Online Supplement

Increasing peripheral insulin sensitivity by PTP1B deletion improves control of blood pressure in obesity

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Short title: Correction of hypertension in *db/db* mice

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Material and Methods

Animal model

To study the cardiovascular consequences of correcting insulin resistance in obese mice, four groups of mice were generated by crossing obese leptin receptor deficient (*db/db*) mice with mice presenting an increased in insulin sensitivity thanks to the deletion of the molecular restraint of the insulin signaling pathway: the protein tyrosine phosphatase 1B[1, 2] (PTP1B, Goodman Cancer Center of McGill University). Because db/db mice are sterile, progeny were generated from dual heterozygotes (H_{db}, heterozygous for mutant leptin receptor, H_{PTP1B}, heterozygous for PTP1B gene). As previously described[3], this breeding strategy yield i) dual heterozygous littermates ($H_{db}H_{PTP1B}$), used as lean control, ii) lean insulin sensitive mice heterozygous for *db* but deficient in PTP1B ($H_{db}K_{PTP1B}$), iii) obese insulin resistant animals deficient in db and heterozygous for PTP1B ($K_{db}H_{PTP1B}$) and iiii) obese insulin sensitive mice deficient in *db* and PTP1B genes ($K_{db}K_{PTP1B}$). Males only were used for the study. Mice were housed in an American Association of Laboratory Animal Care–approved animal care facility at Georgia Health Sciences University, and the Institutional Animal Care and Use Committee approved all protocols.

Metabolic Measurements:

Fasting blood glucose was assessed using a glucometer (Medisense, Bedford, MA, USA). Plasma total cholesterol, triglycerides and NEFA were assessed with colorimetric assays (Wako, Richmond, VA, USA). Plasma insulin, leptin, T3, T4, PTH, C-Reactive Protein and Resistin levels were determined using colorimetric assays from ALPCO Diagnostics (Salem, NH, USA). Plasma aldosterone levels were measured by radioimmunoassay (Siemens Medical Diagnostic Deerfield, IL)[4]. A different set of mice was used to measure food and water intake as well as urine electrolytes concentration and albuminuria (Elisa kit, ALPCO Diagnostics, Salem, NH, USA). Mice form the 4 groups were placed in metabolic cages. After 3 days of acclimation to the cages, food and water consumption were determined and urine collected for 24 hours.

In vivo blood pressure measurement

At 10-12 weeks of age, mice were instrumented with telemetry transmitters to record blood pressure (BP) and heart rate (PA-C10, Data Sciences, Saint Paul, Minn). Transmitters were implanted as described previously [4, 5]. After 7 to 12 days of recovery from surgery, necessary for the mice to gain their initial body weight, baseline data were recorded for 7 days. After one week of treatment, mice were euthanized. Tissues and plasma were collected for later analysis. Blood pressure values were obtained at 10 minute intervals for the duration of the study. Mean values were collected from 24-hour averages. To assess variability in blood pressure, frequency analysis was performed on 7 complete days of telemetry recording (~1400 pts) and the standard deviation of the data as well as the frequency of blood pressure over 140 mmHg were compared. 140 mmHg was selected as a reference for the limit of autoregulation in many vascular beds and a likely threshold of tissue injury. In a separate set of mice, the carotid artery and jugular vein were catheterized under isoflurane anesthesia to measure BP response to ganglionic blockade, as previously described[5].

Renal Morphology

Renal structural alterations were analyzed by the UW Pathology Research Services Laboratory. Briefly, kidneys obtained from $H_{db}H_{PTP1B}$, $H_{db}K_{PTP1B}$, $K_{db}H_{PTP1B}$ and $K_{db}K_{PTP1B}$ mice were immersion-fixed in 10% neutral-buffered formalin. Tissues were embedded in paraffin using standard methods; sectioned (2μm); and stained with silver methenamine. For each animal, section areas were randomly photographed under ×100 magnification. The glomerular cross-sectional area and the amount of silver stained matrix were measured in 15 glomeruli from each animal. The percentage of matrix was calculated using ImagePro Plus image analysis software as described previously[6, 7].

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