Supplemental Data

Fenofibrate inhibits adipocyte hypertrophy and insulin resistance by activating adipose PPARα in high fat diet-induced obese mice

Sunhyo Jeong and Michung Yoon[1]

Methods

Animals

For all experiments, eight-week-old mice (C57BL/6J) were housed and bred at the Korea Research Institute of Bioscience and Biotechnology under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Mice were divided randomly into three groups (n = 5/group), one of which received a low fat diet (4.5% fat, w/w, CJ, Korea). Another group received a high fat diet containing 35% fat (w/w, Research Diets, New Brunswick, NJ), and the final group was fed the same high fat diet supplemented with fenofibrate (0.05%, w/w) for seven weeks (Jeong *et al*., 2004b; Jeong and Yoon, 2007). The composition of high fat diet is shown in Supplemental Data Table S1. In all experiments, body weights were measured daily using a top-loading balance, and the person who measured the body weight was blinded to each treatment group. Animals were sacrificed by cervical dislocation, and tissues were harvested, weighed, snap-frozen in liquid nitrogen, and stored at -80oC until use. Glucose tolerance tests were performed by intraperitoneal injection (IP) of glucose (2 g/kg body weight) in mice on a high fat diet or the same high diet supplemented with fenofibrate for 7 weeks. Blood glucose were measured using a Accu-Chek Performa System (Roche, Germany) at the indicated time intervals.

3T3-L1 differentiation

Mouse 3T3-L1 cells (ATCC) in 6-well plates were proliferated in DMEM containing 10% bovine calf serum (Gibco-BRL, Grand Island, NY). After cells were kept confluent for two days, they were incubated in induction medium (day 0) containing 1 μM dexamethasone, 0.5 mM 1-methyl-3-isobutyl-xanthin, and 1 μg/ml insulin in DMEM with 10% FBS (Gibco-BRL). The cultures were continued for two more days to induce adipocyte differentiation. Thereafter, cells were cultured in DMEM with 10% FBS for the rest of the differentiation process. All other treatments were administered on day 0 to day 2 only, and medium was changed every other day.

Determination of plasma glucose, insulin, triglyceride, and free fatty acid levels

Levels of triglycerides and glucose were measured using an automatic blood chemical analyzer (CIBA Corning, Oberlin, OH). Levels of free fatty acids and insulin were measured using SICDIA NEFAZYME (Shinyang Chemical, Seoul, Korea) and a rat insulin radioimmunoassay kit (Linco, St. Charles, MO), respectively.

Histological analysis

For hematoxylin and eosin (H&E) staining, epididymal adipose tissues were fixed in 10% phosphate-buffered formalin for one day and processed in a routine manner for paraffin sections. Five micrometer-thick sections were cut and stained with H&E for microscopic examination. To quantitate adipocyte number and size, the H&E-stained sections were analyzed using an image analysis system (Image Pro-Plus, Silver Spring, MD).

RT-PCR

Total cellular RNA was prepared using the Trizol reagent (Gibco-BRL, Grand Island, NY). Two μg total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase and an antisense primer to generate cDNA under standard conditions. cDNA samples were amplified by PCR in a MJ Research Thermocycler (Waltham, MA). The PCR primers used for amplication are shown in Supplemental Data Table S2. The reaction consisted of 30 cycles of denaturation for 1 min at 94oC, annealing for 1 min at 58oC, and elongation for 1 min at 72oC. The PCR products were analyzed by electrophoresis on a 1% agarose gel. PCR products were quantified from agarose gels using the GeneGenius kit (Syngene, Cambridge, UK).

Statistical analysis

Unless otherwise noted, all values are expressed as mean ± SD. All data were analyzed by the unpaired, Student's *t*-test for significant differences between the mean values of each group using SigmaPlot 2001 (SPSS Inc, Chicago, IL).

Table S1. Composition of high fat diet

Diet ingredient	gm	kcal
Casein, 80 Mesh	200	800
L-Cystine	3	12
Maltodextrin 10	125	500
Sucrose	68.8	272
Cellulose, BW200	50	0
Soybean Oil	25	225
Lard	245	2205
Mineral Mix S10026	10	0
DiCalcium Phosphate	13	0
Calcium Carbonate	5.5	0
Potassium, $1H_2O$	16.5	0
Vitamin Mix V10001	10	40
Choline Bltartrate	2	0
FD&C Blue Dye #1	0.05	0
Total	773.85	4057
		kcal%
Protein		20
Carbohydrate		20
Fat		60
Total kcal		100

Table S2. Sequences of primers used for the RT-PCR assays

Genes	GeneBank	Primer sequences	Size (bp)
ACOX	J02752	Forward: 5′-actatatttggccaattttgtg-3′ Reverse: 5′-tgtggcagtggtttccaagcc-3′	195
HD	NM_133606	Forward: 5′-caaaaagatcggaaagattg-3′ Reverse: 5′ -ctgataccaccgtttacctg -3′	355
MCAD	NM 007382	Forward: 5′-gacatttggaaagctgctagtg-3′ Reverse: 5′-tcacgagctatgatcagcctctg-3′	321
Leptin	U18812	Forward: 5′-ccaagaagagggatccctgctccagcagc-3′ Reverse: 5′-agaatggggtgaagcccagga-3′	275
TNFα	D00475	Forward: 5′-ctcgagtgacaagcccgtag-3′ Reverse: 5′-ttgacctcagcgctgagcag-3′	387
β-acitn	J00691	Forward: 5′-tggaatcctgtggcatccatgaaa-3′ Reverse: 5′-taaaacgcagctcagtaacagtcc-3′	350