Rutin promotes white adipose tissue “browning” and brown adipose tissue activation partially through the calmodulin-dependent protein kinase kinase β/AMP-activated protein kinase pathway

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Abstract. Promoting white adipose tissue (WAT) “browning” and brown adipose tissue (BAT) activation could contribute to increasing energy expenditure. We explored the mechanisms by which the natural compound rutin induced adipose tissue differentiation and ameliorated obesity in vivo and in vitro. 3T3-L1 preadipocytes were cultured in adipogenic differentiation media with/out rutin. Male C57BL/6 mice (n = 6) were fed a high-fat diet (HFD) for 12 weeks with/out rutin. In HFD-fed mice, rutin treatment significantly inhibited weight gain, improved the metabolic profile of plasma samples, decreased the weights of epididymal WAT (eWAT), inguina WAT (iWAT), and liver, and adipocyte size. Furthermore, rutin also increased the expression of uncoupling protein 1 (Ucp-1) and other thermogenic markers in the WAT and BAT. In 3T3-L1 cells, rutin effectively reduced the formation of lipid droplets, stimulated the expression of thermogenic markers, and reduced the expression of adipogenic genes. Additionally, rutin markedly upregulated the AMP-activated protein kinase (AMPK) pathway, and these effects were diminished by treatment with the AMPK inhibitor compound C (CC). Pretreatment with the calmodulin-dependent protein kinase kinase β (CaMKKβ) inhibitor STO-609 blocked the induction of thermogenic markers in 3T3-L1 cells by rutin. Our results indicated that rutin increased energy consumption, induced WAT “browning” and BAT activation, and thus was a promising target for the development of new therapeutic approaches to improve adipose tissue energy metabolism.

Key words: Rutin, White adipose tissue “browning”, AMP-activated protein kinase, Uncoupling protein 1
and potentially harmless treatment for obesity.

Rutin is a flavonol glycoside extracted from plants such as rue leaves, tomatoes, and buckwheat that has a wide range of pharmacological activities, including antioxidant, anti-inflammatory and anti-diabetic effects [10, 11]. Rutin has been reported to increase mitochondrial biosynthesis, inhibit the expression of lipogenic genes, and improve obesity in obese rats [12]. Increasing evidence has indicated that the effects of rutin on ameliorating obesity are due to regulating adipocyte differentiation. Rutin not only inhibits fat formation of 3T3-L1 cells [13] but also increases the expression of energy expenditure factors in C_{2}H_{10}T_{1/2} cells and adipose tissue of obese mice [14].

AMPK is a key regulator of energy metabolism and mitochondrial biogenesis in eukaryotic cellular and has received intense research scrutiny over the recent years [15]. AMPK activation can improve insulin resistance by transferring glucose transporter-4 to the plasma membrane [16]; thus, AMPK is a molecular target for the treatment of T2DM and obesity [17]. Studies have indicated that some natural products, such as cordycepin and resveratrol, could induce WAT “browning” and improve obesity by activating the AMPK pathway [18, 19]. Meanwhile, reduced AMPK activity in adipose tissue is generally observed in obese and diabetic animals models as well as in obese humans [20-23]. Therefore, AMPK is among the crucial kinase targets of a variety of herbal medicines that regulate fat metabolism. Quercetin and its glycosides (one of the components of rutin) have also been demonstrated to increase AMPK phosphorylation in the WAT of normal and obese mice, and along with phosphorylation of acetyl-CoA carboxylase (kinase downstream of AMPK), improved hyperglycemia and adipose tissue accumulation in diet-induced obese mice [24].

However, the molecular mechanisms through which rutin alters the metabolism of adipose tissue are not fully understood. Due to the central position of AMPK in adipose tissue differentiation, its upstream kinases liver kinase B1 (LKB1) and CaMKKβ are also likely involved and have already been shown to have a key role in adipose differentiation. In this study, we investigated the effects and mechanisms of rutin on WAT “browning” and BAT activation in vivo and in vitro.

Materials and Methods

Chemicals and antibodies

Fetal bovine serum (FBS; PYG0001), Dulbecco’s modified Eagle’s medium (DMEM; PYG0073), dimethyl sulfoxide (DOSO; PYG0040), ELC western blot detection kits (AR1171), penicillin/streptomycin (PYG0016), and trypsin (PYG0015) were purchased from Boster Biotechnology (Pleasanton, CA, USA). Oil Red O staining kit (#1262) and Mito Tracker Red CMXRos (M9940) were purchased from Solarbio (Beijing, China). RIPA lysis buffer (HY-K1001), insulin (HY-P1156), 5-Aminomidazole-4-carb-oxamido ribonucleoside (AICAR; HY-13417), rosiglitazone (Ros; HY-17386), STO-609 (HY-19805), BAPTA-AM (HY-100545), dorsomorphin (HY-13418A), and dexamethasone (DEX; HY-14648) were purchased from MedChemExpress (Monmouth Junction, NJ, USA). Isobutylmethyloxanthine (IBMX; I7018) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibody sources are as follows: Ucp-1 (A5857, ABclonal, Woburn, MA, USA; 1:1,000, 32 kDa), peroxisome proliferator-activated receptor γ co-activator (Pgc-1α; BS91062, Bioworld, Nanjing, China; 1:1,000, 113 kDa), phospho-AMPKα (Thr172) (#2535, 1:6,000, 42 kDa).

Mice

All animal experimental procedures were approved by the local ethical committee of Shanxi Province and by the Shanxi Medical University Animal Ethical Committee. C57BL/6 male mice were purchased at 6 weeks of age from Shanxi Medical University Experiment Center (Taiyuan, China). Animals were housed in plastic cages in an air-conditioned room at 22 to 24°C with a 12 h light/dark cycle and free access to food and water. To induce obesity, the mice were fed a HFD (60% kcal fat, Medicience Ltd., Yangzhou, China). Mice were fed either a normal diet (ND) or HFD for 4 weeks prior to the start of treatment, aspartate transaminase (AST) were assayed by enzymatic analysis. Animals were sacrificed and plasma was immediately separated following blood collection via cardiac puncture. Total cholesterol (TC), total glyceride (TG), low density lipoprotein (LDL), alanine transaminase (ALT), and aspartate transaminase (AST) were assayed by enzymatic methods (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. The liver, eWAT, iWAT, and BAT were collected for molecular and biochemical measures and histological analysis.

Cell culture

3T3-L1 cells were obtained from the China Center for
Type Culture Collection (Wuhan, China) and maintained in basal growth medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) with 5% CO₂ at 37°C. To initiate differentiation, fully confluent cells (defined as day 0) were treated for 2 days with induction medium (growth medium supplemented with 10 μg/mL insulin, 0.5 mM IBMX, and 1 μM DEX). After 48 h, the cells were incubated for another 2 days with growth medium containing 10 μg/mL insulin. Thereafter, the medium was replaced with basal growth medium every other day for the next 4 days (to day 8). HeLa cells were provided by Procell Life Science & Technology Co., Ltd (Wuhan, China) and cultured in basal growth medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) with 5% CO₂ at 37°C. DMSO was used as the vehicle for the different treatments.

**Histology and immunohistochemistry**
Liver and adipose tissues from C57BL/6 mice were immediately fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm thick sections, which were stained with hematoxylin and eosin (H&E) for histological analysis. For immunohistochemical staining, slides were immersed in 0.01 mol/L sodium citrate and heated in a microwave oven for 15 min for antigen retrieval. The sections were blocked in blocking buffer containing 5% bovine serum albumin (BSA), and then incubated overnight with anti-Ucp-1 antibody (1:100) at 4°C. After washing three times with phosphate buffered saline (PBS), slides were incubated with secondary antibodies for 30 min at 37°C.

**Real-time quantitative PCR (qPCR)**
Total RNA was extracted using Trizol reagent (DP424, TianGen, Beijing, China), then 2 μg of RNA was converted cDNA using the BeyoRT II First Strand cDNA Synthesis Kit (D7168L, Beyotime) according to the manufacturer’s instructions. cDNA was synthesized for 60 min at 42°C, and then treated for 10 min at 80°C. qPCR was performed using a QuantiNova SYBR Green PCR Kit (FP205-02, TianGen). Relative genes expression was determined using the 2^ΔΔCT method with normalization to β-actin. The primer sequences of the target genes are detailed in Table 1.

**Western blotting**
Total protein from tissues or cells was lysed in RIPA buffer containing complete protease and phosphatase inhibitors. After separation by 8% or 10% SDS-PAGE (AR0138, Boster Biotechnology) and transfer to nitrocellulose membranes (AR0135-04, Boster Biotechnology), the membranes were blocked with 5% skimmed milk for 2 h at room temperature, and then incubated with the corresponding primary antibodies overnight at 4°C. Then membranes were incubated with HRP-conjugated goat anti-rabbit IgG (H + L) antibody (abs20002ss, Absin Bioscience, Shanghai, China) at room temperature for 2 h. Immunoreactive bands were detected using a western blot detection kit.

**Immunofluorescence**
Plates were inoculated at a density of 1 × 10⁵ cells per well and treated as indicated. Differentiated adipocytes were fixed with pre-cooled 4% paraformaldehyde at room temperature for 20 min, and then permeabilized in 0.2% TritonX-100 for 10 min at 4°C. Subsequently, cells were blocked with 5% BSA in PBS, and then incubated overnight with anti-Ucp-1 antibody in PBS at 4°C. Then, the cells were incubated with fluorescent secondary antibodies for 2 h at room temperature. Nuclei were stained with 4,6-diamidino-2-phenylindole for 3 min at room temperature. A fluorescence microscope was used to observe the fluorescence intensities.

**Cell cytotoxicity assay**
Ninety-six well plates were inoculated with approximately 1,500 3T3-L1 cells per well, which were then treated with medium containing different rutin concentrations. After 48 h, 10 μL of CCK-8 solution was added to each well and incubated for 30 min at 37°C. Absorbance (A) values were measured at 450 nm.

**Oil Red O staining**
Intracellular lipid accumulation was measured with Oil Red O staining according to the manufacturer’s instructions.

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| Gene     | Primer sequence 5' to 3'                                  |
|----------|----------------------------------------------------------|
| Ucp-1    | Forward, ATTCAGAGGCAAAATCAGCTTTG                        |
|          | Reverse, GTTCTTTCTCCTCCTGAAGAGAA                        |
| Pgc-1a   | Forward, GGATATACTTTAAGCAGGTCGA                         |
|          | Reverse, CGTCTGAGTTGATCTAGTC                            |
| AP2      | Forward, GAGAGCTGGAGGAGCTTC                            |
|          | Reverse, GAAGTGCCGTAATCCCACAC                           |
| C/EBPa   | Forward, TATAGACATGCGCCTACATC                           |
|          | Reverse, TTCTTGCCACCGACTTAGC                            |
| PPARY    | Forward, CCAAGAATACAAAGTGCAGTC                          |
|          | Reverse, TCACAGATGAACTCCACATAGT                          |
| β-actin  | Forward, GGAATATCCCCCCTCCATCG                           |
|          | Reverse, CCATGGTGTAACATGCCCATGT                         |

Rutin promotes WAT browning
Microscopic analysis of mitochondria

The cells were fixed and permeabilized, after which 100 nM of a prewarmed (37°C) staining solution containing Mito-Tracker probe was applied for 30 min. After the staining was complete, the solution was replaced with fresh prewarmed media.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Independent samples were statistically analyzed using the Student’s t test or one-way analysis of variance. Statistical analyses were conducted using SPSS v20.0 (IBM, Armonk, NY, USA). p < 0.05 was regarded as statistically significant.

Results

Rutin ameliorated obesity in HFD-fed mice

To investigate the anti-obesity effects of rutin, C57BL/6 mice were fed a HFD containing 0.1% rutin for 8 weeks at ambient temperature. As indicted in Fig. 1A, HFD-fed mice exhibited higher body mass, while weight gains were significantly inhibited in the HFD group with rutin after 12 weeks. Next, we examined the effects of rutin on tissue weights isolated from experimental group. Weights of eWAT, iWAT, and liver were obviously decreased after rutin treatment and no weight differences were detected in BAT (Fig. 1B). The increased levels of TC, TG, LDL, ALT, and AST in the HFD-fed group were significantly attenuated after rutin treatment (Fig. 1C). Histopathology confirmed decreased adipocyte sizes of the eWAT, iWAT, and BAT. Meanwhile, the pathological degeneration of hepatic fat vacuoles was also improved. H&E staining showed reduced triglyceride content after rutin treatment (Fig. 1D).

Rutin regulated WAT “browning” and BAT activation in vivo and in vitro

After confirming that rutin ameliorated obesity, we hypothesized that rutin might facilitate adipocyte differentiation and induce thermogenic procedures. To examine these possibilities, 3T3-L1 cells were treated with different concentrations of rutin. The results showed that rutin concentrations <100 μM had no significant cytotoxicity (Fig. 2A). Western blot analysis revealed that DMSO (vehicle) had no significant effects on the expression of Ucp-1 or P-AMPK (Fig. 2B). For subsequent experiments, 60 μM was used unless otherwise specified. To further determine the influence of rutin on the differentiation of 3T3-L1 cells, we analyzed the expression of thermogenic and adipogenic genes in the presence of rutin. As shown in Fig. 2C and D, rutin increased the expression of thermogenic markers (Ucp-1 and Pgc-1α) in a dose-dependent manner. Meanwhile, rutin decreased expression of the adipogenic genes adipocyte fatty acid-binding protein (AP2), CCAAT/enhancer-binding protein-α (C/EBPα), and peroxisome proliferator-activated receptor γ (PPARγ). Consistently, immunofluorescence results further indicated that rutin intervention increased Ucp-1 protein expression compared with the control differentiation group (Fig. 2E). In vivo results also confirmed increased protein and mRNA expression of Ucp-1 and Pgc-1α in the HFD group after rutin intervention (Fig. 2F–K). Consistent with the suppressive effects of rutin on lipid accumulation, AP2, C/EBPα, and PPARγ levels were reduced during adipogenesis in the eWAT and iWAT (Fig. 2L). Moreover, thermogenic and adipogenic genes were upregulated in BAT of rutin-treated animals (Fig. 2K). In this condition, immunohistochemistry showed that rutin enhanced Ucp-1 expression in the eWAT, iWAT, and BAT of mice (Fig. 2L). Lipid droplet accumulation was dramatically reduced in rutin-treated cells by Oil Red O staining (Fig. 2M). One of the typical characteristics of mature brown adipocytes is high mitochondrial content and activity. We measured mitochondrial content by MitoTracker probes. The results demonstrated higher levels of membrane potential and mitochondrial content upon treatment with rutin (Fig. 2N).

Rutin may induce adipocyte “browning” through AMPK activation

Pgc-1α is a downstream effector of AMPK-dependent metabolic effects [25, 26]. Here, we showed that rutin significantly increased Pgc-1α expression. To elucidate the role of AMPK in the thermogenic effects of rutin, we measured changes in P-AMPK in vivo and in vitro. The results showed that P-AMPK expression was increased in adipose tissue treated with rutin (Fig. 2F–H). Similarly, P-AMPK expression was increased in rutin-treated cells (Fig. 2D). To examine whether AMPK activation was associated with the thermogenic program in rutin-treated cells, 3T3-L1 cells were pretreated with CC, a known AMPK inhibitor, and then with rutin. The data showed decreased expression of thermogenic markers (Fig. 3A–B), which implied that AMPK played a significant role in this process. Following treatment with the AMPK agonist AICAR, there was increased expression of thermogenic proteins in 3T3-L1 cells (Fig. 3C).

Rutin activated the AMPK pathway through CaMKKβ

Because AMPK phosphorylation can be regulated by the upstream kinases LKB1 and CaMKKβ, we investigated whether they were responsible for rutin-induced AMPK activation. We first examined the effects of rutin
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on AMPK activation in HeLa cells, which do not express LKB1. The results showed that rutin increased P-AMPK levels in HeLa cells (Fig. 4A). To detect whether CaMKKβ was responsible for these effects of rutin, we added STO-609 (a CaMKK inhibitor) to the 3T3-L1 cells before rutin treatment. The P-AMPK expression induced

Fig. 1 Effects of rutin on HFD-induced obesity in C57BL/6 mice. Mice were given specific diets with/out rutin for 12 weeks, after which (A) body weights were determined, (B) tissue weights of the eWAT, iWAT, BAT, and liver were measured, and (C) ALT, AST, LDL, total cholesterol, and total glyceride levels were measured in the serum of each group. (D) The eWAT, iWAT, BAT, and liver were stained with H&E staining, and we quantitatively analyzed adipocyte size in the WAT (magnification: 200×, scale bar: 100 μm). Data are presented as mean ± SD; * p < 0.05, ** p < 0.005, *** p < 0.001 vs. ND group; * p < 0.05, ** p < 0.005, *** p < 0.001 vs. control HFD group.
Fig. 2 Effects of rutin on adipocyte differentiation in vivo and in vitro. 3T3-L1 cells after inducing differentiation. (A) CCK-8 assay, (B) levels of thermogenic proteins in DMSO-treated cells, and (C) mRNA levels of thermogenic and adipogenic genes were measured, and expression levels of each mRNA were normalized against β-actin. (D) The expression of thermogenic proteins was measured. (E) Cells were immunostained with Ucp-1 antibody and counterstained with DAPI to visualize nuclei. In each group of mice, western blot analysis was used to determine the expression of thermogenic proteins in the (F) eWAT, (G) iWAT, and (H) BAT. mRNA expression of thermogenic and adipogenic genes in the (I) eWAT, (J) iWAT, and (K) BAT were measured by qPCR. (L) Ucp-1 immunoreactivity signal (brown). (M) Staining of neutral lipids with Oil Red O (magnification: 200×, scale bar: 100 μm). Data are expressed as mean ± SD; *p < 0.05, **p < 0.005, ***p < 0.001 vs. control undifferentiation; *p < 0.05, **p < 0.005, ***p < 0.001 vs. control differentiation. DM, differentiation media.
by rutin was significantly diminished in the presence of STO-609 (Fig. 4B). Additionally, CaMKKβ is activated in response to increased Ca^{2+} levels [27, 28]. Therefore, we further verified whether inhibiting Ca^{2+} attenuated the effects of rutin. BAPTA-AM is a cytosolic calcium chelator that used to pretreat 3T3-L1 cells. Western blot analysis showed that BAPTA-AM diminished rutin-induced AMPK activation (Fig. 4C).

**Rutin affected PPARγ-induced “browning” of 3T3-L1 cells**

To investigate the influence of rutin on PPARγ-induced adipocyte “browning”, 3T3-L1 cells were co-incubated with the PPARγ agonist Ros and rutin. As shown, rutin increased the accumulation of lipid droplets induced by Ros (Fig. 5A), and Ucp-1 and Pgc-1α levels were also significantly increased (Fig. 5B).

**Discussion**

BAT activation can help improve obesity [29]. Therefore, it is important to discover compounds with WAT “browning” effects as an alternative approach to treating these common diseases. In this study, we provided data that rutin increased the expression of energy expenditure factors by promoting WAT “browning” and...
BAT activation through the CaMKKβ/AMPK pathway.

In this study, HFD was used to induce obesity in mice. Mice fed HFD for 12 weeks showed significantly increased body, adipose tissue, and liver weights. Additionally, mice in the HFD group were found to have larger sized adipocytes and hepatic fat vacuoles. However,
animals weight data showed that rutin significantly improved obesity. Furthermore, H&E staining showed that rutin converted large adipocytes into small cells that were filled with multi-localized lipid droplets. Next, we analyzed the effect of rutin on plasma metabolism through the detection of animals plasma metabolism indicators. These data indicated that rutin significantly improved plasma metabolism disorders caused by obesity, which was consistent with the results of Yuan et al. [14]. Oil Red O staining revealed that rutin inhibited lipid droplet accumulation in vitro. Moreover, during adipogenesis, PPARγ and C/EBPα participate in a transcriptional cascade that stimulates expression of downstream adipogenesis-related genes, such as AP2 and fatty acid synthase [30-32]. Our data clearly demonstrated that rutin caused a significant downregulation of adipogenic genes in the eWAT and iWAT. The same results were obtained in 3T3-L1 cells. We also observed that rutin treatment resulted in increased mRNA expression of adipogenesis-related genes in BAT. These results suggest that the effects of rutin on improving obesity may be related to inhibiting adipogenesis.

BAT that is enriched in Ucp-1 expression has been identified as an attractive candidate for increasing energy expenditure in obesity [33]. This study indicated that activated Ucp-1 can increase energy consumption and thermogenesis in BAT as well as being a specific marker of mature brown adipocytes. Elevated Ucp-1 expression increases basal body temperature, cold tolerance, and resistance to HFD-induced obesity in mice [34].
immunohistochemistry results showed that rutin enhanced Ucp-1 expression in the eWAT, iWAT, and BAT of animals. As an energy regulator, Pgc-1α enhances the function and activity of mitochondria to accelerate fatty acid oxidation and increase energy expenditure in the body [35, 36]. Our data showed that rutin stimulated Ucp-1 and Pgc-1α expression in vivo and in vitro. There are natural plants that have been found to induce “browning” such as cordycepin, which can also promote Ucp-1 and Pgc-1α expression in obese mice and 3T3-L1 cells [19]. Thus, rutin also promoted energy expenditure. Moreover, mitochondrial activity is an important indicator of BAT activation. This was also demonstrated by mitochondrial staining, where rutin intervention resulted in enhanced mitochondrial activity. These results established that rutin promoted WAT “browning” and BAT activation.

PPARγ regulates adipocyte differentiation and function. Chronic treatment with PPARγ agonists such as thiazolidinone and Ros can increase the thermogenic capacity of adipocytes [37]. We found that Ros intervention increased Ucp-1 and Pgc-1α protein expression in 3T3-L1 cells, and that rutin and Ros had synergistic effects in upregulating thermogenic proteins.

The role of AMPK in promoting WAT “browning” and BAT activity has been extensively studied. AMPK is a critical regulator of Ucp-1 expression during brown adipocyte differentiation [38]. Research has shown that AMPKα1 knockout mice reduce numbers of BAT progenitor cells and have a block in adipogenesis [39]. Treating obese mice with the AMPK activators metformin or AICAR also increased the number of brown adipose progenitor cells and BAT weights in newborn mice from obese mice [40]. Resveratrol increased the
expression of thermogenic proteins such as Ucp-1 and stimulated fatty acid oxidation in obese mice, inducing WAT “browning” [18], these effects were associated with AMPK activation. Cordycepin promoted WAT “browning” through the AMPK-dependent pathway, increasing energy expenditure and improving metabolism in obese mice [19]. These results suggest that AMPK plays an essential role in regulating WAT “browning” and BAT activation. Our results revealed that rutin increased AMPK activation in vivo and in vitro. Conversely, 3T3-L1 cells treated with the AMPK inhibitor CC showed that AMPK inhibition resulted in downregulation of thermogenic proteins. Meanwhile, the AMPK agonist AICAR increased the expression of Ucp-1 and Pgc-1α in 3T3-L1 cells. These data suggested that the regulation of WAT “browning” and BAT activation by rutin was closely related to the AMPK pathway. Further studies are still needed, such as animal experiments using AMPK knockout mice, to demonstrate that AMPK is responsible for WAT “browning” in vivo.

LKB1 and CaMKKβ phosphorylate Thr-172 of AMPKα, which is essential for AMPK activation [27, 28, 41]. CaMKKβ is a serine/threonine kinase that is present in preadipocytes, where it is part of a calcium-triggered signaling cascade that is also involved in several other cellular processes. It has been shown that there is a potential role for CaMKKβ in the development of obesity. CaMKKβ-null mice fed standard chow showed increases in both the size and number of adipocytes, and in vitro ablation or pharmacological inhibition of CaMKKβ increased adipogenesis [42]. LKB1 is thought to be constitutively-active and plays a critical function in regulating the Thr-172 phosphorylation of AMPKα [43]. In this study, we determined one of the signaling pathways of rutin-induced WAT “browning”, by respectively acting on CaMKKβ and LKB1, the main upstream kinases of AMPK. In HeLa cells (not expressing LKB1), rutin still induced the upregulation of thermogenic proteins, which indicated that LKB1 does not play a critical role in the anti-obesity effect of rutin. In contrast, when the CaMKKβ inhibitor STO-609 was used to treat 3T3-L1 cells, the effect of rutin on promoting the expression of thermogenic proteins was diminished. Further treatment of 3T3-L1 cells with the calcium chelator BAPTA-AM yielded results consistent with previous studies. These data indicated that CaMKKβ was involved in rutin-induced WAT “browning”.

In summary, we have presented evidence showing that rutin ameliorated metabolic disorders and obesity in vivo and in vitro. We revealed that the molecular target and mechanisms through which rutin regulated the expression of thermogenic markers in 3T3-L1 cells was at least in part through the CaMKKβ/AMPK pathway. These results establish an important role for rutin as a natural activator of WAT “browning” and BAT activation, which will provide a new research direction for...
pharmacological agents in obesity and related disorders.

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Disclosure

The authors declare that they have no competing interests.
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