Supporting Information to:

Green Tea Epigallocatechin Gallate Inhibits Insulin Stimulation of Adipocyte Glucose Uptake via the 67-Kilodalton Laminin Receptor and AMP-Activated Protein Kinase Pathways

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Cell culture

Murine 3T3-L1 adipocytes (American Type Culture Collection; CL-173™) and C3H10T1/2 adipocytes (ATCC-CCL-226) were obtained according to a published method [1, 2], in which 2-day postconfluent 3T3-L1 preadipocytes and C3H10T1/2 preadipocytes were treated with DMEM (pH 7.4) containing a final concentration of 100 units/mL penicillin, 100 μg/mL streptomycin, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 5 μg/mL insulin, and 10% fetal bovine serum (FBS) for 48 h in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. The medium was then changed to DMEM containing 10% FBS and 5 μg/mL insulin for an additional 6 days. The medium was replaced every 2 days. Prior to the experiments, adipocytes were incubated with DMEM containing 10% FBS for 2–4 days.

Mouse C2C12 myoblasts (ATCC; CRL-1772), kindly provided by Dr. Shen-Liang Chen (Department of Life Science, National Central University, Jhongli City, Taiwan), were grown in DMEM supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin, 1% L-glutamine, and 20% FBS. Rat H4IIEC3 hepatoma cells (ATCC-CRL-1600™) obtained from the Bioresource Collection and Research Center (Food Industry Research and Development Institute, Hsinchu, Taiwan) were incubated in DMEM containing 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 20% horse serum, and 5% FBS.

Glucose uptake assay

As previously described by Fong et al. [3] and Liu et al. [4], we determined glucose uptake by adipocytes, myoblasts, and hepatoma cells through glucose transporters using the 2-[1,2-³H]-deoxy-D-glucose (2-DOG) method. Briefly, 3T3-L1 adipocytes (1.2 × 10⁶ cells/well),
C3H10T1/2 adipocytes (2 × 10⁶ cells/well), C2C12 myoblasts (1 × 10⁶ cells/well), and H4IIEC3 hepatoma cells (1.5 × 10⁶ cells/well) were plated in triplicate wells of 12-well plates. Adipocytes and myoblasts were washed twice with 1 mL of Krebs–Ringer phosphate Hepes (KRPH) buffer (pH 7.4) containing a final concentration of 136 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, 5 mM KH₂PO₄, 0.1% (w/v) bovine serum albumin (BSA), and 20 mM Hepes. They were then incubated in 500 μL KRPH buffer. To determine the dose- and time-dependent effects of EGCG on insulin-stimulated glucose uptake, cells were treated with EGCG (0–50 μM) in the presence and absence of insulin (100 nM) for the indicated time periods. After 10 min of incubation, cells in each well were supplemented with 30 nM of 2-DOG (specific activity: 8 Ci/mmol; Perkin-Elmer) for an additional 30-min incubation at 37 °C. The tested H4IIEC3 hepatoma cells were serum-starved for 24 h prior to EGCG treatment and incubated with 2-DOG for 5 min. Cellular glucose uptake was stopped by the addition of 1 mL ice-cold KRPH buffer and then washed twice. Cell pellets in each well were broken with 500 μL RadiolImmuno precipitation assay buffer containing 20 mM Tris-HCl, 150 mM NaCl, 1% (w/v) Triton-X100, 0.1% (w/v) sodium dodecyl sulfate (SDS), and 1% (w/v) sodium deoxycholate. An aliquot of 450 μL of cell lysate was transferred to a vial containing the 4 mL scintillation cocktail (Ready Safe; Beckman Coulter), and then the radioactivity was measured using a beta-counter (Beckman LS-6500). Non-specific glucose uptake was determined in the presence of cytochalasin B (10 μM). After normalization to a non-specific value, the amounts of 2-DOG uptake were expressed as fimoles of 2-DOG uptake per minute per milligram of protein. The amounts of protein in the cell lysate were determined in duplicate with the Pierce BCA protein assay reagent (Pierce Biotechnology) at a wavelength of 562 nm using BSA as the standard.

To compare the effects of different catechins on insulin-induced changes in adipocyte glucose uptake, 3T3-L1 and C3H10T1/2 adipocytes were pretreated for 2 h with 10 μM of (+)-
catechin, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallic acid, or EGCG, and then 100 nM of insulin was added. After 10 min of insulin incubation and an additional 30 min of incubation with 30 nM 2-DOG, levels of 2-DOG uptake were measured.

To compare the effects of EGCG with resistin (Rstn) on insulin-induced changes in adipocyte glucose uptake, 3T3-L1 and C3H10T1/2 adipocytes were pretreated for 2 h with either 10 μM of EGCG or 50 ng/mL of Rstn, and then 100 nM of insulin was added. After 10 min of insulin incubation and an additional 30 min of incubation with 30 nM 2-DOG, levels of 2-DOG uptake were measured.

To study the 67LR-dependent effect of EGCG on insulin-stimulated adipocyte glucose uptake, 3T3-L1 and C3H10T1/2 adipocytes were pretreated with 2 μg/mL of either normal rabbit immunoglobulin (IgG) or the 67LR antibody for 1 h, exposed to 10 μM EGCG for 2 h, and then incubated with 100 nM insulin for 10 min. After 30 min of 2-DOG incubation, 2-DOG uptake levels were determined.

To study the AMPK- and GSH-dependent effects of EGCG on insulin-stimulated adipocyte glucose uptake, 3T3-L1 and C3H10T1/2 adipocytes were pretreated with either 10 μM of compound C (an AMPK inhibitor) [5] or 10 mM of N-acetyl-L-cysteine (NAC; a glutathione activator and reactive oxygen species inhibitor) for 1 h, exposed to 10 μM EGCG for 2 h, and then incubated with 100 nM insulin for 10 min. After an additional 30 min of incubation with 30 nM 2-DOG, we measured the amounts of 2-DOG uptake. These compounds were dissolved in 100% DMSO and then added to the culture medium at a final concentration of 0.1% DMSO.

References


3 Fong JC, Kao YS, Tsai HY, Ho LT. Endothelin-1 increases glucose transporter glut1 mRNA accumulation in 3T3-L1 adipocytes by a mitogen-activated protein kinase-dependent pathway. Cell Signal 2001; 13: 491-497
