SUPPLEMENTARY DATA

Oxygen consumption measurements

Cellular oxygen consumption rate (OCR) was measured using an XF24 Analyzer (Seahorse Bioscience) at 37°C. HAEC cells were seeded at 40,000 cells per Poly-D-lysine-coated well 24 h prior to the analysis. On the day of the experiment, cells were treated with EBM-2 medium containing either 5 mM or 25 mM glucose for 5 hours. 1 h before measurement, cells were washed with sodium bicarbonate free DMEM assay medium at pH 7.4 and supplemented with 0.4% (v/v) fetal bovine serum, 5 mM HEPES, 0.4 mM KH_2PO_4, EGM2 growth factor mix from Lonza, 2 mM GlutaMAX, and 5 or 25 mM glucose in the absence of pyruvate (total exposure to 25mM glucose was 6h). After a wash, 675 µL of the assay medium was added to each well and cells were cultured for 1h at 37°C under ambient conditions. A total of 12 OCR measurements at different time points were performed. Oligomycin (5 µg/µL), FCCP (500 nM), and rotenone (5 µM) were sequentially injected after measurements 3, 6, and 9, respectively. These measurements were used to calculate maximal oxygen consumption rate as previously described (1; 2). Data are shown as mean of at least four independent experiments ± SEM.

Determination of Intracellular Free Iron. Free iron was measured by Electron Paramagnetic Resonance (EPR) using a modification of the method described by Srinivasan and colleagues (3). Briefly, HAEC (107/sample) were scraped with a cell scraper in PBS and spun down at 1,000 x g for 10 min. The cell pellet was resuspended in 1ml of PBS and recentrifuged. The cell pellet was then resuspended in 200 µl of assay buffer containing 20 mm Tris-Cl, pH 7.4, 10% glycerol and 2mM deferoxamine mesylate. 200µl of each sample was transferred into an EPR tube, and the sample was immediately frozen on dry ice or liquid nitrogen and stored at –70°C until EPR measurements were performed.

In this study, the g'=4.3 signal was used to monitor the concentration of chelatable free iron present. The amount of the DF:Fe3+ complex was determined by measuring the amplitude of the g'=4.3 EPR peak. Spin concentrations were estimated by comparing the amplitude of the g'=4.3 signal with spectra obtained from standards of known concentrations of DF:Fe3+ in buffer solution. (4).

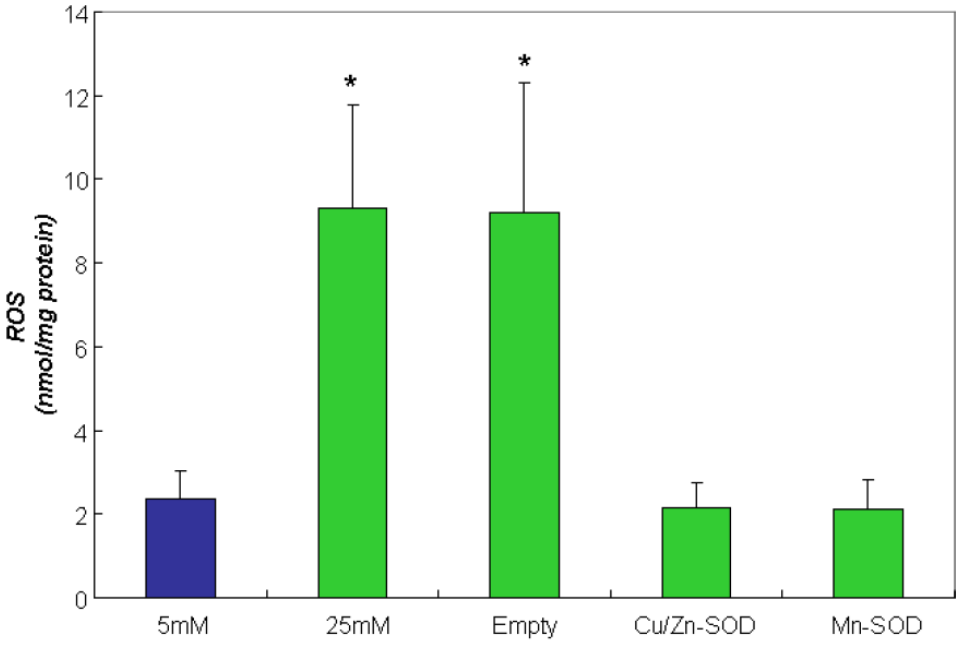
EPR spectra were registered on a Varian E-line 112 X-band spectrometer equipped with a rectangular TE102 resonator. A liquid nitrogen finger dewar was inserted into the resonator to keep the sample at 77K during the measurement. Typical spectral acquisitions parameters were as follows. EPR frequency: 9.11 GHz; power: 20 mW; modulation amplitude: 20 G; time constant: 0.5 seconds; scan time: 2 minutes; number of scans averaged: 4 – 9.

Effect of GLP-1(9-36)^{amide} on Akt1 Activity

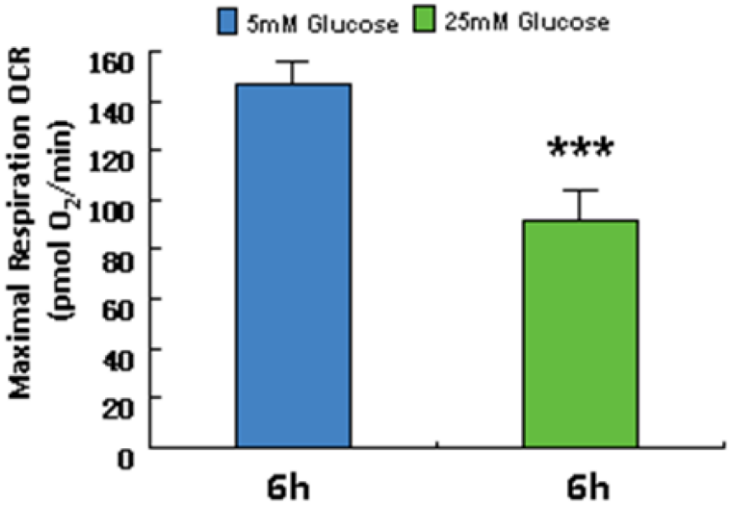
Cells were washed in cold PBS 1X and lysed in 300 µl of lysis buffer (137mM NaCl, 20 mM Tris-HCL pH7.5, 1 mM MgCl2, 1 mM CaCl2, 10% glycerol, 1% NP40, protease inhibitors tablet (Roche), phosphatase inhibitors cocktail 1+2 （Sigma）, 100mM NaF, 10mM NA-Pyrophosphate, 1 µM microcystein. Cells were rocked at 4°C for 20 min, and then scraped off and centrifuged (13000 g, 5mn, 4°C). Protein was quantitated by the Bradford method. 1 mg of cell lysate was added to antibody-protein A agarose mixtures for IP, and rotated overnight at 4°C. Immunoprecipitates were washed with 1 ml of 1% NP40/PBS 1X three times, in ice cold Buffer B (50 mM Tris-HCL, pH7.5, 0.1 mM EGTA, 0.01 % NP40, 0.1% 2-mercaptoethanol) 4 times, and then resuspended in a final volume of 60 µl of ice cold assay buffer (50 mM Tris-HCI, pH7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 10 uM PKA inhibitor peptide, 10 mM Mg Acetate, 7. 5 mM MgCl2). Akt kinase activity was measured in anti-Akt immunoprecipitates using 200 µM Akt substrate crosstide peptide (AKT/SGK). The phosphotransferase reaction began upon the addition of the peptide and 5µl of ATP mix (1 µl of 10 mM unlabeled ATP, 1 µl of 1 mCu [γ ^{32}P] ATP, 3 µl of H20) and was allowed to proceed for 15 min at 30°C. The reaction was stopped by adding 20 µl of 10% TCA and 3 µl of 10% BSA. After 15 min on ice and 1h at 4°C, samples were centrifuged (13000g/min) and 50 µl of the reaction mixture was applied to P81 ion exchange chromatography cellulose phosphate papers. Papers were washed 4 times in 50 mM Phosphoric acid (H3P04) for 30 min each. The phosphocellulose papers were washed briefly in acetone, quick dried, placed in scintillation vials, and radioactivity was measured.

SUPPLEMENTARY DATA

Supplementary Figure S1. ROS levels after 6h of exposure to 25mM glucose. Cells were transfected the night before with empty vector or adenovirus expressing either Cu/Zn-SOD or Mn-SOD and then exposed to 25mM glucose for 6 hours. Data are shown as mean ± S.E.M..* p<0.05



Supplementary Figure S2. Effect of high glucose exposure on maximal mitochondrial respiration. Maximal oxygen consumption rate (OCR) in HAEC exposed to 25mM glucose for 6hr (green bar). Cells exposed to 5mM glucose were used as controls (blue bars). Data are shown as mean ± S.E.M. from at least 4 independent experiments.*** p<0.001 Student's t-test.



SUPPLEMENTARY DATA

Supplementary Figure S3. Effect of transient exposure to GLP-1 (9-36)^{amide} and its precursor peptide GLP-1 (7-36)^{amide} on ROS generation two days after transient exposure to 25mM glucose.
GLP-1 (7-36)^{amide} in the absence of GLP-1 protease inhibitors was able to reverse the increased ROS production (bar 5). However, blocking DDPIV and the membrane metallo-endopeptidases involved in GLP-1 cleavage completely blocked the effect of GLP-1 (7-36) ^{amide} (bar 6), indicating that the peptide responsible for disrupting the ROS generating loop is GLP-1 (9-36)^{amide}. Consistent with this hypothesis, transient GLP-1 (9-36)^{amide} treatment two days after transient exposure to 25mM glucose completely reversed the increased ROS production (bar 4). Data are shown as mean ± S.D. p<0.05*

SUPPORTING REFERENCES

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