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Hyperinsulinemia/Diabetes, Hearing, and Aging in the University of Wisconsin Calorie Restriction Monkeys

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Abstract

The purpose of this study was to determine the effects of hyperinsulinemia/Type 2 diabetes mellitus (HI-T2DM) on hearing impairment using rhesus monkeys to obtain control over diet and lifestyle factors that confound human studies. The study is a retrospective evaluation of rhesus monkeys from the Wisconsin National Primate Research Center (WNPRC) study on caloric restriction and aging. The research questions were the following: 1. Is HI-T2DM related to hearing impairment? 2. If so, what is the site of lesion in the auditory system? and 3. What physiological factors affect the risk of hearing loss in HI-T2DM? Three groups of eight monkeys each were matched by sex and age; the caloric restricted (CR) monkeys had a reduced risk of diabetes, the normal control (NL) group had a normal risk, and the hyperinsulinemia/diabetes (HI-D) group had already developed HI-T2DM. Auditory testing included distortion product otoacoustic emissions (DPOAEs) with f2 frequencies from 2211–8837 Hz and auditory brainstem responses (ABRs) obtained with clicks and tone bursts (8, 16, and 32 kHz). DPOAEs had signal-to-noise ratios 8–17

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dB larger in the NL group than in the HID and CR groups, signifying that cochlear function was best in the NL group. ABR thresholds were 5–8 dB better in the NL group than in the HI-D group, although no significant differences across the groups were evident for the thresholds, latencies, interwave intervals, or amplitudes. Correlations were significant for quadratic relations between body mass index (BMI) and DPOAE, with largest DPOAEs for animals in the middle of the BMI range. ABR thresholds elicited with 16 and 32 kHz signals were significantly correlated, positively with BMI and HbA1c, and negatively with K_G (glucose tolerance), S_I (insulin sensitivity index) and DI (disposition index). These findings suggest that the hearing loss associated with HI-T2DM is predominantly cochlear, and auditory structures underlying the higher frequencies are at risk with HI-T2DM. Loss of auditory function begins in the hyperinsulinemia, pre-diabetic state.

Keywords

Age-related hearing loss; Auditory evoked potentials; Caloric restriction; Distortion product otoacoustic emissions; Hearing; Rhesus macaque; Hyperinsulinemia; Type 2 diabetes

1. Introduction

Diabetes affects approximately 29.1 million people in the United States, and of these, approximately 8.1 million people are undiagnosed (Centers for Disease Control, 2014). Although diabetes is the seventh leading cause of death in the United States, it may be under-reported as the direct or contributing cause of death. The highest rates of diabetes are in the older population as well as in racial and ethnic minorities of all ages (Cowie et al., 2009; Mau et al., 2010; Tucker et al., 2010). Although numerous physiological complications of diabetes are recognized by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, 2015), hearing impairment is not among them. People often do not recognize their own hearing impairment because of its insidious onset, thereby making hearing impairment often an undiagnosed and untreated disorder. The best way to preserve health and quality of life for people with diabetes is to recognize and treat all its complications. The current study focuses on the issue of hearing impairment in hyperinsulinemia or type 2 diabetes (HI-T2DM), which currently is a poorly described complication.

Variable results among studies of diabetes and hearing impairment with human participants have indicated that increased hearing loss occurs in older people with diabetes, younger people with diabetes, people with nephropathy, people who are insulin dependent, and people who are not insulin dependent. Some studies were not able to confirm or identify the type of diabetes in their subjects or match subject groups carefully (e.g., Bainbridge et al., 2008; Dalton et al., 1998). Some studies have reported that Types 1 and 2 diabetes vary in their physiological characteristics (e.g., Durmas et al., 2004). Studies of hearing and diabetes may have used heterogeneous diabetic populations and have not characterized diabetes well in their subjects or its absence in normal controls. Hence, the link between diabetes and hearing impairment remains elusive.

Confounding factors in human studies of diabetes and hearing impairment include individual lifestyle differences among subjects, such as degree of diabetic control, noise exposure, and diet, that cannot be controlled in humans, but do pose independent risks for hearing loss. Other risk factors for health and auditory function, such as cardiovascular disease (Rosen et al., 1965), weight and abdominal fat (Hwang et al., 2009), and hyperglycemia (Jacobs et al., 2012) are avoided with caloric restriction (Colman et al., 2009). Modifiable risk factors, however, can be minimized with an animal model and diseases can be adequately treated, which may lead to more consistent evidence concerning the relations between diabetes and auditory function.

Rhesus monkeys provide a model for human aging and age-associated diseases such as diabetes (Hansen and Bodkin, 1986; Hansen, 1989). The monkeys acquire diabetes spontaneously, and can be monitored closely to obtain accurate data concerning the physiological changes as they progress through diabetes (Hansen and Bodkin, 1986). Rhesus monkeys also provide a model for age-related hearing loss (ARHL). Auditory function in the monkeys is characterized by tympanograms (middle ear), otoacoustic emissions (cochlea), and auditory brainstem responses [auditory thresholds and brainstem neural pathways (e.g., Fowler, et al., 2010; Fowler et al., 2008, Fowler et al., 1999; Lasky, et al., 2000; Torre and Fowler, 2000)]. Hence, the rhesus monkey is an efficient model for studying possible effects of HI-T2DM diabetes on auditory function.

The purpose of the current study was to increase understanding of the effects of HI-T2DM on hearing impairment. The research questions were the following: 1. Is HI-T2DM related to hearing impairment? 2. If so, what are the site/sites of lesion in the auditory system? 3. What physiological factors affect the risk of hearing loss in HI-T2DM? Rhesus monkeys served as the model for aging, HI-T2DM, and hearing impairment. Many of the risk factors for hearing loss in humans were controlled in these non-human primates as they live in the same environment and eat standardized diets.

2. Materials and methods

2.1. Subjects

The study was approved by the Graduate School Institutional Animal Care and Use Committee (IACUC). The study is a retrospective evaluation of rhesus monkeys from the Wisconsin National Primate Research Center (WNPRC) study on caloric restriction and aging (Colman et al., 2009; Kemnitz et al., 1994; Ramsey et al. 2000). Previous reports on auditory function in these monkeys have been published (Fowler et al., 2002; Fowler et al., 2008; Fowler et al., 2010). For the current study, subjects were drawn from the pool of 53 monkeys described in the Fowler et al. (2010) study. The pool included 25 control monkeys that ate ad libitum and 28 monkeys on diets restricted to approximately 70% of the caloric intake of the control monkeys, but with vitamin and mineral supplementation so that the groups were nutritionally matched apart from macronutrient and caloric intake. From this pool, three groups of monkeys were identified. The first group, Hyperinsulinemic-Diabetic (HI-D), comprised subjects that exhibited fasting hyperinsulinemia (>90th percentile) on at least two assessments, which persisted to the time of auditory testing and beyond or had progressed from hyperinsulinemia to glucose intolerance and/or fasting hyperglycemia.

Fasting hyperinsulinemia is readily documented and has proven to be a useful marker of risk for T2DM (Gresl et al., 2001). Group HI-D consisted of 8 monkeys that met these criteria, two of which were receiving insulin therapy. All of these monkeys were in the control group of the original study. The second group (NL) included 8 monkeys without symptoms of nascent T2DM and all were drawn from the original control group. The third group (CR) included 8 monkeys on caloric restriction. None of the monkeys on caloric restriction had developed diabetes. The three groups were matched for age (mean = 22 years, range ~18–26 years) and sex (5 males and 3 females).

2.2. Physical and physiological measures

The animals were weighed and body measurements were taken at 6-month intervals within the parent study. Fasting blood samples were collected to measure blood triglycerides, glucose, and insulin. Frequently sampled intravenous glucose tolerance tests (FSIGT) with minimal model analysis were used for calculation of insulin sensitivity (S_I ; the ability of insulin to promote uptake of glucose and reduce hepatic glucose output) and K_G (glucose tolerance, viz. log-linear rate of decline in plasma glucose concentration following glucose administration) and disposition index (DI; the product of S_I and the acute insulin response to glucose, an integrated indicator of the body's ability to handle a glucose challenge) (Kemnitz et al., 1994; Gresl et al., 2001). Other physiological characteristics, including fat, body weight, and cardiovascular indices, were also measured. For the current study, the physiologic values closest in time to the auditory measures were used. If the auditory tests were midway between two physiological assessments, the two results were averaged.

2.3. Auditory function

Auditory function was measured in the anesthetized monkeys using non-invasive procedures described in Fowler et al. (2010). The monkeys were anesthetized with ketamine and valium for the procedures. All monkeys had otoscopic examinations and tympanometry (Grason-Stadler Model 38 middle ear analyzer) to assure clear ear canals and absence of middle ear effusion. Tympanometry was obtained with a probe in the ear canal, and acoustic admittance was recorded across a pressure range of +400 to –600 daPa (described in Fowler et al., 2008). Monkeys were required to have single-peaked admittance tympanograms for a 226 Hz probe frequency, which signifies normal middle ear function (Torre et al., 2000).

Auditory testing included distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) (Intelligent Hearing Systems SmartOAE 4.31 USBz and SmartEP 3.62 USBz). For the DPOAEs, a soft probe assembly was placed in the ear canal and tone-burst stimuli (1–8 kHz) were presented. Responses were averaged over 64 trials to increase the signal (response)-to-noise ratios. For DPOAE measures, two primary frequencies (f_1 and f_2 , with $f_2 > f_1$) were used with the f_2/f_1 ratio fixed at 1.22. The levels of the primaries were held constant at $L_1=65$ dB and $L_2=55$ dB SPL. The levels of the distortion products of ($2f_1-f_2$) were measured for f_2 frequencies 2211, 3125, 4416, 6250, and 8837 Hz. The DPOAE signal to noise ratios (SNR) were also measured; these measures include the level of the DPOAE relative to the background noise at the same frequency.

ABRs were obtained using clicks and tone bursts (8, 16, and 32 kHz). Clicks and 8 kHz tone bursts were presented through insert earphones (Etymotic ER 3A) and tone bursts at 16 and 32 kHz were presented with high frequency transducers (IHS-3432). For the ABR, needle electrodes were attached at the brow ridge (positive input) and behind the pinnae (negative input) for 2 channel recordings. High level stimuli (100 dB pSPL) were presented to left and right ears individually to measure latencies and amplitudes of evoked potentials (waves 1, 2, and 4, which correspond to I, III, and V in humans) from the eighth nerve and brainstem to determine neural function (See Figure 1 for representative waveform with waves labeled). Neural function was determined by latencies of waves 1, 2, and 4, and interwave latency differences for waves 1–4. Binaural ABR thresholds were obtained, which provided the threshold of the better ear. Stimulus levels were reduced until all waves were eliminated; wave 4 characteristically produced the lowest thresholds. All responses were stored on the computer for later analysis.

2.4. Data analyses

Physiological data were taken every 6 months. Reported data are from the evaluation closest to the time of collection of the auditory data or two time points were averaged if the auditory assessment fell precisely between them. Because of the limited number of subjects, data reduction was required as described in the results. DPOAE and ABR data were evaluated with F-tests for the three groups of subjects, and t-tests were used to evaluate specific differences between the HI-D and NL groups. To determine the relationship of hearing impairment to physiological state, correlations were obtained. Significance was defined at the $p < 0.05$ levels.

3. Results

3.1. Physical/physiological characteristics

Physical and physiological data are shown in Table 1. Data [means and standard deviations (sd)] are divided by categories of tests (left column) for the three groups of monkeys--HI-D, NL, and CR. F values are the results of an F test for the three groups for each of the variables with the accompanying p values; t values are the results of the pairwise t-test and corresponding p values for the comparison of the results from the HI-D and NL groups. The differences for weight, BMI, and abdominal circumferences across the three groups were significant ($p < 0.05$) with the HI-D group having the highest values and the CR group having the lowest. Differences between the HI-D and NL groups, however, failed to reach significance.

Glycosylated hemoglobin (HbA1c), a measure of long-term blood glucose, shows significant differences among the three groups of monkeys and also specifically between the NL and HI-D groups. The highest values were reached by the HI-D group (13.8%). No significant differences among the means for the three groups were noted in the cardiovascular system.

The chemistry panel evaluated fasting glucose, cholesterol, and triglyceride levels. These three measures were highest in the HI-D group and lowest in the CR group. Of these

measures, only glucose level differences among the three groups reached significance, with differences specifically between the HI-D and NL group showing a trend ($p = 0.081$) for the higher levels in the HI-D group. Triglycerides, which had large mean differences in the three groups, failed to reach significance. A distribution analysis showed that the mean differences were biased toward excessively high values in a few monkeys, and the three highest triglyceride levels were obtained from monkeys with diabetes.

For the glucose tolerance test (GTT), significant differences among groups included Gb, Ib, S_I and DI. Only the Ib was significantly different between the HI-D and the NL groups, with the higher concentrations in the HI-D group. There was a trend for the S_I ($p = 0.069$) to be higher in the NL group than in the HI-D group. The lowest values for Ib and S_I , however, were in the CR group, which was not included in the post hoc statistics.

3.2. Auditory Characteristics

Auditory data are shown in Table 2. Data [means and standard deviations (sd)] are divided by categories of tests (left column) for the three groups of monkeys (HI-D, NL, and CR). F values are results of the F test for the three groups with the accompanying p values; t values are the results of the pairwise t test and corresponding p values for the comparison of the HI-D and NL groups. The auditory results are described in terms of cochlear function (DPOAE SNR), auditory sensitivity (ABR thresholds for clicks and high-frequency tone bursts), sensorineural function (click-evoked ABR latencies and amplitudes) and neural function (click-evoked ABR 1–4 interwave intervals). Overall, auditory function was poorest in the HI-D group and best in the NL group.

Figure 2 shows DPOAE SNRs in the right and left ears of the three groups of monkeys. Noise floor levels were not significantly different across groups. DPOAEs showed signal-to-noise ratios from 8–17 dB larger in the NL group than in the HI-D and CR groups, signifying best cochlear function in the NL group. For the average of f2 frequencies for the left and right ears, the differences between the HI-D and NL groups were significant (left ear, $p=0.037$; right ear, $p=0.013$), with the HI-D group having the smaller values. Specifically, significant differences were found for the f2 frequencies 2211 Hz in the left ear and 2211, 3125, and 4416 Hz in the right ear. All the other frequencies showed a trend ($p=0.051$ – 0.10) in the same direction, except for 6250 Hz in the left ear. The HI-D monkeys had the lowest SNR, which is associated with the poorest cochlear function.

ABRs were evaluated for thresholds and for neural transmission times in the three subject groups. Figure 1 shows the ABR waveforms of interest (waves 1, 2, and 4) labeled in a signal level function from 110 to 50 dB pSPL, and with the threshold at 60 dB pSPL. Wave 4 was typically identified at threshold. Figure 3 includes a graph of the ABR thresholds for the three subject groups with thresholds to the click and the individual high frequency tone bursts. ABR thresholds from clicks and tone bursts were 5–8 dB better in the NL group than in the HI-D group, and the CR group had intermediate thresholds consistently across all signals. Latencies and amplitudes were measured for the click-generated ABR waves (1, 2, and 4) and the interwave interval 1–4 was calculated. No significant differences across the groups were evident for any of the thresholds, latencies, interwave intervals, or amplitudes.

3.2. Auditory and Physiological Correlates

Auditory findings in the three groups of subjects were examined in order to identify any physiological correlates of decreased auditory function, which might suggest risk factors for hearing loss in the HI-T2DM populations. In the interest of data reduction for the analyses, only specific physical/physiological measures were used; the specific variables included the BMI, Hb1Ac, Gb, Ib, K_G , S_I , and DI. Because cardiovascular deficits are implicated as factors in auditory impairment, blood pressure and heart rate were also evaluated. All the measures of weight or body size were highly correlated; for example, weight and BMI had a linear correlation of 0.90. Only BMI was used to represent this category. All three groups of subjects were included in each analysis; the CR monkeys represented a group that had a reduced risk of diabetes, the NL group had a normal risk of diabetes, and the HI-D group had already developed persistent HI-T2DM. The assumption that auditory function was worse (higher ABR thresholds and lower DPOAE SNRs) with HI-T2DM justified the use of one-tailed tests. Correlations were considered significant at the $p < 0.05$ level.

BMI correlated significantly with the DPOAE SNRs. The correlations are shown in Figure 4, with the averaged DPOAE from all the f2 frequencies (2211- 8837 Hz) in the left ear (left panels) and in the right ear (right panels). Despite the variability in the data, the monkeys with the lowest (group CR) and highest (group HI-D) BMIs tended to have the lowest DPOAE SNRs, leaving the NL group in the middle of the range with the highest SNRs. One additional correlation, between I_b and the right ear DPOAE SNR at f2 = 4416 Hz, was disregarded because it was an isolated finding.

Physical/physiological factors that correlated significantly ($p < 0.05$) with ABR characteristics are shown Table 3. The most consistent pattern of correlations showed that higher (poorer) thresholds to ABR clicks and 16 kHz and 32 kHz tone bursts were significantly correlated with higher levels of BMI and HbA1c. ABR thresholds to 16 and 32 kHz tone bursts also were significantly and negatively correlated with increases in K_G , S_I , and DI. All correlations for the thresholds were linear. An example of the correlations is given in Figure 5, which shows 16 and 32 kHz thresholds (left and right panels, respectively) increasing with increases in BMI.

Supporting these ABR threshold findings are the correlations showing that the amplitudes of the click-evoked waves 4 (which identified threshold) for both ears were smaller with decreasing K_G , S_I , and DI. Significantly increased latency of click-evoked waves 4 in both ears accompanied the smaller amplitudes. The wave 1–4 latency interval in both ears was correlated only with K_G , such that the latency interval increased as glucose tolerance decreased. These findings suggest that auditory function in the higher frequencies is at increased risk as body mass/weight increases and as the body loses its ability to manage glucose.

Because cardiovascular deficits and central obesity have been associated with poor auditory function, the cardiac values and waist circumference were tested for correlation to ABR thresholds and DPOAE SNR. Auditory thresholds and DPOAE levels were not associated with systolic or diastolic blood pressure. Heart rate was negatively correlated with ABR thresholds for 16 and 32 kHz ($r = -0.469$, $p = 0.012$ and $r = -0.484$, $p = 0.010$, respectively),

revealing that slower heart rate was associated with higher thresholds. Waist circumference was significantly correlated with only one DPOAE SNR (f2 frequency 3125 Hz, $r=-0.377$, $p=0.035$) and no ABR thresholds. Because this was an isolated finding, it was disregarded.

4. Discussion

4.1 Auditory function in HI-T2DM : Thresholds and sites of lesions

Auditory impairment has been reported in human studies of diabetes, but results across studies are highly variable. The current study used an animal model for HI-T2DM to reduce some of the intersubject variability inherent in human studies, and thus, to help clarify the effects of HI-T2DM on hearing impairment. Current findings indicate that the DPOAEs were reduced with HI-T2DM, which suggests a vulnerability of the cochlea, and specifically the outer hair cells, to insults from HI-T2DM. Further, the comparison of DPOAE SNRs between the HI-D and NL groups showed a tendency for the HI-D group to have significantly smaller SNRs and more affected frequencies in the right ear than in the left ear. These results are consistent with the tendency for the right ear in humans to be more affected by hyperinsulinemia or diabetes compared to the left ear as suggested by Frisina et al. (2006).

Despite the significant differences in the DPOAEs, the ABR thresholds were not significantly different between groups. Thresholds were, however, several dB higher in the HI-D group than in the non-diabetic groups (NL and CR), and the amplitudes of wave 4, which identified ABR threshold, were reduced in both ears of the HI-D monkeys. Further ABR thresholds, especially for 16 and 32 kHz, were poorer (higher) for monkeys with poorer glucose control. The elevated thresholds, therefore, were consistent with the reduction in the DPOAE SNRs. The relationship between significant differences with DPOAEs and less-affected auditory thresholds is established in the literature. Behavioral auditory thresholds have been shown to be less sensitive than otoacoustic emissions to early hearing impairment with noise exposure (Uchida et al., 2008) and with insulin-dependent diabetes mellitus (DiNardo et al., 1998). The ABR thresholds are also less sensitive measures of threshold than behavioral measures are, so the otoacoustic emissions are expected to evidence earlier deterioration than ABR thresholds.

Consistent with these findings, loss of outer hair cells, especially in the basal turns of the cochlea, was found on temporal bone studies of adults with type 2 diabetes with a mean age about 53 years (Fukushima et al., 2006). In a rat model, outer hair cell loss occurred early in the development of diabetes (Rust et al., 2006). Although these findings are consistent with the decreased DPOAEs in the current study, longitudinal studies will be necessary to determine if the trends noted in this study are consistent with progressive hearing loss in diabetes.

4.2. Physical/Physiological characteristics and risk for hearing loss in diabetes

The physical/physiological characteristics correlated with the development of hearing impairment were evaluated in the three groups. The quadratic relations between BMI and DPOAE SNRs indicated that both low and high BMIs were associated with reduced

DPOAE, and the middle range of the BMI, regardless of the group assignment, was associated with the largest DPOAEs. No previous studies have linked low BMIs to reduced DPOAEs, but there is support for high BMIs being linked to reduced OAEs. Evans et al. (2007) reported that individuals with chronic hyperlipidemia had lower DPOAE levels than normal controls. Spankovich et al. (2011), in an epidemiologic study of Australians, reported that high levels of fat and cholesterol in the diet were associated with reduced amplitudes for transient otoacoustic emissions (TEOAE). High dietary intake of fat is positively associated with obesity and increased BMI (Gazzaniga & Burns, 1993) as are high levels of cholesterol and low-density lipoprotein cholesterol (Takada et al., 1998). BMI, therefore, has been shown to be associated with cochlear pathology even in the absence of diabetes.

BMI was significantly and positively correlated with the thresholds at 16 and 32 kHz, despite the fact that no significant group differences occurred among the ABR thresholds. Similarly in a study of young, normal adults, Barrenas et al. (2005) found that a higher BMI was associated with more hearing loss. Although the HI-D monkeys dominated the top of the range for both BMI and ABR thresholds, they had BMIs that covered much of the range of BMIs for the entire group. The BMI, rather than the diabetic condition, may be the underlying characteristic that affects the DPOAEs and ABR thresholds in hyperinsulinemia. Regardless of diabetic status, the evidence suggests that a high BMI is a risk factor for hearing impairment.

The ABR latencies were used to estimate neural conduction time in the cochlear nerve and auditory brainstem pathways. In the current investigation, there were no significant latency differences by subject group, but across all groups longer ABR latencies were correlated with higher BMIs. ABR latencies noted in human studies of diabetes have been variable; many find no alteration in ABR latencies, whereas others find significant prolongations. Durmus et al. (2004) reported that only the latencies of ABR waves III and V and the inter-wave interval were significantly prolonged relative to the ABR measures in controls. They suggested that the central auditory system was affected before the thresholds became elevated. Although the Durmus et al. findings provide some support for the latency prolongations in the current study, their control groups were not well-matched by age, which is a possible confounding variable. Vaughan et al. (2007) found statistically significant, but clinically negligible, latency prolongations of ABR waves III and V in their prospective study with a large sample size. The current correlation findings are consistent with the specific ABR waves affected, but not with the diabetic group differences. The numbers of subjects in the current study may be too small to confirm significance of differences in latencies between subject groups.

Human studies have not consistently reported that ABR latency prolongations are associated with diabetic sequelae. Vaughn et al. (2007), despite finding significant latency prolongations, did not identify any diabetic characteristics that were significantly correlated with the latencies. Al-Azzawi and Mirza (2004) also found ABR differences with diabetes, but no significant correlations with type or duration of diabetes. Paring et al. (1990), however, found ABR prolongations in 40% of people with long duration insulin dependent diabetes, but only 5% of those with short duration diabetes, suggesting the increasing

likelihood of development of neural deficits with progression of diabetes. Differences among studies may be due to types of data analysis and definitions of subject and control groups as well as the condition of the auditory brainstem pathways. The current study showed that ABR latencies for wave 4 were correlated with the HI-T2DM characteristics K_G , SI, and DI. Even when the group differences were not significant, data from individuals showed a linear relation with poorer ability of the body to manage glucose associated with longer latencies. The above studies suggest that auditory neural function may be at risk in diabetes, but may remain subclinical for many years in many adults with HI-T2DM.

Limitations of this study include the small sample size and the retrospective analysis. Given the nature of the subject characteristics, the available pool of monkeys was finite. Larger sample sizes and prospective study are needed to confirm the risk factors for hearing in diabetes that were identified in the current study. Although the correlations between physical/ physiological characteristics and hearing were modest, a coherent pattern emerged that suggests that a high body mass index and poor glucose control are detrimental to the auditory system. Longitudinal studies are needed to document the progression of these risk factors and hearing loss.

4.3 Conclusions

The current study was designed to investigate the effects of HI-T2DM on auditory function, and to determine which physical/physiological characteristics of HI-T2DM were associated with impaired auditory function. Rhesus monkeys were used to reduce differences in lifestyle factors that cannot be controlled in human studies, which reduced inter-subject variability. The HI-D monkeys displayed a sensorineural hearing loss that was predominantly cochlear. The ABR thresholds were higher (poorer) for all of the stimulus conditions and correlated with many physical/physiological characteristics of HI-T2DM. Despite the modest size of the correlations, the characteristics of body size and glucose management displayed a coherent pattern across the measures of auditory function that allowed risk factors for hearing impairment in diabetes to emerge. Hearing loss was shown to begin in the hyperinsulinemia, pre-diabetic state. Finally, caloric restriction was shown to preserve hearing in the rhesus monkeys by preventing elevation of risk factors associated with hearing loss and HI-T2DM.

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Abbreviations

ABR	auditory brainstem response
ARHL	age-related hearing loss
BMI	body mass index
DI	disposition index

DPOAE	distortion product otoacoustic emissions
FSIGT	Frequently sampled intravenous glucose tolerance tests
G_b	Basal plasma glucose concentration
HbA1c	glycosylated hemoglobin
HI-T2DM	hyperinsulinemia/Type2 diabetes mellitus
I_b	Basal plasma insulin concentration
K_G	glucose tolerance
S_I	insulin sensitivity index
SNR	signal to noise ratio

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Highlights

- The effect of hyperinsulinemia on auditory function was tested in rhesus monkeys.
- Groups tested were controls, hyperinsulinemia, and caloric restricted monkeys.
- Cochlear pathology correlated with physiological measures in hyperinsulinemia.
- Hearing may be at risk with hyperinsulinemia prior to the diagnosis of diabetes.

Figure 1.

Example of waveforms from a typical monkey in response to click stimuli for signal levels from 110 to 50 dB pSPL. Waves 1, 2, and 4 are labeled on the response at 110 dB pSPL. The threshold is defined as the lowest waveform that contains a repeatable potential, in this case 60 dB pSPL.

Figure 2.

DPOAE signal to noise ratio (SNR) by F2 frequency for the normal (NL), caloric restricted (CR), and hyperinsulinemia/diabetic (HI-D) monkeys for the right and left ears respectively. The error bars represent ± 1 standard deviation. The main effect of group was significant (right ear, $p=0.013$; left ear, $p=0.037$). Specifically between the NL and HI-D groups, in the right ear, 2011, 3125, and 4410 Hz and 2211 Hz in the left ear were significantly different ($p<0.05$). All the other frequencies showed a trend ($p=0.051-0.10$) in the same direction, except for 6250 Hz in the left ear. The HI-D monkeys had the lowest SNR, which is associated with the poorest cochlear function.

Figure 3.

Mean ABR thresholds for the three groups of monkeys to the clicks and the individual tonal thresholds (8, 16, and 32 kHz). The error bars represent ± 1 standard deviation. Although none of these differences reached statistical significance, the HI-D group had the highest thresholds (poorer hearing) for all stimuli.

Figure 4.

The quadratic correlations between DPOAE SNR and BMI for the left and right ears.

Although the correlations are weak (right ear shows significance with $p=0.13$; left ear shows a trend with $p=.059$), the distribution of the individual data points for the monkeys in all three groups shows that the CR monkeys (circles) tend to have low BMI and low DPOAEs, normal monkeys (x) are in the middle range for BMI and have relatively high DPOAEs, and the HI-D monkeys (squares) have the highest BMI and also low DPOAEs. The four-pointed stars represent the responses from the two monkeys with overt diabetes.

Figure 5.

Linear correlations between the thresholds derived from the 16 kHz tone bursts (left) and 32 kHz tone bursts (right) for the caloric restricted (CR), hyperinsulinemia/diabetic (HI-D) and normal control (NL) groups of subjects. The four-pointed stars represent thresholds from the two monkeys with overt diabetes. Both correlations were significant (16 kHz, $p=0.008$; 32 kHz, $p=0.004$) with r^2 values shown on the graphs. Some data points are obscured by others.

Table 1

Means and standard deviations (sd) for the physiological data that were collected on the hyperinsulinemia/diabetic (HI-D), normal (NL), and caloric restricted (CR) monkeys. The “F” column contains values from the F test for the three groups. The “t” column contains values for the pairwise comparison of the NL vs. HI-D groups.

Categories	Variable/Units	HI-D	sd	NL	sd	CR	sd	F	p	t	p
Somatic Data	Weight (kg)	13.02	3.91	11.56	2.25	9.17	1.19	F=4.17	p=0.030*	t=0.92	p=0.374
	Body Mass Index (kg/m ²)	48.42	11.46	41.53	4.27	34.45	2.74	F=7.46	p=0.004**	t=1.59	p=0.146
Glycosylated Hemoglobin	Abdominal Circumference (cm)	60.81	13.73	54.81	7.63	45.38	2.37	F=5.76	p=0.010**	t=1.08	p=0.298
	HbA1c (%)	13.18	4.70	9.00	2.79	8.31	1.03	F=5.37	p=0.013*	t=2.16	p=0.049*
Cardiovascular Data	Systolic Blood Pressure (mmHg)	121.56	15.35	126.31	13.73	124.25	13.07	F=0.23	p=0.797	t=-0.65	p=0.525
	Diastolic Blood Pressure (mmHg)	58.31	4.30	66.13	11.47	60.75	10.88	F=1.43	p=0.262	t=-1.80	p=0.105
	Heart Rate	144.81	18.03	150.69	17.28	156.88	22.76	F=0.76	p=0.478	t=-0.67	p=0.517
	Glucose (mg/dL)	121.00	61.92	75.69	17.29	65.38	7.16	F=5.02	p=0.016*	t=1.99	p=0.081
Chemistry Panel	Cholesterol (mg/dL)	207.63	55.48	188.81	33.93	179.50	31.84	F=0.94	p=0.407	t=0.82	p=0.427
	Triglycerides (mg/dL)	489.39	58.49	169.38	121.31	118.44	84.19	F=2.13	p=0.144	t=1.35	p=0.216
	Glucose tolerance K _G (%)	3.75	1.13	7.00	4.97	6.72	2.72	F=2.32	p=0.123	t=-1.80	p=0.111
Glucose Tolerance Test	Basal plasma glucose G _b (mg/dL)	97.94	50.77	64.88	8.98	58.13	6.16	F=4.04	p=0.033*	t=1.81	p=0.110
	Basal plasma insulin I _b (mU/mL)	79.44	51.01	29.75	9.68	16.69	4.69	F=9.68	p=0.001**	t=2.71	p=0.028*
	S _I *10 ⁻⁴	1.58	1.27	3.40	2.13	6.42	2.95	F=8.86	p=0.002**	t=1.98	p=0.069
	DI	1688.5	2232.3	4561.12	4941.3	5290.89	3394	F=1.90	p=1.75	t=1.41	p=0.181

Significance at p 0.01 is marked with double asterisks, and significance at p 0.05 level is marked with a single asterisk.

Table 2

Means and standard deviation (sd) for the auditory data from the HI-D, NL, and CR monkeys. The column headed "F," contains the results of the F test for the comparison across the three groups. The column headed "t" contains the results of the t value for the pairwise comparison of the NL vs. HI-D groups.

Measure	Ear	Stimulus	HI-D	NL	CR	F, p	t, p
Click ABR Latency (ms)	Better ear	8 kHz	37.14 (11.13)	32.50 (8.86)	33.75 (10.61)	F=0.41, p=0.670	t=-0.90, p=0.385
		16 kHz	51.43 (12.15)	43.75 (10.61)	46.25 (10.61)	F=0.92, p=0.415	t=-1.31, p=0.214
		32 kHz	75.71 (11.34)	67.50 (7.07)	68.75 (13.56)	F=1.19, p=0.325	t=-1.71, p=0.111
		8-32 kHz Average	54.76 (10.34)	47.92 (6.41)	49.58 (9.83)	F=1.17, p=0.332	t=-1.56, p=0.142
		Click	43.75 (9.16)	37.50 (8.86)	38.75 (6.41)	F=1.29, p=0.296	t=-1.39, p=0.187
	Left ear	Wave 1	1.25 (0.11)	1.28 (0.06)	1.24 (0.11)	F=0.43, p=0.655	t=-0.69, p=0.499
		Wave 2	2.28 (0.15)	2.24 (0.06)	2.23 (0.08)	F=0.57, p=0.576	t=-0.80, p=0.441
		Wave 4	3.99 (0.22)	3.95 (0.19)	3.99 (0.28)	F=0.11, p=0.898	t=-0.46, p=0.656
		Interwave 1-4	2.74 (0.18)	2.67 (0.19)	2.76 (0.23)	F=0.47, p=0.633	t=-0.83, p=0.421
		Wave 1	1.27 (0.07)	1.23 (0.08)	1.21 (0.10)	F=0.91, p=0.418	t=-1.18, p=0.258
Click ABR Amplitude (mV)	Right ear	Wave 2	2.28 (0.13)	2.23 (0.06)	2.12 (0.34)	F=1.14, p=0.338	t=-1.03, p=0.327
		Wave 4	4.00 (0.19)	3.91 (0.17)	3.91 (0.21)	F=0.45, p=0.643	t=-0.91, p=0.378
		Interwave 1-4	2.72 (0.17)	2.68 (0.19)	2.70 (0.21)	F=0.08, p=0.923	t=-0.43, p=0.676
		Wave 1	0.50 (0.23)	0.58 (0.28)	0.54 (0.15)	F=0.23, p=0.794	t=-0.60, p=0.558
		Wave 2	0.98 (0.45)	1.31 (0.39)	1.09 (0.37)	F=1.31, p=0.2921	t=-1.52, p=0.151
	Left ear	Wave 4	0.88 (0.18)	0.97 (0.46)	0.91 (0.45)	F=0.13, p=0.875	t=-0.57, p=0.583
		Wave1/4 Ratio	0.56 (0.23)	0.65 (0.23)	0.76 (0.47)	F=0.66, p=0.526	t=-0.69, p=0.501

Measure	Ear	Stimulus	HI-D	NL	CR	F, p	t, p
DPOAE SNR (Ratio)	Right ear	Wave 1	0.43 (0.20)	0.55 (0.31)	0.52 (0.17)	F=0.53, p=0.598	t=-0.89, p=0.387
		Wave 2	0.99 (0.38)	1.20 (0.36)	0.92 (0.45)	F=1.06, p=0.363	t=-1.12, p=0.280
		Wave 4	0.79 (0.13)	0.94 (0.24)	0.83 (0.56)	F=0.36, p=0.699	t=-1.56, p=0.142
		Wave 1/4 Ratio	0.56 (0.27)	0.58 (0.24)	1.12 (0.97)	F=2.24, p=0.131	t=-0.16, p=0.878
		2211 Hz	12.63 (14.11)	30.38 (9.69)	20.50 (8.70)	F=5.15, p=0.015*	t=-2.93, p=0.011*
	Left ear	3125 Hz	14.88 (14.51)	26.50 (7.37)	21.25 (9.65)	F=2.27, p=0.128	t=-2.02, p=0.063
		4416 Hz	8.00 (15.35)	21.50 (10.04)	15.75 (10.15)	F=2.51, p=0.106	t=-2.08, p=0.056
		6250 Hz	13.38 (13.90)	21.88 (12.56)	14.25 (11.56)	F=1.08, p=0.357	t=-1.28, p=0.220
		8837 Hz	12.38 (17.12)	25.13 (10.71)	15.00 (11.84)	F=1.98, p=0.162	t=-1.79, p=0.096
		2211-8837 Average	12.25 (13.65)	25.08 (8.31)	17.35 (8.26)	F=3.09, p=0.067	t=-2.27, p=0.037*
DPOAE SNR (Ratio)	Right ear	2211 Hz	18.00 (13.82)	32.25 (3.81)	21.50 (11.82)	F=3.84, p=0.038*	t=-2.81, p=0.023*
		3125 Hz	14.25 (15.19)	30.00 (7.52)	22.50 (12.22)	F=3.41, p=0.052	t=-2.63, p=0.020*
		4416 Hz	10.13 (13.40)	23.50 (9.37)	17.25 (10.47)	F=2.85, p=0.080	t=-2.31, p=0.036*
		6250 Hz	13.50 (14.53)	27.38 (11.82)	18.75 (8.50)	F=2.78, p=0.085	t=-2.10, p=0.055
		8837 Hz	16.13 (9.64)	24.88 (8.41)	21.63 (9.41)	F=1.86, p=0.180	t=-1.93, p=0.074
		1122-8837 Average	14.40 (11.40)	27.60 (6.52)	20.33 (8.45)	F=4.30, p=0.027*	t=-2.84, p=0.013*

Values significant at the p<0.05 level are marked with an asterisk. No characteristics reached significance at the p<0.01 level.

Table 3

Correlation of the ABR thresholds, latencies, and amplitudes with selected physiological characteristics of HI-T2DM. Thresholds are derived from the responses to the click and tone bursts at 8, 16 and 32 kHz. Latencies and amplitudes are derived from the waves generated by the clicks.

Correlate	Thresholds			Click ABR Latencies								Click ABR Amplitudes							
	Better Ear			Left Ear				Right Ear				Left Ear				Right Ear			
	Click	8 kHz	16 kHz	Wave	Wave	Wave	1-4 ILD	Wave	Wave	Wave	1-4 ILD	Wave	Wave	Wave	1-4 ILD	Wave	Wave	Wave	1-4 ILD
BMI																			
kg/m²	r	.348*	.220	.496**	.540**	.296	.524**	.366*	.270	.372*	.292*	.449*	.282	-.179	-.419*	-.273	-.281	-.214	-.214
	p	.048	.157	.008	.004	.080	.004	.039	.101	.037	.029	.014	.091	.202	.021	.098	.097	.092	.157
HbA1c	r	.376*	.277	.412*	.570**	.280	.325	.226	.121	.360*	.209	.274	.112	.053	-.275	-.225	-.117	-.296	-.191
	p	.035	.101	.025	.002	.092	.060	.144	.287	.042	.164	.098	.301	.403	.097	.146	.294	.080	.186
K	r	-.046	-.148	-.455*	-.442*	-.138	-.437*	-.461*	-.452*	-.368*	-.164	-.563**	-.393*	.173	.246	.374*	.297	.184	.456*
	p	.417	.256	.017	.020	.265	.019	.013	.015	.042	.227	.003	.032	.246	.129	.039	.098	.201	.014
S × 10⁻⁴	r	-.251	-.200	-.471*	-.500*	-.250	-.310	-.411*	-.340	-.353	-.437*	-.422*	-.261	.036	.225	.373*	.114	.213	.413*
	p	.130	.193	.016	.010	.131	.080	.029	.061	.053	.021	.025	.120	.437	.157	.044	.308	.170	.028
DI	r	-.143	-.118	-.388*	-.513**	-.218	-.366*	-.381*	-.323	-.354	-.223	-.498**	-.336	.041	.261	.429*	.142	.239	.452*
	P	.263	.305	.041	.009	.165	.047	.040	.071	.053	.159	.009	.063	.429	.120	.023	.264	.142	.017

Significance at $p < 0.01$ is indicated by two asterisks, and significance at $p < 0.05$ is indicated with a single asterisk. Because no significant correlations existed between ABR characteristics and glucose, Gb, and Ib, these values are not shown.