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Original Article

Differential Functional Deficits in Type 1 Versus Type 2 Diabetic Mice Models

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Abstract

Introduction: Types 1 and 2 diabetes have different causative mechanisms. Hence, different extent of cardiovascular, cognitive and auditory functions could be expected.

Materials and methods: Body weight, blood glucose were measured in Wild type(WT), Akita and db/db mice(n=6 each). Blood pressure, left ventricular ejection fraction (LVEF), Novel object recognition test (NORT), auditory brainstem response(ABR) were measured/elucidated.

Results: Compared to WT, body weight was significantly less in Akita (p=0.004), more in db/db (p=0.001). Blood glucose (p=0.0002 for Akita; p=0.0003 for db/db), blood pressure (p=0.01 for Akita; p=0.02 for db/db), LVEF (p=0.0002 for Akita; p=0.0008 for db/db), NORT-calculated discrimination index (p=0.05 for Akita, db/db) were similar in both types of diabetic mice. ABR threshold was more elevated in Akita (p=0.01) than db/db(p=0.0004).

Conclusion: Cardiovascular/cognitive functions were impaired in both types of diabetics. The significantly greater ABR impairment in db/db mice than in Akita mice is probably due to several concurrent mechanisms affecting the cochlea in db/db mice.

Introduction

The primary physiological characteristic of diabetes mellitus (DM), be it type 1 or type 2, is increased circulating levels of glucose and an inability to utilize this for energy production. The hyperglycemic state in type 1 and type 2 DM is known to result in pathology due to glycation of proteins and oxidative stress (1). The complications of hyperglycemia can be grouped as microangiopathies (e.g. nephropathy, neuropathy, retinopathy and auditory dysfunction) and macroangiopathies (e.g. cardiovascular and cerebrovascular diseases) (2).

Amongst the microangiopathies, the course and

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mechanisms of retinopathy and neuropathy are well-established, but there have been very few studies on auditory dysfunction in diabetes. Dabrowski et al elucidated the involvement of the auditory organ in type 1 diabetes (3). Sanyoura also reported the co-existence of hearing loss with early-onset retinopathy and optic atrophy in a juvenile-onset diabetic (4). Akinpelu et al, in their meta-analysis, concluded that type 2 diabetics had a significantly higher incidence of mild hearing loss which was dependent on duration of diabetes (5). Hence it remains unclear whether mechanism of diabetes mellitus or its duration are the implicating factors for hearing loss.

The macroangiopathies involve the bigger vessels of the heart, brain and kidneys. Type 1 diabetics are known to suffer mild cognitive dysfunction (6). Moran et al showed that type 2 diabetes is associated with neurodegeneration due to cerebral infarcts resulting in cognitive decline (7). But there have been no studies comparing prevalence of these effects in type 1 and type 2 diabetics.

Though all organ systems are affected by macroangiopathies, accelerated cardiovascular pathology is of great concern. One of the major causes of coronary artery disease (CAD) is dyslipidemia, and 97% diabetics exhibit dyslipidemia (8). Not only does diabetes affect the coronary arteries but it also affects the cardiac muscle itself, causing alterations in systolic as well as diastolic blood pressure (9). Obesity, which is associated with type 2 diabetes, is also associated with deranged lipid metabolism. In fact, Denis McGarry, in his Banting lecture 2001, implied that the traditional search for a “glucocentric” metabolic derangement may need to be replaced by a “lipocentric” search (10). But the exact mechanisms of type 2 diabetes remain elusive. Hence, scientists have attempted to identify early markers of vascular pathology so as to prevent the later grave atherosclerotic events. Once again, there have been no studies to demonstrate the differential cardiovascular pathologies in type 1 and type 2 diabetes, considering they have different durations of hyperglycemia and that one is associated with obesity and the other is not.

Hence, this experiment was performed to verify whether or not microangiopathies and macroangiopathies in type 1 and type 2 DM are governed by severity or duration of hyperglycemia (4, 5).

Materials and Methods

Akita mice are insulin deficient and hyperglycemia is evident by the 3rd or 4th week of life. In db/db mice, hyperglycemia is evident by the 4th to 8th week of life. Hence, as a model of type 1 diabetes, we included C57BL/6-Ins2^{Akita} (Akita) mice in our study. To represent type 2 diabetes, the B6.Cg-Dock7^{m} Lepr^{db}/++ (db/db) mice were included (11). C57BL/6J (WT) were used as controls for both types of diabetes as both species of diabetic mice used in this study were on the same background, i.e. C57BL6/J. 6 mice were included in each group). The procedures were in accordance to the institutional animal care guidelines which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication, 2011). All animals were 10 weeks of age. Weight and blood glucose levels were recorded at the beginning of the experiment. The protocol was approved by the institutional ethics committee.

Systolic as well as diastolic blood pressure was measured (not calculated) by the tail-cuff method on Kent Scientific Coda telemeter. Mean blood pressure was calculated from an average of 10 repeats.

Echocardiography of the heart was performed under inhalational anesthesia (isoflurane) on Vevo 2100 system (VisualSonics, Toronto, ON, Canada). The mice were positioned supine on a heating table, temperature maintained at 37.5°C and the Doppler ultrasound done after epilation. During imaging, the transducer, MS250, was held immobile by an integrated rail system (VisualSonics). Images and cine loops were exported and analyzed to obtain calculated indices.

Cognition was assessed by the Novel Object Recognition Test (NORT) performed with the aid of CSI Top View Behaviour Analyzing System. The mice were first acclimatized to a new environment

in several sessions, and then exposed to two similar objects initially and two dissimilar objects subsequently (12). The data was exported to excel files and used to calculate discrimination index (DI).

Auditory Brainstem Response (ABR), also known as auditory evoked potential (AEP), was recorded in a sound-attenuated room on the Tucker Davis Technologies (TDT) SigGenRP apparatus, with acquisition signals of 47 millisecond-long click stimuli delivered at 300-3000 Hz bandwidth. The anesthetic, 2% tribromoethanol (TBE), was administered intraperitoneally in a dose of 240 mg/Kg body weight (13). The stimulus intensity was calibrated at 90 decibels (dB) and signals were delivered starting at 90 dB, followed by subsequent signals at 5 dB decrements. The response was recorded on the TDT system. The lowest sound intensity that produced a recordable waveform was interpreted as the threshold.

Data was subject to statistical analysis by Primer of Biostatistics (7th edition). For differences between groups, ANOVA and unpaired t-test was performed. Spearman's correlation was calculated. A $p<0.05$ was considered as significant. While performing ANOVA, Akita and db/db mice were compared with WT mice as well as with each other.

Results

Our results confirmed the diabetic state in Akita and db/db mice as their mean blood glucose levels were significantly higher in both groups (p=0.0002 in Akita; p=0.0003 in db/db) as compared to WT mice (Fig. 1). Body weight of Akita mice was significantly less (p=0.04) than that of WT, and the body weight of db/db was significantly more (p=0.0001; p=0.0001, respectively)) than that of WT and Akita mice (Fig. 2), which is depictive of the usual trends in type 1 and type 2 diabetes.

Cardiovascular dysfunction was evidenced by raised blood pressure and decreased LVEF:

Mean blood pressure was significantly higher in both Akita (p=0.01) and db/db mice (p=0.02) as compared to the WT mice (Fig. 3a). Ejection fraction of the heart was significantly less in Akita (p=0.0002) as well as in db/db mice (p=0.0008) as compared to that in WT mice, as evidenced by the altered differential ventricular diameter during systole versus diastole (figures 3b and 3c). Thus, the major early indicators of cardiovascular pathology were not differentially expressed in the type 1 and type 2 diabetes, though in both, functional impairment was evident.

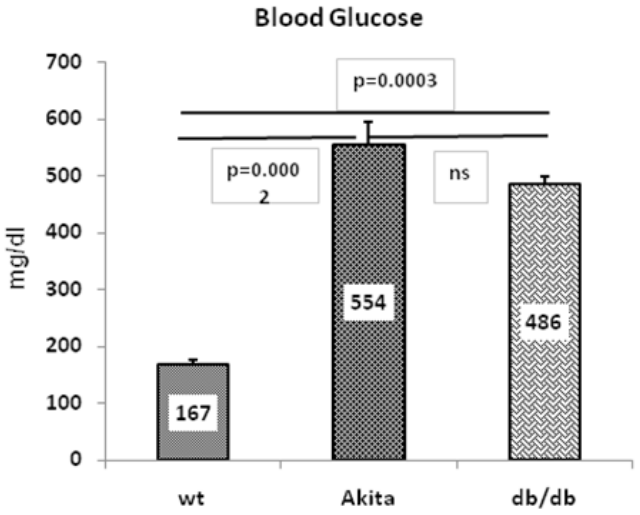

Fig. 1 : Blood glucose levels are significantly higher in both Akita and db/db mice as compared to their controls (WT mice).

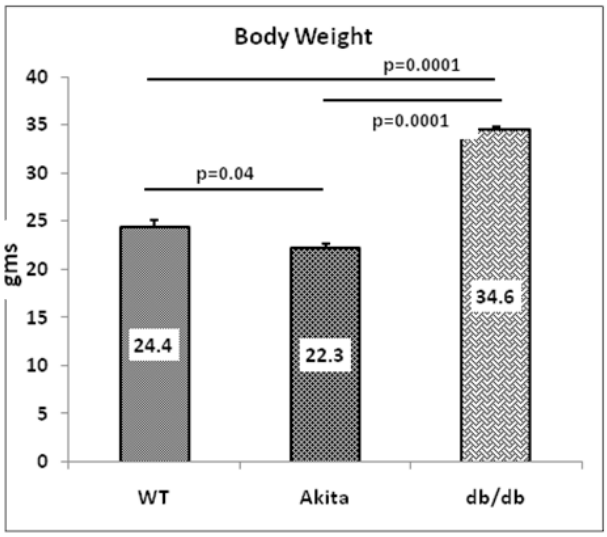

Fig. 2 : Akita mice have significantly lower body weight as compared to WT, and db/db mice have significantly higher body weight as compared to WT and akita.

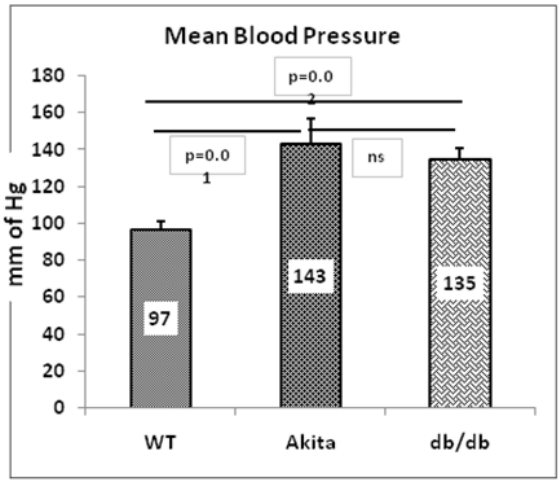

Fig. 3a

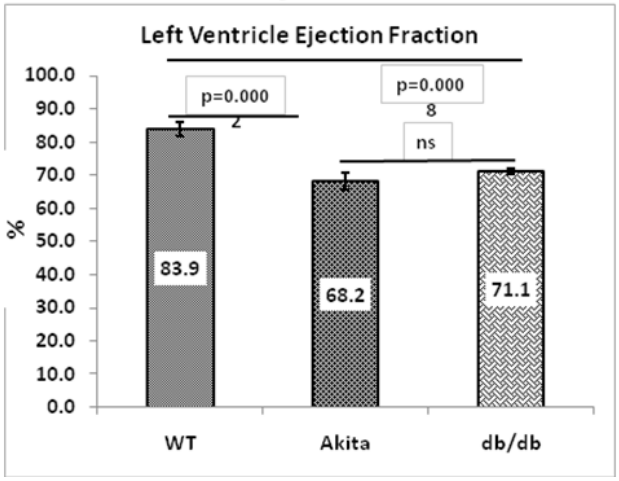

Fig. 3c

M-Mode Echocardiography

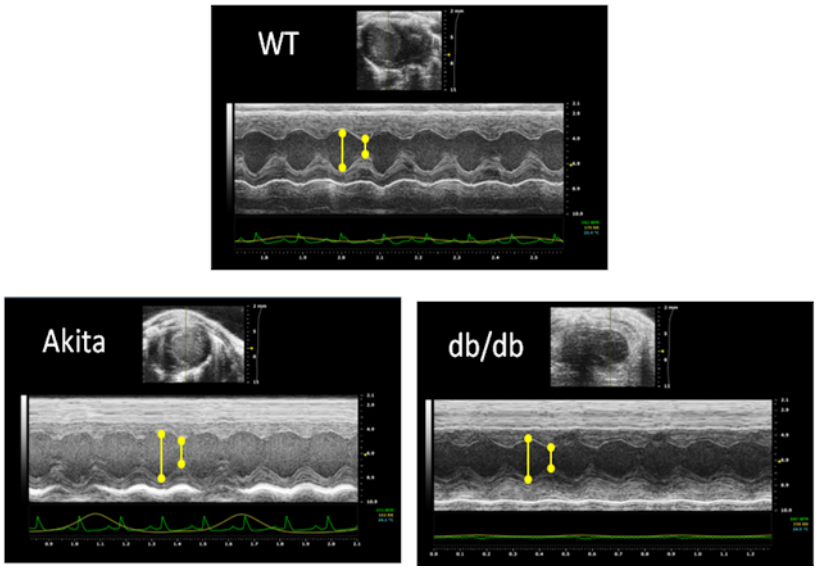

Fig. 3b

Fig. 3 : Cardiovascular parameters

a) Blood pressure was significantly higher in Akita and db/db mice as compared to that in WT mice.

b) M-mode echocardiography showing left ventricular diameter in all groups of mice during systole as well as diastole. In Akita and db/db mice, left ventricular diameter is significantly increased as compared to WT.

c) Left ventricle ejection fraction is significantly less in Akita and db/db mice as compared to WT mice.

Cognitive impairment was observed in both types of diabetes.

Discrimination index is impaired in Akita mice (p=0.05) as well as db/db mice (p=0.05) as compared to WT mice (Fig. 4). As in the case of the cardiovascular dysfunction, cognitive impairment is comparable in both types of diabetic mice (p=ns).

Auditory Brainstem Response was more impaired in type 2 diabetics than in type 1 diabetics.

ABR threshold was significantly higher in Akita mice as compared to WT mice (p=0.01). It was interesting to note that in the db/db mice it was significantly higher than in the WT (p=0.0004) as well as Akita (p=0.05) mice (Fig. 5).

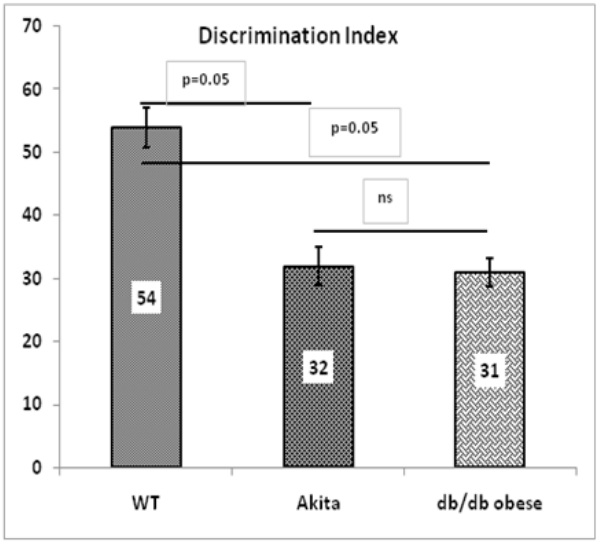

Fig. 4 : Discrimination index is significantly decreased in Akita and db/db as compared to WT, indicating cognitive impairment.

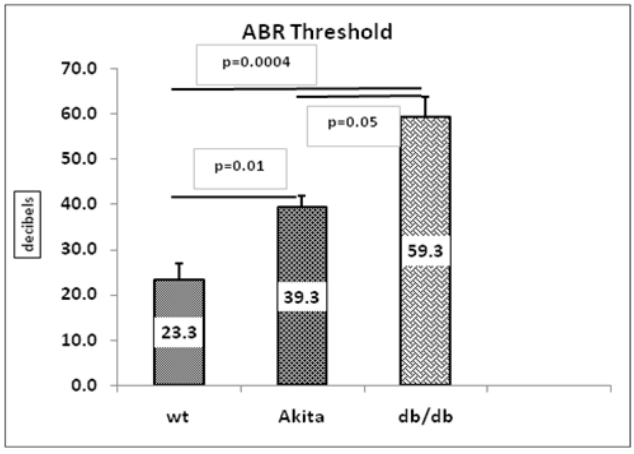

Fig. 5 : ABR threshold is significantly increased in both groups of diabetic mice as compared to the controls. Also, this threshold is significantly higher in db/db mice as compared to Akita and WT mice.

TABLE I : Spearman's correlation studies reveal a significant correlation of blood glucose levels with blood pressure, left ventricular ejection fraction, discrimination index and ABR threshold. There is no significant correlation of blood glucose with body weight.

Correlation of blood glucose level with	*Spearman's correlation 'r'*	*Significance 'p'*
Body weight	0.061	0.839
Blood pressure	0.678	0.017*
Left ventricle ejection fraction	–0.897	0.0001**
Discrimination index	–0.706	0.012*
ABR threshold	0.748	0.007**

*p<0.05 significant; **p<0.005 highly significant.

Spearman's correlation studies are detailed in Table I.

Discussion

In type 1 diabetes mellitus (DM), the basic pathology stems from glutamic acid decarboxylase autoantibodies (GADA), or islet cell autoantibodies (ICA), or insulinoma-associated autoantibodies (IA), or zinc transporter autoantibody (ZnT8), all leading to diminished or absent insulin production and consequent hyperglycemia (14). Type 2 DM results from a peripheral resistance to insulin or a relative insulin deficiency with consequent hyperglycemia. It is also associated with obesity which, in itself, is a risk factor for cardiovascular disease. Since the basic causative mechanisms are different in the 2 types of diabetes and body weight also differs, one would expect differential expression of pathological states, but this was not evidenced in the current study.

Our results indicate that macrovascular functions like left ventricular ejection fraction and cognition are affected by the high glucose concentrations seen in Akita and db/db mice; blood pressure (also a macrovascular function) is raised in both types of diabetic mice, the insulin-deficient Akita mice as well as the obese type 2 diabetic db/db mice. Cochlear function (as denoted by ABR threshold), which is a microvascular function, is significantly compromised in db/db and Akita mice as compared to WT, though db/db mice have greater impairment of hearing than Akita mice and WT.

The Spearman's studies reveal that microvascular as well as macrovascular functional deficits correlate with the blood glucose levels indicating that these deficits depend on the severity of hyperglycemia. Since the duration of hyperglycemia in both types of diabetic mice (Akita and db/db) is different but the macrovascular functions are not significantly different, these cannot be attributed to the duration of hyperglycemia. Also, since the extent of hyperglycemia was not significantly different in the two groups of diabetic mice, the similar deficits in cardiovascular and cognitive functions could be attributes of the hyperglycemia per se.

The decreased ejection fraction seen in the diabetic mice as compared to their controls could be due to decreased vasculature of the heart as demonstrated by us in an earlier study where we demonstrated differential vascular density in different models of mice (including diabetic mice) in various tissues including the heart (15). Ehl et al, in their study on over 2500 diabetics and non-diabetics, concluded that diabetics exhibited a lower LVEF than non-diabetics, irrespective of the presence or extent of CAD (16). This would indicate separate mechanisms affecting blood pressure and coronary arteries, as indicated by Dokken et al (9). Despite the different mechanisms of hyperglycemia in type 1 and type 2 DM, the glycemic status is similar; hence pathologies due to hyperglycemia, too, are similar. This would account for the similar impairment of cardiovascular functions we observed in both types of DM.

Though these diabetic mice (type 1 as well as type 2) are young adults and have had hyperglycemia for just a few weeks, they are already showing impairment of several organ systems.

Streptozotocin-induced type 1 diabetes has also been associated with cognitive decline, in terms of learning and memory impairment, which has been shown to correlate with oxidative stress, hyperglycemia, hypoinsulinemia and decreased number of hippocampal neurons seen in diabetics (17). Type 2 diabetes has also been associated with cortical atrophy similar to preclinical Alzheimer's disease (7). This would account for the decreased cognitive abilities we observed in the Akita and db/db mice. Since cognitive abilities are similar in type 1 and type 2 DM mice, it may be presumed that similar mechanisms are involved in the

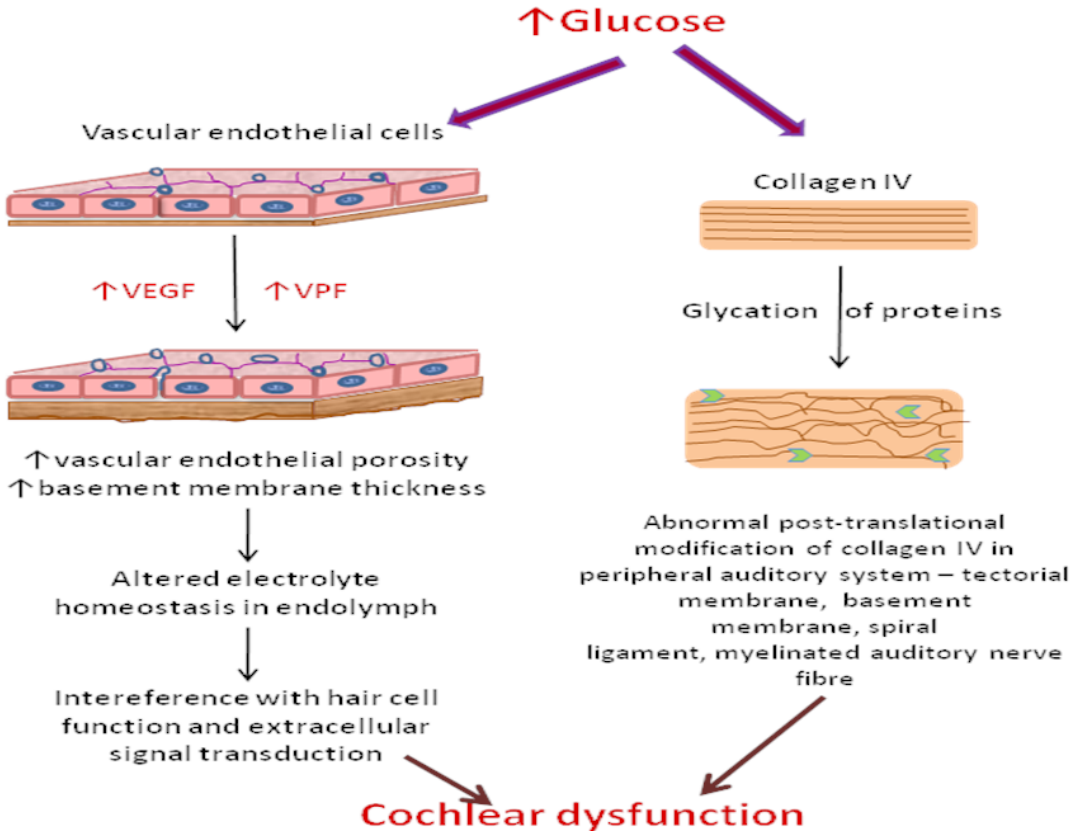

Fig. 6 : **Mechanism of hyperglycemia-induced cochlear dysfunction:** Hyperglycemia results in an accumulation of advanced glycation products which cause an upregulation of vascular endothelial growth factor (VEGF) and vascular porosity factor (VPF). The increased VEGF results in increased endothelial basement membrane thickness, and the elevated VPF results in increased porosity of the basement membrane. These events cause an alteration in the electrolyte homeostasis of the endolymph and, thereby, interference in hair cell function and extracellular signal transduction. Another concurrent mechanism is through the post-translational modification of collagen IV which is a structural protein of the peripheral auditory system that includes the tectorial membrane, basement membrane, spiral ligament and myelinated auditory nerve fibre. This leads to altered conduction through these components of the auditory system. Both these mechanisms have been demonstrated.

modification of cerebral functions in both these situations.

Even though ABR threshold is significantly different in the 2 types of DM, it is not dependent on duration of hyperglycemia as the deficit is more severe in type 2 DM in which hyperglycemia is of a shorter duration than in type 1 DM.

The impaired hearing in both types of diabetic mice as compared to WT mice could probably be accounted for by the expected remodeling of vasculature due to hyperglycemia. Figure 6 shows that hyperglycemia, through a cascade of events, leads to cochlear dysfunction in terms of signal transduction. It has been demonstrated that advanced glycation end-products (AGE) get deposited in collagen especially collagen type IV, which is found in many areas of the peripheral auditory system (e.g. tectorial membrane, basement membrane of striae vascularis, myelinated auditory nerve fibre, spiral ligament, scala media). These AGE deposits result in abnormal post-translational protein modifications causing increased thickness of the basement membrane of the stria vascularis and also increased porosity of their endothelium. The resultant increased permeability of their endothelium alters the electrolyte homeostasis in the endolymph causing interference in hair cell transduction and signal transmission (18, 19).

This, however, would not explain the more severe hearing loss we observed in our db/db obese mice as compared to Akita mice. A possible mechanism that could contribute to the greater hearing loss in db/db obese mice is altered flow mechanics in the small stria vascularis due to increased viscosity, maybe due to higher concentration of lipids in the blood (20). In addition, Miller et al demonstrated structural changes in the cochlea of db/db mice in the form of decreased neuron density in the spiral ganglion, edema of the stria vascularis and endothelial remodeling in the strial vessels which they suggested could account for the severe hearing impairment that they observed in this model of mice (21). Hence the hearing loss in db/db mice is multifactorial.

Yet another mechanism of pathology in db/db mice could be proposed on basis of the observations of Horner et al. They demonstrated increased leptin receptors in the bony labyrinth and described the peripheral role of leptin in osteogenesis. Normal osteoblasts express NF-kB ligand (RANKL) on their cell surface and leptin regulates it by increasing its expression. This binds to its receptor on osteoclast precursors and promotes their maturation, thus maintaining the balance between osteoblastic and osteoclastic activity in normal bone formation (22, 23). Hence, in the db/db mice, the decreased expression of leptin receptors could cause decreased expression of RANKL and consequent decrease in osteoclastic maturation, leading to thickening of the labyrinth. The end-result would be an increased threshold of hearing.

But do other models of type 2 diabetes also exhibit more hearing loss than type 1 diabetics? Such a phenomenon of greater degree of hearing loss in type 2 diabetics (high fat diet) as compared to type 1 diabetics (streptozotocin-induced) was also observed by Vasilyeva et al in a different model (CBA/CaJ) of mice (20). Hence it may be presumed that there is an additional mechanism of hearing loss in type 2 diabetes. Demonstrating this would require additional experiments including various models of type 2 DM.

Conclusion

All parameters of functional deficit (cardiac, cognitive and auditory) are strongly associated to the level of hyperglycemia. This would confirm the major role of severity of hyperglycemia per se as a risk factor for these complications of diabetes. At the same time, no correlation was observed between duration of DM and the severity of complications.

ADA, in its 2013 guidelines, mentions estimation of glycosylated hemoglobin and LDL-cholesterol, and measurement of blood pressure as screening tools for cardiovascular complications (24). It is suggested that measurement of LVEF may be evaluated as a screening tool to enhance the screening process and detect early signs of cardiovascular complications.

Also, assessment of auditory functions should be added to the screening routine of DM.

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Disclosure

None of the authors have any potential conflicts of interest, including specific financial interests and relationships and affiliations (other than those affiliations already listed in the title page) relevant to the subject of this manuscript.

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