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Review

Adipogenesis, Role of Adenosine Monophosphate-Activated Protein Kinase (AMPK) and Use of Plants Products in Combating Obesity

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Abstract: The increasingly widespread emergence of obesity has become a matter of critical concern around the world due to its association with common morbidities including cancer, cardiovascular diseases and diabetes. Over-nutrition and the sedentary lifestyle are considered as the most significant causes of obesity: appropriate lifestyle and behavior interventions are the corner stones of successful weight loss, but to maintain such a lifestyle is highly challenging. There is therefore an urgent need to develop innovative non- or minimally-toxic means to combat obesity. Accordingly, ample natural products from plants (either as crude extracts or purified phytochemicals) have been scrutinized for their anti-obesogenic properties because they are believed to be non-toxic and cost-effective, and frequently well-accepted by patients because of their traditional use. In this review, we will discuss adipose tissue and adipogenesis, signaling pathways involved in the regulation of adipogenesis, role of energy sensor protein of the body AMPK, and recently reported plant products in the management of obesity. We will provide a common platform for understanding obesity, and a possible mechanism of action for anti-obesogenic plant products through activated AMPK, which will be helpful in the scientific development of traditional herbal medicine.

Keywords: adipogenesis; AMPK; plant products; anti-obesogenic; beige/brite adipocytes; natural products; phytochemicals

1. Introduction

Obesity is an increasingly prevalent disorder around the globe promoted by genetic, nutritional, and environmental factors. However, energy imbalance is always a key factor: the excessive consumption of calories compared to utilization results in obesity [1–3]. Obesity is a multifactorial chronic disease, linked to other disorders including type-2 diabetes, insulin resistance, cancer, and cardiovascular diseases [4,5]. Obesity and overweight are the fifth leading cause of death worldwide [3]. According to the World Health Organization (WHO), in 2016 more than 1.9 billion adults were overweight, of whom 650 million were obese, with approximately 2.9 million adults dying each year due to being obese or overweight [6]. Previously, obesity was the concern only of countries with

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higher incomes, but now it is also accelerating in middle and low-income countries [7]. Two causes have been proposed as responsible for this global trend: increased intake of food low in minerals, micronutrients, vitamins, but high in salts, fats and sugars; and a rise in the sedentary lifestyle permitted by easy modes of transportation, office work, and an increase in urbanization. Although, the sedentary lifestyle is considered to be directly linked with obesity, [8] other factors including depression, and social and monetary issues might also contribute [9]. For example, insufficient sleep disturbs the balance of hormones responsible for hunger (leptin and ghrelin) thus increases appetite, ultimately leading to the accumulation of excessive fats in the body [10].

Sedentary lifestyle and other environemntal risk factors discussed above are not the only causes of obesity– mutations in the genetic make up can also lead to the condition [11]. Mutation in the genes such as Leptin (*LEP*), leptin receptor (*LEPR*), prohormone convertase 1 (*PCSK1*), melanocortin 4 receptor (*MC4R*), proopiomelanocortin (*POMC)*, single-minded homolog 1 (*SIM*), and brain derived neurotrophic factor (*BDNF*) have been observed to be associated with obesity [11,12].

Although, it is evident that there are many factors which contribute to obesity, it is also clear that the end product of all these factors is the accumulation of lipids in adipocytes. So adipocytes, their proteins, transcriptional factors and signaling pathways such as Wnt, Notch, Hedgehog signaling pathways are involved in the progression/inhibition of obesity. Consequently, they are all targets of interest for scientific and pharmaceutical industries in the discovery of novel anti-obesogenic drugs with minimal adverse effects. Drugs with anti-obesogenic properties have been extensively studied for some time [7].

Physiological interventions such as exercise and dieting are the preferred ways to fight weight gain, but due to the demands of modern life these activities seem to be difficult to practice and maintain. Therefore, there is also a need for pharmacological intervention - demand for anti-obesogenic drugs and weight loss therapies has escalated and accelerated the development of pharmaceutical industries across the globe [7]. Although these pharamacological drugs help in the management of weight loss by altering processes such as absorption of calories, altering appetite, regulating metabolism of the body etc., their related adverse effects hinder their usage and limit their positive beneficial effects [3]. For example, Sibutramine which inhibits appetite by deactivating neurotransmitters within the brain was withdrawn in 2010 from the market of USA and Canada due to adverse effects on the cardiovascular system resulting in heart attacks and strokes. In the same year, Xenical, another weight loss drug, was withdrawn and sent for revised labelling due to reported adverse effects on the liver [7]. Some others commercially available pharmacological drugs including Metformin, Exenatide, Rimonabant and Pramlintide have also reported to cause abdominal pain, sleeplessness, restlessness, cardiovascular problems, and faulty bowel movements [2]. These adverse effects of synthetic drugs highlight the potentially lower risk associated with the use of natural products which have been consumed for hundreds of years. An ideal weight loss drug should be able to combat weight gain with little or no side effects [13] and pharmacological anti-obesogenic drugs should be prescribed only if their beneficial effects outweigh the adverse effects. As a result, there is a growing emphasis on natural products due to their effectiveness and minimal or complete lack of side effects. Plant-based products, either as standardized extracts or as pure compounds, have provided innumerable opportunities for the discovery of new drugs due to their wide chemical diversity [14].

The aim of this review is to understand the causative key factors (proteins and signaling pathways) involved in the regulation of obesity, and ennumerate (from 2014 onwards) naturally occuring anti-obesogenic bioactive compounds from edible and medicinal plants. Moreover, the

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main targets (mechanism of action) of these naturally occurring anti-obesogenic plant products and in particular the role of activated energy sensor protein '5′adenosine monophosphate-activated protein kinase' (AMPK) in the management of obesity will be discussed. But before heading towards those details it is vital to understand adipocytes and their differentiation process, adipogenesis.

2. Adipose Tissue and Adipogenesis

Adipose tissue is one of the most complex organs, containing adipocytes, immune cells, pre-adipocytes and fibroblasts; it secretes hormones (estrogen, leptin), adipokines and has regulatory roles in endocrine, immune and metabolic systems [15,16]. Adipose tissue is critical for the homeostasis of energy and generally performs three different tasks. Firstly, it is the hub for the synthesis and storage of triglycerides. Secondly, in a situation of increased energy demands, the stored triglycerides are hydrolyzed, and the ensuing free fatty acids are then released into circulation. Thirdly, it acts as endocrine organ, releasing adipokines [17]. Adipose tissue is widely considered as the main therapeutic target of many natural and synthetic anti-obesogenic products. Adipose tissue is divided into subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT). Located centrally or subcutaneously, adipose tissue plays a key role in the survival of an individual because it is the main source of fatty acids for the production of heat and energy. Adipocytes or fat cells are the lipid-laden cells of adipose tissue that acquire the ability to accumulate lipids post differentiation (a process in which the cells from a common ancestor are derived mitotically and become different from one another in morphology and function). This differentiation of adipocytes is determined by the expression of genes which dictate the phenotype of adipocytes. The proteins involved in differentiation then direct the cells to perform their characteristic functions and are also critical for differentiation of pre-adipocytes into mature adipocytes [18]. Collectively, this process of pre-adipocytes differentiating into mature adipocytes is known as adipogenesis. Increase in the size (hypertrophy) and number (hyperplasia) of white adipose tissue (WAT) during adipogenesis (e.g. due to excessive energy intake accompanied by low energy expenditure) may lead to obesity [18,19]. During the process of adipogenesis, the multipotent mesenchymal stem cells (MSCs) are transformed into pre-adipocytes before undergoing secondary differentiation to become mature adipocytes which are laden with lipids and are responsive to insulin [20,21]. Adipogenesis involves three well defined stages: commitment, determination and differentiation. These three stages can be summed up in four substages: (i) commitment of MSCs to adipocyte lineage and growth arrest of pre-adipocytes; (ii) determination of pre-adipocytes to proceed to next stage, involving replication of DNA and duplication of cell, commonly known as mitotic clonal expansion; (iii) early differentiation, involving expression of genes and transcriptional factors such as CCAAT/ enhancer binding proteins (C/EBPs) family and peroxisome proliferator activated receptor–γ (PPAR γ); and (iv) late differentiation, involving significant increase in lipogenesis and induction of mostly lipogenic genes such as Fatty acid synthase (FAS), acetylo CoA carboxylase (ACC), adipose-specific fatty acid binding protein (aP2) [22] etc. The differentiation of MSCs into pre-adipocytes is also influenced by growth factors, hormones and insulin. During the progression of adipogenesis, mitotic clonal expansion of pre-adipocytes which are growth arrested will occur through repetitive rounds of mitotic divisions. Once the pre-adipocytes are released from the cell cycle, they gather cytoplasmic triglycerides and lose their fibroblastic morphology, while gaining the features of mature adipocytes. For successful transformation into mature adipocytes, the transformation of pre-adipocytes into a spherical shape is required, which is an indication of profound changes in the extracellular matrix (ECM),

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morphology of the cell and cytoskeletol components [22]. ECM provides strength, structural support, sites for the attachment of cell receptors, and acts as a source of cell signaling factors. In adipose tissue, ECM interconnects the adipocytes which results in the formation of cluster of fat cells *in vitro* and fat lobules *in vivo* [21]. ECM may also play a key role in modulating the differentiation of adipocytes. For instance, proteolytic degradation of the extracellular matrix of pre-adipocytes by cascade of plasminogen is essential for changes in the expression of adipocyte genes and deposition of fats [18,23]. Recently, it has been revealed that events and changes (molecular and morphological) which are associated with the above-mentioned changes may modulate adipogensis directly, because they alter the expression of positive transcriptional regulators of adipogenesis such as C/EBPα and PPARγ [23]. The components of ECM consist of classes of adhesion proteins such as laminin and fibronectin, and various types of collagen. Type I, III and VI collagens are considered the important isotypes here, due to their usual association with the fibrotic depots of an organ [24]. Inhibition of the synthesis of collagen prohibits the differentiation of adipocytes, demonstrating that active collagen synthesis is also necessary for the differentiation of adipocytes [21].

3. White, Beige and Brown Adipose Tissues

Generally, there are two main types of adipose tissues in mammals; white adipose tissue (WAT) and brown adipose tissue (BAT). The adipocytes of these two tissues possess different functions and morphology. WAT cells each contain a single lipid droplet of triglycerides formed from esterification of fatty acids and glycerol-3-phosphate. BAT contains high numbers of mitochondria and multilocular lipid droplets. The main constituent of adipose tissue is WAT, which is used as an energy substrate when needed. WAT adipocytes have a greater average diamter than those of BAT. WAT stores triglycerides while BAT disperses energy by producing heat, thus there is an antagonistic relationship between the two [2,25]. BAT is known to be protective against hypothermia due to its capacity to break down lipids to generate heat (thermogenesis). Uncoupling protein1 (UCP1) is the key player for thermogenic property of BAT [26]. UCP1, responsible for the uncoupling of electron transport for ATP production, is characteristically expressed by the mitochondria in BAT, thus allowing thermogenesis [27]. Recently, expression of UCP1 in WAT had also been reported by Dempersmier et al. [28]. Over-expression of the transcriptional activator (zfp516) of UCP1 causes 'browning' of WAT, thus known as 'brite' (brown in white) or beige adipocytes. It can be induced by hormonal stimulation, exposure to cold, and innervation. Brown and brite adipose tissues have a similar function in term of lipid breakdown for generation of heat, the only difference being the expression of specific cell surface markers (CD137 and Tmem26) in brite/beige adipocytes [26]. Moreover, the precursor cells giving rise to brite/beige cells in WAT are different to those of BAT, being closer to WAT cell lineage. Under basal conditions, these brite/beige adipocytes show phenotypes similar to white adipocytes: they lack expression of UCP1 and contain one large lipid droplet [29]. However, when exposed to certain stimuli such as exposure to cold [30] and β3-adrenergic activators [31], these brite/beige cells acquire characteristics similar to brown adipocytes including UCP1 expression, and presence of multilocular small lipid droplets. Their thermogenic activity has been reported to act against obesity and increase energy expenditure [32]. Additionally, the prevalance of brite/beige cells is in inverse proportion to obesity, body mass index, and plasma glucose level [33], evidencing the importance of their main role in the regulation of body's metabolism. Some reports had stated that BAT was present only in newborns and small mammals, however recent studies had revealed conclusively the presence and functional relevance of BAT [34]

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and brite adipose tissue in adults as well [35]. Although both brown and white adipocytes originate from MSCs, it is believed that the precursor cells giving rise to brown and white adipocytes are different (Figure 1). MSCs are pledged either to adipogenic (Myf-5 negative) cells and give rise to white adipocytes or myogenic (Myf-5 positive) cells, giving rise to brown adipocytes [36,37]. The Myf- negative precursor cells give rise to white pre-adipocytes through the expression of bone morphogenetic protein-2 and 4 (BMP 2 and 4). Similarly, the Myf-positive precursor cells give rise to brown pre-adipocytes through the expression of bone morphogenetic protein-7 (BMP 7) and trancriptional co-regulator PR-domain containing 16 (PRDM16) [36,38]. The recent rediscovery of effective BAT in adult humans has invigorated interest in it as a viable and novel target for phytochemicals. Upregulating the proteins and transcriptional factors specifically expressed in brown or beige adipocytesis is a highly promising approach in the elimination of obesity. Activation of the thermogenic system in humans, either in WAT or BAT, should correlate well with an increase of energy expenditure. Interest in the prevention of obesity by regulation of non-shivering thermogenesis in BAT and brite adipose tissue through phytochemicals has been increasing rapidly due to their potential for side-effect-minimal medication. Various phytochemicals have been reported (Table 1) to induce browning of white adipose tissue, indicating their thermogenic effects. For example, genistein upregulates the expression of peroxisome proliferator- activated receptor gamma coactivator 1-α (Pgc1α), Ucp1 and Sirt1 [39]. Similarly, Formononetin [40] and Myricetin [41] have also been reported to induce browning of white adipose tissue by upregulating the genes and transcriptional factors specifically expressed in BAT.

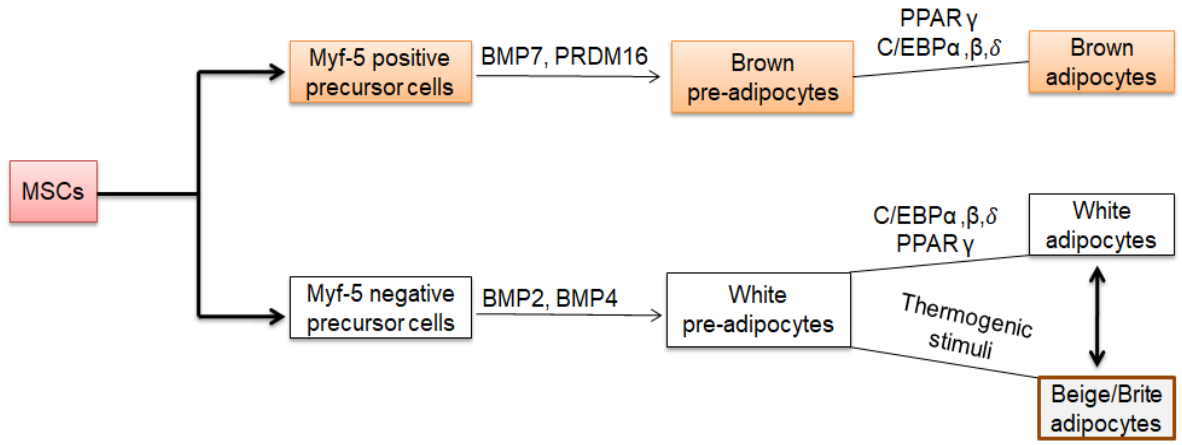

Figure 1. Differentiation of MSCs into white, beige/brite and brown adipocytes
MSCs; Mesenchymal Stem cells, Myf-5; Myogenic Factor-5 protein, BMP; Bone morphogenetic protein, PRDM16; PR-domain containing 16, C/EBPα ,β,δ; CCCAAT/Enhancer Binding Protein α ,β,δ.

Table 1. Some of the recently reported non-shivering thermogenic effects of plant chemicals

Bioactive Compounds	Experimental Model	Positive Regulation of Thermogenic Factors	Reference

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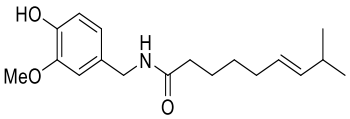
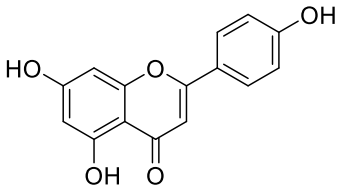
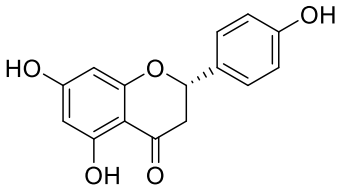
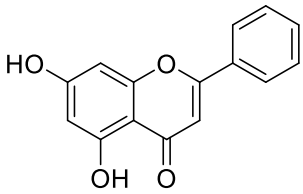
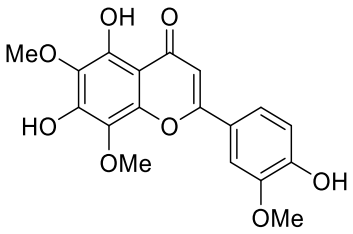
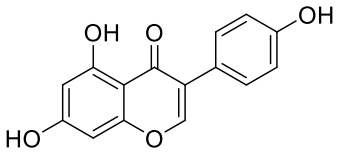
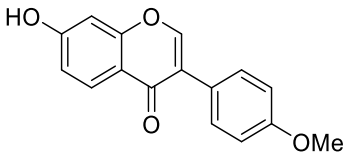
Capsaicin 	WT and TRPV1$^{-/-}$ mice	AMPK, SIRT, PRDM16 and PGC-1α activation	[42]
Apigenin  Naringenin 	Male C57BL/6 mice	Elevation of UCP1 expression in BAT	[43]
Chrysin (5,7-dihydroxyflavone) 	3T3-L1 cells	Increase expression ofTmem26, cited 1 and Tbx1.Upregulation of genes involved in the regulation of brown adipogenesis (Ucp1and Prdm16, Pgc1α)	[44]
Sudachitin 	C57BL/6J mice and db/db mice	Increase in O_2 consumption, energy expenditure and Ucp1 expression in sWAT	[45]
Genistein 	3T3-L1 cells	Upregulation of Pgc1α, Ucp1, Sirt1. Increase in the consumption level of oxygen	[39]
Formononetin 	Male C57BL/6 mice	Activation of AMPK and stabilization of β-catenin	[40]

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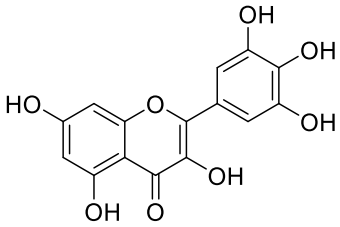
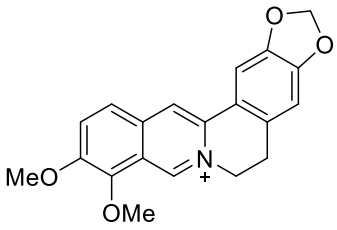
Myricetin 	db/db mice	Increase in the consumption of oxygen and body's temperature. Increase in the browning of IWAT and mitochondrial biogenesis	[41]
Berberine 	Obese C57BLKS/J-Lepr Db (db/db) male mice	Increase in the expression of thermogenic genes and UCP1 in BAT, primary adipocytes and WAT	[46]

Sirt1; Sirtuin 1, PRDM16; PR-domain containing 16, PGC1α; Peroxisome proliferator- activated receptor gamma coactivator 1-α , UCP1; Uncoupling protein 1, BAT; Brwon adipose tissue, scWAT; Subcutaneous white adipose tissue, iWAT; Inguinal white adipose tissue, WAT; White adipose tissue, AMPK; Adenosine monophosphate activated protein kinase.

4.Transcriptional Regulators of Adipogenesis

Adipogenesis requires the activation of several transcription factors including the CCAAT/ enhancer binding protein (C/EBP) family and peroxisome proliferator activated receptor–γ (PPARγ) [18]. Expression of the two members of C/EBP family (C/EBPβ and C/EBPδ) occurs at early stages of adipocyte differentiation and then together, they induce the expression of C/EBPα and PPARγ which are the central positive regulators of adipogenesis [47,48]. PPARγ is the master regulator involved in the differentiation of adipocytes and metabolism [49]. PPARγ and C/EBPα exert positive feedback on each other and regulate the process of adipogenesis positively (Figure 2). Several studies [50,51] have indicated that PPARγ is the key regulator involved in the development and differentiation of adipocytes, and therefore known to be obligate for the differentiation of adipocytes. Expression and normal function of PPARγ is necessary for the progression of adipogenesis: cells deficient in PPARγ cannot differentiate into mature adipocytes even if other powerful pro-adipogenic factors are ectopically expressed [48]. Previous *in vitro* studies have shown that most of the activators and repressors of adipogenesis alter the activity and expression of PPARγ [52]. Transcriptional factors such as C/EBP β, C/EBP δ, kruppel like factor 5 (KLF5) and early β-cell factor 1 (EBF1) are known to directly induce the expression of PPARγ mRNA in adipogenesis [48]. Early β-cell factor 1 and 2 (EBF1 and EBF2) are induced during 3T3-L1 white pre-adipocytes differentiation but their pattern of expression is different [53]. EBF2 has been reported to regulate the expression of brown adipocyte genes such as Ucp1 and Prdm16 and express at higher levels in BAT as compared to WAT [54]. In addition, there are other substantial transcriptional factors that act also as regulators of adipogenesis. Kruppel like factors (KLFs) are either activators or suppressors ofadipogenesis. KLF4, KLF5, KLF6, KLF9, KLF13 and KLF 15 are known to enhance adipogenesis while KLF2, 3, 7 and 16 inhibit

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adipogenesis [17,55–57]. KLF2 directly inhibits the PPARγ promoter. Adenovirus-mediated ectopic expression of KLF2 has been shown to inhibit the expression of PPARγ, C/EBPα and SREBP-1c but did not have any effect on the expression of C/EBPβ and C/EBPδ [58]. KLF3 inhibits the differentiation of 3T3-L1 pre-adipocytes by repressing C/EBPα promoter [57]. Similarly, overexpression of KLF7 is reported to inhibit the differentiation of 3T3-L1 pre-adipocytes [59]. Over expression of KLF7 significantly decreases the expression of PPARγ, C/EBPα, adipsin and adipocyte protein-2 (aP2) [60]. Globin transcription factors GATA2 and 3 are also reported to inhibit adipogenesis by down-regulating the expression of PPARγ [47,61]. Other transcriptional factors such as sterol regulatory binding protein-1 (SREBP1), (which expedites metabolism of fatty acids by inducing expression of PPARγ) and cyclic AMP response binding element (CREB), are needed in the differentiation of pre-adipocytes into mature adipocytes [47]. In pre-adipocytes CREB is required for the induction of differentiation of adipocytes and absence of CREB inhibits differentiation of pre-adipocytes into mature adipocytes [62]. Both PPARγ and C/EBP family members are widely studied targets in *in vitro* and *in vivo* studies of anti-obesogenic medicine due to their role in adipocyte differentiation and energy storage pathways. Strategies to upregulate catabolism related genes and transcriptional factors while downregulating or suppressing genes responsible for anabolic pathways are possible means means to combat obesity. Besides endogenous activators and suppressors of these genes, exogenous activators are requisite to activate them, because in most of the obesogenic conditions, the catabolism related genes, hormones and transcriptional factors are not properly expressed. One such protein, Adenosine Monophosphate Activated Protein Kinase (AMPK) plays a crucial role in the downregulation of transcriptional factors and pathways related to adipogenesis and lipid synthesis. AMPK (discussed in detail in the next section) is the target protein of many obesity and type-2 diabetes related pharmacological studies as it acts as a regulator and sensor of cellular energy metabolism. Additionally, AMPK targets the expression of thermogenesis-causing genes and proteins responsible for generation of heat from stored fat, such as Uncoupling protein1 (UCP1) of BAT, or its over expression in WAT, turning WAT into brite/beige adipose tissue. This may be another strategy to overcome obesity.

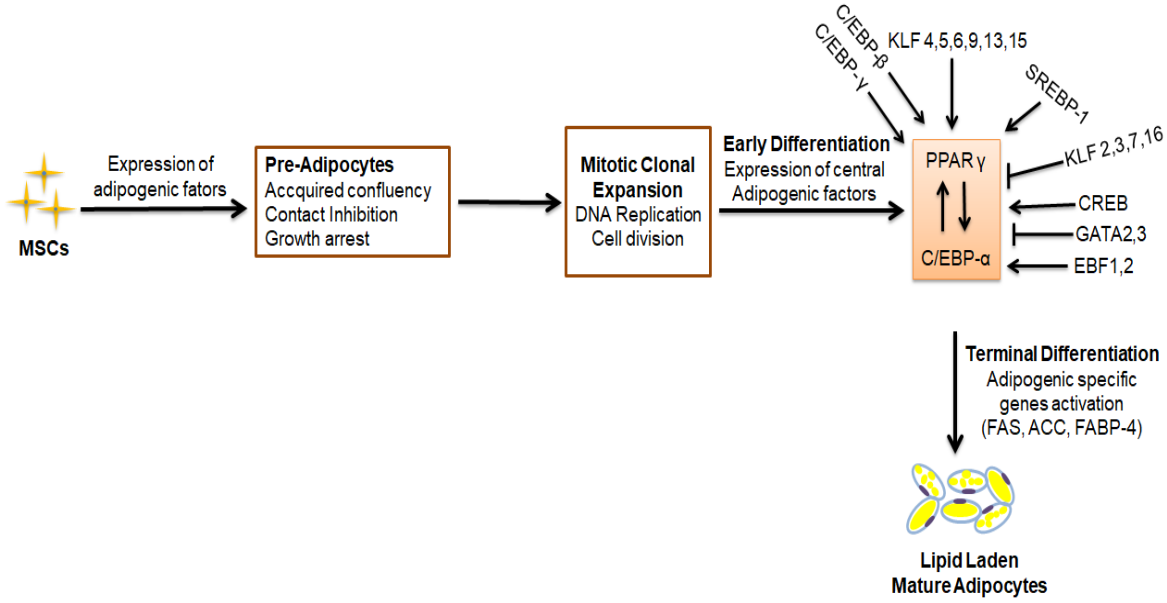

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Figure 2. Transcriptional regulation of adipogenesis. MSCs initially are converted into pre-adipocytes followed by mitotic clonal expansion. This step is followed by expression of central regulators of adipogenesis, PPARγ and C/EBPα. The expression of these two is influenced by various transcriptional factors which are either their positive or negative regulators of adipogenesis. The expression of adipogenic specific genes ultimately leads to the formation of lipid laden mature adipocytes. Arrows represent activation and bars represent inhibition.
MSCs: Mesenchymal Stem cells; DNA; Deoxy ribonucleic acids; C/EBPs: CCAAT / enhancer binding proteins; KLFs: Kruppel Like Factors; SREBP1: Sterol regulatory binding protein-1; CREB: Cyclic AMP response binding element ; GATAs: Globin transcription factors; EBF Early β-cell factor; FAS; Fatty Acids synthase; ACC; Acetyl Co-A carboxylase; FABP-4; Fatty acids binding protein-4.

5. Role of Signaling Pathways in Adipogenesis

MSCs are commited to either osteogenic or adipogenic lineages. This involves discrete signaling pathways including Wnt (canonical and non-canonical), Bone morphogenetic protein (BMP) and Hedgehog signaling pathways. These pathways have very strong influences on the central regulators of both osteogenesis (Runx2) and adipogenesis (PPARγ), two factors which are responsible for the differentiation of MSCs into either osteocytes or adipocytes and work antagonistically with overexpression of one factor repressing the other [63,64].

5.1. Bone Morphogenetic Proteins and Transforming Growth Factors-β Pathway

Bone morphogenetic proteins (BMPs) belong to the superfamily of transforming growth factors-β (TGF-β) and have been identified as regulators of osteogenesis and adipogenesis [63,65]. BMPs are involved in the differentiation of disparate cell types including adipocytes. Initially, BMPs were thought to have a key role only in the formation of bone, but their role in all other organs has been discovered recently [66]. BMPs are known for their regulatory roles in various cellular process including proliferation, apoptosis, differentiation, and determination of cell fate in adulthood and during embryogenesis [65–67]. BMPs play different roles in the differentiation of adipocytes depending on the stage of cells and BMP type and dosage [68]. BMP-2 in particular, inhibits adipogenesis and promotes osteogenic differentiation in bone marrow stromal cells [69] BMP-7 has been reported to induce the differentiation of brown adipocytes [70]. 3T3-F44 2A pre-adipocytes treated with BMP-2 showed a decrease in insulin-induced lipid accumulation [71], but BMP-7 enhanced the differentiation of 3T3-L1 pre-adipocytes, demonstrating the contradictory roles of BMPs in adipogenesis [72,73]. Similarly, BMP-4 regulates the commitment of precursor cells into white adipogenic lineage [74]. BMP-4 and BMP-7 can also activate development of beige adipocytes in human precursor cells [75]. Overexpression of BMP-4 in transgenic mice showed a reduction in the mass and size of white adipose tissue (WAT), and induced browning of WAT (known as britening) [76]. Induced expression of BMP-4 upregulated the expression of key regulators of brown adipose tissue, peroxisome proliferator- activated receptor gamma coactivator 1-α (PGC1α) and its target gene, UCP1 [77,78].

The 33 members of the TGF-β superfamily also have wide roles in different types of cells, including adipocytes. Among all the TGF-β superfamily members, TGF-β1 has a major role in adipogenesis. It inhibits differentiation of 3T3-L1 pre-adipocytes into mature adipocytes [79] by interacting and repressing the expression of essential adipogenic factors PPARγ, C/EBPα and C/EBPβ [65] These pathways are therefore of great interest for the discovery of new and non-toxic

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chemotherapies in preventing obesity through inhibition of WAT development, and promotion of BAT by targeting the key factors of these pathways.

5.2. Hedgehog Signaling Pathway

The Hedgehog (Hh) signaling pathway is involved in the development of both vertebrates and invertebrates [80]. It is known to be one of the important modulators of the stem cell differentiation process and its role in the differentiation of MSCs had been demonstrated in several studies [81,82]. This signaling pathway is downregulated during the differentiation of human adipocytes, and upon activation it reduces the expression of key adipogenic transcription factor C/EBPα and hinders accumulation of lipids and adipogenesis [65,82]. Hh signaling pathway activation in C3H10T1/2 mouse cells was reported to inhibit the expression of PPARγ and C/EBPα, blocked the differentiation of pre-adipocytes into mature adipocytes and increased the commitment of C3H10T1/2 mouse cell lines towards osteogenic lineage [83]. Activation of Hh gene in *B. mori* cell line (*Bm*N) inhibited adipocyte protein 2 (aP2) expression, while knockdown of the Hh gene by RNA interference enhanced the expression of the aP2 gene indicating the regulatory effect of Hh on aP2. Moreover, the blocking of the Hh signaling pathway by antagonist, cyclopamine in silkworm larvae resulted in increased differentiation and size of adipocytes. Inhibition of fat formation by Hh signaling pathway was retained both in vertebrates and invertebrates [80].

5.3. Wnt Signaling Pathway

Wnts (Wingless-type MMTV integration site family members) are secreted glycoproteins that work both in an autocrine manner and a paracrine manner [65]. Wnt signaling pathways are a group of conserved signal transduction pathways and consist of proteins which convey signals through cell surface receptors into the cell. These pathways are involved in processes including cell differentiation and proliferation in adult tissue regeneration, and in embryonic development [65]. These signal transduction pathways can be divided into canonical (β-catenin dependent) and non-canonical (β-catenin independent) pathway. Wnt proteins control the proliferation of cell, cell fate, behaviour and survival by exerting signals through these canonical and non-canonical pathways. MSCs are differentiated into osteocytes and myocytes instead of adipocytes upon activation of Wnt/β-catenin signaling pathway. Conversely, interruption of Wnt/β-catenin signaling promotes the differentiation of pre-adipocytes into mature adipocytes [84]. One important component of the Wnt/β-catenin pathway, Wnt10b, had been reported to be responsible for the anti-adipogenic function of canonical pathway. Wnt10b is highly expressed in pre-adipocytes but its expression declines promptly after induction of differentiation [85]. Its overexpression stabilizes cytoplasmic β-catenin and block adipogenesis in 3T3-L1 pre-adipocytes [86,87]. Moreover, the Wnt/β-catenin signaling pathway also inhibits brown adipogenesis by disrupting the induction of PPARγ and C/EBPα. Wnt10a and Wnt10b, members of the canonical pathway, are possible endogenous inhibitors of BAT. Both Wnt10b and Wnt10a are expressed in pre-adipocytes of BAT but not in differentiated brown adipocytes and their expression reduces with the progression of brown adipogenesis [88]. In addition, Wnt signaling also blocks the thermogenic program of BAT by suppressing the thermogenic protein, UCP1 of BAT through repression of PGC1- α [88]. *In vivo* expression of *Wnt10b* from fatty acid binding protein 4

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(FABP 4) promoter had been shown to reduce total body fat by 50% and provide resistance to WAT accumulation in high fat diets, as well as blocking the development of BAT [89]. These findings had led to the conclusion that the activation of the Wnt/β-catenin signaling pathway during the process of adipogenesis may be a key strategy for anti-obesogenic drugs. For example, 6-gingerol (found in ginger) had been reported to have anti-adipogenic effects, inhibiting adipogenic differentiation through activation of Wnt/β-catenin signaling pathway [90]. Similarly, toosendanin (a triterpenoid) has been reported recently to inhibit adipogenesis through Wnt/β-catenin dependent pathway [91]. Thus, there is a need to investigate further other bioactive compounds and their mechanism of action in inhibiting adipogenesis through Wnt/β-catenin signaling pathway

Besides the canonical pathway, members of Wnt family also activate the non-canonical β-catenin independent pathway. Activation of non-canonical pathway through Wnt5a is reported to antagonize the canonical pathway, promoting the differentiation of pre-adipocytes into mature adipocytes [92].

6. AMPK; Central Target of Many Natural and Synthetic Compounds

5′-Adenosine monophosphate-activated protein kinase (AMPK), is a serine/threonine kinase. The heterotrimeric protein consists of 3 subunits: catalytic subunit α which consists of further two subunits α1, α2 and regulatory subunits β and γ consisting of two subunits (β1, β2) and three subunits (γ1, γ2, γ3) respectively [93,94]. Being expressed in different kind of tissues (liver, adipose, skeletal, kidney and hypothalamus), [95] AMPK plays a central role in controlling and regulating cell cycle and cellular energy homeostasis; it is therefore the most widely studied protein in the investigation and discovery of natural as well as pharmacological compounds against weight loss.

6.1. Metabolic Functions of AMPK and Role in Adipogenesis

Once activated, AMPK directly or indirectly promotes the phosphorylation of downstream targets including transcription and translational factors, metabolic enzymes, epigenetic factors, growth and proliferation pathways. The overall effect of this regulation is to reduce the synthesis of cholesterol, fatty acids, ribosomal RNAs (rRNAs) and proteins [96], making AMPK a potential target of high value for the development of pharmacological and natural anti-obesogenic compounds [97] In adipose tissue, activation of AMPK correlates with decreased level of lipid storage [16]. Regulation of lipid metabolism is the first known function of AMPK. AMPK inhibits *de novo* synthesis of cholesterol, triglycerides (TG), and fatty acids (FAs), and activates FA uptake and β-oxidation (FAO). It inhibits and phosphorylates targets involved in the synthesis of FAs, ACC1, and SREBP1c. SREBP1c is involved in the transcriptional regulation of various lipogenic enzymes including FAS and ACC1. ACC1 converts acetyl-coA to malonyl co-A and hence catalyzes the rate limiting step in the synthesis of FAs [98,99]. Moreover, AMPK inhibits synthesis of cholesterol by phosphorylating and inhibiting HMG-CoA- reductase [98]. It is also known for its inhibitory effects on the mammalian target of rapamycin (mTOR) which is the fundamental cell growth regulator and is highly conserved in all eukaryotes. AMPK remains active under poor energy and nutrient conditions while mTOR remains inactive in such conditions [100]. Thus, the inhibiting effects of AMPK on mTOR define antigrowth and antiproliferative activities under stress conditions. AMPK may therefore play a central role in the inhibition of adipogenesis by inhibiting the mTOR signaling pathway. In the case of obesity, AMPK remains inactive due to the availability of excess nutrients and energy sources, therefore an

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external stimulus is needed to activate AMPK. Much effort has been spent attempting to delineate the exogenous activators of AMPK, and the debate is still ongoing. The net effect of AMPK activation is an increase in body's cellular energy level [95]. Lipid/glucose homeostasis, mitochondrial biogenesis, food intake and insulin signaling are some of the important functions of AMPK. Therefore, the protein is a key therapeutic target for the treatment of major metabolic disorders including obesity and type-2 diabetes [101]. As adipogenesis is an energy consuming process, AMPK regulates the inhibition of expression of FAS, adipocyte specific FA-binding protein (aP2) and induction of C/EBPs and PPARγ. Vingtdeux et al. reported the inhibition of adipogenesis by small-molecule activators (RSVA314 and RSVA405) of AMPK. In that study inhibition of adipogenesis was observed by activation of AMPK via a mitotic clonal expansion (MCE) phase accompanied by reduced expression of C/EBPβ and inhibition of C/EBPα, PPARγ and late adipogenic factors including SREBP1-c, FAS and aP2 [102].

Critically, since activation of AMPK inhibits adipogenesis, there is the exciting possibility of modulating this pathway using phytochemicals, thus harnessing their beneficial properties to address this most important target in anti-obesity research. Indeed, activation of AMPK by phytochemicals such as EGCG, genistein and capsaicin [103], quercetin [104], resveratrol [105], berberine [106] has been reported by many studies. Furthermore, upon activation AMPK inhibited the differentiation of pre-adipocytes into mature adipocytes. He et al. showed that a triterpenoid (ursolic acid) inhibited adipogenesis via the LKB1/AMPK pathway in 3T3-L1 adipocytes [107]. Similarly, Chen et al. identified the phosphorylation of AMPK in 3T3-L1 adipocytes by a phytochemical, resveratrol. Treatment with resveratrol resulted in phosphorylation of AMPKα in a dose-dependent manner, and reduced expression of positive regulators of adipogenesis including PPARγ, C/EBPα and SREBP-1c. Administering AMPKα siRNAs reversed the adipogenesis inhibition, suggesting the inhibition of lipogenesis and differentiation of 3T3-L1 adipocytes were occurring through AMPK activation [97]. AMPK also regulates autophagy by phosphorylating two initiating regulators of autophagy: a protein kinase complex ULK1 and lipid kinase complex PI3KC3/VPS34. Several studies have demonstrated autophagy in lipophagy, glycophagy, adipose tissue differentiation and mass regulation [108] giving heightened importance to the role of AMPK activation in combatting obesity. Kim and Kong. reported the anti-adipogenic effects of dioxinodehydroeckol (DHE) through modulation and activation of the AMPK pathway in 3T3-L1 pre-adipocytes, highlighting the potential role of AMPK in the inhibition of adipogenesis [109]

In addition, AMPK has also been shown to be pertinent in the britening of WAT thereby increasing the energy expenditure through thermogenesis [110,111]. It has also been reported that the activity of AMPK increases during the differentiation of brown adipocytes and that targeting AMPK by short interfering RNAs (siRNAs), inhibits the differentiation of pre-adipocytes into mature brown adipocytes [16,112]. These studies clearly indicate AMPK as a potential therapeutic target in the prevention and treatment of many metabolic diseases including obesity.

6.2. AMPK Activation by Upstream Kinases

AMPK is broadly known as a fuel-sensing enzyme – it is involved in sensitivity to, and the homeostasis of lipids, glucose and insulin [113]. Under different physiological conditions, the subunits of AMPK behave and are regulated differentially. Activation of AMPK can be achieved by either through upstream kinases or allosterically through AMP [95]. It is activated when there is increase in the consumption of adenosine triphosphate (ATP) which leads to an increase in the ratio

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of adenosine monophosphate (AMP) to ATP [114]. The best studied mechanisms of the activation of AMPK are allosteric activation by binding of either AMP or ADP at γ subunit and by phosphorylation of the α subunit [114]. Conditions including hypoxia, exercise, ischaemia and hypoglycaemia usually alter the cellular adenine nucleotides levels (suppress ATP consumption) and subsequently enhance the activity of AMPK [115]. The rise in AMP/ADP and decline in ATP levels cause the activation of AMPK by direct binding of AMP or ADP to the γ subunit of AMPK. This binding prevents access of phosphatases to Thr 17 in the α subunit, and thus maintains a high phosphorylation level of AMPK [98]. Upstream kinases of AMPK include STE-related adaptor (STRAD), mouse protein 25 (MO25) and Liver Kinase B1 (LKB1). Liver Kinase B1 (LKB1) [110,116], is part of a heterotrimeric protein. For activation, it needs the binding of other two upstream kinases of AMPK, STRAD and MO25 to form a heterotrimeric complex. It directly activates AMPK by phosphorylating Thr172 of α subunit. The LKB1/AMPK pathway regulates the metabolic check-points of cells and stops the growth and proliferation of cells in the case of low ATP levels. Previous genetic and biochemical studies in mice, worms and flies have demonstrated that LKB1 was the major phosphorylating agent of AMPK [117]. Shan et al. reported that the presence of LKB1 promoted AMPK activity and its absence worked opposite in HFD-induced mice [26]. Similar, Hawley et al. reported that HeLa cells which were unable to express LKB1, upon exposure to external stimuli (that increase the AMP/ATP ratio) or incubation with 5-amino-4-imidazolecarboxamide riboside (AICAR) which is an analog of AMP and capable of activating AMPK, did not elevate AMPK expression [118]. Based on these observations, LKB1 may be a potential upstream activator of AMPK in case of elevated AMP levels. Additionally, in some of the tissues calcium acts as a trigger, through calcium/calmodulin dependent protein kinase-2 (CaMKK-2) for phosphorylation of AMPK at Thr172 of the α subunit [110]. Contrary to LKB1 complex, CaMMKβ activates AMPK in response to increase in the concentration of cellular Ca^{2+} regardless of changes in ATP/ADP/AMP levels [16]. Presence of Ca^{2+}/CaMMK in adipocytes correspondingly regulates the activation of AMPK [119]. Lin et al. reported the inhibitory effects of CaMMKβ on adipocyte differentiation: differentiation of pre-adipocytes to mature adipocytes was enhanced in a condition of acute inhibition or deletion of CaMMKβ [120], and this condition was reversed by induction of AMPK activation by AICAR 8 [121], confirming the activation of AMPK by CaMMK. Another upstream kinase of AMPK, transforming growth factor-β -activated protein kinase (TAK1) activates AMPK to mediate autophagy induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in cancerous cells. Although, AMPK is phosphorylated and activated by TAK1 in different tissues and organs, LKB1 and CaMMKβ are considered the main upstream kinases of AMPK in adipocytes [121]. Therefore, further study about activation of AMPK by anti-obesogenic products through upstream kinases is required to understand their mechanism of action and develop new anti-obesogenic products specifically targeting these kinases to treat adiposity.

6.3. Exogenous Activators of AMPK

In recent years, much effort had been made to delineate the pathways of AMPK, and to identify both direct and indirect modulators of AMPK for the development of new therapies for disorders including obesity. Therefore, understanding of direct and indirect activators of AMPK is the fundamental step in the development of new therapeutic agents. Many pharmacological and natural exogenous activators from plants had been reported to activate AMPK either directly independent of upstream kinases or indirectly through upstream kinases.

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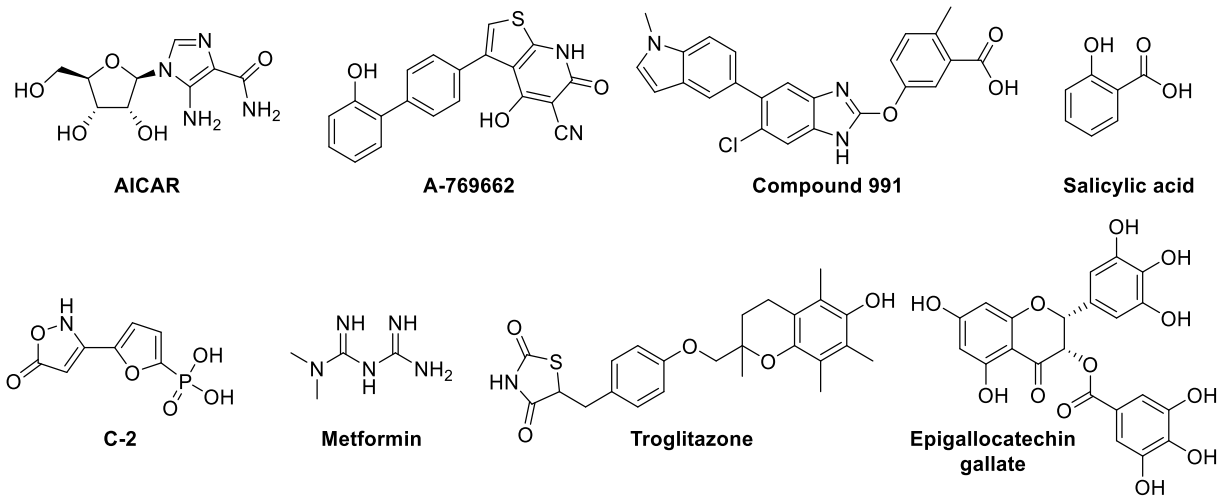

Figure 3. Structures of some of the exogenous activators (discussed in the coming section) of AMPK

6.3.1. Direct Exogenous Activators

Activators that bind directly to AMPK and activate it without significant changes in ATP:AMP ratio are known as direct activators. Direct activators induce conformational changes in the AMPK complex, more specifically by interacting with one of the AMPK subunits. AICAR was the first identified direct activator of AMPK *in vitro* and *in vivo* [122]. To evaluate the downstream effects of activated AMPK in animals, AICAR had been used widely [122]. Structurally AICAR is similar to adenosine, and it is similarly phosphorylated upon entering the cell (via adenosine transporters) to AICAR monophosphate (ZMP) by adenosine kinase. ZMP is an analog of adenosine monophosphate (AMP) and similarly causes allosteric activation of AMPK by binding to its γ subunit. This causes an increase in phosphorylation of Thr172 of α subunit of AMPK [94], and also inhibits the dephosphorylation of AMPK [122]. Treatment with AICAR had been shown to increase glucose tolerance, reduce TGs and free fatty acids (FFAs) level of plasma. Activation of AMPK by AICAR has been reported to suppress the activation of adipogenic transcription factors PPARγ and C/EBPα, and the enzymes acetyl-CoA carboxylase and FAS [123]. AICAR has also been known to cause arrest of cell cycle at the G1 phase by inducing activation of p53 followed by activation of the cell cycle inhibitor protein p21 [124], stopping the growth and proliferation of cell cycle under stress conditions. Although AICAR has promising effects as mentioned above, since it mimics the effects of AMP, it has also other AMPK-independent effects [122]. For instance, it regulates other AMP-regulated enzymes such as fructose-1,6-bisphosphatase (FBPase) and stimulates muscle glycogen phosphorylase [122,125]. In addition, due to short half-life and poor bioavailability, it is unlikely to be used in the treatment of metabolic syndrome and Type-2 diabetes [126] and these are considered to be the main obstacles in the development of AICAR as a promising drug [122].

Other direct activators (i.e. independent of upstream kinases) of AMPK include A-769662 compound (Thienopyridone Family), Compound 991 (Benzimidazole family), and salicylate. A-769662 belongs to thienopyridone family and is a small organic compound which activates AMPK allosterically by phosphorylating AMPK at Ser108 in the AMPKβ subunit, especially β1[94] and inhibits dephosphorylation of Thr172 in AMPKα subunit [127,128]. Activation of AMPK both by AMP and A-769662 have been extensively studied *in vitro* and *in vivo*. AMP and A-769662 compound had been reported to bind to different sites on AMPK and have different mechanism of actions

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[94,128]. Another direct activator of AMPK is referred to as Compound 991 and belongs to benzimidazole family. It is reported to bind the β unit of AMPK and is more effective (5-10 fold) than A-769662 in the allosterical activation and inhibiton of dephosphorylation of AMPK [94,129]. Similar to A-769662, compound 991 failed to activate AMPK complexes which contained mutations in the Ser108 of the β subunit of AMPK. This suggest that both A-769662 and compound 991 show similar mechanism for the activation of AMPK [129]. Salicylate is among the oldest drugs used by humans. It is a phytochemical, produced naturally and obtained from willow bark [126] but for medicinal purposes, it is now used in acetylated form (Aspirin) [127]. Aspirin is easier to take orally than salicylate, and upon entering the blood stream, it is rapidly broken down to salicylate [94]. Salicylate binds to the β1 subunit (the same unit where A-769662 compound binds) of AMPK and thus activates AMPK allosterically, inhibiting the dephosphorylation of Thr172 in the α subunit [130]. Beyond these example, 5-(5-hydroxyl-isoxazol-3-yl)-furan-2- phosphonic acid, termed as Compound-2 (C-2) is the most potent direct activators of AMPK. C-2 binds to the AMPKα subunit, causes allosteric activation of AMPK and prevents the dephosphorylation of Thr172. It mimics AMP's effects in activation of AMPK but unlike AICAR, it does not have any effect on the enzymes which use AMP as a substrate, namely 6-phosphofructo-1 kinase (PFK1), fructose-1,6-bisphosphatase-1 (FBP1) and muscle glycogen β [131]. Compound-2 is known to be two folds more potent than AMP and 20 folds more than A-769662 [94].

6.3.2. Indirect Exogenous Activators

Studies have shown that modulators which can cause calcium or AMP accumulation in the body can activate AMPK [94]. These modulators are known as indirect activators of AMPK and maybe physiological, pharmacological and natural activators. These modulators do not encompass direct interaction with AMPK, they can activate AMPK either by increasing AMP:ATP or calcium accumulation in the bodies [94,126]. Pharmacological and phytochemical activators of AMPK such as metformin, troglitazone, quercetin, genistein, epigallocatechin gallate, resveratrol, berberine, curcumin and α-lipoic acid act as indirect activators of AMPK [94], activating the kinase by expenditure of energy because when ATP is decreased, AMP is increased. Metformin is a biguanide which is found in *Galega officinalis.* It upregulates the activity of AMPK, increases the oxidation of fatty acids, downregulates lipogenic genes, increases the glucose uptake and decreases the production of glucose. Metformin activates AMPK indirectly, by binding and inhibiting the complex I of the mitochondrial respiratory chain, thus increases AMP:ATP ratio. It also inhibits the dephosphorylation of AMPK and increases the phosphorylation of AMPK through upstream kinase of AMPK, LKB1 [127]. Thiazolidinediones (TZDs) are insulin-sensitizing drugs and consist of rosiglitazone, pioglitazone and troglizatone [126]; these compounds indirectly activate AMPK and promote phosphorylation of ACC in various types of tissues including adipose, skeletal muscles and liver [126,132]. They enhance the accumulation of AMP by inhibiting the complex I of the mitochondrial respiratory chain and hence activate AMPK indirectly [133]. Moreover, they enhance the expression of PPARγ which in turn increases the expression and release of adiponectin from adipocytes [132], activates AMPK in liver and skeletal muscle, increases the oxidation of fatty acids and uptake of glucose, and decreases the production of hepatic glucose [94].

Indirect activation of AMPK by phytochemicals had also been demonstrated in numerous studies. Quercetin is one of the most abundant flavonoids found in many plants, food and grains, and is known to activate AMPK indirectly [104]. Exposure of 3T3-L1 cells to quercetin resulted in a

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decrease expression of positive regulators of adipogenesis and attenuation of adipogenesis. This was due to the phosphorylation of AMPK and its downstream substrate ACC [104]. Another indirect activator of AMPK that can be found in grapes is resveratrol. Resveratrol activates AMPK indirectly by increasing AMP:ATP ratio through inhibition of mitochondrial ATP production [105,122,134]. Treatment with resveratrol has been shown to stimulate mitochondrial biogenesis, glucose uptake and reduce accumulation of lipids in different types of cells [126,135–137]. In addition, Curcumin derived from *Curcuma longa* activates AMPK by phosphorylating its α subunit. Exposure of 3T3-L1 adipocytes to curcumin enhanced the phosphorylation and activation of AMPK and decreased the expression of ACC by phosphorylation [138]. Similarly, Hwang et al. reported the inhibition of adipogenesis and apoptosis of adipocytes by genistein, epigallocatechin gallate (EGCG) and capsaicin through activation of AMPK. These phytochemicals activated AMPK through stimulation of reactive oxygen species (ROS) in 3T3-L1 pre-adipocytes [103]. In addition, physiological activators, for instance exercise and calorie restriction induce the increase in AMP: ATP and hence indirectly activate AMPK. Previous studies have revealed that contraction of muscles both in human and rodents activate AMPK and accordingly is the one of the most compelling enzymes through which exercise conveys conclusive effects [126,139].

While intracellular energy level is a crucial determinant in the activity of AMPK, it had been reported that reactive oxygen species (ROS) also induce the activation of AMPK without any decrease in ATP level [94,140,141]. From the pharmacological point of view, there is still much effort needed to combat obesity through direct or indirect activation of AMPK via exogenous activators with no (or minimal) side effects. Studies on direct or indirect activators of AMPK have been performed *in vitro* and *in vivo*, but more efforts are needed to evaluate their anti-obesogenic effects prior to clinical testing in humans. Moreover, these compounds must be evaluated for their toxicity and side effects before forwarding them into market.

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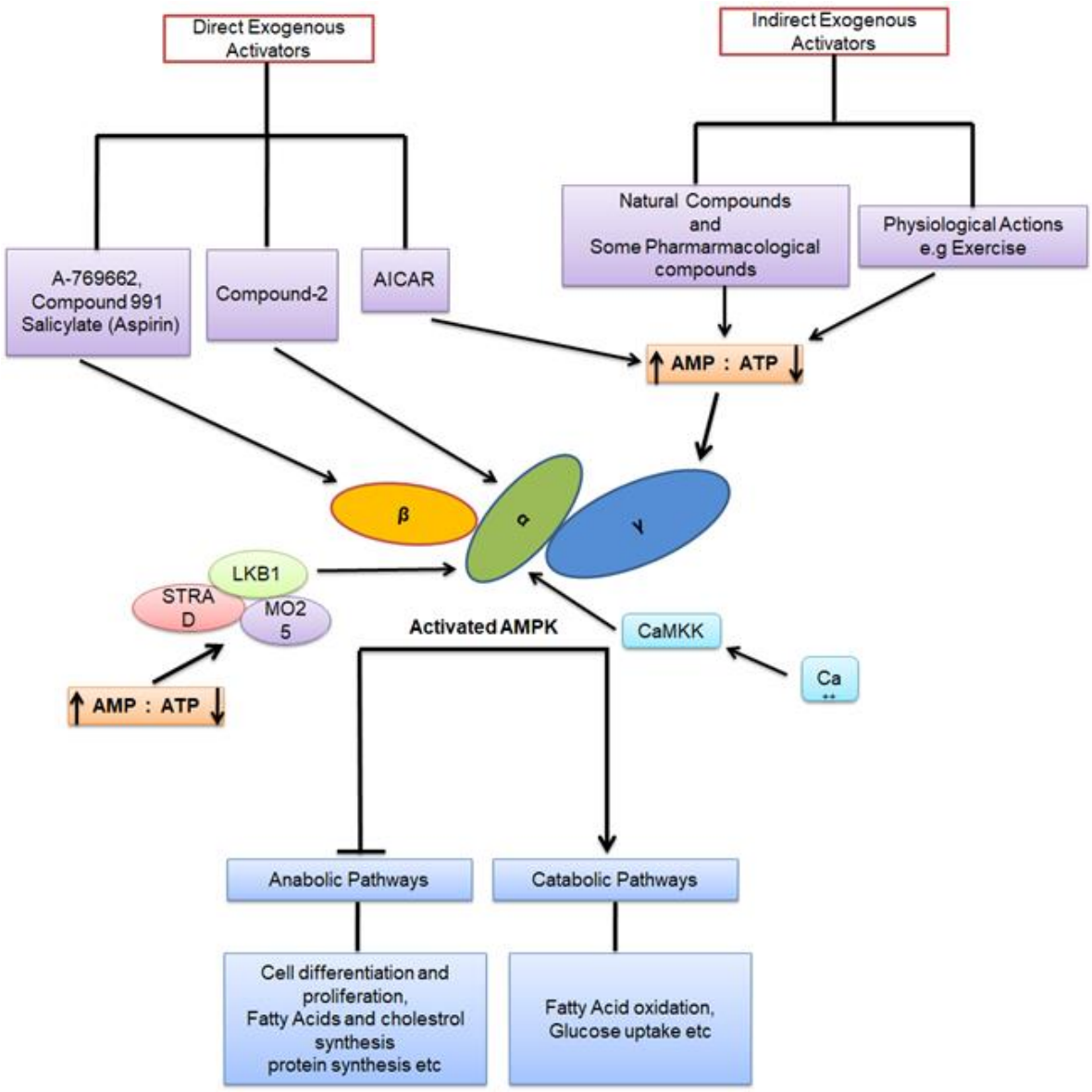

Figure 4. Activation of AMPK by direct and indirect exogenous activators and metabolic functions of activated AMPK

LKB1; Liver kinase B1, STRAD; STE-related adaptor, MO25; Mouse protein 25, AMP; Adenosine monophosphate, ATP; Adenosine triphosphate, AMPK; Adenosine monophosphate-activated protein kinase, CaMMKβ; Calcium/calmodulin-dependent protein kinase kinase 2.

7. Anti-Obesogenic Effects of Plant Products and Their Mechanism of Action

It is evident from the literature that down- or upregulation of hundreds of transcriptional factors, hormones, and enzymes may induce obesity. Accordingly, there is no single mechanism of action of the various phytochemicals/plant products tested in the management of obesity. Different kinds of plant products have different types of mechanisms (Table 2). Their mechanism of action may include: **1. Inhibition of Pancreatic Lipase (PL) Activity.** PL is a crucial enzyme for the hydrolysis of triglycerides into monoglycerides and fatty acids. It hydrolyses 50-70 % of the total dietry fat [142] and the inhibition of this enzyme is among approaches used to combat obesity. The mechanism involves inhibition of absorption of triglycerides in the bodies. PL inhibition is being widely studied

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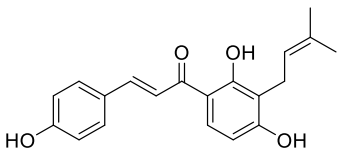
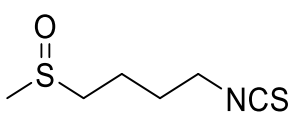
to evaluate the potential of natural products to inhibit dietary fat absorption [143–145]. **2. Increase in Energy Expenditure.** As mentioned above, brown adipose tissues transform energy from stored fat into heat (thermogenesis). UCP1 is the central activator of thermogenic effect and induction of its expression by natural anti-obesogenic plant products is one route to reduce obesity [7,146]. **3. Appetite Suppressors.** State of satiety is regulated by various hormonal and neurological signals in human bodies. Neural signal peptides, for instance, dopamine and histamine are correlated with the state of satiety. Plant products which give a perceived enhancement of satiety through an increase in adrenaline level and activation of the sympathetic nervous system activity are beneficial for controlling weight gain [147,148]. **4. Regulation of Lipid Metabolism.** Hydrolysis of triglycerides into monoglycerides and fatty acids is an important step in the absorption and accumulation of fats. Steering the expression of transcriptional factors and enzymes, for instance AMPK, involved in lipid metabolism (lipolysis) can be achieved by plant products [149]. Similarly, β-adrenergic receptor activation causes non-shivering thermogenesis in brown adipocytes and lipolysis in white adipocytes [150]. **5. Adipocyte Differentiation.** Energy balance and homeostasis of lipids are controlled by adipose tissues. Hypertrophy (increase in size of cells) and hyperplasia (increase in number of cells) are the two primary conditions related to adipose tissue. These cells store triglycerides and release them when needed in response to energy demands. Blockade of the principal and main adipogenic factors PPAR γ, C/EBP, SREBP families along with other proteins and transcriptional factors by anti-obesogenic products may prove fruitful in the development of anti-obesity therapies [7,151].

Table 2. Reported anti-obesogenic effects of plant extracts/phytochemicals **(2014 onwards)**

Plant extracts/Specific Phytochemicals	**Experimental Models**	**Anti-Obesogenic Effects**	**Reference**
Isobavachalcone (IBC) 	3T3-L1 pre-adipocytes	Decreased cell proliferation by 38.6% at day 2 (D2) and 31.0% at day 8 (D8). Reduced intracellular lipids contents by 75%. Decreased protein level of PPARγ and C/EBP α by 85.5 and 97.3%. Decreased the expression of SREBP1c, adiponectin, ACC1, and FAS mRNAs levels	[152]
Suşphoraphane 	3T3-L1 murine pre-adipocytes	Reduced adipocyte differentiation through C/EBPα, PPARγ1, PPARγ2, and GLUT4 mRNA downregulations	[153]
Genistein			

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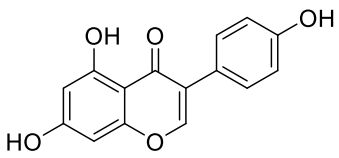
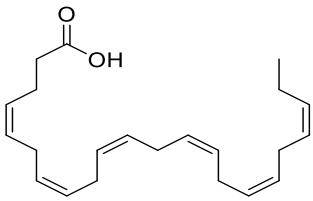
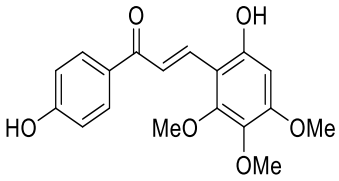
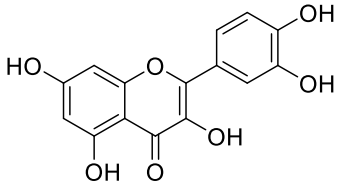
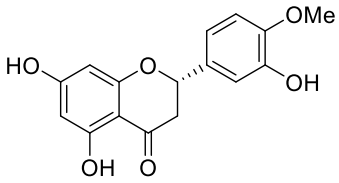
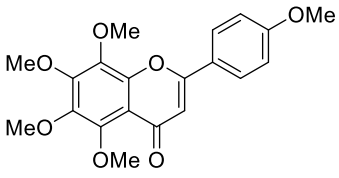
 Docosahexaenoic acid 			
Chromolaena odorata; leaves extracts containing flavonoid (4,2′dihydroxy-4′,5′,6′ trimethoxychalcone) 	3T3-L1 adipocytes	Reduced lipid accumulation by 75 % (30 µmol/L) and 90% (50 µmol/L) in 3T3-L1 adipocytes	[154]
Quercetin-rich supplement 	HFD induce Wister male rats	Reduced lipid accumulation. Reduced size of adipocytes	[155]
Hesperetin  Tangeretin 	3T3-L1 adipocytes	Reduced TG level by 16.08, 23.10 and 45.67 % at 50 µmol concentration. Downregulated expression level of PPARγç	[156]

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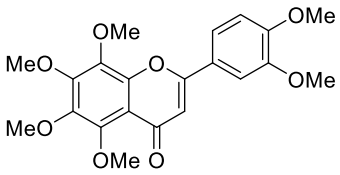
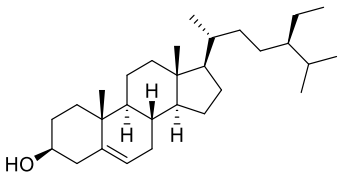
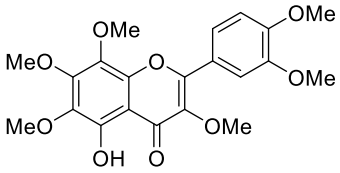
Nobiletin 			
Tropaeolum majus (Extract)	3T3-L1 cells	Reduced TG level up to 25.8% - 54.7%. Decreased PPARγ expression level by 23.0% – 90.4% and C/EBPα by 45.8% and 71.9% at different concentrations	[157]
β -sitosterol and corn silk extracts 	3T3-L1 cell lines and C57BL/6 HFD induced mice.	Reduced the genes and protein expression level of C/EBPα, C/EBP β, aP2, adipsin and PPARγ. In *in vivo* studies, decreased the weight and adipocytes in various organs including adipose tissue and liver	[158]
Anthocyanins from *Vitis coignetiae*	3T3-L1 pre-adipocytes	Enhanced activation of AMPK, decreased the number of lipid droplets. Reduced TG level by 60 % at 200 μg/ml. Inhibited the expression of PPARγ, C/EBP α, - β and SREBP-1c	[159]
5-Hydroxy-3,6,7,8,3',4'-hexamethoxyflavone 	3T3-L1 cells	Reduced lipid accumulation up to 55-60%. Downregulated PPARγ1, PPARγ2, C/EBPα, FAS and ACC	[160]
Oroxylum indicum (L.) extracts	3T3-L1 pre-adipocytes and porcine pancreatic lipase	Decreased cell viability and lipid accumulation by 52% at 200 μg/ml concentration *in vitro* and inhibited the activity of pancreatic lipase (IC_{50} 1062.04 ± 32.21 μg/mL)	[161]
1) *Phyllanthus niruri* (whole plant extract). 2) *Orthosiphon stami neus* (leaves extract).	Porcine pancreatic lipase	>70% inhibition of pancreatic lipase activity with IC_{50} value **1)** 27.7, **2)** 34.7, **3)** 41.5 and **4)** 55.2 μg/ml respectively	[162]

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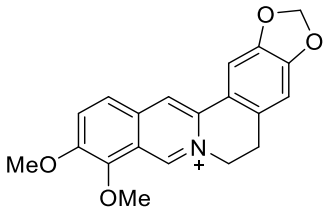
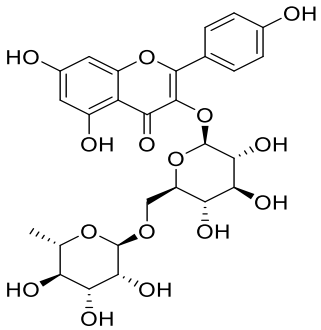
3) *Murraya paniculata* (leaves extract) and 4) *Averrhoa bilimbi* (leaves extract)			
Green coffee, cinnamon and ginger (Combined extracts)	Obese male sprague-dawley rats	Decreased level of TG, LDL and total lipids	[163]
Berberine from *Coptis chinensis* 	3T3-L1 adipocytes	Increased expression of ATGL and HSL. Decreased TG level by 10%	[164]
Açai (*Euterpe oleracea* Mart)/Polyphenols	3T3-L1 mouse adipocytes	Downregulated PPAR γ-2, decreased expression of adipogenic transcription factors (C/EBPα, -β, KLF5 and SREBP1c	[165]
Mulberry leaf extract (MLE) and mulberry leaf polyphenol extract (MLPE)	3T3-L1 mouse embryo cells/C57BL/6 male mice	Inhibited the differentiation of 3T3-L1 pre-adipocytes. Suppressed the expression of SREBP-1c and PPAR-γ proteins and FAS. Increased phosphorylation of AMPK	[166]
Curcuma longa L extracts	Sprague-Dawely (SD) HFD rats	Suppressed adipocyte differentiation and lipogenesis. Decreased mRNA expression of FAS, ACC, Adipocyte protein-2 and LPL	[167]
Kaempferol-3-O-rutinoside from *Solidago virgaurea* 	3T3-L1 fibroblasts	Anti-adipogenic effects through suppression of PPARγ and C/EBPα expression	[168]
Tangeritin	3T3-L1 fat cells.	Inhibited adipogenesis by down-regulating the expression of mRNAs of C/EBPα, C/EBP β and PPARγ	[169]

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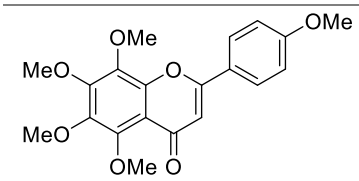
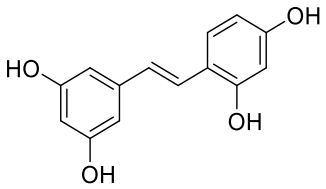
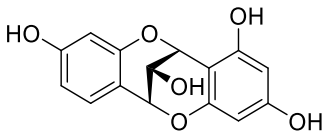
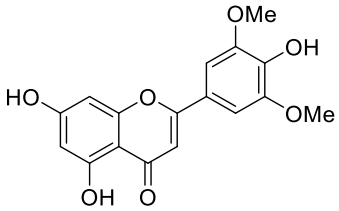
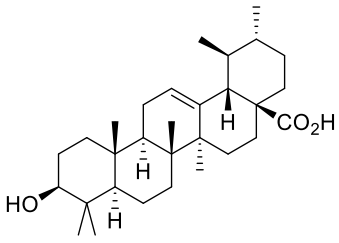
			
Diospyros kaki Fruit and *Citrus unshiu* Peel	Porcine pancreas lipase activity *in vitro* and male HFD induced ICR mice	Inhibited pancreatic lipase activity *in vitro* (IC_{50} 507.01 μg/mL) and significantly reduced serum triacylglycerol, total cholesterol and visceral fat weight in ICR mice	[144]
Oxyresveratrol 	3T3-L1 cells	Reduced TG contents to 68% and 41% of control with 100 and 600 μM concentrations. Inhibited cell proliferation up to 45 % and 67%. Induced cell cycle arrest and down regulated expression of PPARγ and C/EBP α	[170]
Cyanomaclurin 			
Tricin (5,7,4′ trihydroxy-3′,5′-dimethoxyflavone) 	3T3-L1 pre-adipocytes	Inhibited lipid accumulation by 37% at 6 μg/ml. Significantly decreased the mRNA level of PPAR γ, CEBP/ and SREBP1 at 1.5 μg/ml concentration	[171]
Triterpenoid (Ursolic Acid). 	HFD induced Sprague-dawely rats	Enhanced activation of AMPK. Reduced insulin resistance, and body weight (45g average weight loss) Decreased HFD/Body ratio by 17%	[172]
Persimmon Tannin	3T3-L1 pre-adipocytes	Inhibited early stage adipocytes differentiation, reduced triglyceride contents by 6.6 % at 60 μg/ml	[173]

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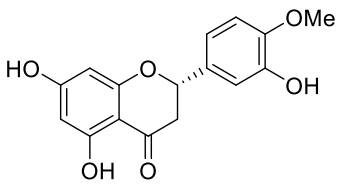
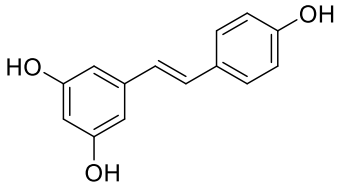
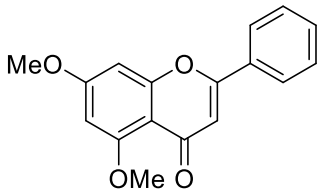
		concentration and suppressed the expression of C/EBP α and PPAR γ	
Hesperetin 	Human Mesenchyma Stem cells (hMSCs)	Decreased lipid contents to 42.6 and 67.82 % in concentration dependent manner. Almost completely suppressed the differentiation of hMSCs to pre-adipocytes. Decreased 1.79- and 1.63-folds expression level of PPARγ and C/EBP β	[174]
Camellia sinensis (polyphenols), polysaccharides)	Sprague-Dawely (SD) HFD rats	Reduced expression of IL-6, TNFα genes, serum leptin level and inhibited fatty acid absorption	[175]
Resveratrol 	HFD induced Kumming mice	Downregulated mRNA level of ACC 1, PPARγ and FAS	[176]
Gyeongshingangjeehwan 18 (GGEx18) from *Rheum palmatum* L, *Laminaria japonica* Aresch, *Ephedra sinica*	3T3-L1 adipocytes and HFD C57BL/6J mice	Reduced lipid accumulation by 41, 54 and 70% at 0.1, 1 and 10 µg/ml concentrations. Decreased visceral adipose tissue weight by 46 and 24% at 250 and 500 mg/kg/d concentration. Increased expression of AMPK	[177]
5,7-Dimethoxyflavone 	3T3-L1 adipocytes and high-fat diet (HFD)-induced obese C57BL/6J mice	Downregulated PPARγ, C/EBPα, SREBP 1-C, HMG-CoA, ACC and FAS. Activated AMPK	[178]

Glut 4: Glucose transporter type 4; LDL: Low Density Lipoproteins; ATGL: Adipose Triglyceride Lipase; HSL: Hormone Sensitive Lipase; hMSCs: Human Mesenchymal Stem cells; IL-6: Interleukin-6; LPL: Lipoprotein Lipase; TG; Triglycerides; AMPK: adenosine monophosphate-activated protein kinase; SREBP-1c: Sterol Regulatory Binding Protein-1c; ACC: Acetyl Co-A Carboxylase; FAS: Fatty Acids Synthase; HFD: High fat diet; TG: Triglycerides.

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8. Summary and Outlook

Obesity is a common disorder caused by the interaction of environmental, genetic and nutritional factors, and its pervasiveness is accelerating worldwide. Changes in lifestyle, extensive consumption of calorific foods, and increasingly sedentary lifestyles are the predominant causative factors for this rise in obesity. Returning to healthy lifestyles, for example daily exercise and consumption of food rich in minerals and fibres is the surest route to successful weight loss, but for many it is extremely challenging to maintain. For decades, synthetic chemicals had been explored to fill this need. Despite generating positive results, they have also caused adverse effects that muted their benefits. Traditionally used medicinal plants which contain a wide range of natural chemicals have been used for the treatment of many infectious as well as chronic disorders including obesity. These natural products from plants (phytochemicals) may play a supportive role in combating obesity by helping obese people to lose weight. A synergestic activity could be conferred by the combination of multiple natural products to increase their anti-obesogenic activities and mode of action on multiple targets, which may prove to be advantageous over other chemical treatments. Anti-obesogenic studies either based on *in vitro* studies or mouse models do not guarantee the same results in humans, and so clinical trials must be performed to evaluate the effectiveness of bioactive compounds in order to understand their safety, bioavailability, pharmacokinetics and efficacy. Clinical trials should be planned carefully to evaluate both immediate and long-term side effects of bioactive compounds. Additionally, studies on phytochemicals and their anti-obesogenic effects on direct or indirect activation of AMPK may expedite the development of anti-obesogenic drugs. Investigation into promising targets and identification of unique regulators of adipogenesis are still needed for formulating appropriate natural drugs against obesity. With the availability of many natural medicinal plants, these will provide scientific bases for the development of anti-obesogenic natural products and play a central role in the management of obesity.

Authors Contributions: Bilal Ahmad and Wong Eng Hwa did the literature search, designed and wrote the draft of the manuscript. Christopher J Serpell and Isabel Fong Lim made editorial changes and reviewed the manuscript. All the authors reviewed and approved the final version of the manuscript before submission.

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Conflict of Interest: The authors declare no conflicts of interest.

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