Feprazone Displays Antiadipogenesis and Antiobesity Capacities in in Vitro 3 T3-L1 Cells and in Vivo Mice

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ABSTRACT: Background and purpose: Excessive lipid accumulation in adipose tissues and deregulation of adipogenesis-induced obesity affect millions of people worldwide. Feprazone, a nonsteroidal anti-inflammatory drug, has a wide clinical use. However, it is unknown whether Feprazone possesses an antiadipogenic ability. The aim of this study is to investigate whether Feprazone possesses an antiadipogenic ability in 3 T3-L1 cells and an antiobesity capacity in mouse models. Methods: An MTT assay was used to determine the optimized incubation concentrations of Feprazone in 3 T3-L1 cells. The lipid accumulation was evaluated using Oil Red O staining. The concentrations of triglyceride and glycerol release were detected to check the lipolysis during 3 T3-L1 adipogenesis. A quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the expressions of sterol regulatory element-binding protein-1C (SREBP-1C) and fatty acid binding protein 4 (FABP4) in treated cells. The expressions of peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer-binding protein α (C/EBP-α), adipose triglyceride lipase (ATGL), and aquaporin-7 (AQP-7) were detected using qRT-PCR and Western blot analysis. After the high-fat diet (HFD) mice were treated with Feprazone, the pathological state of adipocyte tissues was evaluated using HE staining. The adipocyte size, visceral adipocyte tissue weight, and bodyweights were recorded. Results: According to the proliferation result, 30 and 60 μM Feprazone were used as the optimized concentrations of Feprazone. In the in vitro study, lipid accumulation, elevated production of triglycerides, the release of glycerol, upregulated SREBP-1C, FABP4, PPAR-γ, and C/EBP-α and downregulated ATGL and AQP-7 in the 3 T3-L1 adipocytes induced by the adipocyte differentiation cocktail medium were significantly reversed by treatment with Feprazone. In the in vivo experiment, we found that the increased adipocyte size, visceral adipocyte tissue weight, and bodyweights induced by HFD feeding in mice were significantly suppressed by the administration of Feprazone. Conclusion: Feprazone might display anti-adipogenic and antiobesity capacities in in vitro 3 T3-L1 cells and in vivo mice.

INTRODUCTION

Obesity is gradually becoming a global subhealth issue, with steadily increasing prevalence in both the USA and worldwide. The development of obesity is commonly accompanied by metabolic or chronic diseases, including diabetes, cardiovascular diseases, hypertension, and several kinds of malignant tumors, which bring a huge burden to society. As a complicated subhealth disease impacted by lifestyle, genetic factors, and behavior, obesity is a result of energy imbalance due to a sedentary lifestyle and lack of physical activity. Obesity is mainly pathologically derived from excessive adipose accumulation and adipose tissue expansion. The adipose tissue has been regarded as an important energy storage organ and an endocrine organ mediating the metabolism of bioactive substances, which maintains the energy balance and homeostasis of adipocyte-derived pro- and anti-inflammatory adipokines within the body. When the balance of the production and secretion of pro- and anti-inflammatory adipokines are broken, several pathological processes are induced, such as systemic inflammation, insulin resistance, and obesity-related metabolic disorders. Therefore, obesity is also regarded as a chronic low-grade inflammation disease. Lipid metabolism is an important biological process involved in the development of obesity, and lipid metabolism disorder contributes to the accumulation of fat. The sterol regulatory element-binding protein (SREBP) is regarded as the main regulator of liver lipid metabolism, and it mediates the formation of fat by activating the genes involved in the synthesis of fatty acids and triglycerides. The concentration of saturated fatty acids can be elevated by the excessive uptake of fatty acids in the diet, which activates SREBP-1C and induces the synthesis of triglycerides. This results in the expression of microsomal triglyceride transfer protein and the accumulation of fat in the liver. Except for lipid metabolism, the accumulation of adipose tissues can be induced by the transformation of preadipocytes to mature...
adipocytes and is regulated by multiple types of transcriptional factors, such as CCAAT/enhancer-binding protein (C/EBP) \( \alpha/\beta \) and peroxisome proliferator-activated receptor-\( \gamma \) (PPAR-\( \gamma \)).\(^{13,14}\) C/EBP-\( \beta \) is found to be expressed at the early stage of adipocyte differentiation and first triggers the mitotic clonal expansion\(^{5,16}\) to induce the preadipocytes entering the cell cycle. After two cycles of mitosis, the preadipocytes quit the cell cycle and enter the final stage of cell differentiation. Subsequently, the transcriptional factors, such as C/EBP-\( \alpha \) and PPAR-\( \gamma \), that mediate the expressions of adipocyte phenotypic genes (such as 422/ap2, SCD1, and Glut4) are upregulated by C/EBP-\( \beta \).\(^{17,18}\) Therefore, accelerating the process of lipid metabolism and suppressing the differentiation of preadipocytes to mature adipocytes might be two effective ways to ameliorate obesity.

Feprazone (4-prenyl-1,2-diphenyl-3,5-pyrazolidinedione) is a nonsteroidal anti-inflammatory drug widely used in clinics; it has less toxicity and is particularly less ulcerogenic than another pyrazolone derivative, 3,5-dioxo-1,2-diphenyl-4-n-butyl pyrazolidine (phenylbutazone).\(^{19}\) The anti-inflammatory and antipyretic efficacy of Feprazone is similar to that of phenylbutazone.\(^{20}\) In the present study, the in vitro and in vivo antagonogenesis and antiobesity properties of Feprazone will be investigated to explore its new role in the amelioration of adipogenesis.

**MATERIALS AND METHODS**

**Adipocyte Culture and Differentiation.** 3 T3-L1 preadipocytes were purchased from American Type Culture Collection (ATCC, MD, USA) and cultured in the Dulbecco’s modified Eagle’s medium (DMEM) at 37 °C. When grown to confluence on 100 mm culture plates, cells were split using 1 mL of 0.05% trypsin/ethylenediaminetetraacetic acid and seeded on a 6-well plate and cultured with DMEM and 10% bovine calf serum for 3 days to be 100% confluent. For differentiation induction, the culture medium was mixed with an MDI differentiation medium plus 10% fetal bovine serum (FBS), 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 \( \mu \)M dexamethasone, and 5 \( \mu \)g/mL of insulin for 2 days in the presence or absence of Feprazone (30 and 60 \( \mu \)M). A normal culture medium was used as a negative control. Subsequently, the cells were incubated in the DMEM medium containing 10% FBS and 5 \( \mu \)g/mL of insulin in the presence or absence of Feprazone for another 2 days, which was replaced with the regular conditional DMEM medium in the presence or absence of Feprazone every 3 days.

**MTT Assay.** 3 T3-L1 preadipocytes were trypsinized and counted using an automated cell counter (Bio-Rad, USA). The cells were seeded in 96-well plates at a density of 2 \( \times \) 10^4 cells/well. Cells were then treated with Feprazone at the concentrations of 0, 3, 6, 30, 60, 300, and 600 \( \mu \)M for 24 h followed by incubation with 10 \( \mu \)L of 0.5 mg/mL MTT reagent. The composed crystals were then dissolved in DMSO followed by measuring the absorption at 595 nm using a microplate reader (Versamax, California, USA). The cell viability was calculated as the ratio of the OD value of the treated group to the OD value of the control group. Cells treated with 0 \( \mu \)M Feprazone were used as the control group.

**Oil Red O Staining.** After washing with PBS buffer, the adipocytes were fixed with 10% (v/v) formalin for 1 h. Subsequently, 60% isopropanol was used to rinse the cells followed by staining with the Oil Red O working solution for 10 min. The stained oil droplets were extracted using 100% isopropanol and their absorbance was measured at 510 nm using a microplate reader (Versamax, California, USA).\(^{21,22}\) Results were calibrated with isopropanol, and the relative level of lipid accumulation was normalized with untreated control cells.

**Glycerol Determination.** Briefly, 25 \( \mu \)L of a glycerol standard solution at concentrations of 6 to 150 \( \mu \)g/mL was added to the 96-well plates. The corresponding wells were added with 25 \( \mu \)L of the collected cell supernatants followed by the addition of 100 \( \mu \)L of a diluted free glycerol assay reagent (Bioassay, California, USA) and incubation for 15 min. The absorbance was read at 540 nm using a microplate reader (Versamax, California, USA). The standard curve was plotted as a function of glycerol concentration to determine the equation of the line. The glycerol concentration was determined using the formula: glycerol concentration (\( \mu \)g/mL) = [A540 − (y intercept)]/slope × sample dilution.

**Determination of Total Triglycerides.** The concentration of triglycerides was determined using the method described previously. Briefly, the total lipids of the cells were isolated using a commercial kit (Sigma-Aldrich, Missouri, USA), and the concentration of triglycerides was measured using a commercial enzymatic assay kit (Wako, Osaka, Japan). The extraction buffer was used to extract the lipid droplets and triglycerides were then converted to glycerol and fatty acids using the lipase enzyme. The OD value at 570 nm was used to measure the released glycerol. The relative concentration of triglycerides was normalized with the concentration of total proteins, according to the Bradford method, using a Bio-Rad Protein assay kit.\(^{23}\)

**Real-Time Polymerase Chain Reaction Analysis.** Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 \( \mu \)M) for 0, 2, 5, and 7 days, and the total RNAs were isolated from the cells using the RNasy mini kit (Qiagen, CA, USA). The quantity and concentrations of RNA were determined using the NanoDrop 2000. The isolated RNA was stored at −80 °C. RNA was transformed to cDNA using a Revert Aid First Strand cDNA synthesis kit (Fermentas, Vancouver, Canada). A quantitative real-time polymerase chain reaction analysis was performed with the SYBR Green PCR kit (Invitrogen, California, USA) using gene-specific primers. The relative expression levels of related genes were evaluated using the 2^(-ΔΔCt) method, and GAPDH was used as a negative control in the following primers. The following primers were used in this study: SREBP-1C: forward, 5′-CTGGTGTGTGCTGTCTCCT-3′, reverse, 5′-TTTCGATGCTCCAGAAATGT-3′; FABP4: forward, 5′-GATGAAATCACCGCAGACG-3′, reverse 5′-GCCCTTTTCAAACTCTTGG-3′; PPAR-γ; forward, 5′-AGCGGAGAGAGAAGCTTGG-3′, reverse 5′-TGCCACACTTCTTGCTC-3′; C/EBP-α: forward, 5′-TTACCAACAGGCCAGTTCC-3′, reverse 5′-GGCTTGCCGACATACTACA-3′; ATGL: forward, 5′-AACACCGCAGCTCCAGAA-3′, reverse, 5′-GGTCTCGAGGCAATGGCTTC-3′; ACC; forward, 5′-CAGT-3′, reverse, 5′-AGCCAGACAGTCAATGCTTC-3′; AQP-7: forward, 5′-AGCCAGAGCAGTAATCTCCT-3′, reverse, 5′-ACCGAGGAGGCTCC-3′; SREBP-1C: forward, 5′-GATGAAATCACCGCAGACG-3′, reverse 5′-GCCCTTTTCAAACTCTTGG-3′; PPAR-γ; forward, 5′-AGCGGAGAGAGAAGCTTGG-3′, reverse 5′-TGCCACACTTCTTGCTC-3′; C/EBP-α: forward, 5′-TTACCAACAGGCCAGTTCC-3′, reverse 5′-GGCTTGCCGACATACTACA-3′; ATGL: forward, 5′-AACACCGCAGCTCCAGAA-3′, reverse, 5′-GGTCTCGAGGCAATGGCTTC-3′.

**Western Blot Assay.** The proteins were isolated from the adipocytes using the Nuclear and Cytoplasmic Protein Extraction kit (Thermo Fisher Scientific, MA, USA). A 10% SDS-polyacrylamide gel was used to separate the proteins followed by transferring them to a polyvinylidene difluoride
membrane (Millipore, MA, USA). The membrane was incubated with 5% nonfat dry milk in Tris-buffered saline/0.1% Tween-20, pH 7.4 for 1.5 h at room temperature and incubated overnight with primary antibodies to PPAR-γ (Abcam, Cambridge, USA, 1:1000), C/EBP-α (Abcam, Cambridge, USA, 1:1000), ATGL (Abcam, Cambridge, USA, 1:1000), AQP-7 (Abcam, Cambridge, USA, 1:1000), or GADPH (Abcam, Cambridge, USA, 1:1000). This was followed by incubation with a horseradish peroxidase-conjugated secondary antibody against rabbit IgG (Abcam, Cambridge, USA, 1:1000). The bands were visualized using enhanced chemiluminescence (ECL) reagents (Thermo Fisher Scientific, MA, USA) and exposed to a Tanon 5200 Multi for the detection of the protein expression. The software ImageJ was used to quantify the integrated intensity of each band. The statistical significance of the experiments was determined using analysis of variance with the Bonferroni’s post hoc test, using SPSS 22.0 (IBM, Armonk, NY). P < 0.05 was considered as statistically significant.

RESULTS

Effects of Feprazone on Cell Viability of 3 T3-L1 Cells.

The molecular structure of Feprazone is shown in Figure 1A. To evaluate the effect of Feprazone on the proliferation ability of preadipocytes and screen the optimized incubation concentrations of Feprazone, cells were stimulated with 0, 3, 6, 30, 60, 300, and 600 µM Feprazone, and the effects of Feprazone on cell viability were measured using the MTT assay. As shown in Figure 1B, we found that the cell viability decreased greatly as the concentration of Feprazone increased from 300 to 600 µM. Therefore, 30 to 60 µM Feprazone was used in the subsequent experiments.

Histological Analyses of Adipocyte Tissues.

The adipocyte tissues were isolated from each animal followed by washing with PBS for several hours. After being dehydrated with 70, 80, and 90% ethanol solution successively, the tissues were incubated with equal quantities of ethanol and xylene. Following 15 min of incubation, xylene of equal quantity was used in the tissues looked transparent. Then, the samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The images were captured using an inverted microscope (Olympus, Tokyo, Japan).

Statistical Analysis. All the data in the present study were expressed as mean ± SD, and the comparisons between groups were performed using analysis of variance with the Bonferroni’s post hoc test, using SPSS 22.0 (IBM, Armonk, NY). P < 0.05 was considered as statistically significant.
Feprazone Reduced the Triglyceride Content and Increased Lipolysis during 3 T3-L1 Adipogenesis. As shown in Figure 3A, the concentrations of triglyceride in the 3 T3-L1 adipocyte differentiation cocktail medium in the presence or absence of Feprazone (30 and 60 μM). (A) Levels of triglyceride. (B) Glycerol release was used to reflect lipolysis (***, P < 0.001 vs vehicle group; #, ##, P < 0.05, 0.01 vs DMI group, n = 6).

Figure 3. Feprazone reduced the triglyceride content and increased lipolysis during 3 T3-L1 adipogenesis. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (30 and 60 μM). (A) Levels of triglyceride. (B) Glycerol release was used to reflect lipolysis (***, P < 0.001 vs vehicle group; #, ##, P < 0.05, 0.01 vs DMI group, n = 6).

T3-L1 cells incubated with the blank medium, adipocyte differentiation cocktail medium, and adipocyte differentiation cocktail medium in the presence of 30 and 60 μM Feprazone were 9.2, 27.6, 21.3, and 15.8 nmol/mg protein, respectively. Approximately 6.8, 12.3, 16.7, and 19.3 nmol/mg protein/h glycerol were detected in the control, vehicle, and 30 and 60 μM Feprazone, respectively.

The Effects of Feprazone on the Expressions of Adipogenic and Lipogenic Genes. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM) for 0, 2, 5, and 7 days. As shown in Figure 4AB, compared to the vehicle group, the expressions of SREBP-1C and FABP4 were significantly suppressed by treatment with Feprazone on days 5 and 7 postincubation, respectively.

Figure 4. Effects of Feprazone on the expression of adipogenic and lipogenic genes. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM) for 0, 2, 5, and 7 days. (A) mRNA of SREBP-1C and (B) mRNA of FABP4 (###, ####, P < 0.001, 0.0001 vs vehicle group, n = 6).

The Effects of Feprazone on Adipogenic and Lipogenic Genes. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM) for 0, 2, 5, and 7 days. As shown in Figure 4AB, the expressions of SREBP-1C and FABP4 were significantly suppressed by treatment with Feprazone on days 5 and 7 postincubation, respectively.

The Expressions of Proteins Regulating Adipogenesis in Differentiated 3 T3-L1 Adipocytes Were Inhibited by Feprazone. The 3 T3-L1 adipocytes were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM). As shown in Figure 5AB, PPAR-γ and C/EBP-α were significantly upregulated by stimulation with the adipocyte differentiation cocktail medium but greatly downregulated by the introduction of Feprazone.

The Effects of Feprazone on the Expressions of Proteins Regulating Lipolysis in Differentiated 3 T3-L1 Adipocytes. To evaluate the effects of Feprazone on lipolysis processing in differentiated 3 T3-L1 adipocytes, cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM). As shown in Figure 6AB, the expressions of ATGL and AQP-7 were significantly suppressed by incubation with the adipocyte differentiation cocktail medium at both the mRNA and protein levels but elevated by treatment with Feprazone.

The Effects of Feprazone on HFD-Induced Increase in Visceral Adipocyte Tissue and Body Weight in a Mouse Model. Lastly, to verify the effects of Feprazone on obesity, the HFD model was employed on mice treated with Feprazone. As shown in Figure 7AB, according to the images of HE staining on adipocyte tissues, compared to the control, the adipocyte size was significantly elevated in the HFD mice but was greatly inhibited by the administration of Feprazone.

In addition, the visceral adipocyte tissue weights of mice in the control, HFD, and HFD + Feprazone groups were 0.38, 3.51, and 2.37 g, respectively (Figure 7C). As shown in Figure 7D, the average bodyweights of mice in the control, HFD, and HFD + Feprazone groups were 29.6, 41.3, and 34.1 g, respectively.

## DISCUSSION

The differentiation of adipocytes is defined as the process of transformation of preadipocytes to mature adipocytes with an enlarged cell volume. Typically, adipocytes function by uptaking the free fatty acids from the blood to consecutively synthesize triglycerides and produce lipid droplets. The process of adipocytic differentiation contributes to the pathogenesis of obesity, which, on the contrary, is controlled by the lipid metabolism. Under the condition of energy surplus, the triglycerides located in the plasma are degraded by the lipid protein lipase into glycerol and free fatty acids. We used a commercial adipocyte differentiation cocktail medium to induce the differentiation of 3 T3-L1 cells; this was verified by the elevation of lipid accumulation in the Oil Red O staining and the increased production of triglycerides, as well as the decreased release of glycerol. Administration of Feprazone alleviated lipid accumulation, decreased triglyceride secretion, and increased glycerol release, indicating a promising inhibitory and promotional effect of Feprazone on adipocyte differentiation and lipid metabolism, respectively.

Expression of specific genes induced by multiple types of transcriptional factors is involved in the process of cellular differentiation transduction. Therefore, the expression levels and activation of transcriptional factors play an important role in the process of adipocytic differentiation. PPAR-γ belongs to the ligand-activated transcriptional nuclear receptor superfamily and plays an important role in the regulation of adipocyte differentiation and adipogenesis. The PPAR-γ knockdown preadipocyte is reported to lose its lipogenic differentiation ability, thereby inhibiting the adipogenesis process. In vitro studies indicate that lipid accumulation and adipocyte differentiation of 3 T3-L1 cells are induced by lipin-1 phosphorylated PPAR-γ. The elevated expression of PPAR-γ induced by the adipocyte differentiation cocktail
medium was significantly suppressed by treatment with Feprazone, indicating that the regulatory effect of Feprazone on adipocyte differentiation might be related to the down-regulation of PPAR-γ. In our future work, the detailed interaction between Feprazone and PPAR-γ will be further investigated to better understand the function of Feprazone in the process of adipocyte differentiation. C/EBPs are another family of transcriptional factors that regulate the differentiation of preadipocytes; they activate the CCTTA repetitive sequence within the enhancer of specific DNA and are involved in the regulation of preadipocyte differentiation and proliferation. C/EBP-β and C/EBP-δ are found to be first expressed in the early stage of proliferation and differentiation of preadipocytes, and in turn, induce the expression of PPAR-γ to upregulate C/EBP-α. The process of adipocyte differentiation is abolished if both C/EBP-β and C/EBP-δ are knocked down, and C/EBP-α is regarded as a biomarker of the advanced stage of adipocyte differentiation. We found that the elevated expression of C/EBP-α induced by the adipocyte differentiation cocktail medium was significantly inhibited by Feprazone, indicating a promising inhibitory effect of Feprazone on the advanced stage of adipocyte differentiation. In addition to the C/EBP/PPAR-γ axis, SREBP1 is also an important transcriptional factor that induces the proliferation and differentiation of preadipocytes by regulating the glycometabolism. We observed that the mRNA expression of SREBP-1C was suppressed by Feprazone. Therefore, based on our data, it is concluded that Feprazone acts as a mediator of adipocytic differentiation and lipid metabolism by regulating the expressions of several transcriptional factors. Furthermore, adipose triglyceride lipase (ATGL), a key enzyme for the release of fatty acids from triacylglycerol, and aquaporin-7 (AQP-7), an aquaglyceroporin that transports glycerin, were also found to be upregulated by treatment with Feprazone. Combined with the in vivo results that Feprazone suppressed the adipocyte size, visceral adipocyte tissue weight, and

Figure 5. Effects of Feprazone on the expression of adipogenesis-regulating proteins in differentiated 3 T3-L1 adipocytes. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM). (A) mRNA of PPAR-γ and C/EBP-α and (B) protein of PPAR-γ and C/EBP-α as measured using the Western blot analysis (***, P < 0.001 vs vehicle group; ##, P < 0.01 vs DMI group, n = 6).

Figure 6. Effects of Feprazone on the expression of lipolysis-regulating proteins in differentiated 3 T3-L1 adipocytes. Cells were stimulated with the adipocyte differentiation cocktail medium in the presence or absence of Feprazone (60 μM). (A) mRNA expression of ATGL and AQP-7 and (B) the protein expression of ATGL and AQP-7 (*, P < 0.05 vs vehicle group; ##, P < 0.01 vs DMI group, n = 6).

Figure 7. Effects of Feprazone on HFD-induced increase in visceral adipocyte tissue and bodyweight in a mouse model. (A) Histological sections of visceral adipocyte tissue; scale bar, 50 μm; (B) quantification of the adipocyte size; (C) visceral adipocyte tissue weight; and (D) body weight (***, P < 0.001 vs vehicle group; ##, P < 0.01 vs HFD group, n = 8).
bodyweight in HFD mice, the present study provided preliminary data claiming the inhibitory property of Feprazone against adipocytic differentiation and the possible mechanism. In our future work, an accurate target of Feprazone on the preadipocytes will be investigated to further confirm the molecular mechanism by introducing different transcriptional factor plasmids or inhibitors of metabolic enzymes. Taken together, our data indicate that Feprazone might display antiadipogenesis and antiobesity effects in vitro 3 T3-L1 cells and in vivo mice.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Tsai, A. G.; Williamson, D. F.; Glick, H. A. Direct medical cost of overweight and obesity in the USA: a quantitative systematic review. *Obes Rev.* 2011, 12, 50−61.
(2) Elagizi, A.; Kachur, S.; Lavie, C. J.; Carbone, S.; Pandey, A.; Ortega, F. B.; Milani, R. V. An Overview and Update on Obesity and Overweight and Obesity in the USA: a Quantitative Systematic Review. *Obes Rev.* 2019, 20, 174−187.
(3) Bouchard, C. Defining the genetic architecture of the predisposition to obesity: a challenging but not insurmountable task. *Am. J. Clin. Nutr.* 2010, 91, 5−6.
(4) Heber, D. An integrative view of obesity. *Am. J. Clin. Nutr.* 2010, 91, 2805−2835.
(5) Sirivardhana, N.; Kalupahan, N. S.; Cekanova, M.; LeMieux, M.; Greer, B.; Moustaid-Moussa, N. Modulation of adipose tissue inflammation by bioactive food compounds. *J. Nutr. Biochem.* 2013, 24, 613−623.
(6) Kalupahan, N. S.; Moustaid-Moussa, N.; Claycombe, K. J. Immunity as a link between obesity and insulin resistance. *Mol. Aspects Med.* 2012, 33, 26−34.
(7) McCullough, R. L.; McMullen, M. R.; Poulsen, K. L.; Kim, A.; Medof, M. E.; Nagy, L. E. Anaphylatoxin Receptors C3ar and C5ar1 Are Important Factors That Influence the Impact of Ethanol on the Adipose Secretome. *Front Immunol.* 2018, 9, 2133.
(8) Parry, S. A.; Hodson, L. Influence of dietary macronutrients on liver fat accumulation and metabolism. *J Invest Med.* 2017, 65, 1102−1115.
(9) Alves-Bezerra, M.; Cohen, D. E. Triglyceride Metabolism in the Liver. *Compr Physiol.* 2017, 8, 1−8.
(10) Raghov, R.; Yellaturu, C.; Deng, X.; Park, E. A.; Elam, M. B. SREBPs: the crossroads of physiological and pathological lipid homeostasis. *Trends Endocrinol. Metab.* 2008, 19, 65−73.
(11) Kleiner, D. E.; Brunt, E. M.; Van Natta, M.; Behling, C.; Contos, M. J.; Cummings, O. W.; Ferrrell, L. D.; Liu, Y. C.; Torbenson, M. S.; Unalp-Aridan, A.; Yeh, M.; McCullough, A. J.; Sanay, A. J. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005, 41, 1313−1321.
(12) Teran-Garcia, M.; Adamson, A. W.; Yu, G.; Rufo, C.; Suchanka, G.; Dreesen, T. D.; Tekle, M.; Clarke, S. D.; Getty, T. W. Polyunsaturated fatty acid suppression of fatty acid synthase (FASN): evidence for dietary modulation of NF-Y binding to the Fasn promoter by SREBF-1c. *Biochem. J.* 2007, 402, 591−600.
(13) Lin, F. T.; Lane, M. D. CCAAT/enhancer binding protein alpha is sufficient to initiate the 3T3-L1 adipocyte differentiation program. *Proc. Natl. Acad. Sci. U. S. A.* 1994, 91, 8757−8761.
(14) Mandrup, S.; Lane, M. D. Regulating adipogenesis. *J Biol Chem.* 1997, 272, 5367−5370.
(15) Tang, Q. Q.; Lane, M. D. Activation and centromeric localization of CCAAT/enhancer-binding proteins during the mitotic clonal expansion of adipocyte differentiation. *Genes Dev.* 1999, 13, 2231−2241.
(16) Zhang, J. W.; Tang, Q. Q.; Vinson, C.; Lane, M. D. Dominant-negative C/EBP disrupts mitotic clonal expansion and differentiation of 3T3-L1 preadipocytes. *Proc. Natl. Acad. Sci. U. S. A.* 2004, 101, 43−47.
(17) Pan, M. H.; Koh, Y. C.; Lee, T. L.; Wang, B. N.; Chen, W. K.; Nagahushanam, K.; Ho, C. T. Resveratrol and Oxyresveratrol Activate Thermogenesis via Different Transcriptional Coactivators in High-Fat Diet-Induced Obese Mice. *J. Agric. Food Chem.* 2019, 67, 13605−13616.
(18) Hwang, C. S.; Mandrup, S.; MacDougall, O. A.; Geiman, D. E.; Lane, M. D. Transcriptional activation of the mouse obese (ob) gene by CCAAT/enhancer binding protein alpha. *Proc. Natl. Acad. Sci. U. S. A.* 1996, 93, 877−887.
(19) Casadio, S.; Pala, G.; Marazzi-Uberti, E.; Lumachi, B.; Crescenzi, E.; Donetti, A.; Mantegani, A.; Bianchi, C. Terpene compounds as drugs. X. 4-prenyl-1,2-diphenyl-3,5-pyrazolinidione (DA 2370): a new anti-inflammatory drug, with low ulcerogenic effects, derived from a series of terpynyl pyrazolinediones. *Arzneimittelforschung* 1972, 22, 171−174.
(20) Bianchi, P.; Bonardi, G. Pharmacological investigations of 4-prenyl-1,2-diphenyl-3,5-pyrazolinidione (DA 2370): 3. General pharmacological properties. *Arzneimittelforschung* 1972, 22, 196.
(21) Kim, S. P.; Nam, S. H.; Friedman, M. Mechanism of the antiadipogenic-antibesity effects of a rice hull smoke extract in 3T3-L1 preadipocyte cells and in mice on a high-fat diet. *Food Funct.* 2015, 6, 2939−2948.
(22) Hu, J.; Hong, W.; Yao, K. N.; Zhu, X. H.; Chen, Z. Y.; Ye, L. Ursodeoxycholic acid ameliorates hepatic lipid metabolism in L/O2 cells by regulating the AKT/mTOR/SREBP-1 signaling pathway. *World J Gastroenterol.* 2019, 25, 1492−1501.
(23) Figarola, J. L.; Rahbar, S. Smallmolecule COH-SR4 inhibits adipocyte differentiation via AMPK activation. *Int. J. Mol. Med.* 2013, 31, 1166−1176.
(24) Morita, K.; Hayashi, Y.; Ono, T.; Shimakawa, H. Comparison of the effects of feprazone and phenylbutazone on testosterone hydroxylations in mouse hepatic microsomes. Chem. Pharm. Bull. (Tokyo). 1986, 34, 214−220.

(25) Nakamura, K.; Fukunishi, S.; Yokohama, K.; Ohama, H.; Tsuchimoto, Y.; Asai, A.; Tsuda, Y.; Higuchi, K. A long-lasting dipeptidyl peptidase-4 inhibitor, teneligliptin, as a preventive drug for the development of hepatic steatosis in high-fructose diet-fed ob/ob mice. Int. J. Mol. Med. 2017, 39, 969−983.

(26) Poulos, S. P.; Dodson, M. V.; Culver, M. F.; Hausman, G. J. The increasingly complex regulation of adipocyte differentiation. Exp. Biol. Med. (Maywood). 2015, 241, 449−456.

(27) Zechner, R.; Langin, D. Hormone-sensitive lipase deficiency in humans. Cell Metab. 2014, 20, 199−201.

(28) Farmer, S. R. Transcriptional control of adipocyte formation. Cell Metab. 2006, 4, 263−273.

(29) de Mota, S. P.; Richard, A. J.; Hang, H.; Stephens, J. M. Transcriptional Regulation of Adipogenesis. Compr. Physiol. 2017, 7, 635−674.

(30) Astapova, O.; Leff, T. Adiponectin and PPARgamma: cooperative and interdependent actions of two key regulators of metabolism. Vitam. Horm. 2012, 90, 143−162.

(31) Christodoulides, C.; Vidal-Puig, A. PPARs and adipocyte function. Mol. Cell. Endocrinol. 2010, 318, 61−68.

(32) Rosen, E. D.; Sarraf, P.; Troy, A. E.; Bradwin, G.; Moore, K.; Milstone, D. S.; Spiegelman, B. M.; Mortensen, R. M. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol. Cell 1999, 4, 611−617.

(33) Wright, H. M.; Clkh, C. B.; Mikami, T.; Hauser, S.; Yanagi, K.; Hiramatsu, R.; Serhan, C. N.; Spiegelman, B. M. A synthetic antagonist for the peroxisome proliferator-activated receptor gamma inhibits adipocyte differentiation. J. Biol. Chem. 2000, 275, 1873−1877.

(34) Kim, J.; Lee, Y. J.; Kim, J. M.; Lee, S. Y.; Myung-Ae, B.; Ahn, J. H.; Han, D. C.; Kwon, B. M. PPARgamma agonists induce adipocyte differentiation by modulating the expression of Lipin-1, which acts as a PPARgamma phosphatase. Int. J. Biochem. Cell Biol. 2016, 81, 57−66.

(35) Garcia, E.; Lacasa, M.; Agli, B.; Giudicelli, Y.; Lacasa, D. Modulation of rat preadipocyte adipose conversion by androgenic status: involvement of C/EBPs transcription factors. J. Endocrinol. 1999, 161, 89−97.

(36) Yeh, W. C.; Cao, Z.; Classon, M.; McKnight, S. L. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. Genes Dev. 1995, 9, 168−181.

(37) Tanaka, T.; Yoshida, N.; Kishimoto, T.; Akira, S. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. EMBO J. 1997, 16, 7432−7443.

(38) Bays, H.; Blonde, L.; Rosenson, R. Adiposopathy: how do diet, exercise and weight loss drug therapies improve metabolic disease in overweight patients? Expert. Rev. Cardiovasc. Ther. 2014, 4, 871−895.

(39) Kim, J. B.; Wright, H. M.; Wright, M.; Spiegelman, B. M. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. U. S. A. 1998, 95, 4333−4337.

(40) Cerk, I. K.; Wechselberger, L.; Oberer, M. Adipose Triglyceride Lipase Regulation: An Overview. Curr. Protein Pept Sci. 2018, 19, 221−233.