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Sources of Variability in Wideband Energy Reflectance Measurements in Adults

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Abstract

Background: Wideband acoustic immittance measurements of the middle ear, such as wideband energy reflectance (ER), can provide information about how the middle ear functions across the traditional audiometric frequency range. These measurements are being investigated as a new means of evaluating conductive hearing disorders, and studies have been reported on a number of middle-ear disorders. However, the normative database for wideband ER is still being developed, and more information is needed about sources of test variability.

Purpose: The purpose of the present study was to evaluate sources of variability in wideband ER measurements at baseline and across annual tests for up to 5 yr in subjects with normal hearing.

Study Sample: The main group consisted of 112 subjects (187 ears), 24 females and 88 males, with normal hearing and normal 0.226-kHz admittance tympanometry. An additional 24 adults with abnormal 0.226-kHz tympanometry provided baseline comparison data.

Research Design: A longitudinal design was used in obtaining annual measurements of audiometry, tympanometry, and wideband ER at ambient pressure in adults.

Data Collection and Analysis: Clinical audiometry and tympanometry data and 1/3-octave wideband ER measurements were obtained at baseline and annually for up to four additional tests. Descriptive statistics and t-tests were used to explore differences in 1/3-octave baseline ER measures in terms of subject age, test ear, sex, and clinical tympanometry. Longitudinal mixed-effects linear regression models at 1.0, 2.0, and 4.0 kHz were used to examine the different sources of variance affecting ER over time.

Results: There were small but statistically significant mean differences in ER for baseline measurements as a function of ear, sex, and age. Compared with these results, data for 29 ears with abnormal 0.226-kHz tympanometry differed from mean normal data across a broad frequency range by as much as 20%. ER varied as a function of peak compensated static acoustic admittance (Y_{tm}) for measures at 1.0 kHz but was unrelated to Y_{tm} at 2.0 and 4.0 kHz. ER also varied as a function of the test ear, with significantly higher ER on the left at 1.0 and 2.0 kHz, but was not significantly related to the test ear at 4.0 kHz. The

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Douglas H. Keefe is involved in commercializing technology intended to improve the screening and diagnosis of middle-ear disorders.

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standard deviation for test-retest variability was about 0.1 at each frequency, which is consistent with previous studies.

Conclusions: Mean wideband ER at baseline showed small but significant differences related to sex, ear, and age. ER was significantly related to Y_{tm} at 1.0 kHz in the longitudinal data but not at 2.0 or 4.0 kHz and to the test ear at 1.0 and 2.0 kHz but not at 4.0 kHz. When evaluated at ambient pressure, ER for ears with negative middle-ear pressure was similar to that of ears with abnormally low Y_{tm}. Therefore it might be necessary to evaluate wideband acoustic immittance compensated for middle-ear pressure by using tympanometry to obtain an effective differential diagnosis of middle-ear disorders in adults.

Key Words: Wideband acoustic immittance, energy or power reflectance, absorbance, middle ear, tympanometry

Abbreviations: ANOVA = analysis of variance; DPOAE = distortion product otoacoustic emission; ER = energy reflectance; LOWESS = locally weighted scatterplot smoothing curve; SD = standard deviation; SPL = sound pressure level; TPP = tympanometric peak pressure; WAI = wideband acoustic immittance; Y_{tm} = peak compensated static acoustic admittance

Wideband acoustic immittance (WAI) measurements of the middle ear can provide information about how the middle ear functions across the traditional audiometric frequency range. Energy reflectance (ER), also known as power reflectance, is a WAI measure defined as the ratio of the sound power reflected from the middle ear to the incident sound power presented to the sealed ear canal (Keefe and Feeney, 2009). An ER measurement of 0.0 indicates that no sound power is reflected from the middle ear, and a measurement of 1.0 indicates that all sound power is reflected. ER is an attractive way to measure middle-ear function because, unlike traditional admittance tympanometry, it is relatively insensitive to the measurement location in the ear canal (Stinson et al, 1982; Voss et al, 2008). A related WAI term is absorbance, which is simply 1 minus ER. Wideband ER obtained using broad-band acoustic stimuli, such as clicks or chirps, permits characterization of middle-ear function over a large portion of the human range of hearing, including the region important for speech understanding (Keefe et al, 1993). Techniques have been developed to calculate wideband pressure reflectance by quantifying the Thevenin source impedance and sound pressure of a probe assembly and sound source with respect to the characteristic impedance of the ear canal (described for impedance measurements in cats by Allen, 1986). ER is the squared pressure reflectance, which has been measured in human ears across the frequency range of 0.2 to 10.0 kHz (Keefe et al, 1992, 1993; Voss and Allen, 1994).

The normative database for wideband ER is still being developed. Most studies have reported intersubject variability in adults that is greater in the middle to high frequencies than in the low frequencies (Feeney et al, 2003; Keefe and Simmons, 2003; Feeney and Sanford, 2004; Voss and Allen, 1994; Voss et al, 2008; Werner et al, 2010). Voss and Allen (1994) reported that ER measurements in 10 adults tended to be repeatable for a given subject from test to test. A recent study by Werner and colleagues (2010) measured the test-retest reliability of ER measurements in 183 adults (age, 18–30 yr). Absolute test-retest correlations for tests conducted approximately 2 wk apart ranged from 0.95 (3.64 kHz) to 0.28 (5.823 kHz). The absolute test-retest differences in ER were smaller in the midfrequency range than at higher frequencies. Similar results were found by Vander Werff et al (2007) in a subgroup of adults.

Voss et al (2008) characterized some of the sources of intersubject variability in reflectance measurements in temporal bones from adult cadavers. They reported that variation in the volume of the middle-ear space contributed significantly to variability in ER across subjects, although variation in measurement location in the ear canal had only a small effect, which is consistent with previous data (Stinson et al, 1982). Other sources of intrasubject variability in wideband ER in adults include age and sex (Feeney and Sanford, 2004). Another likely source of test-retest variability for wideband ER at ambient pressure is variation in middle-ear pressure from test to test. Negative middle-ear pressure has been shown to result in an increase in ER in the middle to low frequencies (Feeney et al, 2003; Voss et al, 2008). The purpose of the present study was to assess sources of variability in wideband ER across subjects for an initial test and within subjects for repeated annual tests for up to 5 yr.

METHODS

The University of Washington Human Subjects Institutional Review Board approved all methods used in the present investigation. Subjects provided informed consent before undergoing any testing. They received reimbursement for their time and travel at the end of each appointment.

Subjects

All subjects were enrolled in a larger study of noise-induced hearing loss in construction workers (Seixas et al, 2012). One hundred twelve adults with normal hearing and normal middle-ear function, as measured by using 0.226-kHz tympanometry, participated in the present study (24 female subjects [mean age, 35.1 yr; age range, 27.7–45.9 yr] and 88 male subjects [mean age, 33.4 yr; age range, 23.9–51.4 yr]). An additional 24 subjects with abnormal middle-ear status, as measured by using 0.226-kHz tympanometry (described below), also participated in the study and served as a comparison group (2 female subjects [mean age, 35.6 yr; ages, 29.5 and 41.8 yr] and 22 male subjects [mean age, 34.3 yr; age range, 25.3–53.5 yr]). Twenty of these subjects also had normal hearing, as described below.

Equipment

A Grason-Stadler model GSI-39 Tymp Screener was used to obtain 0.226-kHz tympanograms in a sound-treated room. A Grason-Stadler model GSI-61 audiometer with ER-3A insert earphones was used to obtain pure tone audiograms in a double-walled sound suite. The audiometer and the immittance device were calibrated to American National Standards Institute standards S3.6-2010 (American National Standards Institute, 2010) and S3.39-1987, R2012 (American National Standards Institute, 1987), respectively.

Distortion product otoacoustic emissions (DPOAEs) were tested in a single-walled sound-treated booth by using an Etymotic Research ER-10C microphone with a custom measurement system (described in Mills et al, 2007) with a personal computer and sound card.

Wideband ER measurements were obtained by using a personal computer and sound card (CardDeluxe) with the same ER-10C microphone system used for the DPOAE measurements and custom software (Schairer et al, 2007).

Procedures

Before audiometric testing, otoscopy was performed for both ears on all subjects. If subjects were observed to have ears occluded with cerumen, testing was not completed, and the subject was referred for cerumen management and asked to return at a later date. Tympanograms were obtained after otoscopy with a 0.226-kHz probe tone. Subjects with middle-ear dysfunction, as suggested by abnormal tympanometry (e.g., high negative tympanometric peak pressure [TPP]), were referred to their primary care physician, if indicated, based on the clinical judgment of the examining audiologist but continued with the study protocol. These subjects were included in the group of 24 subjects with abnormal tympanometry. Tympanograms were considered to be within normal limits if they had a single-peak shape and met the following criteria: (1) static acoustic admittance at 0.226 kHz $\geq$0.3 mmhos and $\leq$1.7 mmhos (Margolis and Hunter, 1999) and (2) TPP ≥ -100 daPa. Subjects with abnormal tympanometry were grouped into categories for the purposes of comparing their ER measurements with those of subjects who met inclusion criteria. The categories of abnormal tympanometry were as follows: (1) low admittance, which was defined as peak compensated static acoustic admittance (Y_{tm}) <0.3 mmhos; (2) high admittance, which was defined as Y_{tm} >1.7 mmhos; and (3) negative pressure, which was defined as TPP < -100 daPa.

Air conduction audiometry was then conducted for octave frequencies from 0.25 to 8.0 kHz, including interoctaves 3.0 and 6.0 kHz, by using a standard bracketing procedure. Audiograms were considered to be within normal limits if pure tone thresholds were $\leq$25 dB HL at all frequencies tested. Bone conduction thresholds were not tested because of time constraints imposed by the larger study (Seixas et al, 2012).

In addition to having a normal 0.226-kHz tympanogram, subjects in the normal group were required to pass an additional physiologic inclusion criterion. This criterion was the presence of a DPOAE at 2.0 kHz, as described below. This provided additional evidence that forward and reverse middle-ear transduction were normal in the presence of an audiogram in the normal range. DPOAE measurement followed audiometric assessment by using the ER-10C microphone system. DPOAEs were measured at a frequency of 2f1-f2, where f1 and f2 represent the stimulus frequencies (f2 > f1). For the purposes of the present study, DPOAEs were examined with f2 = 2.0 kHz, with f2/f1 set equal to 1.21, the level of f1 set to 65 dB sound pressure level (SPL), and the level of f2 set to 55 dB SPL. Stimulus levels were calibrated in the ear canal, with adjustments to reach the desired levels (Mills et al, 2007). DPOAEs were recorded for a 6-s duration. The presence of a DPOAE at ≥ -10 dB SPL with a signal-to-noise ratio $\geq$6 dB was considered to be within normal limits (Gorga et al, 1997). All subjects who had normal pure tone thresholds and normal tympanometry also met the DPOAE criterion.

The ER stimulus was generated by the digital-to-analog converter in the sound card at a 22.05-kHz sampling rate. The probe stimulus, a series of filtered clicks (0.25–8.0 kHz), was presented at 61 dB peak equivalent SPL, as measured in a 2-cm^3 coupler, and delivered through one of the two receivers in an Etymotic Research model ER-10C microphone system. This was coupled to the ear by using an ER-14A foam insert ear tip unless a small ear canal required the use of a pediatric-sized foam ear tip (ER-14B). All wideband ER measurements were made at ambient pressure.

A two-tube calibration technique based on a model of viscothermal wave propagation in each tube provided the basis for the ER measurements (Keefe and Simmons, 2003; Keefe and Abdala, 2007). As part of this daily calibration, the ER-10C probe was inserted into each of two hard-walled cylindrical tubes closed at one end with a standard ER-14A foam ear tip. The internal diameter of both tubes was 8.0 mm, and the lengths were 290 and 2.5 cm. The calibration process provided an estimate of the incident pressure response and the source pressure reflectance of the probe. The validity of the calibration was evaluated at the time of data analysis based on two additional parameters of the calibration process described in Keefe and Simmons (2003): the root-mean-squared error ΔR in the calibration and an estimate of the match between the measured viscothermal losses in the tubes and the model prediction of those losses. The dimensionless parameter χ equaled 1.0 if the actual viscothermal loss was the same as the model wall loss or exceeded 1.0, indicating that the measured loss exceeded the model. A calibration was considered valid if $\Delta R \leq 0.014$ and $1.0 \leq \chi \leq 1.11$ (Keefe and Abdala, 2007). The median incident pressure response and the source pressure reflectance of the probe were calculated across all calibrations judged to be valid during the course of the study. ER for all tests was calculated based on these median values. The rationale for this approach is that some calibrations were found to be invalid, even though the recordings in the test ears of subjects with a different probe tip were found to be valid. The results of calibrations judged to be valid during the course of the study were found to be highly repeatable (i.e., close to the median values). A soft foam tip used for calibration was sensitive to repeated insertions into the open hard plastic end of each of the individual calibration tubes, and any resulting damage might degrade the validity of the calibration. Because a new soft foam tip was used for each test ear and because the soft foam tip was not damaged by insertion into an ear canal, it was often the case that a calibration used on a particular day might be invalid, even though all the subjects tested on that day had valid recordings based on the criteria in the next paragraph.

After audiometric testing, subjects were asked to sit quietly in a sound-treated booth for experimental measurements. An ER-10C foam ear tip was inserted into the ear canal, and the foam was allowed to expand before data collection. Testing was completed for DPOAE and then ER measurements first in the left ear and then repeated in the right ear. When ER measurement commenced, the fit of the probe was assessed by using a visual display of the wideband ER in the ear. Low ER (<0.8) at 0.25 kHz was used as an indication of a likely leak in the probe fit (Keefe et al, 2000). When this was observed, the probe was removed from the ear canal, reinserted, and then allowed to expand before data collection. If ER did not increase with this procedure, the data were stored, and the measurements were assumed to be valid. However, for the purposes of this study, such measurements were considered to be abnormal if ER was <0.8 at

Table 1. Number of subjects and ears with normal hearing and normal 0.226-kHz tympanometry at baseline stratified by sex

	Subjects	Ears
Total	112	187
Female	24	44
Male	88	143

0.25 kHz (most likely because of an improper probe fit). In such a case none of the measurements for that ear for that test day were included in further analyses. It follows from conservation of energy that ER should not exceed 1.0. However, because of a noisy measurement or small errors in calibration, a measurement of ER might exceed 1.0 (even though energy is conserved). A measurement was excluded from further consideration if ER was >1.024 or <0.0 at any frequency.

Data Analysis

Descriptive statistics were used to characterize the baseline measures in terms of subject age, sex, and mean and standard deviation (SD) of ER as a function of 1/3-octave frequency. A *t* test was used to assess intersubject differences in ER for the test ear and between-subject differences for sex at each frequency. Analysis of variance (ANOVA) was used to assess intersubject differences for age group, and when a significant difference was found for a given frequency, a *t* test was used to test the significance of group differences.

Mixed-effects linear regression models were used to assess the contributions of sex, ear, age, and tympanometric variables to the variability in longitudinal ER measurements, including random effects for subject and ear at three frequencies: 1.0, 2.0, and 4.0 kHz. The inclusion of random effects in these models allows for the characterization of variability in ER

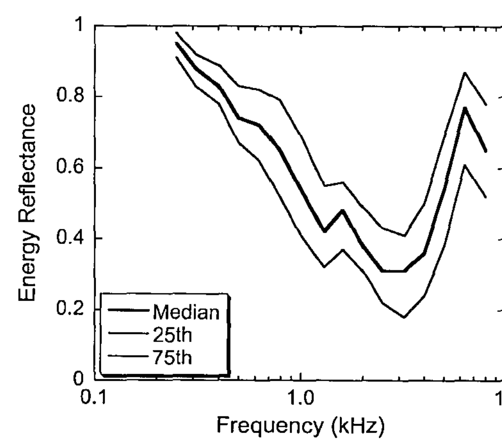

Figure 1. Median baseline 1/3-octave ER (thick line) for 187 ears with normal hearing and normal 0.226-kHz tympanometry. The thin lines represent the 25th and 75th percentiles of the data.

measurements between subjects, ears, and tests over time (test-retest variability).

RESULTS

Baseline Measures

A total of 112 subjects (187 ears) met the criteria of normal hearing and normal 0.226-kHz tympanometry at baseline. Table 1 shows the total number of subjects and ears at baseline stratified by sex. The median 1/3-octave ER from 0.25 to 8.0 kHz and the 25th and 75th percentiles of the data are shown in Figure 1. Median wideband ER had a value slightly less than 1.0 (100%) at 0.25 kHz, decreased to two minima or notches (1.3 kHz and near 3.0 kHz), and then increased at higher frequencies with a local maximum near 6.0 kHz.

Laterality was analyzed to determine differences in ER across frequency for left versus right ears. Figure 2 displays the mean 1/3-octave ER at baseline as a function of ear. The mean was used instead of the median for comparison of groups. The error bars in this and subsequent figures represent ±1 standard error of the mean (standard error). There were 93 left ears and 94 right ears meeting the normal inclusion criteria. At frequencies of less than 4.0 kHz, ER was slightly greater for the left than the right ear. This difference was only significant at two of the 1/3-octave frequencies: 2.0 kHz, with a mean difference of 0.04 (or 4%; $t = 2.29, p = 0.02$), and 2.5 kHz, with a mean difference of 0.05 ($t = 2.45, p = 0.02$). At frequencies of 4.0 kHz and greater, ER was slightly greater for the right ear. This ear difference was only significant at 6.3 kHz, with a mean ER of 0.77 in the right and 0.71 in the left ear ($t = 2.45, p = 0.02$).

There were also slight sex differences in ER at baseline. The 1/3-octave data in Figure 3 show mean ER as a function of frequency for the 44 ears of female subjects and 143 ears of male subjects. Female subjects had slightly higher mean ER than male subjects at frequencies of less than 2.5 kHz and at 8.0 kHz. The differences were not significant for the lower frequencies, but the mean difference of 0.13 at 8.0 kHz was significant ($t = 3.58, p < 0.001$). At frequencies between 2.0 and 8.0 kHz, female subjects had lower mean ER than male subjects. In this frequency range differences in ER were significantly lower for female subjects at 4.0 kHz, with a mean difference of 0.07 ($t = 2.32, p = 0.02$), and 5.0 kHz, with a mean difference of 0.10 ($t = 3.07, p < 0.001$).

The subjects were grouped by test ears into age groups by decade (Table 2). The largest age group was 30 to 39 yr with 63% of ears. Because there were only two ears for subjects in the 50- to 59-yr age group, these data were combined with data from the 40- to 49-yr age group to make one group of 40 to 59 yr for further analyses of age effects. Figure 4 shows mean baseline wideband ER for the three age groups. ANOVA was used to test the significance of the differences in ER among age groups for each 1/3-octave frequency. The ANOVA was significant at all 1/3-octave frequencies between 0.5 and 1.6 kHz but not significant at other frequencies. Table 3 shows the mean ER for each age group at frequencies between 0.5 and 1.6 kHz. The difference in ER between age groups was assessed by using t tests.

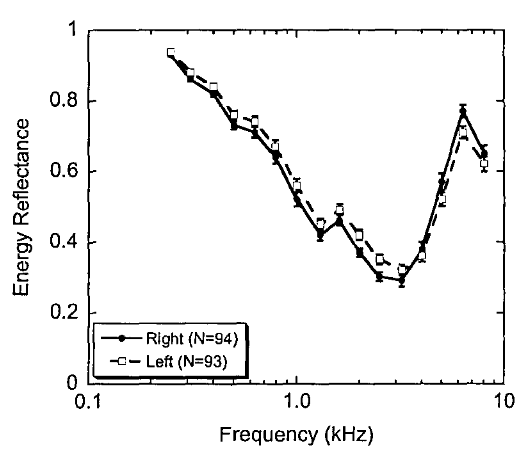

Figure 2. Mean 1/3-octave baseline ER for 187 ears with normal hearing and normal 0.226-kHz tympanometry by ear (n = 94 right and 93 left ears). Error bars represent ±1 standard error.

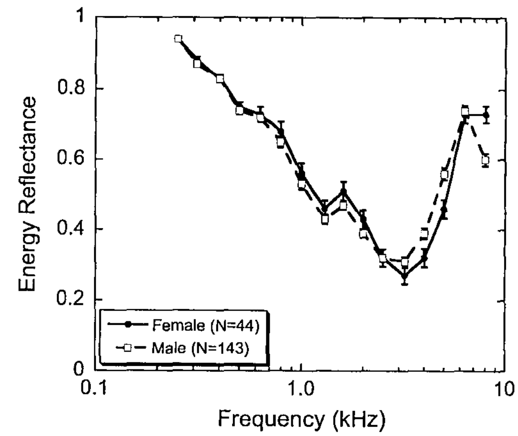

Figure 3. Mean 1/3-octave baseline ER by sex for ears with normal hearing and normal 0.226-kHz tympanometry (N = 44 females and 143 males). Error bars represent ±1 standard error.

Table 2. Age groups by decade for the 187 ears with normal hearing and normal 0.226-kHz tympanometry at baseline stratified by sex

	Age group (yr)				
Sex	20–29	30–39	40–49	50–59	Total
Female	7	23	14	0	44
Male	30	95	16	2	143
Total	37	118	30	2	187

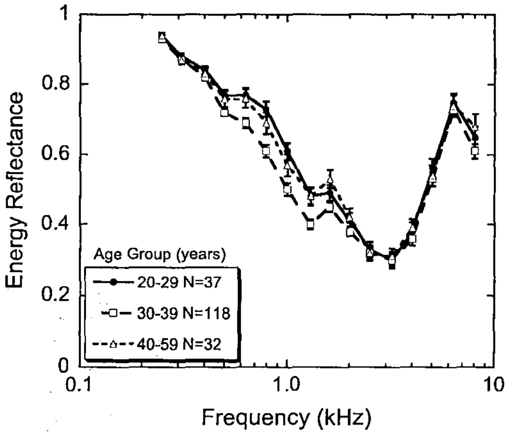

Figure 4. Mean 1/3-octave baseline ER for 187 ears with normal hearing and normal 0.226-kHz tympanometry by age group: 20 to 29 yr (n = 37 ears), 30 to 39 yr (n = 118 ears), and 40 to 59 yr (n = 32 ears). Error bars represent ±1 standard error.

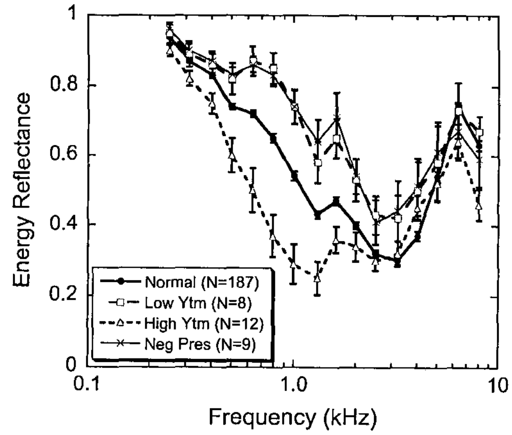

Figure 5. Mean 1/3-octave baseline ER for 187 ears with normal hearing and normal 0.226-kHz tympanometry. Also shown is the mean ER for subjects with three categories of abnormal tympanometry: low Y_{tm} (n = 8 ears), high Y_{tm} (n = 12 ears), and negative TPP (n = 9 ears; see text for details). Error bars represent ±1 standard error.

Table 3 shows the results for *t* tests for comparisons of mean ER between the 20- to 29-yr (young), 30- to 39-yr (middle) and 40- to 59-yr (old) age groups. There were small but significant differences between the young and middle age groups at five of the six frequencies in this range and between the old and middle age groups at four of the six frequencies in this range. None of the *t* tests were significant for comparisons between the young and old age groups at any frequency (not shown).

ER data for the normal group at baseline (n = 187 ears) were compared with those from 29 ears from an additional 24 subjects falling into one of three categories of abnormal 0.226-kHz tympanometry. The low Y_{tm} category (n = 8) was defined as ears having Y_{tm} <0.3 mmhos with normal TPP. The high Y_{tm} category (n = 12) consisted of ears having Y_{tm} >1.7 mmhos with normal TPP. Ears with TPP more negative than −100 daPa formed a separate category of negative TPP (n = 9), regardless of the Y_{tm} value. The results for ears in the three abnormal tympanometric categories are plotted in Figure 5, along with mean data for the normal ears at baseline. Subjects in the high Y_{tm} category had lower ER than the normal group at frequencies of less than about 2.0 kHz. Subjects in the low Y_{tm} or negative TPP categories had higher ER than the normal group at frequencies of less than about 4.0 kHz and greater than 0.3 to 0.4 kHz.

Table 3. Mean ER for three age groups, young (*Y*; 20–29 y), middle (*M*; 30–39 y), and old (*O*; 40–59 y), at 1/3-octave frequencies from 0.5 to 1.6 kHz, at which significant group differences were observed

	Frequency (kHz)					
Measure	0.5	0.63	0.79	1.0	1.3	1.6
Y mean ER	0.77	0.77	0.73	0.61	0.48	0.49
M mean ER	0.72	0.69	0.61	0.50	0.40	0.45
O mean ER	0.76	0.76	0.69	0.57	0.48	0.53
$t_{y\text{-}m}$	2.55*	3.48†	3.77†	3.27†	2.99†	NS
$t_{m\text{-}o}$	NS	2.57*	2.11*	NS	2.61*	2.86*

The *t* test results are shown for comparisons between means for the middle age group with the other two age groups for which differences were significant at the $p < 0.05$ or $p < 0.01$ levels. The symbol $t_{y\text{-}m}$ represents *t* test values for the comparison of means between the young and middle age groups. The symbol $t_{m\text{-}o}$ represents *t* test values for the comparison of means for the middle and old age groups.

NS = not significant.

*$p < 0.05$ and †$p < 0.01$.

Longitudinal Analyses

ER data for both ears of eight subjects obtained at multiple annual test sessions are shown in Figure 6. The data for these subjects were selected because they provide a comparison across three or more annual tests for the two ears of a subject for whom hearing and 0.226-kHz tympanometry were within normal limits on all measurements. The data for left ears of subjects 180 (Figure 6D) and 201 (Figure 6G) consist of five annual tests, the maximum number of tests for this study. The data for these eight subjects illustrate the similarity in ER patterns across ears for many of the subjects, as well as the variability across annual tests for a given ear.

A total of 64 ears met the criteria of three tests for which hearing and tympanometry were within normal limits on each test. The mean ER of the 64 ears for each of the three tests is shown in Figure 7. The mean time between test 1 and test 2 was 423 days (SD = 110 days), with a minimum time of 321 days and a maximum time of 713 days. The mean time between tests 2 and 3 was 396 days (SD = 114 days), with a minimum of 262 days and a maximum of 797 days. As shown in Figure 7, the

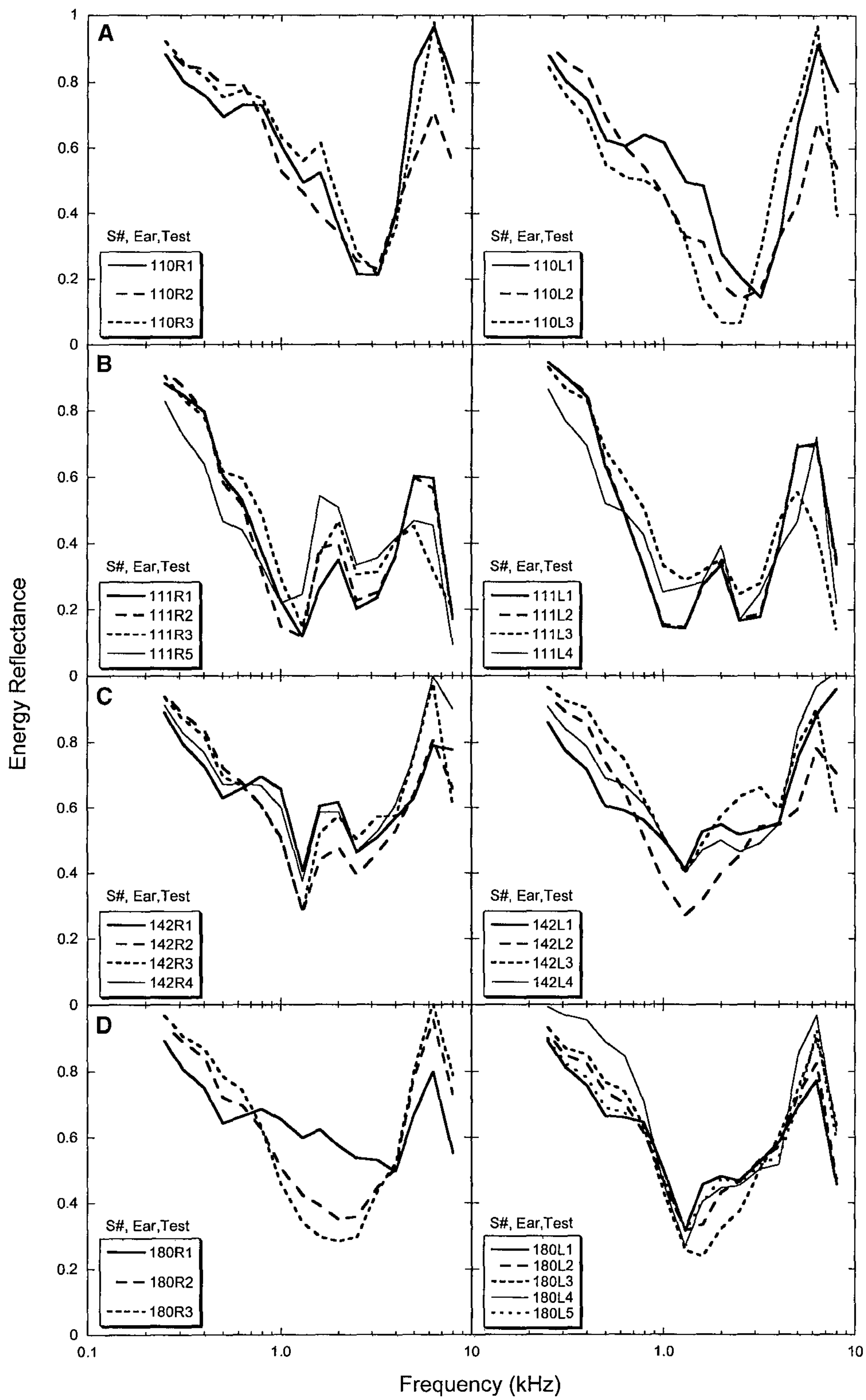

Figure 6. *A-H*, Examples of annual tests for the right and left ears of eight subjects with at least three and as many as five annual tests per ear meeting the requirements of normal hearing and normal 0.226-kHz tympanometry at the time of the test. Each row of two figures shows data for the right and left ears of an individual subject. Note that for *H*, subject 256 has data for a test (256R6). All subjects had only 5 visits, and therefore these are not data for a sixth visit. However, if there were a need to repeat a test (e.g., if cerumen were noted to be partially blocking the probe tip when it was removed from the ear canal), the test number would be increased by one for the repeat test.

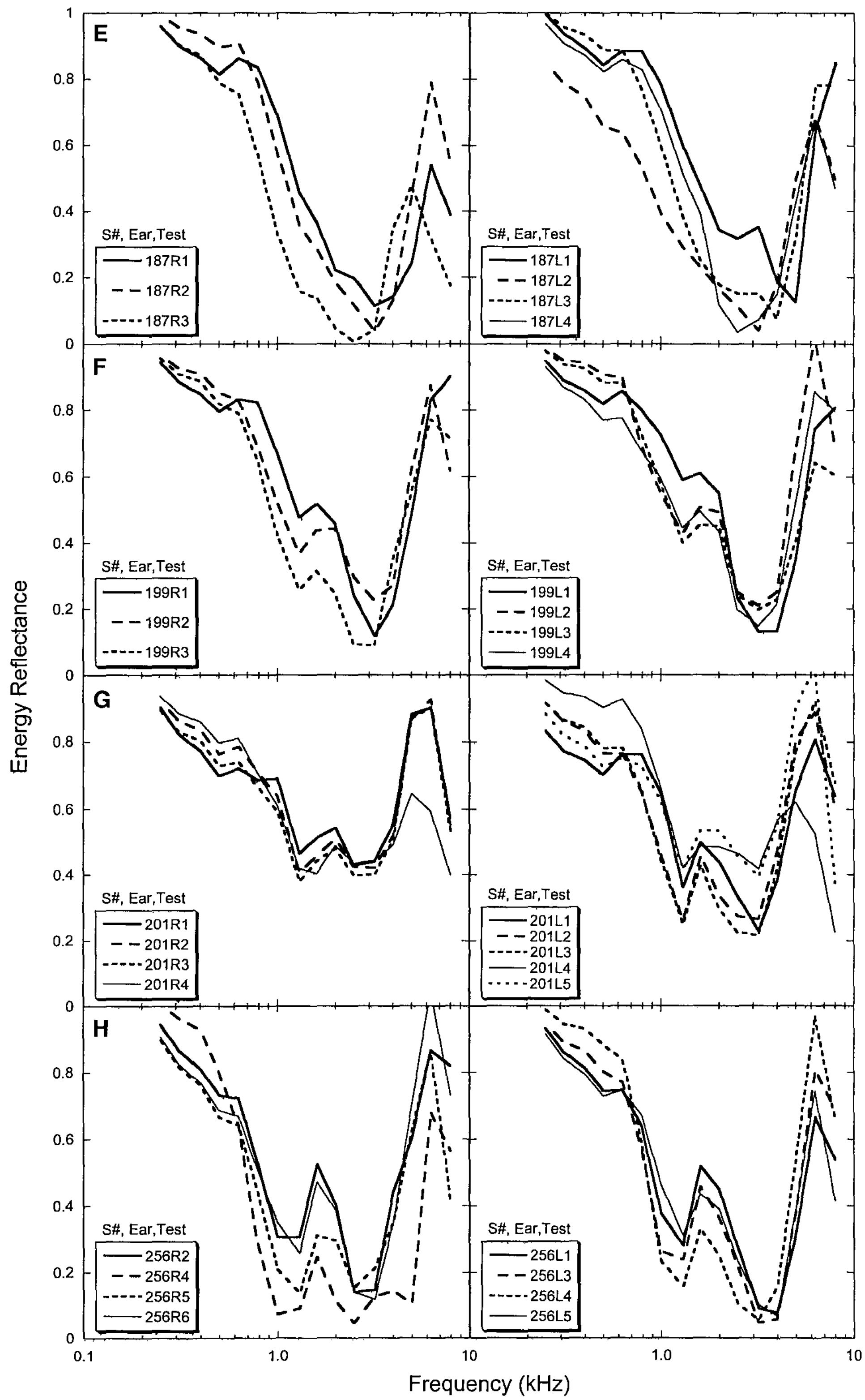

Figure 6. (Continued).

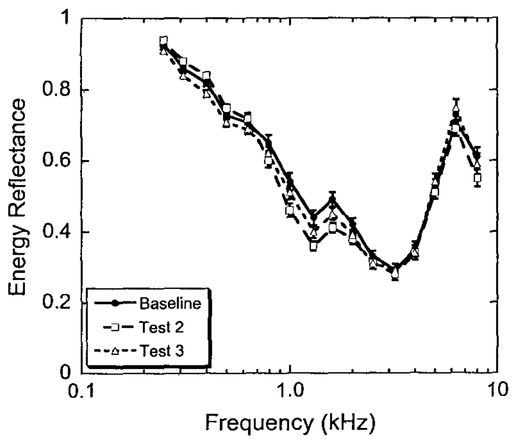

Figure 7. Mean ER for three annual tests for subjects with normal hearing and normal 0.226-kHz tympanometry. The thick solid line represents the mean ER of 64 ears for the first annual test, the long dashed line represents the mean ER for the same 64 ears for the second test obtained an average of 423 days later, and the short dashed line represents the mean ER for the same 64 ears for the third test obtained an average of 396 days later. Error bars represent ±1 standard error.

mean ER across frequency was similar for the three annual tests.

Of the 187 ears meeting inclusion criteria at baseline, 74 had only one test that met the inclusion criteria, and these ears were dropped from longitudinal analyses. Reasons for having only one valid ER test for these ears included loss to follow-up, examiner error, failure to meet the inclusion criteria for normal hearing and/or tympanometry on a given test date, or failure to meet the inclusion criteria for valid ER data, as defined above. The longitudinal analyses were performed for the remaining 113 ears from 75 subjects with multiple annual tests, for a total of 298 tests. This group consisted of 18 female and 57 male subjects with a mean age of 34.9 yr (SD = 5.4 yr). Of the 298 tests, 166 (56%) were from left ears. Linear mixed-effects regression models were used to evaluate the effects on ER at each selected frequency, 1.0, 2.0, and 4.0 kHz, of categorical variables for TPP, Y_{tm}, ear, and age. These frequencies were chosen for the regression models because the variance in ER with respect to differences in middle-ear condition based on 0.226-kHz tympanometry was greatest for this frequency range (Figure 5), and the variance in ER across subjects at these frequencies was large (Figure 1). The analysis partitions the variance associated with these factors (fixed effects) to determine how much of the variability in the repeated annual tests, if any, is associated with each. The analysis partitions the remaining variability in the longitudinal measures into random effects attributable to (1) between-subject differences, (2) between-ear variability (once the between-ear differences are accounted for as a fixed effect), and (3) longitudinal test-retest variability.

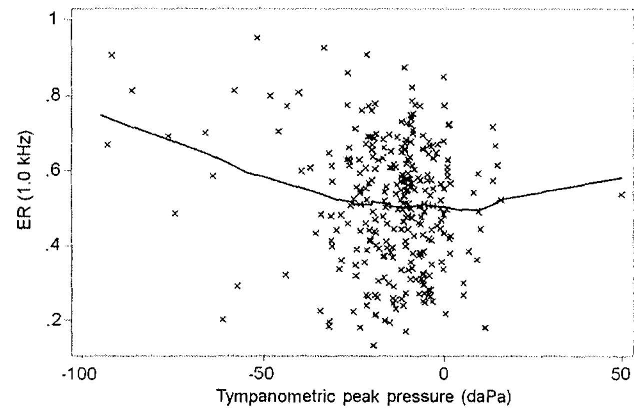

Figure 8. A scatterplot of ER at 1.0 kHz as a function of TPP for the 298 longitudinal observations is shown. The line plot is the LOWESS curve, a nonlinear regression fit to the data.

A scatterplot of ER at 1.0 kHz as a function of TPP for the 298 observations is shown in Figure 8. The line plot in the figure is the locally weighted scatterplot smoothing curve (LOWESS; Ruppert et al, 2003), a moving average of linear regression in a local neighborhood of x values fit to the data. It can be seen that the majority of the data points lie between 0 and about −30 daPa. The ER tends to increase as pressure increases or decreases from 0 daPa but is relatively flat between about 0 and −30 daPa. Because this is a nonlinear relationship and a linear regression model was used, TPP was divided into three categories within the normal range, with roughly equal numbers in each category. These were as follows: category A, ≤−20 daPa to −100 daPa (99 ears); category B, >−20 to ≤−10 daPa (106 ears); and category C, >−10 daPa (93 ears).

A scatterplot of ER at 1.0 kHz as a function of Y_{tm} for the 298 observations is shown in Figure 9. The LOWESS curve shows a locally weighted regression effect of Y_{tm} on ER at 1.0 kHz, which decreases as Y_{tm} increases to greater than 0.3 mmhos to about 1.25 mmhos

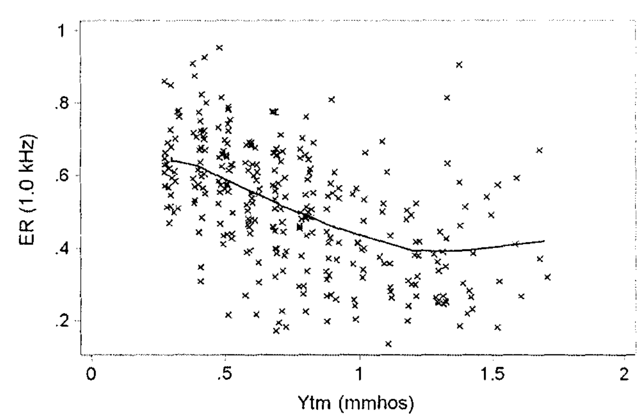

Figure 9. A scatterplot of ER at 1.0 kHz as a function of Y_{tm} for the 298 observations is shown. The LOWESS curve shows a nonlinear effect of Y_{tm} on ER.

and is flat for higher values of Y_{tm} to the limit of 1.7 mmhos. Because of this nonlinear relationship, Y_{tm} in the normal range was also divided into three categories with roughly equal numbers of ears in each: low Y_{tm}, ≥0.3 to ≤0.5 mmhos (96 ears); middle Y_{tm}, >0.5 to ≤0.8 mmhos (101 ears); and high Y_{tm}, >0.8 to ≤1.7 mmhos (101 ears). The age groups used previously for the baseline analyses were also used in the longitudinal models.

The linear mixed model output at each frequency showed that of the fixed-effect factors examined (TPP, Y_{tm}, ear, and age), neither TPP nor age accounted significantly for the variance in longitudinal ER measurements, and the results are not shown. However, ear and Y_{tm} were significant factors in one or more of the models, and those results are shown in Table 4. Ear was a significant factor in the 1.0-kHz model, with 0.038 higher ER for the left ear ($z = 2.16$, $p < .05$), and at 2.0 kHz, with 0.06 higher ER for the left ear ($z = 3.91$, $p < .001$), but was not a significant factor at 4.0 kHz. The 1.0-kHz model showed that Y_{tm} contributed significantly to ER test results. ER was 0.1 lower for tests in the middle Y_{tm} category compared with tests in the low Y_{tm} category and 0.176 lower for tests in the high Y_{tm} category compared with those in the low Y_{tm} category. However, Y_{tm} did not significantly explain ER results in the 2.0- and 4.0-kHz models.

The remaining variability in ER at each frequency that is not accounted for by the chosen fixed effects in the models is partitioned further into three random effects related to the sample: between subjects, between ears, and between tests (Table 4). The between-subject variability has an SD that is about 0.07 ER units for 1.0 and 2.0 kHz but is 0.126 ER units at 4.0 kHz. The remaining random variability of ER between ears, once differences between ears are accounted for as a fixed effect, has an SD of about 0.05 and is similar at each frequency. The residual test-retest variability once all the other factors are accounted for has an SD of about 0.1 or 10% at each frequency.

DISCUSSION

Baseline Analyses

Baseline measurement of ER for adults with normal hearing and normal 0.226-kHz tympanometry, as shown in Figure 1, were consistent with reports in the literature of high ER in the low frequencies decreasing to minima near 1.0 and 3.0 kHz, with higher ER at frequencies of greater than the high-frequency minimum (Voss and Allen, 1994; Margolis et al, 1999; Keefe et al, 1993; Feeney and Sanford, 2004; Shahnaz and Bork, 2006; Werner et al, 2010).

The mean ER for left ears at baseline was higher than the mean ER for right ears at frequencies less than the main minimum at 3.0 kHz but lower at higher frequencies (Figure 2). This is similar to the pattern observed for ambient measures of ER for ears with negative middle-ear pressure or low Y_{tm} (Figure 5) and is also characteristic of patients with ossicular fixation (Feeney et al, 2003; Shahnaz et al, 2009a,b; Voss et al, 2012). However, the difference between ears was only significant for this sample at two frequencies less than the minimum (2.0 and 2.5 kHz) and only one frequency greater than the minimum (6.3 kHz). Keefe et al

Table 4. Results for 1.0-, 2.0-, and 4.0-kHz linear mixed-effects models

	Fixed effect		Random-effects SD		
Model	ER coefficient	p Value	Between subjects	Between ears	Between tests
1.0 kHz			0.071	0.052	0.110
Ear (left reference)	0.038	0.029			
Low Y_{tm} (reference)					
Middle Y_{tm}	−0.100	<0.001			
High Y_{tm}	−0.176	<0.001			
2.0 kHz			0.067	0.061	0.082
Ear (left reference)	0.06	<0.001			
Low Y_{tm} (reference)					
Middle Y_{tm}	−0.033	0.068			
High Y_{tm}	−0.024	0.247			
4.0 kHz			0.126	0.050	0.091
Ear (left reference)	−0.03	0.058			
Low Y_{tm} (reference)					
Middle Y_{tm}	−0.017	0.409			
High Y_{tm}	−0.030	0.242			

The left ear is the reference for the effect of ear, and the low Y_{tm} category is the reference for Y_{tm}. The covariates of TPP category and age category were not found to be significant for any of the three frequencies modeled and are not shown. All models have 298 observations. See text for discussion.

(2000; see their Table 2) reported that infants have higher ER in the left than right ears at 0.353, 0.5, and 0.707 kHz, which is consistent with the present findings in adults, and lower ER at 2.0, 2.8, 4.0, and 5.7 kHz. In comparison, in a study of ER in young and elderly adults, Feeney and Sanford (2004) reported no significant differences between the right and left ears for the young group (n = 40; mean age, 21.4 yr), and there was a significant difference at only one frequency, 6.35 kHz, for the elderly group, with higher ER on the right (n = 30; mean age, 71.6 yr). Werner et al (2010) reported ER results for 92 infants (2–3 mo of age), 110 infants (5–9 mo of age), and 65 young adults in whom both right and left ear data were obtained. In their Figure 10 collapsed across age groups, ER in the right ear trends larger for frequencies from 280 to 600 Hz and again from 2 to 8 kHz. An ANOVA revealed a significant mean difference in ER between ears, with a small but significantly higher mean ER for the right ear of about 0.02 across frequencies, which is the opposite of the finding of higher ER in the left ear in the present study. Their ANOVA found a main effect for ear but no interaction with frequency or age. Of interest is that the right ear was tested first for the subjects in Werner et al (2010), but the left ear was tested first for the subjects in the present study. Different findings of ear effects on ER have been reported; such effects, if significant, are small relative to the difference in ER between groups of subjects with normal or impaired middle-ear responses. Further research is needed to assess how such small ear effects vary within and across subjects, the functional dependence on age, and any possible dependence on which ear is tested first (in a protocol in which both ears are tested).

A pattern of higher mean ER at frequencies less than the minimum at 3.0 kHz and lower mean ER at and greater than the minimum in ER was observed for ears from female subjects compared with ears from male subjects. This difference must be viewed with caution given the relatively small number of female compared with male subjects in this sample. Moreover, this difference might be related to the methodology of how reflectance was calculated using the cylindrical tube area to convert admittance to ER. Inasmuch as the cross-sectional ear-canal area is smaller in ears from female than from male subjects given overall body size, an ER procedure that took size into account would be preferable for analyzing gender differences. Keefe et al (2000) reported that ER was larger for ears from male than from female infants at 0.353, 0.5, 0.707, and 8.0 kHz, which differs from the present adult findings. Sex differences similar to those reported here have been reported for young adults (Feeney and Sanford, 2004), with significant differences at only three of the 1/3-octave frequencies tested: 0.794, 1.0, and 5.04 kHz. Shahnaz and Bork (2006) measured ER in 126 Caucasian and Chinese adult subjects and reported that the effect of sex on ER was not significant. Similarly, Werner et al (2010) found no significant effect of sex on ER in adults.

There were some significant differences among mean ER measurements for the middle age group relative to both the young and old age groups at frequencies between 0.5 and 1.6 kHz: the middle age group had significantly lower ER (Figure 4 and Table 3). There were no significant differences at any frequency between mean ER for the young age group and the old age group. No previous reports have examined age differences in wideband ER across the range from 20 to 59 yr. However, Feeney and Sanford (2004) reported an age effect in wideband ER between a group of young adults (mean age, 21.4 yr) and a group of older adults (mean age, 71.6 yr). The young group had higher ER in the midfrequency range and lower ER at higher frequencies than the old group, which was interpreted to suggest that the wideband ER pattern for the young group was more stiffness dominated than that for the old group. The results of the present study indicate a nonmonotonic change in ER over time with reduced ER between 0.5 and 1.6 kHz for the middle age group, but similar ER for both the young and old groups (Figure 4). A nonmonotonic aging of middle-ear function has been suggested previously in the data of Jerger et al (1972), who reported that Y_{tm} at 0.220 kHz increased to a maximum in the fourth decade and decreased for older subjects. However, the age data from the present study should be interpreted with caution given the disproportionately large number of subjects in the middle age group combined with the sex differences between the age groups (Table 2), which might have contributed to this finding.

When the mean ER for the subjects with normal hearing and normal 0.226-kHz tympanometry was compared with the ER for subjects with abnormal 0.226-kHz tympanometry (Figure 5), there were some systematic differences consistent with the model by Voss et al (2012), which characterizes patterns of ER for various pathologies. Twelve subjects with abnormally low Y_{tm} had mean wideband ER that was higher than the mean normal ER at frequencies from about 0.5 to 4.0 kHz by as much as 18% (1.6 kHz). This is similar to the Voss et al (2012) model for ears with ossicular fixation. This pattern of higher-than-normal low-frequency ER was similar to the case for ears with TPP more negative than −100 daPa, with a difference from normal in ER as great as 24% (1.6 kHz). This is consistent with the Voss et al (2012) model for the effect of negative middle-ear pressure on reflectance and is similar to reported effects of middle-ear pressure on ER (Feeney et al, 2003). Thus when making wideband ER measurements at ambient pressure, adult ears with abnormal TPP might be expected to have ER outside the normal range. This could occur even when pure tone thresholds are within normal limits, as was the case for 20 of the 24 subjects with abnormal tympanometry in the present study. A method for distinguishing otherwise normal middle ears with negative middle-ear pressure from ears with other middle-ear abnormalities would be to perform tests at TPP by using wideband tympanometry so that a subject's ER pattern at TPP can be compared with normative data (Liu et al, 2008).

Longitudinal Analyses

The data from Figures 6 and 7 exhibit the similarity among measures of wideband ER obtained years apart in the same subjects. Ears from the different subjects in Figure 6 suggest the range of patterns that are possible in ears with normal hearing. Figure 6 also shows how the ER pattern for two ears of the same subject might be more similar than the ER pattern for two ears from two subjects. Voss et al (2008) showed in a human temporal bone preparation that large changes in wideband ER can be made by changing the volume of the middle-ear space. Thus one reason for differences in wideband ER between subjects might be related to differences in the middle-ear space, which would be similar for the two ears of a single subject.

Mixed-effects linear regression models of ER at 1.0, 2.0, and 4.0 kHz were used to evaluate the effects of fixed effects of TPP, Y_{tm}, ear, and age on ER over time in ears with normal hearing and normal 0.226-kHz tympanometry. Ear had a significant effect on ER in the 1.0- and 2.0-kHz models, with higher ER on the left, as was the trend for baseline measures. Y_{tm} also had a significant effect on ER for the 1.0-kHz model, with lower ER associated with higher values of Y_{tm}. However, Y_{tm} did not significantly explain ER at 2.0 and 4.0 kHz. This suggests that low-frequency tympanometry cannot significantly account for variation in middle-ear function across the frequency range important for speech perception.

The fact that age did not contribute significantly to ER is not surprising given that there was a minimal effect of age shown in the cross-sectional data (Figure 4). Similarly, TPP was not a significant contributor to ER, which might be expected given the truncated range of pressure values in the normal range. However, as pressure varies from 0 daPa in individual ears, ER is expected to increase at frequencies of less than about 2.0 kHz (Figures 5 and 8, and Feeney et al, 2003). Thus for comparisons with a normative database, it might be advantageous in adults to obtain wideband measurements at TPP.

The test-retest variability was found to have an SD that was about 0.1 at all three frequencies tested (last column of Table 4). This is in good agreement with the data by Rosowski et al (2012), who made four power reflectance measurements separated by a week each on seven adults. The SD for those measurements was also about 0.1 at 1.0, 2.0, and 4.0 kHz (see their Figure 5). This is also in agreement with the data from Werner et al (2010), who reported that the mean absolute test-retest difference between two power reflectance measurements obtained a week apart in adults was approximately 0.1 (see their Figure 6). This suggests that test-retest measurements at these frequencies should fall within ± 0.1 ER units about 70% of the time (± 1 SD = 68.2% of normal distribution). One source of variability in repeated ER measurements might be small acoustic leaks between the foam ear tip and the ear canal (Keefe et al, 2000). This might be overcome by using an impedance probe tip to obtain a hermetic seal, as is done for tympanometry.

CONCLUSION

Annual wideband ER measurements were obtained in a group of adults with normal hearing and normal 0.226-kHz tympanometry. Individual patterns of wideband ER remained stable over multiple annual tests, and the test-retest variance was found to be about 0.1 at 1.0, 2.0, and 4.0 kHz, which is similar to previously published data. In subjects with normal hearing and normal 0.226-kHz tympanometry, ER at 1.0 kHz was affected by Y_{tm}, but this was not a significant effect at 2.0 and 4.0 kHz, suggesting that low-frequency tympanometry does not account for middle-ear function across the frequency range important for speech perception. The relationship between ER in subjects with Y_{tm} outside the normal range or with excessive TPP was higher or lower than normal by 20% or more in the frequency range from about 0.5 to 2.0 kHz (Figure 5). As a result, it might be difficult to distinguish ER patterns associated with middle-ear disorders resulting in low Y_{tm} (e.g., otosclerosis) from the ER pattern associated with negative middle-ear pressure without compensating for the pressure during measurement, as is done currently for single-frequency tympanometry.

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