

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Review Article

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

Yen-Chen Tung^a, Pei-Hsuan Hsieh^b, Min-Hsiung Pan^{a,c,d,*},
Chi-Tang Ho^{b,*}

^a Institute of Food Sciences and Technology, National Taiwan University, Taipei 106, Taiwan

^b Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

^c Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 40402, Taiwan

^d Department of Health and Nutrition Biotechnology, Asia University, Taichung 41354, Taiwan

ARTICLE INFO

Article history:

Received 19 September 2016

Received in revised form

29 October 2016

Accepted 31 October 2016

Available online 7 December 2016

Keywords:

3T3-L1 cells

adipogenesis

lipogenesis

lipolysis

phytochemicals

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.

Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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

* Corresponding authors. Institute of Food Science and Technology, National Taiwan University, Number 1, Section 4, Roosevelt Road, Taipei, 10617, Taiwan (M.-H. Pan); Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA (C.-T. Ho).

E-mail addresses: mhpan@ntu.edu.tw (M.-H. Pan), ho@aesop.rutgers.edu (C.-T. Ho).

<http://dx.doi.org/10.1016/j.jfda.2016.10.018>

1021-9498/Copyright © 2016, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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 15-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/EBP α , C/EBP β , and C/EBP δ), and peroxisome proliferator-activated receptor (PPAR) families (PPAR α , PPAR β/δ , and PPAR γ) [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,

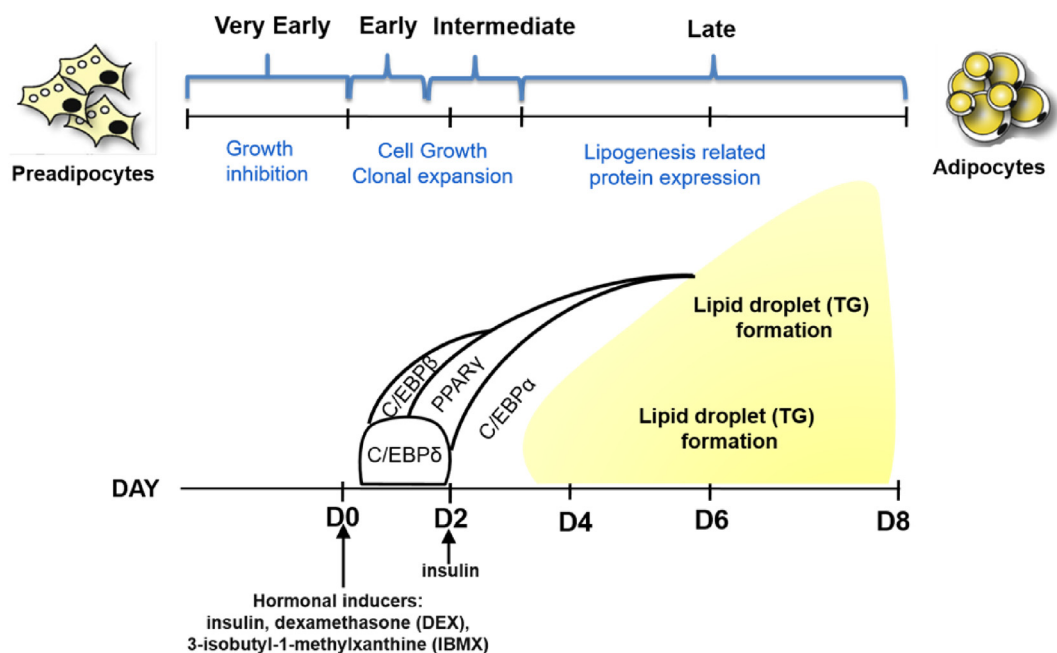


Figure 1 – The differentiation process of 3T3-L1 [9,13].

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 50 μ M apigenin decreased TG content via decreased mRNA and protein expression of PPAR γ , 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 10 μ M 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 PPAR β/δ , γ and C/EBP α at Day 5 and C/EBP β at Day 1. Berberine also decreased the expression of PPAR γ -targeted lipogenic genes such as FABP4, ACC, and FAS [43]. Another study showed that administration of 5 μ g/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, PPAR γ , C/EBP α , 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 (50–250 μ M) 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 PPAR γ , C/EBP α , and leptin protein expression in mature 3T3-L1 adipocytes within 72 hours [46]. Another study showed that 3T3-L1 preadipocytes pretreated with 100 μ M 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 100 μ M EGCG decreased TG content via decreased protein expression of PPAR γ 1/2 and its target genes LXR α during the adipogenesis process [49]. In 2011, Chan et al showed that 10 μ M EGCG decreased adipose conversion by inhibiting 3T3-L1 cell proliferation by arresting cells at the G2/M phase and decreasing C/EBP α and PPAR γ expression [50]. Another study showed that 100 μ M EGCG decreased lipid content via decreased CEBP α , PPAR γ , 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 100 μ M 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 100 μ M 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 CEBP β by increasing CEBP homologous protein expression, then subsequently decreasing CEBP α and PPAR γ expression [52]. Resveratrol (3,5,4'-trihydroxystilbene),

is a natural phytochemical found in grape and red wine [53]. Twenty-five micromoles and 50 μ M of resveratrol significantly decreased TG content via decreased expression of PPAR γ , C/EBP α , SREBP1, and FAS mRNA [54]. Another study investigated the activity of resveratrol (0.03 μ M, 0.1 μ M, 0.3 μ M, 1 μ M, 3 μ M, 10 μ M, 30 μ M, 100 μ M) in 3T3-L1 preadipocytes and mature adipocytes. The results of cell viability tests showed that the 30 μ M and 100 μ M resveratrol had lower cell survival in 3T3-L1 preadipocytes. Based on minimal cytotoxicity, 0.03 μ M, 0.1 μ M, 0.3 μ M, 1 μ M, 3 μ M, and 10 μ M resveratrol were studied. The 3 μ M and 10 μ M resveratrol decreased TG content via decreased PPAR γ 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 100 μ M oxyresveratrol decreased TG content via decreased PPAR γ during the late stage of differentiation and decreased C/EBP α expression during the early stage of differentiation. A concentration of 100 μ M 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 25 μ M curcumin for 48 hours, TG content decreased via blocked differentiation through C/EBP α and PPAR γ , SREBP-1, and FAS expression. 25 μ M curcumin also inhibited phosphorylated mitogen-activated protein kinase, including extracellular signal-regulated kinases (ERK), JNK, and p38 expression during differentiation. A concentration of 25 μ M curcumin restored nuclear translocation of β -catenin [wingless-type MMTV integration site family signaling (Wnt) component] and decreased casein kinase 1 α , glycogen synthase kinase 3 β , 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 30 μ M curcumin decreased TG content via decreased C/EBP β , PPAR γ , and C/EBP α 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/EBP α , and PPAR γ during the early stage of adipocyte differentiation [60]. A similar study showed that 20 μ M curcumin slightly decreased PPAR γ and C/EBP α 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 25 μ M 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 PPAR γ and C/

EBP α , phosphorylated extracellular signal-regulated kinases (ERK1/2), c-Jun amino-terminal kinases (JNK), and Akt expression during adipogenesis [63]. 18 β -Glycyrrhetic acid is a principal active ingredient from the herb licorice root [64]. Twenty micromoles of 18b-GA decreased TG content via decreased PPAR γ and CEBP α 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 100 μ M ginkgolide C decreased TG content via decreased PPAR α , PPAR γ , CEBP α , CEBP β , 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 400 nM of celastrol treated from Day 0 to Day 8 decreased most TG content via decreased PPAR γ 2, C/EBP α , 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 10 μ M capsaicin to full differentiated 3T3-L1 adipocytes for 24 hours showed an increased release of glycerol via increased HSL and CPT1- α [76]. EGCG (10 μ M) 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 10 μ mol/L and 20 μ mol/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 PPAR γ and C/EBP α levels [78]. 18 β -Glycyrrhetic acid increased the release of glycerol via increased HSL, ATGL, and perilipin expression in mature 3T3-L1 preadipocytes [65]. Both 30 μ M and 100 μ M 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 ↓ PPAR γ , FABP4, SCD ↑ Activation of AMPK	[41]
Berberine	↓ TG content ↓ PPAR γ , β , δ , C/EBP α ↓ FABP4, ACC, FAS	[43]
	↓ TG content ↓ PPAR γ , C/EBP α , ADD/SREBP-1c ↓ FABP4, FAS ↑ pAMPK, pACC	[44]
Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide)	↓ TG content ↓ Mitochondria membrane potential ($\Delta\Psi$ m) ↑ Caspase 3, Bax, Bak, the cleavage of PARP ↓ Bcl-2 ↓ PPAR γ , C/EBP α	[46]
	↓ TG content ↑ pAMPK, pACC	[47]
(-)-Epigallocatechin gallate	↓ TG content ↓ PPAR γ 1/2, LXR α ↓ TG content ↓ PPAR γ , C/EBP α ↑ An arrest of cell cycle at G2/M phase ↓ TG content ↓ PPAR γ , C/EBP α ↓ FABP4, FAS ↑ β -catenin, cyclin D1	[49]
	↓ TG content ↓ PPAR γ , C/EBP α	[50]
Genistein	↓ TG content ↑ pAMPK, pACC ↓ TG content ↓ C/EBP β via ↑ CHOP ↓ PPAR γ , C/EBP α	[51]
Resveratrol (3,5,4'-trihydroxystilbene)	↓ TG content ↓ PPAR γ , C/EBP α ↓ SREBP-1, FAS ↓ TG content ↓ PPAR γ	[52]
	↓ TG content ↓ PPAR γ , C/EBP α ↓ Cyclin A, CDK2, cyclin D1, CDK4	[54]
Oxyresveratrol	↓ TG content ↓ PPAR γ , C/EBP α	[55]
Curcumin	↓ TG content ↓ PPAR γ , C/EBP α ↓ SREBP-1, FAS ↓ Phosphorylation of MAPK (ERK, JNK, and p38) ↑ Translocation of β -catenin ↓ CK1 α , GSK-3 β , Axin, FABP4 ↑ Wnt10b, Fz2, LRP5, c-Myc, cyclin D1 ↓ TG content ↓ PPAR γ , C/EBP α , β ↓ KLF5 ↓ TG content ↓ PPAR γ , C/EBP α ↑ Caspase 3, PARP ↑ Cyclin D ↓ Cyclin A ↑ Inhibition of p27 proteolysis by ↓ Skp2, 26S proteasome activity	[57]
Bisdemethoxycurcumin	↓ TG content ↓ Cyclin A, B ↑ p21 ↓ PPAR γ , C/EBP α ↓ Phosphorylated ERK1/2, JNK, Akt	[59]
18 β -Glycyrrhetic acid	↓ TG content ↓ PPAR γ , C/EBP α ↓ Phosphorylated Akt	[60]
		[61]
		[63]
		[65]

Table 1 – (continued)

Phytochemical	Regulation	Reference
Ginkgolide C	↓ TG content ↓ PPAR α , γ , C/EBP α , β , SREBP-1c	[67]
Celastrol	↓ FAS, FABP4 ↓ TG content ↓ PPAR γ 2, C/EBP α ↓ FAS, FABP4	[69]

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 = stearyl-CoA desaturase; TG = triacylglycerols.

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

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

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-1 α) 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

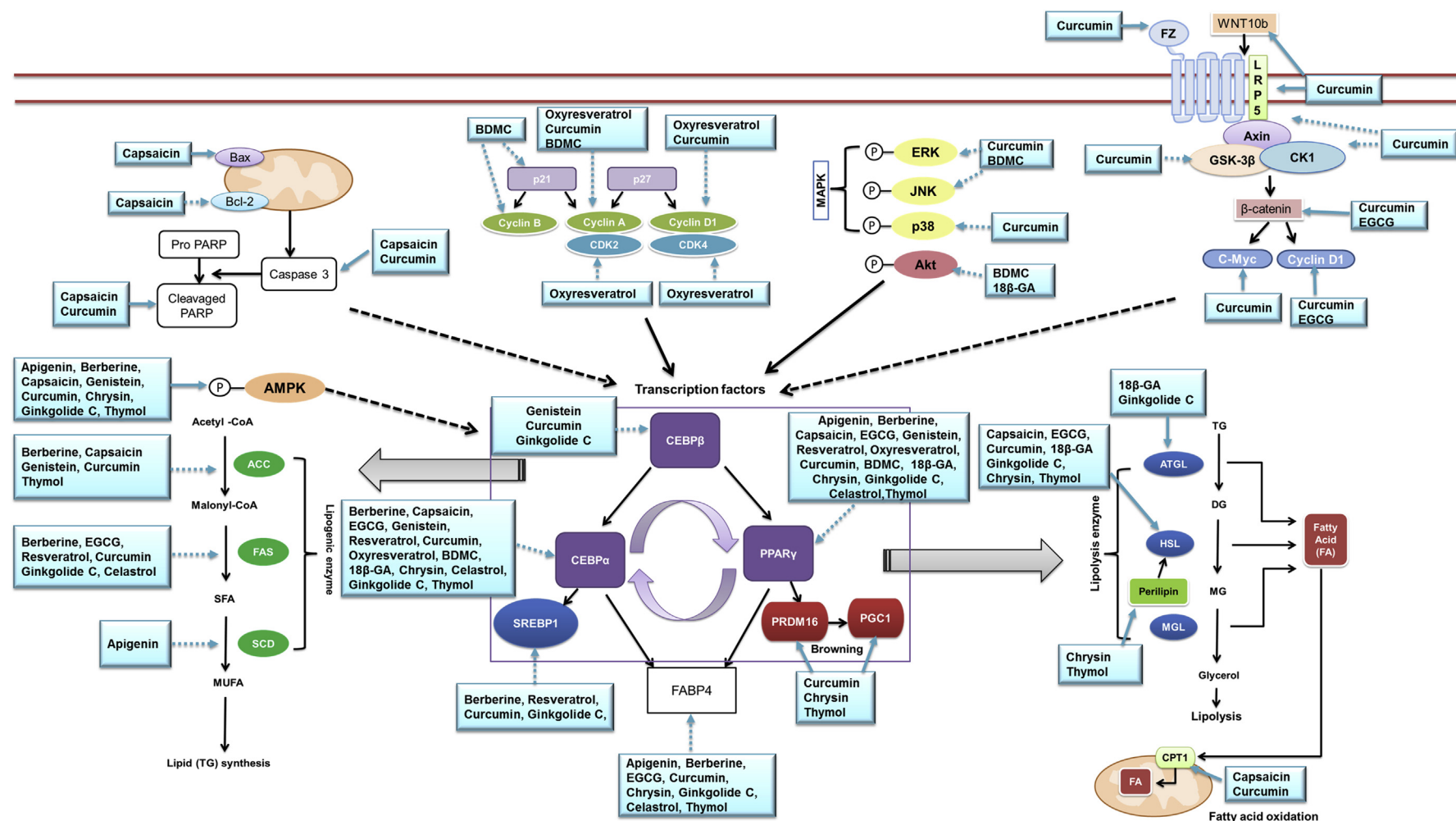


Figure 2 – The possible antiobesity mechanism of phytochemicals in 3T3-L1. 18 β -GA = 18 β -glycyrrhetic acid; ACC = acetyl-Co A carboxylase; AKT = protein kinase B; AMPK = 5'-adenosine monophosphate-activated protein kinase; ATGL = adipose triacylglycerol lipase; BDMC = bisdemethoxycurcumin; CDK = cyclin-dependent 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-3 β = glycogen synthase kinase 3 β ; 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-1 α = 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 PPAR γ or enriched markers such as Cd137, Tbx1, Tmem26, Cited1, and Shox2. C/EBP β , PRDM16, and PGC-1 α 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 50 μ M chrysin increased key brown fat markers PGC-1 α , PPAR γ , and UCP-1 mRNA and protein expression in browning 3T3-L1 adipocytes for 72 hours, and showed that chrysin converts white adipocytes to beige adipocytes. Chrysin decreased TG content in brown-like 3T3-L1 adipocytes. It also affected adipogenesis by decreasing C/EBP α 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 20 μ M curcumin increased brown fat markers PGC-1 α , PPAR γ , UCP1 PRDM16 C/EBP β , Tmem26, Cidea, and Fgf21Tbx1 expression in browning 3T3-L1 adipocytes and showed that curcumin converts white adipocytes to beige adipocytes. Treatment with 20 μ M 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 20 μ M thymol significantly increased expression of the brown fat marker Pgc-1 α , Prdm16, and UCP1 genes, and protein expression showed that thymol converts white adipocytes to beige adipocytes via activated β 3-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-1 α , UCP1, and PRDM16 expression-induced mitochondrial biogenesis. It also decreased C/EBP α 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.

REFERENCES

- [1] WHO. Global health risks: mortality and burden of disease attributable to selected major risks. World Health Organization; 2009.
- [2] Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. A natural solution for obesity: Bioactives for the prevention and treatment of weight gain. A review. *Nutr Neurosci* 2015;18:49–65.
- [3] Lee KH. Current developments in the discovery and design of new drug candidates from plant natural product leads. *J Nat Prod* 2004;67:273–83.
- [4] Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta* 2013;1830:3670–95.
- [5] Moerman DE. Medicinal plants of native America. *Ann Arbor: Museum of Anthropology, University of Michigan*; 1986.
- [6] Santos Ana Paula, Rogero Marcelo M, Bastos Deborah HM. Edible plants, their secondary metabolites and antiobesogenic potential. *Recent Pat Food Nutr Agric* 2010;2:195–212.
- [7] Unger RH. Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology* 2003;144:5159–65.
- [8] Dizdar Ö, Alyamaç E. Obesity: an endocrine tumor? *Med Hypotheses* 2004;63:790–2.
- [9] Ka SO, Kim KA, Kwon KB, Park JW, Park BH. Silibinin attenuates adipogenesis in 3T3-L1 preadipocytes through a potential upregulation of the insig pathway. *Int J Mol Med* 2009;23:633.
- [10] Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep* 2001;2:282–6.
- [11] Sarjeant K, Stephens JM. Adipogenesis. *Cold Spring Harb Perspect Biol* 2012;4:a008417.
- [12] Geloën A, Roy PE, Bukowiecki LJ. Regression of white adipose tissue in diabetic rats. *Am J Physiol* 1989;257:E547–53.
- [13] Ali AT, Hochfeld WE, Myburgh R, Pepper MS. Adipocyte and adipogenesis. *Eur J Cell Biol* 2013;92:229–36.
- [14] Symonds ME, editor. Adipose tissue biology. New York: Springer-Verlag; 2011.
- [15] Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004;84:277–359.
- [16] Farmer SR. Molecular determinants of brown adipocyte formation and function. *Genes Dev* 2008;22:1269–75.

- [17] Armani A, Mammi C, Marzolla V, Calanchini M, Antelmi A, Rosano G, Fabbri A, Caprio M. Cellular models for understanding adipogenesis, adipose dysfunction, and obesity. *J Cell Biochem* 2010;110:564–72.
- [18] Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. *Physiol Rev* 1998;78:783–809.
- [19] Ntambi JM, Young-Cheul K. Adipocyte differentiation and gene expression. *J Nutr* 2000;130:3122S–6S.
- [20] Green H, Meuth M. An established pre-adipose cell line and its differentiation in culture. *Cell* 1974;3:127–33.
- [21] Poulos SP, Dodson MV, Hausman GJ. Cell line models for differentiation: preadipocytes and adipocytes. *Exp Biol Med* (Maywood) 2010;235:1185–93.
- [22] Green H, Kehinde O. An established preadipose cell line and its differentiation in culture II. Factors affecting the adipose conversion. *Cell* 1975;5:19–27.
- [23] Kuri-Harcuch W, Green H. Increasing activity of enzymes on pathway of triacylglycerol synthesis during adipose conversion of 3T3 cells. *J Biol Chem* 1977;252:2158–60.
- [24] Cornelius P, MacDougald OA, Lane MD. Regulation of adipocyte development. *Annu Rev Nutr* 1994;14:99–129.
- [25] Rubin CS, Hirsch A, Fung C, Rosen OM. Development of hormone receptors and hormonal responsiveness *in vitro*. Insulin receptors and insulin sensitivity in the preadipocyte and adipocyte forms of 3T3-L1 cells. *J Biol Chem* 1978;253:7570–8.
- [26] Smith PJ, Wise L, Berkowitz R, Wan C, Rubin C. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. *J Biol Chem* 1988;263:9402–8.
- [27] Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 2000;16:145–71.
- [28] Lefterova MI, Lazar MA. New developments in adipogenesis. *Trends Endocrinol Metab* 2009;20:107–14.
- [29] Moldes M, Zuo Y, Morrison RF, Silva D, Bae-Hang Park BH, Liu J, Farmer SR. Peroxisome-proliferator-activated receptor γ suppresses Wnt/ β -catenin signalling during adipogenesis. *Biochem J* 2003;376:607–13.
- [30] Trayhurn P. Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol Scand* 2005;184:285–93.
- [31] Cowherd RM, Lyle RE, McGehee Jr RE. Molecular regulation of adipocyte differentiation. *Semin Cell Dev Biol* 1999;10:3–10.
- [32] Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J Clin Invest* 1999;103:1489–98.
- [33] Horton JD, Shimomura I, Ikemoto S, Bashmakov Y, Hammer RE. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J Biol Chem* 2003;278:36652–60.
- [34] Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997;89:331–40.
- [35] Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 1996;10:1096–107.
- [36] Mackall J, Student A, Polakis SE, Lane M. Induction of lipogenesis during differentiation in a “preadipocyte” cell line. *J Biol Chem* 1976;251:6462–4.
- [37] Paulauskis JD, Sul H. Cloning and expression of mouse fatty acid synthase and other specific mRNAs. Developmental and hormonal regulation in 3T3-L1 cells. *J Biol Chem* 1988;263:7049–54.
- [38] Spiegelman B, Frank M, Green H. Molecular cloning of mRNA from 3T3 adipocytes. Regulation of mRNA content for glycerophosphate dehydrogenase and other differentiation-dependent proteins during adipocyte development. *J Biol Chem* 1983;258:10083–9.
- [39] Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol Ther* 2001;90:157–77.
- [40] Shukla S, Gupta S. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. *Mol Cancer Ther* 2006;5:843–52.
- [41] Ono M, Fujimori K. Antiadipogenic effect of dietary apigenin through activation of AMPK in 3T3-L1 cells. *J Agric Food Chem* 2011;59:13346–52.
- [42] Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Wang Y, Li Z, Liu J, Jiang JD. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 2004;10:1344–51.
- [43] Huang C, Zhang Y, Gong Z, Sheng X, Li Z, Zhang W, Qin Y. Berberine inhibits 3T3-L1 adipocyte differentiation through the PPAR γ pathway. *Biochem Biophys Res Commun* 2006;348:571–8.
- [44] Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes* 2006;55:2256–64.
- [45] Cordell GA, Araujo OE. Capsaicin: identification, nomenclature, and pharmacotherapy. *Ann Pharmacother* 1993;27:330–6.
- [46] Hsu CL, Yen GC. Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells. *J Agric Food Chem* 2007;55:1730–6.
- [47] Hwang JT, Park IJ, Shin JI, Lee YK, Lee SK, Baik HW, Ha J, Park OJ. Genistein, EGCG, and capsaicin inhibit adipocyte differentiation process via activating AMP-activated protein kinase. *Biochem Biophys Res Commun* 2005;338:694–9.
- [48] Naef R, Jaquier A, Velluz A, Maurer B. New constituents related to 3-methyl-2, 4-nonanedione identified in green tea. *J Agric Food Chem* 2006;54:9201–5.
- [49] Moon HS, Chung CS, Lee HG, Kim TG, Choi YJ, Cho CS. Inhibitory effect of (–)-epigallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells. *Obesity* (Silver Spring) 2007;15:2571–82.
- [50] Chan CY, Wei L, Castro-Muñozledo F, Koo WL. (–)-Epigallocatechin-3-gallate blocks 3T3-L1 adipose conversion by inhibition of cell proliferation and suppression of adipose phenotype expression. *Life Sci* 2011;89:779–85.
- [51] Lee H, Bae S, Yoon Y. The anti-adipogenic effects of (–)-epigallocatechin gallate are dependent on the WNT/ β -catenin pathway. *J Nutr Biochem* 2013;24:1232–40.
- [52] Harmon AW, Patel YM. Genistein inhibits CCAAT/enhancer-binding protein β (C/EBP β) activity and 3T3-L1 adipogenesis by increasing C/EBP homologous protein expression. *Biochem J* 2002;367:203–8.
- [53] Huang JP, Huang SS, Deng JY, Chang CC, Day YJ, Hung LM. Insulin and resveratrol act synergistically, preventing cardiac dysfunction in diabetes, but the advantage of resveratrol in diabetics with acute heart attack is antagonized by insulin. *Free Radic Biol Med* 2010;49:1710–21.
- [54] Rayalam S, Yang JY, Ambati S, Della-Fera MA, Baile CA. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. *Phytother Res* 2008;22:1367–71.
- [55] Chang CC, Lin KY, Peng KY, Day YJ, Hung LM. Resveratrol exerts anti-obesity effects in high-fat diet obese mice and displays differential dosage effects on cytotoxicity, differentiation, and lipolysis in 3T3-L1 cells. *Endocr J* 2016;63:169–78.

- [56] Li H, Cheng KW, Cho C-H, He Z, Wang M. Oxyresveratrol as an antibrowning agent for cloudy apple juices and fresh-cut apples. *J Agric Food Chem* 2007;55:2604–10.
- [57] Tan HY, Iris M, Li ET, Wang M. Inhibitory effects of oxyresveratrol and cyanomaculrin on adipogenesis of 3T3-L1 cells. *J Funct Foods* 2015;15:207–16.
- [58] Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. *Planta Med* 1991;57:1–7.
- [59] Ahn J, Lee H, Kim S, Ha T. Curcumin-induced suppression of adipogenic differentiation is accompanied by activation of Wnt/beta-catenin signaling. *Am J Physiol Cell Physiol* 2010;298:C1510–6.
- [60] Kim CY, Le TT, Chen C, Cheng JX, Kim KH. Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion. *J Nutr Biochem* 2011;22:910–20.
- [61] Ferguson BS, Nam H, Morrison RF. Curcumin inhibits 3T3-L1 preadipocyte proliferation by mechanisms involving post-transcriptional p27 regulation. *Biochem Biophys Res* 2016;5:16–21.
- [62] Gordon ON, Luis PB, Ashley RE, Osheroff N, Schneider C. Oxidative transformation of demethoxy- and bisdemethoxycurcumin: products, mechanism of formation, and poisoning of human topoisomerase II α . *Chem Res Toxicol* 2015;28:989–96.
- [63] Lai CS, Chen YY, Lee PS, Kalyanam N, Ho CT, Liou WS, Yu RC, Pan MH. Bisdemethoxycurcumin inhibits adipogenesis in 3T3-L1 preadipocytes and suppresses obesity in high-fat diet-fed C57BL/6 mice. *J Agric Food Chem* 2016;64:821–30.
- [64] Armanini D, Nacamulli D, Francini-Pesenti F, Battagin G, Ragazzi E, Fiore C. Glycyrrhetic acid, the active principle of licorice, can reduce the thickness of subcutaneous thigh fat through topical application. *Steroids* 2005;70:538–42.
- [65] Moon MH, Jeong JK, Lee YJ, Seol JW, Ahn DC, Kim IS, Park SY. 18 β -Glycyrrhetic acid inhibits adipogenic differentiation and stimulates lipolysis. *Biochem Biophys Res Commun* 2012;420:805–10.
- [66] Yeh KY, Shou SS, Lin YX, Chen CC, Chiang CY, Yeh CY. Effect of *Ginkgo Biloba* extract on lipopolysaccharide-induced anhedonic depressive-like behavior in male rats. *Phytother Res* 2015;29:260–6.
- [67] Liou CJ, Lai XY, Chen YL, Wang CL, Wei C-H, Huang WC. Ginkgolide C suppresses adipogenesis in 3T3-L1 adipocytes via the AMPK signaling pathway. *Evid Based Complement Alternat Med* 2015;2015:298635.
- [68] Tao X, Younger J, Fan FZ, Wang B, Lipsky PE. Benefit of an extract of *Tripterygium Wilfordii* Hook F in patients with rheumatoid arthritis: a double-blind, placebo-controlled study. *Arthritis Rheum* 2002;46:1735–43.
- [69] Choi SK, Park S, Jang S, Cho HH, Lee S, You S, Kim SH, Moon HS. Cascade regulation of PPAR γ 2 and C/EBP α signaling pathways by celastrol impairs adipocyte differentiation and stimulates lipolysis in 3T3-L1 adipocytes. *Metabolism* 2016;65:646–54.
- [70] Rayalam S, Della-Fera MA, Baile CA. Phytochemicals and regulation of the adipocyte life cycle. *J Nutr Biochem* 2008;19:1717–26.
- [71] Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity (Silver Spring)* 2009;17:1872–7.
- [72] Saponaro C, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. *Nutrients* 2015;7:9453–74.
- [73] Greenberg AS, Shen W-J, Muliro K, Patel S, Souza SC, Roth RA, Kraemer FB. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J Biol Chem* 2001;276:45456–61.
- [74] Horton M, Scrimgeour P. Rawn. Principles of biochemistry. 4th ed. Toronto: Prentice Hall; 2006.
- [75] Rupasinghe HV, Sekhon-Loodu S, Mantso T, Panayiotidis MI. Phytochemicals in regulating fatty acid β -oxidation: potential underlying mechanisms and their involvement in obesity and weight loss. *Pharmacol Ther* 2016;165:153–63.
- [76] Lee MS, Kim CT, Kim IH, Kim Y. Effects of capsaicin on lipid catabolism in 3T3-L1 adipocytes. *Phytother Res* 2011;25:935–9.
- [77] Lee MS, Kim CT, Kim IH, Kim Y. Inhibitory effects of green tea catechin on the lipid accumulation in 3T3-L1 adipocytes. *Phytother Res* 2009;23:1088–91.
- [78] Ejaz A, Wu D, Kwan P, Meydani M. Curcumin inhibits adipogenesis in 3T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. *J Nutr* 2009;139:919–25.
- [79] Lone J, Choi JH, Kim SW, Yun JW. Curcumin induces brown fat-like phenotype in 3T3-L1 and primary white adipocytes. *J Nutr Biochem* 2016;27:193–202.
- [80] Choi JH, Yun JW. Chrysin induces brown fat-like phenotype and enhances lipid metabolism in 3T3-L1 adipocytes. *Nutrition* 2016;32:1002–10.
- [81] Choi JH, Kim SW, Yu R, Yun JW. Monoterpene phenolic compound thymol promotes browning of 3T3-L1 adipocytes. *Eur J Nutr* 2016. <http://dx.doi.org/10.1007/s00394-016-1273-2>.
- [82] Rosen ED, Spiegelman BM. What we talk about when we talk about fat. *Cell* 2014;156:20–44.
- [83] Lazar MA. How now, brown fat? *Science* 2008;321:1048–9.
- [84] Parray HA, Yun JW. Cannabidiol promotes browning in 3T3-L1 adipocytes. *Mol Cell Biochem* 2016;416:131–9.
- [85] Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doris A, Kolodny GM, Kahn R. Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* 2009;360:1509–17.
- [86] Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P. Functional brown adipose tissue in healthy adults. *N Engl J Med* 2009;360:1518–25.
- [87] Klingenspor M, Herzig S, Pfeifer A. Brown fat develops a bright future. *Obes Facts* 2012;5:890–6.
- [88] Bartelt A, Heeren J. Adipose tissue browning and metabolic health. *Nature Rev Endocrinol* 2014;10:24–36.
- [89] Ricquier D. Uncoupling protein 1 of brown adipocytes, the only uncoupler: a historical perspective. *Front Endocrinol (Lausanne)* 2011;2:85.
- [90] Vitali A, Murano I, Zingaretti MC, Frontini A, Ricquier D, Cinti S. The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. *J Lipid Res* 2012;53:619–29.
- [91] Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 1998;92:829–39.
- [92] Bonet ML, Oliver P, Palou A. Pharmacological and nutritional agents promoting browning of white adipose tissue. *Biochim Biophys Acta* 2013;1831:969–85.
- [93] Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor γ (PPAR γ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* 2010;285:7153–64.
- [94] Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR,

- Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 2008;454:961–7.
- [95] Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM. Transcriptional control of brown fat determination by PRDM16. *Cell Metab* 2007;6:38–54.
- [96] Hondares E, Rosell M, Díaz-Delfín J, Olmos Y, Monsalve M, Iglesias R, Villarroja F, Giralt M. Peroxisome proliferator-activated receptor α (PPAR α) induces PPAR γ coactivator 1 α (PGC-1 α) gene expression and contributes to thermogenic activation of brown fat involvement of PRDM16. *J Biol Chem* 2011;286:43112–22.
- [97] Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16–C/EBP- β transcriptional complex. *Nature* 2009;460:1154–8.
- [98] Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 2012;150:366–76.
- [99] Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V, Kajimura S. Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS One* 2012;7:e49452.
- [100] Lidell ME, Betz MJ, Leinhard OD, Heglind M, Elander L, Slawik M, Mussack T, Nilsson D, Romu T, Nuutila P, Virtanen KA, Beuschlein F, Persson A, Borga M, Enerbäck S. Evidence for two types of brown adipose tissue in humans. *Nat Med* 2013;19:631–4.
- [101] Martinez de Mena R, Scanlan TS, Obregon MJ. The T3 receptor β 1 isoform regulates UCP1 and D2 deiodinase in rat brown adipocytes. *Endocrinology* 2010;151:5074–83.
- [102] Ohno H, Shinoda K, Spiegelman BM, Kajimura S. PPAR γ agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab* 2012;15:395–404.
- [103] Asano H, Kanamori Y, Higurashi S, Nara T, Kato K, Matsui T, Funaba M. Induction of beige-like adipocytes in 3T3-L1 cells. *J Vet Med Sci* 2014;76:57–64.