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Review Article

Cellular models for the evaluation of the antiobesity effect of selected phytochemicals from food and herbs

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ABSTRACT

Dietary phytochemicals from food and herbs have been studied for their health benefits for a long time. The incidence of obesity has seen an incredible increase worldwide. Although dieting, along with increased physical activity, seems an easy method in theory to manage obesity, it is hard to apply in real life. Obesity treatment drugs and surgery are not successful or targeted for everyone and can have significant side effects. This low rate of success is the major reason that the overweight as well as the pharmaceutical industry seek alternative methods, including phytochemicals. Therefore, more and more research has focused on the role of phytochemicals to alleviate lipid accumulation or enhance energy expenditure in adipocytes. This review discusses selected phytochemicals from food and herbs and their effects on adipogenesis, lipogenesis, lipolysis, oxidation of fatty acids, and browning in 3T3-L1 preadipocytes.

1. Introduction

Obesity has become a critical health problem worldwide, and it is the major risk factors for dyslipidemia, cardiovascular disease, carcinogenesis, and type-2 diabetes [1]. Physical activity, diet modification, drugs, and surgery are common strategies to decrease the incidence of obesity. Although the United States Food and Drug Administration have approved obesity-treatment drugs such as orlistat and lorcaserin to fight obesity, the side effects and the low rate of success of obesity

drugs have made the consumer and pharmaceutical industry seek alternative solutions from natural compounds [2]. Since ancient times, herbs and other plants have been used as medicines for many diseases [3], and they are still the basis of a traditional medicine system in different cultures [4,5]. Natural bioactive substances derived from animal or plants can have different biological benefits in different body systems. Moreover, natural compounds from food are perceived as safe and acceptable for the patient compared with synthetic therapeutics [2]. Dietary phytochemicals may provide a better or more efficient therapeutic approach for treating obesity. Thus, many scientists are interested in the investigation of potential natural products in food or plants to fight obesity [6]. Energy imbalance is the major cause of obesity, and excessive energy causes preadipocyte cells to become adipocytes (hyperplasia) and triacylglycerols (TG) to accumulate in mature adipocytes (hypertrophy) [7—9]. Moreover, the balance of fat accumulation is regulated by fat synthesis and fat breakdown through lipogenesis and lipolysis/fat oxidation pathways [10]. Many studies have investigated the role of dietary phytochemicals in the prevention and treatment of obesity and their effect on decreased energy intake or increased energy expenditures. In this review, we aim to provide an overview of the effect of dietary phytochemicals on regulated adipogenesis/lipogenesis, lipolysis/fatty acid (FA) oxidation, and thermogenesis in vitro and possible mechanisms.

2. Characteristics of adipose tissue

2.1. Adipose tissue

Adipose tissue contains several cell types, including endothelial cells, blood cells, fibroblasts, preadipocytes, macrophages, other immune cells, and mature adipocytes [11,12]. When adipocytes increase in large numbers, they become the main cell type and then form adipose tissue. There are two types of fat in the body; white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is a fuel storage organ; it stores excess energy as TG by a lipogenesis process and uses TG during food deprivation by a lipolysis process. Excessive energy can increase the number and size of adipocytes causing WAT to expand and lead to obesity [11,13]. The most predominant characteristic of BAT is nonshivering thermogenesis, where energy derived from FA oxidation generates heat by mitochondrial uncoupling to maintain body temperature [14—16]. Therefore, understanding the mechanism of adipocytes in both WAT and BAT could lead to an alternative therapy to treat obesity and its metabolic complications [17].

2.2. In vitro model: 3T3-L1 preadipocyte

Various cell culture models, including preadipocyte cell lines and primary cultures of adipose-derived stromal vascular precursor cells, are used to study the molecular mechanisms of adipocyte differentiation. However, the study of preadipocyte differentiation in vivo is very difficult, as human and animal fat tissues are combined with small blood vessels, nerve tissue, fibroblasts, and preadipocytes at various stages of development [12,13,18]. Contamination, the limited life

span, and the low percentage of preadipocytes in the total adipose tissue are big problems for primary preadipocyte cell culture. Therefore, the molecular mechanism of adipogenesis is intensively studied in vitro by using different preadipocyte clonal cell lines from mice or rats [13,19]. The 3T3-L1 cell line, established by Green and Kehinde, is a well-characterized and reliable model for studying the conversion of preadipocytes into adipocytes, derived from disaggregated 17—19-day-old Swiss 3T3 mouse embryos [17,18,20]. 3T3-L1 preadipocyte differentiation has most of the characteristics as it does in animal tissue [21]. When 3T3 lines reach confluence, part of a cell may spontaneously differentiate into rounded cells and contain large droplets of TG, and the cells show 10- to 100-fold increases in de novo lipogenesis; 40—50-fold increases in adenosine triphosphate (ATP)-citrate lyase, acetyl-Co A carboxylase (ACC), and FA synthase (FAS) activity; and 80- and l5-fold increases in glycerophosphate acyltransferase and malic enzyme activity, respectively [22,23]. However, the adipocyte conversion process can occur spontaneously at around 2—4 weeks after confluence, and the conversion process can be accelerated by an adipogenic cocktail including insulin, dexamethasone, and 3-isobutyl-1-methylxanthine; this induces transcription factor expression within 1 hour in 3T3-L1 preadipocytes, which then achieve complete differentiation within 10—12 days [24—26] (Figure 1).

3. Phytochemicals in lipid metabolism

3.1. Characteristics of adipogenesis/lipogenesis

Adipogenesis is a process by which fibroblast-like preadipocytes differentiate into mature adipocytes, which contain large internal fat droplets for lipid storage. The process can continue throughout the whole lifetime in most animals [11,27,28]. There are various stages of adipogenesis: mesenchymal precursors, committed preadipocytes, growth-arrested preadipocytes, mitotic clonal expansion, terminal differentiation, and mature adipocytes [28,29]. The process of adipogenesis involves a series of transcription factors, cell-cycle protein—regulated gene expression, and lipogenesis-related gene and enzyme activity [17,28,30]. The differentiation process is critically modulated through the coordinated regulation of transcription factors, especially cytosine-cytosine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding protein (C/EBP) families (C/EBPα, C/EBPβ, and C/EBPδ), and peroxisome proliferator-activated receptor (PPAR) families (PPARα, PPARβ/δ, and PPARγ) [31]. C/EBP-α and PPAR-γ are both involved in growth arrest in the early stage of adipocyte differentiation. After growth arrest, preadipocytes need mitogenic and adipogenic signals for further differentiation stages. Therefore, mitotic clonal expansion (MCE), induced by hormonal stimulation, is an important process that allows growth-arrested preadipocytes to reenter into the cell cycle, and undergo several rounds of cell division during adipogenesis [28]. In the late process of adipocyte differentiation, de novo lipogenesis intensively increases in adipocytes [18]. Lipogenesis is a process involving FA and TG synthesis, and it can be affected by diet and hormones [10]. For example, carbohydrates stimulate lipogenesis in liver and adipose tissue,
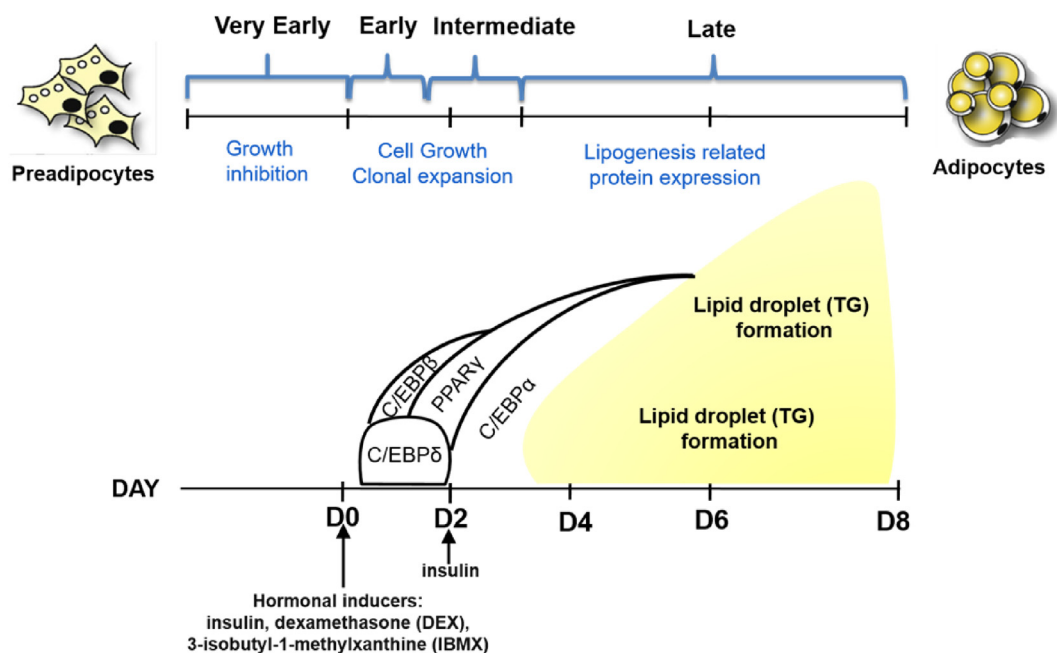
Figure 1 — The differentiation process of 3T3-L1 [9,13].

and fasting changes plasma hormone concentration, thus affecting lipogenesis and lipolysis in the adipose tissue [32]. Sterol regulatory element binding proteins (SREBPs) are another transcription factor related to cellular lipogenesis, lipid homeostasis, and adipocyte differentiation [33,34]. SREBP-1 is a type of SREBP that can induce PPAR-γ expression and regulate lipid biosynthesis in adipocytes, and increase expression of lipogenic genes such as FAS and ACC [14,35,36]. Triacylglycerol metabolism-related enzymes such as stearoyl-CoA desaturase, glycerol-3-phosphate acyltransferase, glycerol-3-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase increase dramatically during lipogenesis [37,38]. Therefore, dietary phytochemicals do have some effects on these stages of adipogenesis and could help treat obesity. Apigenin (4',5,7-trihydroxyflavone) is a flavonoid found in vegetables and fruits [39,40]. Administration of 50μM apigenin decreased TG content via decreased mRNA and protein expression of PPARγ, FA binding protein 4 (FABP4), and stearoyl-CoA desaturase, through an increase in activated 5'-adenosine monophosphate-activated protein kinase (AMPK) [41]. Berberine is a major component from *Cortidis rhizoma* and is an antibacterial drug in traditional Chinese medicine [42]. The results of a study showed that a 10μM berberine decreased TG content by inhibiting clonal expansion during 3T3-L1 adipocyte differentiation. Its possible anti-adipogenic mechanism may be reduced by the mRNA expression of PPARβ/δ, γ and C/EBPα at Day 5 and C/EBPβ at Day 1. Berberine also decreased the expression of PPARγ-targeted lipogenic genes such as FABP4, ACC, and FAS [43]. Another study showed that administration of 5 μg/mL berberine decreased TG content by increasing phosphorylated AMPK (pAMPK) and phosphorylated ACC (pACC) expression and decreasing FAS, adipocyte determination and differentiation dependent factor 1 (ADD1)/SREBP1c, PPARγ, C/EBPα, and FABP4 expression [44]. Capsaicin (8-methyl-N-vanillyl-*trans*-6-nonenamide) is a pungent

source of the hot sensation in red pepper [45]. Capsaicin (50—250μM) decreased TG content by causing apoptosis in 3T3-L1 preadipocytes via affecting the mitochondrial membrane potential ($\Delta\Psi m$); caspase-3, Bax, and Bak, and the cleavage of poly ADP-ribose polymerase protein expression increased and Bcl-2 protein expression decreased within 24 hours of administration. Capsaicin could decrease TG content via decreased PPARγ, C/EBPα, and leptin protein expression in mature 3T3-L1 adipocytes within 72 hours [46]. Another study showed that 3T3-L1 preadipocytes pretreated with 100μM capsaicin for 30 minutes and afterwards went through the differentiation process and decreased TG content via increased pAMPK and pACC protein expression during adipogenesis [47]. The polyphenolic flavonoid (-)-epigallocatechin-3-gallate (EGCG) is one of the catechins present in green tea [48]. Administration of 100μM EGCG decreased TG content via decreased protein expression of PPARγ1/2 and its target genes LXRα during the adipogenesis process [49]. In 2011, Chan et al showed that 10μM EGCG decreased adipose conversion by inhibiting 3T3-L1 cell proliferation by arresting cells at the G2/M phase and decreasing C/EBPα and PPARγ expression [50]. Another study showed that 100μM EGCG decreased lipid content via decreased CEBPα, PPARγ, FAS, and FABP4 expression and increasing β-catenin and its transcriptional product, cyclin D1 expression [51]. Genistein (4',5,7-trihydroxyisoflavone) is an isoflavone from soybean [52]. Administration of 100μM genistein decreased TG content from 24 hours to 72 hours via increased expression of pAMPK and pACC within 24 hours [47]. Another study indicated that, after administering 100μM genistein only at 72 hours during the whole 8 day differentiation process, genistein decreased TG accumulation via blocking the DNA binding and transcriptional activity of CEBPβ by increasing CEBP homologous protein expression, then subsequently decreasing CEBPα and PPARγ expression [52]. Resveratrol (3,5,4'-trihydroxystilbene),

is a natural phytochemical found in grape and red wine [53]. Twenty-five micromoles and 50μM of resveratrol significantly decreased TG content via decreased expression of PPARγ, C/EBPα, SREBP1, and FAS mRNA [54]. Another study investigated the activity of resveratrol (0.03μM, 0.1μM, 0.3μM, 1μM, 3μM, 10μM, 30μM, 100μM) in 3T3-L1 preadipocytes and mature adipocytes. The results of cell viability tests showed that the 30μM and 100μM resveratrol had lower cell survival in 3T3-L1 preadipocytes. Based on minimal cytotoxicity, 0.03μM, 0.1μM, 0.3μM, 1μM, 3μM, and 10μM resveratrol were studied. The 3μM and 10μM resveratrol decreased TG content via decreased PPARγ protein expressions in mature adipocytes [55]. Oxyresveratrol is a natural polyphenol and also an analogue of resveratrol occurring in mulberry (*Morus alba* L.) [56]. Administration of 100μM oxyresveratrol decreased TG content via decreased PPARγ during the late stage of differentiation and decreased C/EBPα expression during the early stage of differentiation. A concentration of 100μM oxyresveratrol also dose-dependently induced cell cycle arrest during the mitotic clonal proliferation stage of differentiation, via decreased cyclin A and cyclin-dependent kinase 2 expression at the S and G2/M phase and decreased cyclin D1 and cyclin-dependent kinase 4 expression at the G0/G1 phase [57]. Curcumin is a major polyphenol and one of the most active ingredients in the rhizome of the perennial herb turmeric (*Curcuma longa*), and it is a dietary spice in curry [58]. When differentiated cells were treated with 25μM curcumin for 48 hours, TG content decreased via blocked differentiation through C/EBPα and PPARγ, SREBP-1, and FAS expression. 25μM curcumin also inhibited phosphorylated mitogen-activated protein kinase, including extracellular signal-regulated kinases (ERK), JNK, and p38 expression during differentiation. A concentration of 25μM curcumin restored nuclear translocation of β-catenin [wingless-type MMTV integration site family signaling (Wnt) component] and decreased casein kinase 1α, glycogen synthase kinase 3β, and Axin expression to activate Wnt signaling. Curcumin also decreased FABP4 and increased Wnt10b, Fz2 (Wnt direct receptor), LRP5 (Wnt coreceptor), c-Myc and cyclin D1 (Wnt targets) mRNA expression during differentiation [59]. Another study showed that 30μM curcumin decreased TG content via decreased C/EBPβ, PPARγ, and C/EBPα expression in the late stages of differentiation. Curcumin also inhibited the MCE process via delayed cell reentry into the S phase through decreased transcription factors, Krüppel-like factor 5, C/EBPα, and PPARγ during the early stage of adipocyte differentiation [60]. A similar study showed that 20μM curcumin slightly decreased PPARγ and C/EBPα within 24 hours of initial differentiation, but induced apoptosis via regulated caspase 3 and poly ADP-ribose polymerase within 20 hours after differentiation. It also arrested cells in the G1 phase via increased cyclin D and decreased cyclin A and inhibited targeted p27 proteolysis through reduced Skp2 and 26S proteasome activity to inhibit cell growth during differentiation within 20 hours [61]. Bisdemethoxycurcumin (BDMC) is one of the curcuminoids in turmeric [62]. A 25μM BDMC decreased TG content more than curcumin and demethoxycurcumin via arresting cells at the G1 phase after differentiation at 18 hours and 24 hours. It affected the MCE process through decreased cyclin A and cyclin B and increased p21 expression. BDMC also decreased PPARγ and C/

EBPα, phosphorylated extracellular signal—regulated kinases (ERK1/2), c-Jun amino-terminal kinases (JNK), and Akt expression during adipogenesis [63]. 18β-Glycyrrhetinic acid is a principal active ingredient from the herb licorice root [64]. Twenty micromoles of 18b-GA decreased TG content via decreased PPARγ and CEBPα expression through decreased phosphorylated Akt expression [65]. *Ginkgo biloba* L. has been used as a medicinal herb in eastern and western medicine. Ginkgolide C is a flavone isolated from *G. biloba* leaves [66]. Administration of 100μM ginkgolide C decreased TG content via decreased PPARα, PPARγ, CEBPα, CEBPβ, and SREBP-1c expression and decreased FAS and FABP4 expression [67]. Celastrol is a triterpene from the vine *Tripterygium wilfordii* (Celastraceae), which has been used as traditional Chinese medicine for hundreds of years [68]. A concentration of 400nM of celastrol treated from Day 0 to Day 8 decreased most TG content via decreased PPARγ2, C/EBPα, and FABP4 expression [69] (Table 1).

3.2. Characteristics of lipolysis/FA oxidation

Breakdown of TG in adipocytes and the release of glycerol and FAs are important for the regulation of energy homeostasis [70]. Lipolysis is a catabolic pathway that mainly happens in adipose tissue to provide energy to peripheral tissues when needed [71]. The process of lipolysis involves the hydrolysis of TGs, which results in the release of FA and glycerol into blood. Various lipases have been discovered to be active during TG hydrolysis. Adipose triacylglycerol lipase (ATGL) is involved in the process of TG hydrolysis into diacylglycerols (DG) and the release of one FA. Subsequently, monoacylglycerol lipase hydrolyzes DGs to monoacylglycerols and releases one FA, or alternatively the DGs are completely hydrolyzed by hormone-sensitive lipase (HSL), releasing two FAs and one glycerol [72,73]. FAs are degraded in the mitochondria and peroxisome through the process of β-oxidation. The long chain FAs are converted to acyl CoA in the cytosol, which needs carnitine acyltransferase-1 or carnitine palmitoyltransferase-1 (CPT1) to convert it to acylcarnitine to enter the mitochondria [74,75]. A treatment of 10μM capsaicin to full differentiated 3T3-L1 adipocytes for 24 hours showed an increased release of glycerol via increased HSL and CPT1-α [76]. EGCG (10μM) in full differentiated 3T3-L1 adipocytes for 24 hours showed an increase in the release of glycerol into the medium via increased HSL expression [77]. Administration of 10μmol/L and 20μmol/L concentrations of curcumin decreased lipid accumulation by increasing palmitic acid oxidation, CPT1 expression, and decreasing glycerol-3-phosphate acyl transferase-1 expression, but did not affect PPARγ and C/EBPα levels [78]. 18β-Glycyrrhetinic acid increased the release of glycerol via increased HSL, ATGL, and perilipin expression in mature 3T3-L1 preadipocytes [65]. Both 30μM and 100μM ginkgolide C increased ATGL and HSL mRNA expression via enhanced Sirt1 and AMPK activation in mature 3T3-L1 adipocytes [67] (Table 2).

3.3. Characteristics of browning

As mentioned above, WAT and BAT are two major adipose tissues that regulate energy balance [82]. In BAT, thermogenesis is activated by characteristics such as uncoupling protein

Table 1 – The effect of phytochemicals in adipogenesis/lipogenesis.

Phytochemical	Regulation	Reference
Apigenin (4′,5,7-trihydroxyflavone)	↓ TG content	[41]
	↓ PPARγ, FABP4, SCD	
	↑ Activation of AMPK	
Berberine	↓ TG content	[43]
	↓ PPARγ,β,δ, C/EBPα	
	↓FABP4, ACC, FAS	
	↓ TG content	[44]
	↓ PPARγ, C/EBPα, ADD/SREBP-1c	
	↓ FABP4, FAS	
	↑ pAMPK, pACC	
Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide)	↓ TG content	[46]
	↓ Mitochondria membrane potential (ΔΨm)	
	↑ Caspase 3, Bax, Bak, the cleavage of PARP	
	↓Bcl-2	
	↓ PPARγ, C/EBPα	
	↓ TG content	[47]
	↑ pAMPK, pACC	
(-)-Epigallocatechin gallate	↓ TG content	[49]
	↓ PPARγ1/2, LXRα	
	↓ TG content	[50]
	↓ PPARγ, C/EBPα	
	↑ An arrest of cell cycle at G2/M phase	
	↓ TG content	[51]
	↓ PPARγ, C/EBPα	
	↓ FABP4, FAS	
	↑ β-catenin, cyclin D1	
Genistein	↓ TG content	[47]
	↑ pAMPK, pACC	
	↓ TG content	[52]
	↓ C/EBPβ via ↑ CHOP	
	↓ PPARγ, C/EBPα	
Resveratrol (3,5,4′-trihydroxystilbene)	↓ TG content	[54]
	↓ PPARγ, C/EBPα	
	↓ SREBP-1, FAS	
	↓ TG content	[55]
	↓ PPARγ	
Oxyresveratrol	↓ TG content	[57]
	↓ PPARγ, C/EBPα	
	↓ Cyclin A, CDK2, cyclin D1, CDK4	
Curcumin	↓ TG content	[59]
	↓ PPARγ, C/EBPα	
	↓ SREBP-1, FAS	
	↓ Phosphorylation of MAPK (ERK, JNK, and p38)	
	↑ Translocation of β-catenin	
	↓ CK1α, GSK-3β, Axin, FABP4	
	↑ Wnt10b, Fz2, LRP5, c-Myc, cyclin D1	
	↓ TG content	[60]
	↓ PPARγ, C/EBPα, β	
	↓ KLF5	
	↓ TG content	[61]
	↓ PPARγ, C/EBPα	
	↑ Caspase 3, PARP	
	↑ Cyclin D	
	↓ Cyclin A	
	↑ Inhibition of p27 proteolysis by ↓ Skp2, 26S proteasome activity	
Bisdemethoxycurcumin	↓ TG content	[63]
	↓ Cyclin A, B	
	↑ p21	
	↓ PPARγ, C/EBPα	
	↓ Phosphorylated ERK1/2, JNK, Akt	
18β-Glycyrrhetinic acid	↓ TG content	[65]
	↓ PPARγ, C/EBPα	
	↓ Phosphorylated Akt	

Table 1 – (continued)

Phytochemical	Regulation	Reference
Ginkgolide C	↓ TG content	[67]
	↓ PPARα,γ, C/EBPα, β, SREBP-1c	
	↓ FAS, FABP4	
Celastrol	↓ TG content	[69]
	↓ PPARγ2, C/EBPα	
	↓ FAS, FABP4	

ACC = acetyl-Co A carboxylase; AMPK = 5′-adenosine monophosphate-activated protein kinase; BDMC = bisdemethoxycurcumin; CDK = cyclin-dependent kinase; CHOP = CEBP homologous protein; C/EBP = CCAAT/enhancer binding protein; ERK = extracellular signal-regulated kinases; FABP = fatty acid binding protein 4; FAS = fatty acid synthase; JNK = c-Jun amino-terminal kinases; KLF5 = Krüppel-like factor 5; LXR = liver X receptor; MAPK = mitogen-activated protein kinase; PPAR = peroxisome proliferator-activated receptor; SCD = stearoyl-CoA desaturase; TG = triacylglycerols.

Table 2 – The effect of phytochemicals in lipolysis/fatty acid oxidation and browning.

Phytochemical	Regulation	Reference
Capsaicin	↑ Glycerol	[76]
(8-methyl-N-vanillyl-trans-6-nonenamide)	↑ HSL, CPT-1-α, UCP2	
(-)-Epigallocatechin gallate	↑ Glycerol	[77]
	↑ HSL	
Curcumin	↓ TG content	[78]
	↓ Palmitic acid oxidation	
	↑ CPT1, GPAT-1	
	↑ pAMPK, pACC	
	↑ White adipocyte become beige cell via ↑ PGC-1α, PPARγ, UCP1, RDM16, C/EBPβ, Tmem26, Cidea, Fgf21, Cited1	[79]
	↓ TG content	
	↑ Fat oxidation via ↑ CPT1, cytochrome C	
	↑ Lipolysis via ↑ HSL	
	↓ Fatty acid synthesis via ↑ pACC, pAMPK/AMPK	
18β-Glycyrrhetinic acid	↑Glycerol	[65]
	↑HSL, ATGL	
Ginkgolide C	↑Glycerol	[67]
	↑HSL, ATGL	
	↑Activation of Sirt1, AMPK	
Chrysin (5,7-dihydroxyflavone)	↑White adipocyte become beige cell via ↑ PGC-1α, PPARγ, UCP1	[80]
	↓TG content	
	↓C/EBPα	
	↑HSL, perilipin,	
	↑CPT1, acyl-coenzyme A oxidase 1,	
	↑pAMPK, pACC	
Thymol (5-methyl- 2-isopropylphenol)	↑White adipocyte become beige cell via ↑ PGC-1α, PPARγ, UCP1	[81]
	↓Lipogenesis via ↑pAMPK/AMPK, pACC	
	↑Mitochondria biogenesis via ↑Tfam, Nrf1, PGC-1α, UCP1, PRDM16	
	↓C/EBPα	
	↑HSL, CPT1, perilipin, ACO	

ACC = acetyl-Co A carboxylase; ACO = acyl-coenzyme A oxidase; AMPK = 5′-adenosine monophosphate-activated protein kinase; ATGL = adipose triacylglycerol lipase; CPT = carnitine palmitoyltransferase; C/EBP = CCAAT/enhancer binding protein; GPAT-1: glycerol-3-phosphate acyl transferase-1; PGC = peroxisome proliferator-activated receptor-γ coactivator; PPAR = peroxisome proliferator-activated receptor; PRDM = PRD1-BF-1-RIZ1 homologous domain containing protein; TG = triacylglycerols; UCP = uncoupling protein 1.

1 (UCP1) through lipolysis [15,83,84]. More and more researchers have found an active BAT in adult human WAT, also called browning of WAT [85,86]. This kind of brown adipocyte is called inducible brown adipocyte (beige, brown-in-white or brite) adipocyte [87,88]. Beige cells have some characteristics such as UCP1 being expressed with thermogenic ability, high mitochondrial content and the expression of brown fat-specific genes including UCP1, Cidea, and encoding PPAR-γ coactivator 1-α (PGC-1α) in WAT [89–91]. Although brown adipocytes and beige adipocytes express UCP1, brown adipocytes always have high levels of UCP1 and other thermogenic genes are under basal control [92]. In beige cells, the
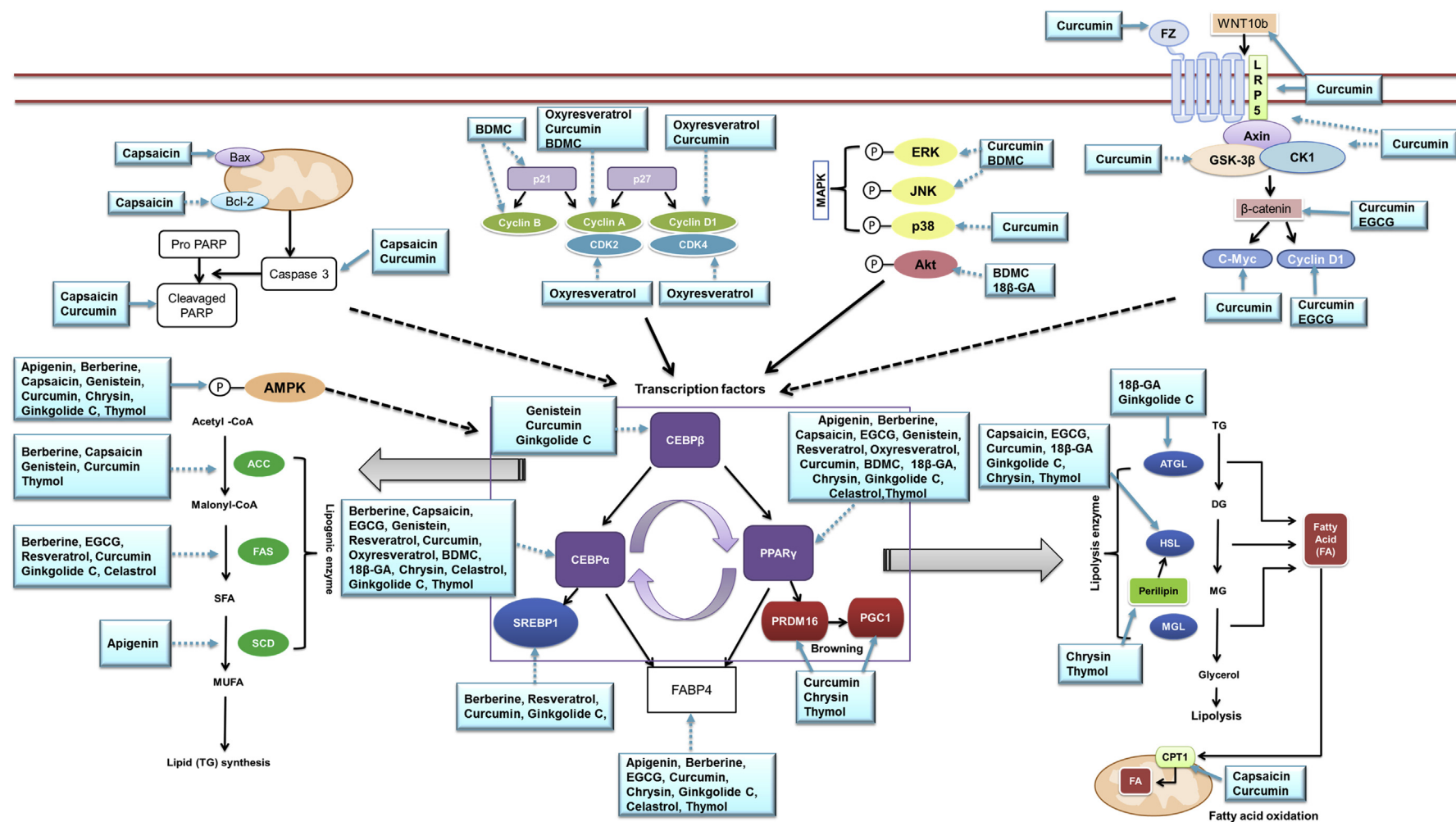
Figure 2 − The possible antiobesity mechanism of phytochemicals in 3T3-L1. 18β-GA = 18β-glycyrrhetinic acid; ACC = acetyl-Co A carboxylase; AKT = protein kinase B; AMPK = 5′-adenosine monophosphate-activated protein kinase; ATGL = adipose triacylglycerol lipase; BDMC = bisdemethoxycurcumin; CDK = cyclin-dependent kinase; C/EBP = CCAAT/enhancer binding protein; CK1 = casein kinase 1; CPT-1 = carnitine palmitoyltransferase-1; DG = diacylglycerols; EGCG = (-)-epigallocatechin-3-gallate; ERK = extracellular signal-regulated kinases; FABP4 = fatty acid binding protein 4; FAS = fatty acid synthase; GSK-3β = glycogen synthase kinase 3β; HSL = hormone-sensitive lipase; JNK = c-Jun amino-terminal kinases; MG = monoacylglycerols; MGL = monoacylglycerol lipase; MUFA = monounsaturated fatty acid; PARP = poly ADP-ribose polymerase; PGC-1α = peroxisome proliferator-activated receptor-γ coactivator 1-α; PPAR = peroxisome proliferator−activated receptor; PRDM = PRD1-BF-1-RIZ1 homologous domain containing protein; SCD = stearoyl-CoA desaturase; SFA = saturated fatty acid; SREBPs = sterol regulatory element binding proteins; TG = triacylglycerol; WNT = wingless-type MMTV integration site family.

expression of these thermogenic genes responds to activators such as agonists of the β-adrenergic receptor or PPARγ or enriched markers such as Cd137, Tbx1, Tmem26, Cited1, and Shox2. C/EBPβ, PRDM16, and PGC-1α are important transcriptional factors in beige cells [93—100]. Therefore, browning, meaning to stimulate the development of beige adipocytes in WAT, could be another strategy to fight obesity. To date, scientists have used a browning medium containing insulin, 3-isobutyl-1-methylxanthine, dexamethasone, triiodothyronine, and rosiglitazone to differentiate 3T3-L1 cells to beige adipocytes [101—103]. Chrysin (5,7-dihydroxyflavone) is a flavonoid found in honeycombs and mushrooms. The addition of 50μM chrysin increased key brown fat markers PGC-1α, PPARγ, and UCP-1 mRNA and protein expression in browning 3T3-L1 adipocytes for 72 hours, and showed that chrysin converts white adipocytes to brite adipocytes. Chrysin decreased TG content in brown-like 3T3-L1 adipocytes. It also affected adipogenesis by decreasing C/EBPα expression and increasing HSL, perilipin, CPT1, and acyl-coenzyme A oxidase 1 enhanced lipolysis as well as increasing pAMPK/AMPK and pACC/ACC expression ratios to alleviate lipogenesis [80]. Administration of 20μM curcumin increased brown fat markers PGC-1α, PPARγ, UCP1 PRDM16 C/EBPβ, Tmem26, Cidea, and Fgf21Tbx1 expression in browning 3T3-L1 adipocytes and showed that curcumin converts white adipocytes to beige adipocytes. Treatment with 20μM curcumin in brown-like adipocytes decreased TG content by increasing mitochondrial CPT1 and cytochrome C protein levels to increase fat oxidation. Moreover, curcumin increased HSL expression to enhance lipolysis and increased pACC and pAMPK/AMPK ratio to suppress FA synthesis [79]. Thymol (5-methyl-2-isopropylphenol) is a monoterpene phenolic ingredient of essential oil from thyme species. Addition of 20μM thymol significantly increased expression of the brown fat marker Pgc-1α, Prdm16, and UCP1 genes, and protein expression showed that thymol converts white adipocytes to beige adipocytes via activated β3-AR, PKA, and p38/mitogen-activated protein kinase expression. Thymol treatment decreased TG content of brown-like 3T3-L1 adipocytes by increasing expression of pAMPK/AMPK and pACC to alleviate lipogenesis and increasing Tfam, Nrf1, PGC-1α, UCP1, and PRDM16 expression-induced mitochondrial biogenesis. It also decreased C/EBPα and LPL expression and increased HSL, perilipin, CPT1, and acyl-coenzyme A oxidase expression to enhance lipolysis during the browning process [81] (Table 2).

4. Conclusion

Dietary phytochemicals from food and herbs have been used as natural therapies for a long time. Obesity is a disease that results from energy imbalance, and it has become an epidemic in modern society over the past decades. Due to side effects and the low success rate of obesity treatment drugs, study of the role of phytochemicals in treating obesity has become more and more important for developing new drugs. More and more scientists focus on the effects of phytochemicals on WAT, especially the process of adipogenesis and lipogenesis, to alleviate the effects of excessive energy on expanding adipose tissue. By contrast, scientists have also investigated the role of phytochemicals on energy expenditure on WAT such as lipolysis and FA oxidation. Moreover, scientists have recently found that beige cells are a kind of brown adipocytes in WAT that showed similar thermogenesis as in BAT. Some phytochemicals do have the ability to convert white adipocytes to beige cells to enhance energy expenditure (Figure 2). Although the safety of phytochemicals and their roles in regulating energy balance remain, further investigation and more and more scientific evidences have proved the antiobesity effectiveness and efficacy of phytochemicals and their potential to be developed as medicines in fighting obesity.

Conflicts of interest

There are no conflicts of interest.

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