Sensorineural hearing loss as a complication of type 2 diabetes mellitus: evidence of several cellular & neural impairments

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Objectives

While retinopathy, nephropathy, and peripheral neuropathy are well-established complications of type 2 diabetes (T2D), **sensorineural deafness** has not yet been considered as a **comorbidity factor** of **diabetes**.

In current clinical practice in France, regular auditory monitoring is not part of the management of diabetic patients, and relatively limited clinical data are available regarding the effects of the patient's diabetes duration and glycemic control on hearing.

Studies suggest that 60% of type 2 diabetes patients aged between 30 and 60 experience some degree of **hearing loss**, with **50% exhibiting moderate to severe impairments** (Al-Rubeaan K. et al., 2021). Various factors including cochlear microangiopathy, chronic systemic inflammation, or auditory peripheral neuropathy (synaptopathy) are proposed as potential contributors to this silent epidemic, leading to early sensorineural deafness.

The development of pharmacological therapies for addressing diabetes-induced hearing loss would significantly benefit from **reliable animal models** representing inner ear damage caused by uncontrolled diabetes.

In this context, we have **characterized auditory functions using an uncontrolled type 2 diabetic murine model**, concurrently evaluating **markers** indicative of this pathology.

Methods

Genetically modified mice (BKS(D)-Lepr^{db}/JOrlRj; Janvier Labs), both homozygous (db/db) or heterozygous (db/+) for the mutated allele on the gene encoding the leptin receptor, were monitored from 5 to 13 weeks of age (n=12/13 per phenotype) for **hearing** and **diabetes biomarkers**.

The study aimed to evaluate diabetes-related **blood markers** (glucose, glycated hemoglobin, biochemical analyses: Urea and Albumin) alongside **auditory acuity** through Auditory Brainstem Response (ABR) and Distortion-Product Otoacoustic Emission (DPOAE) measures (**Figure 1**). Separate cohorts of mice were sacrificed at the age of 11 and 13 weeks to conduct histological analyses of the cochlea.

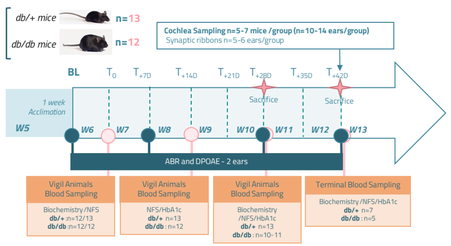


Figure 1. Study schedule to phenotype hearing and diabetes-related blood markers in a mouse model of type 2 diabetes (db/db) comparatively to their littermate control mice (db/+)

Results

Lepr^{db} double mutation induced type 2 diabetes (T2D) phenotype

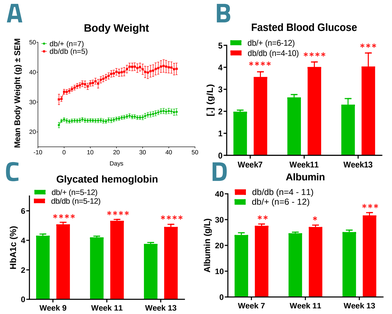


Figure 2. Changes in type 2 diabetes markers in db/db and db/+ mice
Body weight (**A**) was noted from 7 and 13 weeks of age at 7, 11, and 13 weeks of age; concentration of blood glucose (**B**), glycated hemoglobin (HbA1c) and albumin were measured on several fasted mice at different ages (7, 9, 11, and 13 weeks old).
Statistical analysis with One-way ANOVA, Multiple comparisons.

To phenotype T2D, body weight, glucose blood levels, HbA1C and albumin were studied from 7 to 13 weeks of age. All parameters were higher in db/db mice at the age of 7 weeks. Body weight, concentrations of glucose and albumin continued to progessively increase over time, whereas HbA1c remained at a stable, high level.

db/+ mice exhibited a higher blood glucose concentration than expected, based on breeder data.

All these data confirmed the diabetic (hyperglycemia & elevated HbA1c levels) and obese phenotype of the db/db mice, confirming breeder data.

Uncontrolled diabetes was associated with progressive deafness

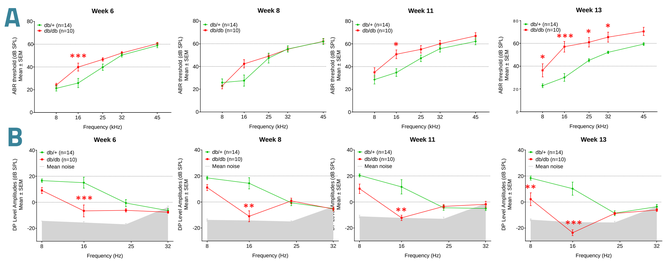


Figure 3. Hearing of db/db and db/+ mice from 6 to 13 weeks of age
ABR (**A**) and DPOAE (**B**) were measured on db/db and db/+ mice at the age of 6, 8, 11, and 13 weeks. For each group, the data corresponded to the average of left and right ear values. Statistical analysis with Two-tailed Dunnett's test.

The db/db mice exhibited an increase in ABR thresholds and a decrease in DPOAE amplitudes, mainly at 16 kHz as early as 6 weeks, indicating **early-onset hearing loss**. With age, a significant progression of ABR & DPOAE impairment was noted, indicating a more pronounced deterioration of hearing, particularly in mid-range frequencies (8 - 25 kHz). The increase in ABR thresholds and decrease in DPOAE amplitudes could suggest a loss of functionality in hair cells (HC) or Stria vascularis. The db/+ mice did not exhibit control values for both ABR & DPOAE, and seemed to exhibit a trend of hearing loss, mainly characterized by very low DP amplitudes at the highest frequencies.

with cochlear synaptopathy ...

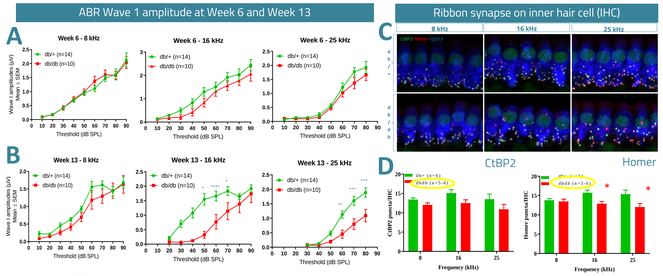


Figure 4. ABR Wave I amplitude and ribbon synapses in db/db and db/+ mice
ABR wave I amplitudes from 10 to 90 dB were evaluated at 6 (**A**) and 13 (**B**) weeks of age at 8, 16, and 25 kHz in db/db and db/+ mice. Statical analysis by Two-way ANOVA. For db/db and db/+ mice at 13 weeks of age at 8, 16 and 25 kHz: (**C**) Representative images of IHC sections immunostained with CtBP2, Homer and Vglut3; (**D**) Number of CtBP2 and Homer punctae per IHC Statical analysis by Two-way ANOVA.

At 6 weeks of age, no differences in wave I amplitudes were observed between db/db and db/+ mice. At 13 weeks of age, at 16 and 25 kHz, db/db mice presented a significant reduction in wave I amplitudes and in postsynaptic connections (Homer) on the IHC, demonstrating cochlear synaptopathy in diabetic mice .

... and cochlear cell loss

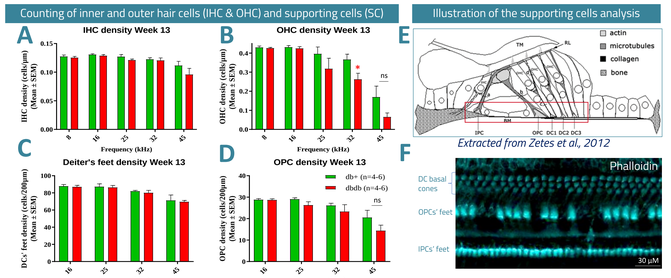
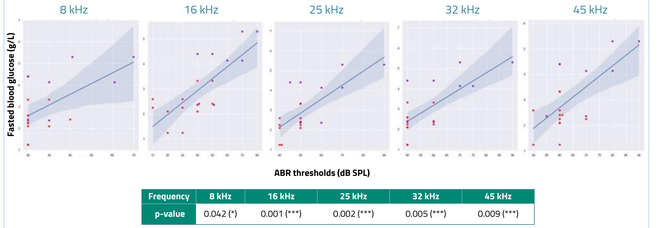


Figure 5. Cochlear cells in db/db and db/+ mice at the age of 13 weeks old
The cochlear cells were counted: IHC (**A**) and OHC (**B**) at 8, 16, 25, 32, and 45 kHz and supporting cells (SC) - Deiters (DC) (**C**) and Outer Pillar Cells (OPC) (**D**) at 16, 25, 32, and 45 kHz on cochleae of 13 weeks old db/db and db/+ mice.
T Test analysis. Diagrammatic cross section of the organ of corti (**E**) illustrating the main cochlear cells. Representative image of a cochlea flat surface preparation immunostained with antibody against phalloidin to highlight SC.

At 13 weeks of age, in db/db mice, cochleograms revealed an OHC loss from 25 kHz, while IHCs remained intact at all analyzed frequencies (8-45 kHz). Regarding SC, a lower density of OPC was observed in diabetic mice at the highest frequencies, corresponding to the base of the cochlea, whereas Deiters' feet and Inner pillars' feet (not illustrated) were all present along the cochleae.

Auditory threshold elevation correlated with blood glucose elevation



Frequency	8 kHz	16 kHz	25 kHz	32 kHz	45 kHz
p-value	0.042 (*)	0.001 (***)	0.002 (***)	0.005 (***)	0.009 (***)

Figure 6. Correlation analyses between hearing acuity and glycemia measured in db/db and db/+ mice
A pearson correlation test was conducted between the concentration of blood glucose in fasted mice and their hearing acuity, characterized by ABR thresholds at different frequencies from 8 to 45 kHz, for 13 weeks old db/db and db/+ mice.

At 13 weeks of age, when hearing impairment was significantly marked from 8 to 45 kHz in diabetic mice compared to heterozygous control mice, a significant and positive correlation was established between the level of blood glucose and ABR thresholds at all tested frequencies (8-45 kHz).
These data clearly demonstrated the correlation of higher glucose concentrations with higher ABR thresholds, suggesting that elevated blood glucose levels are related to a more severe degree of hearing loss, emphasizing the importance of monitoring hearing in diabetic subjects with poorly controlled blood glucose levels.

Conclusion

The db/db (BKS(D)-Lepr^{db}/JOrlRj) mice demonstrated all the main **biomarkers of diabetes**, mainly **obesity** and **hyperglycemia** concommitantly to high blood levels of **HbA1c** and **albumin**, confirming their use as a T2D model. These mice also rapidly presented (as early as 6 weeks of age) **auditory deficits**. Impairment of functional measures characterized this hearing loss: **elevation of ABR thresholds and reduction of DPOAE and ABR wave I amplitudes.** These impairments were progressively aggravated as the mice **aged**. By 11 to 16 weeks of age, diverse **cochlear damage** emerged, including loss of (i) **outer hair cells**, (ii) **synapses** between inner hair cells and auditory nerve fibers, and (iii) **supporting cells**, **outer pillar cells**. This cellular loss lead to early sensorineural deafness, materialized by increased auditory thresholds and diminished auditory nerve sound encoding capacities.

The presented outcomes align with various scientific articles reporting deafness in diabetic mice (Lyu, A.-R. et al. 2021; Lee, Y. Y. et al 2020).

Although the precise cause of this damage remains elusive, the severity of deafness observed in type 2 diabetic animals was significantly correlated with fasting blood glucose levels, suggesting a **link between hyperglycemia and hearing loss.**

This model appears suitable for studying diabetes with insulin resistance and obesity-related hearing impairments, and holds promise for evaluating the **potential effects of specific pharmacological compounds** (oral hypoglycemics, anti-inflammatories, etc.) developped for hearing loss.

The reported findings advocate for a **global raise in awareness** around the **risk of auditory deficits in type 2 diabetic patients**, especially those with inadequate glycemic control, and highlight the importance of implementing **regular hearing assessments in this patient population.**