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Effect of yellow capsicum extract on proliferation and differentiation of 3T3-L1 preadipocytes

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ABSTRACT

Objectives: To evaluate the effect of effect of Yellow Capsicum extract (YCE) that is rich in capsaicin on the proliferation and differentiation of 3T3-L1 preadipocytes in vitro.
Methods: 3T3 L1 cells that were exposed to differentiation-inducing medium containing high glucose DMEM (Dulbecco's Modified Eagle's Medium) and subsequently were treated with capsaicin and YCE for their effect on adipocyte differentiation, changes in their triglyceride content, leptin secretion, expression of lipoprotein lipase, PPARγ, and CCAAT/enhancer-binding protein alpha (C/EBPα).
Results: Both YCE and capsaicin inhibited proliferation and differentiation 3T3-L1 preadipocytes and suppressed accumulation of intracellular triglyceride in a dose-dependent manner. In addition, a significant decrease in the expression of lipoprotein lipase (LPL), leptin, PPARγ, and C/EBPα was noted in 3T3-L1 preadipocytes when induced to differentiate by YCE and Capsaicin.
Conclusions: The potent inhibitory action of YCE and Capsaicin on the differentiation of 3T3-L1 preadipocyte observed suggests that they (YCE and Capsaicin) have the potential to inhibit obesity that needs to be explored in future studies.

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Introduction

Obesity is due to an imbalance between energy intake and energy expenditure, with the balance tilted more toward the former. The clinical significance of obesity lies in the fact that it can result in predisposition for the development of cardiovascular diseases, hypertension, and type 2 diabetes mellitus [1–4]. Hence, methods and strategies need to be developed to control the epidemic of obesity. In this context, it is worthwhile to explore the effect of certain commonly used plant extracts as modulators of adipocyte differentiation and potential candidates for the prevention and treatment of obesity. Capsaicin (8-methyl-*N*-vanillyl-trans-6-nonenamide) is a major pungent component of pepper [5], and is a potent analgesic, anti-inflammatory compound [6] that has the ability to decrease energy intake and adipose tissue weight by decreasing serum triacylglycerol. Yellow capsicum is a special Chinese food and contains high concentrations of capsaicin. Until recently, the effects of yellow capsicum extracts (YCEs) on the proliferation and differentiation of 3T3-L1 preadipocytes were not reported, to our knowledge.

The amount of adipose tissue is tightly regulated and is dependent on the differentiation of preadipocytes to adipocytes [7,8]. Adipocyte differentiation is divided into three stages: contact inhibition of cell growth, clonal proliferation, and permanent growth inhibition. The proliferation of preadipocytes is an important factor for evaluating adipocyte differentiation rates. It has been found that capsaicin decreased cell population growth and induced apoptosis of 3T3-L1 preadipocytes in a time- and dose-dependent manner [9]. In clonal proliferation of preadipocytes, mitosis is closely related to cell cycle. Studies about cell cycle will be beneficial for revealing the mechanism of preadipocyte proliferation.

The differentiation and proliferation of preadipocytes is coordinated and regulated by several adipogenic molecules, which include a variety of growth factors, cytokines, and

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Table 1
Effect of different concentrations of YCE on the proliferation of 3T3-L1 preadipocytes ($\bar{x} \pm s$, n = 6)

	Optical density (λ = 490nm)			
	24 h	48 h	96 h	144 h
Control	0.881 ± 0.024	1.065 ± 0.058	1.249 ± 0.029	1.461 ± 0.054
200 μg/mL	0.813 ± 0.017†	0.965 ± 0.059*	1.125 ± 0.042†	1.239 ± 0.044†
400 μg/ml	0.721 ± 0.013†	0.842 ± 0.026†	0.928 ± 0.038†	1.034 ± 0.058†
600 μg/ml	0.668 ± 0.023†	0.694 ± 0.02†	0.783 ± 0.061†	0.875 ± 0.075†
800 μg/ml	0.533 ± 0.014†	0.596 ± 0.014†	0.654 ± 0.090†	0.724 ± 0.093†
1000 μg/ml	0.463 ± 0.010†	0.499 ± 0.019†	0.558 ± 0.079†	0.606 ± 0.084†

YCE, yellow capsicum extract
Compared with control group *$P < 0.05$; †$P < 0.01$

hormones [10]. Leptin is a specific secretion of adipose tissue, and a dominant regulator of food intake and energy expenditure. Leptin also participates in various other physiological activities, including reproduction, thyroid function, bone density, and immunity [11,12]. Leptin levels in individuals with body mass index (BMI) > 25 kg/m^2 were higher than those in those with BMI < 25 kg/m^2 [13]. One study reported that the expression of leptin was decreased in a time- and dose-dependent manner in 3T3-L1 adipocytes treated with capsaicin [9]. To our knowledge, the effects of YCE on the expression of leptin in 3T3-L1 preadipocytes were not reported.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT- enhancer-binding protein alpha (C/EBPα) are known to play key roles in the regulation of adipogenesis and the modulation of fat cell function in adipose tissue. PPAR-γ and C/EBPα are not expressed in preadipocytes, but are activated during adipocyte differentiation [14]. A study of the effect of YCE on the expression of PPARγ and C/EBPα in 3T3-L1 preadipocytes is needed to delineate whether a change in their expression could be a factor in the induction of differentiation of 3T3-L1 preadipocytes by YCE.

Lipoprotein lipase (LPL) hydrolyzes the triglyceride (TG) component of circulating lipoproteins, and is involved in the delivery of fatty acids to the tissues. LPL is closely related to obesity. The relative levels of LPL activity determine how dietary lipids are partitioned toward storage or utilization and thereby lead to obesity or weight loss [15]. Thus, a reduction of LPL activity in adipose tissue decreases weight gain due to impaired lipid accumulation in adipocytes. In view of this, a study of LPL expression in 3T3-L1 preadipocytes in response to YCE could indicate whether YEC has the potential to prevent obesity induced by a high-fat diet (HFD).

In the present study, we investigated the effects of YCE on the proliferation and differentiation of 3T3-L1 preadipocytes and assessed their TG content, LPL expression, and adipocyte differentiation-related protein level.

Materials and methods

YCE (of which 7% is present as capsaicin as assessed by high-performance liquid chromatography) was obtained from the Zhejiang Zhongwei Company.

Table 2
Effect of different concentrations of capsaicin on the proliferation of 3T3-L1 preadipocytes ($\bar{x} \pm s$, n = 6)

	Optical density (λ = 490nm)			
	24 h	48 h	96 h	144 h
Control	0.607 ± 0.034	0.783 ± 0.044	1.286 ± 0.115	1.737 ± 0.040
8μg/mL	0.560 ± 0.010	0.740 ± 0.015	1.200 ± 0.024*	1.625 ± 0.050*
16μg/mL	0.544 ± 0.006	0.666 ± 0.017*	1.070 ± 0.052*	1.455 ± 0.012†
32μg/mL	0.525 ± 0.010	0.638 ± 0.030*	0.947 ± 0.065†	1.165 ± 0.019†
64μg/mL	0.460 ± 0.010†	0.482 ± 0.101†	0.540 ± 0.102†	0.625 ± 0.055†

Compared with control group *$P < 0.05$; †$P < 0.01$

Table 3
Effect of different concentrations of capsaicin on the distribution of cell cycles of 3T3-L1 preadipocytes (%)

Group	G1 phase	S phase	G2 phase
Control	26.489 ± 0.107	38.813 ± 0.127	34.698 ± 0.225
15μg/mL	30.538 ± 0.142†	23.131 ± 0.126†	46.330 ± 0.263†
30μg/mL	34.127 ± 0.041†	14.820 ± 0.073†	51.052 ± 0.113†
40μg/mL	34.651 ± 0.437†	6.467 ± 0.090†	58.882 ± 0.488†
50μg/mL	33.926 ± 0.084†	1.389 ± 0.043†	64.685 ± 0.119†

Compared with control group *$P < 0.05$; †$P < 0.01$

Capsaicin, MTT dye, Oil Red O, 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), insulin (INS), and trypsin were purchased from Sigma Chemical Co. Neonatal bovine serum was purchased from the Hangzhou Sijiqing Biological Engineering Materials Co. Dulbecco's modified Eagle's medium (DMEM), and the antibiotic mixture Penicillin-Streptomycin (PS) were purchased from the Gibco BRL Co. TG assay kit was purchased from Beijing Beihua Kangtai Clinical Reagent Co. Mouse Leptin, the LEP enzyme-linked immunosorbent assay (ELISA) Kit was purchased from Hefei Bomei Biotechnology Co. Propidium iodide (PI) was purchased from Shanghai Bestbio Co. the Mouse C/EBPα ELISA kit, mouse PPAR-γ ELISA kit, and mouse LPL ELISA kits were purchased from Cusabio Co.

Cell culture

The 3T3-L1 cells were obtained from the Institute of Biochemistry and Cell Biology, Shanghai, China. 3T3-L1 preadipocytes were grown in DMEM containing 10% neonatal bovine serum, and 100 U/mL PS at 37°C under a 5%CO_2 atmosphere.

Adipocyte differentiation

To induce differentiation, 2-d postconfluent preadipocytes (designated day 0) were cultured in a differentiation-inducing medium that contained high-glucose DMEM with 1% PS, 10% fetal bovine serum (FBS), 0.5 mM IBMX, 1 μM DEX, and 1 μM INS for 2 d. Subsequently, cells were cultured for an additional 2 d in high-glucose DMEM containing 1% PS, 10% FBS, and 5μM INS. Thereafter, the cells were maintained in a post-differentiation medium (high-glucose DMEM containing 1% PS and 10% FBS) with replacement of the medium every 2 d. By about 12 to 14 d, about 90% of the 3T3-L1 cells differentiated into adipocytes showing typical adipocyte phenotype.

MTT assay

The assay was performed according to a previous method [16]. Cells were seeded on 96-well microplates at a density of 10^5 cells/well. After 24 h, 200 μL of serial dilutions of YCE (200–1000 μg/mL) and capsaicin (8–64 μg/mL) were added and incubated for 24, 48, 96, and 144 h. At the end of the incubation period, the culture medium was replaced by 20μL MTT solution for 4h. Then 150 μL/well of dimethylsulfoxide was added and measured spectrophotometrically in a Multiskan GO UV/Vis microplate spectrophotometer (Thermo Scientific Ltd, Lafayette, CO, USA). The cell population growth percentage (%) was expressed as the percentage of cell growth compared with the control, and it was calculated as follows: A490 nm/A490 nm [control] × 100.

Cell cycle analyses by PI staining

Cell cycle was assayed using PI staining method [17]. 3T3-L1 preadipocytes were stimulated by YCE and capsaicin for 24 h. Then, cells were harvested and washed twice with phosphate-buffered saline (PBS) and fixed in 80% ethanol at 4°C overnight, followed by incubation with 100 μg/mL RNase for 30 min at 37°C. The cells were then stained with PI for 30 min at 4°C and subjected to flow cytometric analysis of their DNA content using a FC500MCL flow Cytometer (Beckman Ltd, Brea, CA, USA). Approximately 1×10^4 cells were counted in each sample.

Table 4
Effect of different concentrations of YCE on the distribution of cell cycles of 3T3-L1 preadipocytes (%)

Group	G1 phase	S phase	G2 phase
Control	22.886 ± 0.173	17.197 ± 0.082	59.917 ± 0.213
400μg/mL	25.237 ± 0.141†	16.950 ± 0.067	57.813 ± 0.115*
800μg/mL	30.906 ± 0.099†	13.620 ± 0.121†	55.474 ± 0.216†
1000μg/mL	32.679 ± 0.092†	8.108 ± 0.063†	59.213 ± 0.064*

YCE, yellow capsicum extract
Compared with control group *$P < 0.05$; †$P < 0.01$

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