Local anesthetics: New insights into risks and benefits
Lirk, Philipp

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Effects of early and diabetic neuropathy on sciatic nerve block duration and toxicity

Based on
Effects of early and late diabetic neuropathy on sciatic nerve block duration and neurotoxicity.
Introduction

Diabetic peripheral neuropathy (DPN) is a frequent complication of both Type I and Type II diabetes mellitus (DM), and the most prevalent neuropathy in the Western world.\textsuperscript{1} Diabetics undergo surgery more often than non-diabetic patients,\textsuperscript{2} and several surgical procedures for typical complications of long-standing DM, e.g. creation of arteriovenous fistula in patients with end-stage renal disease, might be preferably performed under regional anaesthesia.\textsuperscript{3}

However, diabetic neuropathic nerves may be more sensitive to local anesthetics and their toxicity, and this hypothesis is supported by two lines of evidence. Firstly, regional anaesthesia in diabetic neuropathic patients may be associated with increased risk of neurological injury.\textsuperscript{4} Limited epidemiological evidence suggests higher risk of neurotoxicity in diabetic neuropathic patients,\textsuperscript{5,6} even if experimental evidence has been equivocal.\textsuperscript{7} Secondly, DPN may influence nerve block duration.\textsuperscript{4} Clinical\textsuperscript{8,9} and experimental\textsuperscript{10,11} evidence suggests that block duration may be prolonged in diabetic neuropathic nerves. However, most studies were carried out in models of streptozotocin-induced Type I DM, which does not reflect clinical reality, in which the huge majority of patients suffer from Type II DM.\textsuperscript{5}

Our aim was to determine the impact of regional anaesthesia in DPN in an animal model for Type II DM. We therefore sought to devise a comprehensive model using behavioural, electrophysiological, and histopathological investigations to determine neurotoxicity of a lidocaine 2\% peripheral nerve block and duration of this nerve block in Zucker Diabetic Fatty rats with early diabetic neuropathy, advanced diabetic neuropathy, and advanced diabetic neuropathy under partial glycemic control. Our working hypothesis was that in a rodent model of Type II DM, the presence of advanced (18 weeks) but not early (10 weeks) neuropathy would lead to increased neurotoxicity and block duration following sciatic nerve block with lidocaine as compared to age-matched healthy control animals. The main endpoint was neurohistopathology one week after nerve block.
Materials and Methods

The present study protocol was approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, protocol number LEICA102868-1. Methods and results are reported according to ARRIVE guidelines.12

Animals

Experiments were carried out on the left sciatic nerve of Zucker diabetic fatty (ZDF) rats, obtained from Charles River Laboratories (L’Arbresle, France). This inbred model of Type II DM combines a genetic predisposition (homozygous leptin receptor mutation fa/fa, “diabetic”, or heterozygous mutation fa/+, “control”) with a dietetic component (Purina #5008 diet, Charles River, L’Arbresle, France).11 Animals were obtained at 9 weeks of age and were given one week for acclimatization. For all electrophysiological measurements, sciatic nerve block, and placement of insulin release implants, animals were anaesthetized using isoflurane (Baxter, Utrecht, The Netherlands) with an inspiratory concentration between 2 – 3 Vol%, since this regimen least affects electrophysiological measurements in rodent models.14 Adequacy of anaesthesia was ascertained using sensory testing. We only carried out procedures when the animal did not react to a forceful pinch to the forefoot using a forceps. All procedures were performed percutaneously to minimize animal distress, and analgesic rescue was buprenorphine 0.05 mg/kg body weight. Detailed welfare assessment concerning appearance and behaviour was carried out at least weekly by an animal care technician unrelated to the experiment. After the last measurements, while still under isoflurane anaesthesia, animals were euthanized using CO₂ narcosis.

Experimental groups

The timeline of experimental procedures is given in Figure 1. In all experimental groups, baseline measurements of electrophysiological parameters (see below) were taken at 10 weeks of age.

Group “early control (EC)” were 6 ZDF fa/+ animals, and group “early diabetic (ED)” were 10 ZDF fa/fa animals undergoing left sciatic nerve block
immediately after baseline testing at 10 weeks. One week later, behavioral and
electrophysiologic measurements were repeated, and the left sciatic nerve was
excised for neurohistopathologic evaluation.

The group “late control (LC)” consisted of 10 fa/+ animals kept until 18
weeks of age. The group of diabetic animals for the late experiments were
randomized into one of two groups according to a pre-existing randomization list:
group “late diabetic without insulin (LD)” were 10 ZDF fa/fa diabetic animals kept
until 18 weeks of age. Group “late diabetic with insulin (LDI)” were 10 ZDF fa/fa
animals, which received 1½ subcutaneous insulin implants (LinPlant, LHR-10BV,
LinShin, Toronto, Canada) using a custom-made trocar G12-SS, LinShin) at 10
weeks of age. The latter were dosed according to weight at 10 weeks, approximately
300g, and released approximately 3U insulin per day for a period of 60 days,
covering our experimental period.15 Groups LC, LD, and LDI underwent a second,
“late baseline” electrophysiologic testing at 18 weeks of age, followed by sciatic
nerve block. One week later, behavior and electrophysiology tests were repeated,
and tissue excision for neurohistopathology was performed.

Serum glucose levels were measured in all experimental groups in blood
drawn from the left tail vein, using a commercially available glucose meter (Blue,
FIA Biomed, Emsdetten, Germany), regularly calibrated using the LI and LII
calibration solutions (FIA Biomed). In groups EC and ED, this was done at 10 and
11 weeks, and in groups LC, LD, and LDI, this was done at 10, 14, 16, and 18
weeks of age.

Electrophysiology

With temperature maintained well above 34°C using a warming blanket
(HK25, Beurer, Ulm, Germany), we studied the sciatic and caudal nerve with
monopolar needle electrodes as described previously using a Nicolet Viking IVP
electromyography system (Nicolet, Madison, WI).16 In brief, for motor conduction
studies of the sciatic nerve the recording cathode was placed in the intrinsic muscles
between the hallux and the second digit, and the recording anode was placed
subcutaneously on the lateral surface of the fifth digit. Stimulating electrodes were
inserted 3 mm apart at the medial ankle, and just cranial to the sciatic notch. A
grounding electrode was attached between the stimulating and the recording electrodes. Supramaximal square-wave pulses of 0.1 ms duration were delivered. Supramaximal stimulation was achieved by increasing the intensity by 25-30% above the maximal stimulation. Compound muscle action potential (CMAP) amplitudes (peak to peak) were recorded. Motor nerve conduction velocity (MNCV) was calculated over the segment between the sciatic notch and the ankle, and minimal F-wave latency was measured from seven F-wave recordings.

For studies of the mixed sensory and motor caudal nerve, the recording cathode and anode were inserted at the base of the tail just 5 mm apart. The stimulating cathode was placed laterally in the tail at exactly 3 cm from the base of the tail; the stimulating anode was inserted 5 mm distal to the stimulating cathode. The earth electrode was attached halfway between the stimulating and recording electrodes. Supramaximal square-wave pulses of 0.5 ms duration were delivered. Compound nerve action potential (CNAP) amplitudes (negative peak) were recorded. The nerve conduction velocity (NCV) of the tail nerve was calculated from the latency of the stimulus artefact to the onset of the negative peak of the action potential elicited and the distance between the stimulating and the recording cathodes, which was a standard distance of 3 cm. All measurements were carried out by one investigator (P.L.), and electrophysiological measurements underwent blinded assessment and validation by an experienced neurophysiologist (C.V.).

**Sciatic nerve block**

Nerve block was performed percutaneously combining the technique described by Thalhammer et al.\textsuperscript{18} modified by nerve stimulation as described by Kroin et al.\textsuperscript{10} In brief, a 25G needle was introduced just caudal to the sciatic notch directed cephalad, and connected with a clip to the Viking electromyography system programmed to deliver a pulse of 0.1 ms duration, and 0.6 mA current, triggered manually. Ipsilateral hind-leg kick in the absence of local stimulation was taken as sign of proximity of needle to nerve, and injection of 0.2 mL of lidocaine 2% was performed. We defined a successful nerve block on the basis of three signs:

1) before injection, successful nerve stimulation at 0.6 mA current,
2) gradual disappearance of the CMAP in electrophysiological recordings after injection of lidocaine, and

3) subsequent behavioural testing showing absence of the toe-spreading reflex.

The latter reflex, used to test sciatic nerve fibres, was tested as described before by Kroin et al.\textsuperscript{10} Animals were gently lifted, resulting in a physiological vestibular reflex where toes are extended and spread. We noted the presence or absence of these findings to characterize block duration every 15 minutes until the block subsided. This gross behavioural testing was repeated before the animals were subjected to anaesthesia one week after nerve block to detect any permanent nerve injury.

**Neurohistopathology**

The main outcome parameter was the nerve injury score of the left sciatic nerve one week after nerve block. To this end, after the last electrophysiological measurements and animal euthanasia, left sciatic nerves were excised from animals. The segment proximal and distal from the site of injection was harvested, and fixed with a 2% buffered formalin solution, embedded in paraffin, cut at six microns in longitudinal and transverse sections and stained with hematoxylin-eosin (H.E.) and Masson trichrome. Samples were examined under the light microscope for evidence of inflammation in the epineurial, perineurial and endoneurial compartment, vascular injury and nerve fibre injury. Nerve fibre injury was assessed employing a simple, semi-quantitative four point score where 0 represents a normal nerve and 4 represents extreme injury with inflammation and destruction of all components of the nerve including axons and myelin, extending throughout the nerve bundle.\textsuperscript{10} The pathologist (U. de G.) was blinded to experimental group allocation.

**Statistical analysis**

Semiquantitative neurohistopathological data such as the primary outcome were compared by Friedman test followed - if significant - by Mann-Whitney U test. Power analysis revealed that a group size of 10 animals would have 80% power to reject the null hypothesis that the neurohistopathology score in the one group is
significantly different from another group of nerves using Mann-Whitney U test with a 0.05 two-sided significance level. Body weight, blood glucose level and neurophysiologic data were compared by analysis of variance (ANOVA) between groups followed by posthoc Bonferroni test for multiple comparisons. Variations of neurophysiological data over time were compared by paired samples T test. P < 0.05 was considered significant. Statistical analysis was performed with IBM SPSS® Statistics Version 20 (IBM, San Francisco, CA). Power analysis was done with the aid of nQuery Advisor® 7.0 (Statistical Solutions Ltd., Cork, Ireland).

Results

All animals survived to the end of the experiment, no animal needed analgesic rescue, and no animal fulfilled predefined criteria for termination of experiments (humane endpoints). Welfare assessment showed no abnormalities concerning appearance or behaviour at any time point. All animals showed clinical recovery from sciatic nerve block.

Early diabetes

At baseline (10 weeks), mean glucose value was 7.1 ± 1.0 in EC and 13.1 ± 4.9 mmol/l in ED animals (P < 0.01). Mean body weight was 288.8 ± 8.5 respectively 344.1 ± 16.7 g (P < 0.001). In EC versus ED nerves, sciatic nerve MNCV was 39.3 ± 4.2 versus 35.7 ± 2.5 m/s at baseline (P = 0.02), minimal F-wave latency was 7.3 ± 0.5 ms versus 7.9 ± 0.6 (P < 0.005).

Sciatic nerve block duration was 45 ± 13 min in the EC, and 67.5 ± 27 min in the ED group (P = 0.08).

The differences between electrophysiological parameters at baseline and 1 week after sciatic nerve block were calculated. Mean MNCV was decreased 1 week after block compared to baseline across EC and ED animals (P < 0.01). There was no difference between EC and ED animals in their change over time. We found no significant differences in electrophysiological CMAP parameters for controlled and EC animals at baseline and after nerve block.

In histopathological investigations most specimens in the EC and ED group showed mild chronic inflammation in the epineurium and in the adipose tissue. In
the EC group, 1/6 animals had a minimally elevated nerve injury score of “1+” (out of 4), compared to 3/10 animals in the ED group with an elevated nerve injury score of “1+” (n=1) and “2+” (n=2, n.s.).

Late diabetes

Weight and glucose levels of test animals are given in Table 1. Diabetic animals were randomized at 10 weeks of age to receive insulin treatment (LDI) or no treatment (LD). At 14 weeks of age, the mean glucose values were significantly lower in LDI than in LD animals. However, thereafter the difference became not significant (Table 1). Neurophysiologic data of conduction velocity and minimal F-wave latency are given in Figure 2. Conduction velocity increased over time in the LC group whereas it remained unaltered in LD and LDI animals. The conduction velocity at 18 weeks in the LD animals was significantly lower than in the LC group and remained significantly different one week after sciatic nerve block. After nerve block, conduction velocity tended to decrease in all groups, but this decrease did not reach statistical significance in any of the separate groups nor when data of all groups were pooled (p = 0.15, Figure 2A). We found no significant differences in electrophysiological CMAP parameters for LC and LD animals before and after nerve block. Caudal NCV was slowed significantly when comparing animals in groups LC (65.3 ± 12 m/s) and LD (57.1 ± 8 m/s, \(P < 0.05\)).

At 18 weeks, minimal F-wave latencies were significantly prolonged in the LD as compared to the LC group. At one week after nerve block the latency increased significantly, if data of all three groups (LC, LD, LDI) were pooled (Figure 2B). Decreases in minimal F-wave latency between the three experimental groups were not significant.

Block duration was shortest in the LC group, longest in the LD group, and intermediate in the LDI group (Figure 2C). The LDI animals had a longer block than the LC animals, but there was no significant difference in block duration between LD and LDI animals. LD animals had a block duration significantly longer (94.5 ± 33.2 min) than ED animals (67.5 ± 27 min, \(P < 0.05\)).

In histopathological investigations, we noted only minor changes. LC animals showed minimal, variable, multi-focal chronic inflammatory infiltrates in
epineural connective tissue, generally not extending into the endoneurial or perineurial compartments. One animal out of the LDI group and one animal out of the LD group showed focal oedema or focal area of acute myelin injury and axonal damage, associated with scattered inflammatory cells. The neurohistopathological changes were not significant between groups (Figure 3).

Discussion

Zucker Diabetic Fatty rats at 10 weeks of age did not show prolongation of duration of sciatic block with lidocaine 2% and did not show a discernible impact on nerve damage one week after sciatic nerve block. In rats 18 weeks of age, with more longstanding diabetes and clear signs of neuropathy, sciatic nerve block duration was substantially prolonged, but no gross behavioural signs, or increased neurohistopathological signs of nerve injury were found one week after sciatic nerve block. Electrophysiologic changes suggestive of subtle nerve dysfunction after nerve block were present in all experimental groups, but this was not related to the duration or severity of the neuropathy.

The ZDF model used in the present study represents the important patient collective of Type II DM more accurately than the previously used STZ-induced Type I DM model. Notably, pathogenesis differs considerably between Type I and Type II diabetes in experimental models, and in humans, such that implications for regional anaesthesia should preferably be undertaken in a model most closely resembling the clinical situation. However, all previous investigations had been conducted in models of Type I DM in vivo, or in Type II DM in vitro. Our investigation is the first to investigate the effects of a DPN secondary to Type II DM on toxicology and function of sciatic nerve blockade.

Early diabetes

In our model, ED rats at 10 weeks of age had mildly decreased nerve conduction velocities, indicating a mild diabetic neuropathy. The neuropathy in these rats develops over time and our measurements of neuropathy correspond well with previous literature. Duration of nerve block was not significantly prolonged ($P = 0.08$) in ED as compared to EC animals. We found no significant
neurohistopathologic or gross behavioural signs of nerve damage one week after sciatic nerve block. Our results concerning neurotoxicity correspond to previous \textit{in vitro} investigations in the ZDF model at 12 weeks of age,\textsuperscript{11} and with similar findings in an \textit{in vivo} model of Type I DM.\textsuperscript{23}

\textit{Late diabetes}

LD animals at 18 weeks of age had decreased nerve conduction velocities as compared to LC animals, indicating the development of a more severe diabetic neuropathy over time. This corresponds to previous literature.\textsuperscript{25} We found that while there was a slight increase in MNCV in LC over time, the MNCV of LD did not show such an increase. This is in concordance with previous work by Oltman and colleagues, where a slight increase in MNCV in lean (healthy) animals over time until the 20\textsuperscript{th} week can be observed.\textsuperscript{24} Also, we corroborated our MNCV findings by simultaneous measurements of the caudal NCV, confirming diabetic neuropathy also in this predominantly sensory nerve.

We found no histopathological signs of increased neurotoxicity in LD animals as compared to age-matched LC animals, when lidocaine 2\% was used to elicit sciatic nerve block. Our results mirror previous investigations in type I DM, in which lidocaine at clinical concentrations had limited neurotoxic effects.\textsuperscript{10,21} There have been no \textit{in vivo} local anaesthetic neurotoxicity investigations in type II diabetic models at all, but recent \textit{in vitro} data show only modest neurotoxicity of lidocaine 2\%.\textsuperscript{11} Our data confirm and widen these \textit{in vitro} results using a multifaceted testing setup \textit{in vivo}. Further, epidemiologic clinical outcome data suggest that even when long-lasting local anaesthetics are used, nerve injury following neuraxial blockade in patients with pre-existing neuropathy is rare. Specifically, in one retrospective study, the incidence of apparent neuropathic complications after neuraxial anaesthesia was 2 in 325 patients.\textsuperscript{6} Across the past 20 years, 6 case reports describing association of nerve damage with diabetic neuropathy following regional anaesthesia have been published.\textsuperscript{5}

We note highly significant prolongation of minimal F-wave latency as a subtle marker of nerve dysfunction one week after sciatic nerve block. This has not been previously described, but there was no difference between LD and LC animals,
such that this most likely represents a minor and unspecific sequel of nerve block. The clinical importance of this remains unclear, and is most probably very limited. There has been discussion whether regional anaesthesia in diabetic patients may induce clinically unapparent damage which may promote progression of diabetic neuropathy. However, the changes observed here are very small, and need to be investigated in detail before any clinical relevance can be ascribed.

In LD animals, block duration was significantly prolonged, while LDI animals had an intermediate increase in block duration. This finding was expected on basis of previous findings. Our results differ in magnitude from our previous manuscript, in which several tests were used to quantify motor, deep sensory, and superficial sensory block. In comparison, our rather crude “on/off” testing for a vestibular reflex in this study more closely reflects the data obtained by Kroin, who used the same method. The main difference with the latter study is that we used 0.2 mL of 2% lidocaine as in our previous study, whereas Kroin used 0.1 mL of 1% lidocaine. Several studies assessed block duration in a streptozotocin-induced rat model of type I DM, and while one study found no difference, three studies showed prolonged block duration in diabetic rats. This latter finding was confirmed in the ZDF rat model of type II DM by us. Recently, two clinical studies demonstrated increased sciatic nerve block duration in diabetic patients. Therefore, the evidence strongly indicates that diabetic neuropathy will prolong the duration of peripheral nerve block. This prolongation may be caused by pharmacokinetic or pharmacodynamic (e.g., modulation of sodium channels by neuropathy) mechanisms, but the respective contributions remain unclear.

Potential clinical implications are to consider the diabetic nerve “more sensitive” to the effects of local anaesthetics, and it has been proposed to reduce dose of local anaesthetics when performing nerve blocks for perioperative analgesia. Also, it had been suggested to reduce or omit epinephrine from peripheral nerve blocks in neuropathic patients, and the results obtained in experimental and clinical settings would indicate that nerve block duration in diabetic neuropathy will be prolonged anyway, even without the need to add adjuvant epinephrine.
Limitations

In the LDI rats the effects of insulin were not sufficient to cause glucose levels to be similar to those in control animals, even though the same dose achieved good glucose control in type I and type II models of DM. This may be because insulin was dosed according to body weight at baseline (10 weeks), and diabetic animals were substantially heavier at the end of the experimental period, leading to relative under-dosing towards the end of the experimental period, which is supported by the increasing blood glucose levels over time in these animals. Therefore, our insulin regimen was more representative of loose glycaemic control than of strict glycaemic control.

In diabetic as well as control animals, small inflammatory changes were noted on neurohistopathological investigation in all groups, which may be explained by repeated stimulation. Sciatic nerve inflammation after repetitive stimulation, as occurred in our study due to neurophysiological measurements, has been described in vivo before. The timepoint of excision was chosen on basis of earlier experiments by our group, but differ with the timepoint chosen by Kroin et al. (2 days post block). Seen that the mild changes after block were seen both in the study by Kroin and our study, these results can be interpreted to support and strengthen each other.

The model used by us cannot be extrapolated directly to the clinical situation. First, the age of the experimental rat can be compared to that of a young human adult when estimating on basis of physiological and behavioural parameters. However, it should be noted that we chose the age of our test animals based on the age at which neuropathy typically develops, which is around 20 weeks. Second, the precise interspecies difference between rats and humans concerning neuropathy and toxicity of local anaesthetics is unclear. Nevertheless, rodent models have been used to investigate diabetes and its neuropathy, and determine functional and toxicological aspects of regional anaesthesia in the past.

Lastly, we attest to the fact that the focus of behavioural testing after sciatic nerve block was on the motor component of the nerve block, while DPN profoundly affects sensory function as well. However, in a previous study using the same
model, we obtained comparable prolongations of both motor and sensory blockade upon sciatic nerve blockade.\textsuperscript{11}

\textit{Ethical considerations}

We sought to minimize animal distress by conducting all invasive procedures such as electrophysiology and nerve block under general anaesthesia. To limit tissue injury and prevent post-interventional pain, we performed nerve blocks percutaneously as described by Kroin et al.,\textsuperscript{10} and not open. At the same time, this theoretically entails that the position of injection is less reliable, and intraneural injection is possible. However, the only study directly comparing these two modes of injection found comparable results for both approaches,\textsuperscript{10} such that we felt confident to perform our blocks percutaneously. The same percutaneous approach using thin needle electrodes was chosen for electrophysiological measurements. To avoid repetitive injections of insulin for the animals randomised to the late group, we used subcutaneous implants. To minimize the number of animals used in experiments, we obtained approval to use the heart and the right sciatic nerve of our test animals for pilot experiments investigating enzyme expression and epigenetic markers in type II diabetes, respectively.

\textit{Future perspectives}

We describe a novel comprehensive model to investigate toxicological and functional consequences of diabetic neuropathy \textit{in vivo}, combining behavioural, electrophysiological and histopathology methods. Despite lidocaine being the most widely used local anaesthetic for toxicity research, results obtained by Kroin et al. suggest that longer-acting local anaesthetics such as bupivacaine and/or ropivacaine may be more toxic with respect to neurohistopathology.\textsuperscript{10} We suggest to investigate the neurotoxic potential of long-lasting local anaesthetics such as bupivacaine, and the value of adjuvants, which may increasingly become relevant in clinical practice.\textsuperscript{7,26} Similarly, the pharmacokinetics and –dynamics of the diabetic nerve as relevant to regional anaesthesia warrant further investigation.
Conclusions

Our results suggest increased sensitivity of diabetic nerves for short-acting local anaesthetics without adjuvants \textit{in vivo}, as evidenced by prolonged block duration in a rodent Type II diabetes mellitus model with longstanding diabetic neuropathy. This sensitivity appears to increase with progression of neuropathy. We observed very subtle changes suggestive of nerve injury after nerve block in general, with no correlate in gross behavioural testing or neurohistopathology, and no specific effect of neuropathy. Our results do not support the hypothesis that neuropathy due to Type II diabetes mellitus increases the risk of nerve injury after peripheral nerve block.

Acknowledgments

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200


Table 1

Body weight (gr)

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<th>10</th>
<th>14</th>
<th>16</th>
<th>18</th>
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<tr>
<td>LC (n=10)</td>
<td>282 ± 14</td>
<td>364 ± 17</td>
<td>386 ± 23</td>
<td>402 ± 37</td>
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<tr>
<td>LD (n=10)</td>
<td>345 ± 19</td>
<td>419 ± 39</td>
<td>427 ± 38</td>
<td>435 ± 36</td>
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<tr>
<td>LDI (n=10)</td>
<td>350 ± 20</td>
<td>471 ± 25</td>
<td>484 ± 35</td>
<td>494 ± 41</td>
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<td>p &lt; 0.01</td>
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Blood glucose (mmol)

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<th>Weeks</th>
<th>10</th>
<th>14</th>
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<tr>
<td>LC (n=10)</td>
<td>7.6 ± 1.9</td>
<td>6.0 ± 0.5</td>
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<td>LD (n=10)</td>
<td>11.9 ± 5.3</td>
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<td>n.s.</td>
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Weight and blood glucose levels of all groups over time.
Tested by ANOVA with posthoc Bonferroni correction. n.s. = not significant
Figure legends

Figure 1
Timeline of experimental interventions.

Figure 2
Development of sensible conduction velocity (2A) and minimal F-wave latency (2B) over time in the three experimental groups. After nerve block, conduction velocity tended to decline in all groups, but did not reach statistical significance even if all groups were pooled (p = 0.15).
After the nerve block the minimal F-wave latency increased significantly, when the data of all three groups were pooled.
Analysis of variance (ANOVA) with posthoc Bonferroni test, paired samples T test. * $P < 0.05$; # $P < 0.01$; ## $P < 0.001$.

Figure 3
Sciatic nerve motor block duration in late diabetic animals. Analysis of variance (ANOVA) between groups followed by posthoc Bonferroni correction. * $P < 0.05$; # $P < 0.01$; ## $P < 0.001$.

Figure 4
Neurohistopathology of a healthy control nerve nerve (A), and a nerve from a diabetic animal, showing focal areas of acute myelin injury and axonal damage, associated with scattered chronic inflammatory cells (B).
Figure 1

**Early Diabetes**
- Group Early control (EC)
- Group Early diabetic (ED)

Electrophysiology
Sciatic nerve block

9 weeks old -> 10 weeks old

Electrophysiology
Neurohistopathology

11 weeks old

**Late Diabetes**
- Group Late control (LC)
- Group Late diabetic (LD)
- Group Late diabetic with insulin treatment (LDI)

Electrophysiology
Group LDI: Insulin implant

9 weeks old

//

10 weeks old

18 weeks old

19 weeks old
Figure 2: Neurophysiology of tibial nerve

2A
Regional anesthesia in early and late neuropathy

2C

Block duration

<table>
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<td>LDI</td>
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p < 0.01

p < 0.05
Figure 3