Adipogenesis as a Potential Anti-Obesity Target: A Review of Pharmacological Treatment and Natural Products

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Abstract: Obesity is recognized as a severe threat to overall human health and is associated with type 2 diabetes mellitus, dyslipidemia, hypertension, and cardiovascular diseases. Abnormal expansion of white adipose tissue involves increasing the existing adipocytes' cell size or increasing the number through the differentiation of new adipocytes. Adipogenesis is a process of proliferation and differentiation of adipocyte precursor cells in mature adipocytes. As a key process in determining the number of adipocytes, it is a possible therapeutic approach for obesity. Therefore, it is necessary to identify the molecular mechanisms involved in adipogenesis that could serve as suitable therapeutic targets. Reducing bodyweight is regarded as a major health benefit. Limited efficacy and possible side effects and drug interactions of available anti-obesity treatment highlight a constant need for finding novel efficient and safe anti-obesity ingredients. Numerous studies have recently investigated the inhibitory effects of natural products on adipocyte differentiation and lipid accumulation. Possible anti-obesity effects of natural products include the induction of apoptosis, cell-cycle arrest or delayed progression, and interference with transcription factor cascade or intracellular signaling pathways during the early phase of adipogenesis.

Keywords: adipogenesis, transcriptional cascades, signaling pathways, anti-obesity treatment, bioactive molecules

Introduction

According to the World Health Organization, 39% of adults are overweight, and 13% are obese globally.[1] Obesity has nearly tripled since 1975 and is predicted to triple by 2030.[2] Obesity poses a severe threat to overall human health due to its close association with a cluster of pathological entities globally designated as metabolic syndrome, including type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, and cardiovascular diseases.[3] Obesity is also associated with increased oxidative stress levels due to excessive production of reactive oxygen species (ROS) and dysfunctional antioxidant systems. Adipose tissue is an important energy store and essential regulator of energy balance, but also an active endocrine organ that secretes numerous bioactive peptides and proteins called adipokines.[4] Adipokines generate ROS, reduce the antioxidant capacity, and stimulate the production of pro-inflammatory cytokines such as interleukin 1β (IL-1β) and 6 (IL-6), and tumor necrosis factor-alpha (TNF-α), causing an increase in oxidative stress levels. Thus, obesity induces oxidative stress via low-grade chronic

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inflammation, but also through other mechanisms such as mitochondrial and peroxisomal oxidation of fatty acids and over-consumption or altered O2 metabolism. ROS induces endothelial dysfunction, decreases vasodilator and increases contractile factors, and damages cell structures, causing atherosclerosis, heart failure, hepatic steatosis, and cancer.[5] The link between chronic inflammation and cancer lies in tumor promoters' ability to recruit inflammatory cells and stimulate them to generate ROS. Significant damage may occur to cell structure and functions, inducing somatic mutations, and neoplastic transformation. Oxidative stress causes cancer initiation and progression by interacting with the initiation (gene mutations and structural alterations of the DNA), promotion (abnormal gene expression, blockage of cell- to cell communication, modification of second messenger systems), and progression (further DNA alterations) of cancer. There is a significant contribution to cancer development of the triad of over-weight/obesity, insulin resistance (IR), and adipocytokines. Some of the effects of obesity in cancer etiopathogenesis comprise inducing hyperinsulinemia and subclinical chronic low-grade inflammation and oxidative stress and causing alterations in adipocytokine pathophysiology, and sex hormones biosynthesis.[6]

In light of the current COVID-19 pandemic, it is important to emphasize an association between obesity and infectious diseases, especially pulmonary infections. Obesity increases the risk of hospitalization and admission to intensive care units, in addition to greater severity of the disease and higher mortality in COVID-19 patients. In patients under the age of 60, obesity is the main predictor of severe symptoms and doubles the risk of being admitted to critical care.[7] Underlying mechanisms include obesity-related comorbidities (T2DM, cardiovascular and renal diseases), immune system impairments facilitating a systemic diffusion of infection, increased type 2 inflammation with effects on the lung parenchyma, raised IL-6 levels, and abnormal secretion of adipokines and cytokines inducing low-grade inflammation.[8]

Given the obvious physical constraints and associated psychological stress, as well as the risk of obesity-related health complications, reducing body weight is regarded as a major health benefit. The etiologic-mechanistic perspective of obesity is still poorly understood, and there are very few effective pharmacological approaches for obesity prevention. The etiology of obesity involves an interaction between genetic and environmental factors, the latter being the main reason for the increase in the global prevalence of obesity. Changes in lifestyle and dietary habits have resulted in an outbreak of the obesity pandemic over the last decades. Obesity results from an imbalance between energy intake and expenditure. The increased caloric intake from diets high in saturated fats, sugar, and processed foods, together with reduced physical activity, results in an energy imbalance.[9]

Adipose Tissue and Adipogenesis

Excess energy is stored in the form of lipids in adipocytes, and an excessive accumulation of adipose tissue characterizes obesity. There are two types of adipose tissues, brown (BAT) and white (WAT). BAT is mainly responsible for non-shivering thermogenesis in response to cold stress or β-adrenergic stimulus, while WAT plays a crucial role in lipid homeostasis and maintaining energy balance.[10] Abnormal expansion of WAT associated with obesity involves increasing the cell size of the existing adipocytes (hypertrophy) or increasing the number through differentiation of new adipocytes (hyperplasia).[11] Under normal circumstances, excess energy does not damage the organism as long as it is efficiently stored in the adipose tissue. However, when amounts of fat exceed the adipose tissue's storage capacity and overwhelm adipose tissue's functional capacity, fat begins to deposit ectopically in other metabolically relevant organs, such as the liver, skeletal muscle, kidney, and pancreas.[12] With obesity progression, adipose tissue becomes inflamed and fibrotic, worsening the dysfunction and decreasing the WAT's metabolic flexibility, leading to the development of metabolic abnormalities, such as dyslipidemia and IR.[13] Hypertrophic WAT expansion due to increased white adipose cells, macrophage infiltration, and fibrosis disrupt hormonal balance. The release of inflammatory cytokines and adipokines alters the normal energy homeostasis and causes metabolic syndrome.[14]

Adipocyte hyperplasia, also known as adipogenesis, is a process of proliferation and differentiation of adipocyte precursor cells in mature adipocytes.[15] It is a key process in determining the number of adipocytes, occurring mainly during childhood and adolescence, further determining the lipid-storing capacity of adipose tissue and fat mass in adults.[10] Therefore, the regulation of size and number of adipocytes might be a possible therapeutic approach for obesity, and it is important to understand the molecular mechanisms of adipose tissue formation and alterations in obesity. Identifying potential adipogenic molecular targets

susceptible to modulation by external factors can utilize adipogenesis regulation to control or reverse obesity.

This review focuses on available conventional medicine and natural products to prevent or treat obesity by targeting adipogenesis and discussing potential adipogenesis mechanisms suitable for the therapeutic approach.

Molecular Regulators of Adipogenesis as Potential Therapeutic Targets

Adipogenesis is a complex multi-step process through which preadipocytes convert into mature, lipid-containing adipocytes. It is essential to renew adipose tissue and support adipose dynamics since approximately 10% of our body's fat cells are regenerated each year.[16] Two adipogenesis phases have been recognized: pluripotent stem cells' commitment to a unipotent preadipocyte and terminal differentiation of preadipocytes into mature adipocytes. When pluripotent stem cells commit to the adipocyte lineage, they lose the ability to differentiate into other cell types and undergo morphological and functional changes due to numerous signaling pathways, transcription factors, and genes.[17]

Differentiation of 3T3-L1 preadipocyte fibroblast clonal cell line into mature fat cells is one of the most commonly used in vitro models to study adipose tissue biology. After induction of differentiation by proadipogenic factors, this line shows the morphology and gene expression of white adipose tissue.[18] Adipogenesis in vitro occurs in four steps: growth arrest, mitotic clonal expansion (MCE), early differentiation, and terminal differentiation.[19] After contact inhibition and growth arrest of post-confluent 3T3-L1 preadipocytes, the differentiation is induced by hormonal stimulation with insulin, dexamethasone, and 1-methyl-3-isobutyl-xanthine (IBMX).[20] Dexamethasone and IBMX directly induce genes responsible for the expression of adipogenic transcription factors, while insulin stimulates cells to take up glucose and store it in the form of triacylglycerol.[21,22] After adipogenic cocktail-induced differentiation, cells re-enter the cell cycle synchronously and undergo one or two mitosis cycles, regarded as MCE, when an irreversible commitment to differentiation occurs.[23] MCE is a crucial prerequisite for the differentiation process - if cells are prevented from entering the S phase during MCE, the expression of transcription factors and regulators of adipogenesis will not occur, and differentiation will be blocked.[24] Cell mitosis is required to unwind DNA

allowing transcription factors to access regulatory elements in genes involved in the differentiation process. Therefore, sustaining cells at one point in the cell cycle might be an effective way to block adipogenesis. Cyclin-dependent kinase (CDK) complexes with cyclin D and E are essential for retinoblastoma (Rb) phosphorylation and the reentry into the cell cycle. CDK inhibitors such as p21CIP and p27KIP1 are associated with cell-cycle-arrested preadipocytes, while their degradation results in G1/S phase progression.[25] During early differentiation, a morphological rounding of preadipocytes occurs, and the terminal phase of differentiation is characterized by lipid synthesis and transport, adipocyte-specific proteins secretion, insulin-associated metabolic processes activation, and mature lipid-loaded adipocytes morphology (Figure 1).[26]

Adipogenesis is strictly regulated by the transcriptional cascade and signaling pathways (Figure 1). During the early stages of differentiation, transient high expression of CCAAT/enhancer-binding proteins (C/EBP), C/EBPδ, and C/EBPβ occur. During the intermediate stage of adipogenesis, C/EBPβ/δ stimulate C/EBPα and peroxisome proliferator-activated receptor γ (PPARγ), key transcription factors of adipogenesis. PPARγ and C/EBPα cooperatively promote differentiation and induction of several adipocyte-specific genes, including lipoprotein lipase (LPL), adipocyte protein 2 (aP2), fatty acid synthase (FAS), and perilipin in the terminal stage of differentiation (Figure 1).[27]

Transcription Factors in Adipogenesis
CCAAT/Enhancer-Binding Proteins

C/EBPβ and C/EBPδ are the first transcription factors induced immediately after stimulation by adipogenic hormonal cocktails and play an important role in directing the differentiation process. The localization of C/EBPβ to the nucleus facilitates DNA-binding activity and leads to phosphorylation and transcriptional activation of PPAR and C/EBPα.[28] Decreased nuclear localization of C/EBPβ and disrupted DNA-binding activity inhibits C/EBPα and PPARγ gene expression, thereby suppressing adipogenesis. In C/EBPβ-null mice, adipogenesis is severely impaired, meaning that C/EBPα and PPARγ in the absence of C/EBPβ are not sufficient for complete differentiation.[29] Therefore, C/EBPβ inhibition could be one potential target for preventing or treating obesity, as decreasing early adipogenic transcription markers inhibits subsequent transcriptional cascade and suppresses terminal adipogenic differentiation.
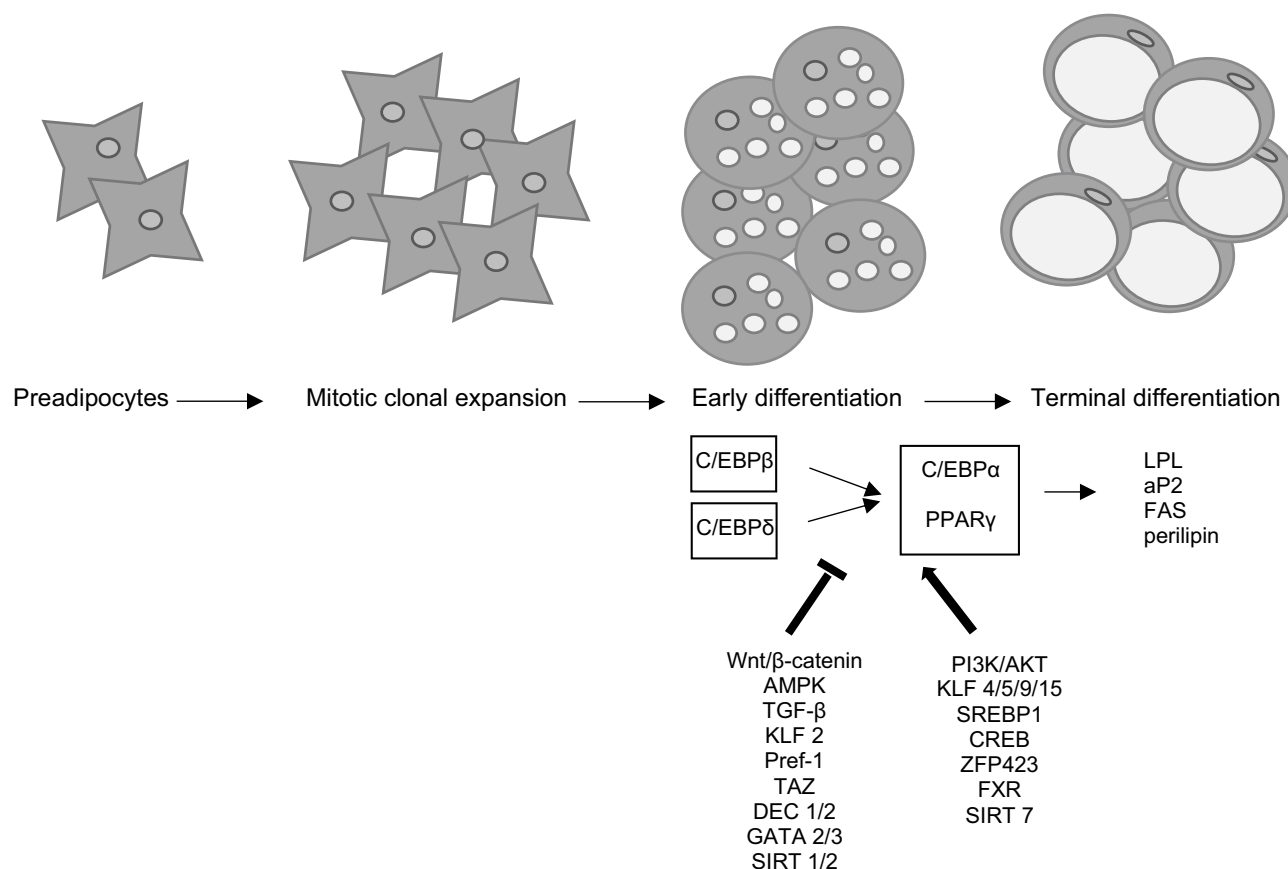
Figure 1 Stages and molecular regulation of adipogenesis.

Some C/EBP family members, such as the C/EBP homologous protein (CHOP), form inactive heterodimers with C/EBPα and C/EBPβ. Heterodimers cannot bind DNA and function as a dominant-negative inhibitor of gene transcription, suppressing adipocyte differentiation.[30] The direct inhibition of C/EBPβ activity by increasing the CHOP level can lead to PPARγ suppression and adipogenesis prevention.

Peroxisome Proliferator-Activated Receptor γ

PPARγ controls gene network expression in adipogenic differentiation, glucose and lipid metabolism, inflammation, and other physiological processes. Protein isoform PPARγ2 is abundantly expressed in the adipose tissue, and it is essential for adipogenesis and the maintenance of the differentiated state. Activation of PPARγ increases the number of small and insulin-sensitive adipocytes and up-regulates adiponectin, improving insulin sensitivity in the liver and muscle.[31] Given its crucial role in adipogenesis, PPARγ has been a critical target in developing anti-obesity drugs. PPARγ binds to specific DNA sequences as a heterodimer with the retinoid X receptor (RXR) and regulates target genes' transcription.[32] The activity of PPARγ can be regulated by binding agonist ligands, such as thiazolidinediones, which improve IR, enhance glucose uptake, and lower glucose concentration. They also exert anti-inflammatory effects, which is potentially important in treating obesity and T2DM.[33] In vitro studies showed that insulin and corticosteroids induce the expression of PPARγ mRNA,[34] whereas TNFα down-regulates the expression of PPARγ.[35] On the other hand, unliganded PPAR/RXR heterodimers interact with co-repressor molecules to repress the transcription of target genes, which means that the investigation of PPAR antagonists may be a logical approach for research of anti-obesity medication.

Signaling Pathways in Adipogenesis

Several signaling pathways are included in the adipogenesis process, such as the insulin and IGF-1-activated phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways, Wnt/β-catenin

signaling, AMP-activated protein kinase (AMPK) pathway, Hedgehog (Hh) signaling, and bone morphogenic protein (BMP) signaling pathways.

PI3K/AKT and MAPK/ERK Signaling Pathways

The hormonal induction cocktail activates PI3K/AKT and MAPK/ERK pathways during the early stage of differentiation. Intracellular MAPK signaling is important for regulating cell proliferation and differentiation, while ERK activation is essential for the induction of MCE and adipogenesis.[36] In 3T3-L1 preadipocytes, inactivation of the PI3K/AKT pathway inhibits adipogenesis, while activation of this pathway contributes to adipocyte differentiation.[37] However, different studies showed that MAPK pathways phosphorylate PPAR and reduce its transcriptional activity and that activation of MAPK antagonizes 3T3-L1 adipocytic differentiation.[38] Clear elucidation of MAPK/ERK signaling pathway effects on adipogenesis could provide another potential target of adipose tissue formation in obesity.

Wnt/β-Catenin Signaling Pathways

Wnt signaling maintains preadipocytes in an undifferentiated state by inhibiting PPARγ and C/EBPα. In 3T3-L1 preadipocytes, Wnt induction inhibits adipogenesis by dysregulation of the cell cycle and blocking PPARγ and C/EBPα expression, while disruption of Wnt signaling leads to adipogenic differentiation.[39] After adipogenesis induction, phosphorylated glycogen synthase kinase 3β (GSK3β) suppresses Wnt signaling by the degradation of β-catenin. Wnt signaling promotes β-catenin stabilization and nuclear translocation, causing the downstream inhibition of C/EBPα and PPARγ.[36] The stabilization and nuclear localization of β-catenin, as an important step in adipogenesis inhibition, might be a potential therapeutic target for the prevention/treatment of obesity.

AMP-Activated Protein Kinase Signaling Pathway

AMPK is a serine/threonine-protein kinase activated by phosphorylation of the α subunit when cellular ATP levels are decreased. When phosphorylated, AMPK promotes catabolic pathways such as fatty acid oxidation and inhibits energy-consuming pathways such as fatty acid synthesis.[10] Since adipogenesis can be considered an energy-consuming process, AMPK acts as a negative regulator of adipogenesis and can be considered a target for the treatment of obesity.[40] The anti-adipogenic effects of AMPK are mediated by suppressing PPARγ via positive regulation of p38 MAPK, which promotes PPAR phosphorylation and inhibits its transcriptional activity.[41] In the 3T3-L1 cell line, AMPK activation inhibits MCE and the expression of C/EBPα, PPARγ, and late adipogenic markers.[42]

Bone Morphogenic Protein Signaling Pathway

BMPs are members of the transforming growth factor-β (TGF-β) superfamily and display varied effects on adipogenesis, depending on BMP's concentration and type. Generally, TGF-β inhibits preadipocyte differentiation in vitro by interacting with C/EBP and repressing its transcriptional activity.[43] TGF-β phosphorylates Smad 2 and Smad 3, and BMP subfamily phosphorylates R-Smads (Smad 1, 5, 8). Phosphorylated R-Smad binds to Smad 4, facilitating the migration into the nucleus and transcriptional activity of the Smad protein. BMPs can also activate the p38MAPK signaling cascade, which regulates mitochondrial biogenesis and insulin-dependent glucose uptake.[44] Some findings suggest a cross-talk between BMP signaling and PPARγ action, which may be explained by the ability of BMP-2 to upregulate PPARγ expression.[45]

Hedgehog Signaling Pathway

Activation of the Hh signaling pathway impairs adipogenesis and lipid accumulation. This pathway is down-regulated during human adipocyte differentiation. Hh protein inhibits adipogenesis by reducing the expression of C/EBPα, PPARγ, and aP2, whereas inhibition of Hh signaling using increases adipogenic differentiation in 3T3-L1.[46] Nearly complete inhibition of adipocyte differentiation is shown by Oil Red O staining, a 40% reduction in triglyceride accumulation, a 50% decrease of adipocyte marker expression, and upregulation of preadipocyte factor-1 (Pref-1).[47] Adipocyte differentiation inhibition occurs even after one hour of Hh stimulation concomitant with adipogenic induction, suggesting that Hh signaling is maintained several days after its stimulation or controls critical early steps of differentiation.[48]

Other Regulators of Adipogenesis
Positive Regulators of Adipogenesis

Several factors are identified as positive regulators of adipogenesis, such as the Kruppel-like factor family (KLF), sterol regulatory element-binding protein 1 (SREBP1), cyclic AMP response element-binding protein (CREB), zinc finger protein 423 (ZFP423), and farnesoid X receptor (FXR). Decreasing adipogenic transcription factors might be a good approach to inhibit adipose tissue development during obesity progression.

Several KLF zinc-finger transcription factors are induced during adipogenesis in 3T3-L1 preadipocytes.[31] KLF4 is an early marker of adipogenesis, expressed within the first 30 min after exposure of 3T3-L1 cells to an adipogenic cocktail.[49] KLF5 is induced by C/EBPδ/β during the early stages of adipogenesis, binds directly to the PPARγ2 promoter, and cooperates with C/EBPs.[22] Inhibition of KLF9, which is usually up-regulated during the middle stage of differentiation and binds directly to the PPARγ2 promoter, inhibits adipogenesis.[50] Inhibition of KLF15 also leads to reduced expression of PPARγ, blocking adipogenesis in 3T3-L1 preadipocytes.[51] SREBP1 regulates the expression of FAS and LPL and increases the activity of PPARγ, augmenting adipogenesis.[52] The expression of CREB is stimulated by the adipogenic cocktail, which can be sufficient to initiate lipid accumulation and the expression of PPARγ and fatty acid-binding protein (FABP).[53] ZFP423 can promote adipogenesis of non-adipogenic NIH 3T3, while its inhibition in 3T3-L1 cells blocks PPARγ expression and adipogenic differentiation.[54] Furthermore, in bovine stromal vascular cells, ZFP423 increases lipid accumulation and expression of PPARγ and C/EBPα.[55] FXR is a nuclear hormone receptor that promotes adipocyte differentiation by partially inducing PPARγ2 and C/EBPα expression. After RXR heterodimerization, the agonist ligand activates FXR, which then binds to FXR response elements and regulates target gene expression.[56] Therefore, investigating FXR activity inhibition by antagonist ligands for FXR can be considered for fighting obesity.

Negative Regulators of Adipogenesis

On the other hand, high and sustained levels of KLF2, Pref-1, transcriptional-coactivator with PDZ-binding motif (TAZ), differentiated embryo chondrocyte (DEC), GATA transcription factors, and histone deacetylase Sirtuin 1 (SIRT1) inhibits adipogenesis and keeps cells at the pre-adipocyte stage. Targeting these factors is a potentially effective therapeutic approach to intervene in obesity.

KLF2 represses adipogenesis by inhibition of PPARγ, C/EBPα, and SREBP1 expression in 3T3-L1 preadipocytes.[57] Pref-1 is highly expressed in preadipocytes and inhibits adipocyte differentiation by preventing lipid accumulation and expression of PPARγ, C/EBPα FAS, and aP2.[58] TAZ suppresses adipocyte differentiation via PPARγ activity repression, and its diminished expression enhances adipogenic differentiation.[59] DEC 1 and 2 are abundantly expressed in preadipocytes and down-regulated during

adipogenesis. They inhibit the transcriptional activity of C/EBPβ/α.[60]

The GATA family of transcription factors play important roles in a variety of biological processes, including adipogenesis. GATA-2 and GATA-3 are predominantly present in white adipose tissue and significantly contribute to adipocyte differentiation regulation. Their constitutive expression inhibits adipogenesis by trapping cells in the preadipocyte stage. This effect could be a result of direct suppression of PPARγ expression through PPARγ promoter or the formation of protein complexes with C/EBPα and C/EBPβ.[61] GATA2 and GATA3 are down-regulated during the terminal differentiation process, and their defective expression is associated with obesity.[62] These findings indicate the crucial role of GATA transcriptional factors during terminal adipocyte differentiation and their potential as targets for the therapeutic intervention of obesity.

Sirtuins (SIRT) and miRNAs are recently discovered modulators of adipogenesis with various effects on adipocyte differentiation progression. SIRT1 is an NAD+-dependent nuclear deacetylase that acts as a negative modulator of adipogenesis in the 3T3-L1 cell line. SIRT1 attenuates adipogenesis by interacting with its cofactors, nuclear receptor co-repressor (NCoR), and the silencing mediator of retinoid and thyroid hormone receptors (SMRT).[63] SIRT1 binds to the same DNA sequences as PPARγ, acting as its co-repressor. SIRT1 can also induce BAT-specific genes while repressing visceral WAT genes and induce the expression of genes involved in fatty acid oxidation.[64] Additionally, SIRT2 exerts an inhibitory effect on adipogenesis by FOXO1 deacetylation and subsequent PPARγ transcriptional activity repression.[65] On the other hand, SIRT7 was shown to be important for PPAR expression and proper adipocyte differentiation. Therefore, activation of SIRT 1 and 2 or inhibition of SIRT7 during adipogenesis could provide a novel therapeutic strategy for obesity. However, it is difficult to find a single compound that would activate some SIRTs while inhibiting others, and it is hard to obtain tissue action specificity.

miRNAs are small non-coding RNAs that bind to specific target mRNAs to promote their degradation or prevent their translation.[66] They have recently been in the focus of obesity research as potent regulators of the post-transcriptional expression of crucial genes in adipogenesis. Some miRNAs (miR-21, miR-29b, miR-144-3p, miR-148a, miR-210, miR-205-5p) enhance adipogenesis by interfering with the expression of anti-adipogenic signaling pathways (TGF-β, TNF-α, Wnt, co-repressors of C/

EBPα). On the other hand, some miRNAs inhibit adipocyte differentiation (miR-27a and b, miR-31, miR-128-3p, miR-130a and b, miR- 146a-5p, miR-155, miR-540) by directly targeting C/EBPs and PPARγ.[11] Additionally, some miRNAs, such as miR-30a, play an important role in mature WAT biology. Lower levels of miR-30a in subcutaneous WAT correlate with IR, while its overexpression improves insulin sensitivity and energy expenditure.[67] miR-30a also exerts an anti-inflammatory effect by targeting signal transducer and activator of transcription 1 (STAT1). Moreover, miR- 103 and miR-107 can regulate the size of the preadipocyte population in WAT, directly suppressing the expression of Wnt3a, an activator of the Wnt/β-catenin pathway, thus promoting stress-mediated apoptosis in preadipocyte.[68] In addition, the miR-128 was found to be one of the most upregulated miRNAs in hypoxic 3T3- L1 adipocytes. It directly targets the 30-UTR sequence of the INSR gene, reducing the expression of plasma membrane tyrosine kinase receptor protein INSR through which insulin exerts its biological effects. It was shown that obesity-induced hypoxia increases the expression of miR-128, which then negatively affects INSR mRNA and protein expression levels in adipose tissue of both obese patients and high-fat diet-fed mice, correlating with the decrease in INSR expression. This result was consistent with the miR-128 expression level in hypoxic 3T3-L1 adipocytes.[69]

miRNAs could be used as clinical biomarkers to predict the development of obesity and related complications or to assess therapeutic anti-obesity strategies' effectiveness due to the normalization of miRNAs levels upon acute weight loss.[70] miRNAs can also regulate SIRTs activity, so there is a possibility to modify the action of SIRTs by specific miRNAs for treating obesity. miR-34a, miR-146b, and miR-181a are negative regulators of SIRT1, and SIRT7 is a metabolic target for miR-93, a negative regulator of adipogenesis.[71] However, safely targeting specific tissue and avoiding side effects using miRNA therapeutics is still a challenge.

Therapeutic Approach – Conventional Medicine with Effects on Adipogenesis

Obesity treatment aims not only for weight reduction but also a reduction of obesity-related complications. The most commonly used therapeutic strategies include lifestyle modification, calorie restriction combined with increased physical activity, while bariatric surgery is limited to morbidly obese patients.[72] Long-term effects of lifestyle modification are frequently disappointing, with 90% of the people returning to their original weight within two to five years.[36] Although anti-obesity drugs might be a promising solution, their limited efficacy, together with possible side effects and drug interactions, highlights a constant need to find novel, efficient, and safe anti-obesity ingredients. For that purpose, it is necessary to identify pathogenic molecular mechanisms that could serve as suitable therapeutic targets.

To observe available medications for treating obesity, one has to bear in mind the connection of obesity with metabolic syndrome and the risk for developing other chronic diseases such as T2DM and cardiovascular disease. Although different pathophysiological mechanisms lead to metabolic syndrome, it is associated with obesity through dysfunction of adipose tissue as the main contributor to obesity and subsequent complications. Disturbed metabolic homeostasis leads to visceral obesity, atherogenic dyslipidemia, arterial hypertension, and IR.[73] Since adipose tissue dysfunction mainly presents as obesity and dyslipidemia, currently approved medications for obesity-related conditions also affect adipose tissue functions and adipogenesis, and knowing the underlying mechanisms of these effects has great clinical value.

Statins

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors that inhibit the conversion of HMG-CoA to mevalonic acid in a competitive manner. They lower total cholesterol, LDL cholesterol, and triglyceride levels while increasing HDL cholesterol levels.[74] In adipose tissue, they enhance lipolysis and decrease lipid accumulation in mature adipocytes, increase mRNA LPL expression in preadipocytes, preventing adipocyte hypertrophy by increasing the number of small adipocytes.[75] Their effect on adipogenesis in vitro goes through down-regulation of C/EBPα, PPARγ, SREBP1, leptin, FABP4, and adiponectin.[76]

Fibrates

Fibrates increase LPL and fatty acids hepatic uptake while reducing hepatic triglyceride production and increasing HDL cholesterol levels. Fibrates act as synthetic ligands for PPARα increasing fatty acids hepatic B-oxidation, LPL activity, and VLDL clearance.[77] Through PPARα stimulation and upregulation of fatty acid oxidation enzymes in adipose tissue, fibrates can decrease body weight and reduce plasma leptin concentration.[76] They also can

increase energy expenditure and even induce the browning of WAT adipocytes.[78] Binding directly to PPARα, fibrates induce adipogenesis and forming small and numerous lipid droplets in adipocytes.[79]

Niacin

Niacin is the most effective for increasing HDL levels and decreases total cholesterol, LDL, triglyceride synthesis, and fatty acid re-esterification.[80] It stimulates adipogenesis throughout increasing PPARγ, FABP4, adiponectin, and leptin expression in 3T3-L1 cells.[81]

Metformin

Metformin is the most commonly used drug to treat T2DM. It suppresses hepatic glucose production and improves adipose tissue metabolism in the liver and muscle, leading to decreased plasma glucose levels.[82] Metformin reduces lipolysis, increases glucose uptake into skeletal muscle cells, increases intestinal glucose utilization, and improves pancreatic β-cell survival.[83] Metformin has a pleiotropic effect in reducing appetite, preventing cardiovascular diseases, improving endothelial function, modulation of inflammation, and cancer prevention.[84] In addition to all of the above, metformin also leads to weight loss severity, although this effect's mechanism is still unknown. The effect of metformin on endocrine adipose tissue function is still a matter of debate, given the fact that it has been shown more effective in obese patients with TMD2 than in those with lower body mass index.[85] Metformin is known to act through AMPK, whose activation inhibits adipogenesis in 3T3-L1 cell culture.[42] Metformin interferes with oxidative phosphorylation in mitochondria and stimulates osteogenic differentiation via AMPK, which is known to promote osteogenic and suppress adipogenic differentiation, although the exact mechanism of this action is unknown.[86]

Liraglutide

Liraglutide was firstly approved as an antidiabetic drug and, but in higher doses, it showed anti-obesity effects. As a glucagon-like peptide 1 receptor agonist, it reduces food ingestion and appetite and can also slow gastric emptying while showing anti-adipogenic, antilipogenic, and prolipolytic effects in human mature adipocytes.[70] Liraglutide reduces total fat mass and can change fat depots' regional distribution.[87] Downregulation of AKT and PI3K pathways and upregulation of AMPK decreases lipogenesis in WAT, reducing lipid storage.[88] Liraglutide also increases energy expenditure by increased thermogenesis.[89] In 3T3-L1 cells, it induces PPARγ, C/EBPβ/δ, and modulates ERK1/2,

PKCB, and AKT pathways.[90] However, in human adipocytes, liraglutide exerts an anti-adipogenic effect through the cAMP pathway. Binding directly to GLP-1R decreases adipogenesis and lipogenesis-related genes while increasing the expression of the lipolytic ones.[91]

Bioactive Molecules with Effects on Adipogenesis

Natural products have played an important role in alleviating a number of health problems throughout history and remained a large portion of pharmaceutical agents nowadays. For example, over 60% of the current anticancer drugs were derived from natural sources.[92] Biomolecules serve as models for the preparation of efficacious analogs, which results in more effective and less toxic targeted therapies. Furthermore, natural products help identify bioactive compounds as a basis for developing anti-inflammatory drugs. The best example is polyphenolics that modulate the inflammatory pathways and serve as biomarkers that can be used to prepare therapeutic agents for treating inflammatory disorders.[93] Natural products have also become a part of our daily diet. Moreover, some antidiabetic agents, such as widely used drug metformin, have been developed from natural sources. Many plants are used traditionally as a part of diabetes treatment throughout the world, and their efficacy and safety have been validated through clinical use over the years. They are commonly considered to be less toxic and with fewer side effects than synthetic medicines.[94] In addition, a variety of natural plants, functional fatty acids, and other natural dietary compounds are ingredients of current anti-obesity products or in consideration as potential ingredients for future anti-obesity products. Active compounds of natural products are mostly derived from plants, including fruits, vegetables, grains, and herbs containing a high amount of phytochemicals, fibers, and unsaturated fatty acids.[95] Mechanisms of action of natural products include interfering with nutrient absorption, decreasing adipogenesis and increasing thermogenesis, suppressing the appetite, and modifying the intestinal microbiota composition.[96]

Recently, numerous studies have investigated the inhibitory effects of natural products on adipocyte differentiation and lipid accumulation. Possible anti-obesity effects of natural products include the induction of apoptosis, cell-cycle arrest or delayed progression, and interference with transcription factor cascade or intracellular signaling pathways during the early phase of adipogenesis. The

following sections briefly discuss some of the natural products, while others are listed in Table 1, grouped by their mechanism of action.

Delphinidin

Delphinidin is an anthocyanin found in pigmented fruits and vegetables with antioxidant, anti-inflammatory, anti-atherosclerosis, and anti-cancer bioactivities. It downregulates the expression of adipogenesis and lipogenesis markers, inhibits lipid accumulation, and upregulates fatty acid metabolism gene expression in 3T3-L1 adipocytes.[97] In addition, the activation of Wnt and nuclear translocation and stabilization of β-catenin decreases lipid accumulation in 3T3-L1 cells. Additionally, delphinidin up-regulates p21CIP or p27KIP1 expression, decreases C/EBPβ expression, and suppresses GSK3β expression.[98]

Genistein

Genistein, an isoflavone found in legumes, by enhancing CHOP blocks the DNA binding and transcriptional activity of C/EBPβ in 3T3-L1 preadipocytes, thus inhibiting protein expression of PPARγ and C/EBPα.[99] Other studies involving 3T3-L1 cells showed that genistein inhibits adipocyte formation through activation of AMPK, induces apoptosis in mature adipocytes,[100] inhibits the phosphorylation of P38 MAPK, and prevents C/EBPα expression.[101] Genistein also suppresses hormonal-induced proliferation and blocks cell entry into the S phase and the S to the G2/M phase transition.[102] In primary human adipocytes, genistein inhibited aP2, SREBP 1, and FAS.[103] In human adipose tissue-derived mesenchymal stem cells, adipogenesis inhibition was associated with the Wnt/β-catenin signaling pathway through the expression of Wnt3, adipogenesis inhibitor, the inhibition of Wnt signaling antagonists, and increase of mRNA and protein levels of β-catenin.[104]

Guggulsterone

Plant sterol guggulsterone by FXR antagonism exhibits cholesterol-lowering activity and prevents preadipocyte differentiation.[52] In 3T3-L1 cells, cis-guggulsterone downregulates PPARγ2, C/EBPα, and C/EBPβ.[105] In addition, the combination of guggulsterone with genistein exerts anti-adipogenic effects more potently than individual compounds.[106] Recently it was shown that guggulsterone exerts its anti-obesity effects by inducing beiging of adipocytes through mitochondrial biogenesis and an upregulation of UCP1 and cellular oxygen consumption in 3T3-L1 preadipocytes.[107]

Berberine

Berberine is an isoquinoline derivative alkaloid that stimulates weight loss, increases insulin sensitivity, lowers blood glucose, and improves lipid metabolism. It increases the mRNA expression of adiponectin and decreases the secretion of leptin and resistin.[108] Although one recent randomized controlled trial reported no change in BMI and body weight, it reported a reduction in hemoglobin A1C (HbA1c) and triglyceride levels.[109] During adipogenesis berberine affects PPARγ transcriptional activity, mRNA, and protein levels in 3T3-L1 cells by interfering with the C/EBPβ signal.[110] Berberine can also increase GATA-2 and GATA-3 mRNA and protein expression in 3T3-L1 cells and reduce expression of PPAR and C/EBPα mRNA and weight gain in high-fat diet-induced obese mice.[111] Other anti-adipogenic mechanisms of berberine include phosphorylation of p38/AMPK, deactivation of PPARγ,[112] and upregulation of DEC2 mRNA levels.[113]

Curcumin

Curcumin, a polyphenol from an Asian spice herb Curcuma longa, exerts anti-adipogenic effects through activation of Wnt/β-catenin signaling in 3T3-L1 cells. During differentiation, curcumin restores β-catenin's nuclear translocation suppresses the expression of β-catenin destruction complex members and increases mRNA levels of cyclin D1.[114] Other anti-adipogenic mechanisms of curcumin include activating AMPK, down-regulating PPARγ transcription,[115] decreasing phosphorylation of MAPKs, altered abundance or phosphorylation of GSK3β,[114] upregulation of KLF4 and KLF5, blocking cell entry into the S phase and the S to the G2/M phase transition, decreasing C/EBPβ expression and suppressing adipogenic cocktail-induced proliferation.[116] In vivo study using mice on high fat diet showed that 500 mg/kg dietary curcumin significantly decreased weight, total body fat and serum cholesterol compared to untreated mice.[96]

Epigallocatechin Gallate

Green tea polyphenol Epigallocatechin gallate (EGCG) is known to possess antiproliferative, antioxidant, antibacterial, and chemopreventive effects.[117] Other health benefits induced by green tea extracts include anti-hypertensive and insulin-sensitizing effects. In humans, EGCG exerts anti-obesity effects through ghrelin secretion inhibition, adiponectin levels increase, appetite control, nutrient absorption decrease, and adipogenesis inhibition.[118] It suppresses adipocyte clonal expansion and inhibits adipogenesis in 3T3-L1

Table 1 Phytochemicals and Their Effect on Adipocyte Differentiation Process

Mechanism of Action	Phytochemicals
Suppressing adipogenic cocktail-induced proliferation	Dehydroleucodine;[143] caffeine;[144] apigenin;[145] rhamnetin;[146] fisetin;[147] sulforaphane;[148] vitisin A[149]
Increasing the cell population in the G0/G1 phase	Dehydroleucodine;[143] apigenin;[145] sulforaphane;[148] vitisin A;[149] sinigrin;[150] bisdemethyoxy-curcumin;[151] curcumin-3,4-dichloro phenylpyrazole;[152] ellagic acid;[153] oleuropein and hydroxytrosol[154]
Arresting the cell cycle at the G1–S checkpoint	Curcumin-3,4-dichloro phenylpyrazole;[152] cocoa;[155] caffeic acid phenethyl ester[156]
Blocking the cell entry into the S phase and the S to G2/mitosis (M) phase transition	Turmeric;[151] piceatannol;[157] dieckol[158]
Upregulating p21CIP and/or p27KIP1 expression	Dehydroleucodine;[143] caffeine;[144] apigenin;[145] sulforaphane;[148] vitisin A;[149] sinigrin;[150] bisdemethyoxy-curcumin;[151] curcumin-3,4-dichloro phenylpyrazole;[152]
Decreasing the phosphorylation of Rb	Sulforaphane;[148] vitisin A;[149] ellagic acid;[153]
Upregulating mRNA and protein expressions of cyclin D1	Coumestrol[159]
Decreasing C/EBPβ expression	Dehydroleucodine;[143] apigenin;[145] piceatannol;[157] dieckol;[158] Rehmannia glutinosa[160]
Decreasing C/EBPβ centromeric localization, DNA-binding activity, and phosphorylation	Apigenin;[145] sinigrin;[150]
Blocking C/EBPβ-induced expression of PPARγ	Retinoic acid[161]
Decreasing mRNA expression and activity of PPARγ	Piperine[162]
Antagonizing PPARγ/RXRα heterodimerization	7-chloroarctinone-b[163]
Suppressing AKT activation	Dehydroleucodine;[143] caffeine;[144] sulforaphane;[148] bisdemethyoxy-curcumin;[151] curcumin-3,4-dichloro phenylpyrazole;[152] cocoa;[155] caffeic acid phenethyl ester;[156] piceatannol;[157] dieckol;[158] coumestrol[159]

(Continued)

Table 1 (Continued).

Mechanism of Action	Phytochemicals
Decreasing the phosphorylation of MAPKs	Sulforaphane;[148] sinigrin;[150] bisdemethyoxy-curcumin;[151] cocoa;[155] caffeic acid phenethyl ester;[156] piceatannol;[157] dieckol[158]
Inhibiting the phosphorylation of ERK	Water extract of Hibiscus sabdariffa L.[164]
Maintaining β-catenin nuclear level and increasing cyclin D1 level	Shikonin[165]
Alteration of GSK3β abundance and/or phosphorylation	Caffeine;[144] coumestrol[159]
AMPK activation	Dieckol;[158] ginsenoside Rh2[166]
Suppressing mRNA expression of KLF2	Caffeine[144]
Upregulating KLF4 and KLF5 expression	Dieckol[158]
SIRT1 activation	Indole-3-carbinol[167]

adipocytes via the PI3K/AKT and MAPK/ERK signaling pathway.[119] Moreover, EGCG blocks cell entry into the S phase and the S to G2/M phase transition and suppresses adipogenic cocktail-induced proliferation.[120] In other studies, using 3T3-L1 cells, EGCG reduced triglyceride accumulation and inhibited the expression of PPARγ and C/EBPα,[121] up-regulated phosphorylation of AMPK and the expression of key Wnt signaling related genes,[120] and increased energy expenditure and thermogenesis.[122] In vivo studies on high fat diet-induced obese mice showed that EGCG reduced body weight and plasma lipids, decreased PPARγ, C/EBPα, SREBP1, aP2, LPL, and FAS in WAT, increased the expression of key genes for lipolysis, β-oxidation and thermogenesis,[123] and attenuated fat tissue formation and body weight gain.[124]

Oxysterols

Oxysterols are oxidized cholesterol metabolites capable of inhibiting the expression of key adipogenic transcripts and adipogenic differentiation. In mouse bone marrow stromal cells, oxysterols inhibited PPARγ2 expression and adipogenic differentiation via Hh signaling pathway;[125] reduced expressions of key adipogenic transcripts through Hh and ERK signaling in hen preadipocytes;[126] and reduced expression of PPARγ2, LPL, and aP2 in MSCs.[127]

Resveratrol

Resveratrol (RSV) is a member of the natural polyphenols found in peanuts, groundnuts, grapevines, and red vines.[128] It is beneficial for human health due to the cardioprotective effect, anti-inflammatory, and anti-cancer properties.[129] In studies using 3T3-L1 cells, resveratrol treatment significantly decreased lipid accumulation, down-regulated the expression of C/EBPα, LPL, FAS, and SREBP-1c through activation of AMPK,[130] decreased phosphorylation of MAPKs, and reduced Rb phosphorylation,[131] suppressed hormonal-induced AKT activation and cell proliferation, and inhibited cell entry into the S phase and the S to the G2/M phase transition.[132] RSV is the first polyphenolic compound that activates SIRT1, inhibiting proliferation and adipogenic differentiation in human preadipocytes.[133] A reformulated version of RSV was tested on obese patients where it showed a reduction of blood pressure, serum glucose, and triglyceride levels and increased the number of small adipocytes in subcutaneous adipose tissue.[134] In another study on patients with T2DM, RSV increased SIRT1 and AMPK expression in muscles.[135] However, its prophylactic administration to non-obese individuals showed no significant effect on insulin sensitivity, blood pressure, and body composition.[136] The effect of RSV in humans still needs further investigations using larger cohorts and longer follow-up time.

Gum

Dietary fibers have recently been recognized as an important ally in the management of hypertension and obesity. Fibers contribute to weight loss, increase insulin sensitivity, have glucose-lowering effects, reduce lipid levels and blood pressure. Dietary gum is rich in fibers and can decrease body weight and blood pressure. Some of the mechanisms include decreased energy intake and adipose tissue accumulation, and downregulation of PPAR-γ. One meta-analysis of 21 studies with 990 participants summarized results on decreased body weight and waist circumference without effect on BMI. That effect was better with shorter administration of ≤15 g/day gum intake.[137]

Phytosterols

Phytosterols are components of the cell membrane in plants structurally similar to cholesterol. The primary dietary sources of phytosterols are vegetable oils, nuts, fruits, and seeds. They have been shown to lower cholesterol absorption in animals, possibly due to the chemical similarity with cholesterol causing cholesterol absorption

inhibition.[138] One study showed that glucose levels decreased and insulin levels increased after oral administration of ß-sitosterol in hyperglycemic rats.[139] Several meta-analyses have concluded that phytosterols use decreases obesity indices, reduces serum lipoprotein and free fatty acid, and improves the lipid profile in humans. A plausible mechanism regarding favorable effects in diabetes might be related to the expression and translocation of GLUT-4 in the skeletal muscle, liver, and white adipose tissue. In vitro studies showed stimulation and phosphorylation of the AMPK pathway enhancing GLUT-4 translocation and expression.[140]

Natural Products and Potential Risks

As the global use of herbal medicinal products increase, and approximately 80% of people today depend upon herbal medication as a component of their primary health care, concerns about their safety and efficacy are more recognized. Only medicines have to be proven safe before being released into the market, and herbal products do not fall under that category. They are readily available in the market without prescription without control for purity and potency. The general perception that herbal remedies are natural, safe, and have fewer side effects is misleading. Knowledge of potential adverse effects are limited as herbal medicines are untested, and their use and effect are not monitored.[141] People often do not consider how natural products may interact with any prescription drug they are taking or with each other. Herbs have been shown to produce a wide range of adverse reactions if taken irregularly, in excessive amounts, or in combination with some medicines. In general, extensive biochemical monitoring and follow-up are necessary for patients taking herbal preparations regularly.[142]

Conclusion

Over the last few years, adipogenesis is considered an important factor in the pathophysiology of obesity and obesity-related complications. As a complex process involving various transcriptional cascades, signaling pathways, and molecular mechanisms, significant effort has been made to identify the most suitable adipogenesis regulators to serve as potential therapeutic targets. Most studies are carried out in vitro, revealing additional mechanisms through which conventional medications that are already in use for treating dyslipidemia or T2DM can also affect adipogenesis. Despite the increase in the number of available drugs, obesity prevalence is still rising. That is why several natural products are

being investigated as anti-obesity agents due to their ability to reduce adipose tissue mass through inhibiting adipogenesis. More evidence indicates that natural products can target more than one known pathway or transcriptional cascade involved in adipogenesis. Our understanding of adipogenesis and its key modulators is still limited. The goal is to increase knowledge on the key adipogenesis determinants' function and relevance to adipose tissue pathophysiology, resulting in new targets for anti-obesity drug development without compromising patients' health. There is still a need to investigate further potential treatment in vivo since their clinical translation is still limited. More studies that will elucidate the best ways for interfering with adipocyte formation, and give precise data on the safety and metabolism of potential anti-obesity treatment, will make adipogenesis molecular mechanisms powerful targets in fighting obesity.

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Disclosure

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