and overnutrition. However, there have been data revealing that an excess of nutrients, as seen in obesity and type 2 diabetes, overwhelms the handling capacity of the mitochondrial metabolic processes, resulting in mitochondrial dysfunction (238). In the mouse preadipocyte cell line, 3T3-L1, mitochondrial dysfunction was manifested as reduced fatty acid oxidation, which resulted in TAG accumulation and increased glucose uptake, that latter of which is suggested to increase glycerol 3-phosphate synthesis, leading to further lipid accumulation. Increased lipid accumulation in adipocytes leads to the eventual loss of the lipotoxicity buffering capacity of these cells. The excess free fatty acids are released into the bloodstream resulting in ectopic fat deposition, which is believed to be the underlying cause of the development of insulin resistance in obesity (239).

In addition to the adverse effects seen from systemic lipid inundation and the subsequent steatosis, mitochondrial dysfunction also results in increased ROS production, as seen in both clinical and experimental studies (240, 241). The electron transport chain (ETC), primarily complexes I, II, and III, are considered to be major sources of ROS generation due to the capacity for electron leakage (242-244). Electron leakage directly correlates with mitochondrial membrane potential (245). Activation of the uncoupler protein (UCP) by ROS serves as a feedback mechanism to lower membrane potential (246). These detrimental by-products of metabolism can induce metabolic dysfunction, inflammation (through upregulation of TNF-alpha), tissue damage, and the development of insulin resistance (38, 39). ROS have also been shown to increase expression of activating transcription factor 3 (ATF-3), a protein responsible for the downregulation of adiponectin expression (33). Though adipocytes, unlike other cell types, can endure high levels of ROS without sustaining substantial damage, chronic ROS elevation is detrimental and decreases adiponectin expression (247). In contrast, increased mitochondrial biogenesis has been correlated with increased adiponectin levels, reduced oxidative stress, improved mitochondrial function, and increased insulin sensitivity (33, 248).

These findings suggest an important link between oxidative stress, mitochondrial dysfunction, and metabolic dysregulation during obesity. Thus, further investigation is warranted to combat obesity and obesity-related dysregulations by targeting any or all of these disorders.

Paradoxically, mild mitochondrial dysfunction, in the absence of oxidative stress, protects against obesity as seen in mice with a fat-specific deletion of the mitochondrial transcription factor A (TFAM). These mice exhibited decreased mitochondrial DNA (mtDNA) copy number and altered expression of ETC proteins, with decreased expression of complex I, the main site of superoxide formation in the ETC (249). These mice also exhibited a compensatory increase in complex II, which resulted in increased oxygen consumption, uncoupling, and decreased ROS production (250). The overall effect of the fat-specific deletion of TFAM in mice challenged with a HFD was higher energy expenditure and

protection from diet-induced obesity, insulin resistance, and steatosis (250).

7.2. Compromised mitochondrial dynamics

Mitochondria are highly dynamic organelles, exhibiting fission, fusion, and mitophagy. Mitochondrial fusion is the process by which two mitochondria physically merge their inner and outer membranes to form a larger, mitochondrion (251). This process is controlled by several proteins, including the dynamin-related GTPases, mitofusin-1 and mitofusin-2 (MFN-1 and MFN-2), and optic atrophy protein 1 (OPA-1), the former are responsible for the outer mitochondrial membrane fusion and the latter is required for the fusion of the inner mitochondrial membranes (252). Mitochondrial fission is the opposite of fusion, and is where a mitochondrion divides to form two mitochondria (251). Fission is controlled by dynamin-related protein 1 (DRP1), a protein recruited from the cytosol to the mitochondrion where it can then bind its receptors, fission 1 (FIS1) and mitochondrial fission factor (MFF) (253). The processes of fusion and fission occur cyclically. Fusion allows two mitochondria to mix their components and is typically followed within minutes by fission, which returns the fused mitochondria back into two distinct organelles (254). This dynamic process of mitochondrial fusion and fission in healthy cells is believed to be critical for cell health and aberrant function of this process is associated with several disease states (255, 256). Mitochondria with high membrane potential will continue the fission/ fusion cycle while those with a low potential will remain depolarized until recovery (254). A continuous, but precisely controlled, cycle of fission and fusion is important for the proper distribution of mitochondria throughout the cell, repair of damaged mitochondria, and for mitochondrial quality control (256). Mitochondria that are damaged/ depolarized and unable to recover, do not undergo fusion and are not incorporated into the healthy mitochondrial network (257). They form autophagosomes that eventually undergo mitophagy (258). Mitophagy is a catabolic process in which damaged mitochondria are degraded by lysosomes (258). This process is initiated by PARKIN and (PTEN)-induced putative kinase 1 (PINK1), which induce ubiquitination and degradation of fusion-promoting proteins MFN-1 and 2 (259).

Mitochondrial dynamics represent cellular adaptation to fluctuations in metabolic demand. Increased energy demand and decreased supply of nutrients are both associated with inhibition of mitochondrial fission and the promotion of fusion, or increased mitochondrial elongation, which allows respiration to be coupled to ATP synthesis (260). During conditions of increased energy expenditure, expression of MFN-2 is increased in skeletal muscles and brown adipose tissue (261, 262). Moreover, exercise improves insulin sensitivity in skeletal muscles of insulin resistant patients and is associated with decreased DRP1 and increased MFN-1 and MFN-2 expression (263). On the flipside, mitochondrial fragmentation and uncoupled respiration predominate under conditions of excess nutrient supply, where the promotion of thermogenesis is necessary to dispose of the caloric excess (264). Increased DRP1 activity in brown adipocytes is associated with increased levels of uncoupling protein-1 (UCP-1) (265). In fact, brown/beige adipocytes rely greatly on fission to enhance the uncoupling process, allowing for increased oxygen consumption. Though mitochondrial fission is considered to be a physiological adaptation to bioenergetic stressors rather than a harmful process, excessive mitochondrial fission events can be deleterious for the cell (264).

Disruption of the tightly regulated mitochondrial dynamics is associated with metabolic disorders and insulin resistance seen in diabetic and obese patients (264). One study showed that mice lacking DRP1 or MFN-1 in the liver were resistant to high-fat diet-induced obesity and insulin resistance (266). Another study reported that increased hepatic levels of PINK1, a protein that promotes mitophagy, were positively correlated with increased insulin sensitivity. This effect is likely due

to successful degradation of dysfunctional mitochondria, which can produce elevated levels of ROS (267-269). In pancreatic beta-cells, exposure to hyperglycemia and high levels of palmitate (obese conditions) resulted in reduced mitochondrial fusion (270). This environment also caused the same effect in leukocytes, which led to their enhanced adhesion to endothelial cells and subsequent inflammation (271). Skeletal muscle of Zucker obese rats showed decreased mitochondrial size, mitochondrial volume/unit of mitochondrial surface ratio, and MFN-2 expression (272). MFN-2 expression was found to be downregulated in human obese and type 2 diabetic patients (273, 274). MFN-2-deficient mice exhibited impaired insulin signaling in the liver and muscle, and increased ER stress through a mechanism involving increased ROS and JNK activation (272). Additionally, adipocyte-specific deletion of MFN-2, but not MFN-1, was associated with brown adipose tissue dysfunction and impaired lipid metabolism (275). Given the importance of proper mitochondrial dynamics in the maintenance of cell health and insulin sensitivity, the mitochondrial fission/fusion cycle is a promising therapeutic target for combating metabolic disorders (276).

7.3. Endoplasmic reticulum stress

The endoplasmic reticulum (ER) is the organelle where protein synthesis, folding, and maturation occurs (277, 278). Accumulation of misfolded proteins in the ER lumen is problematic and can eventually lead to cell death. During ER stress, mammalian cells trigger the unfolded protein response (UPR), a highly conservative response system intended to rectify the aggregation of misfolded proteins in the ER (279). The UPR begins with the activation of signaling pathways involved in either suppression of protein translation, to prevent more proteins from being misfolded, or the upregulation of chaperone protein expression, to coordinate and regulate proper protein folding (280-282). Hypoxia and ROS can increase the production of free fatty acids in adipocytes, oxidize proteins, and decrease calcium levels in ER lumen; all processes that impair ER protein folding in adipocytes and lead to ER stress (33, 283-286). Inflammatory cytokines also trigger ER stress by promoting ROS formation, or by increasing iNOS activity to pathological levels, which impedes the function of the ER Ca^{2+} pump (281, 287-289). Indicators of ER stress have been shown to be elevated in adipose tissue of obese mice and humans (290, 291). Administration of chemical chaperones that block ER stress, like 4-PBA, to mice on a high-fat diet (HFD), reduced adipose tissue inflammation, increased insulin sensitivity, and suppressed HFD-induced weight gain (292). Additionally, weight loss has been shown to be associated with reduced expression of ER stress markers (293).

In addition to triggering the unfolded protein response, ER stress has been shown to disrupt lipid metabolism. This mechanism involves activation of sterol regulatory element-binding protein (SREBP), which induces transcription of fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-COA), genes involved in lipid and cholesterol synthesis, respectively. Upregulation of these genes leads to excessive lipid production, resulting in fatty acid accumulation in the liver (294). Interestingly, deletion of ER stress sensor inositol-requiring enzyme 1 alpha (IRE1 alpha) in mouse adipose tissue macrophages halted the progression of obesity, insulin resistance, and hepatic steatosis when the mice were challenged with a HFD (295). This mutation also increased energy expenditure by inducing the browning of white adipose tissue, increased the metabolic activity of brown adipose tissue, and promoted macrophage polarization to the anti-inflammatory, M2 phenotype (295). Prevention of ER stress activation may be an effective therapeutic strategy for the treatment of metabolic syndrome (294).

8. NITRIC OXIDE SYNTHASE (NOS) DYSFUNCTIONS

Nitric oxide (NO) is well known for its vasodilatory, anti-thrombotic, anti-proliferative,

and anti-inflammatory effect in the vasculature (296-299). NO production and effects occur in a variety of cells and tissues. NO is produced from L-arginine by endothelial, neuronal, and inducible NO synthase (eNOS, nNOS, and iNOS). NO triggers a signal cascade by binding soluble guanylate cyclase (sGC), initiating the conversion of guanosine 5'-triphosphate (GTP) to the secondary messenger, cyclic 3',5'-monophosphate (cGMP) (300). cGMP activates cGMP-dependent protein kinase (PKG) which then phosphorylates target proteins involved in mediating the vasodilatory response (301).

Adipose tissue (AT) from mice lacking eNOS ($eNOS^{-/-}$) was reported to exhibit increased pro-inflammatory gene expression and macrophages, in addition to increased ROS and decreased mitochondrial biogenesis and adiponectin levels (146, 299). In contrast to these findings, another study reported that the lack of eNOS in mice did not promote AT inflammation (302). Use of a phosphodiesterase-5 (PDE5) inhibitor, such as sildenafil, blunted obesity-induced adipose tissue inflammation and macrophage infiltration, through the prevention of PDE5-mediated cGMP degradation (299). PDE5 inhibition resulted in enhanced NO-cGMP-PKG signaling, promotion of vasorelaxation, increased energy expenditure, and elevated insulin sensitivity (303). Other studies have demonstrated that eNOS expression and activity are tightly regulated in adipose tissue and muscle, and that eNOS is necessary for caloric restriction-induced upregulation of SIRT1, a protein involved in promotion of insulin sensitivity and amplification of eNOS activity (235, 304, 305). Further, $eNOS^{-/-}$ mice failed to exhibit the beneficial effects of swim training-induced increases in mitochondrial biogenesis, mtDNA copy number, and glucose uptake in the subcutaneous adipose tissue as compared with wild-type mice. In the same study, the NO donor, DETA-NO, was found to promote mitochondrial biogenesis, glucose uptake, and increased GLUT4 membrane density in cultured murine and human adipocytes (306). These results indicate that physiological levels of NO play a pivotal role in maintaining healthy metabolic function of adipose tissue.

Data from both human and animal studies have shown reduced expression and activity of eNOS and NO production under obese conditions (307-310). Suggested mechanisms for decreased NO levels include upregulation of cell membrane caveolin1 (CAV1), a negative regulator of eNOS activity, and increased levels of ceramide, a disruptor of the eNOS/Akt/HSP-90 complex (311, 312). However, a mechanism involved in the obesity-induced reduction of eNOS-produced NO supported by substantial evidence is the elevation of arginase expression/activity. Arginase is an enzyme that competes with NOS for their common substrate, L-arginine (313). Reduced availability of L-arginine leads to decreased NO production and NOS uncoupling. NOS uncoupling results in production of the ROS superoxide (O_2^-), which can subsequently react with NO to form peroxynitrite ($ONOO^-$), another toxic oxidative species (313). Several studies that used HFD and rodent models of obesity have shown prominent involvement of arginase in both visceral adipose inflammation and vascular dysfunction and inflammation through genetic deletion of arginase or use of arginase inhibitors (14, 73, 314). Our lab also found that mice specifically lacking arginase 1 in endothelial cells were protected from high-fat diet-induced systemic vascular dysfunction, hypertension, reduced vascular NO, elevated ROS levels, adipose tissue inflammation, fibrosis, and reduced vascularity (73, 313).

NO from endothelial or neuronal NOS at low to moderate concentrations stimulates glucose and fatty acid oxidation and inhibits synthesis of glucose, triacylglycerol, and low-density lipoproteins. These beneficial effects are linked to increased mitochondrial biogenesis and oxidative phosphorylation, as well as development and activity of brown adipose tissue (16). NOS function in mitochondria, along with cytoplasmic NO

Table 1. Therapeutic interventions applied to obesity models and the observed effects

Intervention	Model	Effect
Treatment with arginase inhibitor, N^{ω}-hydroxy-nor-L-arginine (nor-NOHA)	C57BL/6 mice fed high-fat diet (HFD)	Prevented HFD-induced increases in body weight, hepatic metabolic abnormalities (323), endothelial dysfunction (324) and adipose tissue inflammation (314).
	Zucker obese rats	Prevented obesity-induced hypertension and endothelial dysfunction (318).
Treatment with arginase inhibitor, S-(2-boronoethyl)-L-cysteine (BEC)	Zucker obese rats	Prevented obesity-induced hypertension and endothelial dysfunction (318).
Treatment with arginase inhibitor, 2-(S)-amino-6-boronohexanoic acid (ABH)	C57BL/6 mice fed high-fat diet (HFD)	Prevented obesity-induced bone loss (325), endothelial dysfunction, hypertension (73) and visceral adipose tissue (VAT) inflammation (14). No effect on body weight.
Endothelial specific Arginase 1 knockout ($eNOS^{-/-}$)	C57BL/6 mice fed high-fat diet (HFD)	Prevented obesity-induced endothelial dysfunction, hypertension (73) and visceral adipose tissue (VAT) inflammation (14). There was no effect on body weight.
Global deletion of Arginase 2°	C57BL/6 mice fed high-fat diet (HFD)	Prevented obesity-induced renal oxidative stress and inflammation (326), endothelial dysfunction (327), hepatic steatosis (150), insulin resistance, adipose tissue inflammation (74), and pancreatic ductal adenocarcinoma growth (328).
Supplement with sepiapterin and L-citrulline; precursor of BH4 (tetrahydrobiopterin) and substrate for L-arginine synthesis, respectively	Db/db mice	Prevented cardiomyopathy in obese T2D mice (329).
L-arginine supplementation (potential mechanism of increased NO availability)	Zucker obese rats	Anti-inflammatory effects in obese rats (330) and decreased macrophage inflammatory response (331).
		Prevented obesity-induced hypertension and endothelial dysfunction (318).
	Healthy patients with metabolic syndrome	Improved endothelial function and glucose metabolism in metabolic syndrome patients (332).
	C57BL/6 mice fed high-fat diet (HFD)	Anti-obesity effects, reduced white fat mass and plasma lipids, increased skeletal muscle and brown fat, insulin sensitivity, and increased energy expenditure (321, 333).
Sodium nitrite treatment	Streptozotocin (STZ)-induced diabetic rats fed HFD	Decreased body weight and induction of white adipose tissue browning (334).
Global $iNOS^{-/-}$ knockout	*Ob/ob* obese mice given high dose of insulin via implanted insulin pump	Prevented hyperinsulinemia-induced inflammation, fibrosis and insulin resistance in adipose tissue (335, 336).
Phosphodiesterase-5 inhibitor (Sildenafil)	C57BL/6 mice fed high-fat diet (HFD)	Long term treatment (12 weeks): anti-obesity and insulin sensitizing (337).
	C57BL/6 mice	Short term (7 days): browning of white adipose tissue (338).
Global $iNOS^{-/-}$ knockout mice	Model of metabolic syndrome - Isolated muscle mitochondria	Lower metabolic activity, reduced mitochondrial density and muscle fatty acid oxidation (339).
Global $iNOS^{-/-}$ knockout mice supplemented with sodium nitrate	Model of metabolic syndrome	Reduced visceral fat mass, circulating triglyceride and glucose levels (340).
eNOS transgenic mice	C57BL/6 mice fed high-fat diet (HFD)	Enhanced fatty acid oxidation in adipose tissue, resistant to diet induced obesity without affecting insulin resistance (20).

production, have been shown to induce mitochondrial biogenesis (235, 315). An *in vitro* study showed that NO acutely inhibits brown adipocyte proliferation but stimulates adipogenesis as shown by increased expression of peroxisome proliferator-activated receptor gamma (PPAR gamma) and uncoupling protein 1 (UCP 1) (316). In spite of the multitude of beneficial effects of NO at low to moderate concentrations, high concentrations of NO produced by iNOS is cytotoxic and can generate detrimental peroxynitrite and hydroxyl radicals (317). Under conditions of low L-arginine bioavailability, such as increased arginase activity, providing supplemental L-arginine restored NO production (318). In Zucker obese/diabetic rats, dietary supplementation with L-arginine suppressed weight gain and other features of metabolic syndrome, while elevating the respiratory exchange ratio (RER) and heat production (319, 320). L-arginine supplementation also improved metabolic disturbances by increasing insulin sensitivity in mice challenged with a low protein diet (321). Human studies also reported the effectiveness of supplemental L-arginine in the improvement of insulin sensitivity in patients with metabolic syndrome (165, 322).

Summaries of studies that have investigated means of enhancing constitutive NO production from NOS to prevent or reduce obesity-induced metabolic and vascular dysfunctions are provided in Table 1.

9. PERSPECTIVE/SUMMARY

Historically, adipocytes were considered to be inert lipid reservoirs, however, recent studies have shown the important, systemic endocrine function of adipocytes, which is intimately involved in the regulation of insulin sensitivity, energy homeostasis, and cardiovascular function. The rapidly growing prevalence of obesity worldwide affects individuals of all genders, ages, ethnic groups, and socioeconomic levels. Obesity greatly increases the risk of developing various comorbidities, indicating the dire need to better understand the intricate mechanisms behind obesity-induced metabolic and cardiovascular dysfunctions. At physiological levels, the vasoprotective molecule, NO, plays a prominent role in maintaining adipocyte and vascular function. However, expression of inducible NOS leads to high levels of NO, which are detrimental to metabolic and cardiovascular function. In obesity, arginase and NOS and are dysregulated. Given the deleterious effects of elevated arginase activity/expression seen in obesity-related metabolic and cardiovascular disorders, targeting this enzyme could be a possible therapeutic strategy in the treatment of obesity-induced diseases. Complicating this potential strategy is the fact that arginase has two isoforms, A1 and A2, and not enough is known about the ability and effect of preferentially targeting them. Additionally, the effect of systemic arginase inhibition in the presence of comorbidities or on other organ systems is not well known. Currently, there are only a few clinical trials testing the efficacy of arginase inhibition in different pathologies. Exploring tissue-specific, cell-specific or isoform-specific arginase inhibitors or modulators may prove to be an effective therapeutic strategy for combating obesity-related disorders. However, further studies of the complex mechanisms behind the development of metabolic and cardiovascular disease induced by obesity are required to address future treatment strategies for this ever-growing health problem.

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Abbreviations: ACC: acetyl-CoA carboxylase; ADD1: adipocyte determination and differentiation-dependent factor 1; ADMA: asymmetric dimethylarginine; AMPK: 5' adenosine monophosphate-activated protein kinase; ANGPTL2: angiopoietin-like protein 2; AT: adipose tissue; ATF-3: activating transcription factor 3; BCAA: branched chain amino acid; BMI: body mass index; BMP4: bone morphogenic protein 4; C/EBP: CCAAT-enhancer-binding protein; CAC: citric acid cycle; CAV1: caveolin1; DDAH: dimethylarginine dimethylaminohydrolase; DRP1: dynamin-related protein 1; eNOS: endothelial nitric oxide synthase; ETC: electron transport chain; FA: fatty acid; FAS: fatty acid synthase; FIS1: mitochondrial fission protein 1; HFD: high-fat diet; HMG-COA: 3-hydroxy-3-methylgutaryl-CoA reductase; HSL: hormone-sensitive lipase; ICAM-1: intracellular adhesion molecule 1; IFN-gamma: interferon gamma; iNOS: inducible nitric oxide synthase; INSR: insulin substrate receptor; IRE1 alpha: inositol-requiring enzyme 1 alpha; IRS-1: insulin receptor substrate 1; IL-1, 6, 10: interleukin 1, interleukin 6, interleukin 10; JNK/AP-1: c-Jun N-terminal kinase–activator protein-1; LPL: lipoprotein lipase; MCP-1: monocyte chemoattractant protein; MFF: mitochondrial fission factor; MFN-1, 2: mitofusin-1, mitofusin-2; mTORC1: mammalian target of rapamycin complex 1; NAD^+: nicotinamide adenine dinucleotide; NF-kappaB: nuclear factor kappa-light-chain-enhancer of activated B cells; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; NOS: nitric oxide synthase; NPY: neuropeptide Y; OPA-1: optic atrophy protein 1; PDE5: phosphodiesterase-5; PI3K: phosophinositide 3' kinase; PINK1: PTEN-induced putative kinase 1; PIP2: phosphatidylinositol (3,4)-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-triphosphate (PIP3); PKG: protein kinase G; PPARgamma: peroxisome proliferator-activated receptor gamma; RBP4: retinol binding protein 4; ROS: reactive oxygen species; SAA: serum amyloid A; SAT: subcutaneous adipose tissue; sGC: soluble guanylate cyclase; SIRT1: sirtuin 1; SOCS3: suppressor of cytokine signaling 3; SREBP: sterol regulatory element-binding protein; T2D: type II diabetes; TAG: triacylglycerol; TFAM: transcription factor A, mitochondrial; TLR-4: toll-like receptor 4; TNF- alpha: tumor necrosis factor alpha; Treg: regulatory T cell; UCP-1: uncoupling protein 1; VAT: visceral adipose tissue; VLDL: very low density lipoprotein; ZNF: zinc-finger protein

Key Words: Obesity, Diabetes, Adipokines, Inflammation, Insulin Resistance, Cardiovascular Disease, Review

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