Supplementary Methods

Reversibility assay. The recovery of enzymatic activity of PDI was determined by incubating 20 μM PDI (a 100-fold the concentration used in the standard assay) with 10 μM quercetin-3-rutinoside (10-fold the concentration required for inhibition). After 30 minute equilibration at room temperature, the PDI-quercetin-3-rutinoside mixture was diluted 100 fold into phosphate-EDTA reaction buffer containing insulin substrate and DTT as a reducing equivalent. The enzymatic progress curve for this reaction was compared to that of a similar sample of PDI incubated and diluted in the absence of quercetin-3-rutinoside, 0.1 μM quercetin-3-rutinoside (9% inhibition), or 10 μM (91% inhibition) of quercetin-3-rutinoside.

Thrombin-catalyzed fibrin polymerization. Thrombin-catalyzed polymerization of human plasma fibrinogen in the presence of quercetin-3-rutinoside was monitored in a 96-well plate as the turbidity changes using SpectraMax PC microtiter plate reader (Molecular Devices, Sunnyvale, CA) as modified from a previous method (50). Briefly, human plasma fibrinogen (Calbiochem, La Jolla, CA) was dialyzed against HBS buffer (20 mM Hepes, pH 7.4, 150 mM NaCl and 1 mM $CaCl_2$). Quercetin-3-rutinoside (Sigma-Aldrich, St. Louis, MO) was added at various concentrations to reaction wells containing 80 μL of 1.25 mg/mL fibrinogen in HBS. Polymerization was initiated by the addition of 10 μL of 1 U/mL human thrombin (Haematologic Technologies, Inc., Essex Junction, VT) and the turbidity at was monitored at 350 nm for 40 minutes at room temperature.

Analysis of quercetin-3-rutinoside plasma levels. For evaluation of plasma levels of quercetin-3-rutinoside following intravenous infusion, either 0.3 mg/kg or 0.5 mg/kg quercetin-3-rutinoside were infused into mice through a jugular cannulus. Blood samples were obtained by cardiac puncture at 5, 30, and 90 min following infusion, since thrombi were formed from 5-90

min following intravenous infusion. For evaluation of quercetin-3-rutinoside following oral infusion, 50 mg/kg quercetin-3-rutinoside was administered by oral gavage. Blood samples were obtained by cardiac puncture at 90, 120, and 180 minutes following oral administration, since thrombi were formed from 90-180 min following oral administration. Quercetin-3-rutinoside levels were measured by LC/MS/MS using an Applied Biosystems MDS SCIEX API 3000 instrument at Integrated Analytical Solutions. Levels were quantified by comparison with a standard curve of quercetin-3-rutinoside and represent the mean+/-SEM of triplicate values for each time point.

Supplementary Table 1: Plasma concentration of quercetin-3-rutinoside after intravenous or oral administration in mice.

Sample	Dose	Time (min)	Plasma Concentration (μM)
Intravenous	0.3 mg/kg	5	0.557 +/- 0.209
		30	0.081 +/- 0.014
		90	0.002 +/- 0.001
	0.5 mg/kg	5	0.363 +/- 0.073
		30	0.084 +/- 0.027
		90	0.002 +/- 0.001
Oral	50 mg/kg	90	0.041 +/- 0.009
		120	0.016 +/- 0.013
		180	0.003 +/- 0.001

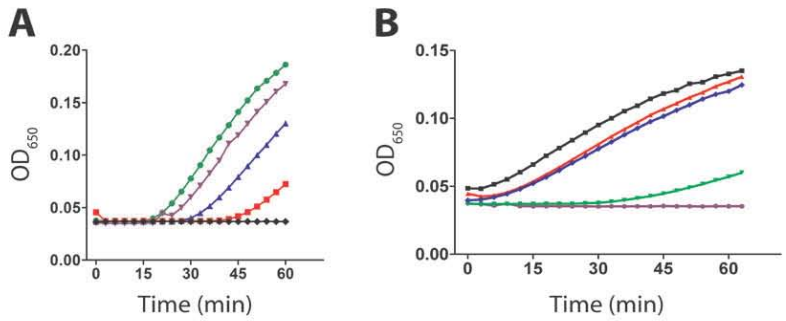
Supplementary Figures

Supplementary Figure 1. (A) PDI activity was measured with the insulin reduction assay in the presence of control buffer (*green*) or presence of 0.3 µM (*purple*), 3 µM (*blue*) or 30 µM (*red*) quercetin-3-rutinoside. The black line indicates the rate of insulin reduction by DTT in the absence of PDI. (B) Reversibility of inhibition of PDI by quercetin-3-rutinoside. PDI (20 µM) was incubated with 10 µM quercetin-3-rutinoside for 30 minutes and subsequently diluted 100-fold into assay buffer (*blue*). Insulin reductase activity was compared to samples containing no PDI (*purple*) or 200 nM PDI in the absence (*black*) or presence of 0.1 µM (*red*) or 10 µM (*green*) quercetin-3-rutinoside. *Blue* line denotes rapid recovery of enzymatic activity after dilution of the PDI-quercetin-3-rutinoside complex.

Supplementary Figure 2.

Quercetin-3-rutinoside does not affect endothelial cell activation or thrombin-catalyzed fibrin polymerization. HUVECs were loaded with Fluo-4-AM (3 μM) and observed using fluorescence microscopy. Representative images of a field of cells before and after a direct laser pulse. An increase in Fluo-4 fluorescence (*green*) reflects a rise in intracellular Ca^{2+} before (A) and after (B) treatment with quercetin-3-rutinoside. (C) Laser-induced activation of Fluo-4 fluorescence in HUVECs is plotted against time showing the mean trace of cells prior to (*black line*) or following (*gray line*) exposure to quercetin-3-rutinoside (n = 5). (D) Effect of quercetin-3-rutinoside on thrombin-catalyzed fibrin polymerization. Human plasma fibrinogen was incubated with 0.1 U/ml thrombin in the presence of vehicle *(black)*, 1 U/ml hirudin *(red)*, or quercetin-3-rutinoside at 15 μM *(blue)* and 75 μM *(green)*. **Original magnification X 60. Scale bars: 10 μm.**

Supplementary Figure 1



Supplementary Figure 2