Review

Citrus peel derived poly-methoxylated flavones (PMF)

A systematic review of the potential bioactive agents against obesity and obesity related metabolic disorders

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Abstract: The prevalence of obesity has increased substantially over the last several decades and several environmental factors have accelerated this trend. Poly-methoxy flavones (PMFs) exist abundantly in the peels of citrus, and their biological activities have been broadly examined in recent years. Several studies have examined the effects of PMFs on obesity and its-related diseases. This systematic review conducted to focus on the effect of PMFs on obesity and its related conditions management. The PubMed, Google Scholar, Scopus, and Science Direct databases were searched for relevant studies published before November 2020. Out of 1,615 records screened, 16 studies met the study criteria. The range of dosage of PMFs was varied from 10 to 200 mg/kg (5–26 weeks) and 1–100 μmol (2h–8 days) across selected animal and *in vitro* studies, respectively. The literature reviewed shows that PMFs modulate several biological processes associated with obesity such as lipid and glucose metabolism, inflammation, energy balance, and oxidative stress by different mechanisms. All of the animal studies showed significant positive effects of PMFs on obesity by reducing body weight (e.g. reduced weight gain by 21.04%), insulin resistance, energy expenditure, inhibiting lipogenesis and reduced blood lipids (e.g. reduced total cholesterol by 23.10%, TG by 44.35% and LDL by 34.41%). The results of the reviewed *in vitro* studies have revealed that treatment with PMFs significantly inhibits lipid accumulation in adipocytes (e.g. reduced lipid accumulation by 55–60%) and 3T3-L1 pre-adipocyte differentiation as well by decreasing the expression of PPARγ and C/EBPα and also reduces the number and size of fat cells and reduced TG content in adipocytes by 45.67% and 23.10% and 16.08% for nobiletin, tangeretin and hesperetin, respectively. Although current evidence supports the use of PMFs as a complementary treatment in obesity, future research is needed to validate this promising treatment modality.

Keywords: Citrus fruits, flavonoids, poly-methoxylated flavones, obesity, lipid metabolism

Abbreviations

ACC, Acetyl-CoA carboxylase; AKT: Protein kinase B; AMPK: AMP-activated protein kinase; C/EBP: CCAAT/enhancer-binding proteins; CAT: catalase; CPT: carnitine palmitoyltransferase; Cox2: cyclooxygenase-2; DAG: diacylglycerol; FA-CoAs: fatty acyl-coenzyme A; FAS: fatty acid synthase; FFA: free fatty acid; IKK/NFKB: IκB kinase/nuclear factor NF-kappa-B; IL: interleukin; LCFA Acyl-CoA: long chain fattyacyl-CoA; LKB1: liver kinase B1; MAPK: mitogen-activated protein kinases; MCP-1: monocyte chemoattractant protein-1; NOX: NADPH oxidase; OMe: methoxy group; PEF2: prostaglandin F2; PGE2: prostaglandin E2; PI3K: Phosphoinositide 3-kinase; PPARγ: Peroxisome proliferator-activated receptors; ROS: Reactive oxygen species; SIRT1: Sirtuin 1; SOD: Superoxide dismutase; SREBP1c: Sterol regulatory element-binding protein 1c; TAG: Triacylglycerol; TC: Total cholesterol; TG: Triglyceride; TNFα: tumor necrosis factor α; XO: xanthine oxidase.

Introduction

Obesity is a serious health problem worldwide and characterized by excess body fat due to increased fat accumulation, usually indicated by a body mass index (BMI)$\geq$30 kg/m^2. Obesity is an important risk factor for several metabolic conditions such as hyperlipidemia, insulin resistance, type 2 diabetes, cardiovascular disease (CVD) and hypertension [1]. The growing number of the people with obesity through the world would be approximately more than 57.8% by 2030 [2, 3]. From an etiological point of view, obesity is a multifactorial disease caused by the interaction of several genetic, environmental and lifestyle factors [4, 5].

Adipose tissue stores excess energy in the form of triglyceride during nutritional excess and releases fatty acids during caloric deficiency [6]. Several investigations suggest that the dysfunction of the adipose tissue in obesity, contributes to the pathophysiology of obesity-associated metabolic disorder such as insulin resistance, glucose intolerance, hyperlipidemia and type2 diabetes [7, 8]. Obesity is caused not only by the hypertrophy of the adipocytes but also by the adipocyte hyperplasia, which causes the differentiation of preadipocytes into adipocytes [6]. Several studies have revealed that the modulation of the adipocyte function and the suppression of the adipogenesis might be potential therapeutic strategies for the decrease of obesity [9, 10].

Furthermore, many conventional drugs used for the treatment of obesity have serious side effects [11]. Over the years, researchers have studied the impact of natural products (resveratrol, quercetin, citrus flavonoids, catechin, spirulina, cumin seeds, thylakoid, rutin and curcumin) in direct relationship to obesity management [11–16]. An inverse relationship has been shown between higher consumption of natural compounds named flavonoids and numerous pathologic conditions such as CVD, insulin resistance, and obesity [16]. Structurally, flavonoids have two benzene rings attached by a propane unit and are derived from flavones [17]. Citrus flavonoids have been of particular attention because of their potential biological action, such as anti-carcinogenic, anti-inflammatory, antithrombogenic, antioxidant, and anti-atherogenic effects [18–21]. Poly methoxy flavones (PMFs), a class of citrus flavonoids with more than two methoxyl groups on the benzo-gamma-pyrone rings and almost exclusively found in the citrus genus, particularly in the peel of mandarin oranges and sweet oranges [18, 22]. So far, there are more than 78 PMFs being isolated and identified from different citrus plants such as nobiletin, tangeretin, sinensetin, and sudachitin [23]. Previous studies suggested that PMFs with more methoxyl groups have a stronger biological action [23, 24].

Nobiletin is one of the most widely studied of all PMFs and has been revealed to improve hyperglycemia, dyslipidemia, insulin resistance and adiposity by regulating lipid and glucose metabolism in animal studies [25–27]. It has been reported that nobiletin can reduce insulin resistance and hyperglycemia in mice [27] and suppress the secretion of an insulin resistance factor in 3T3-L1 adipocytes [28]. Guo et al. [29] also reported that 5-demethylated polymethoxyflavones (5-OH PMF) decreases hyperglycemia, hepatic steatosis, insulin resistance and obesity when administered orally of 0.25% and 0.5% chenpi extract in food over 15 weeks in high fat diet (HFD)-induced obesity/diabetes mouse. Furthermore, Li et al. [30] found that a mixture of tangeretin, synephrine and nobiletin regulated glucose metabolism by modulating adipokines in fructose-induced insulin resistant hamsters. Several studies showed that PMFs could suppress adipogenesis in adipocytes, regulate lipid and glucose metabolism, ameliorate type2 diabetes, and prevent hyperlipidemia, insulin resistance and obesity *in vivo* and *in vitro* [25, 26, 29]. However, to the best of our knowledge, there is no systematic review that has evaluated the findings in this field and summarized the effects of PMFs on obesity and obesity related issues such as insulin resistance, glucose intolerance and hyperlipidemia. Therefore, the current review focuses on the potential beneficial effects of PMFs against obesity and obesity related issues with special emphasis on the mechanistic pathways of PMFs and suggesting the future research windows.

Material and methods

Search strategy

The literature search was performed systematically in electronic databases Scopus, PubMed, Google scholar and Science Direct, using the following descriptors: "poly methoxy flavones" or "citrus fruits" or "flavonoids" or "hydroxylated poly methoxy flavones" and "obesity" or "overweight" or "body fat" or "adiposity" or "adipocyte" or "food intake" or "weight loss" or "dyslipidemia" or "insulin resistance" or "high fat diet" or "inflammation" or "oxidative stress" in the title/abstract. All of the searches were limited to the English language published *in vitro* and animal studies until November 2020. Additionally, the reference lists of selected articles were checked to find other related studies. The extracted articles entered the Endnote software, and similar articles were deleted. This review was conducted according to the Preferred Reporting for Systematic Reviews (PRISMA) Guidelines. In this study, the PICOS (Patient/Population; Intervention; Comparator; Outcome; Study) questions were as follows: the population was cell cultures or animal model; the intervention was a treatment with PMFs; the comparator was no treatment, standard treatment or a placebo; the outcome is the effect of PMFs on obesity and its related metabolic disorders; the study design included animal, and *in vitro*.

Inclusion and exclusion criteria

Original *in vitro* and animal studies published in English language that investigated the effects of PMFs supplementation on obesity and obesity-related conditions, such as insulin resistance, dyslipidemia, inflammation, and oxidative stress were included in this systematic review. Therefore, determined outcome and exposure were obesity and obesity related conditions and PMFs, respectively.

Studies in which PMFs were combined with other supplements or drugs were excluded. Also, studies with insufficient information, and review studies were excluded. Titles and abstracts of all studies had been assessed independently by 2 investigators (MV and MAF). Studies that did not meet the inclusion criteria were excluded. Full texts of potentially relevant articles were retrieved and were then examined to identify any additional relevant studies. Any discrepancies were discussed and resolved through consensus.

Data extraction and quality assessment

Data extraction from the included articles was done by two authors (MV and MAF) and any disagreements between authors were resolved by discussion. The following characteristics were extracted from included studies: publication year, first author's name, country of origin, study design, dosage, duration and route of administration of PMFs. The quality of the animal and *in vitro* studies was assessed using the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk of bias tool [31] and OHAT risk of bias tool [32], respectively. These tools evaluate selection, detection, performance, attrition, and reporting biases. Each domain was given a "high risk" score if the study comprised methodological defects that may have affect its findings, a "low risk" score if there was no defect for that domain and an "unclear risk" score if the information was not sufficient to determine the impact.

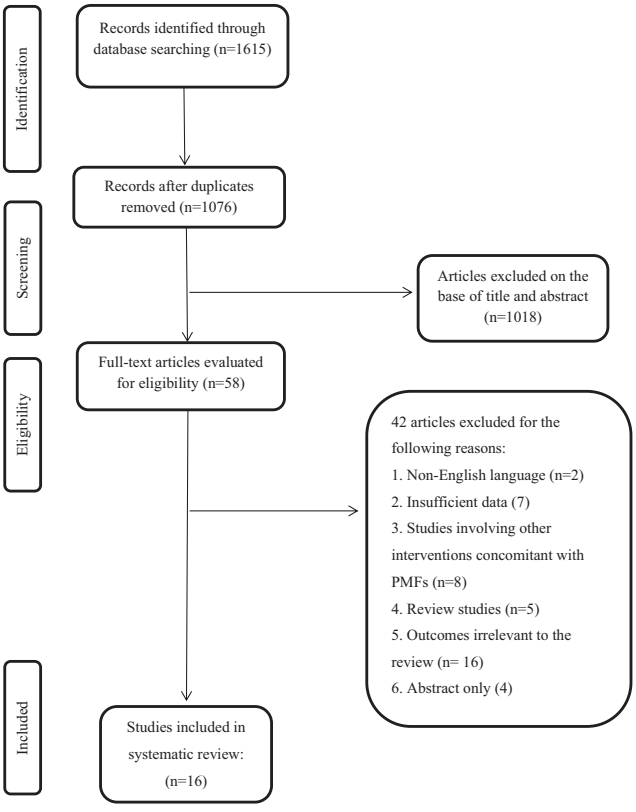


Figure 1. Flow diagram of study screening and selection process.

Results

Study selection

A total of 1,615 studies were retrieved through search databases as shown in Figure 1. After excluding duplicates (n=539), the 1,076 remaining studies were screened by title and abstract which 1,018 of them excluded. In the next step, the remained 58 possible relevant studies were selected for full-text reading. A total of 42 studies failed to meet our inclusion criteria and were excluded. Finally, 16 studies [25, 26, 28, 33–45] met all the inclusion criteria and were included in this systematic review.

Study characteristics

The basic chemical skeleton of PMFs is shown in Figure 2. PMFs, a class of flavonoids, with more than two methoxyl groups on the benzo-gamma-pyrone rings (R_1 to R_3=OH or H or OMe) and almost exclusively found in the citrus peels [18, 22]. The most abundant PMFs in citrus are nobiletin, tangeretin, sinensetin, sudachitin and heptamethoxyflavone (HMF) (Table 1). Characteristics of eligible studies have been summarized in Tables 2 and 3. The relevant studies were arranged into two groups: animal and *in vitro* studies. The studies are presented according to the study design, duration, first author's name, region, main results, dosage and types of PMFs.

Animal studies

Table 2 shows the main characteristics of the selected animal studies [25, 26, 33–37]. These studies were published between 2011 and 2019 and were from the Japan [25, 35, 37], Korea [36], Canada [26], China [34], and USA [33]. About the type of Citrus PMFs, a total of five studies administered nobiletin [25, 26, 33, 36, 37] one study performed the intervention with HMF [34] and one performed the intervention with sudachitin [35]. The dosage of nobiletin supplements wide-ranging from 10 to 200 mg/kg body weight, and duration of intervention extended from 5 to 26 weeks across selected animal studies. Tsutsumi et al. [35] examined the effects of sudachitin on energy metabolism, lipid and glucose in mice with HFD-induced obesity and *db/db* diabetic mice. They found that the administration of sudachitin (5 mg/kg for 12 weeks) significantly reduced

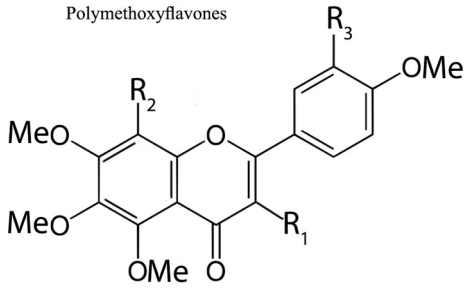


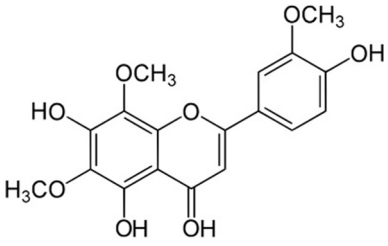
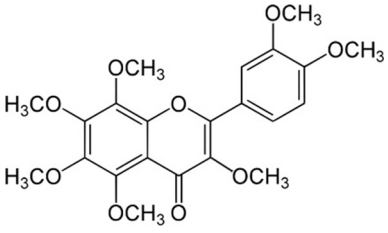
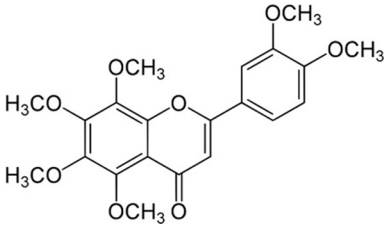
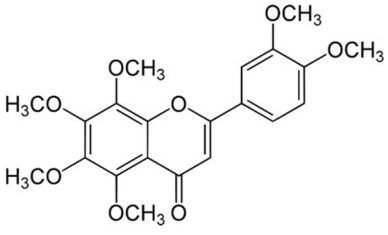
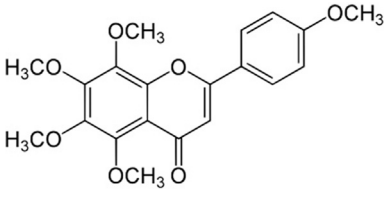
Figure 2. General chemical structures of PMFs.

insulin resistance, weight gain, adipocyte size, body fat, triglyceride (TG), and free fatty acid (FFA) concentrations, and increased fatty acid β-oxidation in HFD treated mice. Feng et al. [34] reported that treatment with HMF (0.02%, 0.04% and 0.08%, w/w) for 6 weeks prevented obesity by a significant reduction in body weight gain, adipose tissues weight, serum levels of total cholesterol (TC), TG, and LDL-C ($p<0.05$) in HFD-fed rats. One work studied the effects of low-dose nobiletin on fat mass in HFD-fed mice. Nobiletin administration (0.02% for 16 weeks) to HFD-fed mice led to a significant decline in total FFA, TC, apo-B, and hepatic TG content. However, nobiletin did not have any significant effects on body weight and food intake in in HFD treated mice [36]. Mulvihill et al. [26] reported that treatment with nobiletin (0.1 or 0.3% w/w) to western HFD-fed mice increased insulin sensitivity, glucose tolerance and reduced TG levels in the liver and intestine and also, prevented obesity by a significant reduction in weight gain, and adipocyte hypertrophy. In another study, Lee et al. [25] also have demonstrated that nobiletin (10 or 100 mg/kg as oral gavage once daily for 5 weeks) decreased weight, white adipose tissue weight, and plasma level of TG, improved adiponectin levels and up-regulated glucose transporter (GLUT)-4 protein expression in HFD-fed male mice. Also, nobiletin administration (50 or 100 mg/kg for 12 weeks) to ovariectomized mice led to a significant decline in body weight gain, white adipose tissue weight, plasma levels of TG, TC and fasting plasma glucose [37]. Nobiletin administration (200 mg/kg body weight for 10 weeks via oral gavage) to HFD-fed male mice resulted in elevated energy expenditure and locomotor activity, reduced body weight gain, and plasma and liver lipid concentrations including TC and TG [33]. All seven animal studies were evaluated for risk of bias using SYRCLE's tool. Qualitative assessment showed that most studies were rated as low risk of bias for the group similarities at baseline category, sequence generation category, and other sources of bias category. In most of these studies randomization in animal housing, random outcome assessment, and blinding of caregivers/investigators and outcome assessor were not mentioned clearly. Methods of allocation concealment were properly described in 72% of the included studies. Risk of incomplete outcome data and selective outcome reporting was identified in two (28.5%) studies and one (14%) of animal studies (Figure 3).

In vitro studies

Table 3 shows the main characteristics of the selected *in vitro* studies [26, 28, 35, 38–45]. Included *in vitro* studies had used human hepatoma cells (HepG2) [26], 3T3-L1 preadipocytes [28, 39–45], primary myoblasts [35], and lipopolysaccharide (LPS)-stimulated RAW264 macrophage cells [38]. These studies were published between 2011and 2018 and were from the Japan [28, 35, 38, 40], Korea [33, 41, 42], Canada [26], Taiwan [44], USA [39], and Malaysia [45]. Namkoong et al. [41] found that administration of nobiletin (10 to 100 μmol for 48 hours) inhibits expression of adipogenic transcription factors, such as CCAAT/enhancer-binding protein-α (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ), in differentiated 3T3-L1 cells. In another study, the roles of sudachitin (30μmol/L) were studied in differentiated myocytes, which was found to significantly increase peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) and sirtuin 1 (SIRT1) mRNA, nuclear factor erythroid 2-related factor 1 (NRF1), NRF2, and uncoupling protein (UCP)-2 expression and in this way; it increased energy expenditure [35]. Mulvihill et al. [26] have shown that administration of nobiletin reduced apoB100 secretion (IC 50=29 μM) from HepG2 cells through activation of mitogen-activated protein kinase extracellular signal–related kinase ($MAPK^{erk}$). Also, nobiletin promoted LDL receptor expression and activity, decreased expression of microsomal triglyceride transfer protein (MTP) and diacylglycerol acyltransferase (DGAT1/2) via activation of $MAPK^{erk}$, all of which are associated with decreased apoB100 secretion. Yuasa et al. [38] investigated the anti-inflammatory effects of PMFs and reported that sudachitin decreased nitric oxide (NO) production by suppressing the expression of inducible NO synthase in LPS-stimulated macrophage-derived mouse cell line. Chen et al. [44] have shown that nobiletin, tangeretin and hesperetin (50 μmol for 24 hours) can decrease lipid accumulation due to alterations in hepatic gene expression related to de novo lipogenesis in 3T3-L1 adipocytes by 35.52% and 35.86% and only 1.05%, respectively and decrease TG content in 3T3-L1 adipocytes by 45.67% and 23.10% and only 16.08% for nobiletin, tangeretin, and hesperetin, respectively. Additionally, Kanda et al. [40] reported that the administration of nobiletin (10 to 100 μmol) to 3T3-L1 cells for 48 hours significantly inhibited differentiation of 3T3-L1 preadipocytes into adipocytes in a dose dependent manner. In another study, the roles of

Table 1. Chemical structure and Source of PMFs

PMFs	Name	Structure	Source
Sudachitin	5,7,4′-Trihydroxy6,8,3′ trimethoxyflavone/ $C_{18}H_{16}O_8$		Skin of the citrus fruit
HMF	3,5,6,7,8,3′,4′heptamethoxyflavone/ $C_{22}H_{24}O_9$		Orange peel
Nobiletin	5,6,7,8,3′,4′Hexamethoxyflavone/ $C_{21}H_{22}O_8$		Citrus peels
Sinensetin	5,6,7,3′,4′-pentamethoxyflavone/ $C_{20}H_{20}O_7$		Orthosiphon stamineus
Tangeretin	5,6,7,8,4′-pentamethoxyflavone/ $C_{20}H_{20}O_7$		Citrus fruit rinds, mandarin orange

tangeretin and nobiletin (0 to 64 μmol) were studied in murine preadipocyte cell line 3T3-L1 for 8 days. They demonstrated that both nobiletin and tangeretin decreased further TG accumulation, decreased the secretion of monocyte chemoattractant protein-1 (MCP-1) and increased adiponectin in 3T3-L1 adipocytes. In addition, nobiletin prevented resistin secretion from mature 3T3-L1 adipocytes [28]. Wang et al. [39] reported a significantly reduces in lipid accumulation by 55–60% in 3T3-L1 adipocytes treatment with 5-OH-HxMF for 8 days. It was also observed that 5-OHHxMF inhibited lipid accumulation 15–20% more than HMF. Kang et al. [43] investigated the effect of sinensetin, at 1 to 40 μmol, on lipid metabolism in mature 3T3-L1 adipocytes for 24 hours. They reported that sinensetin decreased the expression of sterol regulatory element-binding protein 1c (SREBP1c) and increased fatty acid β-oxidation, phosphorylation of protein kinase-A and hormone-sensitive lipase. In another study by Mohamed et al. [45] sinensetin isolated from *Orthosiphon stamineus* (2.5 mg/ml) showed inhibitory activity on α-amylase and α-glucosidase. Additionally, Kang et al. [42] observed that administration of sinensetin (2, 10, and 40 μmol for 2 hours) to 3T3-L1 preadipocytes promoted adipogenesis and lipolysis by increasing cAMP levels in adipocytes, increased

Table 2. Characteristics of animal studies regarding the effect of PMFs on obesity related parameters

First author	Region/Year	PMFs	Dosage/Duration/ Route of administration	Model	Results/Mechanistic pathways
Feng et al. [34]	China/2019	Heptametho-xyflavone	HMF (0.02%, 0.04% and 0.08%, w/w) for 6 weeks given orally	Male rats were fed a normal diet or an HFD with or without HMF.	Rats treated with 0.02, 0.04, and 0.08% HMF in showed a reduction in body weight gain at 8.05, 11.79 and 21.04%, respectively ($p<0.05$). HMF (0.02, 0.04, and 0.08%, w/w) significantly decreased TC by 17.41, 23.61, and 23.10%, TG by 27.14, 34.13, and 44.35%, and LDL-C by 25.00, 28.88, and 34.41%, ($p<0.05$), respectively.
Tsutsumi et al. [35]	Japan/2014	Sudachitin	Sudachitin: 5 mg/kg of body weight and vehicle: 0.2% dimethyl sulfoxide (DMO) for 12 weeks given orally	A group of mice fed a HFD (40% kcal fat) and the other group fed a normal diet treated with sudachitin or vehicle	Sudachitin significantly decreased body weight gain, body fat adipocyte size, TG and FFA concentrations ($p<0.05$). Treatment with sudachitin significantly enhanced insulin resistance, energy expenditure, basal ATP content (1.3-fold) and β-oxidation ($p<0.05$). Treatment with sudachitin increased expression of PPAR-γ in white adipose tissue (1.5-fold) ($p>0.05$).
Kim et al. [36]	Korea/2017	Nobiletin	Nobiletin: (0.02%, w/w) for 16 weeks given orally	mice were fed a HFD, (45% kcal fat) with or without nobiletin	Nobiletin significantly decreased glucose tolerance, hepatic TG, FFA, TC, apoliporotein B concentrations, aminotransferase and inflammation ($p<0.05$). Also, Liver weight was decreased by 19% in Nobiletin-supplemented mice compared to that in the control group ($p=0.068$). Treatment with nobiletin significantly reduced expression of TNF-α, TLR-2 and TLR-4 ($p<0.05$). Food consumption and body weight did not alter following treatment with nobiletin.
Mulvihill et al. [26]	Canada/2011	Nobiletin	Nobiletin: 0.1 or 0.3% (weight/weight) for 8 or 26 weeks given orally	C57BL/6 mice were fed a chow diet, a western HFD or a western HFD with nobiletin	Nobiletin significantly decreased both plasma lipids by 35% ($p<0.05$). 0.1 and 0.3% nobiletin dose-dependently decreased hepatic TG by 44% and 87%, and hepatic cholesteryl ester by 61 and 71%, respectively ($p<0.05$). SREBP1c was dose-dependently reduced by 0.1% (−35%) and 0.3% nobiletin (−80%). Fasting plasma glucose and insulin were significantly decreased by 0.3% nobiletin (6.2 mmol/L and 0.4 ng/mL), respectively ($p<0.05$).
Lee et al. [25]	Japan/2013	Nobiletin	Nobiletin: 10 or 100 mg/kg of body weight as oral gavage once daily for 5 weeks	Male mice were fed a HFD without nobiletin or HFD +10 mg/kg of body weight nobiletin or HFD +100 mg/kg of body weight nobiletin	Nobiletin significantly decreased body weight, WAT weight, plasma level of TG (0.96±0.05 mmol/L), and FPG (3.10±0.42 mmol/L) ($p<0.05$). Treatment with nobiletin significantly enhanced glucose tolerance and adiponectin (5.34±0.97 μg/ml) levels ($p<0.05$). Nobiletin significantly increased expression of PPAR-γ, PPAR-α ($p<0.01$), SREBP1c ($p<0.05$), FAS ($p<0.005$), SCD1 ($p<0.005$), CPT1 ($p<0.05$), UCP-2 ($p<0.05$) and GLUT-4 protein expression ($p<0.05$).
Lee et al. [37]	Japan/2014	Nobiletin	Nobiletin: 50 or 100 mg/kg of body weight. Vehicle: 0.3% carboxyl methyl cellulose/0.5% (DMS) by oral gavage once daily for 12 weeks	Female ovariectomized C57BL/6J mice were fed with 50mg/kg of body weight or 100 mg/kg of body weight nobiletin or vehicle.	Nobiletin significantly decreased body weight gain and WAT weight and plasma levels of TG ($p<0.05$) and tended to decrease plasma glucose and TC levels. Moreover, nobiletin prevented the reduction in bone mineral density of the trabecular region of the femur in ovariectomized mice.
He et al. [33]	USA/2016	Nobiletin	Nobiletin: 200 mg/kg of body weight body weight by oral gavage every other day, Vehicle: DMSO 10 weeks period of treatment	For diet-induced obesity, male mice were fed with HFD until the end of the experimental protocol. The mice were treated with either vehicle or nobiletin	Nobiletin enhanced clock protein levels and elicited pronounced gene expression remodeling ($p<0.01$). Nobiletin significantly increased energy expenditure ($p<0.05$). Treatment with nobiletin significantly prevented obesity and weight gain ($p<0.05$).

ACC: acetyl-CoA carboxylase; CPT1: carnitine palmitoyltransferase-1; DMS: dimethyl sulfoxide; FAS: fatty acid synthase; FFA: free fatty acid; FPG: fasting blood glucose; GLUT: glucose transporter; HFD: high fat diet; HO: Heme-oxygenase-1; IL: interleukin; LPS: lipopolysaccharide; *MCP:* Monocyte chemoattractant protein; NO: nitric oxide synthase; NRF: Nuclear respiratory factor; PPAR: *peroxisome proliferator-activated receptor;* SCD1: Stearoyl-CoA desaturase-1; SREBP1c: sterol regulatory element-binding protein 1c; TC: total cholesterol; TG: triglyceride; TLR: toll-like receptor; TNF: tumor necrosis factor; UCP: uncoupling protein; WAT: white adipose tissue.

Table 3. Characteristics of *in vitro* studies regarding the effect of PMFs on obesity related parameters

First author	Region/Year	PMFs	Dosage/Duration/ Route of administration	Model	Results/Mechanistic pathways
Namkoong et al. [41]	Korea/2017	Nobiletin	Nobiletin: 10 to 100 μmol for 48 hours	A coculture of 3T3-L1 adipocytes and RAW 264.7 macrophages were treated with nobiletin	Treatment with nobiletin significantly decreased PPARγ and C/EBP-α gene expression in adipocyte cell line and inducible NO synthase in macrophage cell line ($p<0.05$).
Tsutsumi et al. [35]	Japan/2014	Sudachitin	Sudachitin: 30 μmol/L or vehicle: 0.2% DMO for 48 hours	Primary myoblasts isolated from the calf region, thigh, and pelvic girdle of 8-week-old mice were treated with sudachitin or vehicle	Treatment with sudachitin significantly increased Sirt-1 and PGC-1α expression, NRF1, 2 (1.5- to 2.5-fold), and UCP2 and 3 ($p<0.05$).
Mulvihill et al. [26]	Canada/2011	Nobiletin	Nobiletin: 10 μmol Insulin: 100 nmol	Human hepatoma cells (HepG2) were treated with either nobiletin or insulin	Treatment with nobiletin significantly decreased lipogenesis and apoliporotein B100 secretion by 50% in HepG2 cells ($p<0.05$).
Yuasa et al. [38]	Japan/2012	Sudachitin vs nobiletin	Sudachitin: Two dosages of 10 and 30 μmol Nobiletin: two dosages of 10 and 30 μmol for 12 hours	A macrophage-derived mouse cell line, RAW264, activated via lipopolysaccharide (LPS) was treated with either nobiletin or sudachitin	Treatment with sudachitin and nobiletin significantly decreased LPS-stimulated iNOS expression (more than 50% by sudachitin) ($p<0.05$). LPS-induced TNF-expression was significantly decreased by treatment with sudachitin ($p<0.05$), but not nobiletin.
Chen et al. [44]	Taiwan/2018	Tangeretin vs nobiletin	Nobiletin: 50 μmol for 24 hours, Tangeretin: 50 μmol for 24 hours Hesperetin: 50 μmol for 24	3T3-L1 preadipocytes (BCRC 60159) was treated with either nobiletin or tangeretin or hesperetin	Treatment with nobiletin, tangeretin and hesperetin significantly reduced lipid accumulation in adipocytes by 35.52% and 35.86% and only 1.05%, respectively ($p<0.05$) and significantly reduced TG content in adipocytes by 45.67% and 23.10% and 16.08% for nobiletin, tangeretin and hesperetin, respectively. Treatment with nobiletin significantly reduced expression of PPARγ in 3T3-L1 preadipocytes ($p<0.05$).
Kanda et al. [40]	Japan/2012	Nobiletin	Nobiletin: 10 to 100 μmol for 48 hours	3T3-L1 preadipocytes were treated with nobiletin under several differentiation conditions	Treatment with nobiletin significantly suppressed the adipogenesis and lipid accumulation in 3T3-L1 preadipocytes ($p<0.05$). Nobiletin did not affect the expression of PPARγ1, but significantly reduced the PPARγ2, C/EBPβ expression ($p<0.05$).
Miyata et al. [28]	Japan/2011	Nobiletin vs Tangeretin	Nobiletin: 16 to 64 μmol for 8 days, Tangeretin: 16 to 64 μmol for 8 days	3T3-L1 preadipocytes were treated with tangeretin, nobiletin, co-calture of both or a vehicle.	Treatment with nobiletin and tangeretin significantly decreased TG accumulation ($p<0.05$), MCP-1 (nobiletin, $p<0.01$; tangeretin, $p<0.001$) and increased adiponectin ($p<0.05$) in 3T3-L1 adipocytes. Also, nobiletin induced caspase-3 cleavage in 3T3-L1 adipocyte.
Wang et al. [39]	USA/2016	5-OH-HxMF and HpMF	1, 5, 10, 20 μmol for 8 days	Mouse 3T3-L1 preadipocytes were treated with 5-OH-HxMF	Treatment with 5-OH-HxMF significantly decreased lipid accumulation by 55–60% ($p<0.05$). At a dose of 20 μmol, 5-OH-HxMF decreased the lipid accumulation by nearly 60%. 5-OH-HxMF administration to 3T3-L1 preadipocytes significantly decreased PPARc, C/EBPs, FAS and ACC levels and activated AMPK signaling and SIRT1 compared to control ($p<0.05$).
Kang et al. [43]	Korea/2013	Sinensetin	1 to 40 μmol for 24 hours	The 3T3-L1 preadipocyte cells were treated with sinensetin or vehicle (DMSO)	Treatment with sinensetin significantly reduced the expression of SREBP1c ($p<0.05$) and also increased the phosphorylation of PKA, HSL and enhanced fatty acid β-oxidation.
Mohamed et al. [45]	Malaysia/2012	Sinestein	2.5 mg/ml sinensetin solution	Sinensetin was isolated from 50% ethanolic extract of *Orthosiphon stamineus*	Treatment with sinensetin significantly inhibited α-amylase and α-glucosidase activities with the IC 50s values of 0.66±0.02 mg/ml and 1.13±0.02 mg/ml, respectively ($p<0.05$).
Kang et al. [42]	Korea/2015	Sinestein	2, 10, and 40 μmol for 2 hours	3T3-L1 preadipocytes	Treatment with sinensetin increased intracellular cAMP levels, enhanced activation of PKA, promoted lipolysis in mature adipocytes and induces adipogenesis in preadipocytes ($p<0.05$).

ACC: acetyl-CoA carboxylase; AMPK: adenosine monophosphate–activated protein kinase; C/EBP-α: CCAAT/enhancer-binding protein-α; DMS: dimethyl sulfoxide; FAS: fatty acid synthase; FFA: free fatty acid; FPG: fasting blood glucose; 5-OH-HxMF: 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone; HFD: high fat diet; HO: heme-oxygenase-1; HSL: hormone-sensitive lipase; GLUT: glucose transporter; IL: interleukin; LPS: lipopolysaccharide; *MCP:* monocyte chemoattractant protein; NO: nitric oxide synthase; NRF: nuclear respiratory factor; PPAR: PGC-1α: *peroxisome proliferator-activated receptor;* PPARγ: peroxisome proliferator-activated receptor γ coactivator 1α; PKA: protein kinase A; SIRT1: Sirtuin 1; SREBP1c: sterol regulatory element-binding protein 1c; TC: total cholesterol; TG: triglyceride; TLR: toll-like receptor; TNF: tumor necrosis factor; UCP: uncoupling protein; WAT: white adipose tissue.

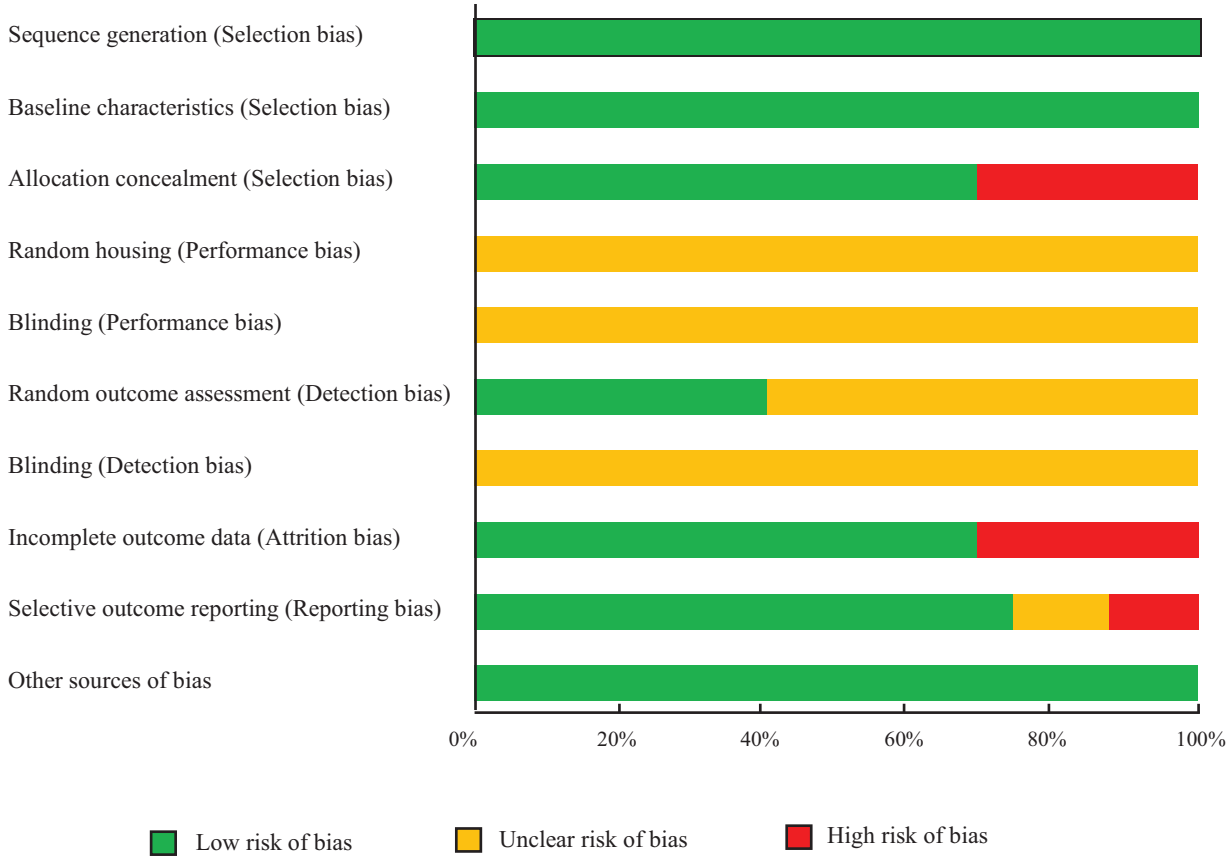


Figure 3. Results of risk of bias assessment for animal studies included in the current systematic review.

expression of the adipogenic transcription factors PPARγ, C/EBPα, and SREBP1c in 3T3-L1 preadipocytes. In addition, sinensetin significantly increased the phosphorylations of cAMP-responsive element-binding protein (CREB) and extracellular signal-regulated kinase (ERK) in 3T3-L1 preadipocytes. The OHAT risk of bias tool was used to evaluate the *In vitro* studies. Qualitative assessment showed that most studies were rated as low risk of bias for the similarity of experimental conditions, incomplete analysis, confidence in the exposure characterization, adequate administration of dose or exposure level, and other sources of bias category. In most of these studies blinding of investigators and outcome assessor were not mentioned clearly. Methods of allocation of groups were properly described in 73% of the included studies. A risk of incomplete outcome data was identified in three (27%) studies (Figure 4).

Discussion

In this systematic review, we evaluated 16 studies with a view to investigating the potential effects of PMFs on obesity and its related conditions. To our knowledge, this is the first systematic review assessing the beneficial effects of PMFs against obesity and its related conditions management. *In vitro* studies have shown evidence of the anti-inflammatory and anti-obesity benefits of PMFs in 3T3-L1 adipocytes [28, 39–44], in primary myoblasts [35] and in HepG2 cells [26]. In most animal studies the anti-obesity properties of PMFs, such as the effect of PMFs on reducing weight gain, adipocyte size, insulin resistance, increasing energy expenditure, and fatty acid β-oxidation, have been demonstrated [25, 33–35, 37]. Graphic summaries of the proposed mechanisms for the beneficial effects of the PMFs are shown in Figure 5.

Beneficial anti-obesity effects of PMFs

Effect of PMFs on body weight and energy expenditure

Increased body weight is generally associated with adipocyte dysfunction and a risk factor for several pathological conditions such as hypertension, insulin resistance, arteriosclerosis, and dyslipidemia. Several studies have suggested that adipocytes and adipogenesis regulation may act as potential targets for anti-obesity strategies

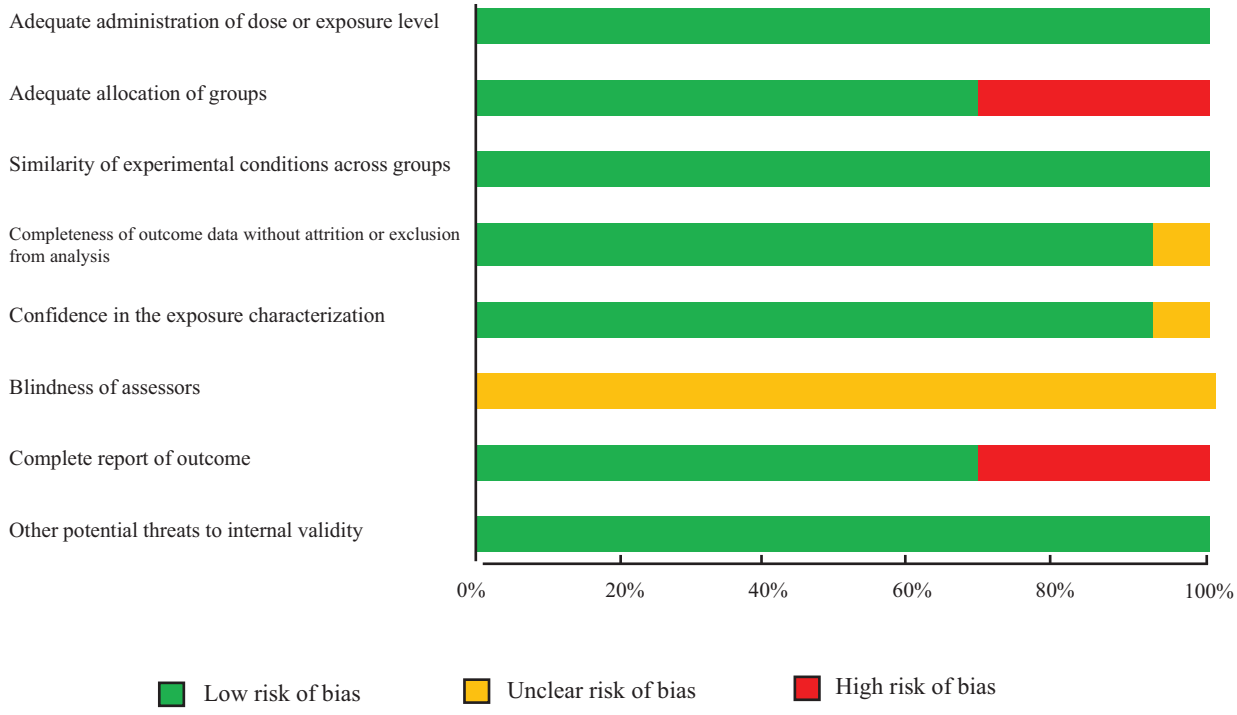


Figure 4. Results of risk of bias assessment for *in vitro* studies included in the current systematic review.

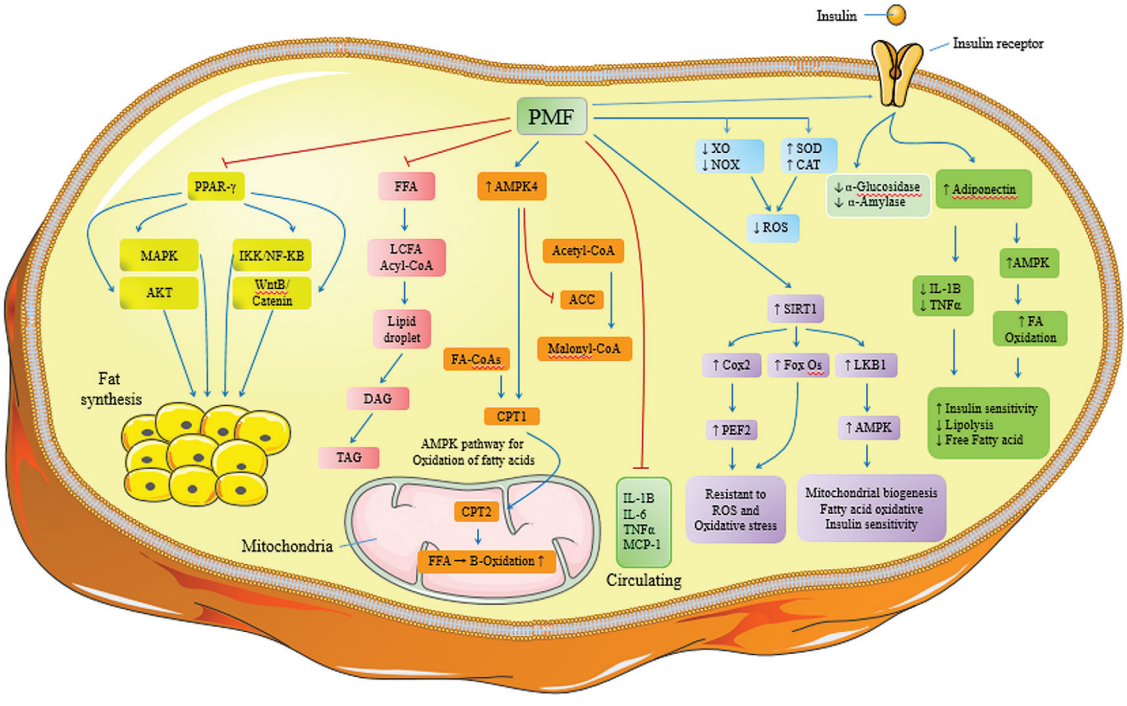


Figure 5. Graphic summaries of the proposed mechanisms for the beneficial effects of the PMFs. Abbreviations: ↑, Increased; ↓, Decreased; AMPK, AMP-activated protein kinase; ACC, Acetyl-CoA carboxylase; AKT, Protein kinase B; CAT, catalase; CPT, carnitine palmitoyltransferase; Cox2, cyclooxygenase-2; DAG, diacylglycerol; FFA, free fatty acid; PPARγ, peroxisome proliferator-activated receptors; MAPK, mitogen-activated protein kinases; IKK/NFKB, Iκ B kinase/nuclear factor NF-kappa-B; LCFA Acyl-CoA, long chain fattyacyl-CoA; FAS, fatty acid synthase; TAG, triacylglycerol; IL-1β, interleukin 1 beta; IL-6, interleukin 6; TNFα, tumor necrosis factor α; SOD, superoxide dismutase; SIRT1, sirtuin 1; PEF2, prostaglandin F2; FA-CoAs, fatty acyl-coenzyme A; ROS, Reactive oxygen species; LKB1, liver kinase B1.

[39, 42]. Citrus PMFs, a class of flavonoids, have been shown to have potent anti-obesity effects and to improve several pathological features of obesity-related diseases. Several PMFs including sudachitin, nobiletin, tangeretin, and HMF are examined to prevent obesity induced by diet [38, 40, 41, 44].

Kang et al. [46] reported that the effects of administration of PMFs significantly decreased body weight gain, adipose tissue weight, serum TC and TG in mice with obesity induced by a HFD. It also improved adipose tissue lipolysis by phosphorylation of hormone sensitive lipase (HSL) and protein kinase A. Sudachitin administration prevented HFD-induced body-weight gain, and improved lipid profiles without affecting food consumption. Sudachitin significantly increased energy expenditure by increasing PGC-1α and SIRT1 expression in myocytes. It proposes that sudachitin could improve energy expenditure, and it was also demonstrated by enhanced mitochondrial function and biogenesis in myocytes cultured with sudachitin [35]. In the study by Li et al. [30] supplementation of citrus PMFs to hamsters, essentially containing nobiletin and tangeretin reduced the weight gain and TG levels in heart and liver compared with the control group. In contrast, Kim et al. [36] identified that long-term low-dose nobiletin supplementation had no effect on food consumption, weight and fat tissue in mice with obesity induced by a HFD. Lee et al. [25] reported that nobiletin significantly reduced weight gain, white adipose tissue weight, and inhibited adipocyte differentiation of 3T3-L1 preadipocytes by down-regulating the PPARγ-C/EBPα pathway in mice with obesity. It was also observed that nobiletin increased phosphorylation of signal transducer and activator of transcription (STAT5) [40]. STAT5 is activated by the growth hormone receptor binding and has been revealed to contribute in the adipogenesis by regulating the PPARγ activity [47] and excessive activation of STAT5 inhibits the differentiation of 3T3-L1 cells by suppress the PPAR-γ gene expression [48].

In vitro studies are useful to further understand the mechanism of the anti-obesity effects of PMFs.

The results from an *in vitro* study by Wang et al. [39] showed that 5-hydroxy-3, 6, 7, 8, 30, 40-hexamethoxyflavone (5-OH-HxMF) administration reduced C/EBPβ, C/EBPα and PPARγ expression in 3T3-L1 preadipocytes. 5-OH-HxMF exhibits anti-obesity effects through up-regulation of AMPK and inhibition of lipid accumulation. Sinensetin inhibited the expression of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC)-1 by down-regulation of SREBP1c, and stimulated fatty acid β-oxidation by increasing the phosphorylation of AMP-activated protein kinase (AMPK) in mature 3T3-L1 adipocytes [43]. PMFs protected against obesity by inhibition of de novo lipogenesis and promotion of FA oxidation in both adipose and liver tissues by down-regulated mRNA levels of SREBP1c and FAS in the liver and FAS in adipose tissue [49]. A HFD has been shown to increase the expression of SREBP1c, which in turn activates FAS transcription, thus increasing fatty acid synthesis, and lastly causes to massive accumulation of fats and fatty liver formation. So, suppression of the expression of SREBP1c and its target genes such as FAS might have positive effects on the improvement of hyperlipidemia and obesity [50]. Another possible mechanism of anti-obesity activity of these compounds is the apoptosis of adipocytes, because it was detected that the administration of PMFs caused an increase in intracellular calcium, which induced the increase expression of calpains and several caspases that mediated apoptosis. Therefore, the reduction in the number of adipose cells through apoptosis could contribute to maintaining weight reduction [18]. Miyata et al. [28] reported that nobiletin induced apoptosis in mature 3T3-L1 adipocytes, while tangeretin suppressed further TG accumulation in mature 3T3-L1 adipocytes without inducing apoptosis. This difference in apoptosis inducing action against adipocytes may be due to variation of the methoxy groups in tangeretin and nobiletin structure. Based on these previous studies, it is likely that increased energy expenditure and lipolysis may be one of the main reasons for the reduced body weight gain and white adipose tissue weight in PMFs-treated animals. Further studies are necessary to explore the effects of PMFs on the expression of lipid metabolism and energy expenditure-associated genes.

Improving effect of PMFs on the lipid metabolism

The dysregulation of lipid metabolism is a common feature of obesity, and it is strongly associated with metabolic disorders. Mulvihill et al. [26] reported that, in HepG2 cells incubated with nobiletin (10 μmol up to 30 min), SREBP1c was dose-dependently reduced by 0.1% (–35%) and 0.3% (–80%) nobiletin, LDL receptor was up-regulated (2.5-fold), MTP (–30%) and DGAT1/2 expression were down-regulated, and apoB-100 secretion was inhibited via activation of MAPKerk signaling. In addition, nobiletin significantly increased carnitine palmitoyltransferase-1 alpha (Cpt-1α) and PGC-1α mRNA expression, resulting in increased cellular FA oxidation. In the study by Kang et al. [42] administration of sinensetin to 3T3-L1 preadipocytes significantly increased adipogenic transcription factors such as PPARγ, C/EBP*α*, C/EBPβ, and SREBP1c. Sinensetin enhanced activation of PKA and increased intracellular cAMP levels in 3T3-L1 preadipocytes. Collectively, these findings propose that sinensetin promotes lipolysis in mature adipocytes and induces adipogenesis in preadipocytes by increasing the intracellular cAMP levels. In a study by Tsutsumi et al. [35] treatment with 5 mg/kg sudachitin for 12 weeks significantly reduced TG and FFA levels as well

as the mRNA transcripts of FAS, and ACC in HFD-fed mouse. Kurowska et al. [51] reported that the levels of serum LDL-C, and TG were significantly reduced by 1% tangeretin or 1% PMFs mixtures (nobiletin and tangeretin) administration to hamster with hypercholesterolemia for 35 days. There is substantial evidence that metabolic disturbances caused by circadian misalignment, and improved circadian could combat metabolic disease. In study by He et al. [33] nobiletin significantly increased clock protein levels and elicited pronounced gene expression remodeling by directly activating the retinoid acid receptor-related orphan receptors. In an animal study by Green et al. [52] in rats with hypercholesterolemia treatment with 1.5% PMFs for seven weeks, the hepatic activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, hepatic TC, serum levels of TC, LDL-C, and TG were significantly decreased, however fecal TC and serum HDL-C levels were significantly increased, proposing that PMFs may exert hypolipidemic effect by decreasing cholesterol synthesis and increasing fecal TC output. In another study by Green et al. [53] administration of 1.5% PMFs lead to a significant decrease in absorption of cholesterol from the digestive tracts with a decreased villi length and number of goblet cells in rats with hypercholesterolemia. Hence, we inferred that treatment with PMFs may regulate the gut microbiota and improve lipid absorption, resulting in significant alteration in gut morphology. Taken together, both *in vitro* and animal studies indicate that the PMFs may be a potentially useful therapeutic agent for obesity-related conditions such as dyslipidemia.

Improving effect of PMFs on the glucose homeostasis and insulin sensitivity

Abnormal lipid and glucose metabolism, commonly observed in insulin-resistant states, can be ameliorated by insulin-sensitizing agents [54]. Several studies highlight PMFs capacity to improve whole-body insulin sensitivity by reducing hepatic-glucose production, ectopic TG accumulation in insulin-sensitive tissues and by enhancing insulin-stimulated glucose uptake by peripheral tissues [25, 26, 36]. Moreover, pyruvate tolerance tests showed that nobiletin could decrease hepatic gluconeogenesis in diet-induced insulin resistance rats [26]. PMFs have been revealed experimentally to enhance glucose uptake in skeletal muscles and to increase insulin sensitivity by improving tyrosine phosphorylation, demonstrating its ability to decrease insulin resistance [35]. A previous *in vitro* study [45] reported that treatment with sinensetin significantly inhibited α-glucosidase and α-amylase activities with the IC 50s values of 0.66±0.02mg/ml and 1.13 ±0.02 mg/ml, respectively. Inhibitors of α-glucosidase and α-amylase enzymes delay the breaking down of carbohydrate and prolong the overall carbohydrate digestion time in the small intestine and consequently blunting the post prandial excursion of plasma glucose. Additionally, Miyata et al. [28] in another *in vitro* study observed that the administration of nobiletin to 3T3-L1 preadipocytes significantly inhibited TG accumulation, increased the secretion of an insulin-sensitizing factor, adiponectin, and decreased the secretion of insulin resistance factors such as resistin and MCP-1, in 3T3-L1 preadipocytes. Tsutsumi et al. [35] demonstrated that administration of sudachitin to differentiated myocytes led to an increase in the PGC-1α and SIRT1 mRNA expression. Also, the genes involved in the mitochondrial biogenesis such as NRF1/2, and mitochondrial transcription factor-A (mtTFA) were increased by sudachitin. Moreover, sudachitin enhanced insulin sensitivity through the activation of Nrf2 signaling pathway [35]. It has been revealed that nobiletin significantly stimulates glucose uptake and GLUT4 translocation in 3T3-F442A adipocytes in a dose dependent manner via the activation of PKA/CREB and PI3K/Akt1/2 signaling pathways [55]. Tangeretin was shown to stimulate glucose uptake in muscle through an AMPK-dependent pathway in both mouse models and cell culture. Kim et al. [56] suggested that tangeretin activates AMPK and increases protein kinase B (Akt) phosphorylation, leading to increased glucose uptake, GLUT4 translocation as well as ameliorated obesity-induced glucose intolerance. The activation of AMPK pathways is commonly considered as a mechanism to increase glucose uptake by PMFs, but its upstream mechanism has not been investigated deeply. Sundaram et al. [57] investigated the anti-hyperglycemic potential of tangeretin on blood glucose levels in rats with diabetes. Daily treatment of tangeretin significantly increased insulin and hemoglobin however the levels of plasma glucose and glycosylated hemoglobin were decreased. Also, in another study by Lee et al. [27] nobiletin improved glucose tolerance, insulin resistance and hyperglycemia in mice with diabetes by regulation of GLUT1, GLUT4 and adipokines expression in muscle and white adipose tissue. It has been stated that nobiletin improve dyslipidemia and insulin resistance by activating the MAPK, and ERK signaling [26]. This mechanism is insulin-independent, therefore nobiletin has the ability to regulate lipoprotein metabolism in the context of insulin resistance. Taken together, the above-mentioned results show that the administration PMFs may have beneficial effects on insulin sensitivity and glucose homeostasis.

Protective effects of PMFs in non-alcoholic fatty liver disease and its related complications

Non-alcoholic fatty liver disease (NAFLD) has been recognized as the hepatic manifestation and is closely associated with the presence of obesity, dyslipidemia, insulin resistance, and diabetes, which are the major characteristics of

metabolic syndrome [58]. There are only limited studies investigating the effects of PMFs on NAFLD and the associated risk factors. In a recent study conducted by Lee et al. treatment with citrus peel extract for eight weeks reduced hepatocyte steatosis, fat accumulation and protected deterioration of liver steatosis by restoring the AMPK activity, mammalian target of rapamycin complex-1 (mTORC1)/endoplasmic reticulum stress response and promoting autophagy in HFD-fed rats [59].

Several studies have proposed that the activation of the Nrf2 pathway plays an important role in the mechanism of hepatocytes resistance to oxidative stress in NAFLD [60–62]. Additionally, Ke et al. [63] observed that the administration of PMFs enriched extract of Daoxianyeju (EDX) (0.2% and 0.5% (w/w) for ten weeks) to HFD-induced obesity C57BL/6 mice significantly improved insulin resistance, glucose tolerance and lipid profiles, and also suppressed hepatic steatosis, and inflammation by suppressing the expression of MCP-1, tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β and oxidative stress via Nrf2 activation. It also induces the expression of genes involved in fatty acid oxidation and cholesterol metabolism, such as CPT-1α, cholesterol 7alpha-hydroxylase (CYP7A1), LDL receptor, and SREBP-2. In addition, EDX significantly increased Glutathione S-Transferase 1/2 (GSTA1/2), and NADPH quinone oxidoreductase (NQO1) in the EDX (0.2 and 0.5%) supplemented mice groups. Collectively, these findings showed that EDX may inhibit the development of NAFLD partially though Nrf2 signaling and reduce inflammatory. Choi et al. [62] have reported that *citrus aurantium* extract, which contains nobiletin and tangeretin, shows antioxidant activity and ameliorates ethanol-induced liver injury by up-regulating phosphorylation of AMPK and Nrf2 in a binge drinking mouse model. In another study done by Feng et al. [34] HMF significantly alleviated hepatic steatosis through down-regulated adipogenesis and inflammatory responses-related gene, enhanced fatty acid oxidation and increased energy expenditure.

Among controlling mechanisms against NAFLD, AMPK play an important role in preventing hepatic lipid accumulation in excess nutrient-treated HepG2 and HFD-fed mice [64, 65]. Citrus peel extracts contribute to the down-regulation of liver X receptor (LXR) and activation of AMPK, which increases fatty acid oxidation and prohibits lipogenesis. It is well-established that a high level of FFAs increases mTORC1 signaling, a pathway associated with the development of diseases such as obesity and NAFLD [66, 67]. Studies have demonstrated that the activation of AMPK inhibits the mTORC1 pathway by phosphorylating and activating the tumor suppressor TSC2 (tuberous sclerosis), is a possible mechanism to explain the inhibition of mTOR/endoplasmic reticulum stress axis in NAFLD [68, 69]. The endoplasmic reticulum stress response is one of the main features of pathological conditions related to NAFLD, and obesity. Li et al. revealed that alteration of AMPK/mTOR pathway activity is involved in the modulation of lipid metabolism, steatosis, and insulin resistance in HFD-induced mice by up-regulation of autophagy [70]. The MAPK signaling pathway may also be a potential therapeutic target for anti-inflammatory treatment of NAFLD. The anti-inflammatory agent applies protective effects against liver injury by down-regulating the ERK signaling and decreasing its downstream effectors of inflammatory responses, including cytokine production and microscopic changes [71]. The main cytokines involved in the pathogenesis of NAFLD are TNF-α, and IL-6. TNF-α and IL-6 inhibit lipoprotein lipase, and TNF-α also down-regulates insulin-stimulated glucose uptake and insulin receptor signaling. TNF-α contributes to the activation of NF-κB signaling in the liver, which is a well-known important regulator of cellular gene expression in response to a wide range of inflammatory stimuli [72]. It is activated in response to the inflammation and controls functions of hepatocytes, hepatic stellate cells and (HSCs) and Kupffer cells by inducing the expression of inflammation-related genes, essentially contributing to liver inflammation in NAFLD development [73].

Previous studies have revealed the role of NF-κB as a central link between hepatic injury, inflammation, fibrosis and hepatocellular carcinoma, since more than 80% of hepatocellular carcinoma is found in patients with hepatic cirrhosis, proposing it as therapeutic target for the treatment from liver fibrosis to hepatocellular carcinoma during NAFLD development [72, 74]. In the study performed by Jiang J et al. oral administration of the total flavonoids of *Qu Zhi Ke* (TFCH) (25, 50 and 100 mg/kg for eight weeks) to rats increased anti-inflammatory and hepatoprotective effects by preventing NF-κB and MAPKs activation and the subsequent production of IL-6, IFN-γ, IL-12, IL-1β, and TNF-α. Also, in their study liver MAPK subfamily, such as ERK and p38 were activated by phosphorylation in NAFLD rats compared to control group. Activation of p38 may lead to liver injury by expression of apoptotic hallmarks including DNA fragmentation and caspase activation. The phosphorylated activations of NF-κB, ERK, and p38 were also reversed via TFCH treatment in the liver of NAFLD rats, indicating an anti-NAFLD effect of TFCH mediated by MAPK and NF-κB signaling [75]. Accordingly, the above-mentioned findings are suggestive of hepatoprotective roles of PMFs through the activation of AMPK, and the inactivation of NF-κB and MAPKs pathways involved in the pathogenesis and progression of NAFLD. It should be noted that, due to the limited number of studies, the effect of PMFs on NAFLD needs to be confirmed in future studies.

Anti-inflammatory properties

It is well known that obesity is a chronic low-grade inflammation in which there are elevated circulating pro-inflammatory cytokines [38, 76]. Adipose tissue produces and releases a variety of adipokines and cytokines, such as leptin, tumor necrosis factor-α (TNF-α), and IL-6, that are involved in the development of obesity and other metabolic diseases [77]. Previously, it has been established that PMFs have strong anti-inflammatory activity [36, 38, 41]. Namkoong et al. [41] explored the plausible anti-inflammatory effect of nobiletin (50 and 100 μmol), and proposed that the raised secretion of nitric oxide (NO) and TNF-α, along with the expression of inducible nitric oxide synthase (iNOS) induced by interaction between adipocytes and macrophages are all weakened by nobiletin treatment. In study conducted by Whitman et al. [21] the administration of nobiletin could inhibit the production of PGE2 induced by LPS and pro-inflammatory cytokines such as IL-6, IL-1β, IL-1α, and TNF-α, in murine J774A.1 macrophages. It also down-regulate cyclooxygenase-2 (COX-2) gene expression instead of COX-1. Additionally, in another study, Xiong et al. [78] reported that nobiletin may exert anti-inflammatory effects on 2, 4, 6-trinitrobenzenesulfonic acid-triggered colitis by decreasing the expression of iNOS and COX-2 proteins. In another animal study [36], it was reported that nobiletin significantly reduced mRNA expression of Toll-like receptor genes and NF-κB in white fat tissue and pro-inflammatory cytokine (TNF-α, IL-6, and IL-1β) levels in plasma. The levels of circulating inflammatory cytokines were increased in animals with obesity [79], and obesity-related chronic systemic inflammation is proposed to play an important role in the pathogenesis of HFD-induced insulin resistance [80]. Study by Hotamisligil et al. [81] showed that TNF-α is highly increased in the adipose tissue of animals with obesity and that it is involved in the induction of insulin resistance in the body. Furthermore, fatty acids, the levels of which are raised in obesity, can stimulate inflammatory pathways through the activation of Toll-likereceptor-2 and-4 in adipose tissue, causes the development of insulin resistance [82]. Mice lacking Toll-like receptor-2 and-4 also show reduced inflammatory cytokine expression in adipose tissue and insulin resistance, proposing a role for Toll-like receptors in obesity-related morbidities [83, 84]. Yuasa et al. [38] in an *in vitro* study investigated the effects of the nobiletin and sudachitin on LPS-induced inflammatory responses in mouse macrophage cells. Both nobiletin and sudachitin inhibited the production of nitric oxide (NO) and the expression of TNF-α in LPS-stimulated mouse macrophage RAW264 cells, nevertheless the inhibitory effect of nobiletin was less than that of sudachitin. Moreover, Shin et al. [85] revealed that sinensetin inhibited the expression of genes related to inflammation, such as COX-2, IL-1, TNF-α, IL-6, and iNOS by regulating the inhibitor κB (IκB)-protein level in LPS-activated macrophages; therefore, these PMFs may be helpful for the treatment of inflammation-associated diseases such as obesity. Collectively, the findings suggest that PMFs may exert anti-inflammatory effects through the inhibition of NF-κB activity and iNOS, and pro-inflammatory cytokines expression.

Future directions

All data eligible for inclusion in the study were based on *in vitro* and animal studies; there were no human studies available. More strong well-designed human studies are needed to assess the effects of PMFs on several aspects of obesity, including adipokines, oxidative stress, body composition, and inflammatory status. Also, more randomized control trials are suggested to compare which types of PMFs have a better effect on obesity. Studies with molecular aspects are proposed to evaluate the effect of the addition of PMFs along with the diet on the several metabolic factors in different patients with chronic metabolic diseases such as hyperlipidemia, diabetes, etc. Given that promising effects have been proposed by *in vitro* and animal studies, it is recommended to conduct clinical trials on the effects of PMFs on the expression of the genes involved in lipogenesis in human adipose tissue.

Conclusion

In this study, we reviewed and summarized current knowledge on the effect of PMFs intake on the various studied parameters related to obesity and suggested mechanisms of action. A variety of PMFs have been used in such studies, which limits direct comparison of study results, but some general trends can be noted. This systematic review suggested beneficial effects of PMFs on obesity, principally through reducing body weight, lipogenesis, improving lipid profile, glycemic index, and also β-oxidation. Treatment with PMFs significantly inhibits lipid accumulation in adipocytes and 3T3-L1 pre-adipocyte differentiation as well by decreasing the expression of PPARγ and C/EBPα and also reduces the number and size of fat cells. Although current evidence indicated a positive link between PMFs as a potential treatment in obesity, further interventional and longitudinal studies are required to validate these findings.

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