Original Study

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Elevation of Serum Prestin in Patients With Tinnitus: Pathophysiological Implications and Biomarker Potential

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Objective: Prestin enables outer hair cell (OHC) function in cochlear amplification and has been implicated in tinnitus. An experimental study of salicylate toxicity, known to cause tinnitus in humans, demonstrated increased expression of prestin. As prestin is quantifiable in the blood, we hypothesized that if prestin expression is increased in tinnitus patients, then serum prestin levels in those with tinnitus compared with those without tinnitus are elevated.
Study design: A prospective, case-control study.
Setting: Single-institution tertiary care center.
Patients: Patients with or without tinnitus.
Intervention: Diagnostic.
Main outcome measure: Serum prestin was quantified through automated Western blot electropherograms. Key covariates, including age, hearing threshold, and daily noise exposure, were accounted for in multivariate analyses.
Results: Eighty-nine participants (49 with chronic tinnitus and 40 controls) underwent audiometry, noise dosimetry, and blood sampling. The metrics of the 97 kDa prestin isoform were significantly increased in the tinnitus group, with differences in age, hearing thresholds, and daily noise exposure between the 2 groups accounted for in multivariate analyses. Correlations between prestin isoform expression and noise exposure seen in controls were disrupted in the tinnitus group, shifting from the 97 kDa isoform to the 140 kDa isoform.
Conclusions: These findings suggest OHC dysfunction involving prestin in those with tinnitus. Furthermore, the 97 kDa isoform of serum prestin represents a promising candidate biomarker in those with tinnitus. Prestin as a biomarker may serve to stratify tinnitus patients according to origin (eg, cochlear vs. central), inform further investigations of the pathophysiology of tinnitus, and potentially develop targeted treatments.

Keywords: Biomarker, Outer hair cell, Prestin, Sensorineural hearing loss, Serum, Tinnitus, Western blot

Introduction

Tinnitus is an auditory disorder characterized by the perception of sound without external stimulation. It affects over 11% of the US population.[1] and ~15% of the global population.[2] Hearing loss is well established to be associated with tinnitus.[3] Other risk factors include noise exposure, ototoxic drugs, and infection.[4,5] Tinnitus is associated with a range of debilitating consequences, including sleep disturbances,[6] anxiety,[7] and impaired cognitive performance.[8] Despite its widespread prevalence and profound impact, the pathophysiological mechanisms underlying tinnitus are unknown, largely due to the absence of objective diagnostic methods.[9] This limitation results in limited treatment options, which are largely palliative.[10]

Recent advancements in the identification of blood-based otologic biomarkers may offer insights. To identify biomarkers, 2 possible strategies could be adopted. One is to apply a shotgun omics (analyzing complex samples without prior knowledge or selection of specific targets) strategy.[11] An alternative approach is to focus on key cochlear proteins that have been implicated in the pathophysiology of tinnitus. Prestin, a transmembrane motor protein highly expressed in cochlear outer hair cells (OHCs), is a promising candidate. Prestin plays a crucial role in regulating cochlear amplification through conformational changes, with experimental results in noise-induced and ototoxin-induced models supporting its potential as a biomarker.[12–18] Furthermore, investigations in humans have successfully quantified serum prestin levels, revealing their stability over time under normal conditions and susceptibility to factors such as age, sensorineural hearing loss, and daily noise exposure.[19–22] Importantly, prestin's cochlear expression is heightened in response to salicylate toxicity, both in the acute and chronic

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experimental models.[23,24] Both acute and chronic salicylate toxicity are associated with tinnitus in humans.[25,26] The upregulation of prestin suggests a possible mechanistic basis for the development of tinnitus.

This study evaluates the potential of serum prestin as a biomarker for tinnitus. We hypothesize that if heightened cochlear expression of prestin is associated with tinnitus, then those with tinnitus will demonstrate elevated serum prestin levels compared with those without.

Materials and methods

Experimental protocol overview

This observational, prospective, case-control study was approved by the Institutional Review Board at the University of Connecticut Health Center (Protocol # 22-165-2) and the University of Connecticut (Protocol # H14-214). Tinnitus subjects were recruited from the patient base at UConn Health. During the initial visit, participants underwent comprehensive audiometric testing. In addition, a tinnitus handicap index (THI) survey.[27] was administered. Participants then wore an Etymotic Noise Dosimeter ER-200D to monitor their sound exposure over a weeklong period and maintained an activity log. Participants returned for their second visit, where blood samples were obtained.

Participants

Eighty-nine adult subjects completed the study. They were assigned to 2 distinct groups: 49 subjects who had been experiencing tinnitus continuously for at least 3 months, and 40 control subjects with no history of tinnitus. Exclusion criteria included exposure to ototoxic drugs; otologic disorders such as otosclerosis, Meniere disease, sudden sensorineural hearing loss; past history of otologic or neurological surgery; hydrocephalus; vertigo; barotrauma; active or recent otitis externa or media; and retrocochlear pathology. All participants completed pure-tone audiometry conducted for standard and extended high frequencies (EHF).

Automated western blot

Quantification of serum prestin level was with Western blot, which was performed by Raybiotech, Inc. (Peachtree Corners, GA), using an automated Capillary Electrophoresis Immunoassay machine (ABBY, ProteinSimple, Santa Clara, CA). Serum samples were diluted ×20. Details of the procedure are described elsewhere.[28]

Noise dosimetry protocol

We gathered one week of environmental sound level data from each participant. The protocol was based on those of our prior studies.[29–32] Details of the protocol are described elsewhere.[22]

Data analysis

The automated Western blots of serum samples were transformed into electropherograms. The electropherogram allows representation of each band by a peak from which metrics such as peak height, width, and area can be calculated.[28] The electropherograms of both groups showed multiple peaks with 3 main peaks, which, on average, were at 46, 97, and 140 kDa, comparable to the results of our recent report of automated Western blots of human prestin in young, healthy subjects.[28] The tinnitus and control groups were compared across height, width, and area under the curve of each of the 3 main peaks.

Prestin levels were compared with average daily noise exposure levels (dB LAeq,8h) from dosimetry, audiometric thresholds in the form of bilateral pure-tone averages (10-frequencies from 0.25 to 10 kHz—BPTA10 in dB HL) measured based on audiograms, and THI scores.[22]

Non-transformed, raw data were used for prestin descriptive statistics and comparisons in all analyses. Statistical analyses were performed using SPSS Statistics 30.0 (IBM, Armonk, NY). Shapiro-Wilk tests of normality were carried out for individual dependent variables. As distributions departed from normality (Fig. 1), nonparametric statistical analyses were carried out, including Mann-Whitney U for independent sample comparisons, Spearman Rho for correlations, and QUADE analysis of covariance (ANCOVA). ANCOVA is more powerful than linear regression in accounting for potential confounders, leading to more accurate results and a reduction of the risk of type I errors.[33] Given the a priori hypothesis of increased prestin expression in the tinnitus group and the directional focus of this investigation, all P-values are 1-tailed for increased statistical power. Statistical significance was set at $P < 0.05$.

Results

Group characteristics

More females (n = 52) than males (n = 37) participated in the study. The control group consisted of 25 female and 15 male subjects. The tinnitus group consisted of 27 female and 22 male subjects. There was no significant difference in the proportion of sexes between the 2 groups ($\chi^2 = 0.496$, $P = 0.481$). The control group had an age range of 28 to 72 years, whereas the tinnitus group was 18 to 89 years of age. The tinnitus group was significantly older (mean rank 50.9 vs. 37.8) (Mann-Whitney $U = 691$, $P = 0.0085$). The tinnitus group had significantly higher bilateral 10-tone PTAs (independent-samples Mann-Whitney U, $P < 0.001$). The mean rank of hearing thresholds for the tinnitus group was 55.33 dB, compared with that of the control group at 32.35 dB ($U = 474$, $P < 0.001$). The tinnitus group had significantly lower daily noise (dB LAeq,8h) exposures (mean rank of 38.33 vs. 53.18) (Mann-Whitney U, $P = 0.0035$). Figure 1 illustrates the key differences in the distributions of the 2 groups.

As expected, age and BPTA10 were strongly and positively correlated (Rho = 0.705, $P < 0.001$) with all subjects pooled. This was also true when the correlation between age and BPTA10 was examined for either the tinnitus (Rho = 0.75, $P < 0.001$) or the control (Rho = 0.515, $P < 0.001$) groups. There were no significant correlations between either BPTA10 or age with average daily noise exposure (LAeq,8hr).

Comparison of tinnitus and control group electropherograms

When comparing automated Western blot results using electropherograms across groups, the tinnitus group had higher values (Fig. 2). These differences were found to be statistically significant in the width of the 46 kDa peak ($U = 620.5$, $P = 0.0015$), and the area ($U = 704$, $P = 0.011$) and width ($U = 369.5$, $P < 0.001$) of the 97 kDa peaks (Supplement Table 1, Supplemental Digital Content 1, http://links.lww.com/MAO/C321). In a Quade ANCOVA, controlling for age, BPTA10, and LAeq,8h, the width of the 46 kDa peak in the tinnitus group was still statistically

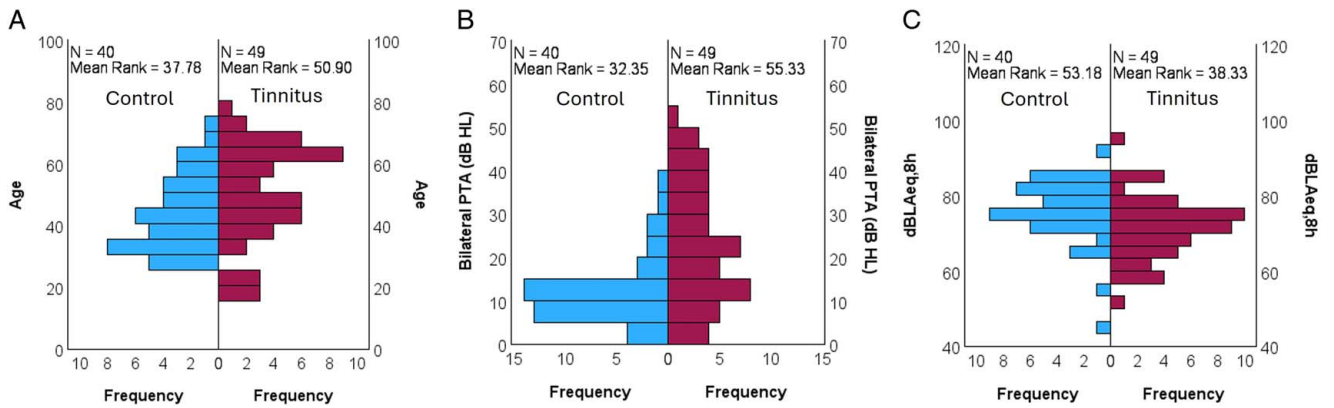

Figure 1. The distributions of age (*A*), bilateral 10-tone pure-tone average (*B*), and average daily noise exposure (dB LAeq,8hr; *C*) for the tinnitus (red) and control (blue) groups.

greater than that of the control group ($F(1,87) = 7.28$, $P = 0.004$). In a Quade ANCOVA, controlling for age, BPTA10, and LAeq,8h, the difference between the 97 kDa area and width for the tinnitus and control groups was still statistically significant ($F(1,87) = 4.52$, $P = 0.016$ and $F(1,87) = 15.99$, $P < 0.001$, respectively).

The significantly greater 97 kDa values for the tinnitus group have important implications warranting further scrutiny given the potential confounding effects of age and hearing threshold. To that end, we examined 97 kDa width in more depth from 2 perspectives. First, the subjects were divided into 3 age groups: younger than 40, 40 to 60, and older than 60 years of age (Table 1). This approach allowed comparison of control and tinnitus groups when age differences were accounted for. Group comparisons were carried out for age, BPTA10, and LAeq,8h, and if significant different then the variable was accounted for in the Quade ANCOVA for that group reported in the right column. Significant differences in 97 kDA width were found across all 3 age groups after accounting for covariates. It was notable that in the youngest group, where age, BPTA10, and LAeq,8h were similar, the difference in 97 kDa width remained robustly statistically significantly different between the tinnitus and control groups. This youngest subgroup had low BPTA10, suggesting hearing loss was not the driver of the difference between the tinnitus and control. Second, we divided all subjects according to hearing thresholds (BPTA10 < 15 or ≥ 15 dB HL, Supplement Table 2, Supplemental Digital Content 2, http://links.lww.com/MAO/C322). This analysis showed that even with hearing thresholds closely matched, the robust statistical difference in the 97 kDa width persisted between the tinnitus and control groups.

We previously showed a relationship between the 97 kDa peak and an OHC functional measure. As this peak is central to our driving hypothesis of prestin disruption at the level of the OHCs, we carried out a more stringent statistical assessment to confirm the differences between the tinnitus and control groups. Specifically, we excluded 4 tinnitus subjects with very large areas (above 1,500,000 square chemiluminescence) in the multivariate analysis. Quade ANCOVA, controlling for age, BPTA10, and LAeq,8h, still showed a significant difference between the 97 kDa width ($F(1,83) = 12.14$, $P = 0.0005$) of the 2 groups, but narrowly missed statistical significance when the 97 kDa area was compared ($F(1,83) = 2.34$, $P = 0.0515$).

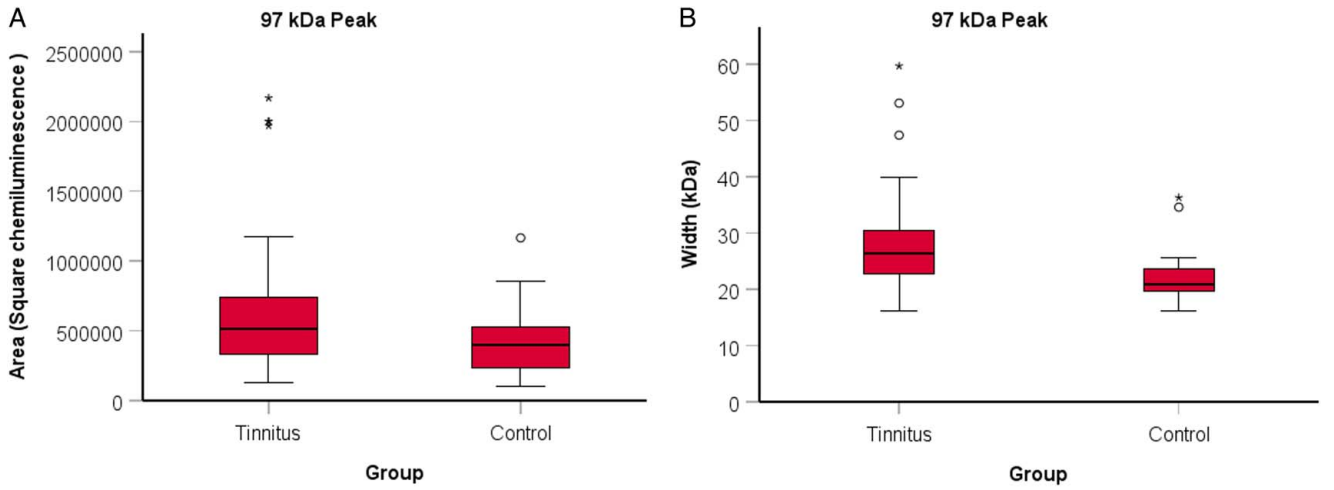

Figure 2. Box diagram comparing the 97 kDa area (*A*) and width (*B*) between the tinnitus and control groups.

Table 1

Comparisons of tinnitus and control groups as a function of age, 97 kDa width, BPTA10, LAeq,8h and multivariate analysis (Quade ANCOVA) of the 97 kDa width controlling for the statistically different independent variables.

	n		Age (Y)		97 kDa Width		BPTA10 (dB HL)		dB LAeq,8h		ANCOVA
Age Group (y)	Control	Tinnitus	Control	Tinnitus	Control	Tinnitus	Control	Tinnitus	Control	Tinnitus	
< 40	16	10	32 (32.3 ± 0.7)	24.5 (26.8 ± 2.4) $P = 0.06$	20.8 (21.1 ± 0.5)	25.6 (28.6 ± 3.3) $P = 0.005$*	7.5 (9.9 ± 1.8)	8.9 (9.2 ± 1.9) $P = 0.45$	74.9 (74.2 ± 2.4)	71.7 (71.9 ± 2.6) $P = 0.15$	
40-60	19	21	49 (48.7 ± 1.5)	48 (48.7 ± 1.3) $P = 0.45$	21.1 (22.6 ± 1.2)	26 (28.2 ± 2.1) $P = 0.002$*	11.7 (12.2 ± 1.3)	18.2 (20.7 ± 2.2) $P < 0.001$*	75.3 (76.4 ± 1.8)	72.9 (71.6 ± 2.2) $P = 0.03$*	F(1,38) = 2.8 $P = 0.025$*
> 60	5	18	62 (64.4 ± 2.1)	65 (66.1 ± 1.2) $P = 0.25$	20.7 (20.4 ± 1.2)	27.1 (27.84+_1.3) $P = 0.003$*	24.2 (22.3 ± 4.2)	35.6 (35.3 ± 2.6) $P = 0.012$*	81.4 (78.8 ± 4.7)	71.5 (71.9 ± 1.7) $P = 0.16$	F(1,21) = 8.33 $P = 0.005$*
All	40	49	42.5 (44.1 ± 2.1)	51 (50.6 ± 2.2) $P = 0.009$*	20.8 (21.7 ± 0.6)	26.4 (28.2 ± 1.2) $P = 0.001$*	11.1 (12.5 ± 1.2)	22.2 (23.7 ± 1.9) $P < 0.001$*	75.9 (75.8 ± 1.4)	72.2 (71.8 ± 1.2) $P = 0.004$*	F(1,87) = 15.99 $P < 0.001$*

For each variable, median (mean ± SEM) is shown. Mann-Whitney *U* test results for control versus tinnitus group comparisons are displayed in the row immediately below. For each age group that had significant differences in the age, BPTA10 and/or LAeq,8h, a Quade ANCOVA was performed with those significant variables included as covariates.
*Statistically significant at $P < 0.05$.

Tinnitus group THI

There was no correlation between THI and 46, 97, or 140 kDa metrics.

Relationship of electropherogram peaks to hearing thresholds, age, and average daily noise exposure (LAeq,8h)

There was a moderate, positive correlation between BPTA10 and the width of the 97 kDa peak width when all subjects were pooled (Rho = 0.32, $P = 0.001$) (Fig. 3).

The correlations between BPTA10 and the width of the 97 kDa peak width did not reach statistical significance when the tinnitus and control groups were examined separately. The analyses were repeated for the age subgroups without any significant findings emerging except in the 40 to 60-year-old group, where a weak positive correlation was found when subjects were pooled (Rho = 0.29, $P = 0.034$), suggesting that the middle age group of the driver of the correlation between prestin and hearing threshold. No significant correlations were found for other peaks or metrics with bilateral PTA.

The 46 kDa width (Rho = 0.2, $P = 0.03$) was weakly correlated with age, with all subjects pooled. When the subjects were divided into tinnitus and control groups, there were no statistically significant correlations between electropherogram metrics and age.

The 97 kDa band area and width were correlated with LAeq,8h (Rho = 0.306, $P = 0.027$; Rho = 0.305, $P = 0.028$, respectively) in the control group, but not in the tinnitus group (Fig. 4).

We also examined the relationship between LAeq,8h and other peaks' parameters. The 46 kDa peak's area and height correlated with LAeq,8h in the control group (Rho = 0.332, $P = 0.018$; Rho = 0.267, $P = 0.048$; respectively), but not for the 140 kDa. In the tinnitus group, LAeq,8h correlated with the 140 kDa area and width (Rho = 0.281, $P = 0.025$; Rho = 0.335, $P = 0.009$, respectively), but not the 97 kDa or 46 kDa metrics.

We explored the relationship between LAeq,8h, and electropherogram parameters further. Among the control subjects, the correlation for LAeq,8h and 97 kDa parameters was the strongest for subjects with BPTA10 $\geq$ 10 dB (n = 23): height (Rho = 0.353, $P = 0.049$), area (Rho = 0.455, $P = 0.015$), and width (Rho = 0.336, $P = 0.06$). In the control group with BPTA10 < 10 (n = 17), correlations were positive, reaching significance only at 140 kDa width (Rho = 0.444, $P = 0.037$).

The relationship between LAeq,8h and 140 kDa parameters was also primarily due to subjects with BPTA10 $\geq$ 10 (n = 40) in the tinnitus group (Fig. 5). The correlations for height, area, and width were Rho = 0.358, $P < 0.012$; Rho = 0.493, $P < 0.001$; and Rho = 0.518, $P < 0.001$, respectively. Interestingly, among the small group of 9 tinnitus subjects with BPTA10 < 10 dB, the relationship between LAeq,8h and nearly all peak parameters was strong and negative. For example, 47 kDa width yields Rho = –0.661, $P = 0.026$; 97 kDa area yields Rho = –0.567, $P = 0.05$; and 140 kDa area yield Rho = –0.667, $P = 0.025$). These results further support the disruption of the prestin-noise relationship in the tinnitus group.

Discussion

The absence of any objective means of assessing the presence and severity of tinnitus, combined with the complexity of the disorder, has been a major barrier to the understanding of its

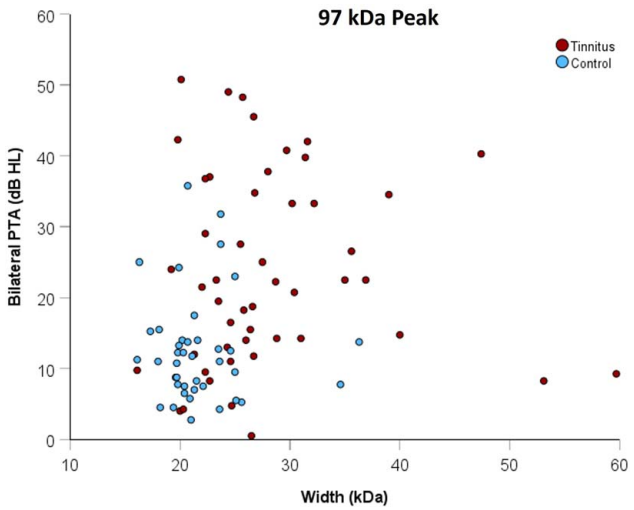

Figure 3. Scatter diagram of the relationship of bilateral 10-frequency (0.25 to 10 kHz) pure-tone average to the width of the 97 kDa peak. There was a moderate positive correlation between the 2 measures with all subjects pooled (Rho = 0.32, $P = 0.001$). Tinnitus subjects are in red and control subjects are in blue.

pathophysiology and the development of direct treatments. The origins of tinnitus are debated, with both peripheral and central auditory system sources suspected,[34] making the separation of the various components difficult.[35] To navigate these complexities, we adopted a reductive approach to tinnitus generation by focusing on its peripheral (ie, cochlear) source. This was motivated by an experimental study that demonstrated salicylate toxicity, well known to cause tinnitus in humans, was associated with increased expression of prestin in the cochlea.[23] We hypothesized that if blood levels of prestin reflect cochlear expression of prestin, then those with tinnitus should have higher levels of prestin. Our results (Fig. 2, Table 1, Supplement Table 1, Supplemental Digital Content 1, http://links.lww.com/MAO/C321 & 2, Supplemental Digital Content 2, http://links.lww.com/MAO/C322) support this hypothesis. The difference in prestin levels of the tinnitus and control groups was modest but statistically significant. Although the current work focused on a peripheral source of tinnitus, we did not specifically recruit those with peripheral sources or exclude those with central sources. If future investigations could subdivide tinnitus patients into peripherally or centrally originating tinnitus (eg, based on OAEs, evoked potentials), we would predict that those who suffer from peripherally originating tinnitus, including patients with well-defined cochlear pathologies (eg, ototoxicity, Meniere disease, sudden sensorineural hearing loss), will have higher serum prestin levels than both controls and patients with centrally originating tinnitus. Thus, the significant difference that we found in the serum levels of prestin between tinnitus and control subjects can be taken as a conservative estimate, making the differences we reported even more meaningful, as no specific tinnitus group was targeted based on origin during recruitment.

A role for OHC prestin in peripheral tinnitus

Our results directly implicate OHCs in the generation of tinnitus, presumably through changes in prestin expression that result in altered electromechanical force generation in the cochlea, that is, altered amplification. A direct role for OHC through prestin upregulation, as a mechanism for peripheral tinnitus, has appeal because it could also explain why normal hearing individuals can experience tinnitus.

To further investigate the relationship between tinnitus and prestin, levels in circulation could be measured serially under conditions that are known to affect the severity of tinnitus. Salicylate is an obvious candidate, but it bears ethical concerns. Other auditory (eg, non-damaging noise) and non-auditory (eg, diet.[36] and sleep.[37]) variables are more practical. A repeated-

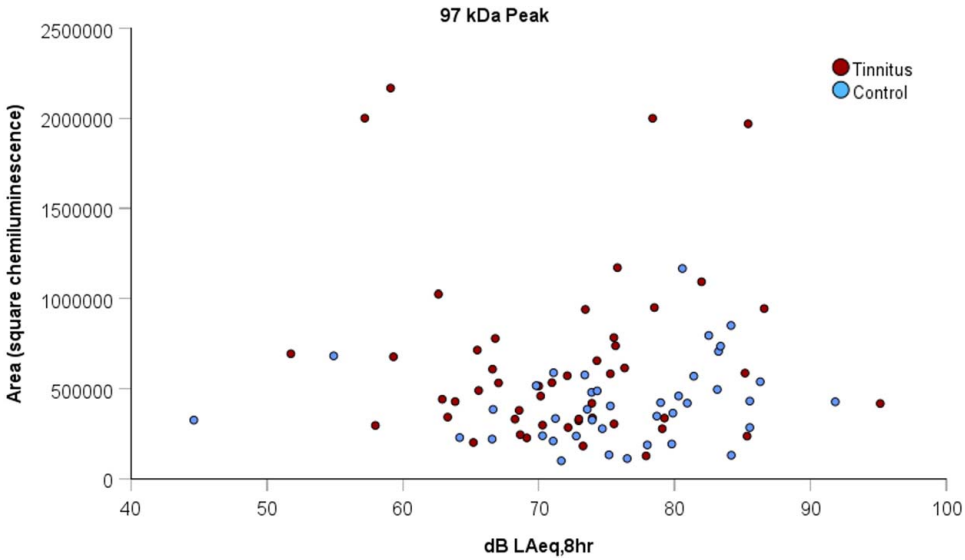

Figure 4. The relationship between average daily noise exposure and the 97 kDa peak area. There is a moderate positive relationship between LAeq,8h, and 97 kDa area (Rho = 0.306, $P = 0.027$) in the control group (blue symbols). This relationship is disrupted in the tinnitus group (Rho = 0.017, $P = 0.454$) (red symbols). Even when the 4 outliers in the tinnitus group with an area > 1,500,000 square chemiluminescence were excluded, no significant correlation emerged (Rho = 0.052, $P = 0.366$).

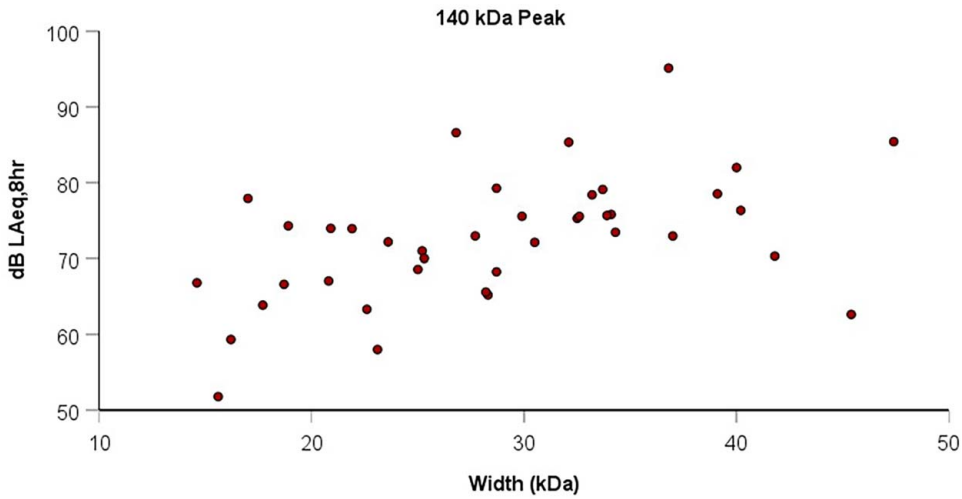

Figure 5. The relationship between the 140 kDa peak width and average daily noise exposure (LAeq,8h) in tinnitus subjects with BPTA10 ≥ 10 dB HL (Rho = 0.518, $P < 0.001$).

measure design employing human participants, including those with and without tinnitus, is feasible. In the experimental setting, we have demonstrated that serum prestin levels gradually decrease after a transient rise in response to a loud noise exposures.[13,17] In ototoxicity models for both cisplatin.[15] and cyclodextrin,[18] we have shown that serum prestin levels start to rise within hours of exposure. This rapid, temporary rise in prestin occurs even when OHCs are not lost—implying a change in expression.[38]

Otoacoustic emission and tinnitus

If OHCs play a role in the generation of tinnitus, otoacoustic emissions (OAEs), representing functional measures of OHCs, may show differences in their characteristics in tinnitus vs. non-tinnitus subjects. It is well established that the 2f1-f2 distortion product OAE (DPOAE), which is the largest and most commonly quantified distortion product, is dependent on prestin-mediated electromotility.[39]

The exploration of a relationship between OAEs and tinnitus dates back to 1986.[40] The most relevant support comes from a salicylate toxicity after overdose case, which resulted in severe hearing loss and tinnitus.[41] Interestingly, during the intoxication state, although 50-dB hearing loss was present, DPOAEs, which should have been expected to be absent, could still be recorded well in the frequency range that corresponded to the appearance of tinnitus. Other data show that in some tinnitus patients, spontaneous OAEs (SOAEs) seemed to be at least partly responsible for their tinnitus.[42] Patients who complained of tinnitus with normal hearing thresholds were shown to have a significantly higher prevalence of SOAEs (100%), and higher transiently evoked OAEs (TOAEs).[43] and DPOAE amplitudes and growth functions.[44] In contrast, a few studies have shown lower TEOAEs.[45] or DPOAEs amplitudes in tinnitus subjects.[46–48] No DPOAE differences have been found across subjects with varying severity of tinnitus.[49] or in the presence of hyperacusis.[50] The conflicting results suggest that the relationship between tinnitus and OAEs is more nuanced. Specifically, the effect of tinnitus on DPOAEs may vary with hearing loss. In one study, DPOAE levels in adults with hearing loss and tinnitus were low, but those with normal hearing and tinnitus had DPOAE levels enhanced relative to the controls.[51] Another study reported that those with chronic tinnitus and elevated hearing thresholds had DPOAEs comparable to those without tinnitus.[52] Taken together, there is support for OAEs being increased in the setting of tinnitus, but confounding factors, such as hearing loss, need to be carefully taken into account, as we have done in this study.

OHC prestin as a biomarker for tinnitus?

It is very tempting to speculate that prestin in the blood may serve as a biomarker for tinnitus, albeit a peripherally generated (ie, cochlear) variety. Having a biomarker for tinnitus is potentially a game-changer. Availability of a biomarker for tinnitus would offer a novel tool in future investigations that may position investigators to successfully gain insights into the mechanisms that contribute to the generation of tinnitus and identify specific targets for intervention. Such a biomarker could also serve to stratify tinnitus patients in the clinical setting (eg, peripheral vs. central) and help hone a targeted treatment.

At this stage, caution is prudent. First, although we found statistically significant differences between those with tinnitus and controls, the magnitude was not overwhelming. As noted above, the results in a subset of tinnitus subjects with peripherally originating tinnitus could be more impressive. Therefore, a larger number of tinnitus subjects would be needed, perhaps stratified according to DPOAE amplitude (ie, peripheral).

Second, our results need to be replicated. Replication and extension of our findings could firmly position serum prestin as a tinnitus biomarker. Such an effort, however, must consider factors that can influence serum prestin levels, such as age, presence of hearing loss, and daily noise exposure, as we have done in this study.

Tinnitus questionnaire

In the absence of objective methods to assess tinnitus, clinicians and researchers have used survey instruments for self-assessment. These surveys aim at assessing primarily the emotional and functional intrusiveness of tinnitus. No single questionnaire is

comprehensive, having differences in format, content, and scales, placing significant limitations on their utility.[53]

Our Western results showed no relationship between prestin levels and THI. This may be because our tinnitus population generally had relatively low THI scores (ie, < 36, suggestive of low distress). In future work, it would be informative to assess serum prestin levels in subjects with THI > 36 or those who are believed to have more severe tinnitus. In contrast, the absence of a relationship between a putative biomarker and THI may not be particularly surprising because THI is more directed at the annoyance features of tinnitus than generation or maintenance attributes.

Conclusions

In this prospective case-control study of tinnitus and control subjects, the tinnitus subjects were found to have statistically higher levels of prestin. The significant difference persisted after accounting for differences between the 2 groups arising from age, hearing threshold, and daily noise exposure. The higher levels of prestin in the blood of tinnitus subjects support a role for outer hair cells in tinnitus generation and raise the promise of developing an objective and measurable biomarker for tinnitus.

References

[1] Batts S, Stankovic KM. Tinnitus prevalence, associated characteristics, and related healthcare use in the United States: a population-level analysis. Lancet Reg Health Am 2024;29:100659.
[2] Jarach CM, Lugo A, Scala M, *et al*. Global prevalence and incidence of tinnitus: a systematic review and meta-analysis. JAMA Neurol 2022;79: 888–900.
[3] Eggermont JJ. Pathophysiology of tinnitus. Prog Brain Res 2007;166:19–35.
[4] Levine RA, Oron Y. Tinnitus. Handb Clin Neurol 2015;129:409–31.
[5] Biswas R, Genitsaridi E, Trpchevska N, *et al*. Low evidence for tinnitus risk factors: a systematic review and meta-analysis. J Assoc Res Otolaryngol 2023;24:81–94.
[6] Gallo KEB, Correa CC, Goncalves CGO, *et al*. Effect of tinnitus on sleep quality and insomnia. Int Arch Otorhinolaryngol 2023;27:e197–202.
[7] Pattyn T, Van Den Eede F, Vanneste S, *et al*. Tinnitus and anxiety disorders: a review. Hear Res 2016;333:255–65.
[8] Neff P, Simoes J, Psatha S, *et al*. The impact of tinnitus distress on cognition. Sci Rep 2021;11:2243.
[9] Cardon E, Joossen I, Vermeersch H, *et al*. Systematic review and meta-analysis of late auditory evoked potentials as a candidate biomarker in the assessment of tinnitus. PLoS ONE 2020;15:e0243785.
[10] Tunkel DE, Bauer CA, Sun GH, *et al*. Clinical practice guideline: tinnitus. Otolaryngol Head Neck Surg 2014;151(2 Suppl):S1–40.
[11] Zeleznik OA, Welling DB, Stankovic K, *et al*. Association of plasma metabolomic biomarkers with persistent tinnitus: a population-based case-control study. JAMA Otolaryngol Head Neck Surg 2023;149: 404–15.
[12] Santos-Sacchi J, Shen W, Zheng J, Dallos P. Effects of membrane potential and tension on prestin, the outer hair cell lateral membrane motor protein. J Physiol 2001;531(Pt 3):661–6.
[13] Parham K, Dyhrfjeld-Johnsen J. Outer hair cell molecular protein, prestin, as a serum biomarker for hearing loss: proof of concept. Otol Neurotol Offic Publ Am Otol Soc Am Neurotol Soc [and] Eur Acad Otol Neurotol 2016;37:1217–22.
[14] Liba B, Naples J, Bezyk E, Campbell C, Mei M, Parham K. Changes in serum prestin concentration after exposure to cisplatin. Otol Neurotol Off Publ Am Otol Soc Am Neurotol Soc [and] Eur Acad Otol Neurotol 2017;38:e501–5.
[15] Naples J, Cox R, Bonaiuto G, Parham K. Prestin as an otologic biomarker of cisplatin ototoxicity in a guinea pig model. Otolaryngol Head Neck Surg Off J Am Acad Otolaryngol Head Neck Surg 2018;158:541–6.
[16] Dogan M, Sahin M, Cetin N, Yilmaz M, Demirci B. Utilizing prestin as a predictive marker for the early detection of outer hair cell damage. Am J Otolaryngol 2018;39:594–8.
[17] Parham K, Sohal M, Petremann M, *et al*. Noise-induced trauma produces a temporal pattern of change in blood levels of the outer hair cell biomarker prestin. Hear Res 2019;371:98–104.
[18] Harrison MS, Driscoll BG, Farnsworth J, *et al*. Automated Western blot analysis of ototoxin-induced prestin burst in the blood after cyclodextrin exposure. Otol Neurotol 2023;44:e653–9.
[19] Parker A, Parham K, Skoe E. Reliability of serological prestin levels in humans and its relation to otoacoustic emissions, a functional measure of outer hair cells. Ear Hear 2021;42:1151–62.
[20] Parker A, Parham K, Skoe E. Age-related declines to serum prestin levels in humans. Hear Res 2022;426:108640.
[21] Al Samarrai ARH, Mahdi BM, Parham K. Relationship of serum prestin levels to the severity of sensorineural hearing loss. Cureus 2024;16: e53763.
[22] Parker A, Parham K, Skoe E. Noise exposure levels predict blood levels of the inner ear protein prestin. Sci Rep 2022;12:1154.
[23] Yu N, Zhu ML, Johnson B, Liu YP, Jones RO, Zhao HB. Prestin up-regulation in chronic salicylate (aspirin) administration: an implication of functional dependence of prestin expression. Cell Mol Life Sci CMLS 2008;65:2407–18.
[24] Yang K, Huang ZW, Liu ZQ, Xiao BK, Peng JH. Long-term administration of salicylate enhances prestin expression in rat cochlea. Int J Audiol 2009;48:18–23.
[25] Temple AR. Acute and chronic effects of aspirin toxicity and their treatment. Arch Intern Med 1981;141(3 Spec No):364–9.
[26] Samlan SR, Jordan MT, Chan SB, Wahl MS, Rubin RL. Tinnitus as a measure of salicylate toxicity in the overdose setting. Western J Emerg Med 2008;9:146–9.
[27] Newman CW, Jacobson GP, Spitzer JB. Development of the tinnitus handicap inventory. Arch Otolaryngol Head Neck Surg 1996;122: 143–8.
[28] McClure HM, Mirza M, Adamczyk P, Skoe E, Parham K. Western blot characterization of human serum prestin, an outer hair cell biomarker. Otol Neurotol Off Publ Am Otol Soc Am Neurotol Soc [and] Eur Acad Otol Neurotol 2025;46:201–5.
[29] Skoe E, Tufts J. Evidence of noise-induced subclinical hearing loss using auditory brainstem responses and objective measures of noise exposure in humans. Hear Res 2018;361:80–91.
[30] Tufts JB, Skoe E. Examining the noisy life of the college musician: weeklong noise dosimetry of music and non-music activities. Int J Audiol 2018;57(sup1):S20–7.
[31] Camera S, Tufts J, Skoe E. Noise exposure and background noise tolerance in listeners with normal audiograms. J Speech Lang Hear Res 2019; 62:2564–70.
[32] Skoe E, Camera S, Tufts J. Noise exposure may diminish the musician advantage for perceiving speech in noise. Ear Hear 2019;40:782–93.
[33] Smith E Ancova vs Regression: Difference and Comparison. Ask Any Difference. Published 2023. Accessed July 3, 2025, 2025. https://askanydifference.com/difference-between-ancova-and-regression/.
[34] Norena AJ. Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches. Audiol Neuro-Otol 2015;20(Suppl 1):53–9.
[35] Shapiro SB, Noij KS, Naples JG, Samy RN. Hearing loss and tinnitus. Med Clin North Am 2021;105:799–811.
[36] Marcrum SC, Engelke M, Goedhart H, *et al*. The influence of diet on tinnitus severity: results of a large-scale, online survey. Nutrients 2022; 14:5356.
[37] Peng J, Dong Y, Luo Y, *et al*. The Relationship between sleep traits and tinnitus in UK Biobank: a population-based cohort study. Ear Hear 2023; 44:53–60.
[38] Harrison MS, Driscoll BG, Farnsworth J, *et al*. Serum prestin after ototoxin exposure is not dependent on outer hair cell loss. Otol Neurotol Off Publ Am Otol Soc Am Neurotol Soc [and] Eur Acad Otol Neurotol 2024; 45:495–501.
[39] Cheatham MA. Distortion product otoacoustic emissions in mice above and below the eliciting primaries. J Assoc Res Otolaryngol 2023;24: 413–28.
[40] Wilson JP. Otoacoustic emissions and tinnitus. Scand Audiol Suppl 1986; 25:109–19.
[41] Janssen T, Boege P, Oestreicher E, Arnold W. Tinnitus and 2f1-f2 distortion product otoacoustic emissions following salicylate overdose. J Acoust Soc Am 2000;107:1790–2.
[42] Norton SJ, Schmidt AR, Stover LJ. Tinnitus and otoacoustic emissions: is there a link? Ear Hear 1990;11:159–66.

[43] Ceranic BJ, Prasher DK, Raglan E, Luxon LM. Tinnitus after head injury: evidence from otoacoustic emissions. J Neurol Neurosurg Psychiatry 1998;65:523–9.
[44] Hesse G, Schaaf H, Laubert A. Specific findings in distortion product otoacoustic emissions and growth functions with chronic tinnitus. Int Tinnitus J 2005;11:6–13.
[45] Nottet JB, Moulin A, Brossard N, Suc B, Job A. Otoacoustic emissions and persistent tinnitus after acute acoustic trauma. Laryngoscope 2006; 116:970–5.
[46] Onishi ET, Fukuda Y, Suzuki FA. Distortion product otoacoustic emissions in tinnitus patients. Int Tinnitus J 2004;10:13–6.
[47] Alshabory HF, Gabr TA, Kotait MA. Distortion product otoacoustic emissions (DPOAEs) in tinnitus patients. Int Arch Otorhinolaryngol 2022;26:e046–57.
[48] Jedrzejczak WW, Pilka E, Ganc M, Kochanek K, Skarzynski H. Ultra-high frequency distortion product otoacoustic emissions for detection of hearing loss and tinnitus. Int J Environ Res Public Health 2022;19:2123.
[49] Tekumalla S, Perlov NM, Gokhale S, *et al*. Evaluation of subjective tinnitus severity and distortion product otoacoustic emissions and extended high-frequency audiometry. Otolaryngol Head Neck Surg 2024;171: 517–20.
[50] Tai Y, Mertes IB, Chappell J, Jeon CH, Husain FT. Comparison of otoacoustic emissions in tinnitus and hyperacusis in adults with normal hearing sensitivity. Int J Audiol 2023;62:442–52.
[51] Husain FT. Effect of tinnitus on distortion product otoacoustic emissions varies with hearing loss. Am J Audiol 2013;22:125–34.
[52] Bhatt IS, Lichtenhan J, Tyler R, Goodman S. Influence of tinnitus, lifetime noise exposure, and firearm use on hearing thresholds, distortion product otoacoustic emissions, and their relative metric. J Acoust Soc Am 2023; 154:418–32.
[53] Storm D. Understanding Tinnitus Assessment Questionnaires. In Tinnitus Today 2018:14–5.