A systematic review of urinary bladder hypertrophy in experimental diabetes: Part 2. Comparison of animal models and functional consequences

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Aims: To explore whether the bladder hypertrophy consistently seen in rats upon streptozotocin injection also occurs in other animal models of type 1 or 2 diabetes and how hypertrophy is linked to functional alterations of the urinary bladder.

Methods: A systematic search for the key word combination “diabetes,” “bladder,” and “hypertrophy” was performed in PubMed; additional references were identified from reference lists of those publications. All papers were systematically extracted for relevant information.

Results: Models other than streptozotocin-injected rats and female animals have been poorly studied. Most animal models of diabetes exhibit less bladder hypertrophy as compared to streptozotocin-injected rats. However, this is not linked to type 1 versus 2 diabetes models, and type 2 models with comparable elevation of blood glucose may exhibit strong or only minor hypertrophy. Bladder dysfunction is frequently observed in experimental diabetes and mostly manifests as increased compliance but does not segregate with hypertrophy. It may at least partly reflect the need to handle large amounts of urine in models associated with major elevation of blood glucose.

Conclusions: To better understand the relevance of bladder hypertrophy in many models of experimental diabetes, more studies in models of type 2 diabetes are urgently needed. Moreover, the role of factors other than hypertrophy in the genesis of bladder dysfunction requires further exploration.

KEYWORDS
animal model, bladder, diabetes, hypertrophy, polyuria

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes (T2DM) is a major global healthcare problem. Lower urinary tract dysfunction and, more specifically, bladder dysfunction occur in 80% and 50% of diabetic patients, respectively. Injection of the pancreatic β-cell-destroying toxin
streptozotocin (STZ) is the most frequently used animal model of diabetes, most often applied to rats. As summarized in the first part of our systematic review, STZ-injected rats consistently exhibit urinary bladder hypertrophy, corresponding to about a doubling in weight. However, the translational value of the rat STZ model remains unclear for multiple reasons: First, STZ is not as selective as previously assumed and can also directly affect other tissues and cell types including afferent nerves, which may directly impact bladder function. Second, injection of STZ is a model of type 1 diabetes (T1DM), but the diabetes pandemic is dominated by T2DM. Moreover, studies in tissues other than the bladder also indicate that STZ injection in rats has limited translational value for the overall diabetic population.

Against this background, this second part of the series systematically reviews data on bladder hypertrophy in animal models other than STZ-injected rats, most importantly in models of T2DM. Moreover, we discuss consequences of diabetes and hypertrophy on bladder function across all animal models. A subsequent third part of the series will discuss the mechanisms underlying bladder hypertrophy and dysfunction in experimental diabetes. The search strategy underlying and other methodological considerations for this article have been explained in part I of the series. We also refer to this previous article for a discussion of the relative value of assessing bladder hypertrophy as bladder weight (BW) or as bladder/body weight ratio (BBW).

2 | COMPARISON OF HYPERPOTROPHY AMONG MODELS OF TYPE 1 DIABETES

STZ injection has been used to induce a T1DM-like state not only in rats but also in mice and rabbits. Four studies in mice, using STZ doses of 125-150 mg/kg (except for one), and one in rabbits, using 65 mg/kg STZ, have reported on bladder hypertrophy (Figure 1, Supplementary Table S1). Studies in mice exhibited a trend for a less pronounced hypertrophy than in rats despite the higher STZ dose (mean BW 164 ± 48% of matched control, BBW 177 ± 42%), while the only study in rabbits found a degree of hypertrophy comparable to that observed in rats (BW 192%, BBW 219%). Thus, bladder hypertrophy upon STZ injection is found in at least three species but the extent may differ.

Alloxan is a toxic glucose analog that, like STZ, destroys pancreatic β-cells. While alloxan-induced diabetes was also associated with bladder hypertrophy in rats and rabbits (Figure 1, Supplementary Table S1), there may be differences as compared to the STZ model. Thus, two studies with alloxan-injected rats reported a BW of 187-196% of control, whereas two later studies observed little hypertrophy (92-118% of control). Interpretation of these differences is complicated by the fact that the latter two studies have not reported on BW but on total bladder thickness. On the other hand, the two studies on alloxan-injected rabbits also reported a modest degree of hypertrophy only based on BW (127-133% of control). Another possible difference is that there was little further bladder enlargement beyond week 1 in the rat STZ model, whereas one study in alloxan-injected rats reported a bladder wall thickness of 553 μm in control and 655 μm in diabetic animals after 6 weeks, which increased further to 899 μm after 20 weeks. Therefore, the alloxan model confirms the existence of bladder hypertrophy in T1DM models but despite a similar extent of blood glucose elevation appears to cause less hypertrophy than injection of STZ.

Bladder hypertrophy has also been studied in two hereditary models of T1DM. The T1DM-like state in BB/Wor rats develops spontaneously at a young age and involves a disorder of the immune system. One group studied diabetes-prone and -resistant BB/Wor rats at ages of 8, 16, and 32 weeks. BW was 139-158% of control and BBW 183-196% of control in the three age groups (Figure 1, Supplementary Table S1). About half of all 129/SvEv mice, also known as Akita mice, have a tyrosine for cysteine mutation in the Ins2 gene encoding insulin two leading to hypoinsulinemia and, consequently, hyperglycemia. Such mice had a BW of 155% of control and a BBW of 164% of control at an age of 20 weeks (Figure 1, Supplementary Table S1). Thus, hereditary rat and mouse models of T1DM also exhibit bladder hypertrophy, although based on a limited number of studies to a lesser extent than the rat STZ model. In conclusion, bladder hypertrophy is a general feature of T1DM animal models but appears to be greater in the rat STZ than in other models.

3 | COMPARISON OF HYPERPOTROPHY AMONG MODELS OF TYPE 2 DIABETES

The prevalence of T2DM is much greater in humans than that of T1DM, making T2DM models potentially more relevant. A wide range of animal models of T2DM exist; many of them, like human T2DM, are based on diets whereas some are hereditary. However, not all T2DM models exhibit a major increase in blood glucose. Moreover, T1DM and T2DM models typically exhibit a reduced and increased body weight, respectively, which leads to an increase or decrease of BBW, respectively, driven by changes of the denominator body weight and not by those of BW.

Fructose-feeding is known to induce metabolic syndrome without overt obesity or diabetes in rats. One group of investigators has fed male rats with a fructose-rich diet for 6, 9, and 12 weeks, and observed a moderate increase in BW to 140-150% of control with little increase in BBW (108-116%
of control). Another group has administered a high-fructose diet to female rats for 13 and 26 weeks but did not observe major changes of BW (97-110% of control) or BBW (93-108% of control). As our analysis of STZ-injected rats has revealed little sex difference in diabetes-associated bladder hypertrophy, it remains to be determined whether male and female fructose-fed rats exhibit a sex difference or these divergent data simply are a chance finding in a single study for each sex only.

High-fat diet also causes only moderate increases in blood glucose and bladder enlargement (Supplementary Table S1). Two groups studying this intervention in mice for 10 or 12 weeks have reported little BW increase (103-112% of control) and, driven by the increase in body weight, a reduced BBW (67-70% of control). Thus, animal models of diet-induced metabolic syndrome with moderate increases of blood glucose but no overt diabetes (Figure 1, Supplementary Table S1) and with and without obesity exhibit little bladder hypertrophy. This may be related to blood glucose levels in these models that remain below the renal glucose threshold.

Hereditary models of T2DM include the Goto-Kakizaki rat, a model with moderately increased blood glucose levels (Supplementary Table S1). Three studies have reported on bladder hypertrophy in Goto-Kakizaki rats as compared to an euglycemic control strain, having looked at 12 and 70 weeks, 10 and 46 weeks, or 18 weeks old animals. They report a BW of 88-132% of control and a BBW of 104-131% of control (Figure 1, Supplementary Table S1), that is, only moderate if any enlargement. Another frequently used hereditary rat model is the Zucker Diabetic Fatty (ZDF) rat, which harbors a mutation of the leptin receptor and exhibits major increases in blood glucose concentration. One study has tested 36 weeks old and another 16 and 27 weeks old ZDF rats, reporting a BW of 215-317% of control and a BBW of 189-306% (Figure 1, Supplementary Table S1). The db/db mouse also harbors a mutation of the leptin receptor and exhibits a pronounced increase in blood glucose as well as in body weight (Supplementary Table S1). The only study reporting on bladder hypertrophy in this model described a BW of 156% of control but a BBW of 90%, the latter obviously driven by the marked obesity.

We conclude that the existing literature on bladder hypertrophy is dominated by T1DM models, which account for 86% (102 of 118) of all reported group comparisons. The T1DM models in turn are dominated by STZ administration (91 of 102), with only six comparisons being based on alloxan injection and five on the hereditary models of BB/Wor rats and Akita mice. Moreover, 86 of the 91 group comparisons with STZ administration come from rats, four from mice and one from rabbits. The mice studies typically used much higher STZ doses than those in other species. Thus, our previous analysis on STZ-injected rats was based on 83 group comparisons, whereas no more than five were available for any other model and often come from only one or two
independent studies. Given the wide range of reported hypertrophy in the rat STZ model (BW 99-499% of matched control), it is difficult to conclude whether apparent discrepancy as compared to other models represent true biological differences or random effects based on small sample sizes in the other models. However, it is noteworthy that two models of T2DM with similar blood glucose levels as STZ-injected rats, ZDF rats and db/db mice exhibited as much or only little hypertrophy as compared to the T1DM models (Figure 1). Therefore, it is a key conclusion of our analysis that we know a lot about STZ-injected rats but far too little about other models, particularly those for T2DM.

4 | ACROSS MODEL COMPARISON OF GLUCOSE LEVELS AND BLADDER HYPERTROPHY

Our previous analysis of STZ-injected rats had not revealed a correlation between blood glucose levels and extent of bladder hypertrophy. In contrast, an analysis of all animal models (Supplementary Table S1) found a moderately strong correlation between blood glucose concentrations and bladder hypertrophy ($r^2$ of 0.1725 and 0.3991 for BW and BBW, respectively; Figure 2). Even this moderate correlation was largely driven by the three models that exhibited a limited increase in blood glucose (<200 mg/dL) but no overt diabetes. For comparison, studies in the rat STZ model have typically excluded animals with blood glucose <300 mg/dL as not being diabetic. Thus, when the three models with moderate glucose elevation were excluded, the correlation coefficient $r^2$ was only 0.0492 and 0.1135 for BW and BBW, respectively, which is more in line with the lack of a relevant correlation between blood glucose and BW or BBW in rat STZ studies. Based on the renal glucose threshold of 160-180 mg/dL, diabetes models with only moderate glucose elevation may not cause polyuria. The implications of this finding for mechanisms leading to bladder hypertrophy will be discussed in detail in the third part of the series.

5 | EXPLORATION OF LINKS BETWEEN HYPERTROPHY AND BLADDER DYSFUNCTION

This section analyzes bladder function associated with bladder hypertrophy in animal models of diabetes. It is based on integration of the findings from STZ-injected rats and the other models of T1DM and T2DM discussed above.

5.1 | Voiding behavior

Micturition patterns have been analyzed in STZ-injected rats to gain indirect insight into bladder function exposed to polyuria. Micturition frequency has consistently been shown to be markedly increased, and this similarly affected voids during daytime and nighttime. This increase occurred similarly in male and female rats and was unaffected by gonadectomy, started within 2 days after STZ injection and was at least partly restored by treatment with insulin. While sucrose feeding mimicked the effects of STZ injection on micturition frequency, a later study from the same group showed that this sucrose effect was driven by an increased frequency during nighttime (active period) with little change during daytime (inactive period).

More interesting as indicator of bladder function is mean voided volume, as it provides indirect information on bladder function.
capacity (BC). Mean voided volume largely followed the observations for micturition frequency, that is, was markedly increased during daytime and nighttime.\textsuperscript{29–31,33–35} This was largely mimicked by sucrose feeding.\textsuperscript{31,33} Maximum voided volume, a more specific indicator of BC, was also consistently increased in STZ-injected rats.\textsuperscript{31–33} Moreover, Brattleboro rats, a model of diabetes insipidus, exhibit a similar increase of diuresis, of micturition frequency, and voided volume as compared to STZ-injected rats.\textsuperscript{31,36} Interestingly, mean voided volume was at least partially restored not only by treatment with insulin\textsuperscript{29,30} but also upon nerve growth factor (NGF) gene transfer into the bladder\textsuperscript{35} in the rat STZ model.

Micturition patterns have also been explored in animal models of T2DM. Among these, ZDF rats and db/db mice exhibit a comparable degree of blood glucose elevation as STZ-injected rats, whereas Goto-Kakizaki rats and Abyssinian-Hartley guinea pigs have a much less severe diabetes (Figure 1, Supplemental Table S1). Although blood glucose was already markedly elevated in 16-week old ZDF rats, micturition frequency was increased only moderately whereas voided volume was markedly reduced; despite similar glucose levels in 27-week old animals, these exhibited a marked increase in both micturition frequency and voided volume.\textsuperscript{26} db/db mice also exhibited a considerable increase in micturition frequency\textsuperscript{27} but data on voided volume have unfortunately not been reported. In contrast, one study in Goto-Kakizaki rats at ages of 12 and 70 weeks did not exhibit alterations of frequency or voided volume, which is in line with the lack of polyuria.\textsuperscript{22} Others have followed Goto-Kakizaki rats over 10 time points ranging from 5 to 44 weeks of age and found no changes in total voided volume but a decrease in frequency accompanied by an increase in voided volume.\textsuperscript{23} The Abyssinian-Hartley guinea pig was reported to have a somewhat increased voided volume during the light but not dark cycle; micturition frequency tended to be decreased but that did not reach statistical significance.\textsuperscript{37} Therefore, based on a limited number of studies with equivocal findings, it is difficult to determine whether T2DM is accompanied by changes in micturition patterns. Taken together, the polyuria seen in animal models of more severe diabetes or induced by osmotic diuretics such as sucrose is mostly handled by the bladder by a combination of greater micturition frequency and BC. Changes of voiding behavior appear to occur less consistently in animal models with moderate elevation of blood glucose and little polyuria. These data are summarized in Supplemental Table S2. Cystometric assessments of BC will be discussed in section 5.2.

### 5.2 Cystometric evaluations

The findings on micturition frequency and voided volume derived from voiding behavior studies (section 5.1) indicate that the T1DM model of STZ-injected rats handles the increased diuresis by having more voids with an increased volume per void, the latter suggesting an enhanced BC. This has been assessed more directly in cystometric studies, which have been performed in vivo in anesthetized and, in some cases, in conscious animals\textsuperscript{34,38,39} and with isolated bladders in vitro.\textsuperscript{40–42}

Similar to voided volume in behavioral studies (see section 5.1), mean micturition volume was also increased in cystometric studies\textsuperscript{29,38,39,43–46} and normalized after insulin treatment.\textsuperscript{29,38,47} Micturition volume was also increased in Brattleboro rats.\textsuperscript{36} Cystometrically-assessed micturition frequency, mostly reported as inter-contraction intervals, typically was lower in diabetic than control rats in vivo\textsuperscript{39,48} and in vitro\textsuperscript{41} when fluid was instilled in the bladder at the same rate in both groups, apparently reflecting the greater BC. In contrast, no difference in frequency was observed when instillation rate was twice as high in diabetic as in control animals, mimicking the greater diuresis in the former.\textsuperscript{29,47} However, one study with equal instillation rate found an increased micturition frequency in STZ-injected and a decreased one in sucrose-fed rats.\textsuperscript{39}

Analysis of micturition data, specifically voided volume, had suggested that BC may be increased in STZ-induced diabetes in rats (see section 5.1). This was confirmed consistently in cystometric studies.\textsuperscript{29,31,34,35,38,39,41,44–48,50} Time course studies have shown that BC is increased as early as 1 week after STZ injection and does not increase substantially thereafter for up to 8 months,\textsuperscript{34,35,50} which is in line with maximum BW at this time point.\textsuperscript{4} Treatment with insulin largely restored BC\textsuperscript{38,45,50} and a partial restoration was also reported with NGF gene transfer.\textsuperscript{35} The increased BC was mimicked by treatment with sucrose or galactose\textsuperscript{29,31,41,47} or in Brattleboro rats.\textsuperscript{31,36} These data suggest that increased BC is a general feature of rat models with polyuria. While these models share bladder hypertrophy, increased BC apparently is not directly related to hypertrophy as treatment with α-lipoic acid\textsuperscript{44} or 3-aminobenzamide\textsuperscript{48} attenuated the increase in BC without affecting hypertrophy. On the other hand, treatment with the aldose reductase inhibitor fidarestat attenuated hypertrophy development without improving BC.\textsuperscript{39} Thus, studies have demonstrated a consistent increase in BC in animals with polyuria. However, it remains unclear whether this is directly related to the bladder hypertrophy observed in these models.

The urinary bladder needs the ability to relax to store urine and to contract to void to fulfill its function. The former can be assessed as compliance, the latter as basal, threshold, and maximum pressure. While one cystometric study did not report a change of bladder compliance,\textsuperscript{39} all others found increased compliance.\textsuperscript{29,31,43,47,48} In one of these studies the increase in compliance was similarly evoked by sucrosefeeding and was normalized by insulin treatment.\textsuperscript{29} Brattleboro rats also had an increased bladder compliance,\textsuperscript{31,36}
indicating that this may be a common feature of conditions with polyuria. A greater compliance in functional studies fits the anatomical finding of reduced collagen content (see part III of series), as the latter is relevant for structural stability of the bladder.51

While there have been exceptions in either direction, basal pressure,38,39,45,46,48 threshold pressure,34,35,38,39,45,48 and peak pressure39,35,38,39,43,45–48 were largely reported to be similar in STZ-treated and control rats. In studies where micturition pressure increased in T1DM, it was normalized by treatment with insulin.38,45 In contrast, one study in Brattleboro rats found basal, threshold, and micturition pressure to be decreased.36 Thus, polyuria appears to be associated with increased bladder compliance but only limited changes of bladder contractility.

Except for some species such as dogs, postvoiding residual urine (PVR) reflects an inability of the bladder to fully expel stored urine. With few exceptions,39 PVR was consistently reported to be increased in STZ-treated rats.28,34,35,38,43,44,46 PVR may worsen with duration of diabetes.44 On the other hand, it can be normalized by treatment with insulin38,46 or NGF gene transfer.35 Voiding efficiency, that is, the ratio between voided volume and BC, is the flipside of PVR. While both micturition volume and BC were consistently increased in STZ-injected rats, voiding efficiency was decreased in most34,44,46 but not all studies.35 As treatment with α-lipoic acid failed to affect bladder hypertrophy but ameliorated PVR and voiding efficiency,44 these may not be directly linked to hypertrophy.

STZ-induced diabetes in mice was also associated with an increased BC and compliance as assessed by in vivo52 or in vitro cystometry.53 It showed unchanged threshold pressure but increased peak pressure, micturition frequency, amplitude of voiding contractions and more frequent and larger non-voiding contractions. The increase in non-voiding contractions are mirrored by increased spontaneous contractile activity in vitro, as observed by other investigators.54 Cystometry has also been reported for another T1DM model, alloxan-induced diabetes in rats. An early study reported an increased threshold volume and markedly reduced contraction frequency; while sucrose feeding mimicked the former, it did not lower contraction frequency.10 A different study reported an increase in BC and contraction frequency along with a reduction of threshold pressure in 44-week-old rats after alloxan injection; while bladder compliance more than quadrupled numerically, this did not reach statistical significance making it an inconclusive study for this parameter.11 Acute surgically-induced vesical denervation markedly increased BC and compliance in control and diabetic animals. A follow-up study performed by the same group at 6 and 20 weeks after alloxan injection found no difference between control and diabetic animals for BC, voiding frequency or maximum pressure.12 Thus, except for the latter study, experiments in STZ-injected mice and alloxan-injected rats exhibited a similar cystometric profile as STZ-injected rats.

Among T2DM models with high blood glucose levels, expected to lead to major diuresis, ZDF rats, Akita mice, and db/db mice have been tested in cystometric studies. Similar to the T1DM models, ZDF rats exhibited increased BC and PVR and a markedly reduced voiding efficiency.25 Acute administration of the α1A-adrenoceptor antagonist silodosin did not markedly alter these three parameters, and the acetycholinesterase inhibitor distigmine had inconclusive effects; combination of silodosin and distigmine, however, reduced BC and PVR and increased voiding efficiency. Akita mice also exhibited an elevated BC and PVR, irrespective whether they had undergone extended infusion with angiotensin II.16 db/db mice also exhibited the increase in BC and bladder compliance consistently observed in STZ-injected rats.27 Thus, animal models of T1DM and those of T2DM with markedly elevated blood glucose concentrations exhibited a rather similar cystometric profile, indicating that this may be a consequence of markedly increased diuresis.

Cystometric investigations have also been reported from T2DM models with only moderate elevations of blood glucose, assumed to exhibit little change in daily urine output. No major differences in cystometric parameters were observed in Goto-Kakizaki rats aged 12 or 70 weeks, except for a reduced PVR in 70-week-old diabetic animals.22 Other investigators also did not observe changes in cystometric parameters in 10-week-old Goto-Kakizaki rats, whereas they noted increased BC, voided volume and bladder compliance accompanied by reduced micturition threshold and peak pressure at 46-week-old animals;23 of note, the changes in the latter group occurred in the absence of bladder hypertrophy. A third study in 18-week-old Goto-Kakizaki rats reported increased BC and micturition volume, unchanged basal or threshold pressure and voiding efficiency and lowered micturition frequency; frequency and amplitude of non-voiding contractions was increased.24 Acute administration of the muscarinic receptor antagonist solifenacin similarly reduced micturition pressure in both strains whereas it reduced duration of micturition only in control and not in diabetic animals. A fourth study differed in design from the other three: it used 12-week-old Goto-Kakizaki rats that had additionally received 4 weeks of high-fat diet to aggravate their metabolic situation; moreover, it did not primarily compare diabetic and control rats but rather explored acute effects of the muscarinic receptor antagonist darifenacin and cold exposure within each strain.55 BC, voided volume and micturition frequency were comparable between the diabetic and the control strain; acute treatment with imidafenacin did not cause major changes of these parameters in either strain. Rats receiving a fructose-rich diet for 6, 9, and 12 weeks exhibited a gradual worsening of bladder function over
time\textsuperscript{19}: while BC remained unchanged, voided volume, voiding efficiency, and maximum pressure decreased while PVR increased; non-voiding contractions, which were absent in control animals, became increasingly frequent. A study with 3 and 6 months of fructose-feeding showed detrusor overactivity at both time points but did not provide quantitative data; of note, bladder hypertrophy was absent in these animals.\textsuperscript{20} A follow-up study from that group also investigating rats after 3 and 6 months of fructose feeding reported a reduced micturition frequency and peak intra-vesical pressure accompanied by basal and threshold pressure.\textsuperscript{18} db/db mice exhibited an irregular micturition pattern with increased frequency of voiding and non-voiding contractions; while BC, bladder compliance, threshold, and peak pressure were not altered, post-void pressure was markedly increased.\textsuperscript{56} Chronic treatment with either metformin or the Ca\textsuperscript{2+}-channel inhibitor amlodipine did not affect cystometric findings in lean mice but normalized the elevated frequency of voiding and non-voiding contractions and of post-void pressure. Abyssinian-Hartley guinea pigs exhibited increases in contractile frequency, peak, and mean intraluminal pressure and rate of pressure increases, whereas basal intraluminal pressure and duration of expulsion activity were reduced.\textsuperscript{37} Thus, T2DM models with only moderate elevations of blood glucose concentration exhibit abnormalities of bladder function. However, these were not consistent across models (or even within models across reports) and clearly did not mimic those observed in T1DM or in T2DM with markedly elevated blood glucose concentrations. These data are summarized in Supplemental Table S3.

5.3 \textbf{In vitro contractility}

Most studies on changes of contractility of bladder smooth muscle have been based on isolated bladder strips, but some investigators have applied whole bladder in vitro preparations.\textsuperscript{31,40,42} Three groups of contractile stimuli have been tested. First, electrical field stimulation (EFS). EFS assesses responses to nerve stimulation, particularly when verified that these are blocked by tetrodotoxin. Accordingly, it is an integrated response to multiple mediators including acetylcholine acting on muscarinic receptors, ATP acting on purinergic receptors and other transmitters. Hence, altered responses to EFS reflect the net effect of changes in transmitter release due to denervation (see part III of series), changes in expression of post-junctional receptors for these transmitters and changes in intrinsic contractile properties of smooth muscle. Using antagonists or desensitization protocols, the relative contribution of various receptors to the EFS response can be estimated.\textsuperscript{9,57} Second, direct receptor agonists. Most studies in this regard have looked at muscarinic receptors using the endogenous agonist acetylcholine or the synthetic agonists carbachol or bethanechol or at purinergic receptors using the endogenous agonist ATP or its synthetic analog β\textsubscript{2}-methylene-ATP. Third, some studies have tested responses to receptor-independent stimuli such as KC\textsubscript{I}\textsuperscript{9,28,58,59} for contraction or aminophylline for relaxation.\textsuperscript{42} These assess intrinsic contractile properties of smooth muscle, that is, events occurring downstream of receptor activation.

While one study from the Longhurst group reported a reduced EFS response in bladder base and dome,\textsuperscript{58} later studies from this group\textsuperscript{30,32,60} and others\textsuperscript{57,61} reported increased EFS responses in STZ-treated rats. Other reports from the Longhurst group\textsuperscript{31,62} and others\textsuperscript{9,63} found no major change in EFS-induced contraction. This group also reported that length-tension relationship may change in STZ-induced diabetes and that detection of an increased EFS responses may depend on the denominator used for normalization.\textsuperscript{59} Reported increases were similarly found in male and female animals, with and without gonadectomy\textsuperscript{32} and were at least partially normalized by insulin treatment.\textsuperscript{30} Some studies have compared changes of EFS responses after STZ administration with those after sucrose feeding, in Brattleboro rats and after induction of bladder outlet obstruction (BOO). While some studies reported similar overall changes after STZ injection and sucrose feeding,\textsuperscript{31,57,59,60} this involved discordant changes in the muscarinic,\textsuperscript{57} the non-adrenergic-non-cholinergic\textsuperscript{57} or the phasic component of contraction,\textsuperscript{60} others reported discordant regulation of overall EFS response.\textsuperscript{59} On the other hand, a report of lack of change of EFS response in STZ-injected sucrose-fed rats reported similar observations in Brattleboro rats.\textsuperscript{31} Two studies from different labs compared two states of bladder hypertrophy, either by STZ injection or by BOO induction. One of them found increased EFS responses after STZ administration, decreased responses after BOO and initially decreased but then recovering responses after a combination of STZ injection and BOO induction.\textsuperscript{61} Others found a lack of change of EFS response after STZ administration, a reduction after BOO and an even greater reduction with the combination of STZ and BOO.\textsuperscript{62} In summary, the clear majority of studies found an increased or unchanged EFS-induced contraction following STZ injection. Findings in STZ-injected rats were often shared by those in sucrose-fed or Brattleboro rats, but in several cases discordant for components of the EFS response. Moreover, in the diuresis-independent BOO model of bladder hypertrophy, EFS responses were always discordant with those of STZ injection. Therefore, EFS-induced contraction can be altered in all models of bladder hypertrophy but extent, composition and even direction of change appears to be model-specific and not a general consequence of hypertrophy. This is in line with the observation that no consistent changes of bladder contractions were observed in cystometric studies in vivo (see section 5.2).
Experiments based on receptor agonists were performed to analyze the underlying components of the EFS response. As muscarinic and purinergic receptors are the quantitatively most important components of EFS responses, most reports have focused on them. Generally, a similar picture emerged for the receptor agonists as compared to EFS. Thus, most studies using muscarinic receptor agonists found increased or unchanged responses in diabetes and only few found decreased responses. While sensitization was most often observed as change in maximum responses, one study found an increased potency without change of maximum responses. As with EFS responses, those to muscarinic agonists were similar in male and female animals with and without gonadectomy and at least partially restored upon treatment with insulin. Enhanced muscarinic responses were also normalized by treatment with hexacosanol; as this did not affect hypertrophy it provides further support for the hypothesis that STZ-induced changes of bladder contractility are not directly linked to hypertrophy. Other models of markedly enhanced diuresis such as sucrose feeding and Brattleboro rats often but not always mimicked findings with STZ injection. BOO as diuresis-independent model of bladder hypertrophy always caused different changes of muscarinic responses as compared to STZ injection and the combination of both pathologies exhibited a complex response. A study comparing two diuresis-independent models of bladder hypertrophy, that is, surgically-induced denervation and BOO also found discordant alterations of muscarinic responses, giving further support to the hypothesis that changes in contractility are related to a specific model and not directly to the presence of bladder hypertrophy. The unchanged to increased responsiveness to muscarinic agonists in the diabetic bladder contrasts with unchanged to decreased responsiveness in euglycemic models of bladder dysfunction.

Changes of responses to purinergic agonists based on a smaller number of studies also followed this pattern, that is, responses were increased in most studies but decreased responses have also been reported. Responses to agonists at other receptors were also reported in several studies but given the heterogeneity of reported EFS, muscarinic, and purinergic responses too few studies were reported for each receptor to allow robust conclusions. This included studies with serotonin, noradrenaline (only a contractile agent in bladder base), prostaglandin F2α, and the peptides substance P, bradykinin and endothelin.

Whether changes in expression of the activated receptor and/or those of intrinsic contractility of the smooth muscle cell underlie those of contractile responses can be assessed by testing contraction in response to a receptor-independent agonist such as KCl or by exploring changes in the expression of the receptor. Changes in KCl responses mimicked those of EFS, muscarinic and/or purinergic stimulation in some cases but yielded discordant results in others, making it inconclusive whether changes in intrinsic contractile properties contribute to those in contractile response to receptor activators.

To explore mechanisms underlying changes in contractile responses to receptor agonists, attempts have been made to quantify the expression of receptors mediating them. This has relied on two approaches for detection at the protein level, each having its limitations. The first approach is based on radioligand binding. This technique has the advantage of providing quantitative information. However, different radioligands can detect different receptor densities when tested side by side, that is, these approaches allow comparisons between groups within a study but not necessarily quantification of absolute numbers. Moreover, most radioligands quantify receptor families, for example, muscarinic receptors, and only additional experiments with subtype-selective competitors can quantify subtypes. On the other hand, antibodies in theory allow highly selective detection of individual receptor subtypes, including their anatomical localization, but are less suitable for quantification. Perhaps even more importantly, it has been shown that the vast majority of antibodies against muscarinic or purinergic receptor subtypes or against G-protein-coupled receptors in general lack useful target selectivity when tested under stringent conditions. A limitation of both quantification approaches is the observation that receptor number may not be the limiting factor for mediation of responses. For instance, M3 muscarinic receptors outnumber M1 receptors in the bladder of humans and most other mammalian species but contraction is mediated by the smaller fraction of M1 receptors. These considerations need to be kept in mind in understanding the data on receptor expression at the protein level.

One radioligand binding study has demonstrated a major increase in overall muscarinic receptors, which was partly restored by insulin treatment, and little change in contribution of subtypes. Two other studies confirmed elevation of muscarinic receptor density without change of contribution of subtypes but found a much smaller magnitude of increase. Rats treated with sucrose also exhibited an increase in muscarinic receptor protein, but to a smaller extent than STZ-injection. Immunoblot data confirmed an increased expression of M2 and M3 receptors in both smooth muscle and urothelium, but are difficult to interpret as they are based on antibodies without sufficient validation. Thus, muscarinic receptors appear up-regulated in the urinary bladder of STZ-injected rats; the sucrose data suggest that this may in part be caused by the increased diuresis. These data are in line with the increased contractile responses to muscarinic agonists observed in many in vitro studies (see above) and indicate that the increased responsiveness may at least in part
be due to enhanced expression of the receptor mediating it. Of note, this up-regulation of muscarinic receptors in the STZ model of T1DM may be specific for the bladder as similar experiments on prostate found the opposite, that is, a reduced muscarinic receptor expression in diabetes.83

To test whether these increases in muscarinic receptor expression in the bladder of STZ-injected rats occur at the level of transcription and/or mRNA stability, corresponding mRNA has been quantified in several studies, but the data are not fully conclusive. While one study reported an increase in M2 receptor expression in smooth muscle and urothelium,81 others confirmed such elevation only at early but not at late time points after STZ administration (3 and 9 vs 20 weeks).84 Similarly, M3 muscarinic receptor expression was found to be elevated in smooth muscle and urothelium in one study,82 but only minor increases were seen in another84 and a study based on gene chip analysis even found a down-regulation.85 Experiments regarding expression of subtypes of P2X purinergic receptors did not reveal a clear picture either.50,84

STZ injection has also been used in mice to induce diabetes. Two studies largely confirm findings in STZ-injected rats, that is, an increased contraction in response to EFS, muscarinic, and purinergic agonists and KCl accompanied by an increased mRNA expression of M3 muscarinic receptors but not M2 or P2X1 receptors.8,53,86 However, two interesting additional observations were made. First, the Ca2+-channel blocker nifedipine and the rho kinase inhibitor Y-27,635 had considerably greater inhibitory effects in diabetic as compared to control mice, which was accompanied by an up-regulation of L-type Ca2+-channel mRNA,53 suggesting that not only an increase in receptor but also in ion channel expression may be involved in the enhanced agonist responses. Second, in vitro addition of the toll-like receptor four antagonist CLI-95 normalized the enhanced carbachol-induced contraction; moreover, the diabetes-associated increase in contractile responses to EFS and carbachol was largely abolished in knock-out mice lacking the toll-like receptor 4.8 Finally, one of these studies suggested that enhancements may not be detectable at early time points (2 weeks after STZ injection) but only at later ones (8-9 or 22-24 weeks), which contrasts the time course of hypertrophy development;4 moreover, enhancements disappeared when contractions were normalized to those elicited by KCl, but interpretation of this finding is difficult as the KCl responses were not reported.86 A second report based on the same animals described a reduced contraction to EFS in M2 receptor knock-out but not wild-type mice, which similarly involved the muscarinic and the purinergic component of contraction,87 pointing to possible compensatory changes in the contributions of M2 and M3 receptors.

Contractile in vitro responses have also been tested in the alloxan-based model of T1DM in rats7-10 and rabbits.13,14 In contrast to the often reported hyperresponsiveness of bladder smooth muscle in rats and mice after STZ injection, alloxan administration did not lead to major alterations of contractile responses of EFS, muscarinic, purinergic or α1-adrenergic or substance P- or KCl-mediated contraction. Similarly, contractile responses to muscarinic agonists or KCl were also largely unchanged in alloxan-injected rabbits.13 However, this study found changes in myosin light chain phosphorylation, and a follow-up study demonstrated slower relaxation by a rho-kinase inhibitor and increased expression of rho kinase and CPI-17 expression, neither being mimicked by sucrose administration.14 Thus, the alloxan model of T1DM exhibits a different pattern of smooth muscle responsiveness in rats and rabbits as compared to the STZ model; which of them, if any resembles the human situation remains unclear. However, difference in contractile responses between STZ and alloxan-based models despite comparable degrees of hypertrophy again highlight that changes in contractility are not necessarily associated with hypertrophy.

Among T2DM models with high glucose levels, one study reported enhanced carbachol-induced contractions in db/db mice.27 Addition of nifedipine markedly inhibited contractions in wild-type and db/db mice, as has also been reported in the absence of diabetes for rat88 and human bladder strips.77 In contrast, mibefradil had little effect in bladder strips from control mice but normalized responses in those from db/db mice. mRNA expression of subunits of L-type Ca2+-channels was unchanged, whereas gene expression of some subunits of T-type channels was increased, which could explain the greater inhibition by mibefradil. In ZDF rats, inconsistent changes of contractile responses were found 16-week old animals; contractile responses were increased for ATP (but not muscarinic agonists or KCl) at 27 weeks but the latter could not be explained by changes in P2X1 receptor mRNA expression.26 Thus, T2DM models with high glucose levels were inconsistent across models for changes in contractility, but too few studies have been reported for robust conclusions. In line with the findings in ZDF rats, contractile response to EFS or the muscarinic receptor agonist carbachol were not altered in Brattleboro rats,36 further supporting the view that neither marked increases in diuresis nor bladder hypertrophy are directly linked to increased sensitivity of contractile responses.

Studies in T2DM models with relatively low blood glucose concentrations, expected to lack polyuria and associated with little bladder hypertrophy, were mostly performed with Goto-Kakizaki rats and mice on a high-fat diet. In Goto-Kakizaki rats, EFS- and KCl-induced contractions were increased whereas those caused by carbachol were unchanged or inhibited minor decreases without major changes of subtypes of muscarinic receptors being involved.22,23 Minor if any changes of M2 and M3 receptor mRNA expression were found at 10-12 weeks of age, but an increase of both was reported at 70 weeks.22,55 Studies with
bladder strips from mice on a high-fat diet were inconsistent; while one reported increased contraction in response to EFS, a muscarinic agonist or KCl but not a purinergic agonist, the other found reduced responses to muscarinic or KCl stimulation. While responses to KCl were restored by in vivo treatment with either metformin or amlodipine in the former study, they were restored by treatment with etanercept in the latter. In fructose-fed rats, contractions in response to EFS remained unchanged after 3 months but markedly declined after 6 months. One study in Abyssinian-Hartley guinea pigs reported decreased contractile response to EFS or acetylcholine in bladder base but not in bladder body. In conclusion, T2DM models without major diuresis or hypertrophy nonetheless exhibited some changes in the responsiveness of detrusor smooth muscle. While the number of studies is too small for robust conclusions on specific models, this reinforces the conclusion that functional changes of detrusor responsiveness are not necessarily related to the presence of hypertrophy. A summary of the main contraction data across all models is provided in Supplemental Table S4.

Increased BC is a fairly consistent feature of animal models of diabetes (see sections 5.1 and 5.2) but in contrast to the many studies on possible alterations of bladder smooth muscle contraction in diabetes, which is required for voiding, only few studies have explored how smooth muscle relaxation may be altered, which is important for urine storage. As β-adrenoceptors are the most important mediator of bladder smooth muscle relaxation, all of them have focused on this receptor family. Of note, bladder relaxation in humans is mediated predominantly if not exclusively by β3-adrenoceptors, whereas that in rats is a mixed response by β2- and β3-adrenoceptors and differentially involves β2- and β3-adrenoceptors in mice depending on the agonist being used. As these two subtypes can be differentially regulated in bladder smooth muscle, the general validity of rat and mouse studies for the human situation is unclear unless specific attempts have been made to separate the role of the two subtypes.

An early study based on in vitro cystometry reported an increased BC upon β-adrenergic stimulation; this was fully mimicked by sucrose feeding or sympathectomy. A study using isolated bladder strips also found increased relaxation responses upon β-adrenergic stimulation after STZ injection but in this setup sucrose feeding caused a considerably smaller enhancement. Finally, a third study reported no changes in relaxation responses at all in STZ-injected rats; rats with BOO exhibited a slight attenuation of relaxation, whereas the combination of both interventions caused a major attenuation. Only one study assessed β-adrenoceptor density in STZ-induced diabetes in rats. Using two different radioligands, each unsuitable to label bladder β3-adrenoceptors under the chosen conditions, an increase in receptor density was observed, probably reflecting mostly a change of β2-adrenoceptors; this was not mimicked by sucrose feeding. For comparison, β-adrenoceptor binding in the prostate of rats injected with STZ was reported to be decreased, an alteration restored by insulin treatment. Therefore, too few studies are available to allow robust conclusions on alterations of expression and function of β-adrenoceptors in the urinary bladder of STZ-injected rats.

6 | CONCLUSIONS AND FUTURE RESEARCH

6.1 | Limitations of analysis

The probably most important limitation of the existing literature on bladder hypertrophy in animal models of diabetes is a heavy focus on T1DM models, mostly STZ-injected rats. This is unfortunate for two reasons. First, studies in other organ systems show that STZ-based models have limited translational value for human diabetes. Second, and probably more importantly, the global diabetes pandemic is dominated by T2DM, but only 14% of the available animal studies were based on T2DM models. Moreover, only five or less group comparisons are available for any of the T2DM models. In light of the wide range of reported extent of hypertrophy in the rat STZ model, it is difficult to determine whether reported apparent differences between STZ-injected rats and T2DM models reflect true differences in biology or simply are a chance finding with limited sample sizes. Therefore, it is a key conclusion of our analysis that we know a lot about bladder hypertrophy in STZ-injected rats but far too little about other models, particularly those for T2DM. This severely limits our ability to extrapolate conclusions from the available animal data to diabetic patients.

A second major limitation of the reported findings is the predominant reliance on male animals. While analysis of STZ-injected rats provides initial evidence that sex of the animal is not a major factor for the development of bladder hypertrophy, the evidence based in other animal models of diabetes does not allow such conclusions. Therefore, we propose that future studies on bladder hypertrophy in experimental diabetes should focus on models of T2DM and include animals of both sexes.

A third major limitation of the existing data is that we do not know whether expressing bladder hypertrophy based on BW or on BBW is more informative. Studies in other diseases, for instance in the cardiovascular system, typically adjust organ hypertrophy based on body weight. However, that may be less valuable in diabetes as models of T1DM typically exhibit decreased body weight, whereas those of T2DM mostly have increased body weight (Supplemental Table S1 and Supplemental Table S1 of). Thus, BBW is
strongly driven by the denominator body weight. Therefore, in contrast to other diseases, correction for body weight may be less informative in diabetes as it leads to an over- and under-estimation of bladder hypertrophy based on BBW as compared to BW in T1DM and T2DM, respectively. Moreover, some species including rats physiologically continue to gain body weight throughout their lifespan whereas this is not necessarily the case for BW. As the available evidence is insufficient, we recommend that studies should report on BW and BBW in parallel, which unfortunately has not been the case in many previous studies.

6.2 | Bladder hypertrophy across animal models

While BW is about doubled in STZ-injected rats, this is quantitatively mimicked or even exceeded in some models of T1DM (STZ-injected rabbit) and T2DM (ZDF rat), whereas others shows less (STZ-injected mouse, alloxan-injected rat or rabbit, BB rat, Akita and db/db mouse) or apparently no bladder hypertrophy (Goto-Kakizaki and fructose-fed rat, mouse on high-fat diet) (Figure 1). The extent of bladder hypertrophy apparently does not depend on T1DM versus T2DM models and among T2DM models ZDF rats and db/db mice exhibit similar blood glucose levels but markedly different extent of bladder hypertrophy (Figure 1). Models with limited glucose elevation (less than 200 mg/dL) typically did not exhibit any bladder hypertrophy. However, all of these conclusions rest on five or less group comparisons for models other than STZ-injected rats. Moreover, we are not aware of data on bladder hypertrophy in diabetic patients. Therefore, it remains unclear which if any of these models are representative for humans.

The correlation between blood glucose levels and extent of hypertrophy was moderate at best and largely disappeared when models with limited glucose elevation were excluded from the analysis (Figure 2), which is in line with our previous analysis in STZ-injected rats.4 Research needs in this area will be discussed along with mechanistic studies on bladder hypertrophy in experimental diabetes in part III of the series.

Oral antidiabetic drugs are the cornerstone of T2DM treatment, but none of them has been tested for effects on bladder hypertrophy. In this regard, SGLT2 inhibitors may be of specific interest because they promote glycosuria.93 More treatment studies, particularly involving drugs in clinical use for the treatment of T2DM, are urgently needed.

6.3 | Functional status of the diabetic bladder

Diabetic cystopathy in patients may manifest in different ways, from overactive bladder syndrome (OAB), its cystometric indicator detrusor overactivity and urgency incontinence on one end of the spectrum to decreased bladder sensation, impaired voiding, increased BC and PVR and even overflow incontinence on the other end.94–97 This may sound contradictory because detrusor overactivity and OAB typically lead to a reduced BC, whereas impaired voiding and increased BC and PVR are mostly found with detrusor underactivity.98 Accordingly, muscarinic receptor antagonists are a cornerstone of the treatment of OAB,99 whereas direct or indirect muscarinic receptor agonists are used in the treatment of detrusor underactivity, although the evidence for their efficacy is questionable.100

These clinical features are also found in animal models of diabetes. Among these the indirect measures of BC such as voided volume or maximum voided volume (see section 5.1) and direct in cystometric experiments (see section 5.2) are one of the most consistent findings. This is likely to be a response to polyuria as similar increases occur in other forms of polyuria, for instance with sucrose or galactose feeding,29,31,41,47 or in Brattleboro rats.31,36 That lowering of blood glucose concentrations by treatment with insulin largely restores BC38,45,50 further supports this idea. On the other hand, BC was at least partially restored by treatments which did not reduce blood glucose (a proxy for diabetes-associated polyuria) or hypertrophy including NGF gene transfer,35 α-lipoic acid,44 or 3-aminobenzamide.48 If an increased BC is required to handle polyuria without too much of an increase in micturition frequency, it can be doubted whether such interventions would be beneficial to diabetic patients. The flipside of this coin is the observation that treatment with the aldose reductase inhibitor fidarestat attenuated hypertrophy development without improving BC,39 which also may aggravate an inability to expel urine.

While the increase in PVR in diabetic patients is considered to reflect detrusor underactivity, increases in BC and PVR in animal models of diabetes are not accompanied by impaired contractility in cystometric or organ bath studies (see section 5), that is, occur mostly in the absence of detrusor underactivity. In contrast, non-voiding contractions have often been reported in STZ-injected rats, both with in vitro preparations40 and in cystometric studies.34,38,44 It is not fully clear why increased PVR in patients appears due to detrusor underactivity, whereas such underactivity is typically not observed in animals with increased PVR; a possible explanation is that underactivity may mostly occur in a late stage of diabetes, whereas most animal studies were performed in early stages of the disease. Moreover, changes in BC and PVR in animal models appear linked to marked polyuria, whereas properly treated patients do not exhibit major polyuria. However, we cannot dismiss the alternative hypothesis that none of the animal models is representative for the clinical situation with regard to bladder function. This deserves additional research.
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CONFLICTS OF INTEREST

EAI and JHE report no conflict of interest. MCM does not report a conflict of interest relative to diabetes but relative to bladder function is a consultant and shareholder of Velicept.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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