SUPPLEMENTARY DATA

Supplementary Table 1. Effect of methazolamide on glucosuria in *db/db* mice.

Parameters measured	Vehicle	MTZ	Metformin	MTZ and Metformin
Body weight (g)	40.8 ± 1.6	41.3 ± 0.9	42.9 ± 0.6	40.5 ± 0.7
Urine glucose (mM)	38.3 ± 2.2	31.3 ± 1.6*	34.3 ± 1.7	34.6 ± 2.0
Total urine glucose excretion (mmol/24h)	0.38 ± 0.10	0.19 ± 0.04	0.18 ± 0.03	0.14 ± 0.02
Urine volume (ml/day)	9.5 ± 2.3	6.0 ± 1.3	5.2 ± 0.9†	3.4 ± 0.7‡
Water intake (ml/day)	11.2 ± 2.3	7.9 ± 1.1	6.6 ± 0.9	5.8 ± 0.8
Urine pH	7.0 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	7.0 ± 0.1

Db/db mice treated with vehicle (50% PEG400), 20 mg/kg/d MTZ, 300 mg/kg/d metformin or MTZ and metformin in combination for 14 days. Data are means ± SE (n=7 per treatment group). Animals were held in metabolic cages for 24 hours. Water intake was measured and urine collected to determine glucose and pH levels. *P=0.014, †P=0.038 and ‡P=0.005 vs. vehicle-treated mice.

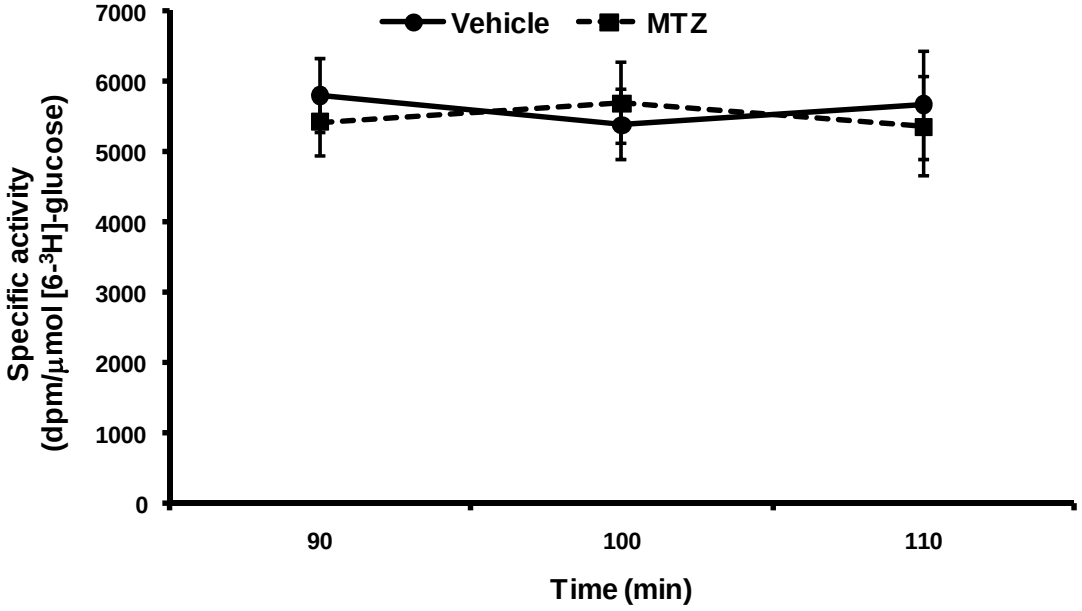
Supplementary Table 2. Effect of methazolamide on whole-body energy balance in DIO mice.

	Vehicle	MTZ
Body weight (g)	29.2 ± 0.8	28.3 ± 1.1
Epididymal fat mass (g)	0.7 ± 0.1	0.6 ± 0.1
Food intake (g/d)	2.8 ± 0.5	2.4 ± 0.3
Water intake (ml/d)	3.1 ± 0.6	4.3 ± 0.7
Cumulative water intake (ml/16 days)	75.5 ± 12.4	85.3 ± 7.8
Urine volume (ml/d)	1.4 ± 0.3	1.5 ± 0.3

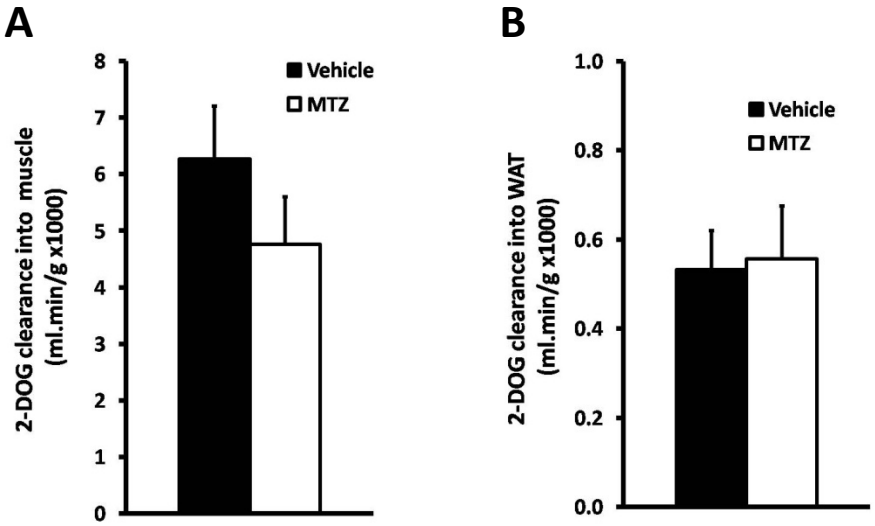
DIO mice treated with vehicle (35% PEG400) or 50 mg/kg/d MTZ for 16 days. Data are means ± SE (n=5-6). Animals were held in metabolic cages for 24 hours. Food and water intake as well as urine excreted was measured. Total water intake during the duration of the study (16 days) is also shown.

SUPPLEMENTARY DATA

Supplementary Figure 1. Specific activity of [^{3}H]glucose in DIO mice treated with vehicle or MTZ. DIO mice were treated with vehicle (35% PEG400; black circles) or 50 mg/kg/d MTZ (black squares) for 14 days. Steady-state was achieved for the glucose infusion rate (GIR) during the hyperinsulinemic-euglycemic clamps in DIO mice. Data are means ± SE (n=10-16).

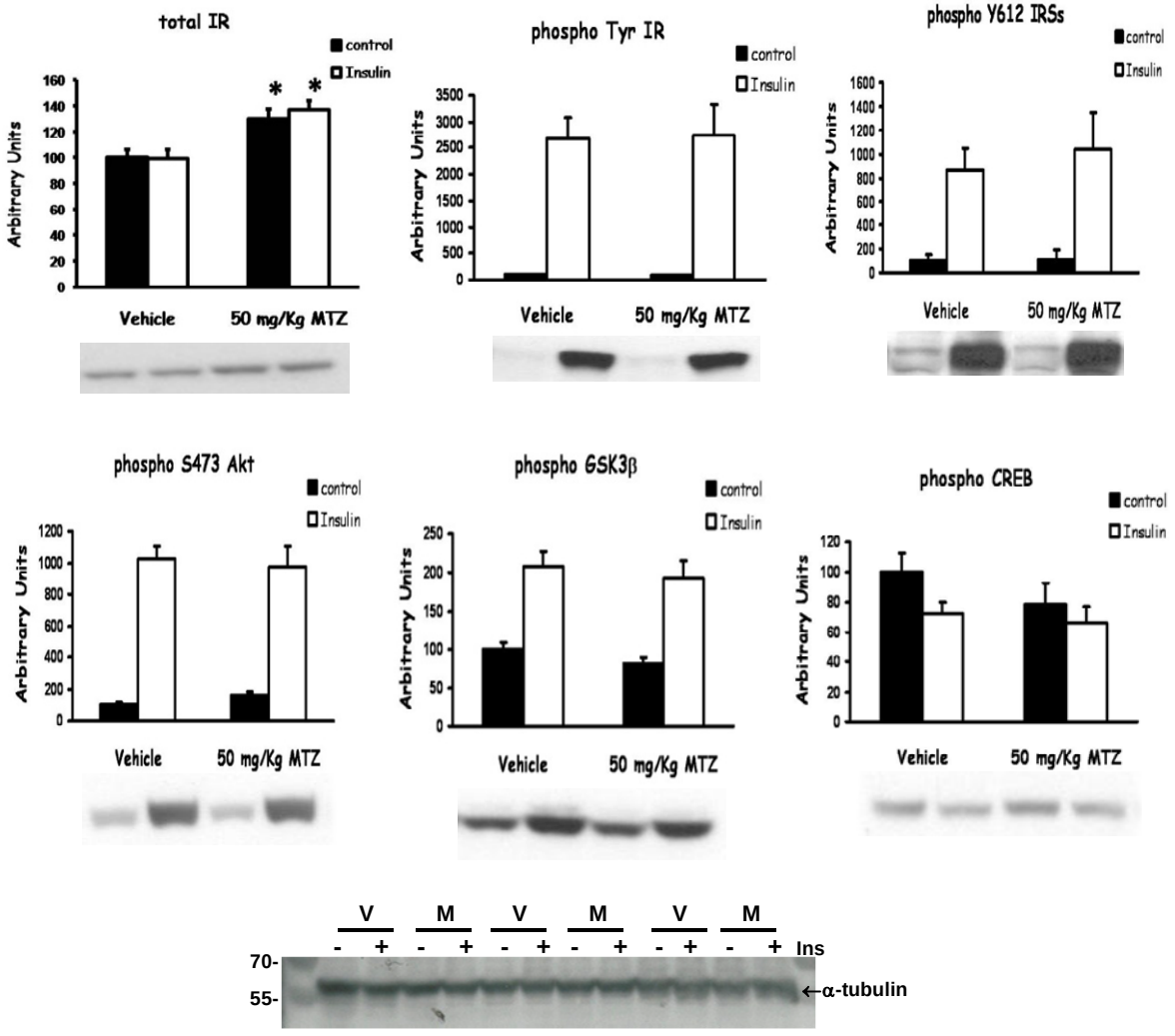


Supplementary Figure 2. Methazolamide did not affect glucose clearance into muscle and white adipose tissue in DIO mice. DIO mice treated with vehicle (35% PEG400) or 50 mg/kg MTZ for 14 days were administered with 10 µCi ^{3}H-2-deoxy-glucose during an i.p. glucose tolerance test (2 g/kg glucose). *A:* Quadriceps and *B:* epididymal white adipose tissues (WAT) were collected after 90 min and 2-DOG content was determined. Data represent the means ± SE of 6-7 animals per group.



SUPPLEMENTARY DATA

Supplementary Figure 3. Methazolamide did not affect the activation of insulin-dependent signalling pathways in liver of DIO mice. DIO mice after 10 weeks of feeding were used. MTZ (M; 50 mg/kg/d) or vehicle (V; 45% PEG400) was administered by a single daily oral gavage for 14 days (n=10 per group) 1 h before the beginning of the dark cycle. Animals were fasted overnight, anaesthetised (Ketamine/Xylasil) and a liver sample was taken (control = black bars). Insulin (0.5 U/kg) was injected via the inferior cava vein and a second liver sample was removed after 3 min (Insulin = white bars). Samples were snap-frozen, pulverised and resuspended in 1 x HES buffer, pH 7.4 with freshly added protease and phosphatase inhibitors to prepare whole cell lysates. Protein concentration was determined by the BCA method. 15 μg of protein was resolved by SDS-PAGE under reducing conditions and the levels of the insulin receptor (IR) protein and phosphorylation on Y1149/Y1150, insulin receptor substrates (IRS) phosphorylation on Y612, Akt phosphorylation on S473, glycogen synthase kinase β (GSK3β) phosphorylation on T38, carbohydrate responsive element binding protein (CREB) phosphorylation on S133 and α-tubulin (additional loading control) was measured by immunoblotting followed by ECL. Intensity of bands was quantified using densitometry and Image J software analysis. A ~30% increase in insulin receptor protein levels was observed in MTZ-treated mice, which may contribute to an enhanced response during high circulating insulin levels. Data represents the means ± SE of 10 animals per group. *$P \leq 0.05$ vs. corresponding vehicle group.



SUPPLEMENTARY DATA

Supplementary Figure 4. Methazolamide did not affect glycogen and triacylglyceride synthesis from glucose in livers from DIO mice. DIO mice treated with vehicle (35% PEG400) or 50 mg/kg MTZ for 14 days were administered with 10 μCi ^{14}C-glucose during an i.p. glucose tolerance test (2 g/kg glucose). Livers were collected after 90 min and the rate of glucose incorporation into *A:* glycogen and *B:* triacylglycerides (TAG) were determined. *C:* Total hepatic glycogen and *D:* total hepatic TAG levels. For determination of glycogen content, liver samples (40-60 mg) were digested in 200 □l 1 M KOH at 70°C for 20 min. 75 μl of saturated Na_2SO_4 and 1.75 ml of 95% ethanol were added to precipitate glycogen. Samples centrifuged at 10,000 x g at 4ºC for 15 min, and the glycogen pellet dissolved in 200 □l distilled water at 70°C for 10 min and then washed with 1.8 ml of 95% ethanol followed by centrifugation at 10,000 x g at 4ºC for 15 min. 1 ml amyloglucosidase buffer (0.3 mg amyloglucosidase per 1 ml 0.25 M acetate buffer, pH 4.75) was added to glycogen pellet and agitated overnight at 37ºC in shaking incubator. 500 μl of resulting glycogen digest was suspended in 7 ml Ultima Gold XR scintillation fluid and counted using a 2900TR-LSA scintillation counter (Packard Bioscience, PerkinElmer Life Sciences, Melbourne, AUS). The [U-^{14}C]-glucose clearance into glycogen was corrected for weight of tissue sample used and area under the curve for blood radioactivity (during the glucose tolerance test) as follows: [U-^{14}C]-glucose clearance into glycogen (ml.min/g x 1000) = ((DPM per 0.5 ml) x 2) / g of tissue) x 1000. The total glucose content in the glycogen digest samples was assayed with Sigma's Glucose Oxidase kit, and data was corrected by the weight of the tissue sample used. For determination of TAG content, 40-60 mg liver was finely chopped and added to 4 ml of chloroform/methanol (2:1) and rotated overnight at 4ºC. 2 ml of 0.6% saline was then added; samples were mixed thoroughly and centrifuged at 2,000 x g at 4ºC for 10 min. The lower chloroform phase (TAGs) was collected and evaporated under nitrogen at 45°C. The dried extract was dissolved in 250 μl of 100% ethanol, and assayed using Triglyceride E kit (Wako Pure Chemical Industries Ltd, Osaka, Japan). Data represent the means ± SE of 6-7 animals per group.