Electronic supplementary material

Methods

Body composition measurements Lean body mass, fat mass and free body fluid were measured in conscious mice using nuclear magnetic resonance (MiniSpec LF50; Bruker Optics, The Woodlands, TX, USA). Gonadal fat weight was recorded as an additional quantitative estimate of body fat.

Immunoblot and immunohistochemistry MIN6 cells and kidneys from wild-type mice were lysed and homogenised in RIPA buffer (Boston Bioproducts, Boston, MA, USA) containing an EDTA-free protein inhibitor cocktail (Roche, Indianapolis, IN, USA). Protein concentration was determined by BCA protein assay (Pierce Biotechnology, Rochford, IL, USA), and 100 μg protein was separated by electrophoresis (4% to 20% TRIS-glycine gel; BioRad, Hercules, CA, USA) and transferred to a polyvinylidene fluoride membrane (Millipore). The membrane was blocked in Odyssey blocking buffer (LI-COR, Lincoln, NE, USA) at room temperature for 1 h, then incubated with MR antibody (Ab2B7, 1:400 dilution; courtesy of C. Gomez-Sanchez and E. Gomez-Sanchez, University of Mississippi, Jackson, MS, USA) in Odyssey blocking buffer + 0.1%Tween-20 and GAPDH antibody (1:5000 dilution; Abcam, Cambridge, MA, USA) at 4ºC overnight. The membrane was washed five times with blocking buffer + Tween-20, incubated for 1 h at room temperature with an IRDye800-conjugated goat anti-mouse IgG secondary antibody and washed with blocking buffer + Tween-20 before detection by infrared imaging system (Odyssey; LI-COR).

Adult pancreases were collected and immunohistochemistry was performed as previously described [1]. Antibodies to insulin (1:500; Dako, Carpinteria, CA, USA), glucagon (1:10,000; Abcam), pancreatic polypeptide (1:200; Bachem, Torrance, CA, USA), somatostatin (1:1000; American Research Products, Belmont, MA, USA) and CD31 (1:200; BD Pharmingen, San Diego, CA, USA) were visualised using appropriate secondary

antibodies conjugated with Cy2, Cy3 and Cy5 fluorophors (all at 1:500) from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Monoclonal antibodies derived in mice against MR peptides 1–18 and provided by E. Gomez-Sanchez and C. Gomez-Sanchez (University of Mississippi) were used at a dilution of 1:1000 [2].

References

1. Berglund ED, Li CY, Poffenberger G et al (2008) Glucose metabolism in vivo in four commonly used inbred mouse strains. Diabetes 57:1790–1799

2. Gomez-Sanchez CE, de Rodriguez AF, Romero DG et al (2006) Development of a panel of monoclonal antibodies against the mineralocorticoid receptor. Endocrinology 147:1343–1348