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Molecular mechanisms underlying the anti-obesity potential of prunetin, an *O*-methylated isoflavone

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ABSTRACT

Prunetin is an *O*-methylated isoflavone, which is a type of flavonoid. There are a limited number of reports detailing the biological activities of prunetin. Although an anti-inflammatory effect of prunetin has been reported in vitro, to our knowledge, there have been no reports on anti-adipogenic effects of prunetin in obese animals. The aims of this study were to determine whether prunetin suppresses high-fat diet (HFD)-induced adipogenesis in the liver and visceral adipose tissues of mice, and to explore the underlying mechanisms mediating the actions of prunetin. To this end, mice were fed a HFD for 10 weeks to induce obesity, and prunetin (10 μg/kg or 20 μg/kg) was administered in the last 3 weeks. Compared to saline-treated mice, mice treated with prunetin showed significantly reduced body weight gain, visceral fat pad weights, and plasma glucose levels. We found that prunetin significantly inhibited the HFD-induced upregulation of the expression of important adipogenic genes (PPARγ, C/EBPα, SREBP, aP2, LPL adiponectin, and leptin), and suppressed HFD-mediated increase in expression of lipid metabolism-related genes (SREBP, PPARγ, LXR, and HMG-CoA) in the liver tissues. Furthermore, prunetin induced expression of adiponectin receptors 1 and 2 (adipoR1, adipoR2), as well as that of AMP-activated protein kinase (AMPK) in the liver and adipose tissue. These results suggest that prunetin mediates anti-obesity/adipogenesis effects by suppressing obesity-related transcription through a feedback mechanism that regulates the expression of adiponectin, adipoR1, adipoR2, and AMPK.

1. Introduction

An excessive increase in adipose tissue mass is defined as obesity [1]. Differentiation of the adipocytes, known as adipogenesis, is a complex process accompanied by changes in morphology, hormone sensitivity, and gene expression [2]. Adipogenesis is a differentiation process in which undifferentiated preadipocytes are converted to fully differentiated adipocytes. This process is regulated by a highly organized cascade of transcription factors such as peroxisome proliferator-activated receptor γ (PPARγ), the CCAAT/enhancer-binding proteins α (C/EBPα), and the sterol regulatory element binding protein (SREBP) [3]. Furthermore, it is widely assumed that lipid metabolism, including lipogenesis, is primarily governed by a number of proteins including SREBP, liver × receptor (LXR), and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) [4,5].

Adiponectin is an adipokine that is secreted by adipocytes. Transcription of adiponectin is under the control of adipocyte transcription factors, including PPARγ and C/EBPα. It is known that secreted adiponectin circulates at relatively high concentrations in the bloodstream, and plays an important role in energy homeostasis and inflammation [6]. Some studies have reported that adiponectin gene expression and its circulating plasma levels are inversely correlated with adiposity [7,8]. Therefore, there have been significant efforts undertaken to identify the roles and the underlying mechanisms whereby adiponectin enhances energy metabolism.

Recently, 2 types of adiponectin receptor were identified. Adiponectin receptor 1 (adipoR1) is ubiquitously expressed, whereas adiponectin receptor 2 (adipoR2) is mainly expressed in the liver [9]. It has also been suggested that AMP-activated protein kinase (AMPK) may be activated by adiponectin and other downstream proteins involved in the adiponectin signaling

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cascade [10,11]. Moreover, the activation of AMPK probably plays a critical role in mediating the stimulative effect of adiponectin on fatty acid oxidation [10,12].

A number of reports indicate that several flavonoids have anti-adipogenesis/anti-obesity effects [13–16]. Prunetin is an *O*-methylated isoflavone, which is a type of flavonoid. Therefore, we hypothesized that prunetin would also have the anti-adipogenesis/anti-obesity effects demonstrated by other flavonoids. Few studies have focused on the biological activities of prunetin except those on its anti-inflammatory effects, and there are no reports on the potential anti-adipogenic effects of prunetin in white adipose tissue (WAT) of obese animals. The present study was designed to investigate prunetin's potential to suppress high-fat diet (HFD)-induced obesity, and to explore the possible transcriptional mechanisms involved in this attenuation.

2. Materials and methods

2.1. Reagents

Dimethyl sulfoxide (DMSO) was obtained from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). Prunetin and all other reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise specified in the text. Prunetin was dissolved in DMSO as a stock solution (70.4 μM) and stored at 4 °C. For intraperitoneal (i.p.) injection, the prunetin stock solution was diluted in distilled water up to 3.52 μM or 7.04 μM.

2.2. Animals

Three-week-old male C57BL/6J mice (15–17 g) were purchased from Daehan Biolink (Daejeon, Republic of Korea). Animals were maintained under conditions that were in accordance with the guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the Institutional Animal Care Committee, Sangji University (Reg. No. 2013-3). Mice were adapted to the modified conditions in 4 weeks prior to the start of the experiment. Thereafter, animals were given free access to food and water for 10 weeks and were housed under a 12-h light/12-h dark cycle at a constant temperature of 22 ± 2 °C and relative humidity of 55 ± 7% throughout the experiment. Mice were randomly distributed into 4 groups of 10 mice each: the normal diet (CON) group, high-fat diet (HF) group, and 2 additional treatment groups that were fed a high-fat diet (HFD) along with daily injections of either 10 μg/kg or 20 μg/kg i.p. prunetin (P10 and P20, respectively). Normal diet and HFD was obtained from Research Diets, Inc. (New Brunswick, NJ, USA). The caloric density and ingredient composition of each diet is shown in Table 1. Body weight and food intake were recorded every week. During the last 3 weeks, prunetin was administered to the P10 and P20 groups, and PBS was administered to the CON and HF groups. At the end of the 10-week period, all animals were fasted for 12 h. The following day, the mice were anesthetized with Zoletil (Virbac, Carros Cedex, France), and blood samples were collected by cardiac puncture. The liver tissue and visceral fat pads were excised, rinsed, weighed, and stored at −70 °C pending further analysis.

2.3. Serum lipid and toxicity markers analysis

Serum concentrations of glucose and total cholesterol (TC) were determined by enzymatic methods using a commercially available assay kit (BioVision Research Products, Inc., CA). Serum concentrations of ALT and CPK were determined with the help of commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA). Serum concentration of BUN was quantified using commercial kit from Bioo Scientific Corp. (Austin, TX, USA).

Table 1
Caloric content and ingredient composition of each diet.

		Normal diet		HFD	
		g%	kcal%	g%	kcal%
Caloric content	Protein	20.3	20.8	24	20
	Carbohydrate	66.0	57.7	41	40
	Fat	11.5	11.5	24	40
Ingredient	Casein-	200	800	200	800
	L-cystine	–	–	3	12
	Corn starch	150	600	315	1260
	Maltodextrin 10	–	–	35	140
	Sucrose	500	2000	350	1400
	Cellulose, BW200	50	0	50	0
	Soybean oil	–	–	25	225
	Corn oil	50	450	–	–
	Lard			20	180
	Mineral mix	35	0	10	0
	Dicalcium phosphate	–	–	13	0
	Calcium carbonate	–	–	5.5	0
	Potassium citrate	–	–	16.5	0
	Vitamin mix	10	40	10	40
	Choline bitartrate	2	0	2	0

2.4. Histological analysis

The liver tissue and visceral fat pads from representative mice in each group were fixed in 4% buffered formalin and embedded in paraffin, and sections of a thickness of 8 μm or 4 μm were cut. The sections were stained with hematoxylin and eosin (H&E) for the histological examination of fat droplets. Images were acquired using an SZX10 microscope (Olympus, Tokyo, Japan).

2.5. Quantitative Real-time PCR analysis

The liver and adipose tissues from each animal were homogenized, and total RNA was isolated using Easy-Blue® Reagent (Intron Biotechnology Inc., Gyeongi-do, Republic of Korea) according to the manufacturer's instructions. Total RNA was quantified using an Epoch® micro-volume spectrophotometer system (BioTek Instruments, Inc. Winooski, VT). Total RNA

Table 2
Primer sequences and PCR conditions.

Gene name	Tm (°C)	Size (bp)	Sequence 5′–3′
PPARγ	55	148	F: TTCGGAATCAGCTCTGTGGA
			R: CCATTGGGTCAGCTCTTGTG
C/EBPα	55	187	F: TCGGTGCGTCTAAGATGAGG
			R: TCAAGGCACATTTTTGCTCC
SREBP1c	55	115	F: ATCGCAAACAAGCTGACCTG
			R: AGATCCAGGTTTGAGGTGGG
LXR	55	119	F: TCCTACACGAGGATCAAGCG
			R: AGTCGCAATGCAAAGACCTG
LPL	55	172	F: TGCCGCTGTTTTGTTTTACC
			R: TCACAGTTTGCTGCTCCCAGC
HMG-CoA	55	285	F: TGGCAGAAAGAGGGAAAGG
			R: CGCCTTTGTTTTCTGGTTGA
aP2	55	128	F: AGCATCATAACCCTAGATGG
			R: GAAGTCACGCCTTTCATAAC
Adiponcetin	55	360	F: GTCACTGTCCCCAATGTTCC
			R: AGAGGCCTGGTCCACATTTT
Leptin	55	143	F: CTCCAAGGTTGTCCAGGGTT
			R: AAAACTCCCCACAGAATGGG
AdipoR1	55	130	F: CCCGTATGATGTGCTTCCTG
			R: GCCAGTCTCTGTGTGGATGC
AdipoR2	55	131	F: CACAGAGACGGGCAACATTT
			R: GGCAGTACACCGTGTGGAAG
AMPK	55	147	F: AAGCCAAATCAGGGACTGCT
			R: TCGAGGGAGGTGACAGATGA