Is bladder enlargement in rodent models of diabetes caused by diabetic polyuria? A mega-study without sacrificing animals for study purposes

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Introduction

As each animal model has limitations, robust conclusions require using various models, which raises ethical questions due to a large number of required animals. The urinary bladder is enlarged in animal models of type 1 diabetes (about doubled weight) and many, but not all models of type 2 diabetes [1].

The prevailing hypothesis explaining bladder enlargement in diabetic animals is that it is caused by diabetic polyuria. This hypothesis is based on repeated observations that sucrose feeding causes a similar degree of diuresis and of bladder enlargement as observed in the rat model of type 1 diabetes induced by injection of streptozotocin (STZ). This hypothesis implies that polyuria and bladder enlargement occur when blood glucose concentrations exceed the renal reabsorption threshold of 9-10 mM, and that blood glucose levels and bladder enlargement should be tightly correlated. However, correlations of glucose levels and degree of bladder enlargement were of moderate strength only in an analysis at the group level dominated by studies in the rat STZ type 1 diabetes model [1], raising doubt about the diabetic polyuria hypothesis and particularly its application to type 2 diabetes.

To test the diabetic polyuria hypothesis, we have performed an analysis of 16 rodent studies with 2-8 arms each (including various dietary interventions and pharmacological treatments) representing 9 distinct rat and mouse models of type 1 and 2 diabetes and obesity and a total of 513 animals. Within each study and in a pooled analysis of all studies, correlations between blood glucose levels and bladder weight were determined based on data from individual animals.

To address the ethical challenge of using data from many animal models and treatments for answering a simple question, we did not perform dedicated studies; rather we built a network of diabetes specialists who contributed data from ongoing studies designed and performed for other purposes; this enabled an unprecedented large number of models to be included without sacrificing a single animal for our scientific purposes.

Methods and Materials

The following models were analyzed:

<u>Type 1 diabetes models:</u>

Two studies in rats injected with STZ, one of them with additional treatment with the SGLT2 inhibitor empagliflozin or the dipeptidylpeptidase 4 inhibitor linagliptin in both control and STZ rats;

Rat insulin promotor lymphocytic choriomeningitis virus (RIP-LMCV) mice as additional type 1 diabetes model.

<u>Type 2 diabetes models:</u>

Two studies in obese as compared to lean ZSF1 rats, being observed up to an age of 20 or 28 weeks, each including multiple treatment arms such as canoletta (hydrogenated rapeseed oil), 0% choline/0.2% methionine, Amylin liver NASH diet, or the PPAR-α/δ agonist elafibranor;

three studies in fructose-fed rats observed for 16 or 20 weeks;

one study in rats with neonatal STZ injection;

one study in insulin receptor substrate 2 knock-out as compared to wild-type mice;

two studies in ob/ob mice with one of them also including an arm with db/db mice;

three studies administered a high-fat diet in mice, one of them having an arm with treatment with the glucagon-like peptide-1 analog semaglutide.

Each of the 16 studies had been approved by the applicable ethical committee.

Presence and extent of bladder enlargement was related to blood glucose levels in two ways: Firstly, presence of bladder enlargement was tested relative to glucose levels exceeding the renal glucose reabsorption threshold. Second, within-study correlation analysis was performed based on all animals of a study. To combine all models and studies, data from the hyperglycemic/diabetic groups were expressed as % of the mean of the corresponding euglycemic control group. r^2 as derived from linear regression analysis served as an indicator of strength of correlation.

Results

Presence of bladder enlargement was not consistently associated with glucose levels above (present in six, largely absent in two studies), around (present in two, absent in three studies) or below the renal reabsorption threshold of 9-10 mM (present in one, absent in three studies).

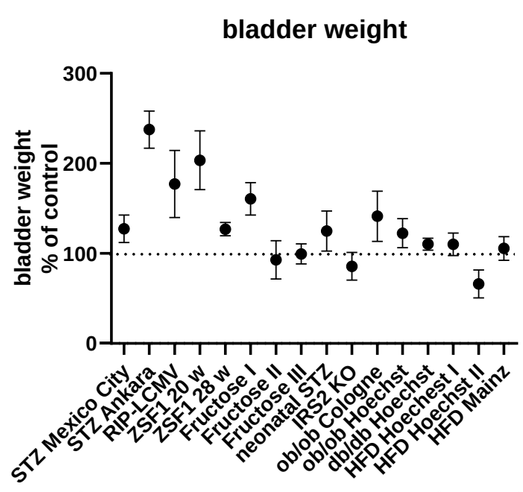

Figure 1. Bladder enlargement across animal models, expressed as % of mean of matched control group.

While correlation between glucose level and bladder weight was strong in one model (RIP-LCMV mice; r^2 0.7226, $p<0.0001$), it was weak to moderate in some (e.g., STZ injection, r^2 0.2346 and 0.3795, $p<0.0001$ each, or in some fructose-fed rat studies) and fully absent in many others (e.g., diet-induced obesity); in one study in fructose-fed rats the correlation numerically was inverse (r^2 0.1979, $p=0.1473$). Within the multi-armed studies, various diets and pharmacological treatments did not consistently change glucose levels and bladder weight in the same way. When looking at all models combined, glucose levels statistically explained only about 5% of the variability in bladder weight (r^2 0.0621, $p<0.0001$).

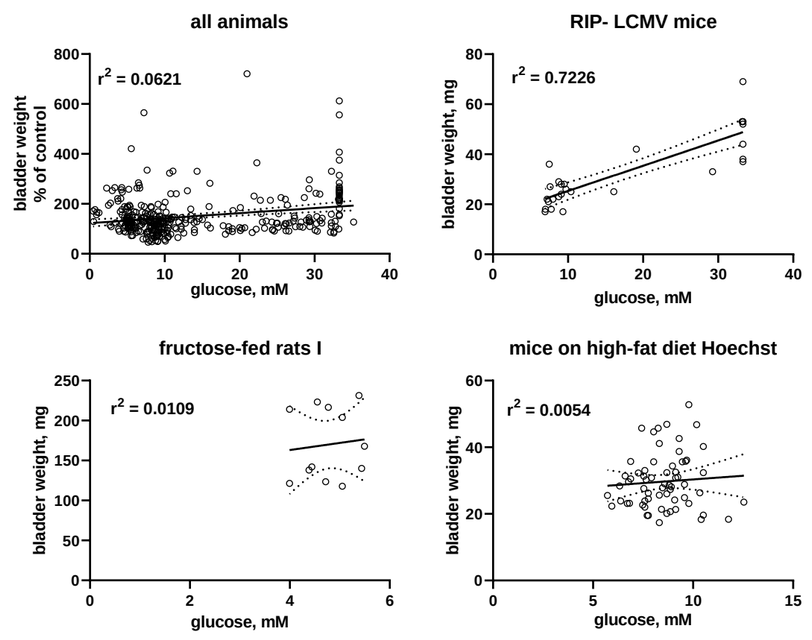

Figure 2. Correlation between glucose level and bladder weight across all studies and in three presentative studies.

Discussion

The weak to moderate correlations between glucose levels and bladder weight and the lack of various diets and pharmacological treatments to consistently affect both in a similar way do not support the diabetic polyuria hypothesis of diabetes-associated bladder enlargement. Importantly, robust conclusions on the diabetic polyuria hypothesis were only feasible by using many distinct animal models and interventions.

Conclusions

Our study does not support the prevailing hypothesis of diabetes-associated bladder enlargement based on an unprecedented number of included models, treatments, and animals.

Based on smart planning and use of animals from studies performed for other aims, this was possible without sacrificing a single animal for the purpose of our study, thereby implementing the 3R principles of animal research in an exemplary manner. This may be an approach suitable for studying the bladder in disease states where multiple animal models exist.

References

[1] Ellenbroek et al. Neurourol Urodyn 37: 2346-2360, 2018.

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