ORIGINAL PAPER

Impairment of forepaw function in type 1 diabetic rats

Susan Amin[1], Ahmed Alekri[1], Ahmed Alaradi[1], Ali Alaysreen[1], Ammar Kheyami[1], Hasan Baksh[2], Khalid Nazzal[2], Abdullah Altamimi[2], O Ali[2], Amer Kamal[2]

[1]School of Medicine, RCSI Bahrain, Bahrain
[2]Department of Physiology, AGU – Arabian Gulf University, Bahrain

ABSTRACT

INTRODUCTION: Hand function disruption occurs in diabetes. We aimed to record this food-handling behaviour in a rat model of diabetes type 1.

MATERIAL AND METHODS: The hand vermicelli test measures practised forepaw use in rats, which can model diseases such as brain injuries. This test demonstrates in a dexterous way how rats handle food items with their paws.

RESULTS: When rats manipulate pasta, such as vermicelli, they adjust their forepaw hold on the pasta. These adjustments can be easily viewed, counted, and recorded. Diabetic type 1 rats experienced statistically significant reductions in adjustments made with the right and left fingers. There was an increase in several atypical handling patterns for the experimental group, an increased eating time of 120 seconds, and increased adjustments made with the left forepaw, compared to the control group.

CONCLUSIONS: The abnormal handling patterns and increased eating time may indicate compensatory measures to cope with diabetes-induced motor impairment.

KEY WORDS: diabetes, forepaw function, compensation.

Current Topics in Diabetes 2022; 2 (3–4): 225–229

CORRESPONDING AUTHOR:
Susan Amin, School of Medicine, RCSI Bahrain, P.O. Box 15503, Building No. 2441, Road 2835, Busaiteen 228, Bahrain, e-mail: susan.amin.uk@gmail.com

Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycaemia. In the long-term, poorly controlled DM can cause peripheral nerve damage (so-called diabetic peripheral neuropathy – DPN), with a prevalence of 30–50% [1]. Both the lower [2] and upper limbs are affected by DPN [2, 3]. Fifty-eight to 82% and 37–69% of DM patients have pain from subclinical nerve injury to the median and ulnar nerves, which restricts the range of movement in the digits and reduces handling, pinch strength, and tactile sensitivity [4].

The mechanisms of how diabetes can affect paw function include oxidative stress, microangiopathy, and polyols accumulation. Oxidative stress is linked to the increased production of mitochondrial superoxide in the endothelial layer of blood vessels, which causes disruptive angiogenesis and expression of pro-inflammatory factors, leading to long-lasting epigenetic events after glycaemia is returned to normal (hyperglycaemic memory) [5]. Functional abnormalities of the vasa nervorum are presented early in DPN models and may predetermine structural changes leading to ischaemic damage of nerve cells [6]. The accumulation of polyols, especially sorbitol, in peripheral nerve cells occurs by increased movement through the aldose reductase pathway. Aldose reductase is located around glial cells that are supporting cells, and increased polyol damages these cells and hence nerve function [7].

Manual dexterity can be studied in rodent models. Rats grip food of varying textures (e.g. pasta such as vermicelli) by asymmetrical forepaw manipulations (i.e. one paw is used predominantly for support) [8–10].

We aim to measure forepaw movements in a rat model of type 1 DM by quantitatively assessing vermicelli manipulation in rats to measure forepaw movements. We defined forepaw corrections as distinct episodes of forepaw release followed by re-handling of the vermicelli strand. We assessed vermicelli gripping techniques as well as forepaw adjustments to evaluate their sensitivity to damage compared to non-DM control rats.

Material and methods

Animals

Type 1 diabetic rats. Male Sprague-Dawley rats (n = 40) aged 5–7 weeks (150–200 g) were housed in pairs on a 12 h light: 12 h dark cycle with unrestricted access to food and water. The rats were randomly assigned to control (n = 20) and DM (n = 20) groups. For diabetes induction, rats (n = 20) were fasted for 4–6 h and then administered a single intraperitoneal (i.p.) bolus injection of 100 mg/kg streptozotocin (STZ) (S-0130, Sigma, UK; dissolved in sodium citrate buffer solution; pH 4.5). Seven days later, blood glucose was measured in a sample of venous blood taken from each animal (OneTouch® Ultra® 2; Lifescan, Inc., USA). All the STZ-treated animals had a blood glucose of > 15 mmol/l (280 mg/dl).

Vermicelli handling test

Stimulus

Rats were provided with uncooked vermicelli sticks 7 cm in length 24 hours after a practice session.

Vermicelli handling trials

Rats were separated from their cage mate during the testing and were assessed in a similar cage. The trials were recorded and then replayed in slow motion for analysis. Data were received blindly. The test comprised 4 to 5 trials with pasta pieces given once per trial.

Normal vermicelli handling patterns

The "grasp" paw grips the pasta at the start of eating. The "guide" paw is held close to the mouth at the start of eating to help guide the vermicelli piece into the mouth. Here, one paw is doing more work than the other; hence, the asymmetrical method.

Forepaw modifications

The main dependent factor collected was the number of adjustments per forepaw for each pasta piece. An adjustment was defined as any release-regrip of the vermicelli piece. Only adjustments made after eating had started were recorded.

We also recorded the duration to eat each vermicelli piece. "Onset of eating" was when the vermicelli piece was held and placed in the mouth. Cessation of eating was when the vermicelli piece was loosened by the paws and disappeared into the mouth. The sounds made during eating were audible and were used to identify eating onset.

Abnormal and general behaviours

The asymmetrical paw hold was the highest vermicelli handling pattern in the control animals. Observations of abnormal vermicelli handling behaviours were noted. These behaviours were

recorded by other authors and are noted in the following list:

- symmetrical handling when both paws are together when the vermicelli piece is long,
- switching of guiding and grasping roles,
- failure to contact with the vermicelli,
- vermicelli piece is dropped,
- paws apart when piece is short,
- mouth pulling,
- the rat hunches over the vermicelli piece,
- one limb holds the piece in an iron grip grasp during eating,
- repeated repositioning of the guide paw around the grasp paw to move the vermicelli,
- the vermicelli is held at an angle.

Data analysis

The data were analysed using Microsoft Excel 2016. Descriptive statistics were used to analyse continuous data. These results were presented as frequencies in graphs and as means ± SEM unless otherwise noted. T-tests were used to confirm differences within and between groups for the vermicelli test variables. The indicator of significance was set at 0.05 ($p < 0.05$).

Data and resource availability

Methods and datasets can be made available from the author upon reasonable request.

Results

Paw behaviour in control vs. diabetic animals

Data from 20 control and diabetic type 1 rats were analysed. The control rats had 12 paw modifications compared to the diabetic rats with 14 paw modifications per vermicelli piece. The control rats made 9 adjustments with the right paw compared to only 5 with the left paw (Fig. 1 B). Diabetic rats made 5.5 adjustments with the right paw compared to only 6.5 with the left paw (Fig. 1 B).

Finger behaviour in control vs. diabetic animals

Data from 20 control and diabetic type 1 rats were analysed ($n = 20$). The control rats had 7 finger modifications compared to the diabetic rats with 3.75 finger modifications per pasta piece. Control rats exhibited 3.75 adjustments with the left paw compared to only 3.25 with the right paw (Fig. 1 A). Diabetic rats exhibited 1.5 adjustments with the right paw compared to only 2.25 with the left paw (Fig. 1 A).

Comparisons of timings to finish the vermicelli piece between the control and the diabetic animals

Control rats ate the vermicelli piece in an average of 59 sec compared to the diabetic rats that needed an average of 120 seconds (Fig. 2 A).

Atypical behaviour vs. typical behaviour in control vs. diabetic animals

All the control rats ate the vermicelli asymmetrically. This pattern was observed in 40 of the trials. There was no partiality between the right and left in terms of this asymmetrical handling. A mixture of both clasping patterns was seen in some rats. The majority of diabetic type 1 rats observed used the symmetrical holding pattern (Fig. 2 B).

In the control group, of the abnormal behaviours viewed, the highest in frequency was having the paws as one when the piece was elongated. This was observed in 3 out of 20 trials. This was

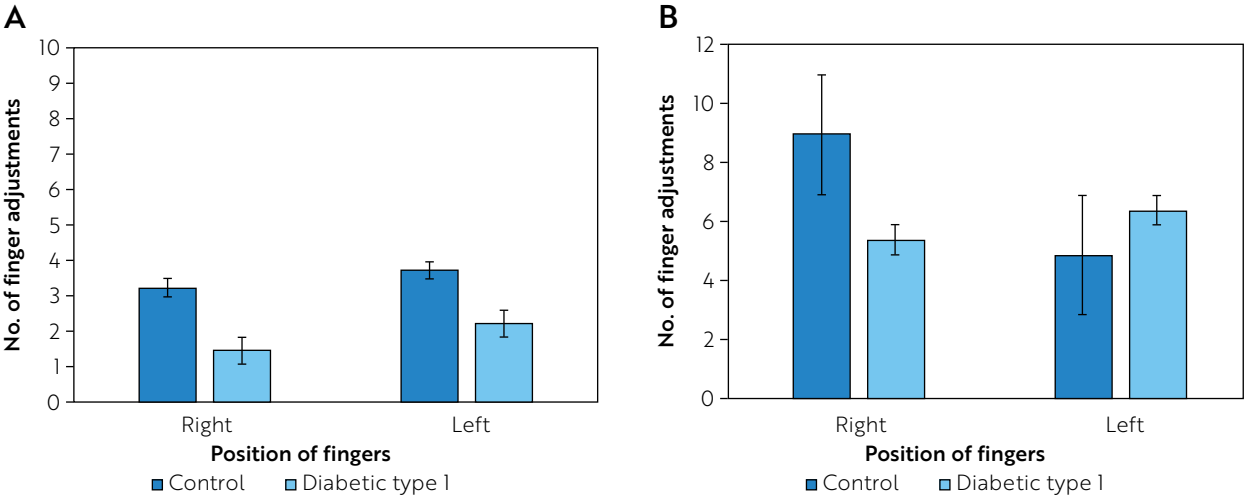

Fig. 1. Comparison of left and right finger adjustments (**A**) and left and right forepaw adjustments (**B**) in the control and diabetic type 1 rats ($p < 0.05$)

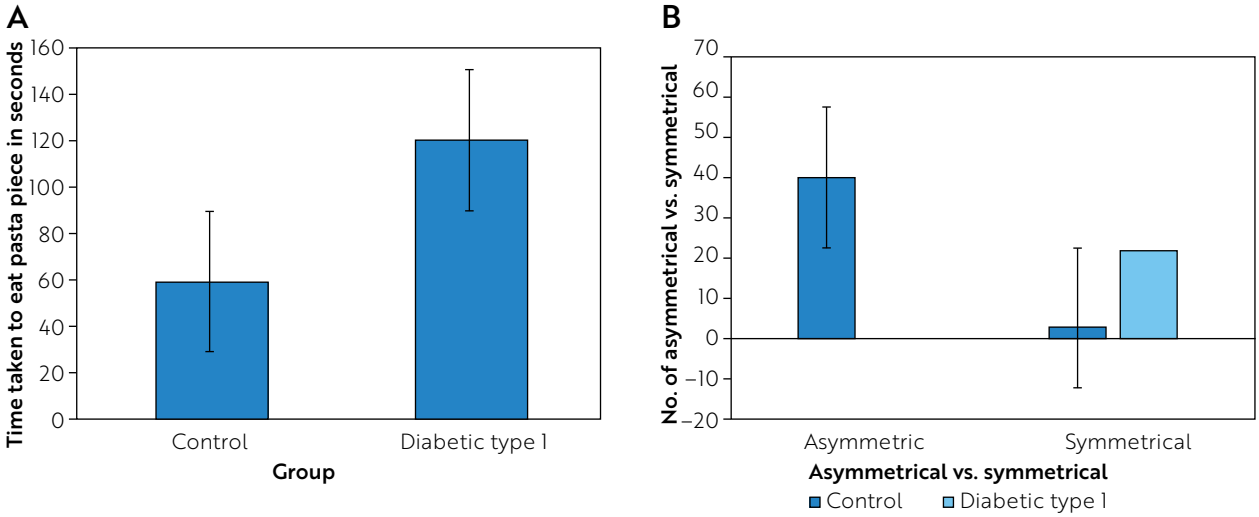

Fig. 2. Comparison of time to finish the vermicelli piece (**A**) and no of symmetrical and asymmetrical adjustments (**B**) in the control and diabetic type 1 rats ($p < 0.05$)

observed an average of 10 times in the diabetic type 1 group. Other behaviours observed in the experimental group in order from the most to the least frequent observations include dropping the vermicelli piece (7 times), iron grip (5 times), paws together when short (4 times), and pulling with mouth (4 times).

Discussion

In this study, we observed that forepaw function was reduced in type 1 DM rats compared to age-matched controls. The eating time, variability with the ipsilateral forepaw, decreased finger movements, and atypical behaviours such as mouth pulling were greater in the DM rats. These results mirror other studies where lesions of the rodent central nervous system disrupted food handling modifications [9–12]. The controls in our study had an asymmetric handling pattern of 89.3%, which is similar to that reported by Allred *et al.* [13].

The increase in forepaw adjustments in one paw compared to the other could also be a compensatory outcome to try and overcome the deficit. In one study, DM was found to disrupt neural route plasticity after stroke [14]. Several studies have documented that the brain's capability to reallocate sensory and motor circuits to similar living regions is important for stroke recovery success [15]. In addition, DM can reduce the brain's ability to repair and rewire during stroke. Functional redistribution of the primary forelimb somatosensory and secondary forelimb somatosensory cortex was absent in diabetic mice, indicating an effect of DM on brain plasticity [16]. Rats with halved dopamine concentration demonstrate decreased movements made by the contralateral paw, whereas the compensatory opposite paw number of modifications remained the same. Diabetes is a risk factor for tardive dyskinesia, a disorder related to abnormal dopamine regulation [17]. Another study reports that in DM the associated sensory deficits decreased the capacity of online motor modifications in response to sensory feedback [18].

The successes of this study include having a convenient data collection, in which videotapes of the eating can be replayed back in slower motion and the assessors could then count the number of adjustments in the right and left limbs. Ratters could watch previous videos to familiarise themselves with the way of counting. This type of study could be useful in the future for looking at other disease models and making comparisons.

The limitations in our study included some rats taking a long time to participate in the vermicelli trial. Some rats starting a vermicelli piece, then abandoned it, and then started again.

Future studies could look at brain imaging of the rats to visualize any sensory and motor regions that DM may have impacted, and histology to look at the sizes of the regions that may be impacted as well as the structural decline of the paws. The groups of forepaw adjustments could be distinguished, as well as looking at this skill in other diabetic models.

Conclusions

The manipulation test with the vermicelli gave us a picture of the extent of damage to the paws in the rats in this study. The technique was dependable and convenient. The abnormal handling patterns and increased eating time may indicate

compensatory measures to cope with the diabetes-induced motor impairment.

Acknowledgments

We would like to thank Ebrahim Rajab for reviewing and editing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Callaghan BC, Little AA, Feldman EL, Hughes RA. Enhanced glucose control for preventing and treating diabetic neuropathy. Cochrane Database Syst Rev 2012; 6: CD007543.
2. Hicks CW, Selvarajah S, Mathioudakis N, et al. Burden of infected diabetic foot ulcers on hospital admissions and costs. Ann Vasc Surg 2016; 33: 149-158.
3. Rota E, Cocito D. Electrodiagnostic testing in diabetic neuropathy: which limb? Diabetes Res Clin Pr 2015; 110: 95-97.
4. Ochoa N, Gorniak SL. Changes in sensory function and force production in adults with type II diabetes. Muscle Nerve 2014; 50: 984-990.
5. Giacco F, Brownlee M. Oxidative stress, and diabetic complications. Circ Res 2010; 107: 1058-1070.
6. Dyck PJ, Dyck PJ, Larson TS, O'Brien PC, Velosa JA. Patterns of quantitative sensation testing of hypoesthesia and hyperalgesia are predictive of diabetic polyneuropathy: a study of three cohorts.. Diabetes Care 2000; 23: 510-517.
7. Bril V, Buchanan RA. Long-term effects of ranirestat (AS3201) on peripheral nerve function in patients with diabetic sensorimotor polyneuropathy. Diabetes Care 2006; 29: 68-72.
8. Ivanco TL, Pellis SM, Whishaw IQ. Skilled forelimb movements in prey catching and in reaching by rats (Rattus norvegicus) and opossums (Monodelphis domestica): relations to anatomical differences in motor systems. Behav Brain Res 1996; 79: 163-181.
9. Whishaw IQ, Coles BL. Varieties of paw and digit movement during spontaneous food handling in rats: postures, bimanual coordination, preferences, and the effect of forelimb cortex lesions. Behav Brain Res 1996; 77: 135-148.
10. Whishaw IQ, Sarna JR, Pellis SM. Evidence for rodent-common and species-typical limb and digit use in eating, derived from a comparative analysis of ten rodent species. Behav Brain Res 1998; 96: 79-91.
11. Whishaw IQ, Coles BL, Pellis SM, Miklyaeva EI. Impairments and compensation in mouth and limb use in free feeding after unilateral dopamine depletions in a rat analog of human Parkinson's disease. Behav Brain Res 1997; 84: 167-177.
12. Gomez C, Santiago-Mejia J, Ventura-Martinez R, Rodriguez R. The sunflower seed test: a simple procedure to evaluate forelimb motor dysfunction after brain ischemia. Drug Develop Res 2006: 67: 752-756.
13. Allred RP, Adkins DL, Woodlee MT, et al. The vermicelli handling test: a simple quantitative measure of dexterous forepaw function in rats. [Internet]. J Neurosci Methods 2008; 170: 229-244.
14. Zhang L, Chopp M, Zhang Y, et al. Diabetes mellitus impairs cognitive function in middle-aged rats and neurological recovery in middle-aged rats after stroke. Stroke 2016; 47: 2112-2118.
15. Alia C, Spalletti C, Lai S, et al. Neuroplastic changes following brain ischemia and their contribution to stroke recovery: novel approaches in neurorehabilitation. Front Cell Neurosci 2017; 11: 76.
16. Sweetnam D, Holmes A, Tennant KA, et al. Diabetes impairs cortical plasticity and functional recovery following ischemic stroke. J Neurosci 2012; 32: 5132-5143.
17. Mohapatra S. Successful management of tardive dyskinesia with quetiapine and clonazepam in a patient of schizophrenia with type 2 diabetes mellitus. Clin Psychopharmacol Neurosci 2016;14:18-220.
18. Li K, Wei N, Cheng M, Hou X, Song J. dynamical coordination of hand intrinsic muscles for precision grip in diabetes mellitus. Sci Rep 2018; 8: 4365.