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Research paper

# Interactions of hearing loss and diabetes mellitus in the middle age CBA/CaJ mouse model of presbycusis

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ABSTRACT

Recently, we characterized the more severe nature of hearing loss in aged Type 2 diabetic human subjects [Frisina, S.T., Mapes, F., Kim, S., Frisina, D.R., Frisina, R.D., 2006. Characterization of hearing loss in aged type II diabetics. Hear. Res. 211, 103–113]. The current study prospectively assessed hearing abilities in middle age CBA/CaJ mice with Type 1 diabetes mellitus (T1DM) (STZ injection) or Type 2 diabetes mellitus (T2DM) (high fat diet), for a period of 6 months. Blood glucose, body weight and auditory tests (Auditory Brainstem Response-ABR, Distortion Product Otoacoustic Emissions-DPOAE) were evaluated at baseline and every 2 months. Tone and broad-band noise-burst responses in the inferior colliculus were obtained at 6 months. Body weights of controls did not change over 6 months (~32 g), but there was a significant (~5 g) decline in the T1DM, while T2DM exhibited ~10 g weight gain. Blood glucose levels significantly increased: 3-fold for T1DM, 1.3-fold for T2DM; with no significant changes in controls. ABR threshold elevations were found for both types of diabetes, but were most pronounced in the T2DM, starting as early as 2 months after induction of diabetes. A decline of mean DPOAE amplitudes was observed in both diabetic groups at high frequencies, and for the T2DM at low frequencies. In contrast to ABR thresholds, tone and noise thresholds in the inferior colliculus were lower for both diabetic groups. Induction of diabetes in middle-aged CBA/CaJ mice promotes amplification of age-related peripheral hearing loss which makes it a suitable model for studying the interaction of age-related hearing loss and diabetes. On the other hand, initial results of effects from very high blood glucose level (T1DM) on the auditory midbrain showed disruption of central inhibition, increased response synchrony or enhanced excitation in the inferior colliculus.



## 1. Introduction

Diabetes mellitus (DM) is a prevalent metabolic disease of middle age and older adults worldwide (about 7.0% and rising). There are two major types of diabetes: Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM). It is estimated that 5–10% of Americans who are diagnosed with diabetes have T1DM. Most common is T2DM, affecting more than 20% of people over age 60 (American Diabetes Association, 2006). Both types of DM can cause serious health complications that involve multiple organs and physiological systems. Many T2DM complications in older people are associated with natural aging, but they appear earlier in diabetic patients (Biessels et al., 2002).

Studies that have attempted to characterize hearing loss in diabetics show inconsistent results (for reviews see Tay et al., 1995; Fowler and Jones, 1999; Maia and Campos, 2005). Specifically, some studies find bilateral hearing loss at high frequencies for children with T1DM (Elamin et al., 2005), while others did not find hearing loss (Dalton et al., 1998; de Espana et al., 1995), or found hearing loss in middle age and older T2DM patients (Rózańska-Kudelska et al., 2002; Diaz de Leon-Morales et al., 2005; Sakuta et al., 2007). However, Tay et al. (1995) showed hearing impairment at low and middle frequencies in diabetics. In contrast, Salvinelli et al. (2004) found no effect on hearing for either type of diabetes,

*Abbreviations:* ANOVA, analysis of variance; ABR, auditory brainstem responses; BW, body weight; DPOAEs, distortion product otoacoustic emissions; DM, diabetes mellitus; EEG, electro-encephalography; GM, geometric mean; HINT, hearing-in-noise test; IC, inferior colliculus; IHC, inner hair cells; IP, intraperitoneal injection; NFAEP, near field auditory evoked potential; OAEs, otoacoustic emissions; OHC, outer hair cells; RM, repeated measures; RMS, Root mean square voltage; IAC, soundproof acoustic chamber; SPL, sound pressure level; SPF, specific pathogen free; STZ, streptozotocin; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus

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and evoked otoacoustic emissions (OAEs) were not affected at most frequencies in T2DM subjects (Di Leo et al., 1997; Erdem et al., 2003). However, Sasso et al. (1999) reported a significant drop in OAE amplitudes for diabetic subjects. To evaluate brainstem auditory function, auditory brainstem response (ABR) latencies were used (Di Leo et al., 1997; Sasso et al., 1999), to show prolonged activation of central auditory pathways. Except for the ABR latency experiments, these human studies used simple, limited auditory measures that evaluated peripheral hearing.

Recently, we showed a significant effect on both peripheral and central hearing functions in older T2DM subjects by using a comprehensive test battery (Frisina et al., 2006). Peripheral hearing function was assayed by pure tones, otoacoustic emissions (transient and distortion product), and speech thresholds, revealing more significant losses in the T2DM group compared to age-matched controls. For the first time, effects of T2DM on hearing tests involving central auditory processing in aged human subjects were evaluated by measuring hearing in-noise (HINT) and supra-threshold gap detection thresholds. As for the inner ear findings, the T2DM group showed significantly worse performance on these tests compared to age-matched non-diabetic controls, suggesting that the CNS is susceptible to the damaging effects of the diabetes conditions.

There are several animal models of diabetes: animals that develop diabetes by specific experimental procedures and animals that develop diabetes spontaneously, owing to a genetic predisposition (Mordes and Rossini, 1981; Shafrir, 1997). Streptozotocin (STZ) induced diabetes is a T1DM model and is widely used for longer (6 months) duration studies of diabetes. For example, this T1DM model has been used to evaluate alteration of auditory function with ABRs. Threshold elevations and first-peak latency prolongation appeared in rodents (Biessels et al., 2001; Manschot et al., 2003; Biessels et al., 2005). Also, significant prolongation of auditory evoked potentials was reported for STZ-treated rats by 3 months of diabetes duration, suggesting changes in central auditory regions (Biessels et al., 1999). In genetically predisposed young adult rats (T2DM, 13 months of age) evaluation of auditory function showed elevation of ABR thresholds (Ishikawa et al., 1995) as compared to non-susceptible strains. A main limitation of these previous studies that attempted to identify the effect of diabetes on hearing function was that hearing ability of the rodent strains were not well characterized initially, making it difficult to identify effects of hearing impairment, diabetes, and chemical side effects (those induced by streptozotocin, alloxan).

The necessity of utilizing an appropriate mouse model for studying effects of diabetes (prevalent in older populations) on age-related hearing loss is obvious from scientific and clinical perspectives. The most commonly used animal model of T1DM, STZ injection, may interfere with metabolism of inner ear hair cells as well as with auditory neurons in the brain. Another, important aspect of evaluating mouse models of diabetes is selection of an appropriate mouse strain to avoid genetic effects on presbycusis. For example, C57Bl/6 and DBA strains of mice have the ahl allele, inducing a rapid, high-frequency hearing loss (Willott and Carlson, 1995; Willott et al., 2001), making them unsuitable for studies in middle age and old animals of the interaction of diabetes and presbycusis. Much evidence suggests that the CBA/CaJ serves as an excellent model for many cases of human presbycusis since it shows hearing loss that progresses on a time frame similar to human's, when one corrects for the different absolute lifespans of mice and men. The age-related hearing loss of the CBA/CaJ strain most likely corresponds to the sensory-neural type of human presbycusis (high-tone frequency loss with changes in central auditory regions) (e.g., Spongr et al., 1997; Frisina et al., 1998; Parham et al., 1999; Willott et al., 2001; Jacobson et al. 2003).

Anatomical analysis of the cochlea has shown stria vascularis degeneration and outer hair cell (OHC) loss for both types of diabetes in humans (Fukushima et al. 2005, 2006). However, studies on diabetic animals reveal mixed results. For example, sometimes examination of diabetic animal models has found loss of OHCs (Nakae and Tachibana, 1986; Rust et al., 1992; Triana et al., 1991; Raynor et al., 1995) and inner hair cells (IHC) (Nakae and Tachibana, 1986), as well as changes in intermediate (Nakae and Tachibana, 1986; Ishikawa et al., 1995) and marginal cells of the stria vascularis. Some authors reported degeneration of spiral ganglion cells (Raynor et al., 1995; Ishikawa et al., 1995) and thickening of the basement-membranes of capillaries (Smith et al., 1995). Others did not find any of these associations in diabetic animal models (Nageris et al., 1998).

The aim of the present investigation was to induce two types of diabetes in middle age CBA/CaJ mice and evaluate effects on age-related hearing loss using different hearing tests. The effect of a long duration (6 months) metabolic stress on the progression and time course of age-related hearing loss – presbycusis, was characterized by hearing assessments for both peripheral (cochlea) and central (inferior colliculus – IC) portions of the auditory pathway. Age-matched non-diabetic control mice were utilized for comparison with hyperglycemic T1DM mice (STZ induced) and for T2DM (high fat diet) to maximize effects for future studies of possible anatomical, genetic and neurochemical mechanisms of diabetes on hearing ability with age.

## 2. Methods

### 2.1. Animals

Twelve month old CBA/CaJ males mice were used ($N = 36$). The breeding pairs were purchased from Jackson Laboratories and subjects of the present study were bred and housed at the University of Rochester. Animals were kept under specific pathogen free (SPF) conditions in 12-h light/dark cycle with chow and water ad libitum in a low noise environment. All procedures were approved by the University of Rochester Animal Care Committee.

### 2.2. Experimental design

Male mice were randomly divided into the 3 experimental groups: control, $n$=14, T1DM, $n = 11$, and T2DM, $n = 11$. Females were excluded from this study based on estrogen-related decreases in susceptibility to diet-induced obesity (Wade et al., 1985; Zhang et al., 2005). T1DM was induced by a single intraperitoneal injection (IP) of streptozotocin (STZ, at 200 mg/kg of body weight; Sigma Chemical, St. Louis, MO) dissolved in 0.1 M citric buffer (pH 4.5) (Paik et al., 1980). T2DM was induced with a high fat, high simple carbohydrate, low fiber diet (Diet #5800-B, Test Diet, Richmond, IN) (Surwit et al., 1988). Controls were injected with citrate buffer and fed standard Purina rodent chow and water ad libitum. There was about a 20% mortality rate due to late middle age in the T2DM group, and about 50% in the control and T1DM groups. The rate of mortality went even higher during survival surgery prior to the IC recording due to age, obesity, hyperglycemia, and hyperlipidemia in T2DM (5 mice) and T1DM (2 mice). Whereas the control mice all survived the IC surgery. Data from the mice that died were not included in the longitudinal results or statistics, e.g., for body weights, glucose levels, ABRs, DPOAEs. For cross-sectional IC recordings, the total number of animals was fewer.

### 2.3. Blood glucose levels evaluation

Mice fasted for 4 h before the blood glucose test. Blood samples were obtained by puncture of the tail vein, and blood glucose level