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The Effect of Fraction 5 of Theabrownin from Pu-erh Tea on 3T3-L1 Preadipocyte Proliferation and Differentiation

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Abstract Pu-erh tea, a well-known tea from ancient times, was originally produced in the Yunnan province of China. Theabrownin of pu-erh tea was successfully separated into six fractions by using a sephadex LH-20 column. A previous study showed that the inhibition of α-glycosidase and pancreatic lipase activities was enriched in theabrownin fraction 5 (F5). Here, the effects of F5 on the proliferation and differentiation of 3T3-L1 preadipocytes were investigated. Results showed that F5 suppressed 3T3-L1 preadipocyte proliferation and during 3T3-L1 preadipocyte differentiation, F5 dose-dependently promoted lipid accumulation and significantly decreased the expression of PPARγ2, PTP1B mRNA and protein and Glut4 protein as well as increased the expression of Glut4 mRNA. The findings suggested that F5 act to maintain a reasonable lipid metabolism balance by regulating the mRNA and protein expression of PPARγ2, and allow the activation of insulin receptor proteins to promote Glut4 translocation to the cell membrane and glucose intake by decreasing PTP1B protein expression. Together, these activities may improve Insulin resistance (IR) and prevent metabolic syndrome, which is important for establishing a basis for the development of F5 into a functional food.

Keywords: *pu-erh tea, theabrownin, fraction 5, 3T3-L1 preadipocyte, PPARγ2, PTP1B,Glut4*

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1. Introduction

Tea plants (*Camellia sinensis*) are widely cultivated in China, Sri Lanka, Indonesia, Japan, India, Taiwan and Central Africa. Tea has an attractive aroma, distinctive taste, and health benefits, so it has become one of the most popular beverages in the world. Based on the degree of fermentation, tea can generally be classified into three major categories: non-fermented green tea, partially fermented oolong or paochong tea, and fully fermented black or pu-erh tea [1]. Pu-erh tea, a well-known tea from ancient times, was originally produced in the Yunnan province of China through a special post-fermentation process, using crude green tea prepared from the leaves of Camellia sinensis var. assamica as raw materials. It is consumed widely in southwest China, Japan, South Korea, Taiwan, Hong Kong, and other regions [2]. In the last decade, some studies have reported that pu-erh tea has biological activities, such as anticancer, antioxidant, increased metabolism, antimicrobial, antimutagenic, and arteriosclerosis resistance [3,4,5,6,7], so pu-erh tea has been attracted more attention because of such health benefits.

Insulin resistance (IR) is a pathological condition in which cells and tissues lose sensitivity and/or reactivity to insulin; it predisposes individuals to develop diabetes mellitus, obesity, coronary heart disease, hypertension, atherosclerosis, and other metabolic diseases [8,9,10]. Fat cells are one of the most important targets of insulin and are the main site of glycolipid metabolism. Because of this important link to IR, fat cells have been used to screen for drugs with insulin sensitization, fat reduction, and weight loss effects. The 3T3-L1 preadipocyte cell line, cloned from 3T3 mouse embryo fibroblasts, differentiate in monolayer cultures into cells with morphological and biochemical characteristics of adipocytes. Thus, the 3T3-L1 cell line is one of the most widely used to study glycolipid metabolism by the scientific community.

Because of its long fermentation time and high degree of polyphenol oxidation, Pu-erh tea contains less theaflavins and thearubigins than black tea [11]. In pu-erh tea, the theaflavins and thearubigins are further oxidized and polymerized to the main pigment, theabrownine. However, compared with the theaflavins and thearubigin, less is known about the fractionation, chemical composition, properties, and biological activities of theabrownin. Referencing previous separations and

preparations of theaflavins and thearubigins, the authors successfully separated theabrownin into six fractions using a sephadex LH-20 column [12]. A previous study showed that theabrownin could inhibit α-glucosidase and pancreatic lipase, and this activity was enriched in Fraction 5 (F5). Here, the effect of F5 on the proliferation and differentiation of 3T3-L1 preadipocytes was investigated to better understand the hypoglycemic and lipid-reducing activities of F5. These data will be important for establishing a basis for the development of F5 into a functional food.

2. Materials and Methods

2.1. Materials

HPLC grade N,N-Dimethyl formamide and methanol were purchased from Changsha Keningxinye Bioengineering Co., Ltd, China. Insulin, dexamethasone(DEX), 3-isobutyl-1-methylxanthine(IBMX), bis-acryamide, ammonium persulfate, N,N,N′,N′-tetramethylethylenediamine, phenylmethanesulfonyl fluoride (PMSF), acrylamide, ethylenediaminetetraacetic acid disodium, sodium dodecyl sulfate (SDS), dithiothreitol, dimethyl sulfoxide (DMSO) and trypsin were purchased from Sigma-Aldrich(St. Louis, MO, USA). Diethylpyrocarbonate, trizol, oligo(dT), reaction buffer, RNase inhibitor, dNTP mix, M-MLV reverse transcriptase and SYBR Green dye were purchased from Fermentas of Thermo Fisher Scientific (Waltham, MA, USA). Antibodies that recognize β-actin, PPARγ2, PTP1B and Glut4 were purchased from Abcam (Cambridge, UK). Antibodies that recognize rabbit IgG conjugated with horseradish peroxidase (HRP) were purchased from Beijing Zhongshanjinqiao Bioengineering Co, Ltd, China. Chemiluminescent HRP substrate for western blotting was purchased from Millipore (Billerica, MA, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and non-fat dried milk were purchased from Amresco (Solon, OH, USA). All other reagents were from vendors in China.

Pu-erh tea producted in 2007 was provided by the Longrun Tea Group (Yunnan Province, China). 3T3-L1 preadipocyte cells were purchased from the cell resource center at the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences Institute and Sephadex LH-20 from Beijing Hui De Yi Technology Co. LTD, China.

2.2. Preparation of F5 of Theabrownin from Pu-erh Tea

F5 of theabrownin from pu-erh tea was prepared by using previously published methods [12]. Pu-erh tea (30 g) was extracted with 90°C hot water (750 mL) for 70 min. The filtrate was condensed to a 150 mL volume by rotary vacuum concentration. Then, the filtrate was successively extracted six times with chloroform, ethyl acetate, and *n*-butanol, and the remaining water layer was condensed in using rotary vacuum concentration until the odor of the solvent vanished. The product was freeze dried to obtain a crude theabrownin extract. To fractionate theabrownin, the crude extract (350 mg) was dissolved in distilled water, filtered, and loaded onto a sephadex LH-20 column. The column was eluted with 40% acetone in water. Six fractions of eluate were collected at a speed of 0.05 bed volume per hour. Each fraction collected corresponded to an eluted pigmented band and the solution containing the fifth pigmented band was condensed by rotary vacuum concentration until the organic solvent smell disappeared. The product was freeze dried to obtain a powder, termed F5.

2.3. Analysis of Conventional Ingredients in F5 of Theabrownin from Pu-erh Tea

Total polyphenol content was determined by the Folin–Denis method using gallic acid as a standard [13]. Total carbohydrate content was determined by the phenol-sulfuric acid method using glucose as a standard [14]. Total protein content was determined by the Coomassie Brilliant Blue method using bovine serum albumin as a standard [15]. The concentration of catechins and caffeine were determined using a HPLC system (LC-2010A; Shimadzu Corp., Kyoto, Japan) equipped with a Shim-pack VP-ODS C18 column (5 mm, 4.6×150 mm, 35°C) at 278 nm, as previously described with some modifications [16]. Solvents A (water) and B (N,N-Dimethylformamide: methanol: acetic acid, 20:1:0.5, v/v) were run on linear gradients, with B increasing from 14% to 23% within 13 min, and from 23% to 36% within the next 12 min. Columns were maintained for 3 min thereafter at a rate of 1.0 mL min^{-1}. The concentrations of catechins and caffeine were quantified by their peak areas compared with those of standards prepared from validated compounds.

2.4. MTT Assay

3T3-L1 preadipocytes were seeded into 96-well plates at 1×10^4 cells per well and grew them in 100 μL Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS). When the cells were about70% confluent, they were cultured with DMEM containing 10% FCS and various concentrations of F5. After cells were cultured for 24, 48, or 72 h, they were incubated with 20 μL of MTT solution (5 mg/mL in PBS) that was added to media for 4 h at 37°C. Next, media was discarded and cells were treated with 100 μL DMSO per well. The 96-well plates were gently shaken for 10 min in the dark and then relative cell viability was determined by measuring absorbance at 490 nm using a Varioskan Flash Multimode Reader (Thermo Scientific, Country).

2.5. Oil Red O Staining

3T3-L1 cells were seeded in triplicate in six-well plates at 2.5×10^5 cells per well and grew them to confluence in high glucose DMEM for 2 days. Cells were then induced to differentiate using the following schedule of media and hormone additions: 0.5 mM IBMX, 1 μM DEX, and 10 μg mL^{-1} insulin. Various concentrations of F5, 5 μM fenofibrate (Fen), or 5 μM rosiglitazone (Ros) was also added to media of the treatment groups throughout the entire differentiation process. Control groups contained identical media but no added compound. On day 2, the media was replaced with high glucose DMEM containing 10 μg mL^{-1} insulin. On days 4 and 6, the media was replaced with high glucose DMEM containing 10% FCS. On day 8, the media was replaced with 4% paraformaldehyde, and cells were incubated for 1 h at

room temperature, washed with 60% isopropanol, and then allowed to dry. Oil red O working solution was added to each well for 45 min, and then cells were washed with a deionized water. Cell images were obtained using an Olympus BX51TF microscope with imaging software. To quantify lipid accumulation, 1 mL isopropanol was added to dissolve the oil red O, then added 100 µL isopropanol containing oil Red O to each well of 96-well plates in triplicate that were used to determine the optical density (OD) by measuring absorbance at 510 nm with a Varioskan Flash Multimode Reader (Thermo Scientific).

2.6. Quantitative Real-time PCR Analysis

Total RNA using Trizol reagent was extracted from 3T3-L1 preadipocytes that were treated with 50 µg/mL F5, 5 µM Fen, or 5 µM Ros throughout the differentiation process. cDNA was synthesized by using a M-MLV Reverse Transcriptase Kit following the manufacturer's specifications and 2 µg of total RNA. Gene expression in 3T3-L1 cells treated with 50 µg/mL F5, 5 µM Fen, or 5 µM Ros was assessed by quantitative RT-PCR. The primers (Table 1) were designed for qPCR with Primer3^{+} software using cDNA sequences as templates. Reactions were carried out with the Rotor-Gene Q6200 Real-Time PCR System (Qiagen) using three-step cycling conditions of denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 20 s. The reaction mixture (20 µL) contained 0.5 µL cDNA solution, 10 µL Platinum SYBR® Green qPCR SuperMix-UDG, 0.4 µL of each primer (10 µM/L), and 8.7 µL double-deionized water purified with a Millipore filter. Each reaction was performed in triplicate and the results expressed were the fold-change relative to the controls after normalizing to β-actin expression levels.

Table 1. Primer Sequences for quantitative PCR

Gene Name	Froward primer(5'-3')	Reverse primer (3'-5')	Product size (pb)
β-actin	5-agccatgtacgtagccatcc-3	5-ctctcagctgtggtggtgaa-3	228
PPARγ2	5-accactcgcattcctttgac-3	5-ccacagactcggcactcaat-3	265
PTP1B	5-cggaacaggtaccgagatgt-3	5-gaagtgcccacatgttttg-3	159
GLUT4	5-gattctgctgcccttctgtc-3	5-attggacgctctctctccaa-3	168

2.7. Western Blot Analysis

To extract total protein from 3T3-L1 preadipocytes that had been treated with 50 µg/mL F5, 5 µM Fen, or 5 µM Ros throughout the differentiation process, cells were lysed for 30 min in ice-cold RIPA buffer (10 mM Tris-HCl, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 1 mM PMSF). Cell lysates were centrifuged and protein content was determined in the supernatant by using a protein assay kit (Bio-Rad, Hercules, CA, USA). Proteins between 50 and 100 µg were resolved by SDS-polyacrylamide gel electrophoresis and transferred them to a polyvinylidene fluoride membrane. Membranes were blocked for 1 h at room temperature in 10 mM PBS containing 0.2% Tween 20 (TBST) and 5% non-fat milk and then incubated them with target protein-specific antibodies for 12 h at 4 °C. Afterwards, membranes were washed three times with TBST for 5 min, and then incubated them with HRP-conjugated goat anti-rabbit IgG for 1 h. All primary antibodies were used at a dilution of 1:1000 and HRP-conjugated goat anti-rabbit IgG was used at a dilution of 1:10,000. Reactive bands were visualized with an enhanced chemiluminescence detection system at room temperature according to the manufacturer's directions. Band intensities with a FluorChem FC2 universal gel imaging system (Alpha Innotech Corp, San Leandro, CA, USA) were detected and quantified.

2.8. Statistical Analysis

Statistical analyses were conducted using SPSS version 13.0 for Windows software (IBM, Armonk, NY, USA). Each data set is presented as the mean ± standard deviation (SD). Differences between groups were considered to be statistically significant when $P < 0.01$ or $P < 0.05$.

3. Results

3.1. Determination of Conventional Ingredients in F5

The content of caffeine, tea polyphenols, protein, tea polysaccharides, epigallocatechin gallate (EGC), DL-catechin (DL-C), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), and epicatechin gallate (ECG) in F5 was 0.47%, 56.28%, 14%, 17.66%, 0.555%, 0.511%, 0.024%, 0.083%, and 0.032%, respectively.

3.2. F5 Inhibits the Proliferation of 3T3-L1 Preadipocytes

The concentration-dependent and kinetic effects of F5 on the proliferation of 3T3-L1 preadipocytes were shown in Figure 1. The addition of 10, 20, 30, 50, or 100 µg/mL of F5 to 3T3-L1 preadipocyte cultures for 24 h only slightly inhibited cell proliferation. The average inhibition rate of each treatment group was 0.45%, 0.62%, 4.54%, 2.94%, and 6.89%, respectively; the no added F5 control group was used for comparison and none of these differences reached the level of statistical significance. After 48 h of culture with F5, when compared with the control group the average inhibition rate was 7.26%, 16.03% ($P < 0.05$), 17.64% ($P < 0.05$), 21.83% ($P < 0.01$), and 28.40% ($P < 0.01$), respectively. After 72 h of culture with F5, when compared with the control group the average inhibition rate was 2.70%, 10.22%, 15.63% ($P < 0.05$), 9.07%, and 20.24% ($P < 0.05$), respectively.

3.3. F5 Promotes Lipid Accumulation during 3T3-L1 Preadipocyte Differentiation

The lipid content of 3T3-L1 preadipocytes treated for 48 h with different concentrations of F5, 5 µM Ros, or 5

μM Fen was shown in Figure 2. The lipid content of the 5 μM Fen treatment group was the lowest, whereas the lipid content of the 5 μM Ros treatment group was the highest. F5 promoted lipid accumulation in a dose-dependent manner that was significantly ($P < 0.01$) higher than the control group (0 μg/mL F5) and 5 μM Fen treatment group, but significantly ($P < 0.01$) lower than the 5 μM Ros treatment group.

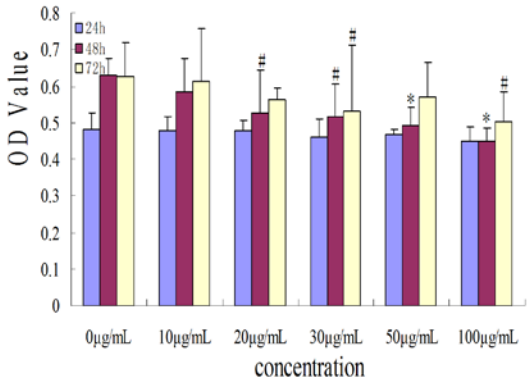

Figure 1. F5 inhibits the proliferation of 3T3-L1 preadipocytes compared with the control group (0 μg/mL F5), *=P<0.01 and #=P<0.05

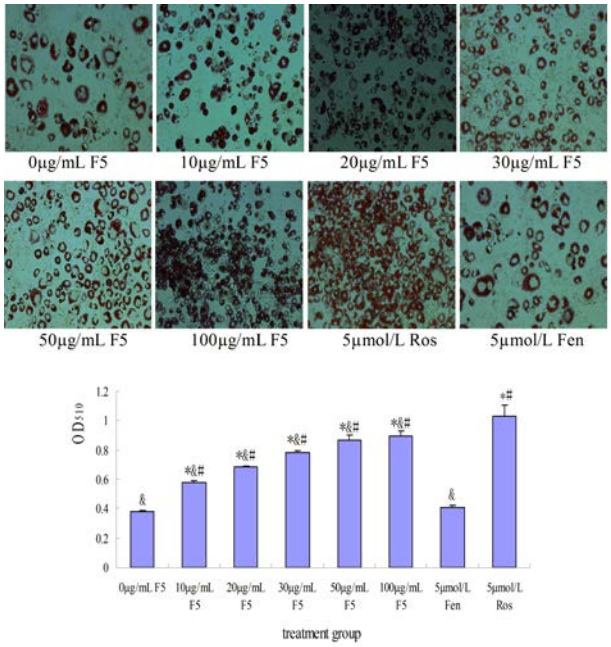

Figure 2. F5 promotes lipid accumulation during 3T3-L1 preadipocyte differentiation compared with the control group (0 μg/mL F5), #=P<0.01; compared with the Ros group, &=P<0.01; compared with the Fen group, *=P<0.01

3.4. F5 Decreases PPARγ2 and PTP1B and Increases Glut4 mRNA Expression Levels

The expression levels of PPARγ2, PTP1B and Glut4 mRNA in differentiated 3T3-L1 preadipocytes treated with 50 μg/mL F5, 5 μM Ros, or 5 μM Fen were shown in Figure 3. For PPARγ2, both the 50 μg/mL F5 and 5 μM Fen treatment groups showed significantly less ($P < 0.01$) PPARγ2 mRNA expression compared with the control group. However, compared with the control and 50 μg/mL F5 treatment groups, the 5 μM Ros treatment group showed significantly increased expression of PPARγ2 mRNA ($P < 0.01$).

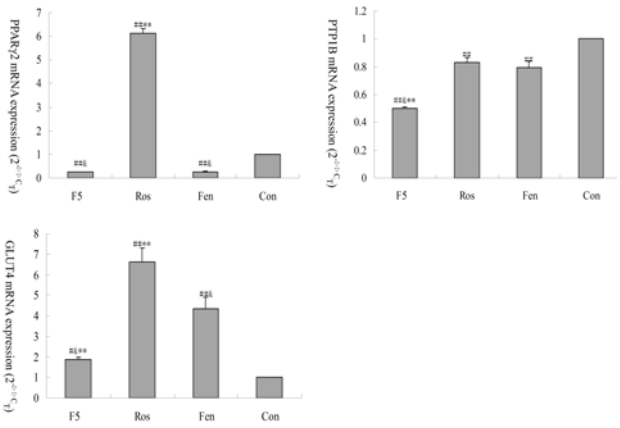

Figure 3. The effects of F5, Ros, and Fen on the expression level of PPARγ2, PTP1B and GLUT4 mRNA compared with the control group (0 μg/mL), #=P<0.05 and ##=P<0.01; compared with the Ros group, &=P<0.01; compared with the Fen group, *=P<0.05 and **=P<0.01

For PTP1B, compared with the control treatment group, the 50 μg/mL F5, 5 μM Fen and 5 μM Ros treatment groups all significantly ($P < 0.01$) decreased the expression level of PTP1B mRNA. The 50 μg/mL F5 treatment group showed the most obviously decreased PTP1B mRNA expression among the three treatments and the difference was very significant ($P < 0.01$) compared with the 5 μM Fen and 5 μM Ros treatment groups.

For Glut4, compared with the control treatment group, the 50 μg/mL F5, 5 μM Fen, and 5 μM Ros treatment groups all significantly ($P < 0.01$) increased the expression level of Glut4 mRNA. The 50 μg/mL F5 treatment group showed the smallest increase ($P < 0.05$) in Glut4 levels, which was significantly lower ($P < 0.01$) than the 5 μM Fen and 5 μM Ros treatment groups.

3.5. F5 Decreases PPARγ2, PTP1B, and Glut4 Protein Levels

The expression levels of PPARγ2, PTP1B, and Glut4 proteins in differentiated 3T3-L1 preadipocytes treated with 50 μg/mL F5, 5 μM Ros, or 5 μM Fen were shown in Figure 4. For PPARγ2, compared with the control treatment group, both the 50 μg/mL F5 and 5 μM Fen treatment groups showed significantly ($P < 0.01$) decreased expression of PPARγ2 protein, and also a significant ($P < 0.01$) difference between these groups. The 5 μM Ros treatment group showed significantly ($P < 0.01$) increased expression of PPARγ2 protein.

For PTP1B, compared with the control treatment group, both the 50 μg/mL F5 and 5μM Fen treatment groups showed significantly ($P < 0.01$) decreased expression of PTP1B protein, while the 5 μM Ros treatment group showed only slightly decreased expression of PTP1B protein. the difference in the expression levels of PTP1B protein was significantly ($P < 0.01$) reduced in the Ros compared with the 5 μM Fen and 50 μg/mL F5 treatment groups.

For Glut4, compared with the control treatment group, both the 50 μg/mL F5 and 5 μM Fen treatment groups significantly ($P < 0.01$) decreased the expression level of Glut4 protein, whereas the 5 μM Ros treatment group showed significantly ($P < 0.01$) increased Glut4 protein expression. The level of the 50 μg/mL F5 treatment group was significantly ($P < 0.01$) different from the 5 μM Fen and 5 μM Ros treatment groups.

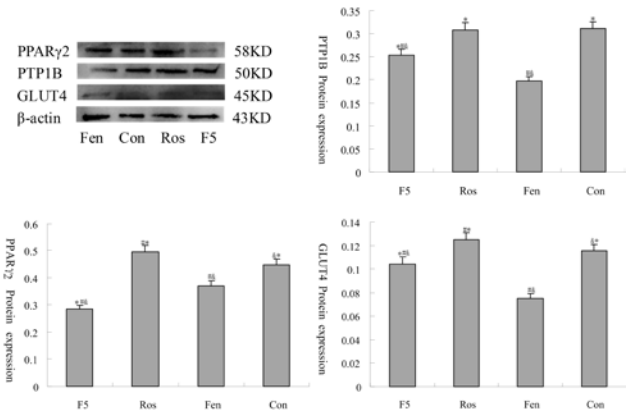

Figure 4. The effect of F5, Ros, and Fen on the expression level of PPARγ2,PTP1B and GLUT4 protein compared with the control group (0 μg/mL), #=P<0.01; compared with the Ros group, &=P<0.01; compared with the Fen group, *=P<0.01

4. Discussion

Effectively inhibiting adipocyte proliferation is very important as it may allow for the prevention of diseases such as hypertension, type-2 diabetes, and dyslipidemia-a condition closely related to obesity and IR [17]. Currently, adipocyte proliferation and differentiation cannot be directly studied in vitro. The inhibitory effect on preadipocyte proliferation has been generally studied using pharmacological stimuli that could reduce or prevent preadipocyte differentiation into adipocytes. It was speculated that such drugs could inhibit adipocyte proliferation. Here, the inhibition of preadipocyte proliferation by F5 might represent a mechanism that can promote weight loss and consequently improve IR.

Adipocyte differentiation involves a series of changes in morphology and function of preadipocytes. This complicated process is regulated by conserved transcription factors, including PPARγ/Rxr heterodimers, C/EBP and adipocyte differentiation decision factor/sterol regulatory element binding protein 1 (Add1/Srebp1). PPARγ is thought to be one of the main transcription factors that regulate adipocyte differentiation [18,19]. The results showed that F5 significantly promoted lipid accumulation in cells during 3T3-L1 preadipocyte differentiation and also significantly lowered PPARγ2 mRNA and protein levels. However, the drug Ros, commonly used to treat IR, also significantly promoted lipid accumulation during 3T3-L1 preadipocyte differentiation, but markedly increased PPARγ2 mRNA and protein levels. Therefore, F5 promotes adipocyte differentiation by a different mechanism than Ros. Determining whether F5 regulates adipocyte differentiation and promotes lipid accumulation through mechanisms involving transcription factors other than PPARγ family members will require further research. However, the results showed that Fen slightly promoted lipid accumulation in 3T3-L1 adipocytes and significantly inhibited PPARγ2 mRNA and protein expression, which was similar to the effects of F5 on 3T3-L1 adipocytes. These findings indicated that the mechanisms of action of F5 and Fen on adipocyte differentiation could be similar. Some studies have reported that pu-erh tea may have a multiple effects on lipid and glucose metabolism [20], resulting in the reduction of lipids, improved IR, and less cardiovascular disease [21]. PEF8 components isolated from the ethyl acetate layer of pu-erh tea can promote 3T3-L1 preadipocyte differentiation and the lipolysis of mature 3T3-L1 adipocytes [22]. Additionally, large molecular polymeric pigments isolated from fermented Zijuan pu-erh tea have some hypolipidemic effects and can significantly enhance Hsl mRNA expression in rat liver and adipose tissue, thereby increasing Hsl activity [23]. A previous study from the same authors showed that F5 could inhibit the activities of α-glycosidase and pancreatic lipase enzymes. Therefore, F5 may effectively reduce lipid content and hyperglycemia, while not causing the side effects associated with Ros and Fen. Testing this hypothesis will require additional study, including measurements of the expression of other genes and proteins, especially cytokines, related to the regulation of glycolipid metabolism in adipocytes.

Fatty acids connection protein (AP2), phosphoenolpyruvate kinase (PEPCK), glucose transporter 4 (Glut4), and insulin receptor are expressed because of the synergistic effects of PPARγ and C/EBPα during 3T3-L1 preadipocyte differentiation. Among them, research on Glut4 has mainly assessed the expression level of Glut4 and signal transduction pathways affecting Glut4 translocation; such research will be very important for selecting future drug targets. Many reports have shown that the expression level of Glut4 can be affected by many factors, such as physiological, pathological, and pharmacological effects [24,25,26]. Here, Ros could clearly promote the expression of Glut4 mRNA and protein, similar to the effects of thiazolidinediones [27]. Fen and F5 increased the expression level of Glut4 mRNA and decreased the expression level of Glut4 protein, which may be related to both transcriptional and post-transcriptional effects directed towards Glut4. It is known that Glut4 translocation can be regulated by the AMPK, Ca^{2+}, and insulin signal transduction pathways. Among them, the insulin signal transduction pathway is the most important in adipocyte and muscle cells. Glut4 translocation and the absorption of glucose are greatly reduced when the insulin signal is weakened or blocked. Many factors can act on intermediates of the insulin signal transduction pathway to influence Glut4 translocation [28,30]. In addition, insulin signal transduction can be reduced by PTP1B [31,32,33], which is a negative regulator of insulin signal transduction [34] and will likely to become a new target for the treatment of type 2 diabetes and obesity. Overexpressing PTP1B in cells can significantly reduce Glut4 translocation to the cell membrane and the intake of glucose, which leads to post-receptor IR. This study showed that the expression level of Glut4 and PTP1B protein in cells treated with the Fen, F5, and Ros was clearly decreased. Additionally, Fen could improve IR by reducing blood fat and decreasing the expression of PTP1B protein, which accelerated Glut4 protein translocation to the cell membrane. Presumably, F5 may also decrease PTP1B protein expression and activate the insulin receptor signal to promote Glut4 translocation to the cell membrane and the intake of glucose. These activities may prevent or ameliorate diseases with post-receptor IR and deserve future exploration.

The significant suppression of FAS in the livers of rats fed pu-erh tea may occur through the downregulation of

the PI3K/AKT and JNK signaling pathways. Because the bioactive ingredients in pu-erh tea are different from green, black, and oolong tea, they may be related to polymer compounds generated during the fermentation process [35].Fraction 5 from the ethyl acetate extract of pu-erh tea showed hypolipidemic effects on the human hepatoma HepG2 cell line and contained EGCG and ECG hypolipidemic ingredients [36]. In this study, the authors found that F5 contained a very low content of catechin and caffeine, but the total tea polyphenol was as high as 56.28%. Therefore, determining whether F5 contains specific polyphenols with bioactivity and its mechanism will require further research.

5. Conclusion

F5 can inhibit 3T3-L1 preadipocyte proliferation, which may be the reason why F5 can reduce obesity and improve IR without causing adipocyte accumulation. It has been suggested that F5 may be suitable for the prevention or treatment of metabolic diseases related to obesity and IR. Compared with control sample, F5 promoted lipid accumulation in a dose-dependent manner during 3T3-L1 preadipocyte differentiation, but the lipid accumulation effect of F5 on 3T3-L1 preadipocytes was lower than Ros. F5 may act to maintain a reasonable lipid metabolism balance by decreasing PPARγ2 mRNA and protein expression, and may decrease PTP1B protein expression allowing the activation of insulin receptor proteins to promote Glut4 translocation to the cell membrane and glucose intake. Together, these activities may improve IR and prevent metabolic syndrome.

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