JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Determination of peripheral neuropathy in high-fat diet fed low-dose streptozotocin-treated female C57Bl/6J mice and Sprague–Dawley rats

Lawrence J Coppey[1], Hanna Shevalye[1], Alexander Obrosov[1], Eric P Davidson[1], Mark A Yorek[1,2,3,4]*

[1]Department of Internal Medicine, The University of Iowa, [2]Department of Veterans Affairs, Iowa City Health Care System, [3]Iowa City Veterans Administration Center for the Prevention and Treatment of Visual Loss, and [4]Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa, USA

Keywords
Diet-induced obesity, Peripheral neuropathy, Type 2 diabetes

***Correspondence**
Mark A Yorek
Tel.: +1-319-338-0581 ext. 7696
Fax: +1-319-339-7162
E-mail address:
mark-yorek@uiowa.edu

J Diabetes Investig 2018; 9: 1033–1040

doi: 10.1111/jdi.12814

ABSTRACT

Aims/Introduction: Peripheral neuropathy is a common complication of diabetes and also occurs in 30% of human obese individuals with impaired glucose tolerance. Even though peripheral neuropathy affects both sexes, most pre-clinical studies have been carried out using male rodents. The aim of the present study was to create diet-induced obesity and type 2 diabetes in female rats and mice in order to examine the development of peripheral neuropathy.

Materials and Methods: At 12 weeks-of-age, rats and mice were separated into three groups. Two groups or rats and mice were fed a 60-kcal% high-fat diet for 12 weeks (rats) or 8 weeks (mice). To induce type 2 diabetes, one group of high-fat diet-fed rats and mice were treated with a low dose of streptozotocin. Analyses of multiple neural end-points were carried out 12 weeks later.

Results: Glucose utilization was impaired in diet-induced obese female rats and mice, as was a number of neurological end-points including nerve conduction velocity, intraepidermal and subepithelial corneal nerve fiber densities, and thermal and mechanical sensitivity. When female diet-induced obese rats or mice were made hyperglycemic, glucose utilization and sensory nerve density of the skin and cornea, as well as thermal and mechanical sensitivity, were more significantly impaired compared with diet-induced obese female rodents.

Conclusions: These studies show that diet-induced obese and type 2 diabetic female rodents develop peripheral neuropathy that is similar to that occurring in male rodents. However, for female rats, more aggressive treatment is required to induce dietary obesity.

INTRODUCTION

In 2014, Dr Clayton and Dr Collins of the National Institute of Health created a policy requiring gender inclusion in pre-clinical research[1]. As stated, the intention of this policy is to ensure that the health of the people of the USA is being served by supporting science that meets the highest standards of rigor.

In human studies of diabetic neuropathy, Aaberg *et al.*[2] reported that men developed neuropathy earlier than women. In contrast, Javed *et al.*[3] found no statistically significant sex differences in diabetic peripheral neuropathy in respect to frequency, age at diagnosis or duration of diabetes before onset. However, these authors state that more studies are required to settle whether sex-based differences in onset and progression of diabetic neuropathy exist[3].

To date, most studies of rodents on peripheral neuropathy have been carried out in male mice or rats. Several likely reasons for the predominate use of male rodents is to avoid the potential complicating effect of the estrogen cycle in studies, as well as male rodents being more abundant from breeders than female animals. Another issue that could impact outcomes in

Received 5 December 2017; revised 10 January 2018; accepted 1 February 2018

female rodents is determining the age of the animal to use for the study. Selection of virgin animals versus animals that have been allowed to breed or have passed their breeding age can affect outcomes. To begin to address this issue, we characterized diabetic peripheral neuropathy in two common rodent models: female C57Bl6/J mice and Sprague–Dawley rats. We initiated our studies with virgin rodents that were aged 12 weeks. This is the same age that we have used for our male rodent studies. Analyses were carried out in both diet-induced obese and diabetic female C57Bl6/J mice, and Sprague–Dawley rats. To induce a form of late-stage type 2 diabetes, these rodents were fed a high-fat diet to create insulin resistance followed by a low dose(s) of streptozotocin in order to destroy a portion of the β-cells and render the mouse or rat diabetic[4,5]. Twelve weeks later, analyses of multiple nerve-related endpoints were carried out. We have previously used this approach in both male C57Bl6/J mice and Sprague–Dawley rats to examine the effect of diet-induced obesity and type 2 diabetes, and treatment on peripheral neuropathy[4–6].

Results from these studies show that once hyperglycemia is established, female C57Bl6/J mice and Sprague–Dawley rats develop peripheral neuropathy that in severity is similar to male C57Bl6/J mice and Sprague–Dawley rats, respectively. Creating insulin resistance through a high-fat diet in female C57Bl6/J mice and the outcome was similar to male C57Bl6/J mice[5]. However, creating obesity through a high-fat diet in female Sprague–Dawley rats required a greater percentage of fat in the diet and longer duration compared with male Sprague–Dawley rats[4].

METHODS

Unless stated otherwise, all chemicals used in these studies were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

Animals

Female Sprague–Dawley rats were purchased from Harlan Teklad (Madison, WI, USA), and female C57Bl/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Rats and mice were housed in a certified animal care facility, and a standard diet (Harlan Teklad, #7001) and water provided *ad libitum*. At 12 weeks-of-age, rats and mice were separated into three groups. Two of these groups were fed a 60-kcal% high-fat diet (D12492; Research Diets, New Brunswick, NJ, USA) for 12 weeks (rats) or 8 weeks (mice). The other group of female rats or mice remained on the standard diet. After the initial phase of high-fat diets, one group of rats and mice was treated with streptozotocin, as previously described, to create a model of type 2 diabetes[4,5]. All high-fat diet-fed rats and mice remained on the high-fat diets for the duration of the study. The high-fat diet-fed rodents that did not receive streptozotocin are referred to as the non-diabetic high-fat diet-fed group and are considered as the diet-induced obese rats or mice. Analyses were carried out at 12 weeks after hyperglycemia induction.

Glucose utilization

Glucose utilization was determined by injecting mice or rats with a saline solution containing 2 g/kg glucose i.p. after an overnight fast, as previously described[4,5]. Circulating blood glucose levels were measured immediately before the glucose injection and at different intervals afterwards depending on whether it was a mouse or rat being examined[4,5].

Thermal nociceptive response and mechanical allodynia

Thermal sensitivity was measured using the Hargreaves method, with instrumentation provided by IITC Life Science (model 390G; Woodland Hills, CA, USA). This procedure was initiated by placing the rodents in the observation chamber. The animals were allowed to acclimatize to the warmed glass surface (30°C) and surroundings for a period of 15 min. Afterwards, the heat source was maneuvered so that it was under the heel of the hindpaw and activated, a process that turns on a timer and locally warms the glass surface, and when the rodent withdrew its paw, the timer and the heat source were turned off[4,5]. After an initial recording, which was discarded, four measurements were made for each hindpaw, with a rest period of 5 min between each examination. The mean of the measurements, reported in seconds, was used as a measure of the thermal nociceptive response latency. Mechanical allodynia was evaluated by quantifying the withdrawal threshold of the hindpaw in response to stimulation with flexible von Frey filaments, as previously described[4,5]. The data were reported in grams. The tactile response tests were repeated at least three times with a rest period of 10 min between tests. The behavioral examinations were carried out in a masked fashion on different days and completed immediately before the terminal procedures.

Motor and sensory nerve conduction velocity

On the day of terminal studies, rodents were weighed and anesthetized with Nembutal i.p. (75 mg/kg for mice and 50 mg/kg for rats i.p.; Diamondback Drug, Scottsdale, AZ, USA). Motor and sensory nerve conduction velocities were determined, as previously described, using a non-invasive procedure in the sciatic-posterior tibial conducting system and digital nerve, respectively, and reported in m/s[4,5].

Corneal innervation

Subepithelial corneal nerves were imaged using the Rostock cornea module of the Heidelberg Retina Tomograph confocal microscope, as previously described[5,7]. Briefly, the anesthetized rodent was secured to a customized platform that allowed adjustment and positioning in three dimensions. Then, five and 10, for mice and rats, respectively, random high-quality images, without overlap of the subepithelial nerve plexus of the central cornea, were acquired by finely focusing the objective lens to maximally resolve the nerve layer just under the corneal epithelium. The investigator acquiring these images was masked with respect to the identity of the animal condition. The corneal nerve fiber length was defined as the total length of all nerve fibers and

branches (in mm) present in the acquired images standardized for the area of the image (in mm^2). The corneal fiber length for each animal was the mean value obtained from the acquired images and expressed as mm/mm^2[8]. Based on receiver operating characteristic curve analysis, corneal nerve fiber length is the optimal parameter for diagnosing patients with diabetic neuropathy and has the lowest coefficient of variation[9,10].

Intraepidermal nerve fiber density in skin from the hindpaw

As previously described, immunoreactive nerve fiber profiles innervating the skin from the hindpaw were determined using standard confocal microscopy[4,5]. Immunostained nerve profiles were counted by two individual investigators that were masked to the sample identity. All immunoreactive profiles were normalized to epidermal length.

Additional parameters

In addition to non-fasting blood glucose, serum was collected for determining levels of free fatty acid, triglyceride and free cholesterol using commercial kits from Roche Diagnostics (Mannheim, Germany), Sigma Aldrich Chemical Co. and Bio Vision (Mountain View, CA, USA) respectively. To examine steatosis, liver samples were frozen in optimal cutting temperature compound (Sakura FineTek USA, Torrance, CA, USA) at –80°C. Liver sections of 5 μm were incubated with BODIPY (Molecular Probes, Carlsbad, CA, USA) at a 1:5,000 dilution in 1% bovine serum albumin for 1 h at room temperature. After washing, liver sections were mounted using ProLong® Gold antifade reagent (Molecular Probes) and covered with a glass coverslip. Images were collected using Zeiss LSM confocal laser scanning microscope (Carl Zeiss Microscopy, Thornwood, NY, USA) and analyzed for percentage area fraction of lipid droplets using Image J software (National Institute of Health, Bethesda, MD, USA).

Statistical analysis

Results are presented as mean ± standard error of the mean. Comparisons between groups were carried out using a one-way analysis of variance (ANOVA) and Dunnett's pairwise test for multiple comparisons (Prism software; GraphPad, San Diego, CA, USA). A P-value of <0.05 was considered significant.

RESULTS

Tables 1 and 2 provide data on the start and end weights, blood glucose and serum free fatty acid, triglyceride, and cholesterol levels of rats and mice, respectively. All female rats and mice weighed the same at the beginning of the study. At the end of the study, non-diabetic high-fat diet-fed rats and mice weighed significantly more than the control and diabetic rats and mice, respectively. Non-fasting blood glucose was significantly increased in female diabetic rats and mice. Serum free fatty acids were increased in diabetic rats compared with control and non-diabetic high-fat diet-fed rats. Serum triglyceride levels were unchanged in control, non-diabetic high-fat diet-fed and diabetic female rats. Serum cholesterol was significantly increased in non-diabetic and diabetic high-fat diet-fed rats. Serum free fatty acid and triglyceride levels were unchanged in control, non-diabetic high-fat diet-fed and diabetic female mice. Serum cholesterol was significantly increased in diabetic high-fat diet-fed mice compared with control and non-diabetic high-fat diet-fed female mice. Non-diabetic high-fat diet-fed rats and mice, and diabetic rats and mice had fatty livers compared with control rats and mice (Tables 1 and 2).

Table 1 | Effect of high fat diet and type 2 diabetes in female Sprague–Dawley rats

Determination	Control (12)	High fat (12)	Diabetic (12)
Start weight (g)	208 ± 3	209 ± 3	209 ± 2
End weight (g)	291 ± 3	328 ± 5†	294 ± 6‡
Blood glucose (mg/dL)	140 ± 6	140 ± 4	283 ± 35†‡
Serum free fatty acid (mmol/L)	0.20 ± 0.04	0.27 ± 0.04	0.33 ± 0.03†
Serum triglycerides (mg/mL)	0.38 ± 0.04	0.33 ± 0.03	0.46 ± 0.05
Serum free cholesterol (mg/mL)	0.79 ± 0.07	1.13 ± 0.07†	1.11 ± 0.09†
Steatosis (%)	4.8 ± 0.5	24.4 ± 1.9†	22.7 ± 1.6†

Total duration of high-fat diet and hyperglycemia (post-streptozotocin injection) was 24 and 12 weeks, respectively. Data are presented as the mean ± standard error of the mean. †$P < 0.05$ compared with control; ‡$P < 0.05$ compared with high fat. Parentheses indicate the number of experimental animals in each group.

Table 2 | Effect of high fat diet and type 2 diabetes in female C57Bl/6J mice

Determination	Control (11)	High fat (12)	Diabetic (13)
Start weight (g)	20.4 ± 0.4	20.1 ± 0.4	20.7 ± 0.3
End weight (g)	23.6 ± 0.7	43.0 ± 2.1†	27.0 ± 0.7‡
Blood glucose (mg/dL)	219 ± 9	229 ± 10	418 ± 21†‡
Serum free fatty acid (mmol/L)	0.32 ± 0.03	0.34 ± 0.03	0.29 ± 0.02
Serum triglycerides (mg/mL)	0.56 ± 0.05	0.67 ± 0.04	0.47 ± 0.04
Serum free cholesterol (mg/mL)	1.14 ± 0.22	2.11 ± 0.29	3.40 ± 0.33†‡
Steatosis (%)	6.4 ± 0.6	54.3 ± 1.7†	49.0 ± 1.9†

Total duration of high-fat diet and hyperglycemia (post-streptozotocin injection) was 20 and 12 weeks, respectively. Data are presented as the mean ± standard error of the mean. †$P < 0.05$ compared with control; ‡$P < 0.05$ compared with high fat. Parentheses indicate the number of experimental animals in each group.

Data in Figure 1 show that glucose clearance is impaired in non-diabetic high-fat diet-fed rats (Figure 1a) and mice (Figure 1b), and to even a greater extent in diabetic high-fat diet-fed rats and mice compared with the control rats and mice.

A standard outcome measure of neurological function is motor and sensory nerve conduction velocity. Data in Figure 2

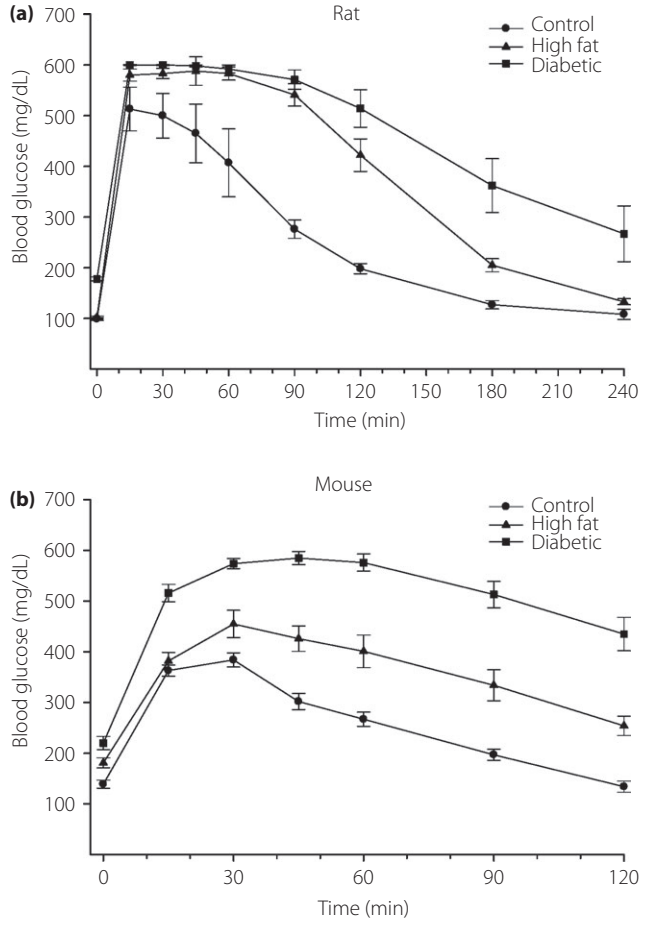

Figure 1 | Glucose clearance in normal, dietary-obese, and type 2 diabetic (a) rats and (b) mice. Glucose clearance was determined as described in the Methods section. Data are presented as the mean ± standard error of the mean for glucose utilization in mg/dL. The fasting blood glucose levels for the control, non-diabetic high-fat diet-fed and diabetic high-fat diet-fed rats and mice were 99 ± 3, 102 ± 3 and 178 ± 11*,$^{+}$; and 138 ± 8, 181 ± 10* and 220 ± 13*, respectively. The number of rodents in each group was the same as described in Tables 1 and 2 for rats and mice, respectively. *$P < 0.05$ compared with control rodents; $^{+}P < 0.05$ compared with high-fat diet-fed rodents. *Significantly different from control. $^{+}$Significantly different from high fat fed.

show that in non-diabetic high-fat diet-fed female rats, only sensory nerve conduction velocity is impaired compared with control rats. In contrast, in diabetic high-fat diet-fed rats, both motor and sensory nerve conduction velocity is slowed compared with control rats. In female mice, unlike female rats, both motor and sensory nerve conduction velocity is decreased in non-diabetic high-fat diet-fed and diabetic high-fat diet-fed mice compared with control mice.

Examination of the density of sensory nerves and their function has become another reliable determination of neurological function. In data reported in Figure 3, we investigated the density of intraepidermal nerve fibers in the skin (Figure 3a,b), and thermal and mechanical sensitivity of these nerves (Figure 3c,d) in non-diabetic high-fat diet-fed and diabetic high-fat diet-fed female rats (Figure 3a,c) and mice (Figure 3b,d). In female rats, intraepidermal nerve fiber density was decreased in non-diabetic rats fed a high-fat diet and in diabetic rats fed a high-fat diet. Latency to a thermal stimulation was impaired in diet-induced obese and diabetic female rats. Sensitivity to a mechanical stimulus was increased in female rats fed a high-fat diet and to a greater extent in diabetic female rats. In female mice, intraepidermal nerve fiber density was decreased in non-diabetic mice fed a high-fat diet and to a greater extent in diabetic mice fed a high-fat diet. Both thermal nociception and mechanical allodynia are impaired in diet-induced obese female mice and to a greater degree in diabetic female mice.

Examination of density sensory nerves in the subepithelial layer or the cornea has been promoted to be an early marker of peripheral neuropathy[11,12]. It has been shown that the density of corneal nerves is decreased in humans with insulin resistance, as well as in pre-diabetic and diabetic animal models[7,8,13]. In humans with diabetes, a decrease in corneal nerves has been shown to correlate with the severity of peripheral diabetic neuropathy[9,10]. Data in Figure 4 provide representative images of subepithelial corneal nerves from a control rat (Figure 4a) and mouse (Figure 4b), with the red arrows pointing toward the nerves. Analysis of subepithelial corneal nerves in non-diabetic high-fat diet-fed and diabetic high-fat diet-fed female rats and mice showed that diet-induced obesity causes a significant decrease in density of these nerves that is worsened 12 weeks after the induction of hyperglycemia.

Provided as supporting information, Tables S1 and S2 provide data on neural end-points for male rats and mice, respectively, including motor and sensory nerve conduction velocities, thermal nociception, mechanical allodynia (mice only), intraepidermal nerve fiber density and corneal nerve fiber length[5,7,14]. For male rats and mice, high-fat diets with 45% and 60% kcal as fat, respectively, were initiated when the animals were 12 weeks-of-age. After 8 weeks on these diets, low-dose protocols of streptozotocin were used to induce a form of late-stage type 2 diabetes[5,7,14]. Analyses of the neural end-points were completed 12 weeks later. These supporting data show that the peripheral neuropathy that existed at the end of the study period for male and female rats and mice were general similar.

DISCUSSION

The main findings from these studies of female Sprague–Dawley rats and C57Bl/6J mice were that the creation of diet-induced obesity or type 2 diabetes in these rodents was accompanied by impaired glucose clearance and characteristics of peripheral neuropathy. Furthermore, the peripheral neuropathy that developed in the female rodents was similar to the neuropathy we have determined in age- and protocol-matched male rats and mice with diet-induced obesity or type 2 diabetes[5,15]. The primary difference we observed in the present study was the challenge encountered in inducing dietary obesity and impaired glucose

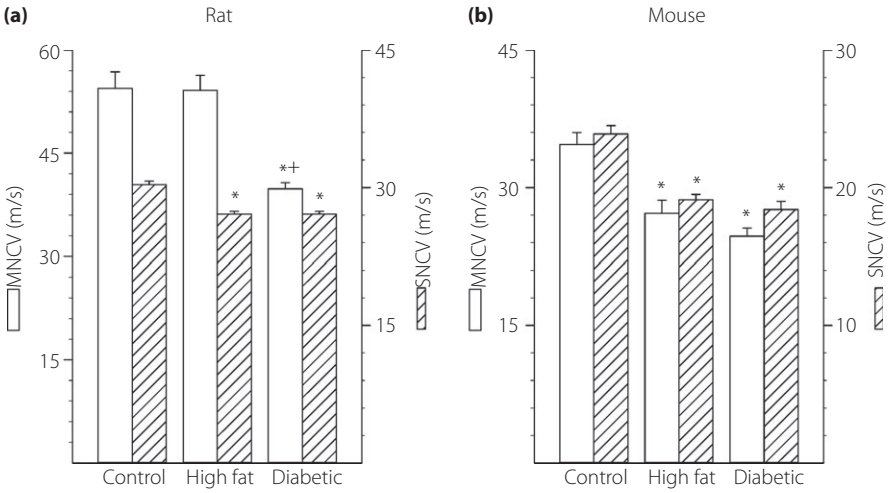

Figure 2 | Motor and sensory nerve conduction velocity in normal, dietary-obese and type 2 diabetic (a) rats and (b) mice. Motor (MNCV) and sensory nerve conduction velocity (SNCV) was examined as described in the Methods section. The number of rodents in each group was the same as described in Tables 1 and 2 for rats and mice, respectively. Data are presented as the mean ± standard error of the mean in m/s. *$P < 0.05$ compared to control rodents; $^{+}P < 0.05$ compared with high-fat diet-fed rodents.

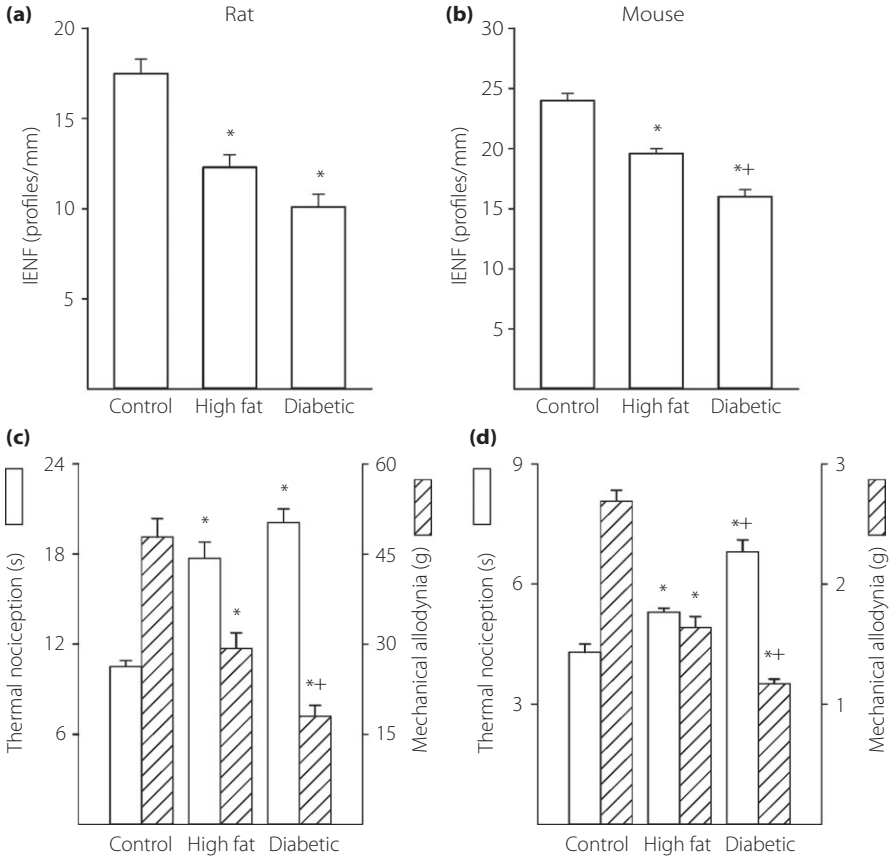

Figure 3 | (a,b) Intraepidermal nerve fiber density (IENF), (c) thermal nociception and (d) mechanical allodynia in normal, dietary-obese, and type 2 diabetic rats and mice. IENF density, thermal nociception and mechanical allodynia were examined as described in the Methods section. The number of rodents in each group was the same as described in Tables 1 and 2 for rats and mice, respectively. Data are presented as the mean ± standard error of the mean for intraepidermal nerve fiber density as profiles per mm, thermal nociception in seconds and mechanical allodynia in grams. *$P < 0.05$ compared with control rodents; $^{+}P < 0.05$ compared with high-fat diet-fed rodents.

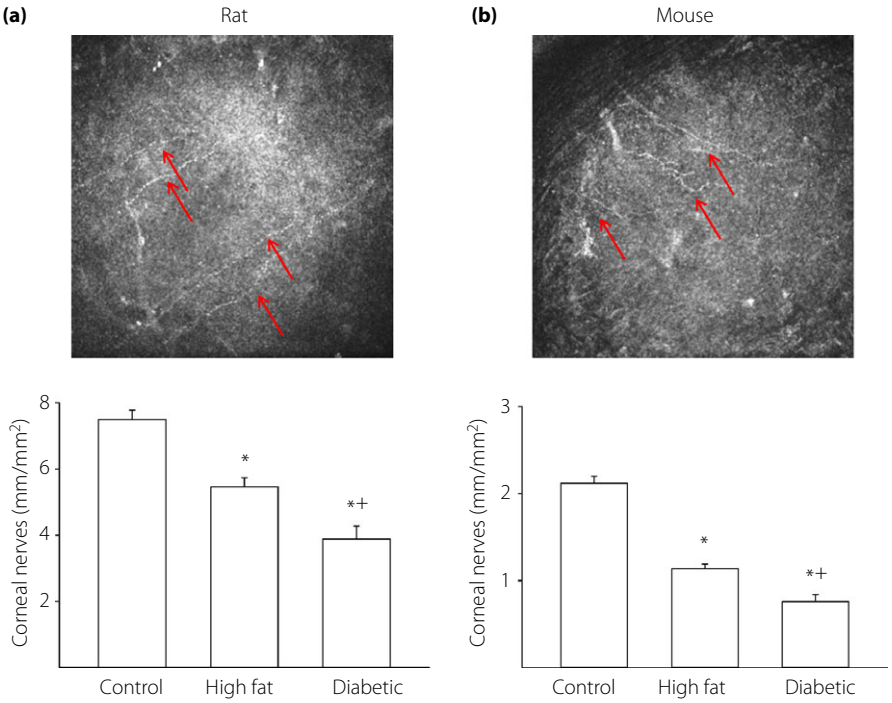

Figure 4 | Corneal nerve fiber length in normal, dietary-obese, and type 2 diabetic (a) rats and (b) mice. Corneal nerve fiber length was examined as described in the Methods section. Inserts are representative images of the subepithelial layer of the cornea from (a) Sprague–Dawley rats and (b) C57Bl/6J mice. The red arrows point out the corneal nerves. The number of rodents in each group was the same as described in Tables 1 and 2 for rats and mice, respectively. Data are presented as the mean ± standard error of the mean for corneal nerve fiber length in mm/mm^2. *$P < 0.05$ compared with control rodents; $^+P < 0.05$ compared with high-fat diet-fed rodents.

tolerance in female Sprague–Dawley rats. We experienced no difficulty in creating diet-induced obesity with impaired glucose tolerance in female C57Bl/6J mice. The protocol we used to induce diet-induced obesity with impaired glucose tolerance in male C57Bl/6J mice had the same effect on female mice, with the female mice gaining approximately the same amount of weight over the course of the study as male mice[3]. Feeding male Sprague–Dawley rats at 12 weeks-of-age a high-fat diet consisting of 45% kcal as fat (Research Diets D12451) for 8 weeks caused significant weight gain and impaired glucose clearance[6,7,15]. However, female Sprague–Dawley rats at 12 weeks-of-age weighed approximately 100 g less than male rats of the same age, and after 8 weeks on the 45% kcal fat diet failed to gain any weight compared with the age-matched controls, and glucose clearance was the same as the control female rats (data not shown)[15]. It was our observation that female rats were considerably more active compared with male rats; however, this was not quantified. In order to induce obesity with impaired glucose tolerance, female Sprague–Dawley rats were fed a 60% kcal as fat diet for 12 weeks, and this only caused an increase of 37 g compared with the control rats. In comparison, male Sprague–Dawley rats fed a 45% kcal fat diet for 8 weeks weighed approximately 60 g more than age-matched controls[15]. Other laboratories have had a similar experience. It has been reported that a 30% kcal high-fat diet did not cause obesity in female Wistar rats[16]. In addition, Taraschenko *et al.*[17] reported that a 45% kcal high-fat diet induced obesity in male Sprague–Dawley rats, but not female rats. Once diet-induced obesity was established in female Sprague–Dawley rats and C57Bl/6J mice, the same low dose of streptozotocin regimen that we used in male Sprague–Dawley rats and C57Bl/6J mice, respectively, was effective in inducing hyperglycemia[5–7,15].

Glucose utilization was impaired in diet-induced female rats and mice, and this was worsened after the onset of hyperglycemia. We have previously reported similar results for glucose utilization with male rats and mice with diet-induced obesity or type 2 diabetes[5–7,15]. Pettersson *et al.*[18] also reported impaired glucose utilization in female C57Bl/6J mice 14 weeks after a 60% kcal high-fat diet. They also reported that diet-induced obese female mice were protected from other metabolic changes and displayed anti-inflammatory properties compared with male mice[18].

Non-alcoholic fatty liver disease in patients with type 1 or type 2 diabetes is associated with an increased prevalence of peripheral neuropathy[19,20]. As part of our evaluation of peripheral neuropathy and related end-points, we examined the livers of obese and diabetic female rats and mice, and found steatosis to be significantly increased. We have previously reported similar results in both obese and diabetic male rats and mice[5–7,15]. Choi *et al.*[21] reported that a diet high in fructose promotes liver steatosis and hepatocyte apoptosis in female C57Bl/6J mice. Female Sprague–Dawley rats developed steatosis when fed a

diet consisting of 71% kcal fat, 18% kcal protein and 11% kcal saccharides at 5 weeks-of-age for 3 weeks[22]. Different models of type 2 diabetic female rats and mice have also been shown to develop steatosis[23–27].

The occurrence of peripheral neuropathy in diet-induced obese and diabetic male rodents has been studied extensively (see Tables S1 and S2). In the present study, we wanted to determine whether diet-induced obesity and/or type 2 diabetes in female rats and mice causes peripheral neuropathy. After examining multiple end-points including motor and sensory nerve conduction velocity, thermal algesia and mechanical allodynia, and nerve density of the skin and cornea, we found that both obese and diabetic female rats and mice develop peripheral neuropathy similar to diet-induced obese and diabetic male rats and mice. The only difference we found was the more intensive dietary approach required to induce obesity in female rats compared with male rats[6,7]. However, once chronic obesity was established, the occurrence of peripheral neuropathy was detected with progression of severity in end-points measuring thermal and mechanical sensitivity, and nerve density in the skin and cornea after the onset of hyperglycemia. Like in male diet-induced obese rats, and unlike female mice, we did not detect a slowing of motor nerve conduction in diet-induced obese female rats[6,7]. However, once hyperglycemia was established through treatment with a low dose of streptozotocin, a decrease in motor nerve conduction occurred. In contrast, sensory nerve conduction velocity was slower in both obese and diabetic female rats and mice. Overall, we found the development of peripheral neuropathy in diet-induced obese or type 2 diabetic female rodents to be similar to male rodents. O'Brien *et al.*[28] reported that both male and female BTBR *ob/ob* mice develop robust diabetic peripheral neuropathy with the sole difference that intraepidermal nerve fiber density was greater in female *ob/ob* mice compared with male mice. In has been reported that pain-related behavior is similar in male and female type 2 diabetic rats, but not in type 1 diabetic rats[29]. In type 2 diabetic Goto–Kakizaki rats, male animals with diabetes have a higher frequency of neuropathy than females with diabetes, and nerve regeneration after sciatic nerve injury shows sex differences[30].

In summary, we have shown that peripheral neuropathy develops and is progressive in Sprague–Dawley female rats and C57Bl/6J female mice after inducement of obesity and creation of type 2 diabetes, and except for the more aggressive approach required to induce obesity in female rats, the presence of peripheral neuropathy and progression in female rodents is similar to male rodents. Sprague–Dawley rats and C57Bl/6J mice are common rodent models used for studies of diet-induced obesity and diabetes. The high-fat diet-fed low-dose streptozotocin-treated rodent is also becoming a popular model that represents late-stage type 2 diabetes[4,31–35].

ACKNOWLEDGMENTS

This material is based on work supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK107339 from NIH and the Department of Veterans Affairs, Veterans Health Administration, Rehabilitation Research and Development (Merit award: RX000889-05). The content of this manuscript is new and solely the responsibility of the authors, and does not necessarily represent the official views of the granting agencies.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Clayton JA, Collins FS. NIH to balance sex in cell and animal studies. *Nature* 2014; 509: 282–283.
2. Aaberg ML, Burch DM, Hud ZR, *et al.* Gender differences in the onset of diabetic neuropathy. *J Diabetes Complications* 2008; 22: 83–87.
3. Javed A, Furqan A, Zaheer M, *et al.* Gender based differences in diabetic peripheral neuropathy. *Pakistan J Neurolog Sci* 2014; 9: 20–24.
4. Davidson EP, Coppey LJ, Holmes A, *et al.* Effect of treatment of high fat fed/low dose streptozotocin-diabetic rats with Ilepatril on vascular and neural complications. *Eur J Pharmacol* 2011; 668: 497–506.
5. Yorek MS, Obrosov A, Shevalye H, *et al.* Effect of diet-induced obesity or type 1 or type 2 diabetes on corneal nerves and peripheral neuropathy in C57Bl/6J mice. *J Peripher Nerv Syst* 2015; 20: 24–31.
6. Davidson EP, Coppey LJ, Calcutt NA, *et al.* Diet-induced obesity in Sprague-Dawley rats causes microvascular and neural dysfunction. *Diabetes Metab Res Rev* 2010; 26: 306–318.
7. Davidson EP, Coppey LJ, Kardon RH, *et al.* Differences and similarities in development of corneal nerve damage and peripheral neuropathy and in diet-induced obesity and type 2 diabetic rats. *Invest Ophthalmol Vis Sci* 2014; 55: 1222–1230.
8. Davidson EP, Coppey LJ, Holmes A, *et al.* Changes in corneal innervation and sensitivity and acetylcholine-mediated vascular relaxation of the posterior ciliary artery in a type 2 diabetic rat. *Invest Ophthalmol Vis Sci* 2012; 53: 1182–1187.
9. Quattrini C, Tavakoli M, Jeziorska M, *et al.* Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes* 2007; 56: 2148–2154.
10. Tavakoli M, Quattrini C, Abbott C, *et al.* Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. *Diabetes Care* 2010; 33: 1792–1797.
11. Hossain P, Sachdev A, Malik RA. Early detection of diabetic peripheral neuropathy with corneal confocal microscopy. *Lancet* 2005; 366: 1340–1343.
12. Pritchard N, Edwards K, Vagenas D, *et al.* Corneal sensitivity as an ophthalmic marker of diabetic neuropathy. *Optom Vis Sci* 2010; 87: 1003–1008.

13. Azmi S, Ferdousi M, Petropoulos IN, *et al.* Corneal confocal microscopy identifies small-fiber neuropathy in subjects with impaired glucose tolerance who develop type 2 diabetes. *Diabetes Care* 2015; 38: 1502–1508.
14. Obrosov A, Shevalye H, Coppey LJ, *et al.* Effect of tempol on peripheral neuropathy in diet-induced obese and high-fat fed/low-dose streptozotocin-treated C57Bl6/J mice. *Free Radic Res* 2017; 51: 360–367.
15. Holmes A, Coppey LJ, Davidson EP, *et al.* Rat models of diet-induced obesity and high fat/low dose streptozotocin type 2 diabetes: effect of reversal of high fat diet compared to treatment with enalapril or menhaden oil on glucose utilization and neuropathic endpoints. *J Diabetes Res* 2015; 2015: 307285.
16. Bartness TJ, Polk DR, McGriff WR, *et al.* Reversal of high-fat diet-induced obesity in female rats. *Am J Physiol* 1992; 263: R790–R797.
17. Taraschenko OD, Maisonneuve IM, Glick SD. Sex differences in high fat-induced obesity in rats: effects of 18-methoxycoronaridine. *Physiol Behav* 2011; 103: 308–314.
18. Pettersson US, Walden TB, Carlsson PO, *et al.* Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. *PLoS ONE* 2012; 7: e46057.
19. Mantovani A, Rigolon R, Mingolla L, *et al.* Nonalcoholic fatty liver disease is associated with an increased prevalence of distal symmetric polyneuropathy in adult patients with type 1 diabetes. *J Diabetes Complications* 2017; 31: 1021–1026.
20. Williams KH, Burns K, Constantino M, *et al.* An association of large-fibre peripheral nerve dysfunction with non-invasive measures of liver fibrosis secondary to non-alcoholic fatty liver disease in diabetes. *J Diabetes Complications* 2015; 29: 1240–1247.
21. Choi Y, Abdelmegeed MA, Song BJ. Diet high in fructose promotes liver steatosis and hepatocyte apoptosis in C57Bl/6J female mice: role of disturbed lipid homeostasis and increased oxidative stress. *Food Chem Toxicol* 2017; 103: 111–121.
22. Stoppeler S, Palmes D, Fehr M, *et al.* Gender and strain-specific differences in the development of steatosis in rats. *Lab Anim* 2013; 47: 43–52.
23. Ishii Y, Motohashi Y, Muramatsu M, *et al.* Female spontaneously diabetic Torii fatty rats develop nonalcoholic steatohepatitis-like hepatic lesions. *World J Gastroenterol* 2015; 21: 9067–9078.
24. Zhong J, Gong W, Lu L, *et al.* Irbesartan ameliorates hyperlipidemia and liver steatosis in type 2 diabetic db/db mice via stimulating PPAR-γ, AMPK/Akt/mTOR signaling and autophagy. *Int Immunopharmacol* 2017; 42: 176–184.
25. Itoi-Babaya M, Ikegami H, Fujisawa T, *et al.* Fatty liver and obesity: phenotypically correlated but genetically distinct traits in a mouse model of type 2 diabetes. *Diabetologia* 2007; 50: 1641–1648.
26. Yu F, Takahashi T, Moriya J, *et al.* Angiotensin-II receptor antagonist alleviates non-alcoholic fatty liver in KKAy obese mice with type 2 diabetes. *J Int Med Res* 2006; 34: 297–302.
27. Sahai A, Malladi P, Pan X, *et al.* Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G1035–G1043.
28. O'Brien PD, Hur J, Robell NJ, *et al.* Gender-specific differences in diabetic neuropathy in BTBR *ob/ob* mice. *J Diabetes Complications* 2016; 30: 30–37.
29. Ferhatovic L, Banozic A, Kostic C, *et al.* Sex differences in pain-related behavior and expression of calcium/calmodulin-dependent protein kinase II in dorsal root ganglia of rats with diabeter type 1 and type 2. *Acta Histochem* 2013; 115: 496–504.
30. Stenberg L, Dahlin LB. Gender differences in nerve regeneration after sciatic nerve injury and repair in healthy and in type 2 diabetic Goto-Kakizaki rats. *BMC Neurosci* 2014; 15: 107–117.
31. Reed MJ, Meszaros K, Entes LJ, *et al.* A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000; 49: 1390–1394.
32. Srinivasan K, Viswanad B, Asrat L, *et al.* Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; 52: 313–320.
33. Zhang M, Lv XY, Li J, *et al.* The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp Diabetes Res* 2008; 2008: 704045.
34. Arulmozhi DK, Kurian R, Bodhankar SL, *et al.* Metabolic effects of various antidiabetic and hypolipidaemic agents on a high-fat diet and multiple low-dose streptozotocin (MLDS) mouse model of diabetes. *J Pharm Pharmacol* 2008; 60: 1167–1173.
35. Mali VR, Ning R, Chen J, *et al.* Impairment of aldehyde dehydrogenase-2 by 4-hydroxy-2-noneal adduct formation and cardiomyocyte hypertrophy in mice fed a high-fat diet and injected with low-dose streptozotocin. *Exp Biol Med* 2014; 239: 610–618.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | Effect of high-fat diet and type 2 diabetes on neural-related end-points in male Sprague–Dawley rats.
Table S2 | Effect of high-fat diet and type 2 diabetes on neural-related end-points in male C57Bl/6J mice.