Skin electrodes transduced signals to the bladder resulting in ameliorated hypomotility in a rabbit model of diabetes*☆○

Xinmin Wang¹, Qirui Fu¹, Qingmei Zhang¹, Ping Xu¹, Lin Cao¹, Meng Xue¹, Wei Wang²

¹Department of Endocrinology and Metabolism, the Second Clinical Medical College of Jinan University (Shenzhen People’s Hospital), Shenzhen 518020, Guangdong Province, China
²Department of Physics & Astronomy, McMaster University, Medical Physics, Hamilton L8S 4L8, Ontario, Canada

Abstract
Electric signals from a chest skin electrode can be conducted to the heart and activate contraction. In the present study, normal and diabetic rabbits were stimulated by skin electrode on the abnormal bladder projection area using three levels of exporting voltage (5.84 V, 8.00 V, and 11.00 V). Results demonstrated significantly attenuated electric signals from both groups, in particular the diabetes group. The skin electrode signals were conducted to the bladders, and all vesical signals increased according to strength of stimulating signals from the skin electrode. However, vesical signals from diabetic rabbits were less than those from normal rabbits at the same stimulating strength of exporting voltage. Vesical pressures from the two groups increased along with increased vesical signals, but vesical pressure was less those from diabetic rabbits than in normal rabbits (basic status and different stimulating levels). Linear correlation analysis showed a significantly positive correlation between vesical pressure and signal. These results demonstrated that electric signals from skin electrodes resulted in increased vesical pressure, and vesical pressure increased along with stimulation strength.

Key Words: diabetes mellitus; peripheral neurogenic bladder; vesical pressure; vesical stimulation

INTRODUCTION
The incidence of diabetic cystopathy (DCP), which is primarily caused by damage to the peripheral nerve below the spinal neuron and is defined as peripheral neurogenic bladder, is 40-60% in diabetic patients [1]. DCP, a type of peripheral neurogenic bladder, presents as vesical hypomotility upon diagnosis. Patients with DCP ultimately develop persistent urinary tract infections, sepsis, or renal failure due to invasive treatments, such as continuous catheterization or cystostomy as a result of bladder over-expansion and persistent urinary retention. This results in significantly decreased quality of life and life span [1,2]. To date, microelectrode nerve stimulation is commonly used to treat central neurogenic bladder resulting from spinal cord injury (presents as overactive bladder), but this treatment is virtually ineffective for peripheral neurogenic bladder patients with DCP [2]. Bypassing the damaged bladder innervation has been shown to reduce or eliminate residual urine in DCP patients; urination is even restored by direct stimulation to the bladder with a skin electrode [3]. Therefore, skin electrode stimulation to the bladder projection area could serve as the most effective non-invasive treatment for DCP, although the mechanisms of action remain poorly understood [3-4]. Electric signals from a chest skin electrode conduct signals to the heart and subsequently activate contraction upon cardiac arrest. Therefore, it was assumed that electric signals from successive skin stimulation on an abnormal bladder projection area could conduct signals to the vesical, thereby inducing contraction and ameliorating hypomotility. The present study analyzed signal transduction via skin electrodes attached to bladders of normal rabbits and diabetic rabbits, thereby inducing mechanical vesical contraction.

RESULTS
Quantitative analysis of experiment animals
A total of 30 male, New Zealand rabbits were randomly assigned to a control group (normal saline injected via the helix vein; n = 10) and diabetes mellitus (DM) group (DM was induced by alloxan injected via the helix vein; n = 20). A total of 11 rabbits from the
DM group died from intestinal infections and were eliminated, and 19 rabbits were included in the final analysis.

**General rabbit information**
Weight and fasting blood glucose in the normal control rabbits prior to experimentation were 1.77 ± 0.30 kg and 5.76 ± 0.64 mM, respectively. These values were 1.88 ± 0.18 kg and 5.61 ± 0.69 mM, respectively, in the DM rabbits, with no significant differences between normal control and DM rabbits (P > 0.05). Normal control rabbit body weight after 4 weeks was 2.31 ± 0.16 kg, which was greater than 2.10 ± 0.18 kg in the DM group. However, fasting blood glucose levels in the normal control rabbits were less than in the DM group (5.70 ± 0.59 mM vs. 24.56 ± 8.32 mM; both P < 0.01).

**Signal conduction and attenuation of abdominal muscle and bladder in rabbits post-stimulation**
A BL-410 Biological Experimental System was used to measure received signals from abdominal muscles and bladders in both groups under three levels of exporting voltage. Voltage to the abdominal muscles and bladders increased in both groups (P < 0.01) when the level of exporting voltage to the skin increased from low (5.84 V) to middle (8.00 V) and high (11.00 V) voltage; bladder voltage was significantly less than abdominal muscle voltage (P < 0.01). In addition, attenuation was associated with the transduced signals via the abdominal wall, and the signals decreased to millivoltage levels with attenuation > 99.8 ± 0.0%. Conversely, the attenuation percentage increased with stimulation strength. From the abdominal muscle to the bladder, the percentage of attenuation was > 22.8 ± 1.0%, but attenuation decreased with stimulation strength. However, the total attenuated percentage within a group, from skin to bladder, was not significantly different (P > 0.05; Tables 1, 2).

### Table 1 Signal transduction and total attenuation percentage (TAP) of exporting voltage (EV) in the skin

<table>
<thead>
<tr>
<th>EV</th>
<th>Control group</th>
<th>Diabetes mellitus group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM (mV)</td>
<td>BL (mV)</td>
</tr>
<tr>
<td>Low</td>
<td>11.18±1.00</td>
<td>7.09±0.29</td>
</tr>
<tr>
<td>Middle</td>
<td>13.09±0.49</td>
<td>8.99±0.35</td>
</tr>
<tr>
<td>High</td>
<td>16.99±0.52</td>
<td>12.82±0.37</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, with 10 rabbits in the control group and nine rabbits in the diabetes mellitus group. All significances between groups were determined using the t-test for each received voltage, and analysis of variance was used to determine significances within a group.

*P < 0.01 vs. low EV; *P < 0.01 vs. middle EV; **P < 0.01 vs. AM; *P < 0.01 vs. control group. TAP = (EV – BL)/EV × 100%. AM: Abdominal muscle voltage; BL: bladder voltage.

### Table 2 Signal attenuation percentage of electric stimulations from the skin to the vesica

<table>
<thead>
<tr>
<th>EV</th>
<th>Control group</th>
<th>Diabetes mellitus group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(EV-AM)/EV×100%</td>
<td>(AM-BL)/AM×100%</td>
</tr>
<tr>
<td>Low</td>
<td>99.81±0.00</td>
<td>36.5±1.3</td>
</tr>
<tr>
<td>Middle</td>
<td>99.84±0.00</td>
<td>31.3±1.5</td>
</tr>
<tr>
<td>High</td>
<td>99.85±0.00</td>
<td>22.8±1.0</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, with 10 rabbits in the control group and nine rabbits in the diabetes mellitus group. All significances between groups were determined using the t-test for each received voltage, and analysis of variance was used to determine significances within a group.

*P < 0.01 vs. low EV; *P < 0.01 vs. middle EV; **P < 0.01 vs. control group. AM: Abdominal muscle voltage; BL: bladder voltage; EV: exporting voltage.

Both Table 1 and Table 2 show that voltage to the abdominal muscles and bladders of diabetic rabbits was less than in normal control rabbits (P < 0.01), while the attenuated percentage in normal control rabbits was significantly less than in diabetic rabbits (P < 0.01).

### Table 3 Vesical voltage and pressure under basic and excited conditions

<table>
<thead>
<tr>
<th>EV</th>
<th>Control group</th>
<th>Diabetes mellitus group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BLv (mV)</td>
<td>BLp (mm Hg)</td>
</tr>
<tr>
<td>Basic</td>
<td>0.00</td>
<td>43.78±3.12</td>
</tr>
<tr>
<td>Low</td>
<td>7.09±0.29</td>
<td>44.33±3.13</td>
</tr>
<tr>
<td>Middle</td>
<td>8.99±0.35</td>
<td>50.59±3.27</td>
</tr>
<tr>
<td>High</td>
<td>12.82±0.37</td>
<td>57.40±3.41</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, with 10 rabbits in the control group and nine rabbits in the diabetes mellitus group. All significances between groups were determined using the t-test for each received voltage, and analysis of variance was used to determine significances within a group.

*P < 0.01 vs. low EV; *P < 0.01 vs. middle EV; **P < 0.01 vs. control group. BLv: Voltage on bladder; BLp: pressure on bladder.
Results demonstrated significant pressure in the bladder ($P < 0.01$) under middle and high levels of stimulation in the control group, but only under high levels in the DM group. In addition, total bladder pressure in the DM group was less than corresponding values in the control group ($P < 0.01$).

**Correlation between signals and pressure in normal control and diabetic rabbit groups**

As shown in Figure 1, vesical voltage in both groups increased linearly with stimulation signal, where the coefficient was 0.869 in the control group and 0.750 in the DM group ($r = 0.869, 0.750; P < 0.01$).

**DISCUSSION**

In contrast to central nerve injury cystopathy (central neurogenic bladder), DCP is a peripheral nerve injury cystopathy or peripheral neurogenic bladder, which is not easily treated by metabolic control, neurotroph (i.e., Methyl-B12 and ganglioside), nerve growth factor infusion, or gene therapy.$^{[1, 2]}$. Patients with urinary retention either suffer a difficult life or possibly die of intractable urinary tract infections and renal failure due to continuous catheterization or cystostomy. Treatments, such as electrical stimulation of neural pathways (including pudendal nerve stimulation, sacral root nerve stimulation, and implant microelectrode stimulation), as well as the establishment of artificial somatic nerves and visceral reflex pathways, have been shown to effectively ameliorate central neurogenic bladder due to spinal paraplegia. However, effective treatment of peripheral neurogenic bladder due to diabetes has not been shown, because the peripheral nerve is damaged in DCP patients.$^{[3, 6-9]}$. In addition, the ability for a bladder to empty could be improved through direct electrical stimulation to the bladder via the body cavity or laparotomy$^{[8, 10]}$. Previous results have shown that stimulation with a skin electrode on the abdominal wall of the bladder projection area is an effective and non-invasive treatment for DCP patients. In severe DCP patients, residual urine volume is reduced or eliminated through supplemental measures, such as continuous open catheter, which restores the urinary retention to urinate, even though the mechanisms remain unclear$^{[11]}$. Results from the present study demonstrated that signals produced by all three levels of exporting voltage (5.84 V, 8.00 V, and 11.00 V), with a frequency of 960 times/min, was transduced into the bladder following significant attenuation via the abdominal wall. Abdominal pressure from the skin to abdominal muscles was > 99.8%, which might be related to maximum impedance of skin and subcutaneous adipose tissue. However, abdominal pressure from abdominal muscles to the bladder was 22.8% greater than signals received at abdominal muscles, which might be due to reduced impedance of body fluids and serosa. However, the mechanisms for reduced received signals in diabetic rabbits, whose weight and abdominal subcutaneous fat was less than normal rabbits, remains poorly understood. This could be associated with increased resistance due to increased lipid content of body fluids (e.g., plasma and tissue fluid) as a result of metabolic disorders$^{[12]}$.

Several studies have reported that electrical stimulation induces bladder detrusor contraction and results in urination, although these studies did not perform direct stimulation to the bladder through the skin and body cavity, but rather stimulated the neural pathway$^{[3, 8, 10-12]}$. Results from the present study demonstrated that skin electrode signals were transduced to the bladder following significant attenuation due to abdominal wall impedance, which subsequently led to increased bladder pressure. However, bladder signals must be at least 8.75 ± 0.44 mV to induce significantly increased pressure in bladders of both normal and DM rabbits. Therefore, signal transductions through the skin to stimulate the bladder can result in coupled bladder mechanical contraction. Nevertheless, bladder pressure in the diabetic rabbits was less than in normal rabbits under basic and stimulated states, which could be due to injury to the tissue structure of the bladder detrusor.
and possibly an underlying molecular pathology, such as reduced expression of the cholinergic M₃ receptor[4,10]. Skin electrode signals directly stimulating the abdominal wall bladder projection area were transduced to the bladder subsequently inducing a coupling mechanical contraction of the detrusor. This contributed to urination following significant attenuation as a result of abdominal wall impedance. Furthermore, this non-invasive and effective method could be used to treat hypodynamic peripheral neurogenic bladder, such as DCP.

**MATERIALS AND METHODS**

**Design**
A randomized, controlled, animal experiment.

**Time and setting**
Experiments were performed in the Animal Room of Shenzhen People’s Hospital, China in 2009.

**Materials**
A total of 30 four-month-old, purebred, male, New Zealand rabbits (Medical Laboratory Animal Center of Guangdong Province, China; License No. SYXX (Yue) 2005-0061), weighing 1.2–1.4 kg, were housed in individual cages and were allowed free access to food at room temperature. Animal treatments were in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, issued by the Ministry of Science and Technology of China[10].

**Methods**

**Grouping and modeling**
After a two-week adaptation period, 10 rabbits were randomly assigned numbers from 1 to 30, which were divisible by 3, and were assigned to the control group. The remaining 20 rabbits were assigned to the DM group. After ten-hour fasting, a 5% alloxan (Sigma, St. Louis, MO, USA) solution diluted with normal saline was injected into the ear vein of rabbits in the DM group within 30 seconds (120 mg alloxan/kg). The same amount of sterile saline was injected into rabbits in the control group using the same method. At 72 hours after injection, fasting blood glucose was monitored to select successful models with fasting blood glucose levels ≥ 16.7 mM on consecutive 5 days[4].

**Signal and pressure recordings in the bladders of rabbits**
Four weeks after infusion, all successful models were anesthetized by 20% urethane (Sinopharm Chemical Reagent, Beijing, China) solution diluted with normal saline, which was injected into the ear vein (5 mL urethane/kg). Then, 8% sodium sulphide (Guangzhou Liqiang Chemical Plant, Guangdong Province, China) solution diluted with normal saline was used to remove hypogastric hair and fat, and a 3-cm horizontal incision was made in the fasting rabbits at approximately 4.5 cm above the pubis to expose the abdominal wall muscles, as well as the bladder. Urine was excreted via a F8 2-way catheter (Shenzhen Banghua Electric Equipment, Guangdong Province, China), which was fixed inside the urethra, and the ureter was ligated. Then, three pairs of electric probes were vertically attached to the three layers via the abdominal wall, where a pair of stimulating probes was attached at 2 cm above the cut on the skin of both sides at 1 cm from the abdominal midline. The probes were connected to Low-Frequency Electrical Bladder Therapeutic Equipment (Shenzhen Lihe Medical Instrument, Guangdong Province, China), and a pair of receiving probes was placed at the same position, but in the abdominal muscle layer. The other pair of receiving probes, with a 5-mm separation, was placed in muscle at the top of the bladder, and both receiving pairs were connected to a BL-410 Biological Experimental System (Thai Union Technology, Chengdu, Sichuan Province, China). The incision was closed layer-by-layer, but circulation was maintained between the atmosphere and the abdominal cavity. Subsequently, a F8 2-way catheter was connected to the system to form an enclosed space to maintain bladder volume after 40 mL normal saline was injected into the bladder to maintain contact with the abdominal wall. According to results obtained from the pre-experiment, three levels of voltage from [low (5.84 V), middle (8.00 V), and high (11.00 V)], with a fixed frequency of 960 times/min, were applied as stimulation to each rabbit during a 30-minute interval. At the basic state of 0 V, the rabbits in both and groups received voltage to the abdominal wall and the top muscle layers of the bladder, and the signals of receiving probes were recorded. In addition, bladder pressure was measured using a BL-410 Biological Experimental System connected to the F8 2-way catheter.

**Statistical analysis**
All data were presented as mean ± SD. The t-test was used to compare between groups, and a one-way analysis of variance was used to analyze parameters within a group. The linear correlation and regression relationship was analyzed to determine independent and dependent variables. SPSS 13.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis of data. Statistical significance was set to P < 0.05.

**Author contributions:** Xinmin Wang was responsible for the study design and funding. Qirui Fu, Qingmei Zhang, Ping Xu, Lin Cao, and Meng Xue participated in the animal experiments. Ping Xu was responsible for statistical analysis. Wei Wang composed the manuscript.

**Conflicts of interest:** None declared.

**Funding:** This research was supported by a grant from the Science and Technology Bureau of Shenzhen (Shenzhen Project of Science and Technology in 2010), No. 201001003.

**Ethical approval:** Animal procedures were in accordance with Animal Ethics Committee of Shenzhen People’s Hospital in China.

**Acknowledgments:** Many thanks to all of our colleagues from the Endocrinology Department and Animal Room of Shenzhen People’s Hospital in China.
REFERENCES


