

1 Alpha-motoneurons maintain biophysical heterogeneity in obesity and diabetes in Zucker rats.

2

3

4 Christopher W. MacDonell, Jeremy W. Chopek, Kalan R Gardiner, Phillip F Gardiner

5

6

7 Spinal Cord Research Centre, Department of Physiology & Pathophysiology, Rady Faculty of Health,
8 University of Manitoba, Winnipeg, MB, R3E 0J9

9

10 Abbreviated Title: Obese and diabetic motoneurons

11

12

13 Keyword: motoneuron, obesity, diabetes, electrophysiology, neurophysiology.

14

15

16

17

18

19

20 Correspondence to:

21 Christopher W. MacDonell, Ph.D.

22 402 Basic Medical Science Building

23 Spinal Cord Research Centre, Department of Physiology & Pathophysiology, Faculty of Medicine,

24 University of Manitoba

25 Winnipeg MB, Canada, R3E 0J9

26 Telephone: 204.977.5622

27 Facsimile: 204.789.3930

28 Email: cmacdon@scrc.umanitoba.ca

29 ABSTRACT

30 Small diameter sensory dysfunction resulting from diabetes has received much attention in the
31 literature, while the impact of diabetes on alpha-motoneurons (MN) has not. In addition to this, the
32 chance of developing insulin resistance and diabetes is increased in obesity. No study has examined the
33 impact of obesity or diabetes on the biophysical properties of MN. Lean Zucker rats and Zucker Diabetic
34 Fatty (ZDF) rats were separated into Lean, Obese (ZDF fed standard chow) and Diabetic (ZDF fed high fat
35 diet that led to diabetes) groups. Glass micropipettes recorded hind-limb motoneuron properties from
36 identified flexor and extensor motoneurons. Motoneurons were separated within their groups based on
37 input conductance, which created high and low input conductance subpopulations for each. A
38 significant shorter (20%) afterhyperpolarization half-decay (AHP_{1/2}) was found in low conductance
39 motoneurons for the diabetic group only, while the AHP_{1/2} tended to be shorter in the Obese group
40 (19%). Significant positive correlations were found among Rheobase and input conductance for both
41 lean and obese animals. No differences were found between the groups for the afterhyperpolarization
42 amplitude (AHPamp), input conductance (IC), rheobase or any of the rhythmic firing properties
43 (Frequency-Current slope and spike frequency adaptation index). Motoneuron properties continue to
44 be heterogeneous in obese and diabetic animals. Obesity does not seem to influence lumbar
45 motoneurons. Despite the motoneurons resistance to the impact of diabetes, the reduced AHP $\frac{1}{2}$ decay
46 and the tendency for a reduction in AHPamp may be the first sign of change to motoneuron function.

47 **NEW & NOTEWORTHY**

48 Knowledge about the impact of obesity and diabetes on the biophysical properties of motoneurons is
49 lacking. We found that diabetes reduces the duration of the afterhyperpolarization and that
50 motoneuron function is unchanged by obesity. A reduced afterhyperpolarization may impact discharge
51 characteristics and may be the first sign of change to motoneuron function.

52 **INTRODUCTION**

53 Health concerns related to obesity and diabetes are growing. In Canada, adults that are overweight or
54 obese (BMI greater than 25) constitute 54% of the population (Statistics Canada, 2014). Further to this,
55 obesity is associated with increased risk of developing diabetes in persons greater than 18 years of age
56 (Millar and Young, 2003). It has been shown by many that diabetes disrupts the normal physiological
57 function of small-diameter sensory nerves over time, leading to increased pain and/or loss of sensation
58 (see review, DUBY et al. 2004). However, there are few studies that have examined how diabetes affects
59 alpha-motoneurons (hereafter referred to as motoneuron) in the lumbar spinal cord.

60 A properly functioning sensory system seems to be necessary for producing optimal motor output. In a
61 spinal transected animal model, with either reduced or eliminated sensory feedback, basic and rhythmic
62 lumbar motoneuron properties show less excitability (Button et al. 2008; Beaumont et al. 2004).

63 However, when spinal transected rats go through a passive cycling exercise regime, proper spinal
64 motoneuron function is restored (Beaumont et al. 2004). Part of this restoration in function may be due
65 to the sensory feedback created by the movement of the lower limbs. As such, any change in small
66 diameter sensory afferents via diabetes, may impact motoneuron output.

67 In humans, studies have shown that motor units from limb muscles in both Type I and II diabetics show
68 deficits when compared to non-diabetics. Motor axons were shown to have a decreased conduction
69 velocity, while compound muscle action potentials were smaller in amplitude and longer in duration in

70 diabetics (Brown and Feasby, 1974; Hansen and Ballantyne, 1977). In addition, motor unit number
71 estimates showed estimated decreases in the thenar and extensor digitorum brevis muscles of adult
72 diabetics (Brown and Feasby, 1974); a finding that has been confirmed in additional muscles in adults
73 (Hansen and Ballantyne, 1977; Allen et al. 2013;) and type I diabetic children (Toth et al. 2014).
74 Functionally, motor unit firing rates, peak force production and time to fatigue parameters were
75 reduced in human Type I diabetics (Almeida et al., 2008); while in type II diabetics, both motor unit firing
76 rate and the stability of the force signal was reduced during isometric contraction (Watanabe et al.
77 2013). Finally, a redistribution of muscle fiber type to a fast-twitch phenotype has been shown to occur
78 as a result of diabetes (Oberbach et al. 2006).

79 In Type I diabetic rodent models, similar changes in the neuromuscular system have been found.
80 Following streptozotocin (STZ) injection (Type I diabetes model), mice showed a decrease in motor unit
81 number estimates, increased single motor unit potentials (Souayah et al. 2009), decreased motor axonal
82 conduction velocity, and reduced compound muscle action potential (CMAP) amplitude (Ramji et al.
83 2007). In addition, neuromuscular junction numbers were reduced by 60% (Ramji et al. 2007) and
84 miniature endplate current amplitudes and acetylcholine quantal release were reduced in response to 1-
85 Hz nerve stimulation (Souayah et al. 2009). In Type II diabetic rodents, the Zucker Diabetic Fatty rat
86 (ZDF) model suffers from slowed motor nerve (Coppey et al., 2002) and sensory nerve conduction
87 velocity, as well as decreased CMAP and sensory nerve action potential amplitudes (Russell et al. 2008).
88 While Type I myosin heavy chain (MHC) content decreases, fast Type II MHC content increases (Kim et
89 al. 2015). Finally, changes to thermal and mechanical nociception occur (Sugimoto et al., 2008).

90 At the spinal motoneuron and motor nuclei level, available literature suggests that diabetes may impact
91 motor nuclei size and number. Dorfman et al., (2004) found a decrease in spinal nuclei volume and a
92 shift towards smaller nuclei area in the bulbocavernosus motor nucleus of induced diabetic rats (4

93 weeks post STZ). However, Ramji et al. (2007) found no difference in either the number or
94 morphological characteristic of motor nuclei in the lumbar spinal cord of mice (8 months post STZ) but
95 did find an increase in cellular markers of neuronal stress and protection. In addition, Muramatsu et al.,
96 (2011) found that both size and number of presumed gamma-motoneurons were reduced (22-weeks
97 post STZ) in rats, but later reported (Muramatsu et al., 2017) that large (presumed alpha) and small
98 motoneurons of the MG nucleus had less cross-sectional area (12 weeks post) and were fewer in
99 number (12 and 24 weeks post STZ). Although slightly variable, the balance of results from the available
100 literature suggests motoneurons are susceptible to the effects of diabetes in a Type I diabetic model.

101 A strong association exists between obesity and insulin resistance that leads to type II diabetes,
102 which is likely mediated by widespread chronic inflammation (Hotamisligil et al. 1993; Hotamisligil,
103 2006). Given the association between obesity and diabetes, any change in motoneuron function may
104 exist along a continuum and therefore be evident in obese non-diabetics. In the ZDF model, decreased
105 oxidative capacity and increase in fast Type II muscle fibers have been found compared to control rats
106 (Acevedo et al., in press). In humans, with regard to obesity and the neuromuscular system, the
107 reported impact of being overweight or obese on motoneuron function is limited to voluntary activation
108 assessed via the twitch interpolation technique and muscular strength. Blimkie et al. (1990) showed
109 voluntary activation in adolescent obese males to be lower during isometric knee extensor contractions
110 compared to age-matched lean adolescents. In contrast, Garcia-Vicencio et al. show that adolescent
111 girls have increased voluntary activation of knee extensors during fatiguing isometric contractions
112 (2015) and during isometric contractions (2016) in both knee extensor and plantar flexor muscle groups.
113 In addition, greater absolute knee extensor strength has been shown in obese adolescent males
114 (Abdelmoula et al., 2012) and females (Garcia-Vicencio et al. 2015) compared to lean individuals, while
115 relative strength has been shown to be higher (Abdelmoula et al., 2012) less (Maffiuletti et al., 2008) or
116 similar (Blimkie et al. 1990) compared to lean adolescents. Relative plantar flexor strength has been

117 evaluated only in females and was recently shown to be greater in obese adolescent girls (Garcia-
118 Vicencio et al. 2016). Although variable, these results may imply that increased body mass may confer
119 a neuromuscular overload effect consistent with changes seen in the neuromuscular system as a result
120 of increased activity in an animal model (wheel and treadmill running; Beaumont and Gardiner, 2002;
121 2003).

122 Healthy motoneurons have distinct heterogeneous properties that operate over a continuum and relate
123 to the type of muscle fiber innervated. For example, motoneuron afterhyperpolarization amplitude and
124 duration are greater, rheobase current is less, and input resistance is larger in motoneurons that
125 innervate slow twitch muscle fibers compared to those that innervate fast twitch muscle fibers (Eccles et
126 al. 1958; Gardiner and Kernell, 1990; Zengel et al. 1985; Fleschman et al. 1981). Motoneuron
127 biophysical properties are also not static and respond to exercise (Beaumont and Gardiner, 2002, 2003;
128 Beaumont et al. 2004; MacDonell et al., 2012), sedentary activity (Cormery et al. 2005), as well as
129 eliminated descending and afferent input (Button et al., 2008). To our knowledge, no studies have
130 examined the impact of diabetes or obesity on motoneuron biophysical function. The purpose of this
131 investigation was to establish the impact of diabetes and obesity on flexor and extensor lumbar
132 motoneurons in diabetic and obese rats. Given the reported changes in motor units and reduced
133 motoneuron number and area (Muramatsu et al. 2017; Dorfman et al., 2004), altered
134 electrophysiological properties mentioned above should be evident from the sampled motoneuron
135 pool. In addition, with obesity, the extra mass may confer a training overload effect that would
136 translate to changes in motoneuron properties similar to that seen with exercise trained rats (Beaumont
137 and Gardiner, 2002, 2003).

138 **METHODS**

139 ***Experimental Animals***

140

141 Female Zucker Diabetic Fatty (ZDF) and Zucker Lean rats were received from Charles River at six
142 weeks of age. The animals were divided into three groups: 1) Zucker Lean (Lean) group that were fed
143 standard chow, 2) Zucker Obese (Obese) group that were of the ZDF strain but were fed standard chow
144 and 3) Zucker Obese-Diabetic (Diabetic) group that were fed a high fat diet (D12468, Research Diets Inc.,
145 NJ, USA) that has been shown to consistently induce a diabetic phenotype (Mulder et al., 2010). Rats
146 were caged in pairs in the Animal Care facility at the University of Manitoba.

147 A subset of animals had their blood glucose tested (Table 1) at the beginning of the experiment,
148 immediately after induction of a surgical plane using a mixture of Isoflurane (5%) and pure oxygen.
149 Following induction, anesthesia was maintained (1-2.5% Isoflurane), and verified by monitoring heart
150 rate and testing bilateral toe-pinch and eye-lash reflexes. Isoflurane delivery occurred until the
151 completion of a precollicular-postmamillary decerebration, after which ventilation of the animal
152 occurred with pure oxygen until the termination of the experiment. The animals had a mean age of 9.2
153 months at the time of data collection. In accordance with the University of Manitoba animal ethics, at
154 the termination of data collection, animals were killed by an IV injection of potassium-chloride and a
155 bilateral pneumothorax. The authors confirm that the present research was carried out in accordance to
156 the animal ethics committee for the University of Manitoba, which met the guidelines set forth by the
157 Canadian Council of Animal Care.

158 ***Surgical Procedures***

159 Immediately following the induction of anaesthesia and glucose testing, an IP injection of
160 atropine (0.05 mg·kg⁻¹ atropine within 5% dextrose physiological saline) was administered to minimize
161 airway secretions. Following atropine administration bilateral toe-pinch reflexes were re-assessed to

162 ensure the animal was in a surgical plane of anaesthesia. The surgical procedures, in order included: 1)
163 left tibial (extensor) and peroneal (flexor) nerves exposure for antidromic stimulation, 2) insertion of a
164 tracheal tube for ventilation (Harvard Apparatus, CA; rate: 60-80 strokes min⁻¹; tidal volume range 2.0 to
165 2.5 mL), and expired CO₂ monitoring (levels ranged 3-4%; CAPSTAR 100 CO₂ analyzer, CWE Inc., USA); 3)
166 cannulization of the right carotid artery to monitor mean arterial blood pressure (MAP) and provide an
167 infusion port; 4) dexamethasone administration (0.1 mL) via the carotid artery cannula to reduce
168 swelling of the brain; 5) occlusion of left carotid with a suture (2-0) prior to dissection of the back
169 musculature in preparation for a laminectomy (T12 to S1); and 6) laminectomy within a stereotaxic
170 frame. Upon completion of the laminectomy, the dorsal roots were brushed aside, a mineral oil bath
171 was formed and the parietal bones of the skull were exposed and excised to prepare for a precollicular-
172 postmamillary decerebration.

173 In an attempt to reduce bleeding, both carotid arteries were previously tied off. The skull was removed
174 bilaterally, leaving the central and inter-parietal sutures intact. This resulted in small oval holes in the
175 skull superior to the zygomatic arches with the inter-parietal suture, the central suture and 2mm rostral
176 of bregma as the borders. The sutures and dura were cauterized to reduce bleeding. Under low suction
177 the cortex was removed in the parietal regions while controlling bleeding with a combination of Surgicel,
178 (Johnson and Johnson USA); Instat, (Johnson and Johnson, USA); Gelfoam, (Pharmacia and Upjohn,
179 USA); and Surgi absorbent swabs, (Kettenbach GmbH and Co., DE). The remaining skull along the central
180 suture was removed and the remaining tissue was aspirated until the superior colliculi and thalamus
181 were exposed. At this point, a pre-collicular cut was made and the hypothalamus, thalamus, and
182 forebrain removed. Absorbable hemostat was applied to control bleeding throughout the procedure.
183 Typically, MAP decreased to 50 mmHg during the procedure, but rebounded to 80 mmHG or more
184 within minutes. However, if MAP did not restore due to excessive hemorrhaging, a saline-alginate
185 (0.07%) solution was administered intravenously to expand plasma volume and restore MAP (Cabrales

186 et al. 2004). Following decerebration, isoflurane delivery was discontinued and the animal ventilated
187 with pure oxygen for data collection. To eliminate movement of the animal from antidromic
188 stimulation of the peripheral nerves a neuromuscular junction blocker (Pancuronium bromide, 2mg/mL)
189 was administered to paralyze the animal and a unilateral pneumothorax helped reduce movement of
190 the spinal cord related to ventilation. Following the pneumothorax, both tibial and peroneal nerves
191 were mounted using silver-chloride hook electrodes and the micropipette was positioned along the L3-
192 L4 dorsal root.

193 ***Intracellular Recordings***

194 Glass micropipettes (1.0 mm thin-walled, World Precision Instruments, USA) were formed with a 1-2 μm
195 diameter (resistance 7 – 12 $\text{M}\Omega$; Kopf Vertical Pipette Puller, David Kopf Instruments, USA) and filled
196 with a two-molar solution of K^+ Citrate. The use of bilateral flexor (peroneal) and extensor (tibial) silver-
197 chloride hook electrodes allowed for peripheral nerve stimulation to identify spinal motoneurons
198 antidromically. Stimulation of the peroneal and tibial nerves occurred at a frequency of 2 – 3 Hz (0.1-0.2
199 mA for 0.1 ms) whereby the field potentials produced were monitored continuously during micropipette
200 advancement through the spinal cord. Intracellular motoneuron records were collected at 20 KHz by an
201 Axoclamp intracellular amplifier system (Axoclamp 2B, Axon Instruments Inc., USA) used either in bridge
202 or discontinuous current-clamp mode (DCC; 3-10 kHz switching), with capacitance maximally
203 compensated. Evidence of successful motoneuron impalement included a sudden increase in
204 membrane potential to at least 50 mV, an antidromic action potential (AP) spike amplitude greater than
205 55 mV with a positive overshoot and a reproducible latency of less than 2.5 ms from the stimulation
206 artifact. Upon completion of data collection from a motoneuron, confirmation of the resting membrane
207 potential occurred by backing the micropipette out of the cell using steps of five- μm .

208 ***Intracellular Data***

209 The following intracellular data were collected in DCC mode (3-8 kHz) from antidromically
210 identified hindlimb motoneurons: 1) rheobase, defined as the current amplitude of a 50-ms depolarizing
211 pulse that caused an action potential 50% of the time; 2) input conductance, defined as the reciprocal of
212 the motoneuron input resistance calculated from the average membrane response to 25 or more 150-
213 ms 1-nA hyperpolarizing current pulses, 3) the discharge response to a ten-second triangular
214 depolarizing ramp current injection, to calculate the frequency-current (F/I) slope to slow input; 4) a
215 series of 500-ms depolarizing current pulse injections to calculate the F/I slope to a fast input and 5) an
216 adaptation index from the F/I slope calculated from the reciprocal of the averaged last 3 ISI to the first
217 ISI (see below). In addition, resting membrane potential was measured before a short 0.5 ms
218 intracellular depolarizing pulse in bridge mode. An average of at least 30 of the resulting action
219 potentials, the afterhyperpolarization (AHP) amplitude (AHPamp), AHP half-decay (AHP_{1/2}), spike height,
220 and spike duration were measured. Except for the adaptation index and frequency-current slope
221 relationship (described below), the groups were subdivided into high and low conductance
222 subpopulations based on the 50th percentile value of each group (Figure 1) Those motoneurons with an
223 IC greater than the 50th percentile were designated as high input conductance motoneurons, while
224 those less than or equal to the 50th percentile were designated as low input conductance motoneurons.

225 ***Frequency-Current Relationship Slope***

226 The slope of the frequency current (F/I) relationship was calculated by applying linear regression to the
227 data obtained from a slow depolarizing triangular (5-s ascending and 5-s descending) intracellular
228 current injection and a fast depolarizing (500-ms) intracellular current injection.

229 *Slow depolarizing triangular current injection (slow F/I)*: The reciprocal of the inter-spike interval from
230 action potentials produced by current injection were plotted against current amplitude to obtain the
231 slow F/I relationship, wherefrom the slow F/I slope was calculated.

232 *500-ms depolarizing current injection (fast F/I):* A series of increasing amplitude 500-ms depolarizing
233 current steps were delivered (0.3 Hz) until the motoneuron failed to discharge the entire 500-ms epoch.
234 The reciprocal of the first inter-spike interval produced from each current step (with exception of the
235 step where discharge failed) was plotted against the current amplitude to obtain the F/I relationship,
236 wherefrom the fast F/I slope was calculated.

237 ***Spike Frequency Adaptation Index***

238 Spike frequency adaptation (SFA) is the time dependent decrease in discharge rate during a constant
239 depolarizing current injection (Granit et al. 1965). SFA was assessed by creating an index of the F/I
240 slopes calculated from the initial firing frequency and steady-state firing frequency of the action
241 potentials collected during a series of 500-ms depolarizing current injections. The initial firing
242 frequency comprised the first two spikes (Initial), while the steady-state firing frequency (SSFF)
243 contained the last four spikes. As done previously (MacDonell et al., 2012), the ratio of the SSFF F/I
244 slope to the Initial F/I slope yielded the SFA adaptation index (AI).

245 ***Statistics***

246 All statistical tests were computed in MATLAB (R2013a, Mathworks Inc., MA) and figures were created
247 with Origin (version 7, OriginLab Corp., MA). The first step in statistical analysis was to determine
248 whether the dependent variables were distributed normally. For the normality test and subsequent
249 analyses, a p-value of less than or equal to 0.05 determined significance.

250 The following variables were found to be not normally distributed according to the Kolmogorov-Smirnov
251 normality test: $AHP_{1/2}$, AHPamp, AI, IC, rheobase, fast F/I and slow F/I (p-values < 0.0001). Due to the
252 decision to separate the motoneurons into high and low conductance groups, the Kolmogorov-Smirnov
253 two sample test was used to determine if the distribution of IC values were different between the
254 groups. The Kolmogorov-Smirnov test returned non-significant probability values for each comparison
255 (Lean-Obese, p = 0.184; Lean-Diabetic, p = 0.452; Obese-Diabetic, p = 0.1334).

256 Given the above, non-parametric statistics were chosen to determine if differences existed between
257 Lean, Obese and Diabetic hindlimb motoneuron properties. Kruskal-Wallis analysis of variance (KW-
258 ANOVA) on ranks tested whether the dependent variables (see above) were significantly different
259 between the groups (Lean, Obese and Diabetic) but is limited to one level. Ranksum tests evaluated
260 which groups differed following a significant KW-ANOVA test and were also used to determine
261 significance between two groups. Spearman's Rho (ρ) tested the magnitude and direction of any
262 correlation between variables.

263 Differences between groups for mass and blood glucose levels were tested with parametric tests.
264 Significant differences were tested with separate one way independent ANOVA. Upon finding a
265 significant result, a student's t-test tested for any differences in means between the groups. Since the
266 variances between the groups were unequal, the unequal variance t-test determined the t-critical value.
267 The increase in familywise error rate due to multiple comparisons was not corrected for because it was
268 deemed that avoiding a type II error (failing to reject the null hypothesis when it should have been
269 rejected) was more important than inflating the type I error rate (rejecting the null hypothesis instead of
270 failing to reject the null hypothesis). Data are presented as the median with the interquartile range
271 (IQR) in brackets for non-parametric data and mean (SD) for parametric data.

272 **RESULTS**

273 In total, data were collected from 195 hindlimb motoneurons from 50 animals (17 Lean; 17 Obese; 16
274 Diabetic). Motoneuron properties were collected from both flexor and extensor motoneuron pools. No
275 significant difference between motoneuron pools was evident. Therefore, flexor and extensor data was
276 pooled. Average mass and glucose levels for each group are displayed in Table 1. Significant main
277 effects for mass ($F_{(2,45)} = 3.2, p < 0.00001$) and blood glucose levels ($F_{(2,18)} = 3.5, p = 0.00013$) were found.
278 For blood glucose, all groups had a significantly different blood glucose level, where Lean (7.3 mmolL^{-1}) <

279 Obese (10.2 mmolL^{-1}) < Diabetic (20.5 mmolL^{-1}) ($p < 0.001$). For mass, Obese (359 g) and Diabetic (367 g)
280 animals had a similar mean mass, but both were heavier than lean (249 g) animals ($p < 0.00001$).

281 Figure 1 shows the cumulative distribution of the input conductance for each group, and the 50th
282 percentile cut-off that separated the data into high and low conductance motoneuron groups. Those
283 cells with an IC above the 50th percentile was categorized as high IC cells, while those at or below the
284 50th percentile was categorized as low IC cells. Table 2 contains median (IQR) biophysical properties for
285 hindlimb motoneurons. Median values for IC, AI, fast F/I, slow F/I, and rheobase were found to be
286 similar, whereas the AHP1/2 was shorter in low conductance cells in diabetic animals compared to
287 control.

288 Figure 2 illustrates the AHP1/2 decay and amplitude for high IC and low IC motoneurons in each of the
289 three groups. The AHPamp and AHP1/2 for high IC cells showed no difference and the AHPamp of low
290 conductance cells tended to be smaller in amplitude ($p=0.067$). Significant main effects were found for
291 the AHP1/2 ($\chi^2_{(2, 76)} = 7.73, p = 0.021$) in low conductance cells (Figure 3), whereby a longer duration
292 AHP1/2 was found in the Lean (15.35 ± 6.9) group compared to the Diabetic group ($12.3 \pm 3.8; Z = 2.67,$
293 $p = 0.0076$). The AHP1/2 of the Obese (12.5 ± 8) group tended to be shorter than the lean group ($p =$
294 0.065), but did not differ from the Diabetic group. When motoneurons are separated into low IC and
295 high IC regardless of experimental group, the low IC group had a greater AHPamp ($1.7 \text{ mV} \pm 3.1$ vs 1.2
296 $\text{mV} \pm 0.55; p = 0.018$) and AHP1/2 durations ($15.3 \text{ ms} \pm 6.9$ vs $12.2 \pm 2.8; p = 0.0009$) than those of high
297 input conductance cells.

298 Moderate positive correlations (Figure 4) between IC and rheobase were found for Lean ($\rho = 0.65, p <$
299 0.00001), Obese ($\rho = 0.46, p = 0.003$) and Diabetic ($\rho = 0.51, p = 0.0002$) animals, indicating that the
300 relationship between excitability and ion conductance was not appreciably altered in the experimental
301 groups. Significant moderate negative correlations were also shown for Lean ($\rho = -0.51, p = 0.00001$) and

302 Obese ($\rho = -0.39$, $p = 0.0109$) animals for the relationship between AHP1/2 and IC, while Diabetic ($\rho = -$
303 0.14 , $p = 0.3257$) animals showed no correlation between AHP1/2 and IC (Figure 5). Diabetic animals
304 seem to lack the same number of long duration AHP1/2, compared to that seen in the Lean and Obese
305 animals, despite having a similar range of IC.

306 **DISCUSSION**

307 This investigation is the first to report on biophysical motoneuron properties in diabetic and obese
308 animals. The main finding of this investigation is spinal lumbar motoneurons are not appreciably
309 impacted by diabetes or obesity, showing that motoneurons maintain their biophysical heterogeneity. A
310 decrease in the half-decay duration of the afterhyperpolarization in diabetic animals was the only
311 difference found among the animals and is discussed below. The finding that all other properties
312 measured were similar across groups suggests that changes in motor unit physiology are not necessarily
313 due to a widespread change in function of motoneurons, at least at the level of the lumbar spinal cord.
314 These finding do not preclude changes to motoneuron morphology but does indicate that most
315 electrophysiological parameters remain intact in the Zucker Type II diabetes model. As for obese rats,
316 motoneurons were not impacted by increased body mass.

317 *Afterhyperpolarization*

318 This investigation found the afterhyperpolarization half-decay to be reduced in Diabetic rats (3-ms
319 median reduction) and a tendency for the amplitude to be reduced in Diabetic animals (0.6 mV median
320 reduction). When the motoneuron is driven to fire action potentials via depolarizing current injections,
321 the AHP duration is correlated to the cells minimum rate of discharge (Kernell, 1965) and to the type
322 (fast or slow) of motoneuron (Eccles et al. 1958; Gardiner 1993). When the motoneuron is driven to
323 fire by way of mesencephalic locomotor region stimulation, the AHP is largely reduced (cats and rats)
324 and motoneuron firing is more variable (Brownstone et al. 1992; MacDonnell e al. 2015). These two
325 scenarios, discharge during quiescence and discharge during motor output, represent two highly

326 different states of synaptic drive but the average discharge rate between the two scenarios has been
327 found to be similar (Brownstone et al. 1992), suggesting that the AHP may not govern discharge during
328 motor behaviour. The faster AHP_{1/2} in diabetic motoneurons found herein, may represent a transition
329 to more excitable state. This could allow for faster motoneuron discharge at lower levels of drive (a
330 reduced AHP is known to increase the gain of the motoneuron; Lape and Nistri, 2000; Miles et al. 2007)
331 and impact the ability of the motoneuron to maintain low rates of firing during fine motor movements/
332 low synaptic drive.

333 Another possibility is that muscle remodelling influenced the afterhyperpolarization duration of the
334 innervated muscle unit. While fiber-type distribution was not measured in this study, a shift in fiber
335 type distribution in human and animals has been shown. Humans with non-insulin dependent type II
336 diabetes show increased Type IIx muscle fibers and decreased Type I muscle fibers compared to control
337 (Marin et al., 1994; Oberbach et al., 2006). In both ZDF (Kim et al. 2015) and STZ rats (Snow et al. 2005),
338 increases in Type II MHC (associated with fast type muscle fibers) and decreases in the Type I MHC
339 (associated with slow type muscle fibers) compared to control rats have been shown to occur by 12
340 (STZ) and 13 weeks (ZDF). Finally, Russell et al. (2008) showed decreased sensory nerve conduction
341 velocities, CMAP amplitudes and sensory nerve synaptic potential amplitudes following 10 weeks of
342 hyperglycemia. Muscle and sensory changes documented above, all occurred before the mean age
343 (36.8 weeks) of the ZDF rat used in the current study.

344 If there were a similar re-organization of fiber type in the diabetic animals, motoneurons may have
345 adapted to the change in phenotype due to the speed-match that exists between motoneurons and the
346 muscle fibers they innervate (Gardiner and Kernell, 1990; MacDonell et al. 2008). A retrograde influence
347 of muscle on motoneuron AHP following muscle denervation/reinnervation has been demonstrated
348 (Foehring and Munson, 1990). In these experiments, Foehring and Munson (1990) cross-innervated the

349 medial gastrocnemius and soleus nerves. The electrical properties of the medial gastrocnemius
350 motoneuron pool innervating the soleus muscle changed and became more *slow-like*. That is, the AHP
351 duration and input resistance increased and rheobase decreased. In addition, Cormery et al. (2000)
352 showed that AHP durations in slow motoneurons are shorter in rats after four weeks of tetrodotoxin
353 induced hindlimb paralysis. A change in muscle phenotype, therefore, may explain the change in AHP,
354 if a change in fiber type distribution occurred.

355 We used the input conductance 50th percentile value to separate the groups into high and low input
356 conductance subpopulations. This was done to ensure a change in the AHP parameters was not missed,
357 given that the AHP is mutable (Gardiner and Beaumont, 2002; 2003; Cormery et al. 2003). Had we used
358 the AHP ½ duration criteria to separate putative fast and slow motoneuron types used by Gardiner
359 (1993), any change in AHP may have been masked. Since the cumulative distribution of the input
360 conductance between Lean, Obese, and Diabetic groups did not differ (Figure 1.), and input conductance
361 is a robust measure that relates well with motoneuron type (Zengel et al., 1985), it provided a way to
362 compare motoneurons without missing any potential change in AHP parameters.

363 *Unchanged motoneuron properties in diabetic animals*

364 In a Type I diabetic animal model, Ramji et al. (2007) showed little change to motoneuron morphology
365 or number in the lumbar spinal cord but that the tibialis anterior muscle and NMJ were adversely
366 affected by diabetes. Similarly in a Type I diabetes model, Muramastu et al. (2012) also reported that
367 STZ did not alter alpha-motoneuron numbers but did suggest that gamma motoneurons were lost.
368 However, a follow-up study by the same group (Muramastu et al. 2017) showed evidence for reduced
369 motoneuron numbers in the gastrocnemius motor nuclei at 12 and 24 weeks, with both the smallest and
370 largest motoneurons being reduced in number, demonstrating variability in assessing motoneuron
371 changes in diabetic models. The ZDF rat is an animal model for Type II diabetes. The ZDF rat has been
372 shown to have elevated levels of blood glucose at 8 weeks that continues to increase up to 20 weeks

373 before plateauing (Sugimoto et al. 2008). In addition, at 16 weeks the ZDF rat developed an increased
374 sensitivity to thermal nociceptive stimuli and, at 18 weeks, a decreased response to mechanical
375 nociceptive stimuli occurred (Sugimoto et al. 2008). Other deficits, such as a decreased motor nerve
376 conduction velocity, decreased endoneural blood flow (Coppey et al., 2002), and, as mentioned above,
377 changes in muscle phenotype occur as early as 13 weeks in ZDF rats.

378 In relation to this, three reasons may be considered for the lack of widespread difference in the
379 biophysical properties of motoneurons in our study. First, our investigation used a Type II model of
380 diabetes, while the investigations noted above used a Type I model of disease. Second, the change in
381 cross-sectional area and motoneuron number found by Muramastu et al., (2017), although significant,
382 may be too modest to effect biophysical properties. Gardiner (1993) showed that rat motoneurons
383 have a considerable range of properties that include both slow and fast motor units. Given this wide
384 range, the effect of type II diabetes on motoneuron numbers may be too modest to detect and thus a
385 sampling of the available motor pool reveals little difference. Third, the full impact of diabetes on spinal
386 cord motoneurons might not be fully realized until much later. Diabetic neuropathy is polymodal and
387 the progression of diabetic neuropathy includes changes to both peripheral nerve fibers (Dyck et al.,
388 1986) and ion channel changes at the DRG (Hong et al., 2004). The progression of neuronal dysfunction
389 in efferent somatic neurons may not be realized at a mean age of 9.2 months. As such, the reduced AHP
390 $\frac{1}{2}$ decay and the tendency for a reduction in AHPamp shown herein may be the first sign of change.

391 A probability value of 0.05 set the level of significance (i.e. Type I error rate); despite performing
392 multiple comparisons, no adjustment to the probability value was made. As the number of comparisons
393 increase, so does the likelihood of incorrectly rejecting the null hypothesis (false discovery). Given that
394 this is the first report examining motoneuron biophysical properties in obese and diabetic animals, the
395 authors chose a more liberal level of significance. Had we adjusted for the false discovery using the

396 equation described by Hassard and Baker (1986), an adjusted p-value of 0.026 (20 comparisons) would
397 have been set. In relation to the data presented herein, this would have impacted only the tendency of
398 the AHP_{1/2} decay to be different between Obese and Lean groups. Although adjusting the false
399 discovery rate is an important practice, its use needs to be evaluated with the type of study being
400 conducted.

401 *Lack of change in obese Zucker Rats*

402 To our knowledge, this is the first study to examine the effects of obesity on motoneuron properties.
403 We hypothesized that the sampling distribution of electrophysiological properties may be shifted
404 towards those properties consistent with exercised (running) motoneurons (Beaumont and Gardiner,
405 2002, 2003). The only relevant literature known to the authors that examined how the neuromuscular
406 system responds to obesity looked at adolescent boys and girls. These studies suggest that the
407 neuromuscular system adapts to obesity (Garcia-Vicencio et. al., 2015; 2016; Abdelmoula et al. 2012). In
408 this, adolescent boys and girls tend to have increased muscle strength and total muscle activation;
409 although there is discrepancy in the literature (Blimkie et al. 1990; Maffioletti et al., 2008). Testing
410 whether obesity changed motoneuron properties was important due to the association between obesity
411 and developing Type II diabetes (Hotamisligil, 2006). The lack of a significant results found in this
412 investigation may indicate obesity simply does not confer any benefit/detriment to motoneurons.

413

414 *Conclusion*

415 Motoneuron properties continue to be heterogeneous in obese and diabetic animals. While obesity did
416 not influence motoneuron properties significantly, a tendency towards an altered AHP existed. Despite
417 the motoneurons overall resilience, for diabetic neurons the reduced AHP $\frac{1}{2}$ decay and the tendency for
418 a reduction in AHPamp shown herein may be the first sign of change to motoneuron function.

419 FUNDING SOURCES

420 This research was supported by grants from the Canadian Institutes of Health Research (CIHR) Team
421 NERVE grant and the Canada Research Chairs program. Financial support was provided by CIHR Doctoral
422 award (J. W. Chopek).

423

424

425 **References**

- 426 Abdelmoula A, Martin C, Bouchant A, Walrand S, Lavet C, Taillardat M, Maffiuletti N, Boisseau N,
427 Duche P and Ratel S. Knee extension strength in obese and nonobese male adolescents. *Appl.*
428 *Physiol. Nutr. Metab.* 37: 269–275, 2012.
- 429
- 430 Acevedo LM, Raya AI, Ríos R, Aguilera–Tejero E, River JL. Obesity-induced discrepancy between
431 contractile and metabolic phenotypes in slow- and fast-twitch skeletal muscles of female obese
432 Zucker rats. *J Appl Physiol*, in press,2017.
- 433
- 434 Allen MD, Choi IH, Kimpinski K, Doherty TJ and Rice CL. Motor Unit Loss & Weakness in Association
435 with Diabetic Neuropathy in Humans. *Muscle and Nerve*, 48: 298-300, 2013.
- 436
- 437 Almeida S, Riddell MC, Cafarelli E. Slower conduction velocity and motor unit discharge frequency
438 are associated with muscle fatigue during isometric exercise in type 1 diabetes mellitus. *Muscle*
439 *and Nerve.* 37:231-40, 2008.
- 440
- 441 Button DC, Kalmar JM, Gardiner K, Marqueste T, Zhong H, Roy RR, Edgerton VR, Gardiner PF. Does
442 elimination of afferent input modify the changes in rat motoneurone properties that occur
443 following chronic spinal cord transection? *J Physiol.* 586:529-544, 2008.
- 444
- 445 Beaumont E and Gardiner P. Effects of daily spontaneous running on the electrophysiological
446 properties of hindlimb motoneurons in rats. *J Physiol* 540, 129-138, 2002.
- 447
- 448 Beaumont E and Gardiner PF. Endurance training alters the biophysical properties of hindlimb
449 motoneurons in rats. *Muscle Nerve* 27, 228-236, 2003.

450

451 Beaumont E, Houlé JD, Peterson CA, Gardiner PF. Passive exercise and fetal spinal cord transplant
452 both help to restore motoneuronal properties after spinal cord transection in rats. *Muscle*
453 *Nerve*, 29:234-242, 2004.

454

455 Blimkie CJ, Sale DG, Bar-Or O. Voluntary strength, evoked twitch contractile properties and motor
456 unit activation of knee extensors in obese and non-obese adolescent males. *Eur J Appl Physiol*
457 *Occup Physiol*. 61: 313-8, 1990.

458

459 Brown WF and Feasby TE. Estimates of Functional Motor Axon Loss in Diabetics. *Journal of the*
460 *neurological Sciences*, 1974, 23:275-293, 1974.

461

462 Brownstone RM, Jordan LM, Kriellaars DJ, Noga BR and Shefchyk SJ. On the regulation of repetitive
463 firing in lumbar motoneurons during fictive locomotion in the cat. *Exp Brain Res* 90, 441-455,
464 1992.

465

466 Cabrales P, Tsai AG & Intaglietta M. Alginate plasma expander maintains perfusion and plasma
467 viscosity during extreme hemodilution. *Am J Physiol*, **288**: H1708- H1716, 2005.

468

469 Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Yorek MA. Changes in endoneurial blood flow, motor
470 nerve conduction velocity and vascular relaxation of epineurial arterioles of the sciatic nerve in
471 ZDF-obese diabetic rats. *Diabetes Metab Res Rev*, 18: 49–56, 2002.

472

473 Cormery B, Marini JF, & Gardiner PF. Changes in electrophysiological properties of tibial
474 motoneurons in the rat following 4 weeks of tetrodotoxin-induced paralysis. *Neurosci Lett* 287,
475 21-24, 2000.

476

477 Cormery B, Beaumont E, Csukly K, and Gardiner P. Hindlimb unweighting for 2 weeks alters
478 physiological properties of rat hindlimb motoneurons. *J Physiol* 568, 841-850, 2005.

479

480 Dorfman VB, Vega MC, Coirini H. Reduction of the spinal nucleus of the bulbocavernosus volume
481 by experimental diabetes. *Brain Res*, 1019:265-269, 2004.

482

483 Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen, KA. Diabetic neuropathy: An intensive
484 review. *American Journal of Health-System Pharmacy*, 61: 160-176
485 Delayed depolarization and the repetitive response to intracellular stimulation of mammalian
486 motoneurons. *J Physiol* 168: 890-910, 1963.

487

488 Dyck PJ, Lais A, Karnes JL, O'Brien P, Rizza R. Fiber loss is primary and multifocal in sural nerves in
489 diabetic polyneuropathy. *Ann Neurol.*, 19: 425-39, 1986.

490

491 Eccles JC, Eccles RM, Lundberg A. The action potentials of the alpha motoneurons supplying fast
492 and slow muscles. *J Physiol* 142, 275-291, 1958.

493

494 Fleshman JW, Munson JB, Sybert GW and Friedman WA. Rheobase, input resistance, and motor-
495 unit type in medial gastrocnemius motoneurons in the cat. *J Neurophysiol* 46, 1326-1338, 1981.

496

497 Foehring RC, Munson JB . Motoneuron and muscle-unit properties after long-term direct
498 innervation of soleus muscle by medial gastrocnemius nerve in cat. J Neurophys 64: 847-861,
499 1990.

500

501 Garcia-Vicencio S, Coudeyre E, Kluka V, Cardenoux C, Jegu AG, Fourot AV, Ratel S, Martin V. The
502 bigger, the stronger? Insights from muscle architecture and nervous characteristics in obese
503 adolescent girls. Int J Obes (Lond). 40:245-251, 2016.

504

505 Garcia-Vicencio S, Martin V, Kluka V, Cardenoux C, Jegu AG, Fourot AV, Coudeyre E, Ratel S.
506 Obesity-related differences in neuromuscular fatigue in adolescent girls. Eur J Appl Physiol. 115:
507 2421-2432, 2015.

508

509 Gardiner PF. Physiological properties of motoneurons innervating different muscle unit types in rat
510 gastrocnemius. J Neurophysiol 69, 1160-1170, 1993.

511

512 Gardiner PF and Kernell D. The "fastness" of rat motoneurons: time-course of
513 afterhyperpolarization in relation to axonal conduction velocity and muscle unit contractile
514 speed. Pflugers Arch 415, 762-766, 1990.

515

516 Hansen SH and Ballantyne JP. Axonal dysfunction in the neuropathy of diabetes mellitus: a
517 quantitative electrophysiological study. J Neurol Neurosurg Psychiatry, 40: 555-564, 1977.

518 Hassard T and Becker A. Faulty statistical analysis. J. Pediatr, 109: 1075–1076, 1986.

519

520 Hotamisligil GS. Inflammation and metabolic disorders. Nature, 444: 860-867, 2006.

521

522 Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis
523 factor- α : direct role in obesity-linked insulin resistance. *Science* 259, 87–91, 1993.

524

525 Hong S, Morrow TJ, Paulson PE, Isom LL, Wiley JW. Early painful diabetic neuropathy is associated
526 with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root
527 ganglion neurons in the rat. *J Biol Chem.* 279: 29341–29350, 2004.

528

529 Kalmar JM, Button DC, Gardiner K, Cahill F, Gardiner. Caloric restriction does not offset age-
530 associated changes in the biophysical properties of motoneurons. *PF. J Neurophysiol.* 2009 101:
531 548-557, 2008.

532

533 Kernell D. The limits of firing frequency in cat lumbosacral motoneurons possessing different time
534 course of afterhyperpolarization. *Acta Physiol Scand* 65, 87-100, 1965.

535

536 Kim J-Y, Choi MJ, So B, Kim H-J, Seong JK, Song W. The preventive effects of 8 weeks of resistance
537 training on glucose tolerance and muscle fiber type composition in Zucker rats. *Diabetes Metab*
538 *J*, 39:424-433, 2015.

539

540 Lape R , Nistri A. Current and voltage clamp studies of the spike medium afterhyperpolarization of
541 hypoglossal motoneurons in a rat brain stem slice. *J Neurophysiol* 83:2987–2995, 2000.

542

543 MacDonell CW, Ivanova TD, Garland SJ. Afterhyperpolarization time-course and minimal discharge
544 rate in low threshold motor units in humans. *Exp Brain Res.* 189:23-33, 2008.

545
546 MacDonell CW, Button DC, Beaumont E, Cormery B, Gardiner PF. Plasticity of rat motoneuron
547 rhythmic firing properties with varying levels of afferent and descending inputs. *J Neurophysiol.*
548 107:265-272, 2012.
549
550 MacDonell CW, Power KE, Chopek JW, Gardiner KR, Gardiner PF. Extensor motoneurone properties
551 are altered immediately before and during fictive locomotion in the adult decerebrate rat. *J*
552 *Physiol.* 593:2327-2342, 2015.
553
554 Maffiuletti N, Jubeau M, Agosti F, De Col A, Sartorio A. Quadriceps muscle function characteristics
555 in severely obese and nonobese adolescents. *Eur J Appl Physiol.* 103: 481-484, 2008.
556 Mårin P, Andersson B, Krotkiewski M, Björntorp P. Muscle fiber composition and capillary density
557 in women and men with NIDDM. *Diabetes Care*, 17:382-386, 1994.
558
559 Miles GB, Hartley R, Todd AJ, Brownstone RM. Spinal cholinergic interneurons regulate the
560 excitability of motoneurons during locomotion. *Proc Natl Acad Sci USA* 104: 2448–2453, 2007.
561
562 Millar WJ and Young TK. Tracking diabetes: Prevalence, incidence and risk factors. *Health Reports*,
563 14: 35-47, 2003.
564
565 Mulder GB, Luo S and Gramlich P. The zucker diabetic fatty (ZDF) rat: Diet evaluation study for the
566 induction of type 2 diabetes in obese female ZDF rats. *Charles River Technical Sheet*: 1-4, 2010.
567

568 Muramatsu K, Niwa M, Nagai M, Kamimura T, Sasaki S, Ishiguro T. The size of motoneurons of the
569 gastrocnemius muscle in rats with diabetes. *Neurosci Lett.* , 531:109-113, 2012.
570

571 Muramatsu K, Niwa M, Tamaki T, Ikutomo M, Masu Y, Hasegawa T, Shimo S, Sasaki SI. Effect of
572 streptozotocin-induced diabetes on motoneurons and muscle spindles in rats. *Neurosci Res.*
573 115:21-28, 2017.
574

575 Toth C, Hervert V, Gougeon C, Virtanen H, Mah JK and Pacaud D. Motor unit number estimations
576 are smaller in children with type1 diabetes mellitus: A case-cohort study. *Muscle and Nerve*, 50:
577 593-598, 2014.
578

579 Oberbach A, Bossenz Y, Lehmann S, Niebauer J, Adams V, Paschke R, Schön MR, Blüher M, Punkt K.
580 Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal
581 muscle of patients with type 2 diabetes. *Diabetes Care*, 29:895-900, 2006.
582

583 Ohinmaa A, Jacobs P, Simpson S and Johnson JA. The projection of prevalenc and cost of diabetes in
584 Canada: 2000:2016. *Canadian Journal of Diabettes*, 28:1-8, 2004.
585

586 Ramji N, Toth C, Kennedy J and Zochodne DW. Does diabetes target motor neurons. *Neurobiology*
587 *of Disease* 2007, 26:301-311, 2007.
588

589 Russell JW, Berent-Spillson A, Vincent, AM, Freimann CL, Sullivan KA, Feldman EL. Oxidative injury
590 and neuropathy in diabetes and impaired glucose tolerance. *Neurobiol Dis*, 30:420-429, 2008.
591

592 Statistics Canada (2016-03-07). Body mass index, overweight or obese, self-reported, adult, by sex,
593 provinces and territories (Percent). CANSIM, table 105-0501 and Catalogue no. 82-221-X, 2014
594 [Online]. [http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health82b-
596 eng.htm?sdi=body%20mass%20index](http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/health82b-
595 eng.htm?sdi=body%20mass%20index), 2014.

596
597 Snow LM, Sanchez OA, McLoon LK, Serfass RC, Thompson LV. Myosin heavy chain
598 isoform immunolabelling in diabetic rats with peripheral neuropathy. *Acta Histochem*, 107:221-
599 229, 2005.

600
601 Souayah N, Potian JG, Garcia CC, Krivitskaya N, Boone C, Routh VH and McArdle JJ. Motor unit
602 number estimate as a predictor of motor dysfunction in an animal model of type 1 diabetes.
603 *American Journal of Physiology - Endocrinology and Metabolism* , 297:E602-E608, 2009.

604
605 Sugimoto K, Rashid IB, Kojimaa, K, Shoji M, Tanabe J, Tamasawa N, Suda T, Yasujima M. Time
606 course of pain sensation in rat models of insulin resistance, type 2 diabetes, and exogenous
607 hyperinsulinaemia. *Diabetes-Metab Res*, 24: 642-650, 2008.

608
609 Watanabe K, Gazzoni M, Holobar A, Miyamoto T, Fukuda K, Merletti R, Moritani T. Motor unit firing
610 pattern of vastus lateralis muscle in type 2 diabetes mellitus patients. *Muscle Nerve*, 48:806-
611 813, 2013.

612 Zengel JE, Reid SA, Sybert GW and Munson JB. Membrane electrical properties and prediction of
613 motor-unit type of medial gastrocnemius motoneurons in the cat. *J Neurophysiol* 530, 1323-
614 1344, 1985.

615

616

617 **Table 1. Zucker rat mass and blood glucose level means (SD).** Bold indicates a significant difference
618 from other groups. Data are presented as mean (SD).

619 **Table 2. Motoneuron properties separated by group.** Data are presented as the median value and
620 interquartile range (IQR) in brackets. **Bold** indicates significant from Lean $p < 0.05$. Underline indicates a
621 tendency to differ from Lean $p < 0.07$.

622 **Figure 1. Cumulative Distribution function (CDF) of input conductance for the groups.** Input
623 conductance (IC) for Lean (solid line), Obese (dashed line) and Diabetic (dotted line) groups are shown.
624 The 50th percentile is marked by the vertical hatched line. The 50th percentile, indicated above, for each
625 group was used to separate motoneurons into high input conductance (H-IC) and low input conductance
626 (L-IC) cells. Those motoneurons with an IC greater than the 50th percentile were designated as high input
627 conductance motoneurons. No difference existed between the distributions of IC values between each
628 group.

629 **Figure 2. Post-spike afterhyperpolarization (AHP) amplitude and half-decay time.** Data were
630 separated into high and low input conductance (IC) categories according to the 50th percentile of the IC
631 for each group (see Figure 1). The AHP $\frac{1}{2}$ decay time (A) and AHP amplitude (B) for the low IC are
632 shown on the left panels, while AHP $\frac{1}{2}$ decay time (C) and AHP amplitude (D) for the high IC category
633 are displayed on the right. Whiskers represent the range of values, the 25th and 75th percentile are
634 indicated by the top and bottom of the box, the median is the horizontal line within the box, while the
635 symbol in the centre indicates the mean. * denotes significant difference from diabetic $p = 0.0026$.

636 **Figure 3. Post-spike afterhyperpolarization (AHP) comparison between Lean and Diabetic groups.**
637 Magnified AHP tracings representative of the median value from the significantly different ($p < 0.007$)
638 Lean (solid line) and Diabetic (dotted line) groups of low conductance cells. The inset shows the full

639 action potentials of each group (solid line, Lean; dotted line, Diabetic) generated from a supramaximal
640 orthodromic depolarizing pulse (0.5-ms).

641 **Figure 4. Rheobase versus input conductance according to groups.** Rheobase as a function of input
642 conductance for Lean (open triangle; $\rho=0.65$, $p < 0.00001$), Obese (shaded triangle; $\rho=0.47$, $p = 0.0028$),
643 and Diabetic (square; $\rho=0.51$, $p = 0.000217$) groups. Inset shows the lines of best fit for each group.

644 **Figure 5. Input conductance as a function of the afterhyperpolarization half-decay.** Spearman's rank
645 correlation coefficients for Lean ($\rho = -0.51$, $p < 0.0001$), Obese ($\rho = -0.39$, $p = 0.01$), and Diabetic ($\rho = -$
646 0.14 , n.s.).

647

648

649

Table 1.

Measure	Mass (g)	Glucose (mmolL ⁻¹)
Lean	249.2 (24.2)	7.26 (0.9)
Obese	358.6 (70.2)	12.0 (6.4)
Diabetic	367.6 (43.9)	20.5 (4.9)

650

651

Table 2.

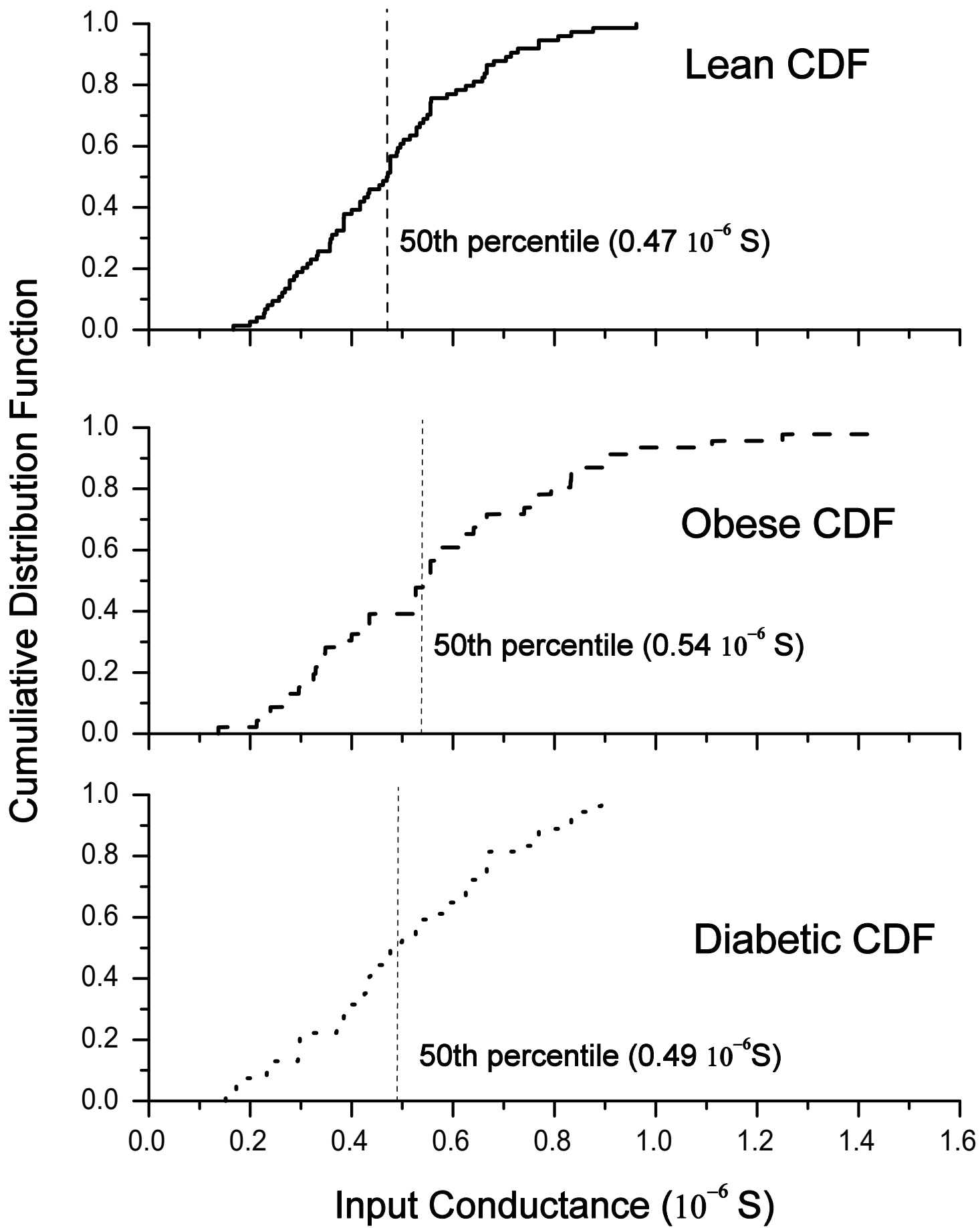
	Lean	Obese	Diabetic
<i>Input Conductance</i> ($10^{-6} S$)	0.48 (0.2); N = 66	0.53 (0.42) N = 43	0.48 (0.28) N = 54
<i>IC-High</i> ($10^{-6} S$)	0.56 (0.16) N = 34	0.78 (0.29) N = 20	0.67 (0.18) N = 27
<i>IC-Low</i> ($10^{-6} S$)	0.33 (0.11) N = 32	0.35 (0.16) N = 23	0.39 (0.19) N = 27
<i>Rheobase</i> (mV)	7.2 (7.3) N = 58	7.8 (8.1) N = 40	7.5 (6.6) N = 49
<i>Rheobase – High IC</i> (mV)	10.25(5.8) N = 29	10.7 (5.8) N = 19	8.5 (5.3) N = 25
<i>Rheobase –Low IC</i> (mV)	4.75 (3.0) N = 29	4.5 (6.8) N = 21	5.6 (7.8) N = 24
<i>AHP_{AMP} – High IC</i> (mV)	1.22 (0.55) N = 34	1.25 (0.60) N = 20	1.19 (0.70) N = 26
<i>AHP_{1/2} – High IC</i> (ms)	12.20 (2.8) N = 34	11.3 (2.8) N = 20	12.0 (2.4) N = 26
<i>AHP_{AMP} – Low IC</i> (mV)	1.8 (2.8) N = 32	1.5 (1.5) N = 22	1.2 (0.9) N = 25
<i>AHP_{1/2} – Low IC</i> (ms)	15.35 (6.7) N = 32	<u>12.5 (8.0)</u> N = 22	12.3 (3.8) N = 25
<i>Adaptation Index</i> (a.u.)	0.21 (0.25) N = 25	0.15 (0.27) N = 21	0.19 (0.14) N = 27
<i>F/I Fast</i>	<i>One linear range = 9</i> <i>Two linear ranges = 15</i>	<i>One linear range = 9</i> <i>Two linear ranges = 15</i>	<i>One linear range = 12</i> <i>Two linear ranges = 16</i>
<i>F/I Slope</i> (Hz·nA)	29.75 (12.43)	30.12 (24.33)	34.77 (16.35)
<i>I_{PK}</i> (nA)	12.87 (10.70)	11.10 (11.00)	14.96 (11.01)
<i>I_{MN}</i> (nA)	7.03 (7.04)	9.20 (9.59)	8.26 (11.12)
<i>Rate_{PK}</i> (Hz)	246.31 (194.84)	187.34 (200.15)	206.19 (187.34)
<i>Rate_{MN}</i> (Hz)	38.74 (56.27)	59.99 (84.22)	56.98 (60.88)
<i>F/I Slow</i>	N = 43	N = 30	N = 40
<i>F/I Slope</i> (Hz·nA)	9.71 (8.15)	11.05 (4.89)	10.38 (6.2)
<i>I_{PK}</i> (nA)	10.08 (9.76)	10.5 (8.93)	14.08 (9.14)
<i>I_{MN}</i> (nA)	7.17 (9.72)	7.75 (8.77)	11.28 (9.2)
<i>Rate_{PK}</i> (Hz)	48.40 (19.34)	50.53 (29.64)	56.61 (22.30)
<i>Rate_{MN}</i> (Hz)	10.87 (13.69)	9.54 (10.9)	9.23 (12.71)

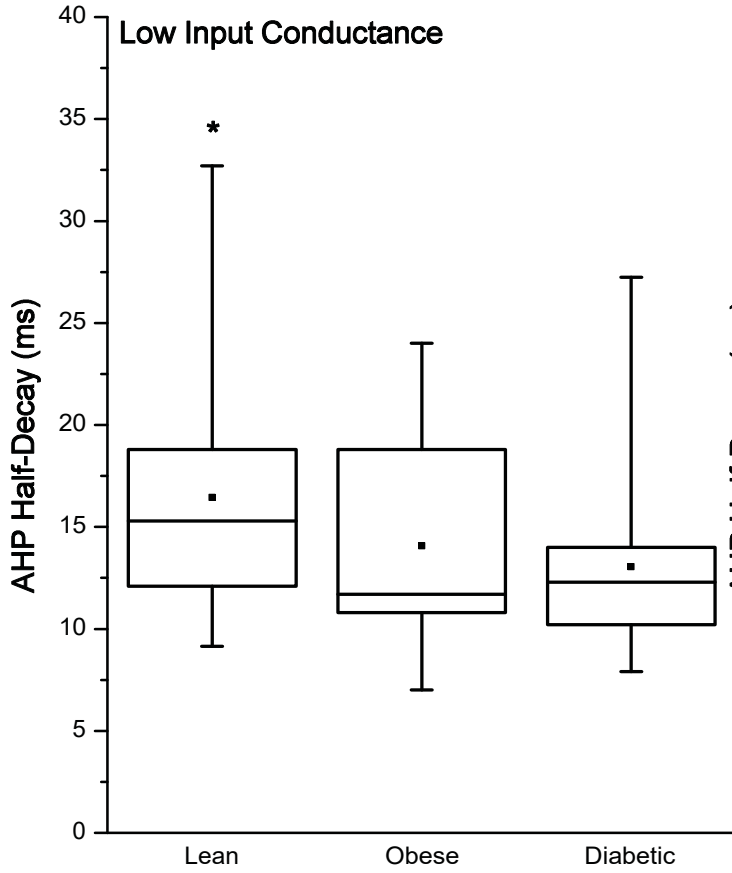
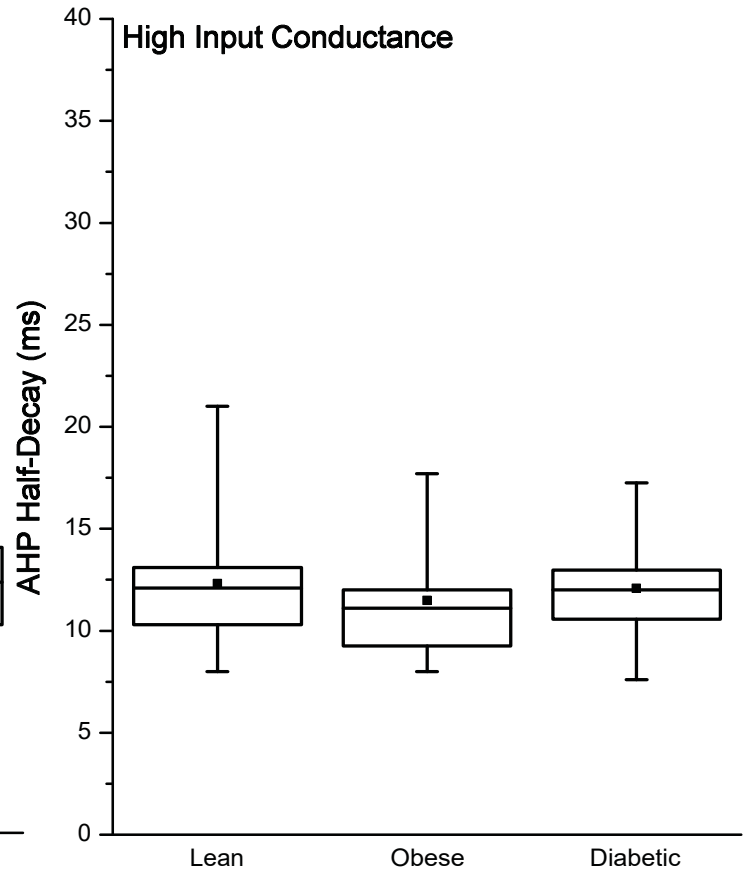
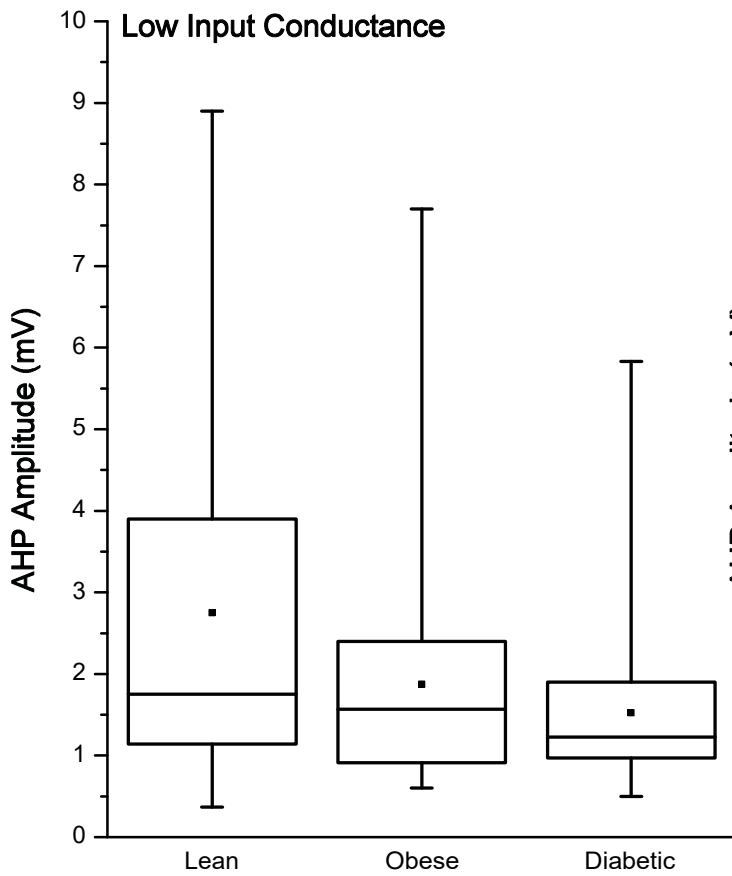
Table 1.

Measure	Mass (g)	Glucose (mmolL ⁻¹)
Lean	249.2 (24.2)	7.26 (0.9)
Obese	358.6 (70.2)	12.0 (6.4)
Diabetic	367.6 (43.9)	20.5 (4.9)

Table 2.

	Lean	Obese	Diabetic
<i>Input Conductance</i> ($10^{-6}S$)	0.48 (0.2); N = 66	0.53 (0.42) N = 43	0.48 (0.28) N = 54
<i>IC-High</i> ($10^{-6}S$)	0.56 (0.16) N = 34	0.78 (0.29) N = 20	0.67 (0.18) N = 27
<i>IC-Low</i> ($10^{-6}S$)	0.33 (0.11) N = 32	0.35 (0.16) N = 23	0.39 (0.19) N = 27
<i>Rheobase</i> (mV)	7.2 (7.3) N = 58	7.8 (8.1) N = 40	7.5 (6.6) N = 49
<i>Rheobase – High IC</i> (mV)	10.25(5.8) N = 29	10.7 (5.8) N = 19	8.5 (5.3) N = 25
<i>Rheobase –Low IC</i> (mV)	4.75 (3.0) N = 29	4.5 (6.8) N = 21	5.6 (7.8) N = 24
<i>AHP_{AMP} – High IC</i> (mV)	1.22 (0.55) N = 34	1.25 (0.60) N = 20	1.19 (0.70) N = 26
<i>AHP_{1/2} – High IC</i> (ms)	12.20 (2.8) N = 34	11.3 (2.8) N = 20	12.0 (2.4) N = 26
<i>AHP_{AMP} – Low IC</i> (mV)	1.8 (2.8) N = 32	1.5 (1.5) N = 22	1.2 (0.9) N = 25
<i>AHP_{1/2} – Low IC</i> (ms)	15.35 (6.7) N = 32	<u>12.5 (8.0)</u> N = 22	12.3 (3.8) N = 25
<i>Adaptation Index</i> (a.u.)	0.21 (0.25) N = 25	0.15 (0.27) N = 21	0.19 (0.14) N = 27
<i>F/I Fast</i>	<i>One linear range = 9</i> <i>Two linear ranges = 15</i>	<i>One linear range = 9</i> <i>Two linear ranges = 15</i>	<i>One linear range = 12</i> <i>Two linear ranges = 16</i>
<i>F/I Slope</i> (Hz·nA)	29.75 (12.43)	30.12 (24.33)	34.77 (16.35)
<i>I_{PK}</i> (nA)	12.87 (10.70)	11.10 (11.00)	14.96 (11.01)
<i>I_{MN}</i> (nA)	7.03 (7.04)	9.20 (9.59)	8.26 (11.12)
<i>Rate_{PK}</i> (Hz)	246.31 (194.84)	187.34 (200.15)	206.19 (187.34)
<i>Rate_{MN}</i> (Hz)	38.74 (56.27)	59.99 (84.22)	56.98 (60.88)
<i>F/I Slow</i>	N = 43	N = 30	N = 40
<i>F/I Slope</i> (Hz·nA)	9.71 (8.15)	11.05 (4.89)	10.38 (6.2)
<i>I_{PK}</i> (nA)	10.08 (9.76)	10.5 (8.93)	14.08 (9.14)
<i>I_{MN}</i> (nA)	7.17 (9.72)	7.75 (8.77)	11.28 (9.2)
<i>Rate_{PK}</i> (Hz)	48.40 (19.34)	50.53 (29.64)	56.61 (22.30)
<i>Rate_{MN}</i> (Hz)	10.87 (13.69)	9.54 (10.9)	9.23 (12.71)



A.**C.****B.****D.**