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ARTICLE

Animal Models

Polymethoxyselenoflavones exert anti-obesity effects through activation of lipolysis and brown adipocyte metabolism

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Abstract

Background/objectives Polymethoxyselenoflavone (PMSF) is a compound that substitutes the oxygen atom in a flavonoid with selenium. This study aimed to investigate the effects of PMSFs on lipid metabolism in adipocytes and their anti-obesity potential.

Subjects/methods To test lipolytic and thermogenic effects of the compounds in vitro, adipocytes differentiated from immortalized pre-brown adipocyte progenitors and pre-white adipocyte cell lines were treated with 19 PMSFs. The expression levels of brown adipocyte markers and genes related to mitochondrial metabolism were analyzed by qPCR and western blot. In vivo anti-obesity effect was investigated using diet-induced obesity mouse models and adipocyte-specific ATGL knockout mice.

Results The qPCR analysis identified 2-(3,4-dimethoxyphenyl)-4H-selenochromen-4-one (DMPSC) as the most potent brown adipogenic candidate among the 19 compounds tested in this study. DMPSC treatment significantly increased the mitochondrial content and oxidative metabolism in adipocytes in vitro. Mechanistically, DMPSC treatment increased lipolysis through activation of PKA downstream signaling. Consistently, the in vivo treatment of DMPSC increased energy consumption, reduced body weight, and improved glucose tolerance in mice fed with high-fat diets. Moreover, DMPSC treatment increased brown adipocyte marker expression and mitochondrial content in adipose tissue of mice. The anti-obesity effects were absent in adipocyte-specific ATGL knockout mice, indicating that the DMPSC effect is mediated by cytosolic lipase-dependent mechanisms.

Conclusions Collectively, our results indicated that DMPSC exerted anti-obesity effects partially through the PKA signaling-mediated activation of lipolysis and brown adipose tissue metabolism.

Introduction

The adipose tissue plays an essential role in regulating energy homeostasis and can be classified into two types: brown adipose tissue (BAT) and white adipose tissue (WAT) [1]. WAT is further subcategorized into subcutaneous and visceral WAT [2, 3]. In general, excess accumulation of visceral WAT is associated with high incidence of metabolic disease, while subcutaneous WAT has been found to be beneficial for the maintenance of metabolic homeostasis [2, 4]. In contrast, the anatomical location of BAT is more specific around the neck and clavicle [2], containing multilocular small lipid droplets (LDs) and a large number of mitochondria [5]. BAT possesses specialized thermoregulatory functions and its unique expression of uncoupling protein 1 (UCP1) is responsible for non-shivering thermogenesis [5]. Thus, molecular

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mechanisms controlling brown adipocyte thermogenesis have been investigated as a potential therapeutic target to counteract obesity and metabolic diseases [5, 6].

Besides classic brown and white adipocytes, beige adipocytes have been characterized as UCP1 + adipocytes that appear in WAT, under thermogenic stimuli including cold stress and beta3-adrenergic stimulation [7]. These adipocytes have a higher number of mitochondria and smaller diameter multilocular LDs compared with white adipocytes [3, 8]. The appearance of beige adipocytes contributes to beneficial phenotypical changes that improve metabolic profiles and augment energy expenditure [1, 3, 5, 6].

Flavonoids, more than 8000 in numbers, are phenolic compounds isolated from a variety of vascular plants [9–11]. Flavonoids have been found to possess health benefits, including the regulation of energy homeostasis [12]. Polymethoxyselenoflavone (PMSF) is a flavonoid compound in which the oxygen atom is replaced with selenium [13]. We have reported that selenium substitution improved polarity and lipophilicity [14]. Furthermore, PMSFs exhibited potent antioxidant properties [4], suggesting that PMSFs could be used as a promising agent that can target adipose tissue and mitochondrial metabolism. Accordingly, we tried to characterize the thermogenic effects of PMSFs on adipocytes and its anti-obesity potential.

The current study demonstrated that, among the 19 selnoflavanone compounds tested in this study, 2-(3,4-dimethoxyphenyl)-4H-selenochromen-4-one (DMPSC) significantly increased brown adipocyte marker expression, mitochondrial content and oxidative metabolism in adipocytes. Mechanistically, DMPSC increased cAMP-dependent protein kinase A (PKA) downstream singling pathway that regulates lipolysis. Consistent with these results, in vivo treatment of DMPSC reduced adipose tissue mass and increased energy expenditure. Moreover, DMPSC treatment increased brown adipocyte marker expression and mitochondrial contents. Collectively, our results demonstrated anti-obesity effects of DMPSC through the activation of lipolysis and BAT metabolism.

Methods

Animals

All protocols of animal studies were reviewed and approved by the Institutional Animal Care and Use Committees of Yonsei University (IACUC-20170621901) and Seoul National University (SNU-191118-3, SNU-191025-2). All animal experiments were conducted according to the guidelines for humane care and use of laboratory animals (Ministry of Food and Drug Safety). Mice were fed a rodent chow diet and were kept at 22 ± 2 °C under a 12 h light/dark cycle and free access to water and food. After acclimatization, mice were randomly assigned to experimental groups. All experiments were performed using male mice, and all analyses were carried out by scientists blinded to the group allocation. For high-fat diet (HFD) experiment, C57BL/6 mice (Central Lab. Animal Inc., Seoul, South Korea) were fed with HFD (60% fat, #D12492, Research Diets, NJ, USA) at 7 weeks of age, and HFD feeding was continued for 8 weeks. 3′4′-dimethoxyflavone (DMF, 10 mg/kg body weight, p.o.) or DMPSC-4A (4A) (10 mg/kg body weight, p.o.) was treated for up to 2 weeks [14]. For indirect calorimetry analysis by a single treatment, DMPSC-4A was treated via intraperitoneal injection (10 mg/kg mouse). Energy expenditure and oxygen consumption rates were monitored by using indirect calorimetry system (TSE PhenoMaster, Bad Homburg, Germany), as previously described [15]. Adipoq-CreER (stock# 024671, Jackson Laboratory) mice were crossed with Pnpla2-floxed mice (stock# 024278, Jackson Laboratory) to generate adipocyte-specific adipose triglyceride lipase (ATGL) knockout mice [16]. Mice were treated with tamoxifen (Sigma, 75 mg/kg/day, p.o.) by oral gavage for 5 days to induce Cre recombination. Control groups treated with vehicle were included.

For intraperitoneal glucose tolerance test (GTT), mice were intraperitoneally injected with 200 mg/ml D-Glucose (Sigma, 2 g/kg body weight) [17]. Glucose meter (Accu-Check Performa, Roche) was used to measure glucose concentration. Ex vivo electron transport activity related to mitochondrial oxidative phosphorylation was evaluated by monitoring the reduction of triphenyltetrazolium chloride (TTC, Sigma), as described previously [18]. For cold tolerance test, mice were injected with DMPSC-4A and vehicle, and then placed in a 4 °C cold room where body temperature was measured by rectal thermometry, as described in the previous works [17].

Cell cultures

3T3-L1 (ATCC), C3H10T1/2 (ATCC) and immortalized brown pre-adipocytes were cultured as described previously [19]. For adipogenic differentiation, 3T3-L1 cells or immortalized brown pre-adipocytes [19] were cultured in growth medium (10% FBS DMEM (Gibco)). After reaching confluence, cells were cultured in adipogenic differentiation medium (10% FBS DMEM) with isobutylmethylxanthine (2.5 mM, Sigma), insulin (1 μg/ml, Sigma), indomethacin (0.125 mM, Cayman), dexamethasone (1 μM, Cayman), and triiodothyronine (1 nM, Sigma) for three days, and then maintained in 10% FBS DMEM supplemented with insulin (1 μg/ml) for four days. The fully differentiated cells were treated with compounds for up to 24 h in growth medium. C3H10T1/2 cells were treated with BMP4 (20 ng/ml: R&D systems, #314-BP) before exposure to adipogenic

differentiation medium. For ATGL knockdown, siRNA targeting Pnpla2 (encoding ATGL) (Bioneer) and Negative Control siRNA (Bioneer) were transfected to C3H10T1/2 adipocytes, using jetPRIME reagent (#114-75, Polyplus Transfection). Oxygen consumption rate (OCR) was measured by Seahorse XF Analyzers (Agilent) as previously described [20]. MitoTracker™ Red CMXRos (#M7512, Thermo scientific) was used for mitochondrial staining. Glycerol levels in conditioned media were measured using glycerol reagent (Sigma), as described previously [15]. Intracellular cAMP levels were determined by using cAMP ELISA Kit (Enzo), according to manufacturer's instructions.

mRNA expression analysis

Quantitative real-time polymerase chain reaction (qPCR) was performed, as described previously. TRIzol® reagent (Invitrogen) was used for RNA extraction. Applied Biosystems High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific), IQ-SYBR Green Super Mix (Bio-Rad) and CFX Connect Real-time PCR Detection system (Bio-Rad) were used. All primers used in experiments were described previously [15].

Protein extraction and Western blot

Protein extraction and Western blot analysis were proceeded, as described previously [8]. The following primary antibodies were used: anti-hormone sensitive lipase (HSL) antibody (Rabbit, #4107, 1:1000, Cell Signaling), phosphor-HSL(Ser660) antibody (p-HSL, Rabbit, #41126, 1:1000, Cell Signaling), UCP1 antibody (Rabbit, #UCP11-A, 1:2000, Alpha Diagnostic International), α/β tubulin antibody (Rabbit, #2148, 1:1000, Cell Signaling), p-CREB (Ser133) (87G3) antibody (Rabbit, #9198, 1:1000, Cell Signaling), COXIV (3E11) antibody (Rabbit, #4850S, 1:1000, Cell Signaling), Phosphor-(Ser/Thr) PKA substrates antibody (p-PKA, Rabbit, #9621, 1:1000, Cell signaling) and Total OXPHOS Rodent western blot antibody Cocktail (Mouse, #110413, 1:1000, Abcam: CI subunit NDUFB8-20kDa (ab110242), CII-SDHB-30kDa (ab14714), CIII-Core protein 2–48 kDa (ab14745), CIV subunit I-40 kDa (ab14705) and CV alpha subunit −55 kDa (ab14748)).

Histology and immunohistochemistry

Histological analyses were performed, as previously described [8]. Briefly, adipose tissues were fixed in formalin solution (Sigma, neutral buffered, 10%) and subjected to paraffin embedding and histological sections. Anti-F4/80 antibody (Rat, #MCA497GA, 1:100, AbD Serotech) was used for immunohistochemistry.

In silico prediction of physicochemical property

The topological polar surface area (tPSA) of the compounds was calculated by Cambridgesoft ChemBioDraw (Waltham, MA, USA) [14].

Statistical analysis

GraphPad Prism 5 software was used for statistical analyses. Mean ± Standard Errors of the means (SEMs) was used for data presentation. Statistical significance was determined by *t*-test, two-way analysis of variance (ANOVA), or one-way ANOVA with Bonferroni post hoc tests, as appropriate.

Results

Effects of polymethoxyselenoflavones on brown adipocyte marker expression

In order to search for candidates with browning potential, the effects of 19 selenoflavonoids (Table S1) on gene expression were tested using qPCR. Chemical structures of 19 selenoflavonoids are shown in Table S1. Calculated values of tPSA indicated a reduction in the polarity of most of the selenoflavonoids compared with the counterpart flavonoids (Table S1). Fully differentiated brown adipocyte precursor cells were treated with each of the selenoflavonoid compounds for 24 h. The expression of UCP1, a brown adipocyte-specific thermogenic gene, increased significantly upon treatment with 2-(3,4-dimethoxyphenyl)-4H-selenochromen-4-one (DMPSC; compound code:4A) and 2-(3,5-dimethoxyphenyl)-4H-selenochromen-4-one (DMPSC; compound code: 4C). The expression levels of peroxisome proliferator-activated receptor (PPAR) γ coactivator 1 (*Ppargca1*) and cytochrome c oxidase subunit 8B (*Cox8b*) were also significantly increased by 4A (Figs. 1a and S1a). We selected the top 3 compounds, 4A, 4C, and 4H, with brown adipogenic activity, and examined additional brown adipocyte marker expression. Data indicated that 4A was the most potent brown adipogenic compound (Fig. S1b).

We also confirmed the browning effect of the compounds by using white adipocyte cell lines, C3H10T1/2 and 3T3-L1 (Fig. S1c, d). Although the extent of browning effects by the compounds was lower in case of white adipocyte cell lines compared with brown adipocytes, brown adipogenic markers (*Elovl3, Acadm, Dio2*) in C3H10T1/2 adipocytes were significantly increased by 4A (Fig. S1c). Similarly, *Cox8b* and *Ppargc1a* expression levels in adipocytes differentiated from 3T3-L1 were significantly increased by 4A (Fig. S1d).

Effects of DMPSC on mitochondrial content and mitochondrial oxidative metabolism in brown adipocytes

The effect of browning is characterized as an increase in the activity and content of mitochondria; thus, effects of these compounds on mitochondrial levels were examined by western blot analysis. As indicated in Fig. 2a, 4A significantly increased the levels of COXIV and mitochondrial enzymes involved in mitochondrial oxidative phosphorylation (ATP5A, UQCRC2, NDUFB8) (Fig. 2a). In addition, 24 h of 4A treatment increased MitoTracker staining in brown adipocytes from immortalized precursor cells (Fig. S2b). Furthermore, 4A treatment increased basal, maximal, uncoupled, and ATP-linked oxygen consumption rates of brown adipocytes differentiated from immortalized precursor cells (Figs. 2b and 2Sc).

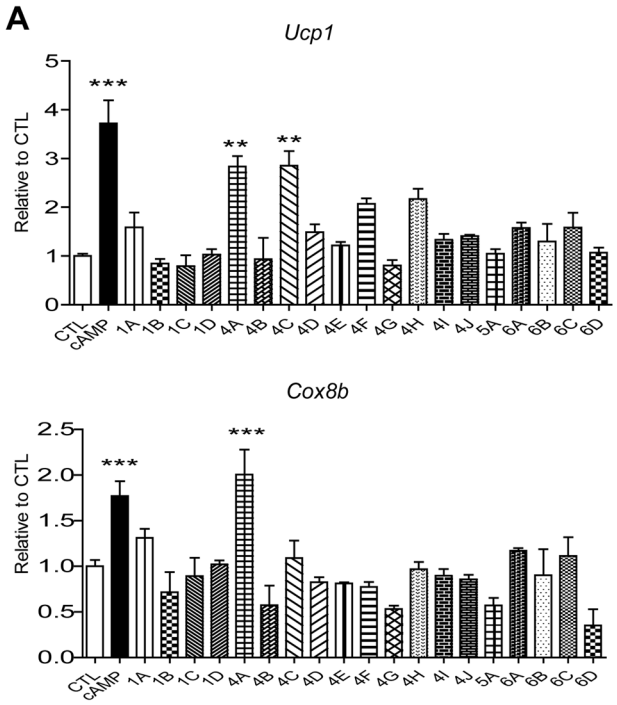

Fig. 1 In vitro effects of polymethoxyselenoflavones on brown adipogenic marker expression in brown adipocytes. a qPCR analysis of brown adipogenic gene expression in adipocytes differentiated from immortalized pre-brown adipocytes treated with selenoflavones (1 μM) for 24 h. The chemical structures of selenoflavones are provided in Table S1. 8Br-cAMP (1 mM) was used for positive controls ($n = 6$ per condition, mean ± S.E.M, *t*-test, $^{**}p < 0.01$, $^{***}p < 0.001$).

Effect of DMPSC on PKA signaling and lipolysis in brown adipocytes

Sympathetic activation through beta3-adrenergic receptor is the major regulator of brown adipocyte metabolism, including thermogenesis and lipolysis [21]. Beta3-adrenergic receptor mainly acts downstream of cAMP-dependent PKA pathway [22, 23] and a representative target of PKA is HSL [22], which is a rate limiting step of cytosolic lipolysis [22]. We found that 4A treatment increased phosphorylation levels of PKA substrates and HSL, as confirmed by western blot analysis (Figs. 3a and S2d). Also, 4A treatment increased lipolysis and cAMP levels in brown adipocytes in vitro (Figs. 3b and S2e), suggesting that 4A regulated cAMP/PKA-dependent lipolysis.

In vivo anti-obesity effect of DMPSC

To test the effects of DMPSC in vivo, we first measured oxygen consumption and energy expenditure using indirect calorimetry. As indicated in Fig. 4, 4A treatment increased oxygen consumption and energy expenditure. In addition, 4A treatment reduced respiratory exchange rate (Fig. S3a), indicating that treatment with 4A increased lipid utilization. When mice were acutely challenged with cold (4 °C) stress for 4 h, 4A-treated mice exhibited similar levels of cold tolerance compared with vehicle-treated control (Fig. S3b). Data suggested that 4A may act through common pathways that are engaged with cold stress to increase thermogenesis.

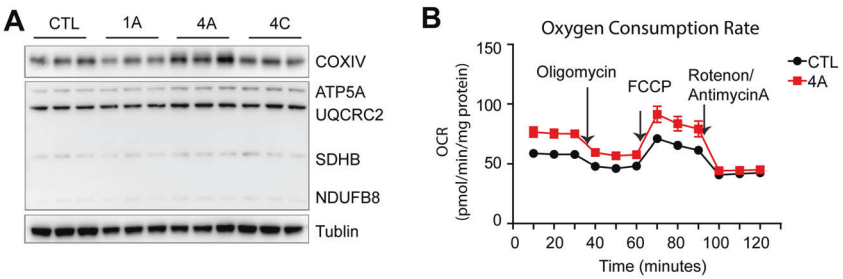

Fig. 2 4A increases mitochondrial content in brown adipocytes. a Immunoblot analysis of mitochondrial proteins in brown adipocytes (BA) differentiated from immortalized brown adipocyte precursors. Differentiated BA cells were treated with each compound (1 μM) for 24 h. Tubulin was used as loading controls. **b** Oxygen consumption rate (OCR) in differentiated BA cells with a series of treatments of indicated reagents (oligomycin, FCCP, antimycin A and rotenone) measured by Seahorse XFp Extracellular Flux Analyzer. Differentiated BA cells were pre-treated with 1 μM 4 A for 4 h ($n = 6$, means ± SEM, also see Fig. S2).

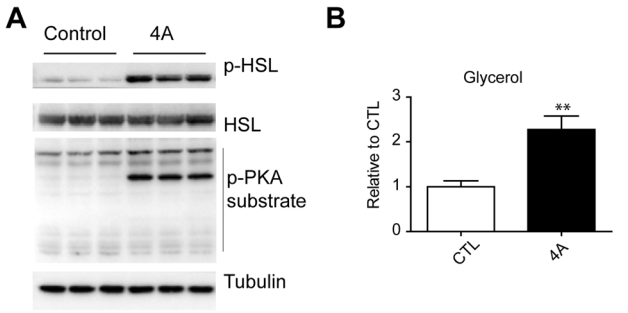

Fig. 3 4A increased PKA downstream signaling pathway related to lipolysis. a Immunoblot analysis of phosphorylation of HSL and PKA substrates in brown adipocytes differentiated from pre-brown adipocyte precursor cells treated with DMPSC-4A for 4 h. **b** Analysis of glycerol levels in conditioned media levels of brown adipocytes treated with DMPSC-4A for 4 h ($n = 6$, t-test, mean ± S.E.M, $^{**}p < 0.01$, $^{***}p < 0.001$).

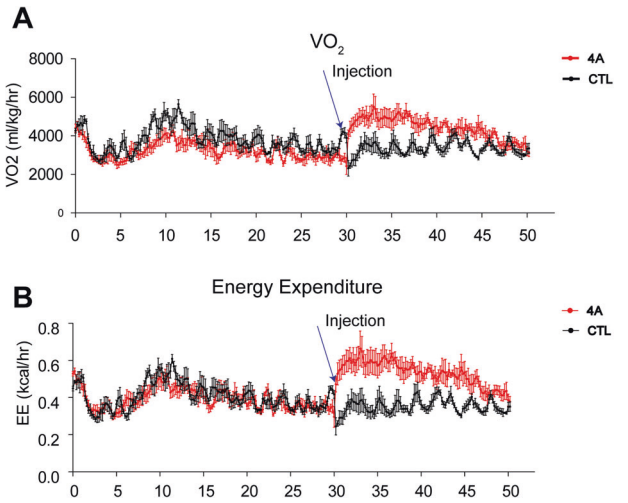

Fig. 4 4A increased oxygen consumption and energy expenditure of mice. Indirect calorimetry analysis of oxygen consumption rate (VO_2: **a**) and energy expenditure (EE: **b**) of mice treated with 4A and vehicle. Arrows indicate time of the treatment (10 mg/kg, i.p.). Average values after 4A injection were presented as bar graphs in Supplemental Fig. S3 ($n = 5$–6 per condition, t-test, mean ± S.E.M, ** $p < 0.01$).

To determine whether 4A treatment has an anti-obesity effect, we used HFD-induced obesity mouse models [17]. We also used non-selenium substitution counterpart compound (3′4′-dimethoxyflavone: DMF) to examine the effects of selenium substitution. Fourteen days of treatment showed a reduction in the body weight (Fig. 5a). Especially, the weight of visceral adipose tissue gWAT was significantly reduced (Fig. 5b). In addition, glucose tolerance was improved by 4A treatment (Fig. S4a). Consistent with the in vitro data, 4A treatment increased UCP1, COXIV, p-HSL, mitochondrial enzymes involved in oxidative phosphorylation and p-PKA substrate levels in BAT (Fig. S4b, c). Moreover, qPCR analysis indicated that 4A treatment increased brown adipocyte marker expression (*Ucp1, Cidea, Dio2, Elovl3*) (Fig. S4d). Treatment with 4A increased COXIV and UCP1 expression levels in inguinal subcutaneous WAT (iWAT) and COXIV expression levels in gonadal WAT (gWAT) (Fig. S5a, b), indicating browning of WAT. Consequently, induction of mitochondrial metabolism by 4A reduced serum FFA and TG levels (Fig. S5c, d), implying improvement in blood lipid profiles. Higher intensity of eosin staining in paraffin sections (Fig. S5e) further supported that 4A treatment increased mitochondrial content in BAT and WAT. Immunostaining of UCP1 on paraffin sections showed its induction by 4A treatment in BAT and iWAT (Fig. S5f). Furthermore, TTC staining was used to measure mitochondrial oxidative metabolism of adipose tissue isolated from mice treated with 4A, DMF, and vehicle, and it demonstrated that 4A treatment increased oxidation in BAT, iWAT and gWAT (Fig. S6). The effects of DMPSC on mitochondrial content and oxidative capacity were more potent than the effects of the corresponding flavone DMF (Figs. 5 and S4–S6).

Finally, to test if the effects of 4A required cytosolic lipase pathway, we used adipocyte-specific ATGL knockout mice models [23]. ATGL is the first lipase that initiates the hydrolysis of triglycerides, and its activity is regulated by the cAMP/PKA-dependent phosphorylation of perilipin1 and interaction with α/β hydrolase domain containing 5 (ABHD5) [22]. Adipocyte-specific ATGL knockout was confirmed by western blot (Fig. 6a). Consistent with previous reports [23], BAT of ATGL knockout mice had greatly reduced expression of UCP1 (Fig. 6a). Treatment of 4A neither increased UCP1 expression nor reduced body weight (Fig. 6). To determine whether the effects of 4A were mediated by ATGL-dependent lipolysis in adipocytes, we performed in vitro siRNA knockdown experiments (Fig. S7). As shown in Fig. S7a, siRNA knockdown of ATGL attenuated the effects of 4A on the upregulation of UCP1 and COXIV expression in adipocytes. qPCR analysis also confirmed that ATGL knockdown inhibited the role of 4A in inducing brown adipocyte marker expression (Fig. S7b). Collectively, the data indicated that ATGL-dependent lipolysis is the major mechanism of anti-obesity effects of 4A.

Discussion

Flavonoids are bioactive secondary metabolites found in a wide variety of plants [9], and have health benefits owing to their antioxidant, anti-inflammatory, and anticancer properties [24–26]. Polymethoxyflavones (PMFs) are a subclass of flavonoids [27], and this study focused on the selenium-substituted forms of PMF (PMSFs). The potential benefits of PMFs include anti-obesity and anti-diabetes effects [28–32].

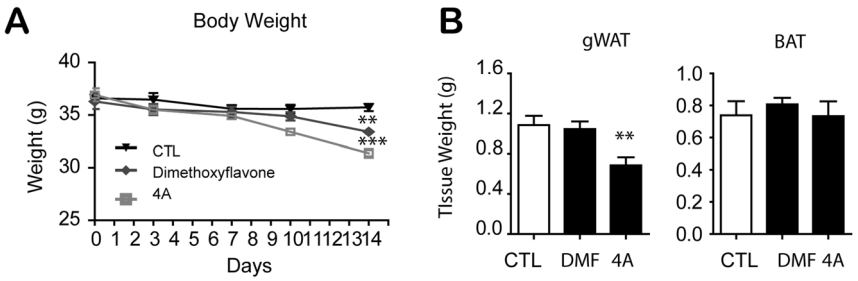

Fig. 5 In vivo anti-obesity effects of 4A in high-fat-diet-fed obese mice. Mice were fed with high-fat diet for 8 weeks and then treated with DMPSC-4A (4A), 3′4′-dimethoxyflavone (DMF), and vehicle for two weeks. **a** Body weight monitoring during treatment. **b** Tissue mass analysis of gonadal white adipose tissue (gWAT) and interscapular brown adipose tissue (BAT) of mice treated with DMPSC-4A (4A), 3′4′-dimethoxyflavone (DMF), and vehicle for two weeks ($n = 6$, one-way ANOVA with Bonferroni posttests, mean ± S.E.M, $^{**}p < 0.01$).

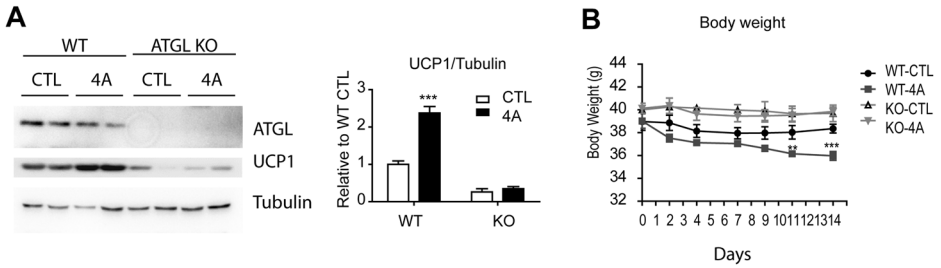

Fig. 6 ATGL knockout attenuates effects of 4A on body weight and brown adipocyte marker expression. a Immunoblot analysis of ATGL and UCP1 in brown adipose tissue of WT controls and adipocyte-specific ATGL KO mice treated with DMPSC-4A or vehicle (CTL) ($n = 6$ per condition, two-way ANOVA with Bonferroni posttests, mean ± S.E.M, $^{**}p < 0.01$, $^{***}p < 0.001$). B. Body weight monitoring of WT controls and adipocyte-specific ATGL KO mice during 4A treatment.

For instance, citrus PMFs (mainly nobiletin and tangeretin) have been shown to elevate lipid homeostasis and regulate adipokine secretion [29], In addition, 5,7-dimethoxyflavone exhibits antidiabetic and hypo-lipidemic activities in diabetic animal models [31], and its anti-obesity effects are associated with anti-adipogenic effects [30]. Also, nobiletin is known to induce lipolysis through the cAMP/PKA signal pathway in vitro [32]. Anti-obesity effects of heptamethoxyflavones also involve anti-adipogenic activity through the PKA signaling pathway [28]. Although the browning effects of PMFs have not been completely investigated, several flavonoids have been reported to play a role in inducing browning of WAT [12]. For example, rutin exerts anti-obesity effect through BAT activation [33] and quercetin induces thermogenic marker expression in white adipocytes in vitro [34] and in vivo [35].

The current study demonstrated that the effects of DMPSC on lipolysis and adipose browning were mediated by PKA downstream signaling. In line with our current study, the PKA signaling pathway has been shown as one of the main pathways to activate the browning of WAT and lipolysis by natural and synthetic compounds [21, 36–38]. Other major pathways involved in flavonoid-induced browning include AMP-activated protein kinase (AMPK)/Sirtuin1 [33] and PPAR-related signaling pathways [12].

In the current study, in vitro screening of PMSFs for brown adipogenic activity identified the candidate compounds for treatment of obesity. Around 1 μM of the selected compound DMPSC-4A was effective enough to induce thermogenic gene expression in adipocytes, and it was within physiologically achievable concentrations of flavonoids (0.1–10 μmol/L) [39]. Anti-obesity effects of DMPSC were examined using diet-induced obesity mouse models. As various dosing protocols have not been evaluated in this study, does-response relationship studies and pharmacokinetic analyses [40] of DMPSC deserve further investigation to determine the effective dose and administration routes for clinical applications. In addition, the chronic oral administration [41] of DMPSC has other side effects on food consumption or energy extraction in the digestive system that might affect weight gain. Further studies are required to examine the effects of DMPSCs on food intake and absorption.

Chemical modifications of candidate bioactive compounds are necessary to optimize the therapeutic efficacy and reduce the adverse effects. For instance, changes in pharmacokinetic profiles would improve the efficacy of

the drug and increase its specificity to target organ, thus reducing adverse effects [42]. In this regard, our group synthesized a compound that replaced the flavonoid's oxygen atom with selenium, leading to improved physicochemical properties and increased biological activity [4, 14]. Epidemiologic studies have shown that selenium has strong associations with protective effect on metabolic dysfunction, and neurodegeneration [13]. Moreover, selenium increases lipophilicity [14], which might enhance adipose tissue-targeting of the compound. Our study indicated that selenium-substituted compound is more efficient to reduce body weight and browning phenotype of adipose tissue. Thus, we speculated that the increase in lipophilicity may increase adipocyte-specific targeting of the molecules and this can be applied to other agents that potentially target adipocyte-specific molecular players. Although this study focuses on the molecular mechanisms of anti-obesity effects of DMPSC, it would be highly important to understand the pharmacokinetic profiles and tissue distribution of DMPSC.

Although there is a lack of large-scale clinical studies on drugs that induce browning [21], browning-inducing reagents have been investigated as promising candidates to combat obesity and related metabolic diseases [21, 38]. Research on optimization and strategies for personalized medicine would facilitate the development of novel anti-obesity drugs that target adipose tissue browning.

Collectively, our results indicate that DMPSC exerts anti-obesity effects partly through the activation of brown adipocyte metabolism and browning of WAT. Mechanistically, DMPSC effect is mediated by PKA signaling. Further studies will be required for the characterization of molecular targets of DMPSC and their development into anti-obesity drugs.

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Conflict of interest The authors declare that they have no conflict of interest.

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