Title: Age and gender effects on wideband absorbance in adults with normal outer and middle ear function

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Short Title: Wideband Energy Absorbance in Adults

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Abbreviations:

EA	Energy absorbance	TW	Tympanometric width
ER	Energy reflectance	Vea	Ear canal volume
OAE	Otoacoustic emission	WAI	Wideband acoustic immittance
RMS	Root-mean-squared	Y_{tm}	Peak compensated static admittance
TPP	Tympanometric Peak Pressu	re	

ABSTRACT

Objectives: This study examined the effects of age and gender on wideband energy absorbance in adults with normal middle ear function. Design: Forty young adults (14M/26F, aged 20-38 yr), 31 middle-aged (16M/15F, aged 42-64 yr), and 30 older adults (20M/10F, aged 65-82 yr) were assessed. Energy absorbance (EA) data were collected at 30 frequencies using a prototype commercial instrument developed by Interacoustics. **Results:** Results showed that young adults had significantly lower EA from 400 Hz to 560 Hz than the middle-aged group. However, the middle-age group showed significantly lower EA between 2240 Hz and 5040 Hz than the young adult group. Additionally, the elderly had significantly lower EA than the young adult group between 2520 to 5040 Hz. No significance difference in EA was found at any frequency between middle-aged and older adults. Across age groups, gender differences were found with males having significantly higher EA values than females at lower frequencies, whereas females had significantly higher EA at higher frequencies. **Conclusions:** This study has provided evidence of the influence of gender and age on EA in adults with normal outer and middle ear function. These findings support the importance of establishing age- and gender-specific EA norms for the adult population.

INTRODUCTION

With aging, changes may occur in the human auditory system including the outer and middle ear. Changes in the outer and middle ear associated with aging have been documented in the literature. These may include collapsing of the cartilaginous external auditory canal (Randolph and Schow 1983), stiffening of the tympanic membrane and ossicular chain (Etholm and Belal 1974; Ruah et al. 1991). Hinojosa and Naunton (1980) summarized a number of tissue changes in the aging ear canal including a reduction of epithelial cell production, aging of extracellular substances (elastic tissue, cartilage, and bone), and extracellular deposition of various materials. Weinstein (1994) further remarked that aging may lead to degeneration of the outer and middle ear with structural changes to the tissue lining, ceruminal gland atrophy, degeneration of the incudomalleal and incudostapedial joints of the ossicles, and tensor tympani and stapedius muscle atrophy. These changes may decrease the efficiency of sound transmission into the middle ear, resulting in reduced hearing sensitivity.

The aging effects on the outer and middle ear system have been studied using conventional 226-Hz tympanometry. Holte et al. (1996) measured peak compensated static admittance (Y_{tm}) in adults aged 20 to 79 years and found no significant age effect. This finding is supported by Uchida et al. (2000) who obtained tympanometric data from adults ranging in age from 40 to 70 years, and Margolis and Heller (1987) who reported data on adults aged between 19 and 61 years. However, when Margolis and Heller's (1987) data were compared with Wiley et al.'s (1996) data obtained from adults aged 48 to 92 years, the participants in Margolis and Heller's (1987) study had significantly greater Y_{tm} , smaller ear canal volume (V_{ea}) and larger tympanometric

width (TW). In another study, Roup et al. (1998) found that their participants, aged 20 to 30 years, had significantly higher V_{ea} and smaller TW than the participants in Margolis and Heller's (1987) study. Nevertheless, these differences in 226-Hz tympanometric parameters across age groups between studies do not provide sufficient evidence of an age effect on the outer and middle ear because of the differences in subject samples, equipment and experimental design.

Besides age, gender may be an influencing factor. Roup et al. (1998) and Wiley et al. (1996) found that Y_{tm} and V_{ea} were higher in males than females, while TW was higher in females than males. Shahnaz and Bork (2006) reported a similar effect with females having significantly smaller Y_{tm} than males. In contrast, Wan and Wong (2002) observed an opposite effect with females having significantly greater V_{ea} than males. Surprisingly, Margolis and Heller (1987) did not find any significant gender effect for Y_{tm} and TW. In summary, the effects of age and gender on tympanometric measures are inconclusive. These conflicting findings may be attributed to a combination of influencing factors including subject sampling, test protocol and the use of a single frequency probe tone, which provided limited information about the mechano-acoustic properties of the outer and middle ear system.

In recent years, wideband acoustic immittance (WAI) measures have been developed to evaluate the mechanical properties of the conductive pathway (i.e. outer and middle ear) over a wide frequency range (Keefe et al. 1993; Keefe and Levi 1996). Of the WAI measures, energy reflectance (ER) was most commonly investigated by researchers. ER can be expressed as a ratio of the energy reflected back from the middle ear to the incident energy at the probe tip (Voss and Allen 1994). ER ranges from 0 to 1, with 0 meaning that all of the sound energy is absorbed by the middle ear, and 1 indicating that all of the energy is reflected (Stinson 1990). Provided that the

probe tip is fitted snugly in the ear canal with no leakage, the measurement of ER does not depend on the distance of the probe tip from the eardrum (Voss and Allen 1994).

The main advantages of measuring ER over the conventional 226 Hz tympanometry are: (1) it does not cause discomfort to patients because it can be conducted at ambient pressure, and (2) it captures responses over a wide frequency range (0.25-8 kHz) which is important for speech perception (Piskorski et al. 1999; Feeney et al. 2003; Keefe and Simmons 2003).

To date, only a few normative ER data sets measured at ambient pressure for normally hearing adults have been published (Keefe et al. 1993; Voss and Allen 1994; Feeney and Sanford 2004; Shahnaz and Bork 2006). Typically, the results show that the ER is high (more energy being reflected) at frequencies below 1 kHz, decreases to a minimum between 2 and 4 kHz, and increases beyond 4 kHz. While normative ER data for adults are available, reports on the effect of age and gender on ER are scant in the literature. The majority of studies provided ER norms for young adults aged from 18 to 30 years (e.g., Voss and Allen 1994; Feeney and Sanford 2004; Shahnaz and Bork 2006). Feeney and Sanford (2004) were the first to compare ER among young and older adults. They found a significant decrease in ER from 0.8 to 2 kHz, but an increase near 4 kHz in older adults (60-85 years) when compared to that in young adults (18-28 years). These results suggest a decrease in stiffness of the outer and middle ear with age. The authors also found differences in the ER pattern with 15 percent of young adults versus 63 percent of older adults showing two minima (i.e. one between 1 and 2 kHz, and the other between 4 and 5 kHz). They suggested that the secondary low-frequency ER minimum found in the majority of the older adults may be associated with reduced middle-ear stiffness in older adults when compared to young adults. In 2012, Rosowski et al.'s analysed ER in 29 adults aged 22 to 64 years

with normal middle ear function, as determined by a combination of otoscopic examination, pure tone audiometry and tympanometry. Rosowski et al. (2012) did not find any significant difference in ER as a function of age, except at 1000 Hz. They also found positive significant correlation between ER and age, and umbo velocity at 1000 Hz, which was consistent with an increase in tympanic membrane mobility with age. In another study, Carpenter et al. (2012) reported a significant age effect for ER from 1007 to 5039 Hz with young adult (18-25 years) having significantly lower ER than the older groups (50-65 years). They suggested that hormonal influences might account for differences in ER with age.

The effect of gender on ER in the adult population has not been extensively investigated (Shahnaz et al. 2013). Feeney and Sanford (2004) found a significant gender effect on ER in the young adult group, with females having higher ER in the low-to-mid frequencies, but lower ER at 4 kHz than males. However, no significant gender effect was demonstrated in the older adult group. Although Rosowski et al. (2012) reported that ER measured from females were generally higher than males at frequencies \leq 2000 Hz and lower from 3000 to 6000 Hz, only ER at 4000 Hz was found to be statistically significant with gender. Besides the two aforementioned studies, gender differences have also been reported by Carpenter et al. (2012). In Carpenter et al.' study, females had significantly higher ER than males from 1500 to 2531 Hz, but lower ER from 1007 to 1265 Hz and from 3000 to 5039 Hz. In contrast, Shahnaz and Bork (2006) reported no significant gender effect on ER for both Caucasian and Chinese adults. To date, the results have not provided convincing evidence on the effect of gender on ER findings.

To sum up, the above overview of the literature indicates a scarcity of normative data in the adult population. While an aging effect of the outer and middle

ear system is proposed based on the ER results obtained from young and older adults (Feeney and Sanford 2004), it is not clear if there are any changes in ER for adults in the intermediate age range (e.g., between 42 and 64 years), when compared to both the younger and older adult groups. There is presently no information about the possible gender effects for this intermediate age group. Therefore, the specific goal of the present study was to extend Feeney and Sanford's (2004) study by investigating possible age and gender effects in adults across three age groups.

Materials and Methods

Subjects

A total of 101 adults participated in this study. Participants were divided into groups based on age: Group 1 consisted of 40 young adults (14 men and 26 women) aged between 20 and 38 years (mean = 25.7 years; SD = 4.9), Group 2 consisted of 31 middle-aged adults (16 men and 15 women) aged from 42 to 64 years (mean = 55.5 years; SD = 5.4), and Group 3 consisted of 30 older adults (20 men and 10 women), with age between 65 and 82 years (mean = 72.8 years; SD = 4.9). These age ranges were categorized according to Erikson's definition with young adults defined as between 20 and 40 years of age, middle-aged adults as between 40 and 64 years, and older adults as 65 years and older (Erikson 1963). In regard to ethnicity, participants in the present study were made up of 24 Malays, 66 Chinese, and 11 Indians. The distribution of participants by age group and gender for each ethnic group is shown in Table 1.

Participants from the three groups were included in the study if they met the following inclusion criteria: (1) negative history of otologic disease; (2) normal ear canal and tympanic membrane as documented by otoscopic examination; (3) normal

226-Hz tympanogram defined as having a single peak between +50 and -100 daPa with Y_{tm} between 0.3 and 1.8 mmho (Margolis and Hunter 1999); (4) passed an ipsilateral acoustic stapedial reflex screening test at 95 dB HL for 0.5, 1 and 2 kHz stimuli; (5) pure tone air conduction thresholds better than 25 dB HL from 0.25 to 8 kHz with an air-bone gap of \leq 10 dB at octave frequencies from 0.25 to 4 kHz.

Instrumentation

Pure tone audiometry was conducted using a Grason-Stadler GSI 61 Clinical Audiometer. Supra-aural headphones (TDH-50P) or insert earphones (ER3A) and a bone vibrator (B71) were used to measure a participant's air and bone conduction hearing thresholds. Tympanometry and acoustic stapedial reflex screening was performed using a Grason-Stadler GSI Tympstar Middle Ear Analyzer. Instruments used for pure tone audiometry and acoustic immittance screening were calibrated according to American National Standards Institute (ANSI) specifications, S3.6-1996 and S3.6-1987, respectively.

Measures of WAI were made using a wideband tympanometry research system (Interacoustics, Version 3.2.1). This system consists of a custom WAI ear probe with two receiver ports and one microphone port, an Interacoustics AT235 Middle Ear Analyzer that contains a pressure pump and controller system, a CardDeluxe professional sound card with 24 bits of resolution, a dedicated PC with Windows XP operating system, and the Reflwin software. Calibration was performed every day (Keefe and Simmons 2003) at ambient pressure to determine the source reflectance and incident sound pressure associated with the probe and its transducers based on acoustic measurements in two rigid-walled, cylindrical calibration tubes that were open at one end and closed at the other end with a steel rod. The adult calibration

tubes had lengths of 290.50 and 8.10 mm, each with a diameter of 7.9 mm. Rootmean-squared (RMS) reflectance error function was generated to determine the error in the acoustical estimate of the length of each tube relative to the acoustic wave propagation model. An RMS reflectance error of less than 0.009 was required for a successful calibration.

Procedures

Ethical approval for this study was obtained from the Universiti Kebangsaan Malaysia Ethical Review Committee. The aim of the study and the test procedures were explained to all participants verbally and in written form prior to data collection. Participants were also required to sign a consent form for participation in this study.

All testing was conducted by a final year undergraduate audiology student under the supervision of an experienced clinical audiologist. Training was provided to the audiology student until she was familiar with the test procedures. All measurements were performed in a double-walled sound-treated room at the Audiology Clinic of Universiti Kebangsaan Malaysia Medical Centre with an ambient noise of less than 30 dBA as measured by a Quest 2900 sound level meter. Before testing began, a visual inspection of the ear canal and tympanic membrane was conducted by an experienced otologist using an otoscope to check for any abnormalities of the outer and middle ear. Only one ear, chosen at random, was tested. In instances where abnormalities of the ear canal or tympanic membrane were detected in one ear, the other ear was considered for testing and inclusion in the study. Pure tone audiometry was always performed before acoustic immittance screening. Air and bone conduction thresholds were obtained using the modified Hughson-Westlake technique (Carhart and Jerger 1959). Conventional tympanometry with a probe tone of 226 Hz was used to assess a participant's middle ear status. Tympanogram was obtained by sweeping the pressure from +200 daPa to -400 daPa at a speed of 200 daPa/sec. Using the same equipment and immediately following tympanometry, an ipsilateral acoustic stapedial reflex test was performed at a fixed intensity level of 95 dB HL for the 0.5, 1 and 2 kHz stimuli to ensure normal middle ear function.

Participants who met the inclusion criteria were assessed using the WAI device. Energy absorbance (EA), which is defined as one minus ER, was measured. EA rather than ER was recommended to evaluate the conductive characteristics of the outer and middle ear (Feeney et al. 2013). To start EA measurements, a clean rubber tip of appropriate size was placed on the probe assembly to achieve a hermetic seal. EA was obtained under ambient ear canal pressure by recording acoustic response to clicks, presented at 55 dB SPL at a rate of one click per 46 ms, to the participant's ear. Responses from a total of 32 clicks were averaged for each measurement. During testing, a visual display with high absorbance at frequencies below 1 kHz served as an on-screen prompt that informed the tester for a possible probe leak. The WAI device automatically generates EA data as a function of frequency from 220 Hz to 8000 Hz. However, only EA data between 280 Hz and 8000 Hz were analysed in this study.

Statistical Analysis

Descriptive statistics of EA, including mean and 90% range, at 30 one-sixthoctave frequencies from 280 Hz to 8000 Hz were provided. A repeated measures analysis of variance (ANOVA) was used to evaluate the effects of age, gender and

frequency on EA. The Greenhouse and Geisser (G-G) approach (Greenhouse and Geisser 1959) was used to compensate for the violation of compound symmetry and sphericity and also to reduce Type 1 error. The significance of any main effects or interactions was assessed at 0.05 significance level.

RESULTS

Tympanometric data obtained from each age group and gender are shown in Table 2 and Table 3, respectively. A factorial model, which included two factors [age group (young adult/middle-age/elderly) and gender (male/female)], and all interactions, was fitted to the data to investigate the effects of these potentially influencing variables on the measured tympanometric parameters [i.e. Y_{tm}, V_{ea}, and tympanometric peak pressure (TPP)]. Results from this analysis revealed a statistically significant gender effect for the V_{ea} parameter [F(1,93) = 4.941, p = 0.029], with mean V_{ea} for females were significantly smaller than for males (1.15 vs 1.29 mmhos). No other significant effects or interactions were found. Using ANOVA analyses of Y_{tm}, V_{ea} and TPP on gender, no significant difference in these parameters was found for each age group.

The descriptive statistics of EA data obtained at ambient pressure across age groups are shown in Figure 1. The mean EA results for the three groups show a similar characteristic trend with minimum mean EA at 280 Hz, increasing with frequency to reach a maximum between 1590 Hz and 3170 Hz, and then decreasing until it saturates at 6350-7130 Hz. The 95th percentile EA results also show the same trend for the three age groups. However, there are large differences in the 5th percentile EA results in the high frequencies (> 4 kHz) among the age groups, despite having a similar trend to the mean EA results.

(Insert Figure 1 about here)

The ANOVA results revealed a significant main effect for frequency [F(29, 350) = 139.979, p < 0.001], indicating that EA varied across the frequencies tested. Additionally, the interaction between frequency and age [F(58, 350) = 2.367, p = 0.02] and the interaction between frequency and gender [F(29, 350) = 3.352, p = 0.013] were significant. These results indicate that the variation of EA across frequencies differed among age groups and across genders. The main effects of age [F(2, 95) = 0.613, p = 0.544] and gender [F(1, 95) = 0.075, p = 0.784] did not reach significance (p > 0.05). The interaction between age and gender was not significant [F(2, 95) = 0.375, p = 0.689].

To further investigate the interaction between frequency and age, a post hoc Tukey Honestly Significantly Different (HSD) test was performed. The results showed that middle-aged adults had significantly higher mean EA from 280 Hz to 500 Hz than young adults (p < 0.05) (see Figure 1). However, at frequencies between 2000 Hz and 5040 Hz, young adults showed significantly higher mean EA than middleaged adults. Young adults also showed significantly higher mean EA from 2240 to 5040 Hz than the elderly group. No significance difference in EA was found in this frequency range between the middle-aged and elderly groups.

In examining the mean EA pattern obtained from the three age groups (Figure 1), it was noted that the young adults had a single EA maximum pattern with a peak occurring at a frequency of 2830 Hz, while both middle-aged and elderly groups showed two peaks of EA. Furthermore, these two peaks for both middle-age and elderly groups occurred at the same frequencies which were at 1780 Hz and 2830 Hz, respectively.

In view of the significant frequency and gender interaction, a Tukey's HSD post-hoc test was applied to the EA data collapsed across all age groups. The results show that females had significantly lower EA between 280 and 790 Hz than males (p < 0.05), while females had significantly higher EA between 2830 and 4490 Hz than males (p < 0.05) (see Figure 2).

(Insert Figure 2 about here)

To further analyse the gender effect, the mean EA values of the males are compared with that of the females for each age group using ANOVA analysis. Figure 3-5 shows the mean EA values against frequency for both genders for each age group. Results from ANOVA analysis showed no significant differences in EA between males and females at any frequency in both young adult and elderly groups. However, significant gender differences in EA were observed for middle-aged group from 500 to 790 Hz. In specific, males in the middle-age group had significantly higher EA values than females at this frequency range.

(Insert Figures 3-5 about here)

DISCUSSION

One of the aims of the present study was to investigate the effect of aging on the acoustic-mechanical properties of the outer and middle ear via wideband energy absorbance measures. The main results of this study revealed that EA results changed depending on the age of the participants (Figure 1). While young adults had slightly lower mean EA than the middle-aged and older adults from 280 to 1780 Hz, they attained significantly higher EA from 2000 Hz to 5040 Hz than the other two groups. This implies that more power is absorbed by the middle ear of young adults than older adults. This finding suggests that anatomical and physiological differences, which

may influence sound transmission into the middle ear, might exist among the age groups. The present findings are in agreement with Feeney and Sanford (2004) who compared EA results between young and older adults and found that young adults had significantly higher EA (lower ER) at 4000 Hz than older adults. Similar patterns of higher EA among young adults relative to both older groups reported in the present study are also consistent with Carpenter et al. (2012) who found significant differences in ER from 1007 to 5039 Hz.

The present study noted the small variation of EA between 794 and 2000 Hz with no significant difference in EA at these frequencies among the age groups. This result is not in agreement with the finding of Feeney and Sanford (2004) who found that older adults had significantly higher EA (lower ER) in this frequency region than young adults. The discrepancy in findings may be attributed to differences in middle ear pressure between two studies. The effects of middle ear pressure on ER have been investigated in a recent study by Shaver and Sun (2013). They demonstrated that ER increases for low-to-mid-frequencies (~0.2 between 1000 Hz and 1500 Hz) and decreases for frequencies above 3000 Hz (>0.10 between 4500 Hz and 5500 Hz) when the middle ear pressure is varied by as low as -40 to -95 daPa. In this particular study, the TPP was required to be within ± 25 daPa of ambient pressure. Feeney and Sanford (2004) adopted a more stringent subject inclusion criterion which was TPP of ± 10 daPa of the ambient pressure, whereas the present study included TPP between +50 daPa and -100 daPa as a criterion. As shown in Table 2, there were large variation in TPP value among age group. It is possible that the difference in EA did not reach statistical significance in the current study because of the large intersubject variability in TPP.

Overall, the observed differences in EA in the high frequencies among the three age groups found in the present study suggest that ER changes associated with aging can be attributed to the loss of stiffness in the middle ear as proposed by Feeney and Sanford (2004) in their simple model of the middle ear. Although this particular finding is in contrast with anatomical studies suggesting increased middle ear stiffness with age (e.g. Etholm and Belal 1974; Randolph and Schow 1983; Ruah et al. 1991), Doan et al. (1996) proposed that a reduction in tonic middle ear muscle contraction and changes in the rotational axis of the ossicles could contribute to reduced stiffness in the aging middle ear. In addition, the assumptions of uniform cross-sectional area and rigid ear canal walls in deriving EA may potentially contribute to differences in EA between groups in the present study. This notion is based from Voss et al. (2008) who reported small but consistent differences in ER that vary with the cross-sectional area of the ear canal. With regard to rigidity of the ear canal wall, this assumption may be violated because the ear canal wall of the adult human ear canal is composed of cartilage and soft tissues making it not perfectly rigid and subjected to small deviation during EA measurement (Rosowski et al., 2013).

An important finding from the present study is that there was no significant difference in EA at any frequencies between the middle-aged and elderly groups while the young adult group had higher mean EA than the other aged groups. When these results are interpreted in terms of aging, there is evidence to showed that aging of the outer and middle ear might have started from middle age (i.e., 42 years and older) in a sample of non-Caucasians with the majority being Chinese (i.e. 66 out of 101 ears). Further research is needed to investigate the effect of aging in a middle-aged group of Caucasians.

The mean of EA from the young adults group in the current study is plotted alongside with the normative values developed by Keefe et al. [1993], Feeney et al. [2004], and Shahnaz et al. [2006] (see Figure 6). These three studies were chosen for comparison because the participant fall within the specified age range of the young adults used in the present study. In specific, the study by Shahnaz et al. [2006] were obtained from a total of 237 ears from 126 subjects aged 18 to 32 years, while Feeney et al. [2004] and Keefe et al. [1994] established their normative data from young adults aged 18 to 28 years.

(Insert Figure 6 about here)

The data in Figure 6 show good agreement at all frequencies of the present study and those from Shahnaz et al. (2006) and from Keefe et al. (1994). However, the present study shows lower mean EA at frequencies between 1000 Hz and 4000 Hz than Shahnaz et al. (2006), the mean EA data from this study are within ± 1 SD of the current study. On the contrary, the mean EA data for the young adults in the present study are significantly higher than Feeney et al. (2004) from 250 Hz to 3175 Hz, but the pattern reverse for frequency above 3175 Hz. In examining the ER pattern, the young adult in the present study demonstrated a single minimum pattern which is similar to those obtained from the study by Feeney et al (2004) and Shahnaz et al (2006). For instance, 34 of the 40 young adults (85%) in Feeney et al.'s study showed the single minimum pattern, while Shahnaz et al. (2006) reported that the single minimum pattern emerges once the norms from the Caucasian are pooled with the Chinese young adults.

The other aim of the present study was to investigate the effect of gender on the conductive properties of the outer and middle ear as measured using EA. In general, there was evidence of a gender effect on EA measured at ambient ear canal

pressure (see Figure 2). Specifically, males had significant higher EA than females for frequencies below 1000 Hz, although the differences were small and might not be clinically significant. In contrast, females had higher EA from 2830 Hz to 4490 Hz than males. When gender difference was examined within each age group, differences in EA were found to be significant only for the middle-aged group between 500 Hz and 790 Hz (see Figure 4). The gender effect demonstrated in the present study is consistent with Feeney and Sanford (2004) who reported significant gender differences with females having lower EA (higher ER) than males at 794 and 1000 Hz, but higher EA (lower ER) at 5040 Hz. Carpenter et al. (2012) also found significant gender related differences in EA from 1007 to 1265 Hz and from 3000 to 5039 Hz. Shahnaz and Bork (2006) suggested that difference in EA between males and females may be attributed to differences in both middle ear and ear canal volume due to difference in body size between the two genders. As shown in Table 2, males showed generally higher V_{ea} values than females, but a gender difference in the present study was observed only for V_{ea} (male = 1.30 mmho, female = 1.14 mmho) when data were pooled across age group. In addition, the present study revealed no significant differences in Vea between age groups. Therefore, it may be reasonable to suggest that gender-related differences in EA demonstrated in the present study were contributed by significant differences in Vea between the two groups.

Margolis and Hunter (1999) analyzed the effects of gender on acoustic impedance and found that males had less resistance between 2000 Hz and 4000 Hz than females. Based on this finding, they proposed that males had ear drums that were less stiffness- dominated relative to females at frequencies below 1000 Hz. Based on the hypothesis put forward by Margolis and Hunter (1999), the findings in the present study show that females may have stiffer middle ear system than males. Moreover, it

was indicated in many studies that the hearing acuity in males was poorer than females at higher frequencies (Gates et al., 1990; Pearson et al., 1995; Wilson et al., 1999; Uchida et al., 2000). One of the confounding factors for such findings could be related to males being exposed more to work or social noise than females. Since the present study screened out participants from noise-induced hearing loss and otological disease in various ways, the effect of gender on ER demonstrated in the present study can be considered to be genuinely related to differences in the acoustic-mechanical properties of the middle ear between the genders. One of the limitations in conducting the present study was related to the inclusion criteria of an air-bone gap of \leq 10 dB HL to rule out significant middle ear pathology. It was reported by Nixon et al. (2004) that an average air-bone gap of 12 dB was measured from their normal hearing older group with age ranging between 50-59 years. Thus, it is possible that the present study underestimated the effect of aging on the middle ear by excluding those with greater high frequency air-bone gaps, which may be the norm for the older age groups.

Another limitation of this study is the use of 226-Hz tympanogram to quantify the middle ear status of each participant. It was shown in previous studies that normal 226-Hz tympanogram had been observed in the cases of middle ear disorders such as ossicular disarticulation, otoslcerosis and otitis media with effusion (Hunter and Margolis 1997; Feeney et al. 2003), which will affect middle ear stiffness and resulting in significant differences on acoustic mechanical properties due to aging and gender effects. To control for this confounding variable, the ipsilateral acoustic stapedial reflexes, which has been found to be sensitive in ruling out the middle ear pathology (Gelfand 2009), was used in the present study to further verify the condition of the middle ear.

In summary, the present study provides important information regarding ageand gender-related changes on EA measurements in the adult population. It is demonstrated in the present study that the acoustic-mechanical properties of a healthy middle ear differ between: (1) young and middle-aged, (2) young and elderly, and (3) males and females. The present study also confirmed that EA between middle-age and elderly group was not significantly different. In addition, this study provided a preliminary EA data for the intermediate age range in the adult population, which have not been reported in the literature. As the present study was performed in a multiracial country which composes of three main ethnic of Malays, Chinese, and Indians, it is imperative to investigate the effect of ethnicity on EA measures in the future study.

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Figure Legends

Figure 1: The mean and 90% range of EA for the three age groups as a function of frequency. The vertical bars indicate ± 1 SE.

Figure 2: The group mean one-sixth-octave EA for males (N=50, solid line and filled squares) and females (N=51, dashed line and filled triangles) as a function of frequency. The vertical bars indicate \pm 1SE.

Figure 3: The group mean one-sixth-octave EA for young adult males (N=14, solid line and filled squares) and young adult females (N=26, dashed line and filled triangles) as a function of frequency. The vertical bars indicate \pm 1SE.

Figure 4: The group mean one-sixth-octave EA for middle-aged males (N=16, solid line and filled squares) and middle-aged females (N=15, dashed line and filled triangles) as a function of frequency. The vertical bars indicate \pm 1SE.

Figure 5: The group mean one-sixth-octave EA for elderly males (N=20, solid line and filled squares) and elderly females (N=10, dashed line and filled triangles) as a function of frequency. The vertical bars indicate \pm 1SE.

Figure 6: Comparison of group EA mean for the young adult group as a function of frequency between Shahnaz et al. (2006), Feeney et al. (2004), Voss et al. (1994), and the current study. The vertical bars indicate \pm 1SD.

Table 1: Distribution of participants by age group, gender, and ethnicity.

Male					
Age group	Malays	Chinese	Indians		
Young adults	4	10	2		
Middle-aged	4	5	5		
Elderly	4	14	1		

Female

Age group	Malays	Chinese	Indians
Young adults	9	16	1
Middle-aged	2	11	1
Elderly	1	10	1

Table 2: Tympanometric parameters for the young adults, the middle-aged, and the elderly groups. The means, standard deviations (SDs) and ranges of values are shown for peak compensated static admittance (Peak Ytm), equivalent ear canal volume (Vea) and tympanometric peak pressure (TPP).

T	Young Adults		Middle-aged		Elderly	
Tympanometric _ Parameter	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Peak Ytm (mmhos)	0.57 (0.26)	0.30 - 1.20	0.54 (0.23)	0.3 – 1.20	0.60 (0.63)	0.3 – 1.7
Vea (mmhos)	1.15 (0.26)	0.6 - 1.90	1.22 (0.33)	0.6 - 2.00	1.31 (0.31)	0.78 - 2.00
TPP (daPa)	16.59 (11.06)	-23 - 25	10.16 (19.6)	-55 - 25	12.33 (24.77)	-70 - 45

Table 3: Tympanometric parameters for the young adult, the middle-aged, and the elderly groups stratified by gender. The means and standard deviations (SDs) are shown for peak compensated static admittance (Peak Ytm), equivalent ear canal volume (Vea) and tympanometric peak pressure (TPP).

Tymponomotric	Young Adults		Middle-aged		Elderly	
Tympanometric Parameter	Male (n = 14) Mean (SD)	Female (n= 26) Mean (SD)	Male (n = 16) Mean (SD)	Female (n = 15) Mean (SD)	Male (n = 20) Mean (SD)	Female (n = 10) Mean (SD)
Peak Ytm (mmhos)	0.68 (0.34)	0.52 (0.19)	0.57 (0.21)	0.52 (0.25)	0.70 (0.77)	0.45 (0.22)
Vea (mmhos)	1.22 (0.33)	1.12 (0.21)	1.32 (0.34)	1.13 (0.29)	1.33 (0.32)	1.22 (0.26)
TPP (daPa)	16.92 (13.16)	16.42 (10.13)	12.50 (13.66)	7.67 (24.70)	11.58 (23.28)	12.5 (29.56)