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Original Article

Effect of ear canal pressure and age on wideband absorbance in young infants

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Abstract

Objective: The study investigated the effect of ear canal pressure and age on wideband absorbance (WBA) in healthy young infants. *Design*: Using a cross-sectional design, WBA at 0.25 to 8 kHz was obtained from infants as the ear canal pressure was swept from +200 to −300 daPa. *Study sample*: The participants included 29 newborns, 9 infants each at 1 and 4 months and 11 infants at 6 months of age who passed distortion product otoacoustic emissions test. *Results*: In general, negative-ear canal pressures reduced WBA across the frequency range, while positive-ear canal pressures resulted in reduced WBA from 0.25 to 2 kHz and above 4 kHz with an increase in absorbance between 2 and 3 kHz compared to WBA at ambient pressure. The variation in WBA below 0.5 kHz, as the pressure was varied, was the greatest in newborns. But, the variation was progressively reduced in older infants up to the age of 6 months, suggesting stiffening of the ear canal with age. *Conclusions*: Significant changes in WBA were observed as a function of pressure and age. In particular, developmental effects on WBA were evident during the first six months of life.

Key Words: Wideband tympanometry, wideband absorbance, tympanometry, middle ear, ear canal pressure

Introduction

Outer and middle ear of infants undergo rapid developmental changes in the first few months of life. Some of these developmental changes include increase in length and diameter of the external auditory canal (Qi et al, 2006), decrease in the cartilaginous portion and increase in the bony portion of the canal wall (Fung, 1993), changes in tympanic membrane orientation (Ikui et al, 1997), reduction in the thickness of the tympanic membrane (Ruah et al, 1991) and vascular and cellular content (Richany et al, 1954), increase in the volume of the tympanic cavity (Ikui et al, 2000) and increase in weight and size of the ossicles (Anson & Donaldson, 1981). Assessment of the conductive system (outer and middle ears) using a single-frequency measure, such as high-frequency tympanometry (HFT), does not reflect the maturational changes in infants. On the other hand, wideband immittance that uses a click signal and measures acoustic responses from 0.25 to 8 kHz has emerged as a promising tool for the assessment of middle ear function in neonates (Sanford et al, 2009; Hunter et al, 2010; Aithal et al, 2015).

Wideband absorbance (WBA) is one of the wideband acoustic immittance measures, which were designed to assess the function of the outer and middle ear. WBA represents the sound energy absorbed by the middle ear when a wideband stimulus is presented in the ear canal. It ranges from 1 (where the majority of the sound energy is absorbed by the middle ear) to 0 (where the majority of the energy is reflected back from the middle ear) (Stinson, 1990). Several studies have also shown that WBA could be used to detect middle ear disorders or conductive hearing loss in children (Keefe et al, 2000, 2003; Vander Werff et al, 2007; Hunter et al, 2008; Beers et al, 2010; Ellison et al, 2012; Feeney and Sanford, 2012; Keefe et al, 2012; Prieve et al, 2013).

WBA can be studied under both ambient and pressurised conditions. Several researchers have described developmental characteristics of WBA at ambient pressure in infants between birth and 12 months of age (Keefe et al, 1993; Hunter et al, 2008; Merchant et al, 2010; Werner et al, 2010; Aithal et al, 2014; Shahnaz et al, 2014). Nevertheless, ambient aural acoustic measures do not directly account for middle ear pressure effects. Measurement of WBA under pressurised condition [known as wideband tympanometry (WBT)] provides information about WBA under various ear canal pressures. WBT could provide a better

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Abbreviations

AABR	Automated auditory brainstem response
ANOVA	Analysis of variance
DPOAE	Distortion product otoacoustic emissions
HFT	High frequency tympanometry
OAE	Otoacoustic emissions
SPL	Sound Pressure Level
TPP	Peak tympanometric pressure
WAI	Wideband acoustic immittance
WBA	Wideband absorbance
WBA_{SP}	Wideband absorbance at a given static ear canal pressure
WBA_0	Wideband absorbance at ambient pressure
WBR	Wideband reflectance
WBT	Wideband tympanometry

understanding of the variations in acoustic measures caused by rapid developmental changes in the outer and middle ears compared to ambient WBA measures. It is important to distinguish between variations in WBA attributable to maturation aspects and those attributable to disorders in the conductive system (Kei et al, 2013).

To date, there have been very few studies that have systematically investigated the effect of ear canal pressure on WBA in newborns and infants (Sanford & Feeney 2008; Sanford et al 2009; Hunter et al, 2016). Sanford et al (2009) reported that WBA under pressurised conditions performed better than WBA at ambient pressure in predicting the conductive status of 230 neonates who passed or failed a distortion product otoacoustic emission (DPOAE) test. Nonetheless, they did not describe the variations of WBA under various ear canal pressures. Sanford and Feeney (2008) investigated the effect of ear canal pressure on WBA measurements in 4-, 12- and 24-week-old infants. They found minimal WBA changes with positive and negative pressure from 0.75 to 2 kHz for all age groups. However, between 2 and 6 kHz, negative pressures were associated with decreased absorbance and positive pressures were associated with increased absorbance. As such effects were not observed in other infant groups, the authors concluded that the outer and middle ear system of 4-week-old infants was still developing.

In a recent study, Hunter et al (2016) followed 182 infants longitudinally from birth to 12 months of age using WBA under ambient pressure and WBT. Their results showed large effects of age in the first six months. In particular, immature absorbance and group delay patterns were apparent in the low frequencies at birth and one month, but changed substantially to a more adult like pattern by 6 months of age for both ambient pressure and WBT.

To date, there have been very few studies on the effects of ear canal pressure on WBA in newborns and young infants at various age intervals during the first 6 months of life. Investigation of effects of pressure on WBA in young infants not only advances knowledge in the acoustic-mechanical properties of the outer and middle ear, but it also provides useful information on the maturation aspects of the outer and middle ear in this population. This information is very useful in differentiating normal from abnormal ears. Hence, it is necessary to understand variations in WBA as a function of ear canal pressure in healthy infants. The objective of the present study was to investigate the changes in WBA as a function of ear canal pressure using WBT in healthy newborns and young infants.

Methods

Ethical approval for the study was obtained from the Townsville Hospital and Health Service Institutional Ethics Committee and the University of Queensland Behavioural and Social Sciences Ethical Review Committee. Infants were recruited from the maternity unit at the Townsville Hospital. All the parents consented via a consent form approved by the Institutional Ethics committees.

Participants

All infants were born at term without any risk factors, with normal birth weight and no medical complications. The number of participants included in the data analyses were 28 newborns (36 ears), 9 infants aged 1 month (13 ears), 9 infants aged 4 months (13 ears) and 11 infants aged 6 months (16 ears). Details of participants included in the study are provided in Table 1.

Test environment

Testing was offered at birth with follow up testing at 1, 4 and 6 months of age. When an infant attended more than one appointment, data obtained at only one of the appointments were included for analysis. Hence, this study provides cross-sectional WBA data of infants at various age intervals. All tests with newborns were performed in a quiet room in the maternity unit. Evaluations with infants aged 1 to 6 months were performed in a sound treated booth at the Audiology department. The mean ambient noise level in the maternity room and the sound booths were 35.7 and 32.0 dBA, respectively. Infants at all ages were seen after feeding while in natural sleep or in an awake but quiet state.

For infants in each age group, only ears that passed a DPOAE test were included in the study. Although the number of infants recruited for this study included 23 infants (42 ears) at 1 month, 28 infants (52 ears) at 4 months and 30 infants (55 ears) at 6 months, the final numbers of ears available for analysis were low due to several factors, including inclusion of data from only one visit when the infant attended appointments at various ages, unsettled infant and incomplete test results. Our experience testing infants revealed that the older the infant, the more difficult it was to complete all testing (Table 1).

Test battery

DPOAE testing was performed using a Biologic Navigator Plus. DPOAEs were measured in response to pairs of primary tones with $F2$ set at 2, 3, 4 and 6 kHz. The $F2/F1$ ratio was 1.2 for each primary pair. The level of $F1$ was 65 dB SPL and $F2$ was 55 dB SPL. Pass criteria included (i) DPOAE-to-noise ratio of at least 6 dB in at least three out of four frequencies from 2 to 6 kHz (Sanford et al, 2009; Hunter et al, 2010) and (ii) DPOAE amplitude of at least -6 dB at 2, 3, 4 and 6 kHz (Sanford et al, 2009; Merchant et al, 2010).

WBT was carried out using a research prototype, Interacoustics Reflwin research system, which consisted of a Windows-based computer, a 24-bit resolution sound card, a pressure pump and controller system contained in an acoustic immittance instrument (AT235), and custom software for stimulus generation and data acquisition. Calibration was performed every day to determine the source reflectance and incident sound pressure associated with the probe and its transducers based on acoustic measurements in two

Table 1. Details of infants included in the study (inclusion criteria – pass in DPOAE).

	Number of subjects			*Number of ears*			*Age*		
Age group (Month)	*Male*	*Female*	*Total*	*Right*	*Left*	*Total*	*Mean*	*SD*	*Range*
0	18	10	28	19	17	36	37.42 h	13.8	11.30–64.38 h
1	3	6	9	7	6	13	33.4 days	3.9	31–42 days
4	7	2	9	5	8	13	125 days	10.9	113–147 days
6	10	1	11	8	8	16	180 days	8.4	169–190 days

rigid-walled cylindrical calibration tubes of lengths 226.7 and 61 mm, and same diameter of 4.76 mm as described by Keefe and Simmons (2003). The adequacy of each calibration was determined by the algorithm of the software. A calibration was accepted as long as the root-mean-squared reflectance error (ΔR) did not exceed 0.009 and the loss parameter χ was in the range from 1 to 1.09 (Keefe & Simmons 2003; Sanford et al, 2009). If the results of calibration were not accepted, the calibration was repeated until it was judged acceptable.

WBT measures were obtained under dynamic pressure condition wherein the ear canal pressure was swept from +200 to −300 daPa at a rate of 75 daPa/s. During the pressure sweep, acoustic responses were measured for a train of clicks with an inter-click interval of 46 ms. Approximately 145 acoustic responses were obtained across a pressure change of 500 daPa. During the test, visual display of the tympanometric curve alerted the tester for any leaks in the pressure seal. In addition, a visual prompt also alerted the tester for leaks with an option to either save the results or redo the test.

Data analysis

WBA was measured as a function of both frequency and ear canal pressure. The frequency grid consisted of a total of 60 frequencies from 0.25 to 8 kHz that were analysed in 1/12 octave intervals. The pressure grid included 101 pressures, spaced in 5-daPa increments, from +200 to −300 daPa. Thus, WBA was specified across 6060 pairs of frequency and pressure. For the purpose of analysis of this study, one-third-octave averaged WBA results were considered at seven ear canal pressures [−300, −200, −100, 0. +100 and +200 daPa and tympanometric peak pressure (TPP)]. This resulted in 112 (16 frequencies ×7 pressures) WBA data points for each ear. Mean WBA for each of the one-third octave frequency at each of the seven different ear canal pressures was determined for all four age groups.

Given the difficulty of completing all tests for infants, it was decided to include all ears that passed the test battery in the analyses. Given the relatively small numbers in all age groups except for the newborns and the large number of independent variables (4 age groups, 16 frequencies, 2 ears and 2 genders) for evaluation, it would be more appropriate to apply parametric statistical procedures, while acknowledging that the dependent variables (WBA under each condition) might not necessarily be normally distributed and of equal variances.

Results

An analysis of variance (ANOVA) with repeated measures was applied to the data to evaluate the effects of ear on WBA. In this model, for each pressure condition, ear served as between-subject factors, and frequency (16 levels) as a within-subject factor. The Greenhouse and Geisser (G–G) (1959) approach was used to compensate for the violation of compound symmetry and sphericity. The ANOVA results revealed that there was no significant main effect for ear ($p > 0.05$) across all age groups. In view of the insignificant ear differences, data were pooled across ears across all age groups.

Similarly, effect of gender was analysed using an ANOVA with repeated measures for each pressure condition in 0- and 1-month-old infants. Gender served as between subject factor and frequency served as within subject factor. The effect of gender was not significant ($p > 0.05$) for either the 0- or 1-month-old infants. The effect of gender was not analysed in the 4- and 6-month-old infants since there were more males than females in these age groups.

Figure 1 illustrates mean WBA plotted against one-third octave frequencies from 0.25 to 8 kHz for each age group at +200, +100, 0, −100, −200, −300 daPa and TPP. The TPP was obtained from the peak pressure that was automatically provided with the absorbance plot. The mean TPP was 4.86 daPa (SD =47.83 daPa, range 80 to −105 daPa) for 0-month-old infants, −7.69 daPa (SD =28.69 daPa, range = −50 to 35 daPa) for 1-month-old infants, −53.0.8 daPa (SD 57.17 daPa, range = −150 to 10 daPa) for 4-month-old infants and −31.6 daPa (SD =421.2 daPa, range = −105 to 30 daPa).

The mean WBA at TPP for 0-, 1- and 4-month-old infants exhibited multi-peaked patterns across various ear canal pressures except for the 0-month group for negative pressures. The multi-peaked pattern was less distinct for the 6-month-old group. The two WBA maxima for newborns (0-month) occurred at 1.25 and 5 kHz, while the maxima for the 1- and 4-month-olds occurred at around 2 and 5 kHz. In comparison, the WBA maxima for 6-month-olds occurred at 2 and 4 kHz. The mean WBA at ambient pressure (WBA_0) was very similar to that at TPP (WBA_{TPP}) throughout the frequency range for infants in all the age groups.

Standard deviations (SDs) were calculated as a measure of variability (Figure 2). In general, across all ages, SDs were slightly higher for negative-middle ear pressures for all age groups relative to ambient and positive-ear canal pressures. SD of WBA remained steady across the frequency range except at 6 and 8 kHz for 1-month-old infants, while there was an increase in SD with frequency for 0- and 6-month-old infants. The SD of WBA was highest between 0.8 and 2 kHz for the 4-month-old infants compared to other age groups.

In order to examine the effect of ear canal pressure on WBA, the difference between WBA at a given static pressure condition (WBA_{SP}) and WBA at 0 daPa (WBA_0) were analysed. The *Y* axis for the panels in Figure 3 illustrates WBA difference ($WBA_{SP}-WBA_0$) plotted against frequencies from 0.25 to 8 kHz for each age group. A positive value on the *Y* axis for right panel in Figure 3 indicates an increase in WBA at a given static pressure relative to WBA at 0 daPa, whereas a negative value indicates a decrease in WBA relative to WBA at 0 daPa. Generally, the

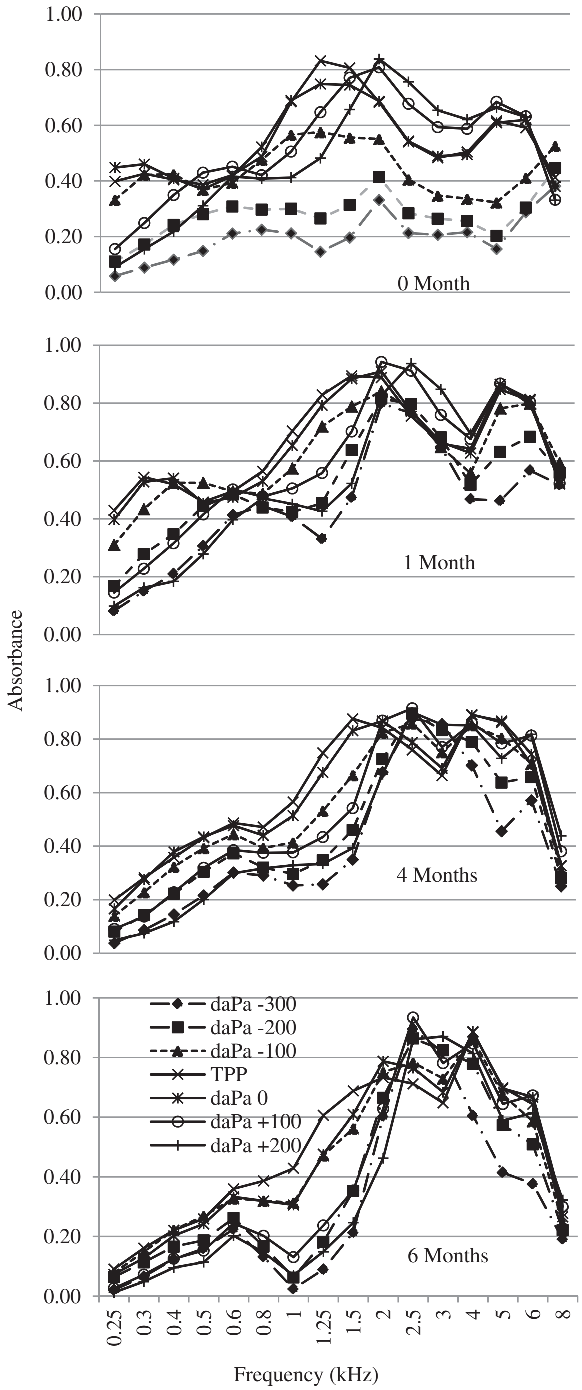

Figure 1. Mean WBA plotted as a function of frequency across various ear canal pressures for different age groups (0-, 1-, 4- and 6-month).

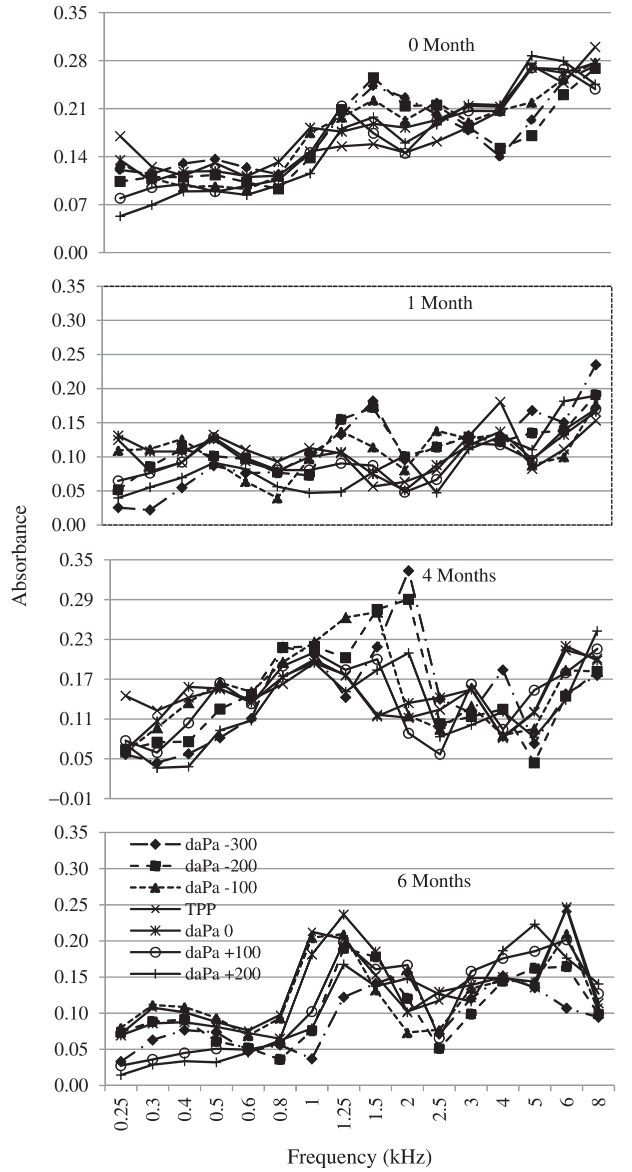

Figure 2. Standard deviation of WBA plotted as a function of frequency across various ear canal pressures for different age groups (0-, 1-, 4- and 6-month).

introduction of ear canal pressure (positive or negative) resulted in a reduction of WBA_{SP} relative to WBA_0. The greatest pressure induced difference in WBA for all age groups occurred between 1 and 1.5 kHz, and to a lesser extent between 4 and 6 kHz. In particular, positive-ear canal pressures resulted in reduced absorbance (relative to WBA_0) from 0.25 to 1.5 kHz and 4 to 8 kHz and increased absorbance between 2 and 3 kHz for infants aged between 0 and 6 months. The greatest reduction was observed between 1.25 to 1.5 kHz.

In comparison, negative-ear canal pressures demonstrated a different pattern of WBA across various age groups. WBA_{-100} showed only slight reduction of WBA compared to WBA at other negative-ear canal pressures. WBA_{-100} was reduced between 0 and 10% relative to WBA_0 across all age groups except for 0-month-old infants between 3 and 6 kHz (Figure 3). WBA_{-200} and WBA_{-300} were reduced compared to WBA_{-100} across the entire frequency range for 0-month-old infants. For 1-month-old infants, WBA_{-200} was reduced between 0.25 and 2 kHz whereas WBA_{-300} was reduced across the entire frequency range when compared to WBA_0. With 4- and 6-month-old infants, WBA_{-200} and WBA_{-300} were reduced from 0.25 to 2 kHz and 4 to 8 kHz, but increased at 2.5 to 3 kHz relative to WBA_0.

As illustrated in Figure 3, the WBA difference ($WBA_{SP}-WBA_0$) across the frequencies reduced with age.

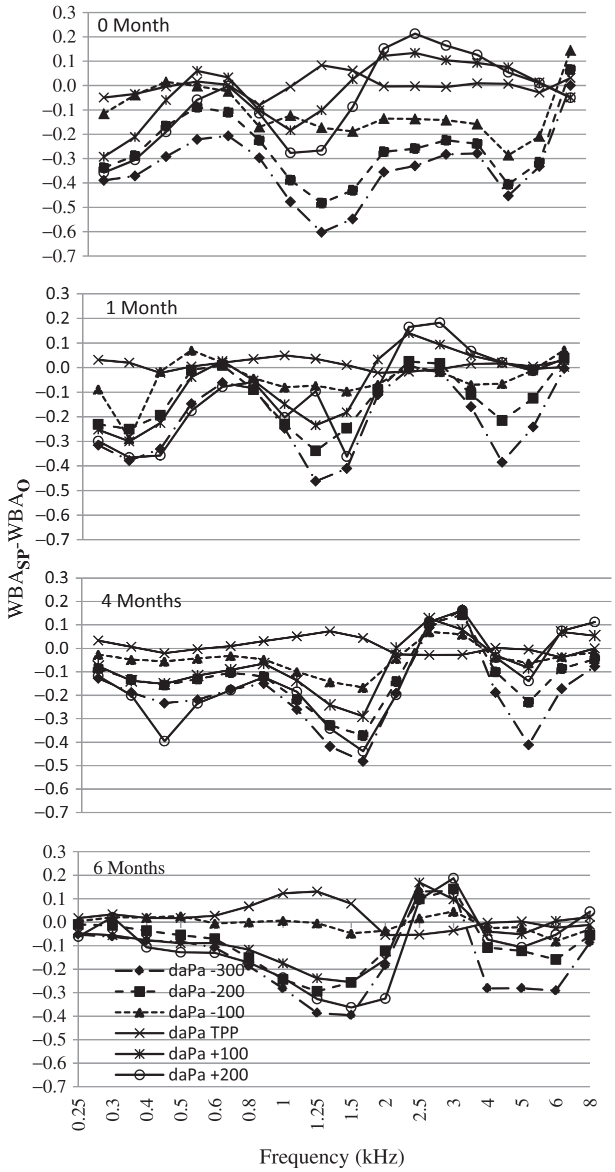

Figure 3. Mean WBA differences plotted as a function of frequency across various ear canal pressures for different age groups (0-,1-,4-,6-months and 4 to 6 years).

The 0-month-old infants demonstrated the largest variation of WBA (40 to 60%), while the 1-, 4- and 6-month-old infants' demonstrated moderate WBA variations (10 to 40%).

Mean WBA data were plotted along with standard errors of the mean for all age groups for 0daPa and TPP (Figure 4), positive pressures (+100 and +200) (Figure 5) and negative pressures (−100, −200 and −300 daPa) (Figure 6). The standard error of mean was large between 0.8 and 2 kHz with the 4-month-old infants especially for all the positive-pressure conditions.

A two-way analysis of variance (ANOVA) (age × frequency) with repeated measures was performed at each ear canal pressure to analyse the effect of (i) ear canal pressure on mean WBA, and (ii) pressure differences at various frequencies. An α level of 0.05 was used for all analyses. Frequency was used as a within-subject variable, and age and pressure difference as between-subject variables. The results indicated a significant age effect across the pressures and frequencies. *Post hoc* analyses using Bonferroni's correction were computed to test for significant differences between age groups for each frequency, and results are summarised in Table 2.

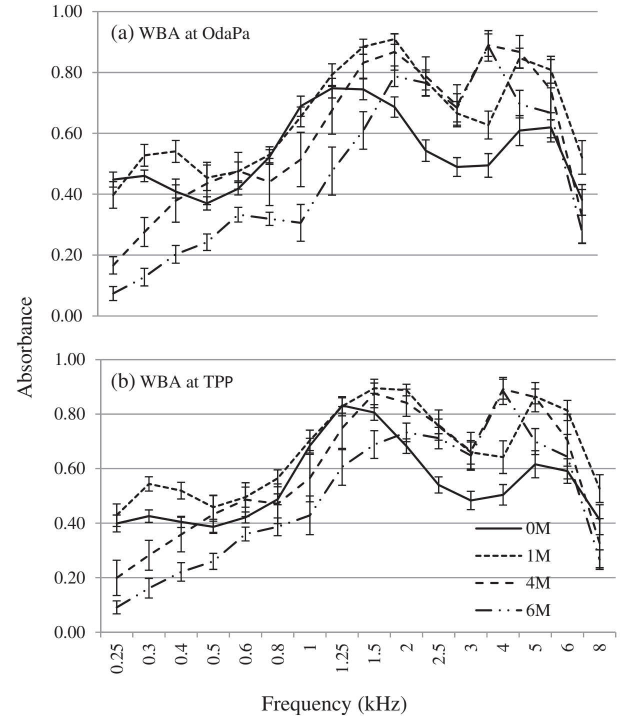

Figure 4. Mean WBA as a function of frequency for all age groups at 0 daPa and TPP. Error bars indicate ±1 standard error of mean.

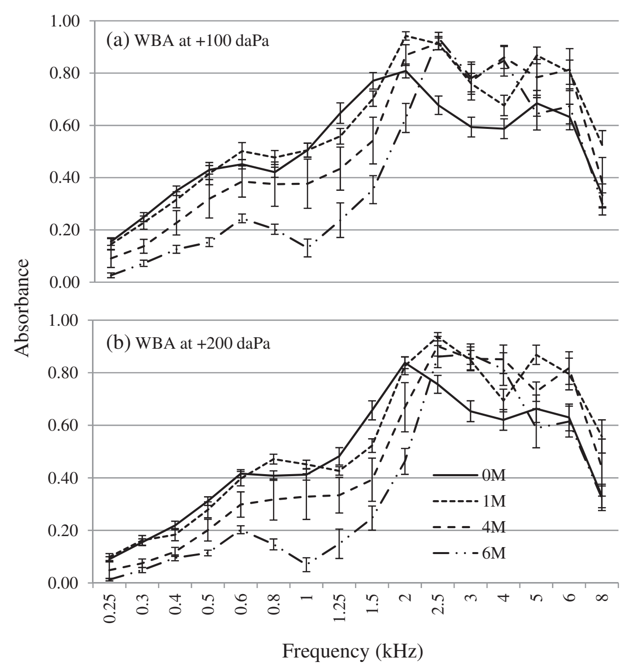

Figure 5. Mean WBA as a function of frequency for all age groups at positive-ear canal pressures, namely, +100 and +200 daPa. Error bars indicate ±1 standard error of mean.

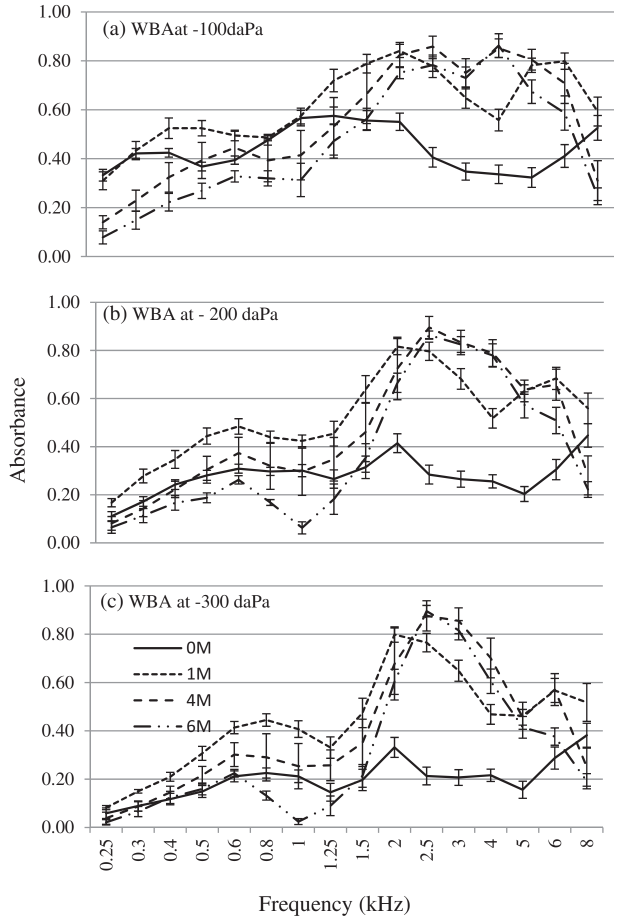

Figure 6. Mean WBA as a function of frequency for all age groups at negative-ear canal pressures, namely, −100, −200 and −300 daPa. Error bars indicate ±1 standard error of mean.

Table 2 illustrates the frequencies and pressures at which the mean WBA were significantly different between the age groups. In general, across all the pressure conditions, the WBA of newborns (0-month-old group) was significantly different between 0.25 and 5 kHz from WBA of 4- and 6-month-old infants, and was significantly different between newborns and 1-month-olds from 1.5 to 6 kHz (Figures 4–6). WBA of 1-month-old infants was significantly different from that of 4- and 6-month-old infants predominantly between 0.25 and 2 kHz. WBA of 4-month-old infants was also significantly different from that of 6-month-old infants between 0.25 and 2 kHz.

Next, an ANOVA with WBA differences between a given ear canal pressure and ambient pressure conditions as dependent variable, and age and frequency as independent variables was performed. The results indicated a significant age effect across the pressures and frequencies. *Post hoc* analyses with Bonferroni's correction were applied to test for significant differences between age groups for each frequency and results are summarised in Table 3.

As illustrated in Table 3, significant age effects were observed across WBA differences between ambient and ear canal pressure conditions except between WBA_{TPP} and WBA_0. Across all the infant age groups, significant differences were observed below 0.8 kHz and between 2 and 4 kHz. The difference in WBA was most evident between the 0- and 6-month-old infants compared to other infant groups.

Discussion

The present study described changes in WBA as the static ear canal pressure was varied between −300 to +200 daPa in 0-, 1-, 4- and 6-month old infants. Although, there is adequate research on WBA at ambient pressure (Keefe et al, 1993; Hunter et al, 2008; Merchant et al, 2010; Werner et al, 2010; Aithal et al, 2014; Shahnaz et al, 2014), to date, there have been very few developmental studies that describe WBA under pressurised conditions (Sanford & Feeney, 2008; Hunter et al, 2016).

The middle ear is expected to be more efficient at absorbing sound energy at TPP, where the mobility of the eardrum is maximal (Feeney & Keefe, 2012). WBA_0 was similar to WBA_{TPP} at most frequencies with the infants. This finding is similar to that reported by Sanford and Feeney (2008) and Hunter et al (2016). The similarity between WBA_{TPP} and WBA_0 is attributed to the fact that the mean TPP was close to the ambient pressure for all the infants (4.86 daPa in 0-month-old, -7.69 daPa in 1-month-old, -53.1 daPa in 4-month-old and -31.6 daPa in 6-month-old infants). It will be interesting to compare the WBA_{TPP} and WBA_0 in infants with negative-middle ear pressures. Margolis et al (1999) suggested that WBT is sensitive to middle ear status and therefore, with negative-middle ear pressures, it may be advantageous to assess the middle ear system at both ambient pressure and TPP.

Development of the infant's external and middle ear strongly influences impedance and absorbance measures (Keefe et al, 1993). Results of the present study concur with the findings of other developmental studies (Sanford & Feeney, 2008; Aithal et al, 2014; Shahnaz et al, 2014; Hunter et al, 2015) and illustrate maturational effects on WBA under various ear canal pressures. First, multi-peaked WBA curves that were observed at different ear canal pressures for 0-, 1- and 4-month-old infants were less clear in 6-month-old infants. Second, the WBA for 0- and 1-month-old infants differed significantly from 6-month-old infants, especially at frequencies below 800 Hz and between 2 and 4 kHz. Third, with younger infants (0- and 1-month-old), negative-ear canal pressures resulted in reduced WBA across the frequency range while positive pressures resulted in reduced WBA up to 2 kHz and increased WBA from 2 to 4 kHz. There were significant differences in WBA between each of the age groups in the study. However, the true nature of difference between the age groups may not be evident due to the small number of ears in infants in 1-, 4- and 6- month age groups. Fourth, the magnitude of change in WBA with both positive- and negative-ear canal pressures at and below 0.5 kHz was greatest for 0-month-old infants and reduced as the age increased from 0 to 6 months.

The differential effects of pressure found in the present study concur with the findings of Sanford and Feeney (2008) and Hunter et al (2016) who have reported similar findings with WBT in infants. The differential pressure effects in young infants are due to anatomical differences in their outer and middle ears. The decrease in WBA in low frequencies with age has been attributed to the stiffening of the compliant ear canal wall due to the development of the osseous portion of ear canal, which becomes stiffer with the introduction of ear-canal pressure and in turn absorbs less acoustic energy (Keefe et al 1993; Keefe and Simmons, 2003; Sanford and Feeney, 2008).

Table 2. Results of *post hoc* analyses with Bonferroni's correction indicating the ages (in months) at which mean WBA of infants were significantly different from each other ($\alpha = 0.05$).

Frequency (kHz)	*Ear canal pressure (daPa)*						
	−300	*−200*	*−100*	*0*	*TPP*	*100*	*200*
0.25	1–6	0–6,1–4,1–6	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–6,1–6,4–6	0–4,0–6,1–6
0.3	1–6	0–1,0–6,1–4,1–6	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6	0–1,0–4,0–6,1–4,–6,4–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6, 4–6
0.4	0–1,1–6	0–1,1–4,1–6	0–1,0–4,0–6,1–4,–6,4–6	0–1,0–4,0–6,1–4,1–6	0–1,0–6,1–4,1–6,4–6	0–4,0–6,1–6,4–6	0–4,0–6,1–4,1–6
0.5	0–1,1–6,4–6	0–1,0–6,1–4,1–6,4–6	0–1,0–6,1–6,4–6	0–1,0–4,0–6,1–4,1–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6, 4–6
0.6	0–1,0–4,1–6,4–6	0–1,0–6,1–6,4–6	0–1,0–6,1–6,4–6	0–6,1–6,4–6	0–6,1–6,4–6	0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6, 4–6
0.8	0–1,0–6,1–6,4–6	0–1,0–6,1–6,4–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6,4–6	0–6,1–6,4–6	0–6,1–6,4–6	0–1,0–6,1–4,1–6, 4–6
1	0–1,0–6,1–6,4–6	0–6,1–6,4–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6,4–6	0–6,1–6,4–6
1.25	0–1,0–4,1–6,4–6	1–6,4–6	0–6,1–6	0–4,0–6,1–4,1–6	0–6,1–6,4–6	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–6,4–6
1.5	0–1,0–4,1–6,4–6	0–1,0–4,1–6,4–6	0–1,1–6	0–1,0–4,0–6,1–4,1–6	1–6,4–6,4–6	0–4,0–6,1–4,1–6,4–6	0–1,0–4,0–6,1–4,1–,4–6
2	0–1,0–4,0–6,1–6	0–1,0–4,0–6,1–6	0–1,0–4,1–6,4–6	0–1,1–4,1–6	0–1,0–4,1–6	0–1,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6,4–6
2.5	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6,4–6	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–6,1–4
3	0–1,0–4,0–6,1–4	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6,1–6	0–4	0–1,0–6
4	0–1,0–4,0–6,1–4,4–6	0–1,0–4,0–6,1–4	0–1,0–4,0–6,1–4,1–6	0–4,0–6,1–4,1–6	0–1,0–4,0–6,1–4,1–6	0–4,0–6,1–4	0–4
5	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6,1–6,4–6	0–1,0–6	0–1,1–6	0–1,1–6	0–1,1–4,1–6,4–6
6	0–1,0–4,1–6,4–6	0–1,0–4,1–6,4–6	0–1,0–4,1–6,4–6	0–1,1–6,4–6	0–1,1–4,1–6	0–1,1–6	0–1,1–6
8	1–4,1–6	0–6,1–4,1–6	0–4,0–6,1–4,1–6	0–1,1–4,1–6	0–6,1–4,1–6	0–1,1–4,1–6	0–1,1–6

An increase in WBA at high frequencies (above 2 kHz) with positive-ear canal pressures that is reported in 4-week old infants in Sanford and Feeney (2008) study concurs with results of the present study and Hunter et al (2016). Sanford and Feeney hypothesised this marked increase in WBA to a combination of factors associated with ossicular chain, namely, its coupling, histological immaturity and changes in orientation, compared to older infants. They suggested that the negative-ear canal pressures in these infants could lessen the efficiency of middle ear system by functionally disarticulating the ossicular chain as the tympanic membrane is pulled outward into the ear canal, thus, accounting for decrease in WBA with increasing negative pressure. They also suggested that positive-ear canal pressure could enhance ossicular coupling due to the ossicles being pushed more closely together. This could result in a more acoustically efficient middle ear transmission system due to better coupling of the ossicles in the pressurised infant middle ear and thus more closely matching the impedance of the ear canal that is shown as an increase in WBA between 2 and 6 kHz.

The compliant properties associated with the ear canal wall motion of neonates with the introduction of ear canal pressure are thought to influence the interpretation of WBA measurements, particularly, at frequencies below 1 kHz. Therefore, researchers recommend that WBA results at frequencies below 1 kHz should be weighted less strongly in predicting middle ear dysfunction (Piskorski et al, 1999; Keefe et al, 2000; 2003). Sanford and Feeney (2008) found that WBA in the range of frequencies from 0.8 to 2 kHz did not change significantly with age, and therefore, suggested that this frequency range may be the frequency region of interest in future studies. The results of the present study also found that the effects of pressure were most noticeable below 0.8 kHz and above 2 kHz. Several ambient WBA studies in neonates have reported that the absorbance was highest between 1 and 4 kHz compared to that at frequencies below 1 kHz and above 4 kHz (Keefe et al, 2000; Vander Werff et al, 2007; Shahnaz, 2008; Sanford et al, 2009; Hunter et al, 2010; Aithal et al, 2013). In view of differential frequency effect on WBA, future studies could focus on the changes in absorbance at frequencies between 1 and 4 kHz during evaluation of middle ear function in neonates and young infants. Previous studies have indicated that WBA at this frequency range provide the greatest discriminability between the ears with a DPOAE pass or refer result (Sanford et al, 2009; Hunter et al, 2010; Aithal et al, 2015).

WBA attained the lowest values at −200 and −300 daPa with the neonates with a relatively flat pattern (approximately 0.3 at −200 and 0.2 at −300 daPa) across frequencies. Highly reduced WBA at these extreme negative-ear canal pressures suggest the possibility of ear canal collapse in neonates. Murakoshi et al (2013) and Aithal et al (2016) studied dynamic behaviour of conductive system using sweep frequency impedance and reported that more than 90% of ears showed signs of collapsed ear canals at −200 daPa.

The present study demonstrated significant changes to WBA as a function of pressure in infants who passed a battery of tests. The differential effects of pressure and frequency suggest that the outer/middle ears of young infants respond differently to changes in pressure compared to older infants. It is important to distinguish between variations in WBA attributable to maturation aspects and those attributable to disorders in the conductive system (Kei et al, 2013). Since the maturational effects have been described in this study, the results can be used to differentiate the variations in WBA

Table 3. Results of *post hoc* analyses with Bonferroni's correction indicating the ages (in months) at which WBA differences ($WBA_{SP}-WBA_0$) in infants were significantly different from each other ($\alpha = 0.05$).

	Ear canal pressure (daPa)					
Frequency (kHz)	*−300*	*−200*	*−100*	*TPP*	*100*	*200*
0.25	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6,4–6	0–6		0–4,0–6,1–4,1–6,4–6	0–1,0–4,0–6,1–4,1–6.4–6
0.3	0–4,0–6,1–4,1–6,4–6	0–4,0–6,1–4,1–6,4–6	1–6	0–4	0–1,0–4,0–6,1–4,1–6	0–4,0–6,1–4,1–6,4–6
0.4	0–4,0–6,1–4,1–6,4–6	0–6,1–6			0–1,1–4,1–6	0–1,0–6,1–4,1–6,4–6
0.5	0–1,0–6,4–6	0–6,1–4,4–6			0–1,0–4,0–6,1–4,4–6	0–1,0–4,4–6
0.6	0–1,0–4,0–6,1–4	0–1			0–1,0–4,0–6,1–4,1–6	0–1,0–4,0–6,1–4,4–6
0.8	0–1,0–4,0–6	0–1,0–4,0–6		0–1,0–4,0–6	0–4,1–6,4–6	1–6
1	0–4,0–6,1–4	0–4,0–6		0–4,0–6		0–4
1.25	0–4,0–6,1–4,1–6	0–4,1–4	0–6	1–4	0–4	
1.5	0–1,0–4,0–6,1–4,4–6	0–1,0–4,0–6	0–1,0–4,0–6	0–4,1–4,4–6	0–4,0–6,1–6,4–6	0–1,0–4,0–6
2	0–1,0–40–6,1–6	0–1,0–4,0–6	0–1,0–4		0–1,0–6,1–6	0–1,0–4,0–6,1–4,1–6
2.5	0–1,0–4,0–6	0–1,0–4,0–6,	0–1,0–4,0–6		0–6	0–4
3	0–1,0–4,0–6	0–1,0–4,0–6	0–1,0–4,0–6		0–4,1–4	0–4,4–6
4	0–1,0–4,1–6	0–1,0–4,0–6	0–1,0–4,0–6		0–1,0–4,0–6,1–4	0–4,0–6,1–4,1–6,4–6
5	0–6	0–4,0–6	0–1,0–4,0–6		0–4,0–6,1–4,1–6	0–4,0–6,1–4,1–6
6		0–4,0–6	0–1,0–4		0–6	
8			0–1,0–4,0–6,1–4		0–1,0–4,0–6,1–4	0–1,0–4,0–6

A blank space indicates the lack of a statistically significant difference between any of the infant age groups.

due to conductive pathology during assessment of middle ear function in infants.

While such qualitative measures may be useful for comparison of individual cases, further quantitative measures are needed for interpretation of results in a clinical setting (Sanford et al, 2013). Lack of clinical use of WBT in neonates can partly be attributed to the lack of fast and efficient methods for interpreting WBA results. If WBT is to be used as an effective clinical tool, it is important to develop efficient approaches to interpret results quickly as well as identify predictive indices from the large amount of data obtained from WBT.

Strengths and limitations

This is one of the few studies to analyse the effects of ear canal pressure on WBA in healthy newborns and compare with infants who passed both the HFT and DPOAE tests. Since the WBT provides information about the middle ear over a range of pressures and frequencies, the normative patterns provided in the present study may be useful in understanding age-related changes while assessing middle ear function in this population.

The study had some limitations that could have been affected the interpretation of results. First, the sample size was small for infants at 1, 4 and 6 months of age. Furthermore, there were more males than females in the 4- and 6-month-old groups. Consequently, this limits the generalisation of the results to this population. Hence, it is necessary to develop age-specific normative data by using large sample sizes with equitable gender distribution and establish middle ear reference standards, such as acoustic reflex threshold testing along with tympanometry, as well as diagnostic otoacoustic emission tests.

Second, when an infant passed the test battery in both ears, data from both ears were included since analyses showed no significant differences between right and left ears. Although this maximised available data, analyses should be interpreted with the knowledge that there could be some correlation between measurements from right and left ear of an infant.

Third, in the study, only a few pressure and frequency points from a large data set of 6060 samples were selected. However, it is not known whether significant diagnostic information is thereby lost in this process. Further studies are needed to determine if the seven pressure values used in the present study are adequate to differentiate ears with and without middle ear dysfunction.

Lastly despite the similarity of results, there were methodological differences between the present study and other developmental studies. While the present study measured WBA at absolute ear canal pressures, Sanford and Feeney (2008) measured WBA at ear canal pressures relative to TPP. The present study studied WBA in a cross sectional design while Hunter et al's (2016) study was longitudinal. Further, age range of participants in each of the age groups in Hunter et al's (2016) study was larger than the present study.

Summary

In conclusion, the present study demonstrated effects of ear canal pressure on WBA in young infants and children with normal middle ear function. Positive- and negative-ear canal pressures had differential effects on WBA in younger infants compared to older infants and children, suggesting a maturational effect due to development of outer and middle ear system in the first few months of life. Future studies need to develop normative quantitative WBT measures that can assist in the quick and easy interpretation of the middle ear status in neonates.

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